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Progress in fur animal science

Proceedings from the Vth International Scientific Congress in Fur Animal Production

Organiced by IFASA

Norwegian Agricultural Advisory Service, Ås, Norway

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Preface

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The International Fur Animal Scientific Association (IFASA) was established in 1989, following a proposal made by the Fur Animal Division of the Scandinavian Association of Agricultural Scientists at the IVth International Scientific Congress in Fur Animal Production in Canada in 1988.

The objective of IFASA is to promote knowledge of all aspects of fur animal science and the fur industry by encouraging the exchange of information among scientists with an interest in fur animals. An important function of the IFASA is therefore to organize international cooperation in fur animal science and to coordinate and arrange international scientific meetings and congresses. This congress is the Vth International Scientific Congress in Fur Animal Production, the first having been held in Helsinki in 1976. However, it is the first international congress to be arranged by IFASA.

These Proceedings from the Vth International Scientific Congress in Fur Animal Production contain the full text of contributions accepted for oral presentation or as posters. A total of 90 papers are published in this volume, and this reflects the large body of information resulting from research in the area of fur animal science. The papers cover a wide range of topics relating to the five working groups of IFASA: (1) Breeding, reproduction and genetics, (2) nutrition, (3) pathology and diseases, (4) ethology and welfare and (5) fur properties. The members of the Scientific

Committee which is responsible for the scientific programme and the Proceedings are:

Prof. Dr. Anders Skrede (Chairman)
Dr. Bjarne O. Braastad
Prof. Dr. Einar J. Einarsson
Dr. Jan A. Fougner
M.Sc. Kai-Rune Johannessen
Prof. Dr. Adrian Smith
M.Sc. Morten Bakken (Secretary)

We thank all authors who submitted manuscripts. Unfortunately some manuscripts could not be accepted mainly because of late submission, and a large number of editorial changes have been made. We trust that this will be accepted by authors and readers.

We take this opportunity to thank the Norwegian Agricultural Advisory Service for financial support and excellent cooperation during the preparation of the proceedings. In particular, we thank the managing editor of the Norwegian Journal of Agricultural Sciences, Jan A. Breian, for his enthusiasm and great flexibility. Thanks are also extended to George Drennan for language corrections.

Breeding, reproduction and genetics

Progress and challenges in the physiology of reproduction in furbearing carnivores

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There has been substantial progress in the understanding of the reproductive biology of furbearing carnivores in recent years. However, much of the physiology remains unknown and information in a number of areas could be used in strategy to improve production. It has been determined that melatonin is the endocrine message which regulates seasonal breeding, but it is not known on which tissues it acts in furbearers, nor by what mechanisms its effects are mediated. The dynamics of follicle development are not well understood in any species of carnivore and these are of particular interest in mink, as these animals appear to be able to ovulate on almost any day of the breeding season. The processes of embryonic diapause, embryo attachment and invasion of the uterus at implantation require investigation. This article describes these problems in further detail and discusses potential approaches to aid in their resolution.

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The methods for breeding of fur animals were derived empirically by those who domesticated these species during the last century. An understanding of the biological basis for reproduction came much later. At the First International Congress of Fur Animal Production in 1976 the late Dr. Cyril Adams identified the principal problems in mink reproduction (Adams 1976). Sixteen years have passed and, despite substantial progress in several areas, many of these problems remain unsolved. Reproduction in the mink is yet restricted to a brief annual window and there remains a high incidence of both embryo mortality and male infertility. Perinatal mortality approaches 25%, far beyond levels deemed acceptable in other domestic animals. In the face of considerable progress in manipulation of reproduction in other species, there has been little improvement or technological change in mink reproductive management.

The picture is somewhat brighter with respect to the fox. Advances have been made in artificial insemination of foxes (Fougner 1984), and for many years it has been possible to produce hybrid animals and to freeze semen. Yet, relatively little is known about ovulation, ovum and embryo transport, and embryo attachment. There is also a high incidence of embryonic and perinatal mortality in the fox.

There have been some recent reviews on mink reproduction, notably those by

Sundqvist et al. (1988, 1989), and Murphy (1988). No such summaries appear to have been published on the topic of fox reproduction. In the present review, I will try to summarize the new findings in furbearer reproduction of the last few years. Then I will attempt to delineate some of the important areas that require further investigation, and discuss approaches to meeting these challenges.

NEW INFORMATION AND UNRESOLVED PROBLEMS

Seasonal breeding of carnivores

Most mammals in temperate latitudes display an annual cycle of reproductive function, entrained by photoperiod. In the cow and the domestic dog, centuries of domestication have resulted in selection for animals that breed the year around. To alter the physiology of carnivores for breeding throughout the year is an important, if overly optimistic goal. Its achievement would facilitate the application of modern biotechnology to fur animal production.

The breeding season of the mink occurs over a 3-4-week period around the vernal equinox. The process of testicular development in male mink requires short-day photoperiod, and there has been significant progress in determining the mechanisms by which photoperiod induces reproductive events in this species. The resonant photoperiod model, which states that the coincidence of daily light with a photosensitive period in the circadian rhythm of the animal is responsible for photoperiod-influenced events, has been shown to be valid for the male mink (Boissin-Agasse et al. 1986).

The *zeitgeber* in mink, as in most other species, is the pineal gland. Its ablation (Boisson-Agasse et al 1988), or deafferentation (Maurel et al. 1990) profoundly affects the development of annual reproductive function, if performed before the beginning of testicular recrudescence (15 September). Pinealectomy in July completely prevents the development of the testes and, thus, reproductive function (Boisson-Agasse et al. 1988). Melatonin, which has been localized in the mink pineal (Tillet et al. 1989), has been generally accepted as the pineal effector in mink. It induces precocious maturation of the mink testis, if administered chronically beginning within the first 10 weeks of life (DiGregorio & Murphy 1987). However, when chronic melatonin is administered beginning at the time of initiation of testicular growth (1 October), there is no effect on gonadal development, or on circulating levels of luteinizing hormone, prolactin or testosterone (DiGregorio et al. 1992). Melatonin probably acts, at least in part, on the central nervous system in mink, via the superchiasmatic nucleus of the hypothalamus, which has been shown to be necessary for photoperiod-induced alterations in reproductive function in this species (Maurel et al. 1990).

The recent literature contains similar studies in silver and blue foxes. Smith et al. (1987a) reported profound changes in testicular cycles, largely the prolongation of the breeding condition following treatment of blue foxes with exogenous melatonin. Studies in the silver fox demonstrated that chronic treatment with melatonin induces precocious development of the fox testis (Forsberg et al. 1990), and that short days inhibit the annual regression of the testis (Forsberg et al. 1989). Melatonin alters the patterns of prolactin and the response to GnRH in the blue fox (Smith et al. 1987a), and the pattern of plasma testosterone in the silver fox (Forsberg et al. 1990).

In female mink, chronic treatment with melatonin beginning at ten weeks of age had no effect on the attainment of puberty (Murphy et al. 1990). Implantation of the mink embryo is induced by prolactin (Murphy et al. 1981), and influenced by photoperiod (Murphy & James 1974). Melatonin inhibited the secretion of prolactin necessary for the initiation of implantation (Murphy et al. 1990), an effect that could be reversed by administration of exogenous prolactin. The pivital role of melatonin in gestation was demonstrated in elegant experiments by Bonnefond et al. (1990). They showed that short photoperiods inhibited prolactin secretion during gestation. This inhibition could be reversed by deafferentation of the pineal, and replaced by infusion of melatonin.

In spite of this array of information, it is not known how darkness induces melatonin synthesis in carnivores, whether melatonin acts solely in the brain (Meunier et al. 1988; Bonnefond et al. 1990) or also has pituitary and peripheral effects on reproductive function. Labelled melatonin binds to the pars distalis of the pituitary of the sheep (Morgan et al. 1989) to the pars tuberalis and pars distalis, but not the brain, of the ferret (Weaver & Reppert 1990). Nothing is known about melatonin receptors in the mink or fox. There is little information on the mechanism of action of melatonin in any species, let alone the carnivores. It is not clear how melatonin inhibits prolactin secretion, or induces early furring. The mechanisms by which melatonin sustains testicular function, and the regression which will subsequently occur despite the presence of chronically elevated melatonin levels, are other mysteries which remain to be solved.

Ovarian function in mink

Some progress has been made in the understanding of the follicular and luteal events which occur in the mink ovary during the breeding season. In most species of mammals, the ovarian events of the reproductive cycle can be inferred from behavioral events. This approach has not been successful in mink because the female displays few overt signs of estrus, and can be mated at almost any time during the breeding season. The phenomenon of superfetation is another interesting character of mink reproduction, and the minimum interval for successful breeding of six days between ovulations was established many years ago by Hansson (1947).

The follicular events during the breeding season (Lagerkvist et al. 1992), and between matings (Douglas et al. 1992) have recently received attention. It would appear from both these studies that there are always follicles of 0.6-0.8 mm diameter present in the ovaries of unbred animals, and from this pool come the ovulatory follicles which develop as a result of the mating stimulus. If the process of follicular development is synchronized by hormonal induction of ovulation, a pattern emerges (Douglas et al. 1992). The number of small antral follicles (0.4-0.6mm diameter) increases soon after ovulation,and these are in greatest abundance by day 4 after the ovulation. Follicles of 0.8-1.0 mm diameter are most frequent on the ensuing three days (days 5-7 after ovulation). Follicles with a diameter $>$ 1.0 mm occur only after the ovulatory stimulus has been applied. Thus, there appears to be a recruitment phase, beginning two days after ovulation, when the complement of follicles begins to grow. There is then a selection phase, beginning on day 4 or 5, and the selected follicles grow to the size at which they can respond to the ovulatory stimulus by day 6. Both Lagerqvist et al. (1992) and Douglas et al. (1992) identified luteinization as the process by which follicles larger than 0.5 mm in diameter become inactive and subsequently degenerate.

Mink can breed and ovulate any time during the breeding season except for the first six days following mating. The question of whether follicles develop and remain large, whether there is continuous development, or whether there are discrete or overlapping waves of follicle development during the breeding season in the mink, remains unanswered. The occurrence of luteinized follicles undergoing degeneration in unmated mink (Lagerqvist et al. 1992; Douglas et al. 1992) strongly suggests that there is turnover of follicular populations during the breeding season. A pattern of elevated estradiol concentrations prior to the breeding season and some two weeks after mating, further led Lagerqvist et al. (1992) to postulate that there were waves of follicular development in the mink.

Uterine-maternal interactions and delayed implantation

The mammalian embryo is transported to the uterus at a stage of development which is characteristic for each species. In the mink, the process of preimplantation development has been delineated in the epic works of Hansson (1947) and Enders (1952) and more recently by Gustafsson et al. (1987). Fertilization takes place in the oviduct, approximately 50-60 h after mating. Mink embryos progress rapidly down the oviduct during the first 12 h after fertilization, to arrive in the upper reaches of the uterine horns six or seven days later. During this transit, the zygote develops into a blastocyst of 200-500 cells, and mitotic division ceases. Embryonic development is arrested, a condition known as embryonic diapause.

The endocrine factors which control embryonic diapause, or delayed implantation, have been partially determined. As noted above, the increasing photoperiod associated with the vernal equinox is the proximal influence, but is not absolutely essential (Murphy & James 1974). Implantation is mediated by a reduction in melatonin (Bonnefond et al. 1990; Murphy et al. 1990), and expressed as an increase in hypophysial release of prolactin (Martinet et al. 1983). This prolactin reactivates the corpus luteum (Papke et al. 1980). The luteal compartment of the ovary produces progesterone and other factor(s) necessary for the induction of implantation (Murphy et al. 1983). The elevations in prolactin and progesterone precede implantation by a few days (Martinet et al. 1981).

A number of mysteries remain. Daniel (1971) demonstrated that stress during diapause terminates gestation. A further delay in implantation ensues in animals handled daily for blood sampling, in spite of the occurrence of the prolonged elevation in both prolactin and progesterone (Murphy 1983). It has been previously shown that the pituitary is necessary for the support of the mink corpus luteum after implantation (Murphy et al. 1980), but the nature of this luteotrophic effect is not known. Luteal function, as indicated by progesterone levels, spontaneously declines over the period of postimplantation gestation, and the mechanisms of its demise remain undiscovered.

Reciprocal transfers by Chang (1968) demonstrated that mink embryos in diapause resumed development in the ferret uterus. Further, there was an arrest in development of ferret embryos, which do not normally display diapause, when they were transferred to the mink uterus. Thus, the control of delayed implantation appears to reside in the uterus. The nature of this control is unknown, but two possibilities come to mind. First, embryo development may be inhibited by a uterine factor, resulting in embryonic diapause. Alternatively, the embryo may be inherently inhibited at the blastocyst stage, and a stimulatory uterine signal is necessary to reinitiate development. The effects of stress may be

visited directly on the uterus, preventing the appearance of the signal which reinitiates embryogenesis.

NEW TECHNOLOGY

Investigators of fur animal reproduction have been quick to employ new technology as it has become available. Radioimmunoassay (Möller et al. 1973), immunolocalization (Murphy et al. 1976) and DNA flow cytometry (Smith et al. 1987b), to mention but a few, have been used to resolve questions in the physiology of fur animals. In recent years there has been an explosion of technology in molecular biology. As noted below, molecular methods are powerful tools to answer many questions relevant to carnivore reproduction. For example, Southern and Northern hybridizations can be used to identify specific gene (DNA) and RNA sequences, and ribonuclease protection assays can be employed to quantify RNA, in order to evaluate the rate of expression of any genes in which we are interested. The translation of mRNA to protein can be evaluated by Western blotting, and the site of production of a particular mRNA or protein, can be ascertained respectively by *in situ* hybridization and immunolocalization. The amplification and identification of specific DNA sequences by the polymerase chain reaction (PCR) is a method without parallel in sensitivity for the determination of the presence of specific gene products. By this means, a single transcript can be amplified and identified.

The possibility of altering the embryonic genome by insertion of genes has been well established in the mouse and has been used as a research tool to investigate the tissue specific expression of genes. The obvious application for this technology in fur animals is in the making of specific changes to the inheritance of individual animals. One of few genes that has been cloned in mink is for growth hormone (Harada et al. 1990). Insertion of further growth hormone genes has been shown to increase the size of offspring in mice (Palmister et al. 1982). Development of transgenic mink that secrete growth hormone from extrapituitary tissues may be an effective way to increase the size or feed efficiency of the animals.

Transgenic technology can also be used as a tool to study the expression or the role of specific genes. Mice have been rendered transgenic for growth hormone or insulin-like growth factors in order to study the interrelationship of these two growth promoting agents (Behringer et al. 1990; Camacho-Hubner et al. 1991). The study of virtually any gene can be undertaken by inducing its overproduction or its absence by transgenic technology.

EXPERIMENTAL APPROACHES

A few of the unresolved problems of fur animal reproduction have been described in the earlier portions of this review. Some, but by no means all, experimental approaches to their solution can be proposed.

Seasonal breeding

The foregoing discussion demonstrates that the key to understanding the basics for seasonal

reproduction in carnivores is melatonin. Continued studies of the neuroendocrine characteristics of seasonal breeders are essential to the understanding of this regulation. There is a need for better definition of the sequence of induction of seasonal gonadal development, including the changes in gonadotrophin secretion, the potential feedback effects of steroids and gonadal proteins, and the short-term effects of melatonin in initiation of gonadal recrudescence. By frequent monitoring of circulating testosterone, it may be possible to determine the beginning of testicular regression, and then the concurrent neuroendocrine conditions, such as response to GnRH, prolactin levels, etc., can be studied to evaluate that crucial moment.

It is essential to determine whether melatonin acts on single or multiple loci in carnivores, whether it acts only on the brain, or on the pituitary and gonads as well. The investigation should include determination of the location of melatonin receptors by autoradiography or by ligand-binding studies. As noted above, the role of melatonin on gestation in mink appears to be solely the suppression of the secretion of prolactin. The effects on males are more complicated, as they cannot be reversed by excess prolactin (DiGregorio et al. 1992). Further *in vitro* investigation to determine whether melatonin directly affects gonadotrophic hormone synthesis or steroidogenesis could enlighten us about its action.

Ovarian events

The experimental means to meet the abundant challenges in ovarian function in carnivores are not entirely clear. The sequence of folliculogenetic events has been studied by determination of peripheral hormone levels, and by histologic examination of ovaries. Neither of these approaches is without drawbacks. In larger species, it has been possible to study follicular changes by serial ultrasonographic examination of the ovaries (Pierson & Ginther 1984), but current ultrasound equipment lacks the sensitivity for similar studies in mink. Molecular methods provide the potential for more definitive determination of the state of follicle development. For example, the onset of the expression of the genes for the LH and FSH receptors can be determined by Northern and *in situ* analysis. The onset of steroid synthesis and the product of steroid synthesis (androgens vs estrogens) can be studied by analysis of the expression of genes for steroidogenic enzymes. A better indication of the mechanisms of atresia would result from molecular investigation of paracrine and autocrine effectors of follicle function such as angiotensin (Li et al. 1992), or epidermal growth factor (Lindsell 1992).

Embryo-uterine interactions

The invasion of the uterus by embryonic components requires intimate interaction between the individual cells of the embryo and those of the endometrium. The mink embryo, quiescent for the period of delayed implantation, responds to some endometrial signal, that reinitiates, or allows the reinitiation of, its development. Adhesion of the carnivore embryo to the uterus precedes invasion (Enders & Schlafke 1972) but the nature of this attachment is obscure. The simplest hypothesis is that the uterus produces a surface glycoprotein which adheres to the glycoprotein zona pellucida of the blastocyst. This hypothesis could be tested by determining whether any of the several glycoprotein adhesion molecules, such as fibronectin, laminin or elastin (Carey 1991; Findlay et al. 1990) are present on the luminal

surface of the endometrial epithelium at the time of attachment, and whether any of these interact with the mink zona pellucida.

Embryo adhesion may also be a receptor interaction, and there is ample evidence to indicate that extracellular matrix glycoproteins bind to membrane receptor proteins, including integrins, laminin binding protein, and cell surface proteoglycans (Carey 1991). The hypothesis in this case is that the endometrial receptor recognizes the acellular mucoprotein coat of the blastocyst. It has been shown that the endometrial glycoprotein, laminin interacts with trophoblast proteins in humans (Lala & Graham 1990). Fractionation and labelling of zona pellucida components, followed by assessment of binding of these potential radioligands to endometrial sections or homogenates may resolve this issue. The best known of the membrane glycoprotein binding proteins are the integrins, a large family of proteins for which many of the gene sequences are known (Ruoshlati 1988; Mecham 1991). *In situ* hybridization, and/or PCR amplification will determine the occurrence of the genes for integrin or other extracellular matrix binding proteins in the endometrium at the time of attachment of the mink embryo.

A more perplexing question is whether the mink uterus inhibits development, or whether it overcomes embryonic diapause by sending a signal to reinitiate embryonic mitosis. Support for both views can be derived from studies of embryo-uterine interactions in other species. There are at least two types of potential uterine inhibitors of embryonic development, cytokines and uterine proteases (Lala & Graham 1990). Leukemia inhibitory factor (LIF) is a cytokine first detected in blood cells, which suppresses mitosis (Gearing et al. 1987). It has been shown to prevent the differentiation of mouse trophoblast cells (Williams et al. 1988). It is produced by the endometrial glands of the mouse uterus (Croy et al. 1991). It therefore has the potential to inhibit embryonic development in the mink. A second potential inhibitor has been found in the cat. Throughout preimplantation in this species there is a specific, progesterone dependent protein (PDP) produced by the uterus. PDP disappears rapidly at the time of implantation (Li et al. 1992). We have found PDP present in the mink uterus during all phases of gestation; however, it is present in reduced amounts in implantation sites and placenta (Song et al. 1992). PDP alone, or in combination with other factors, may prevent the regrowth and invasion of the mink trophoblast.

The cytokine literature describes agents which may be responsible for the stimulation of the reinitation of development of the blastocyst in diapause. Colony-stimulating factor-1 (CSF) is a steroid-regulated protein, produced by the mouse uterus (Arceci et al. 1989). It is present on the mouse uterine epithelium prior to implantation, and its expression is increased during trophoblastic invasion of the uterus. Pollard et al. (1987) reported that CSF-1 is the stimulator of trophoblastic invasion of the maternal epithelium. A receptor for CSF-1 has been found on trophoblast cells (Pollard et al. 1987). A second cytokine, granulocyte-macrophage colony-stimulating factor (GM-CSF) is produced by the uterine glands, and is known to induce the growth of mouse trophoblast (Kanzaki et al. 1991). GM-CSF is also involved in the process of implantation of the mouse embryo (Crainie et al. 1990).

The evidence argues for paracrine signals, produced in small amounts, which control the inhibition and/or proliferation of the mink embryo during diapause. Thus, investigation of these interactions requires the methods of molecular biology. PCR amplification is the most effective means to determine the presence of putative stimulatory and inhibitory agents, such as the cytokines described above. Immunolocalization and *in situ* hybridization

will be important tools to establish the site of synthesis (embryo, uterine epithelium, uterine glands) of the potential regulators of implantation.

SUMMARY AND CONCLUSIONS

The topic for this paper, reproduction in furbearing animals, is broad, and has allowed me to undertake a wide-ranging discussion of current literature, of my views on the most important problems in carnivore reproduction, and my opinions about experimentation that may help toward resolving these problems. It should be noted that the presentation has a strong leaning toward physiology, and there are many other areas of reproduction that are equally unknown and have not been discussed herein.

The seasonality of furbearing carnivores remains an impediment to maximum production of these animals. It may be possible to extend the breeding intervals by selection of animals or by alteration of photoperiods. A more productive approach would be to manipulate melatonin levels to alter the seasons in both male and female animals. To accomplish this aim, experimentation is deemed necessary to determine the mechanism and sites of action of melatonin.

A number of problems in ovarian function have been identified, in particular, the dynamics of follicular development remain to be understood. The mechanisms of activation of the corpus luteum and its endocrine and paracrine control require further investigation.

Little is known about the interactions between the embryo and the uterus which result in implantation and invasion of the endometrium by the trophoblast. The delayed implantation which characterizes gestation in mink, and a number of other mustelid species, appears to be due to uterine influences on the embryo. Further investigation, using the methods of molecular biology, is required to determine the nature of this influence.

The information that has been gathered about reproduction in furbearing carnivores, as well as the investigations that have been proposed, have significance beyond the husbandry of fur animals. The breeding of a number of endangered carnivores is currently being performed using methods only slightly more sophisticated than the emperical methods used by the early fur ranchers. The information from studies of carnivore reproduction is relevant not only to fur farming, but also to the survival of many species of carnivores which are on the brink of extinction.

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History, progress and challenges in the breeding and genetics of mink and foxes

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For many years the genetics of mink and foxes was concentrated on colour genetics and the breeding work was based on phenotype selection, mainly for fur characters. The mutants and combinations have been well described and the colour genetics explained by Mendel's law of inheritance. However, a more detailed and better explanation is discussed, based on the theory of homology of colour loci between species and using new biotechniques for gene-mapping. For quantative genetics it is important to have welldefined breeding goals, using modern methods of breeding value estimation and developing an optimum breeding programme. It is necessary to search for objective and representative fur characteristics. The importance of international cooperation and cooperation between scientific fields is underlined.

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The first serious attempts at farming mink and foxes took place around the last century. The primary goals for the farmers were to feed the animals properly and to reproduce them in captivity. The farming was based on phenotype selection, while only minimal effort was put into selection and breeding strategies, based on a total breeding value estimation. Mink and foxes have been farmed for about 40 generations, which in human terms is commensurate with going back to the time of the Vikings.

Colour genetics have played an important role in fur production. In mink production there was a great enthusiasm about the new colour types, from the first mutant, bluefrost, in 1931. Knowledge about the genetics of colour in mink and foxes came from both the farmers' experiences and from the scientists.

The breeding of mink and foxes has, more than for most of the other domestic animals, been characterized by individuality, where fur characters have been emphasized. The genetic gain depended on the farmers ability to judge their animals accurately, and on the possibility of achieving new genetic material. In recent years new methods and new techniques have been included to develop modern breeding programmes, including the different traits of economic value.

QUALITATIVE GENETICS

Colour genetics

Qualitative genetics, for mink and foxes known as colour genetics, has a long tradition within this kind of production. The explanation of the inheritance of the colours is based on a traditional model, Mendel's law of inheritance, through Mendelian segregation. This is a good model, especially for practical use in the farms. It has also been under strict control, where all new mutants and symbols have to be approved (Nes et al. 1988).

Nes et al. (1988) have given a good review of the colour genetics in mink and foxes, describing all the mutants and several of the combinations. In mink, 30 different mutants have been approved, within 21 loci, while 12 mutants within 8 loci are approved in blue and silver fox, respectively. The number of mutants gives a wide range of combinations with different colours, patterns and shades. The variations in types within the species have been, and are, important for the whole production, offering the buyers many different natural colours. The colour spectrum is also widened by the effect of darkness and clarity within the colour type, caused by polygenes.

The hybrids from the crosses between the two fox species stimulated interest in the search for new explanation models for the inheritance of colour gentics. It was difficult to foresee the colour of the progenies from interspecies crosses, mainly because it was not known which colour genes were paired in the hybrid. The theory about homology of colour loci between species was presented by Searle (1968), and was later also discussed for foxes (Adalsteinsson et al. 1987). One example of homology between loci is known from fox, namely the sapphire frost fox, a result from crossing a pearl silver fox and the Swedish sapphire bluefox. The homology of loci between species is one of the fields where more scientific effort is required, both between mink and foxes and with other domestic animals.

Several loci and several alleles within these loci have been shown to affect the colour, for example in sheep, where Adalsteinsson (1983) found 13 alleles in the A-locus. He also described the effects of the different alleles, especially on melanin. So far, the main focus has been on the loci A, B, C, D and E, and Nes et al. (1988) have given the following description of the groups of the colour system. The A system is the wild or agouti system, controlling the regional distribution of both eumelanin and pheomelanin. The brown system (B) controls the form of pigment granules. The albino system (C) controls the number and intensity of pigment granules. D is the diluting system, affecting the distribution and clumping of granules, and, finally, the extention system (E) controls the amount of eumelanin. It is also shown that there are other loci involved, like dsu, BG, Mi, Sl, W, G and S (reviewed by Lønne 1992). This may make it easier to explain the homology of the many colour genes found in mink.

There are also new biotechniques that should be used to make a colour-gene map for mink and foxes. However, first of all it has to be assumed that we have relevant probes and restriction enzymes. When the probes are marked with radioactive phosphorus, they behave like a gene-searcher in the hybridization. The restriction enzymes recognize and cut the DNA-thread.

On a chromosome level, *in situ* hybridization could be used as a starting point for gene-mapping in mink and foxes in order to discover where the colour loci are located. On a DNA-level, one of the most frequently used techniques is the RFLP-diagnostic

(Restriction Fragment Length Polymorphism), however, relevant restriction enzymes and probes are required. Other techniques are VNTR (Variable Number Tandem Repeat) and the PCR-technique (Polymerase Chain Reaction). It may also be possible to make use of the MHC-system (Major Histacompatability Complex), which has been used for several other domestic animals. The marker-genes should also be mentioned, as we have pleiotropic effects in mink and foxes, for example deafness in white Hedlund mink. Several of the mentioned techniques are well known and used for other domestic animals. It should therefore not be too difficult to adapt them for use with mink and foxes. This is a field that should be given high priority and could be financed within international scientific programmes.

Other qualitative genes

In recent years more attention has been drawn to the effect of single genes on quantitative traits, known as major-genes. It is shown that single genes may have an influence on quantitative traits, such as growth and fertility. In bluefox a major-gene for growth is known (Joutsenlahti et al. 1988) and a giant gene is also well known in mink. It is, however, somewhat complicated to include major-genes in a breeding programme. When these genes are fixed, only a low variation occurs and genetic gain from normal selection is difficult to achieve. It must also be added that in fur production there have been ethical arguments against the use of so-called abnormal animals for growth.

Genebanks

There is, or rather has been, a wide range of different colour types. Many of the mutants and colour types, especially in mink, have become lost over the years. It may even be difficult to find skins from some of the older types. It is therefore important that a genebank is established, primarily on an international basis. This could prevent the loss of colour types of foxes, as we have seen for mink. The most convenient and simplest way is to establish the genebank on frozen semen. Other possibilities, better but more complicated and expensive, are frozen embryo or live animals. This is also one of the challenges in fur production, and it should be added, one that also could be of economic interest to the industry.

QUANTITATIVE GENETICS

Briefly, the purpose of the breeding work is to define the breeding goals to be achieved, the breeding value estimation (the selection programme) and, finally, how to use the breeding animals in the complete breeding programme. The main goal of the breeding work is to increase the genetic gain towards the defined goals. The components that affect the genetic gain are the security of the breeding value estimate (r_{TI}), the selection intensity (i), the variance (σ_G) and the generation interval (L).

$$\Delta G = (r_{TI} * i * \sigma_G)/L$$

Breeding goals

A characteristic of the breeding work with mink and foxes is there have been no well-defined breeding goals. The farmers themselves have used their own discretion about the breeding goals for their farm, with the result that the strategies have not been an optimum, where goals are concerned, also because the goals have varied over a short time. This is very different from the breeding programmes of other domestic animals.

In 1979 Olausson discussed breeding goals for mink, including fertility, body size, pelt quality and disease resistance. In 1983 the first national breeding plan was set up in Norway where the breeding goals were defined as a basis for outlining the breeding strategies (Einarsson et al. 1983). It is important to define as many of the breeding goals as possible on a long-term basis, as the breeding strategies give results over the long term. It is, however, necessary that some goals are also short-term, such as those for clarity and darkness. It is important that the breeding goals are not only accepted by all levels in the production, but also that the involved persons identify themselves with the goals. The breeding goals, even though they are long-term, must come under continual evaluation.

Genetic parameters

Knowledge about genetic parameters for the traits included in the breeding goals and breeding strategies is essential before a breeding programme can be designed. It was not until the 1970s and 1980s that these parameters were estimated for fertility, size, fur- and pelt characteristics in mink and foxes (estimated and reviewed by Johansson 1965; Jonsson 1971; Olausson 1976; Reiten 1977a, b, 1988; Einarsson 1981, 1987, 1988a; Lagerkvist & Lundeheim 1985; Kjær 1988; Kenttamies 1988).

Selection experiments give realized heritabilities of the selection traits and are very valuable, both to show the direct response and the correlated responses. Therefore more selection experiments should be conducted. Although they can only analyse one or a few direct traits at a time, they give very good and exact results.

The experiments and investigations on genetic parameters that were lacking in the 1970s are now relatively well known, and provide a good basis for developing effective breeding programmes. They have, among other things, provided the basis for developing a selection index for litter size (Einarsson & Elofson 1988).

Reproductive traits

The physiology and endocrinology of mink and foxes are relatively well known, particularly for mink, starting with the works of Hansson (1947) and Enders (1952). A trait like litter size is an index in itself, determined by several subtraits, depending on the sire, the dam and the foetus or the progeny itself. These effects have both a genetic and an environmental background, as we can see for the maternal effects. In several multiparous species the maternal, or more precisely, the negative covariance component between direct and maternal effect for litter size is negative, causing problems in achieving a high genetic gain. Such an effect is not found for mink (Einarsson 1981) and is probably not present for foxes either.

A selection experiment for mink has shown a good response when selecting for litter size, with a corrected realized heritability of 0.16 (Einarsson 1987). This single trait selection did not show any negative correlated effects on other economically important

traits. Under practical conditions it should be possible to obtain a genetic gain of 0.1 kits per year. It is also shown that the litter size increases under practical conditions when using a selection index (Børsting 1989; Johannessen 1990). However, it is important to concentrate more on an optimum litter size rather than a maximum one. The loss of foetus and kits should be given more attention, together with kits' birthweight, maternal ability and number of normal teats.

Fur characteristics and size

Several experiments and estimations on genetic parameters have been conducted concerning the characteristics and size of live animals and pelts. It has been concluded that all records should be made on the winter coat and/or on the pelt. The main problems in using live grading are variations within judges (farmer), a short scale where only a few characters are used and the practical solution of recording and calculating breeding values on all relevant animals within a short time. The main problem of using classifications from the auction are that skins may be held over from one year to the next and that the pelted animals may not be representative of the farm, especially if there is a large sale of breeding animals.

The methods used for estimating the breeding values are based on normal distribution. When we characterize live animals or skins, we express the underlying normal distribution as a discontinued distribution with few classes. On the top of this, we may also, by judging, make a very skewed distribution, especially when the deviation of the trait is calculated from the country-average. This may be a poor basis for an accurate estimation of the breeding value. It is therefore a great challenge to search for a good way to characterize what is going to be a skin of high economic value and to be able to include these characteristics in the breeding programme.

Computer programs

In the 1970s several computer programs in mink breeding were developed, in both Scandinavia and North America (reviewed by Einarsson & Lohi 1990). There are two different policies in using computer programs, based on a central system or by PC. The Norwegian field control (Fimland & Einarsson 1988) and DanMink (Børsting 1988) are good representatives for these two directions. It must be borne in mind that these two systems are based on different assumptions within the two countries concerning the structure of the production and main production in foxes or mink. The good Nordic cooperation has been to the general benefit of both these systems.

It has to be aasumed that the computer programs are practical and based on modern knowledge and techniques from animal breeding programmes. The selection indexes used have mainly been traditional (Hazel 1943), however, BLUP-based indexes have been developed.

Breeding structure

The breeding structure for mink and foxes has been very open-ended. In principle, there is no nucleus, as the breeding strategies are conducted on every farm. However, it is a fact that some farms have a high sale of breeding animals, and in a way act as nucleus herds. It is important that the breeding value of the animals is estimated accurately, especially when breeding animals are sold out of the farm.

The use of specialized lines has been briefly discussed, mainly for mink. The use of linecrossing is favourable when we select for different traits in different lines, especially traits with negative genetic correlations and when utilizing non-additive effects (heterosis). If we want to increase the use of heterosis, one should use the Reciprocal Recurrent Selection method. However, this will complicate the breeding programmes and increase the generation interval. The low reproduction rate in mink and foxes is also a disadvantage when using line-crosses. In addition to the scientific evaluation of using lines, there is also a political discussion, among other things, that the breeding structure will be changed into a pyramid form.

Biotechnology

During recent years considerable progress has been made within the fields of biotechnology. However, practical adaptions have been made to breeding programmes in only few areas. Einarsson (1988b) discussed the use of biotechniques in mink and fox breeding, grouped in sementechniques, embryotechniques DNA-techniques and other techniques. The use of artificial insemination is well known, and has been adapted to breeding programs within the fox-circles, allowing progeny testing for fertility and fur characteristics (Einarsson 1983). Gene-mapping and possible use of major-genes have been discussed earlier, while only a few of the other biotechniques are of practical interest in fur animal breeding programmes.

Cooperation

It is a challenge to increase cooperation within the scientific areas. It is necessary that we, for example, adapt ethological traits in the breeding programmes, as well as increase the cooperation between the science of reproduction and genetics. It is through these multifields that we scientists are able to contribute to better production of skins from mink and foxes.

To utilize the resources as much as possible it is necessary to cooperate closely on an international basis. We have seen great benefits from the Scandinavian cooperation, and let me add, we hope also to see this on a worldwide basis, within IFASA.

SUMMARY

In the early years of mink and fox farming the concentration was on reproducing and feeding the animals. Later the main efforts within breeding and genetics were concentrated on producing the right type of good pelt quality, according to the farmers judgement. Many practical and scientific investigations clarified the colour genetics of mink and foxes. It has, however, only been during the last 10 to 15 years that quantitative genetics have been given higher priority. Through estimation of genetic parameters and experience from other domestic animals more modern breeding programmes have been developed. The challenges of the future is to define, qualitatively and quantitatively, the breeding goals, and to use strategies that are in accordance with these defined goals. It is important that new techniques are included, both within qualitative and quantitative genetics, such as gene-mapping of colour genes, optimum breeding value estimation and the use of defined and representative fur characteristics. It is also important that experience from other scientific

fields, such as ethology and reproduction, are used in revising the breeding programs.

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Selection for fertility, body size and pelt quality in mink and effects of crossing

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In 1984, a five generation selection experiment with standard mink was initiated. The lines were selected for fertility (F-line - litter size at three weeks of age), body size (BS-line - September weight), pelt quality (P-line - underfur density) and combined selection for fertility and body size. A control group with random mating was established. Each line comprised 80 females but the combined line 160 females. In 1987 and 1988 reciprocal crossings between two-year-old animals from the F- and BS-lines were performed. In 1990, the experiment was extended to include corresponding crossings in yearlings and two-year-old animals in order to evaluate the effects of age and heterosis. Litter size at three weeks was in the last generation 5.3 in the F-line and 3.7 in the control ($p < 0.01$), yearling females. Male September weight was in the BS-line 2254 g and in the control 1989 ($p < 0.001$). Underfur density scores (5-point score) were 4.1 in the P-line and 2.9 in the control ($p < 0.001$). Heritabilities and breeding values were estimated by using an animal model; $h^2 \pm$S.E was 0.05 ± 0.03 for litter size at three weeks , 0.54 ± 0.09 for male September weight and 0.21 ± 0.06 for underfur density. Crossing between F-females and BS-males gave the best reproductive results, 6.2 (1987) and 7.2 (1988) kits per litter at three weeks of age. Crossing between yearling F-females and BS-males (1990) gave 1.2 kits more than the average of the single lines while the reciprocal crossing gave 0.8 kits below the average. The effect of crossing in two-year-old females was +0.6 and -0.1 kits, respectively. The project will be extended to include field experiments on back-crossing of F X BS females to BS males, in order to take advantage of maternal heterosis.

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During recent years emphasis has been placed on the development of national mink breeding programmes and computers have therefor become powerful tools for selection. Consequently, it has become a matter of urgentcy to increase our knowledge of basic genetic concepts for economically important traits, as to how they respond to intensive selection and if there exist genetic relationships between them. It is also of the utmost importance to elucidate how such selection affects traits that are less exposed to selection today but that may become important in the future.

Conventional selection index theory (Hazel & Lush 1942) was late in being introduced

into mink breeding. Rønningen et al. (1980) constructed an index that combined fertility and overall impression, comprising fur quality and body size. Einarsson & Elofson (1988) developed a selection index for litter size in mink, and a possible genetic gain in litter size of 0.1 kits per year was predicted. This was later realized in a divergent selection experiment on litter size in mink (Einarsson 1987).

Heterosis in characteristics involved in the reproductive process is a common observation in many species when unrelated strains are being crossed (e.g. Gowen 1952). In mammals, the superiority in litter size of crossbreds could be due to higher ovulation rate or embryo survival. Bradford & Nott (1969) suggested that embryo survival, particularly at the preimplantation stage, exhibits more heterosis than ovulation rate. The effect of heterosis has not been evaluated in mink, but there is reason to believe that such information is important in order to develop efficient crossbreeding programmes.

On this background, a five generation selection experiment with standard mink was initiated at the Funbo-Lövsta Research Centre, Swedish University of Agricultural Sciences. The experiment comprised selection for fertility, body size, underfur density and combined selection for fertility and body size. The objectives were to study direct the responses in each trait and correlated effects. The purpose was further to compare the effects of selection for fertility and body size in separate lines, followed by crossing, to simultaneous selection for both traits. The project was extended with reciprocal crossings between the fertility and body size lines, in order to evaluate effects of heterosis, in yearlings and two-year-old females.

MATERIALS AND METHODS

Selection in separate lines

In November 1984 when the selection lines were established, 849 males and 885 females of standard type (yearlings) were available for the experiment. The outline of the experiment is presented below:

Line	Selection criteria	No. females
F	Fertility - litter size at three weeks	80
BS	Body size - body weight in September (around the 20th)	80
P	Pelt quality - underfur density	80
F+BS	Combined selection for fertility and body size	160
C	Unselected control	80

Females were randomly allocated to each line, full sisters being distributed over the lines. No selection was performed in females in 1984. All males were likewise distributed and within each line the best 20 males were thereafter selected according to the selection criteria. Selection for fertility was performed by using a pedigree index (Elofson & Einarsson 1984; Einarsson & Elofson 1988). For body size and pelt quality indices were constructed based on information from the individual itself and from full and half sibs. The

parameters used were estimated in the base population (Lagerkvist 1988; Lagerkvist & Lundeheim 1990). In the combined line the separate indices were equally weighted together into a combined index, after standardization of the variances of the indices. The number of full sibs selected was maximized to four females and two males. The lines were kept closed throughout the experiment.

The generation interval was set to one year with the exception of in the control line, where all fertile females were kept for the first three years. This was done in order to reduce the risk of inbreeding and genetic drift. Thereafter the generation interval was set to one year also in this line. The lines were distributed over the sheds in order to reduce environmental differences.

After the weighing in September, registrations were performed in males only,because of the work involved and also since around 40% of the females were kept for breeding. However, in the P-line, also females were graded for fur traits. A detailed description of the experimental design and traits registered were given earlier (Lagerkvist 1988). This report will focus on response to selection and the effects of crossings.

Reciprocal crossings

The design of the crossing experiments is presented below:

Line	(No. females)	Model of crossing
1987 and 1988		
HF	(40)*	F-females (2 yrs) mated to BS-males
HBS	(40)*	BS-females (2 yrs) mated to F-males
1990		
HF	(43)	F-females (yearlings) mated to BS-males
	(18)	F-females (2 yrs) mated to BS-males
HBS	(27)	BS-females (yearlings) mated to F-males
	(12)	BS-females (2 yrs) mated to F-males

* per year

In 1990, a higher number of females were actually reserved for the experiment, but in the HBS lines many females rejected mating and these were excluded. Yearling females were mated 1+9, matings starting around 7 March. The two-year-old females were mated 1+1, matings starting around 17 March. This system was applied to maximize reproductive output following the method of Elofson et al. (1989).

Heritabilities and breeding values were estimated by use of an animal model, single trait analysis (Johansson 1992). Each line was analysed together with the control. The programme includes all animals without ancestors in the base population , for which breeding values are set to zero. Consequently, genetic trends represent the divergence from the base population. Standard errors of the heritabilities were calculated as $\tilde{A}\ 32 \cdot h^2 / T$ (Falconer 1989), where T is the total number of observations. Since the formula is based on half-sibs families, but the actual population structure is a mixture of full- and half-sib families, the standard error may be overestimated.

RESULTS

Fertility

The reproductive result after five generations of selection (1989) are presented in Table 1. It is obvious that the best result was obtained in the F-line. In litter size at three weeks, the result was 5.3 kits in the F-line versus 3.7 in the control ($p < 0.01$). The corresponding figures from the previous year were 5.2 (F-line) and 4.6 (C-line), the difference being close to significance. A high rate of empty females and stillborn kits was observed in the heavy females (BS- and BS+F- lines). In recent years it was found that both females and males in these lines were unwilling to mate and some males were found to be sterile, despite a positive sperm test. Fat animals were put on a restrictive diet before the breeding season, but these lines did not seem to respond as well as the other lines.

Table 1. Reproductive results in 1989, after five generations of selection (yearling females)

Line	Empty females, %	Total born	Stillborn (%)	Kit mortality day 1-3 (%)	No. kits at 3 weeks per litter	per mated female
F	0	6.6	7	13	5.3	5.3
BS	15	5.0	25	10	3.0	2.6
P	2	5.9	9	10	4.7	4.6
F+BS	11	5.7	12	18	3.9	3.4
C	3	5.4	19	14	3.7	3.6

The heritability estimates ($h^2 \pm$S.E) for the trait litter size at three weeks were found to be low; 0.05 ± 0.03. Average breeding values were in the last generation 0.18 (F-line), 0.04 (F+BS-line) and 0.02 in the control.

Body weight - skin size

Body weight, body length and skin size in the last generation are listed in Table 2. Males in the BS-line were 280 g heavier in September than the control males. The BS-line differed significantly ($p<0.01$) from all other lines in 1985. There were considerable variations in average weights between years. In 1988 the BS-males weighed 2400 in September and 2730 at pelting, about 300 g more than control males. Skin lengths were 79.7 (BS-line) and 76.7 cm (control). The response of the combined lines was remarkable - the weight was about the same as that in the BS-line. The body length measurements reveal that the higher weights do not just reflect fatness - the animals are also of larger size.

The heritability estimate for the September weight of the males was 0.54 ± 0.09. As the programme does not handle litter effects, heritability was also estimated by a REML program by Meyer (1988), which gave $h^2 = 0.36 \pm 0.09$ and $c^2 = 0.19$. Breeding values for the last generation were 365 g in the BS-line, 315 g in the F+BS-line and 100 g in the control group. Obviously, there was also a positive genetic trend in the control group.

Table 2. Body weight (g) and length of body and skin (cm) in males after five generations of selection

Line	Body weight		Length of	
	in September	at pelting	body	skin
F	1900	2130	44.7	72.9
BS	2250	2650	46.8	77.1
P	2010	2340	45.3	75.3
F+BS	2190	2630	46.3	77.5
C	1980	2300	45.0	74.4

Table 3. Average scores for fur traits after five generation of selection (5-point score)

Line	Underfur density		Guard hair quality		General impression	
	live	pelts	live	pelts	live	pelts
F	3.0	3.0	3.0	3.2	3.1	3.4
BS	2.9	2.3	2.8	3.1	3.1	3.0
P	4.1	3.6	3.7	3.8	4.2	4.0
F+BS	2.8	3.0	3.0	3.0	3.3	3.3
C	2.9	3.0	3.0	3.3	3.3	3.4

Fur quality

The scores for underfur density, guard hair quality and overall impression, (body size excluded) are listed in Table 3. The animals were graded in a 5-point score, half point scores for overall impression. The P-line is the most superior in all traits, differing from all other lines ($p < 0.001$) since 1986. Underfur density was poor in the BS-line, when graded as pelts ($p < 0.001$). Otherwise, differences between lines were of minor importance.

The heritability for underfur density was estimated to 0.21 ± 0.06. The breeding values in the last generation were 0.98 points in the P-line and 0.15 in the controls. A correlated positive effect on the fur defect metallic sheen was observed in the P-line, the incidence diminishing steadily over the years. In the last generation, the incidence was 3% (live animals) and 1% (pelts) in the P-line while it varied between 19% and 37% in the other lines.

Effects of crossing on reproductive traits

The reproductive results and kit mortality of the single lines and reciprocal crossings between F- and BS-lines in 1987 and 1988 are outlined in Fig. 1. It is obvious that the crossings gave the best result, but since the crossed females were two years of age while those in the single lines were yearlings, the effects of crossing and female age cannot be fully separated. The best result was observed in the HF-crossing, in terms of litter size, rate of empty emales, stillborn kits and lost litters. The BS-line and HBS-crossing gave the highest rates of empty females and, in 1988, a high percentage of stillborn kits (Table 4; Fig. 1).

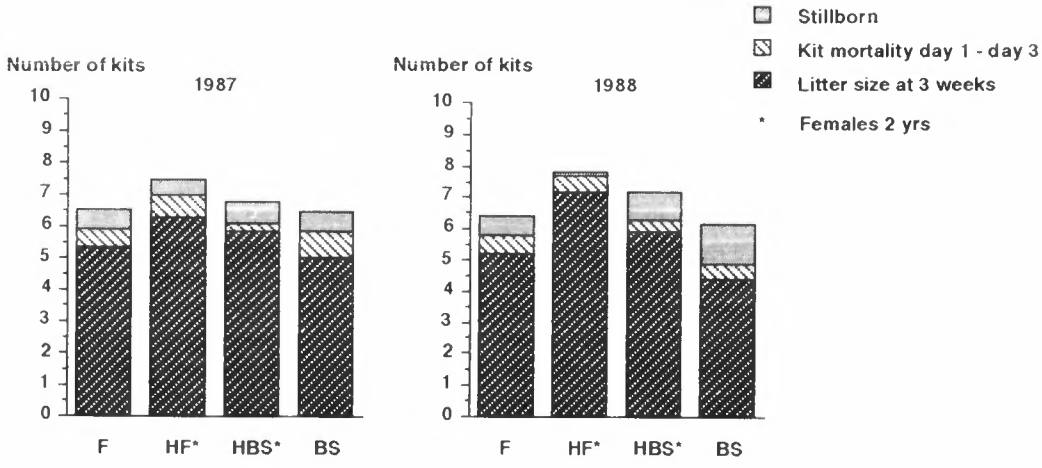

Fig. 1 Kit mortality and litter size in single lines and crossings in 1987 and 1988

Table 4. Frequency of empty and cannibalistic females in 1987 and 1988

Line/ Crossing	Emty (%) 1987	Emty (%) 1988	Cannibalistic (%) 1987	Cannibalistic (%) 1988
F	4	12	4	0
BS	6	18	4	6
HF*	0	5	0	0
HBS*	8	15	0	6

* Females 2 yrs

In 1990 the crossings were performed within age category. Fig. 2 illustrates the reproductive results and kit mortality, within age category and mating combination, and the rate of empty females and percentage of lost litters are listed in Table 5. Maternal ability and survival of the kits were extremely poor for yearlings in the heavy lines (BS and HBS). The total kit mortality was about the same in both lines. The larger number of kits lost was registered as stillborn in the BS-line whereas in the HBS-crossing kits was lost during the first three days. The F-line and HF-crossing had the best reproductive performance and a positive effect of crossing on litter size was observed in the latter. In the two-year-old females, the total number of kits born varied little between lines/crossings. It is clear however, that the maternal ability and/or fitness of the kits was best in the F- and HF-groups. In the crossings, it seems as though the rate of stillborn kits decreases while, on the other hand, the number of kits lost during the first three days becomes higher.
The effects of crossing on litter size, calculated as deviation from the means of the single lines, are listed in Table 6. The superiority of the HF-crossing is prominent, especially in

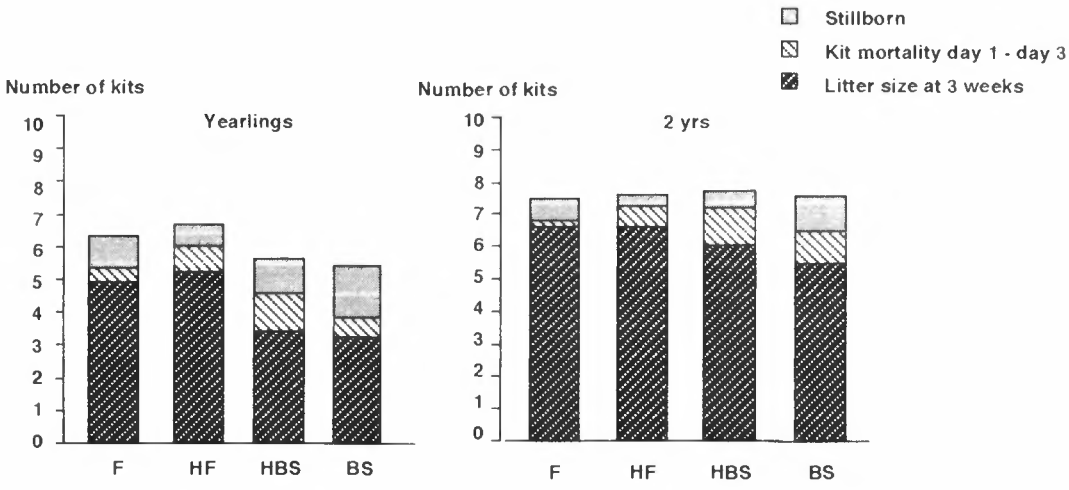

Fig. 2. Kit mortality and litter size in single lines and crossings, yearlings and two-year-old females in 1990

Table 5. Frequency of empty and cannibalistic females, yearlings and two-year-old females in 1990

Line/ crossing	Empty (%) yearlings	2 yrs	Cannibalistic (%) yearlings	2 yrs
F	12	14	2	0
BS	38	8	20	9
HF	30	0	7	0
HBS	25	25	25	9

Table 6. Effects of crossing on litter size, in terms of deviations from the average of single lines within age category, 1990

Age category/trait	Average of F-and BS-lines	Deviations of crossings HF	HBS
Yearlings			
Total born	6.0	+0.9	⁻0.2
Liveborn	4.7	+1.5	±0
At 3 weeks	4.2	+1.2	⁻0.8
Females 2 yrs			
Total born	7.5	+0.1	±0
Liveborn	6.6	+0.6	+0.4
At 3 weeks	6.0	+0.6	⁻0.1

yearlings, while the positive effect of heterosis in the kits is concealed by the poor maternal performance of the HBS-females. In the older HBS-females, a positive effect on number of live born kits was revealed.

DISCUSSION

Crossbreeding in order to take advantage of hybrid vigour has not been applied in mink breeding. The exception might be the production of Scanbrown type mink, by the crossing of two recessive mutants. However, the main purpose has not been to improve fitness traits but to produce a new colour type. Under practical conditions, however, an increase in litter size and body size compared to the single recessive types has been observed. When the increase is prominent, it is taken as a sign of inbreeding in the recessive lines, the fitness lost being restored by the crossing.

Positive effects of crossing were observed in terms of increased viability of the kits. The larger litter size in the HF-crossing is most likely caused by reduced losses during gestation. Also the low rates of still born kits indicate an increased fitness of the kits. The heavy yearling females, however, have a poor maternal ability that is not compensated by the effect of heterosis in the kits.

It would seem that females selected for body weight have an extremely poor reproductive performance during the first year, but "recover" in their second breeding season. Since the selection was performed in yearlings, it is logical that one should find the greatest differences in yearlings. There is reason to believe that yearling fertility and that of older females in some respects should be regarded as different traits, i. e. in part controlled by different gene systems. Significant effects of interaction between female age and reproductive parameters were earlier demonstrated by Elofson et al. (1989).

It is sometimes argued that the difference in reproductive performance between yearlings and two-year-old females is mainly an effect of selection and does not represent a biological difference. In the control line, where all females giving birth were kept for three years, the 1st and 2nd litters of the same females were compared for size. Averages for 1st and 2nd litters, respectively, were 5.8 and 7.2 (total born), 5.5 and 6.7 (liveborn) and 5.1 and 6.1 (at three weeks). Litters were significantly larger in the second year.

In experiments with mice selected for growth rate, a decrease in fertility and fitness is a common observation (Bradford 1971; Barria & Bradford 1981), but in other studies an increase in litter size has been observed (Eisen et al., 1973). Land (1970) concluded that there is a consistent positive genetic relationship between body weight and ovulation rate, while body weight seems to be negatively correlated to embryo survival. This may explain the inconsistency between investigations.

The project will be extended to include field experiments on back-crossing of F X BS females to BS males, in order to evaluate maternal heterosis.

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Development of an animal model for multi-trait analysis in mink

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Genetic analysis using the Animal Model is becoming widely used in the cattle and swine industries. Adaptation of current swine models is a natural starting point for utilization with mink and other litter-bearing animals. A single trait model was adapted from the from the work carried out at the University of Guelph. This was implemented using data from over 15 fur farms. This early model included ranking and indexing of analyzed traits. In 1991 a multi-trait analysis which supports adjusting, indexing and ranking of traits was completed. Modifications were made for speed and efficiency so that up to six traits can be analyzed simultaneously. Indexing and ranking on up to nine traits can be performed. An efficient, fast, practical working model has been developed and is now in use.

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The goal of any commercial breeding program is profitability. This is maximized when the pelt crop is superior and the number of pelts per breeder female is increased. Uniformity of the pelt crop can also improve income by reducing the number of low end pelts. The use of an animal model can accelerate genetic progress and enhance uniformity. A realistic program for commercial farms must take into account both the long- and short-term goals. It should allow for weighting of traits to a ranking.

Studies have shown that improvement of genetic gain through use of "Family Indexes" can be as much as 60% depending on the heritability (h^2) of a trait and the type of analysis used (Børsting 1988). A practical program must allow for evaluation of commonly accepted practices, such as multiple sire matings, etc.

Following a *Mink Computer Selection Conference* sponsored by the National Research Foundation on 18 January 1989, development of such a "Program" was undertaken. From 1989 to 1991 the initial development system was offered as a service only system. More than a dozen farms in North America participated. All work and processing was carried out at one central location. During the fall of 1991 and spring of 1992 the development of a stand alone software package based on the latest computer technology was tested and completed. This paper describes the theory and rationale behind the Animal Model and its use in practical ranch management.

MATERIAL AND METHODS

Background

The core of the software centers around the use of Best Linear Unbiased Prediction (BLUP) or, as it is more commonly known, the "Animal Model". The basic model uses information from ALL recorded relatives to derive a genetic value for each trait on each individual (Schaeffer & Kennedy 1986; Henderson 1988; Van Vleck 1988). The methods used in this study are based on programs developed at the University of Guelph for the Canadian Swine industry (Schaeffer & Kennedy 1986; Kennedy 1988; Kennedy 1989). Conversion to the "C++" programming language and modifications for special cases were made and tested. The greatest difficulty experienced was in the recording and translating of data from the farm into a format that the model could handle. A database developed by means of DataEase was used.

Pre-Process

The individual records in the database were extracted and converted to pure numerical data. Either all litter traits or all animal traits are prepared at one time. For this study, litter size at birth, litter size at weaning and weaning weights were integrated. Up to six graded traits can be analyzed, these are user definable and vary from ranch to ranch.

Necessary information was extracted from the database and put in a form the genetics program could handle. Records are classified acccording to type. All recorded animals and their parents (if identified) are coded as type 1. Type 2 denotes a phantom parent group (Quaas 1988; Westell 1988; Wiggans et al. 1988). All genetic lines eventually end with a type 2 phantom group. Litter weights are adjusted for the number of male and female kits (Westwood 1989). Graded traits are usually stored in human understandable format (non-numeric and converted). After extraction, litter data were put through a program that performed maximum likelihood variance components estimations (Kennedy 1985) and adjusted the data for:(A) parity; B) color type of dam by color type of sire; (C) mating type within age group and (D) number of kits in weaning weight (when applicable). Adjustments are performed within color types. Then the data are run through a program that renumbers the herd-year-season, litter, animal, sire and dam numbers from 1 to n consecutively.

Processing

The genetic program was then executed. First it read in the control records for traits, then it read in the data records. Necessary items were stored in memory in two ordered lists. Prior to 1992 these items were sorted and stored on disk, making analysis a very lengthy process. Once the records were loaded into memory, the analysis began. Records were read in sorted order and solutions determined. This continued until convergence was achieved. Normally this occurred in less than 30 iterations.

Post-Process

After convergence, records were mapped back to their original numbers and stored in the database. The selected animals were marked and indexes and rankings were generated. The ranking program generates indexes using a base of 100 and a standard deviation of 10

(Christensen et al. 1984). Individual genetic values were weighted and combined to form combined indexes for production and quality and for an overall index and ranking. Ranking involves simply sorting animals within sex and color on the overall raw value.

RESULTS

Not enough time has passed to ensure effective evaluation of all aspects of the program. The auction house data from the Westwood farm are presented in Table 1. Because of the subjective nature in auction house grading, especially for quality, these results were not assumed to be highly accurate. However, Westwood's own observations followed the same trend. From 1989 to 1990 male size in darks and demis increased by 112 and 271 g, respectively. Quality and color followed the same trend. Initially, the biggest improvement noted was the elimination of the lower end pelts. This was probably due to mating to maximize strengths and cover up weaknesses in the kit crop using the indexes on each of the traits.

Table 1. Westwood farm auction results for 1990 to 1992

		Size	Quality	Color
Dark Males:		0/1	1st section	Xdark/Dark
	1990 -	82%	41%	14%
	1991 -	84%	60%	16%
	1992 -	91%	74%	57%
Dark Females:		3/2	1st section	Xdark/Dark
	1990 -	70%	60%	38%
	1991 -	73%	69%	55%
	1992 -	80%	70%	54%
Demi Males:		1/0/00	1st section	
	1990 -	86%	46%	
	1991 -	92%	78%	
	1992 -	98%	87%	
Demi Females:		3/2	1st section	
	1990 -	80%	52%	
	1991 -	88%	83%	
	1992 -	96%	89%	

DISCUSSION

The basic model as applied in 1989 had some weaknesses that were addressed in the latest version. Some of these were: full and cross-bred mink in the same data set, more than one color type per data set, different grading of the same trait in different color types, missing parents, missing observations, inclusion of test group effects, handling of purchased mink and phenotypic productivity attributable to age.

An index of estimated future production was generated for use in culling older females (Cassell 1988). This allowed maximization of the number of kits (short-term gain) as well as future genetic merit of the herd for litter size (long-term gain). In practice, the genetic values for litter size should generally be better for the kits than for the older females when breeding best to best, but a high percentage of kit females is counterproductive (in the short term) due to their inexperience in raising kits.

A ranking of males on quality and females on production as well as overall genetic merit helped maximize the short-term gain without sacrificing the long-term gain. The top end males and females were selected on the overall ranking. These animals should produce the most of the future breeders. The rest of the breeders should produce primarily the pelt crop. Thus, the highest producing females were selected to maximize kits per litter and were bred to the highest quality males in order to maximize the pelt crop. While the gains for each of these improvements were small, the cumulative effect should be significant.

An unexpected plus came when analyzing the herd-year-season solutions from the Animal Model. Test groups and yearly gain can be effectively evaluated with these figures. They were already adjusted for the genetic level of the animals in each group.

Mixed Sires

The wide use of triple mating to different sires was a hinderance due to the difficulty in identifying accurately the correct sires of individual kits. At present, mixed sires in the final mating cycle were replaced with phantom groups. Thus, all indexes were based on known conditions. Modifications to allow for inclusion of a percentage from each sire would not be particularly difficult, but the memory requirements and time needed to run the model would greatly increase. Many farms are mating old females once to a single sire and kits twice eight days apart.

The large amount of information, the number of times data must be read and the raw computing power needed are probably the primary reasons the Animal Model has not been more widely used outside of government, university and research applications. For example, on a farm of 3000 breeder females with four years worth of data, or about 60,000 animal records, it takes approximately eight megabytes of main memory to load the needed records, then 10-12 h to run all the necessary steps, depending on the computer hardware. The advances in computing speed and the drop in price for large amounts of high speed memory have opened the door to economical on-farm processing.

Educating the ranchers in the use and understanding of practical genetic analysis is still a major hurdle. Newly purchased breeders always raise questions. These animals cannot be effectively evaluated for at least two years, as daughters do not produce until then. The incorporation of repeatabilities would help as well. Vocal input of data will greatly simplify input. This is currently under development.

CONCLUSION

Proper use of computers and the Animal Model can accelerate genetic progress, although an understanding of genetic principles and practical ranch management is important. As computer technology improves, additional enhancements for on-farm use with mink records will occur.

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An adviser based databank for support of commercial breeding programs

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The extensive use of personal computers in Danish mink breeding has made it possible to develop a data bank system run by the local advisory service. The users of the DanMink system send a copy of their files to the local adviser twice a year. The adviser transfers the data to SAS data sets and runs a set of statistics on the data. A report is then issued and given to the farmer. It gives the farmer an overview of the breeding work and estimates of the latest genetic progress. The normal genetic parameters are also estimated. The adviser helps the farmer to use the report for improvements in his breeding program and the adviser's own know-how is enhanced by the system. New statistics are developed every year, and calculation of inbreeding in each line will be one of the future tools in the system.

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Breeding work on Danish mink farms is carried out on the basis of the individual farmer's own decisions. The method used has primarily been that of mass selection based on the phenotype of the kits. With regard to pelt traits, the selection is made on the basis of subjective grading of the live animals. The number of kits per the litter is the criterion for selection for fertility, while pedigrees have been used to a certain extent to select families.

In the 1970s, computers were introduced as a means of registering pedigrees and printing cage cards and general statistics on mating and birth results. The systems were run on large, centrally located computers.

At the beginning of the 1980s, the central computer systems were expanded to include calculation of breeding value for female fertility. The breeding value was expressed as an index for litter size.

The Danish Fur Breeder Association expanded its activities in the area of breeding during the 1980s (Børsting 1990). Two breeding advisers were hired in 1984 and a breeding research farm, the Fur Animal Research Farm SYD, was established in 1986. A decentralized breeding program for personal computers (PC's), called DanMink®, was developed and marketed in 1988 (Clausen 1990a) and (Clausen 1990b). The decentralized location means that the program can be used in connection with selection of breeding animals.

The breeding program DanMink is used by the individual farmers on their own farms.

The program is user-friendly and includes the possibility of calculating indices based on subjective gradings of the kits' pelt traits. A total breeding value can be calculated on the basis of litter size index and index for various pelt traits. The total breeding value is used to select new breeding animals.

A normal part of efficient breeding work is planning followed up by a check on the attained results. In connection with the increasing distribution of DanMink, a data bank was established on a trial basis in Funen and South Jutland Fur Breeder Association (Kjær & Børsting 1989).

The local adviser is responsible for building up and managing the data bank. The aim of the data bank is to support the regional association's DanMink users in their breeding work and to strengthen the adviser's counselling.

MATERIALS AND METHODS

The data bank builds upon data received from the DanMink users. The farmers send a copy of their DanMink files on a diskette twice yearly. The first delivery occurs in July, after data on mating and whelping have been recorded. The second dispatch occurs in December, after data relating to grading of the kits' pelt traits have been entered.

A prerequisite for participation in the data bank is that the farmer has used LIVE ANIMAL EVALUATION in at least one line. The live animal evaluation requires that all of the kits in a line are graded, after which an index-based selection is made.

Breeding reports on mating and whelping data and live animal grading data, respectively, are prepared.

The DanMink files, which consist of Dataflex® files, are transferred to SAS® data sets. It is the intention that both the DanMink files and the SAS data sets shall remain in the data bank for several years.

The breeding report on mating and whelping data contains, among other things, the following statements:

- mating and whelping results dependent on number of matings and the age of the female and male
- birth year of the mother for all young females
- number of times the female was mated in the first and second breeding year
- origins of breeding animals
- litter size of young females dependent on the litter size they were born in
- litter size of young females dependent on their litter size index as kits

The breeding report on live animal grading contains the following statements:

- overview of the average grades per line
- overview of the distribution of grades in the lines
- live animal grading results according to the mothers' and fathers' birth years
- live animal grading results according to parents' gradings
- heritability (h^2) of the individual traits, calculated per line

Summaries of the results from the other participants in the breeding data bank are also included in both reports.

In 1991, 54 farms have had breeding reports prepared on the basis of information sent in. The data bank contains 88,500 breeding females, corresponding to 15% of Funen and South Jutland Fur Breeder Association's females.

The breeding reports are supplemented with one to two annual visits by the adviser, when the reports are looked through, and the future breeding work is discussed and planned.

RESULTS

The reports have contributed to a wider understanding of concepts such as:

- female fertility
- influence of age on litter size
- family selection
- index construction and reliability

The efficiency of the litter size indices can be shown by dividing the young females into groups according to the size of the litter they were born in or their own litter size index as kits. In Table 1. it can be seen that 2377 females are divided into three groups according to original litter size and the kit litter size index, respectively. The 792 young females from the largest litters gave birth to 0.15 kits more than the average. The 792 young females with high litter size indices gave birth to 0.26 kits more than the average. It can be seen that the litter size index is a more reliable expression of breeding value than litter size.

The family structure in a line can reveal the selection method and, for many users, the report has given new insight into how the previous selection has functioned. An example of a population where 25.4% of the breeding males have only one progeny in the existing population is presented in Table 2. It can be seen that family selection has been practised to only a limited degree.

Table 1. Litter sizes of young females according to original litter size or kit litter size index

Young females grouped according to original litter size	No. of young females	No. of kits at second count
High	792	6.13
Medium	792	5.96
Low	793	5.84
Total	2377	5.98
Young females grouped according to kit litter size index	**No. of young females**	**No. of kits at second count**
High	792	6.24
Medium	792	5.87
Low	793	5.82
Total	2377	5.98

The table indicates, for example, that there are 116 males that have only one progeny among existing breeders.

Table 2. Origins of the breeding animals "Fathers/kits in existing population"

No. of kits	No. of fathers	No. of fathers in %	No. of fathers accum.	No. of fathers in % accum.
1	116	25.4	116	25.4
2	79	17.3	195	42.8
3	67	14.7	262	57.5
4	32	7.0	294	64.5
5	30	6.6	324	71.1
6	37	8.1	361	79.2
7	17	3.7	378	82.9
8	16	3.5	394	86.4
9	11	2.4	405	88.8
10	15	3.3	420	92.1
11	9	2.0	429	94.1
12	7	1.5	436	95.6
13	8	1.8	444	97.4
14	3	0.7	447	98.0
15	3	0.7	450	98.7
16	2	0.4	452	99.1
17	1	0.2	453	99.3
19	1	0.2	454	99.6
21	1	0.2	455	99.8
24	1	0.2	456	100.0

A condition for using subjective gradings to calculate the family index is that the gradings have a more or less normal distribution. An example of a farmer's quality grading is given in Table 3. It can be seen that the grade scale has been used appropriately.

Table 3. Overview of the distribution of grades in the lines

Live animal evaluation Kit quality		Males No.	Males %	Females No.	Females %
Line no.					
30	1	18	7.73	4	1.68
	2	89	38.20	58	24.37
	3	76	32.62	95	39.92
	4	39	16.74	59	24.79
	5	11	4.72	21	8.82
	6	.	.	1	0.42
	Total	233	100.00	238	100.00

The distribution of the live animal gradings per line is indicated. A normal distribution is desirable, so that most animals receive, for example, grade 3, fewer 2 and 4, and the fewest 1 and 5. A normal distribution of the grading serves as the basis of the calculations in the live animal evaluation.

The heritability can be expressed as parent-progeny regression. An example of the regression is given in Table 4. It can be seen that there is a positive correlation between the average grades of the parents and their progeny.

The average kit grades are placed under each combination of young mothers' and fathers' grades. Naturally top males mated to top females should, on average, result in the best kits. The statement is made per line.

Table 4. Live animal grading results according to parent gradings

Kits' average colour	Mothers' colour 2	3	4	5
Fathers' colour				
3		2.95	3.00	3.00
4	2.80	3.21	3.33	3.33
5		3.52	3.48	3.45

The grouping of the graded kits according to the birth year of the parents indicates the development of the population. Clear progress in line 1 can be seen in Table 5, whereas in line 5 there is no development.

The kits' average grades are shown under each combination of the mothers' and fathers' birth years. The statement is made per line.

DISCUSSION

Work with the data bank suggests that a combination of breeding reports and individual counselling results in an improved control and a better overview of the farm breeding work. The yield from the investment in the breeding work is increasing. The data bank provides a better foundation for counselling, since the advice is more factually based than that previously offered. The continuing building-up of the data bank will increase its value, since it will, among other things, be possible to use it for ad hoc analyses. The interaction between farmer and adviser widens the understanding of the practical effects of breeding theories. Moreover, the areas where there is a lack of theoretical knowledge will be highlighted.

Table 5. Live animal grading results according to birth year of the father and mother

Live animal grading average quality		Mother 1988	1989	1990	Total
Line no	Father				
1	1990	2.53	2.66	2.79	2.69
	Total	2.53	2.66	2.79	2.69
5	Father				
	1990	2.80	2.78	2.81	2.80
	Total	2.80	2.78	2.81	2.80

In order to attain interaction between farmer and adviser, it is necessary that the adviser has responsibility for the data bank. A natural consequence of the continuing contact between farmer and adviser with regard to the subject of breeding is that the adviser becomes a specialist in both genetics and computer work.

The breeding reports will undergo a continuous development as it becomes possible to undertake calculations on several generations. Experience from work with the data bank will have an effect on the future development of DanMink and interaction between the field and the Research Farm SYD's work is also likely to take place.

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DanMink® is the registered trade mark of the Danish Fur Breeder Association.
Dataflex® is the registered trademark of the Data Access Corporation.
SAS® is the registered trade mark of the SAS Institute Inc., Carry, N.C., USA.

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Reliability of subjective grading in foxes

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The association between repeatedly scored body size and fur characteristics evaluated by several judges was studied in silver foxes (*Vulpes vulpes*) and blue foxes (*Alopex lagopus*). The grading in different environments was studied for blue foxes. Differences between various colour types of the fox were compared. There were obvious differences between the various viewpoints of the judges in repeatabilities. In blue foxes, differences between judges appeared to be greater than differences between environments. Grading outside the cages, particularly in daylight, was found to be more reliable than grading inside the cages, while colour tended to be easier to evaluate than the other traits. In the comparative gradings, caged gradings produced higher repeatabilities for colour in silver foxes than in blue foxes. The most uniform repeatabilities were obtained for assessment of body size, the most varying ones were those for clarity. The "silver type" mutants seemed to be easier to judge for general appearance than the "golden type" animals. The most reliable results were achieved when the same judge evaluated the animals in the same environmental conditions.

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It has been found that the reliability of subjective grading for fur-bearing animals is highly dependent on the animal species, the skill of the judges and the existing grading conditions (Jonsson 1970; Reiten 1977a; Jeżewska & Maciejowski 1982). Subjective scoring is greatly dependent on the personal points of view of the judges which partly accounts for the fact that the traits can sometimes be imprecisely determined. The inherent difficulty of the uncertain evaluation is reflected in the low heritability estimates previously found in some traits for quality and clarity (e.g. Einarsson 1988; Jonsson 1971; Reiten 1977b; Kenttämies 1988; Lagerkvist & Lundeheim 1990; Rosberg & Olausson 1978). Nevertheless, these traits are used for the evaluation and selection of breeding animals. Numerous farmers evaluate the animals themselves or at least put the animals into groups to be used for breeding or pelting.

In the present study the association between repeatedly scored body size and fur characteristics was investigated in silver foxes (*Vulpes vulpes*) and blue foxes (*Alopex lagopus*). The differences between judges' assessments were studied. Gradings in different environmental conditions were compared for blue foxes. Among various colour types of fox, repeatability estimates were studied. In the silver fox type single evaluations were compared with the repeated ones in order to assess their accuracy (r_{TI}).

MATERIALS AND METHODS

Experiments were carried out in which foxes were repeatedly graded on a private farm. Five persons were appointed to judge 248 male and female silver foxes (Sample 1), and one judge evaluated 2063 males of various colour mutants of the silver fox (Sample 2). These experiments with foxes are described in more detail by Kenttämies & Käyhkö (1991).

An experiment for grading blue foxes in different environmental conditions was carried out at the Agricultural Research Centre, the Research Station for Fur Animals. Three persons judged a total of 77 males and females (Sample 3). Each judge conducted the grading three times in cages in daylight and two times outside the cages both in daylight and by lamplight. The study is more closely reported by Kenttämies & Smeds (1992). In each sample the animals were graded according to a scale from 1 to 5. The experiments are described in the following scheme.

Colour type	Sample	No. anim.	Trait	Grad.turns	No. judges
Silver fox	1	248	Body size Colour	3	5
Various foxes	2	2063	General appearance	3	1
Blue fox	3	77	Body size Colour Underfur density Guardhair density Clarity	7	3

Statistical methods

The materials were edited and analysed using a statistical program WSYS (Vilva 1989). Repeatabilities for the traits were analysed from the components of variance. In silver foxes (Sample 1) the following model was used:

Model I:

$$Y_{ijk}=\mu+a_i+b_{ij}+\epsilon_{ijk}$$

where Y_{ijk} = individual observation
μ = general mean
a_i = effect of the *i*th judge; i=1-5
b_{ij} = effect of the *ij*th animal; j=1-248
ϵ_{ijk} = random error

The effects of the judge were considered to be fixed and the animal effects to be random. Model I was also used in analysing the repeatability for general appearance in various

colour types of the fox (Sample 2). The model included colour type and animal.

In blue foxes (Sample 3) repeatabilities were analysed within judges and grading conditions according to the following model:
Model II:

$$Y_{ijkl}=\mu+a_i+b_{ij}+c_{ijk}+\epsilon_{ijkl}$$

where Y_{ijkl}= individual observation
μ = general mean
a_i= effect of the *i*th judge; i=1-3
b_{ij}= effect of the *ij*th condition; j=1-3
c_{ijk}= effect of the *ijk*th animal; k=1-77
ϵ_{ijkl}= random error

The effects of judge were considered to be fixed, the other effects to be random. In order to analyse repeatabilities separately within grading conditions or judges, Model I was applied. In order to obtain the repeatabilities separately for judges (Sample 1), colour types (Sample 2) and for judges and environments (Sample 3), a model including random effects attributable to the animal was used.

The accuracy of the predicted breeding value (r_{TI}) was estimated for the general appearance of silver fox type in Sample 2. Heritabilities needed for estimations were analysed from the sire components of variance (39 sires, 361 dams, 28 kits/sire). For a single grading r_{TI}) was estimated as a square root of heritability and for the average scores of the repeated turns according to the following formula (Van Vleck 1974):

$$r_{TI}=\sqrt{\frac{nh^2}{1+(n-1)\,r}}$$

where n = number of repeated scorings
h^2 = heritability of the trait
r = repeatability of the trait

RESULTS AND DISCUSSION

In both species colour tended to be easier to evaluate than the other traits (Table 1). The results are in accordance with previous studies (Jonsson 1970; Reiten 1977a; Jeżewska & Maciejowski 1982). In the present study, the reliability of grading for the traits, apart from colour, tended to be much the same as that for gradings conducted by the same judges in the same conditions. In the corresponding environments, in cages, higher coefficients of repeatability were obtained for the colour of silver foxes (r = 0.74) than those for blue foxes (r = 0.63) whereas similar results were found for body size (r = 0.55 vs 0.56).

The heritability estimate for general appearance of silver foxes was 0.17 ± 0.08 for the first time of grading. Using the obtained repeatability, 0.57, a higher accuracy was

found on the basis of repeated grading as compared with a single grading (r_{TI} = 0.48 vs 0.41). Therefore repeated scoring, especially for imprecisely evaluated traits is of value for higher accuracy in the prediction of breeding value.

Table 1. Repeatabilities ± standard errors (r ± SE) for the scores of the traits graded in foxes and blue foxes

Trait	Repeatabilities ± standard errors	
	Silver foxes	Blue foxes
Colour	0.74 ± 0.01	0.67 ± 0.02
Body size	0.55 ± 0.02	0.59 ± 0.03†
Underfur density		0.56 ± 0.02
Guardhair density		0.56 ± 0.02
Clarity		0.53 ± 0.03
General appearance	0.57 ± 0.01	

† males

Differences between judges

In silver foxes and blue foxes differences between judges were obvious (Table 2). Jeżewska & Maciejowski (1982) also observed remarkable differences between judges in their competence of grading. The greatest differences between judges in the present study were found for the clarity of blue foxes. In the grading for colour, similar differences between judges were found for silver foxes (r = 0.68-0.82) as for blue foxes in the corresponding environment (r = 0.57-0.72). The differences were also much the same for body size.

Table 2. Differences between judges in the grading of silver foxes and blue foxes

Trait	Repeatabilities ± standard errors	
	Silver foxes	Blue foxes
Colour	0.68 - 0.82	0.65 - 0.68
Body size	0.51 - 0.59	0.56 - 0.60†
Underfur density		0.53 - 0.64
Guardhair density		0.47 - 0.61
Clarity		0.20 - 0.67

† males

Differences between environments

In the grading of blue foxes, differences between judges seemed to be greater than between environments. The grading which took place outside the cages, particularly in daylight, was found to be more reliable than that inside the cages (Table 3). The most uniform results were obtained for body size and guardhair density in the grading outside the cages in daylight. The most variable repeatabilities were found for clarity in blue foxes in each environment.

Differences between colour types

In various colour types of fox, a repeatability of 0.57 was obtained for general appearance. The same value was found for silver foxes (Table 1). The repeatabilities seemed to be greater for silver foxes and platinum and arctic marble mutants (r = 0.59) as compared

with gold foxes (r = 0.55) and various cross-fox types, particularly gold and silver cross-foxes (r = 0.50). Therefore the "silver type" mutants seemed to be easier to grade than the "golden type" animals.

Table 3. The repeatability coefficient ± standard errors for scores of the traits in live blue foxes analysed by grading conditions

Trait	Repeatabilities ± standard errors		
	Cages	Corridor	
	daylight	daylight	lamplight
Colour	0.63 ± 0.03	0.76 ± 0.03	0.64 ± 0.04
Bocy size, males	0.47 ± 0.06	0.73 ± 0.05	0.65 ± 0.06
Underfur density	0.51 ± 0.04	0.71 ± 0.03	0.48 ± 0.05
Guardhair density	0.45 ± 0.04	0.70 ± 0.03	0.56 ± 0.04
Clarity	0.46 ± 0.04	0.59 ± 0.04	0.60 ± 0.04

SUMMARY

Colour was found to be easier and clarity more difficult to evaluate than the other traits. Differences in the reliability of grading appeared between species and various colour mutants. Obvious differences between the various viewpoints of the judges and grading conditions were found as well. The most reliable results were obtained when the same judge evaluated the animals in the same environmental conditions. High r_{TI}-values based on repeated gradings in comparison with single ones indicate that repeated judging for traits with low repeatability of grading is of use in order to obtain more accurate predictions for breeding value.

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The comparison of two exterior evaluation methods of foxes

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The results of exterior evaluation of red and pastel foxes conducted according to two different methods were compared. The authors have put forward a modified evaluation system, whose essence is the reduction of the number of evaluated traits as well as the narrowing of the assessment scale. The proposed system contributes to better underlining of animal variability, and what this involves, but it should contribute to an easier and more effective selection for utility traits in carnivorous fur animals.

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The results of other studies by the authors (Jeżewska et al., in print; Maciejowski et al. 1990) definitely indicate that the existing and applied system of evaluation and selection of carnivorous fur animals does not come up to expectation. High divergence, and what this involves, low recurrence of exterior features assessments (Dembowski et al. 1987; Jeżewska & Maciejowski 1983) is a result of too high a number of traits being simultaneously taken into consideration. This being the case, licence judges pass their assessments mainly on the basis of general impression, and they are not always able to point out particular traits responsible for the lowering of overall assessment. The recurrence of total licence assessment is generally satisfactory; however, the recurrence of assessments concerning particular traits is considerably lower. This highlights the necessity of reducing the number of evaluated traits so that the total assessment of a particular animal should in fact be the sum of detailed assessments.

The authors have established a new exterior evaluation system for red and pastel foxes, which is intended to reduce the number of evaluated traits and to increase the variability of assessments.

The aim of the present study is to compare the convergence and divergence of assessments obtained by means of the old and the new system, assumed as a selection criterion.

MATERIAL AND METHODS

Animals were evaluated in November during the period of full maturity of their hair cover. The evaluation comprised two colour varieties - red and pastel foxes.

The animals were evaluated twice. The first evaluation was conducted according to the existing pattern of exterior evaluation (the exterior evaluation pattern for common foxes, 1988) which takes into account seven specific traits with the following scale of assessment: body length (measured from the tip of the nose to the base of the tail) - 0-3 points, colour type 0-3; silver purity and veil 0-6; colour purity of pigmented hair 0-3; hair density 0-6; length, silkiness and resilience of hair 0-6; general appearance 0-3. Maximum scale is thus 30 points. The second evaluation was based on the new method suggested by the authors. This evaluation took into consideration three basic traits with the following system of assessment: body length 0-3 points; hair cover colour 0-3; hair cover structure 0-4; maximum of 10 points.

The proposed method might seem to narrow rather than widen the assessment range (lower number of evaluated traits, smaller scale of assessments, lower total). In practice, however, when using the traditional system with a 30-point maximum, judges tended to use only the uppermost 3-5 points, whereas lower assessments were almost never used (Jeżewska & Maciejowski 1983). Obtained results were processed according to commonly applied statistical methods, separately for males and females.

RESULTS AND DISCUSSION

The results of the traditional evaluation method for both colour varieties and sexes are listed in Table 1. The mean assessment values and variability characteristics are also given. The results for the same animals,obtained by means of the new method put forward by the authors are presented in Table 2. Regardless of the assumed method, the assessments of body length are obviously the same, since it is the only measurable trait whose assessment is independent of the judges' subjectivism. The high number of traits taken into account in the traditional method jeopardizes its objectivism and also the recurrence of the total assessment. For example, the animals' hair colour is evaluated as actually three different traits: colour type, silver purity and veil, and colour purity of pigmented hair. Hair cover structure is viewed as two traits: hair density and hair length, the latter, in addition, including silkiness and resilience of hair.

The modified 10 point evaluation method is considerably stricter. This fact can be seen when comparing the assessments of articular traits in relation to possible maximum.

Two evaluation systems with different point scales and an unequal number of evaluated traits cannot be compared directly. Consequently, in order to make such a comparison, deviations of mean assessments for the investigated traits are shown graphically (see Fig.1) in relation to the maximum, assumed to be 100. The system of comparing results obtained by neans of both evaluation methods, presented here, makes it possible to determine to what extent mean assessments are different from the hypothetically possible maximum. The scores in the traditional evaluation of such traits as hair cover colour and structure resulted from the summing up of the obtained assessments. The devi-

Table 1. Average scores for exterior traits in common foxes - traditional method

Evaluated traits	Max. score	Variability measures	Variety			
			Red		Pastel	
			♂♂	♀♀	♂♂	♀♀
Number of animals			213	197	231	221
Body length	3	$\bar{x}$	2.13	2.12	1.94	1.99
		s	0.51	0.53	0.46	0.53
		v	23.94	25.00	23.71	26.63
Colour type	3	$\bar{x}$	2.87	2.81	2.67	2.61
		s	0.34	0.39	0.48	0.52
		v	11.85	13.88	17.98	19.92
Silver purity and veil	6	$\bar{x}$	5.28	5.39	5.65	5.75
		s	0.96	0.92	0.76	0.65
		v	18.18	17.07	13.45	11.30
Pigmented hair purity	3	$\bar{x}$	2.97	2.95	2.93	2.91
		s	0.18	0.22	0.25	0.28
		v	6.06	7.46	8.53	9.62
Hair density	6	$\bar{x}$	5.48	5.39	5.38	5.33
		s	0.53	0.53	0.58	0.60
		v	9.67	9.83	10.78	11.26
Hair length, silkiness and resilience	6	$\bar{x}$	4.85	5.84	4.78	4.84
		s	0.55	0.55	0.58	0.61
		v	11.34	11.36	12.13	12.60
General appearance	3	$\bar{x}$	2.99	2.98	2.98	2.96
		s	0.12	0.14	0.15	0.20
		v	4.01	4.70	5.03	6.76
Total	30	$\bar{x}$	26.57	26.48	26.33	26.39
		s	1.26	1.11	1.35	1.33
		v	4.74	4.19	5.13	5.04

$\bar{x}$ - mean
s - standard deviation
v - variability

ations of the mean assessment values from the maximum are - for all presented cases - considerably lower in the modified system. This is a positive phenomenon since it points to, first, a fuller utilization of the possible assessment scale, and, secondly, to the fact that the obtained assessment distribution approximates the Gaussian curve. The distribution in the traditional system, where mean assessment values approximate the maximum, is definitely skewed to the left. This fact does not make the proper selection of animals for a breeding stock easy. Lower assessment variability also adds to the difficulty. Assessment variability coefficients presented in Tables 1 and 2 are considerably higher for the modified system. The diagram in Fig.2 gives an example of assessment distribution in 30 -, and 10 -

Table 2. Average scores for exterior traits in common foxes - modified method

Evaluated traits	Max. score	Variability measures	Variety			
			Red		Pastel	
			♂♂	♀♀	♂♂	♀♀
Number of animals			213	197	231	221
Body length	3	$\bar{x}$	2.13	2.12	1.94	1.99
		s	0.52	0.53	0.45	0.53
		v	24.65	25.00	23.40	26.58
Hair cover colour	3	$\bar{x}$	2.29	2.34	2.37	2.40
		s	0.72	0.65	0.62	0.58
		v	31.31	27.61	26.08	24.38
Hair cover structure	4	$\bar{x}$	2.88	2.86	2.60	2.66
		s	0.67	0.59	0.69	0.68
		v	23.13	20.80	26.54	25.75
Total	10	$\bar{x}$	7.30	7.32	6.91	7.05
		s	1.11	1.12	1.13	1.19
		v	15.27	15.37	16.38	16.86

$\bar{x}$ - mean
s - standard deviation
v - variability

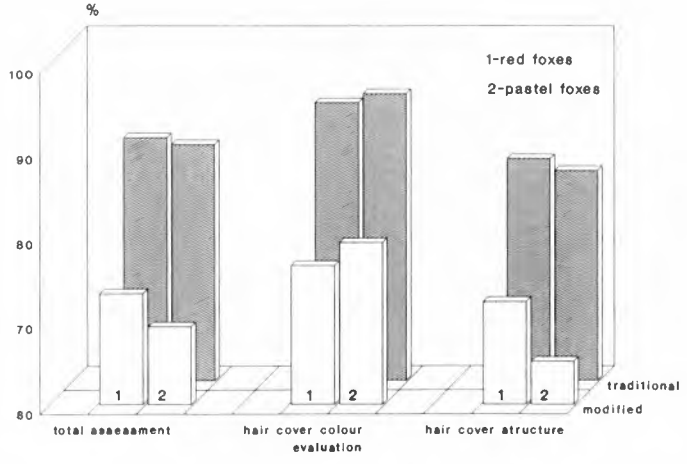

Fig. 1. Average assessments of evaluated traits expressed as deviations from the max. assessments of 100

point scales. The skewness of the traditional method distribution is much greater than that of the respective distribution in the modified method. Both distributions involve the assessment results of pastel males. The tendencies in the distributions of the remaining groups were similar.

The question that arises is, why the assessment scale range causes the differentiation in the way point are awarded to the animals evaluated. According to the authors

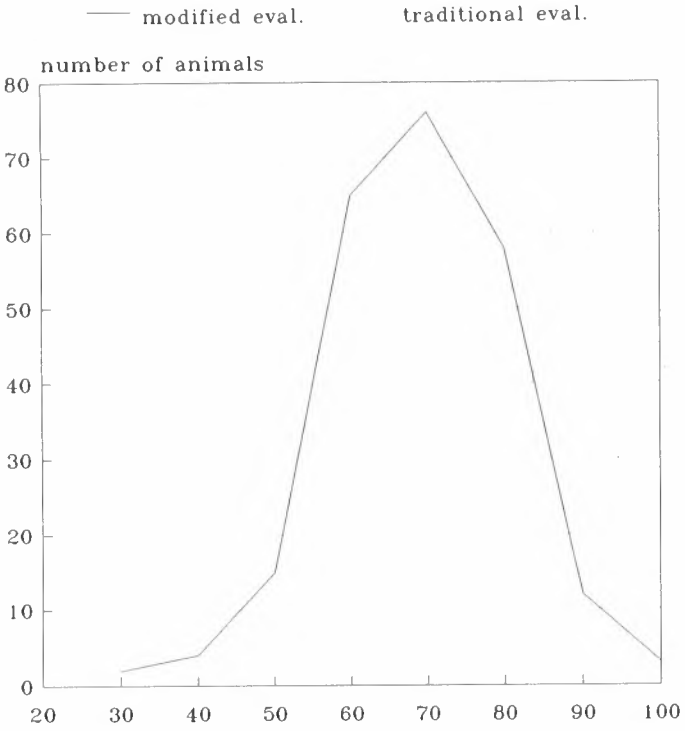

Fig. 2. Comparison of assessments distribution in traditional and modified system on a standardized scale, expressed as a percentage of max. assessments

it results from the fact that it is much easier for a judge to make a decision when he has only four points to consider. In that situation he awards the "marks" in a "teacherish way": unsatisfactory, satisfactory, good, very good. When he has a much greater number of points at his disposal, a judge, as a rule, uses only the upper two points in his assessments, and a zero to disqualify an animal (Jeżewska & Maciejowski 1983), a fact that causes unsatisfactory utilization of a vast point scale without its contributing to any substantial differentiation of the animals.

Both methods compared generally evaluate the same utility traits. The calculated correlations between the total traditional assessment and the modified one are relatively high, r_{xy} = 0.62-0.71. The new evaluation method, which increases the variability of the evaluated animals, should make breeding decisions easier and should contribute to greater selection efficiency.

CONCLUSIONS

This comparison of the two evaluation systems has led us to draw the following conclusions:

(1) The proposed selection system, reducing both the number of evaluated traits and the assessment scale,contributes to better underlining of the variability in the evaluated animals.
(2) The assessment distribution in the modified system approximates the Gaussian curve more closely.
(3) The narrower assessment scale is wholly utilized and makes judges' decisions easier.
(4) The differentation of evaluated animals should make their selection for a breeding stock easier.

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On-farm animal-model estimation of breeding values

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An algorithm for animal model evaluations for a typical fur animal population is described. This algorithm iterates on data so that mixed model equations are not set up explicitly. It is reasonable to assume that at maximum, one observation is recorded on fur animals with no offspring. This allows for an efficient way of "absorbing" non-parent breeding values. This gives an efficient algorithm for large-scale animal model evaluations on small to moderate computing capacities (e.g. PC's). This makes it useful for on-farm evaluations of breeding values using an animal model. A small numerical example is given.

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Animal model evaluation of breeding values is the preferred method in many different species of farm animals in many countries. Animal models utilize all available data, provide simultaneous evaluation of all animals and correction for fixed effects, and account for selection and non-random mating included in the data. Direct solution of animal model equations is normally computationally very demanding, as the order of the equations can be several times larger than the number of records. However, iterative solutions can be obtained even with a small computing capacity. This paper describes how an iterative algorithm works with a reduced animal model. Iteration is only on breeding values of parents, and after convergence breeding values of non-parents (animals without offspring) are obtained from own records and parental breeding values. This strategy minimizes the number of breeding values kept in core and avoids iteration on a large fraction of breeding values. This means that animal model evaluations in large populations are possible with small to moderate computing capacity (e.g. PC's).

METHODS

Data are described by the mixed model in matrix notation

$$Y = X\beta + Za + Wc + e \quad (1)$$

where β is a vector of fixed effects, **a** is a random vector of breeding values, and **c** and **e** are vectors of other random effects and residuals, respectively. **X** , **Z** and **W** are design matrices relating explanatory variables to the observations **Y**.

$$E(Y) = X\beta, \quad Var(a)=A\sigma_a^2, \quad Var(c)=I\sigma_c^2, \quad Var(e)=I\sigma_e^2$$

A is the numerator relationship matrix and **I** an identity matrix of proper order.

Assume that the matrix of observations is sorted by parents and non-parents. Parents are all animals with offspring, and non-parents are all animals without any offspring. Then model (1) can be rewritten

$$\begin{bmatrix} Y_p \\ Y_o \end{bmatrix} = x\beta + \begin{bmatrix} Z_p a_p \\ Z_o a_o \end{bmatrix} + Wc + \begin{bmatrix} e_p \\ e_o \end{bmatrix} \quad (2)$$

where subscripts p and o refer to parent- and non-parent (offspring) records, respectively.

Assume that non-parents have no records or one record, i.e. Z_o is a diagonal matrix with 1 in the diagonal for non-parents with records and 0 in the diagonal for non-parents without records.

The breeding values of offspring can be written as

$$a_o = \frac{1}{2}a_s + \frac{1}{2}a_d + z \quad (4)$$

where subscripts o,s and d represent offspring, sire and dam, respectively. z is the Mendelian sampling term. In matrix notation.

$$\mathbf{a}_o = \mathbf{T}\,\mathbf{a}_p + \mathbf{z} \quad (5)$$

$Var(z)=D\sigma_a^2$, where **D** is a diagonal matrix with elements d_i equal to ½, ¾ and 1 if both, one or neither parent is known, respectively. **T** is a design matrix with ½ in the columns corresponding to parents of the animal in row i.

Using (5) in (2) we get

$$Y = X\beta + \begin{bmatrix} Z_p \\ Z_o T \end{bmatrix} a_p + Wc + \begin{bmatrix} e_p \\ e_o + Z_o z \end{bmatrix} \quad (6)$$

which can be written

The models in (1) and (7) are equivalent models as defined by Henderson (1985), as first

$$Y = X\beta + Z^*a_p + Wc + \epsilon \tag{7}$$

and second moments are the same. Solutions can be obtained by the mixed model equations (Henderson 1973)

$$\begin{bmatrix} X'R^-X & X'R^-Z^* & X'R^-W \\ Z^{*'}R^-X & Z^{*'}R^-Z^*+A^-\lambda & Z^{*'}R^-W \\ W'R^-X & W'R^-Z^* & W'R^-W+I\delta \end{bmatrix} \begin{bmatrix} \hat{\beta} \\ \hat{a}_p \\ \hat{c} \end{bmatrix} = \begin{bmatrix} X'R^-Y \\ Z^{*'}R^-Y \\ W'R^-Y \end{bmatrix} \tag{8}$$

$$\textit{where} \quad \lambda = \frac{\sigma_e^2}{\sigma_a^2} \; ; \quad \delta = \frac{\sigma_e^2}{\sigma_c^2}$$

This is equivalent to the reduced animal model proposed by Quaas & Pollak (1980). Non-parents breeding values are absorbed which gives

$$R^- = I - Z_o(Z_o'Z_o + D^-\lambda)^-Z_o'$$

and back solving for non-parents breeding values gives

$$\hat{a}_o = T\hat{a}_p + (Z_o'Z_o + D^-\lambda)^-Z_o'(Y_o - X_o\hat{\beta} - Z_oT\hat{a}_p - W_o\hat{c}) \tag{9}$$

However, these equations do not need to be set up explicitly. Solutions to the mixed model equations can be obtained using an iterative method, iterating on data (Schaeffer & Kennedy 1986; Misztal & Gianola 1986). When convergence is reached solutions for non-parents are obtained using back-solving techniques while reading the data-file once again.

To simplify notation and to facilitate presentation of the ideas, the algorithm is given in scalar notation for a simple case with only one fixed effect and with breeding values of the animals in the model (1).

A mixed Gauss-Seidel and second order Jacobi algorithm as proposed by Misztal & Gianola (1987) is used. Gauss-Seidel is used for all fixed effects and yields the following iterative equation

$$\hat{\beta}_l^{t+1} = \frac{1}{n_l}\sum_{1}^{n_l} r_i\{Y_{ijk} - k_o\hat{a}_j^t - (1-k_o)(\frac{\hat{a}_{js}^t + \hat{a}_{jd}^t}{2})\} \tag{10}$$

where k_o is equal to 1 if it is a record for a parent and equal to 0 if it is a non-parent

record. a_{js} (a_{jd}) is the breeding value of sire (dam) of animal j in round t. Superscript t represents the iteration number. That is, a new estimate of β_i is obtained as a weighted average of records related to β_i adjusted for the present breeding values of animals. Breeding values are the breeding values of parents for parent records and the average parental breeding value for non-parent records. Weights are elements of R^{-}, which is a diagonal matrix with elements

$$r_t = \begin{cases} 1 & \textit{if parent record} \\ \dfrac{\lambda}{\lambda + d_t} & \textit{if non-parent record} \end{cases}$$

The iterative process, using second order Jacobi, for breeding values yields the following equation

$$\hat{a}_j^{t+1} = \alpha(\hat{a}_j^{t} - \hat{a}_j^{t-1}) + \frac{Q^t}{D} \qquad (11)$$

where the first term is a contribution from the last change in the estimate for a_j ($0 < \alpha < 1$). α equal to 0 gives first order Jacobi, and $\alpha > 0$ gives second order Jacobi. Second order Jacobi is preferable in models with a relationship matrix to aid convergence.
Further

$$D = n_j + \frac{1}{4}(n_2 + n_1) + \lambda\left(\frac{n_2}{2} + \frac{n_1}{3}\right) + k_o f\lambda$$

f is equal to 2, 4/3 or 1 if two, one or neither parent is known, respectively. λ is the ratio of residual variance over additive genetic variance. If there are no other random effects in the model, $\lambda = (1-h^2)/h^2$. n_1 and n_2 are the numbers of offspring with one and both parents known, respectively. The factor D is the effective number of observations on animal j, weighting information from own records, from offspring and from parents.

$$Q^t = \begin{aligned} &\sum_1^{n_j} r_j \frac{1}{2}(1-k_o)\{Y_{ijk} - \hat{\beta}_i^t - (1-k_o)\left(\frac{\hat{a}_{jm}^t}{2}\right)\} \\ &-\lambda k_o \sum_1^{n_2} -\hat{a}_{jo}^t + \frac{\hat{a}_{jm}^t}{2} \\ &-\lambda k_o \sum_1^{n_1} -\frac{2\hat{a}_{jo}^t}{3} \\ &-\lambda k_o g \end{aligned}$$

where

$$g = \begin{cases} -\hat{a}^t_{js} - \hat{a}^t_{jd} & \text{if both parents are known} \\ -\frac{2}{3}\hat{a}^t_{jp} & \text{if one parent is known} \\ 0 & \text{if neither parent is known} \end{cases}$$

This is the accumulated information on animal j in round t. The first line represents information from own and offspring records corrected for fixed effects. Offspring records are also corrected for the mate (a_{jm}, the breeding value of the other parent of the offspring). The rest represents information from relationships to other animals. The second line is information from offspring with the other parent known. The third line is information from offspring with the other parent unknown. The last line is information from parental breeding values.

Predicted breeding values are weighted averages of (1) own records adjusted for fixed effects and progeny records adjusted for fixed effects and breeding value of mate and (2) information from relatives, i.e. parental and progeny breeding values.

After convergence, the solutions for non-parents are obtained by back-solving

$$\hat{a}_{jo} = \frac{1}{2}(l_s\hat{a}_{js} + l_d\hat{a}_{jd}) + c_j\frac{d_j}{d_j+\lambda}(Y_{ij} - \hat{b}_i - \frac{1}{2}(l_s\hat{a}_{js} + l_d\hat{a}_{jd})) \qquad (12)$$

where l_s (l_d) is equal to 1 if the sire (dam) is known and 0 if unknown. c is equal to 1 if that non-parent has a record or 0 if it has no record. Breeding values of non-parents are simply obtained as the average parental breeding value plus a contribution from their own record, if they have one. This contribution is a function of their deviation from their expected performance multiplied by a constant which depends on the relative variances. With both parents known and $\lambda=(1-h^2)/h^2$ this is $h^2/(1-\frac{1}{2}h^2)$.

The simple method of computing the inverse of the numerator relationship matrix is used above (Henderson 1976), but an algorithm can be included to take inbreeding into account (Quaas 1976).

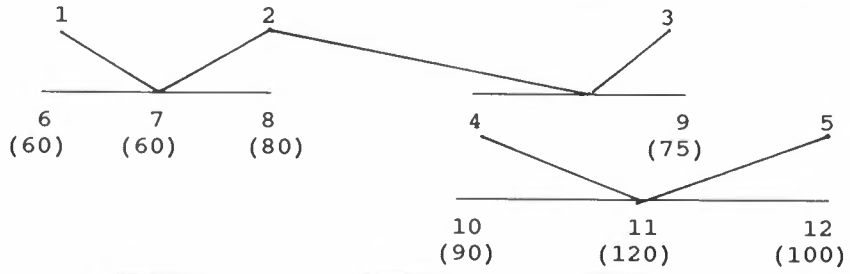
NUMERICAL EXAMPLE

To illustrate the algorithm a small pedigree is shown in Table 1. Animals with records (leather thickness in 1/100 mm) have the record in parentheses. Data are extracted from a data set where leather thickness is measured on skins from male kits (data set I in Berg & Lohi 1991). Leather thickness is only measured in non-parents. In this example there are two fixed effects (year), five parents (1-5) and eight non-parent animals (6-12). If we denote solutions for year i by P_i and solutions and observations for animal j by a_j and Y_j,

we get a set of equations (13) to iterate on.

Superscripts refer to iteration number. Estimates without superscripts refer to round t. The ratio of residual to genetic variance, λ, is equal to 1 ($h^2=0.50$).

Table 1. Pedigree and data (in parentheses) for a numerical example



$$
\begin{aligned}
\hat{P}_1^{t+1} &= \frac{Y_6+Y_7+Y_8+Y_9-\frac{1}{2}(3\hat{a}_1+4\hat{a}_2+\hat{a}_3)}{4} \\
\hat{P}_2^{t+1} &= \frac{Y_{10}+Y_{11}+Y_{12}-\frac{3}{2}(\hat{a}_4+\hat{a}_5)}{3} \\
\hat{a}_1^{t+1} &= \frac{Y_6+Y_7+Y_8-3\hat{P}_1-\frac{3}{2}\hat{a}_2}{4.5}+\alpha(\hat{a}_1-\hat{a}_1^{t-1}) \\
\hat{a}_2^{t+1} &= \frac{Y_6+Y_7+Y_8+Y_9-4\hat{P}_1-\frac{3}{2}\hat{a}_1-2\hat{a}_3-\frac{3}{2}\hat{a}_4}{6.5}+\alpha(\hat{a}_2-\hat{a}_2^{t-1}) \\
\hat{a}_3^{t+1} &= \frac{Y_9-\hat{P}_1-2\hat{a}_2-3\hat{a}_4}{5}+\alpha(\hat{a}_3-\hat{a}_3^{t-1}) \\
\hat{a}_4^{t+1} &= \frac{Y_{10}+Y_{11}+Y_{12}-3\hat{P}_2+3(\hat{a}_2+\hat{a}_3)-\frac{3}{2}\hat{a}_5}{7.5}+\alpha(\hat{a}_4-\hat{a}_4^{t-1} \\
\hat{a}_5^{t+1} &= \frac{Y_{10}+Y_{11}+Y_{12}-3\hat{P}_2-\frac{3}{2}\hat{a}_4}{4.5}+\alpha(\hat{a}_5-\hat{a}_5^{t-1})
\end{aligned}
\qquad (13)
$$

The estimate for P_1 is the mean of records in year 1 adjusted for the breeding values of animals recorded in year 1.

The estimate for a_2 is the mean of its offspring corrected for the fixed effects of year 1 and corrected for the dams of its offspring plus a contribution from the breeding value

of its son, animal 4, corrected for the breeding value of the dam of animal 4, animal 3.

The results from selected rounds of iteration are presented in Table 2 with $\alpha=0.5$.

Non-parent breeding values are simply calculated as the average of their parental breeding values plus

$$\frac{2}{3}(Y_i-\hat{P}_i-\frac{1}{2}(\hat{a}_s+\hat{a}_d))$$

Breeding values for non-parents are a weighted average of the average parental breeding values and their phenotypic deviation from their expected value.

Table 2. Iteration history of the numerical example in Table 1, using equations (13)

Round	P_1	P_2	a_1	a_2	a_3	a_4	a_5
1	68.75	103.33	-1.39	0.00	1.25	0.00	0.00
2	69.11	103.33	-2.33	-0.29	1.80	0.50	0.00
3	69.54	103.08	-2.29	-0.42	1.78	0.96	0.00
4	69.59	102.86	-1.79	-0.16	1.81	0.96	0.00
5	69.28	102.85	-1.44	0.10	1.80	0.86	0.00
.							
.							
20	69.17	102.92	-1.67	0.00	1.67	0.83	-0.00
.							
.							
100	69.17	102.92	-1.67	-0.00	1.67	0.83	0.00

DISCUSSION

The presented algorithm is especially efficient in multiparous species, like fur animals, where the number of parents is considerably lower than the number of animals recorded. Up until convergence, iteration is only on parent breeding values and after convergence breeding values for non-parents are found.

This has two advantages. In each round of iteration calculations are reduced to those involved in estimation of parent breeding values.

This also means that this algorithm has to keep fewer solutions in memory than full animal model applications, so larger problems can be solved within a given computer capacity. This makes it useful in on-farm breeding value estimation for fur animals.

A further simplification is possible. In fur animals most traits are measured only once, either in kits as live animals or on skins. This means that the datafile and relationship file can be combined, which reduces the number of files read in each round and hence speeds up the algorithm. This could also be done if the combined file was sorted by animal id. This can, however, be a problem in large data sets.

Reproductive traits are only measured in parent animals, so non-parents only have information from their parents. In this case the algorithm excludes these non-informative animals from iteration and after convergence calculates their breeding value as the average

of their sires' and dams' breeding values.

The presented algorithm covers the most important situations in fur animals, but the algorithm can be extended to include multiple observations on non-parents and maternal effects.

CONCLUSION

An algorithm for animal model evaluation is presented which utilizes a reduced animal model parameterization. However, the algorithm iterates on data and the reduced animal model equations are not set up explicitly.

This algorithm is useful in fur animal applications, where traits are measured only once in non-parents, as is the case for most if not all traits in fur animal breeding. Furthermore the proposed algorithm is efficient as it can handle large populations within a small computing capacity. This makes it useful for on-farm evaluations of fur animals.

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Norwegian breeding programme for mink and foxes

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The national breeding programme for mink and foxes was established in Norway in 1983, based on a national breeding plan. The breeding goals and strategies are presented here together with an evaluation of the results up until 1992. The most important strategy is the field recording system (Pelsdyrkontrollen). About 25% of farmers are participating in the field control, representing 17.8%, 26.4% and 26.6% of the mink, silver fox and blue fox populations, respectively. The results of the breeding strategies are discussed and related to the assumptions that were put forward. The practical adaptations of the programme are also discussed and alterations to the breeding programme proposed.

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The farming of mink and foxes started at the end of the last century in North America. The first attempts were sporadic and unsuccessful. The start of modern farming could therefore be defined as the start at Prince Edward Island, on the east coast of Canada, by Dalton, Oulton and Rayner. The first pair of silver foxes came to Norway in 1914, Amund and Amanda, and the first mink about ten years later.

Einarsson (1988) has divided the history of mink and fox breeding in Norway into three periods. During the first period, up until 1926, there was no organized breeding work carried out with defined breeding goals, and there was an uncritical distribution of breeding animals. During the next period, up until 1983, strict criteria were established for the approval and registration of breeding animals. Exhibitions of live animals and skins were also introduced during this period, and gave the farmers good standards for their own selection of breeding animals. The national breeding plan was presented in 1983 (Einarsson et al. 1983), describing the breeding goals and strategies to be used. The field programme for mink and foxes was developed and introduced, together with the fox circles that were based on artificial insemination.

BREEDING GOALS AND BREEDING PROGRAMME

The breeding goals are discussed and defined in the national breeding plan. The general

breeding goal is to achieve the best economic return per breeding unit. This is divided into long-term, short-term and future breeding goals, as follows.

Long-term	Short-term	Future
Increase no. of kits per breeding unit	The right colour.	Better feed conversion
Animals with good liveability, growth and development	Good clarity	Better disease resistance
Increased pelt size		
Good temper		
Right type of characteristics		

The developed field recording system (Pelsdyrkontrollen) is based on the information on the animals registered by the farmer and the skin registrations, or classifications, from the auction house. The data flow and the different units involved in the control are shown in Fig. 1. The control is based on cage-cards, on which all information about the animals is given (identification, indexes, results in recent years etc.). The farmer reports back to the central databank through a slip on the cage-card or through the telephone line. The programme includes calculation of two indexes, one for litter size and one for skin quality, as classified at the auction house. Besides these two independent indexes, several other traits are included through independent culling level. For further description of the system, see Fimland & Einarsson (1988) and Johannessen (1990).

The fox circles are based on the use of artificial insemination, and allow a progeny test for fertility and fur characteristics (Einarsson 1983). This programme is not presented or discussed further in this paper (see Johannessen (1989) for presentation and results).

The members in the field control, as a percentage of the Association's members, have increased by up to about 25% (Table 1). As observed in other countries the number of members in the Norwegian Fur Breeders Association has decreased during the recent years (Table 1).

In Table 2 the numbers of breeding females from the field control farms are presented, where a farm can have one or more of the three species. The breeding females included in the control have increased for all species, and in 1991 are 17.8%, 26.4% and 26.6% for mink, silver fox and blue fox, respectively. A total of 34 093 breeding females were included in the control in 1991.

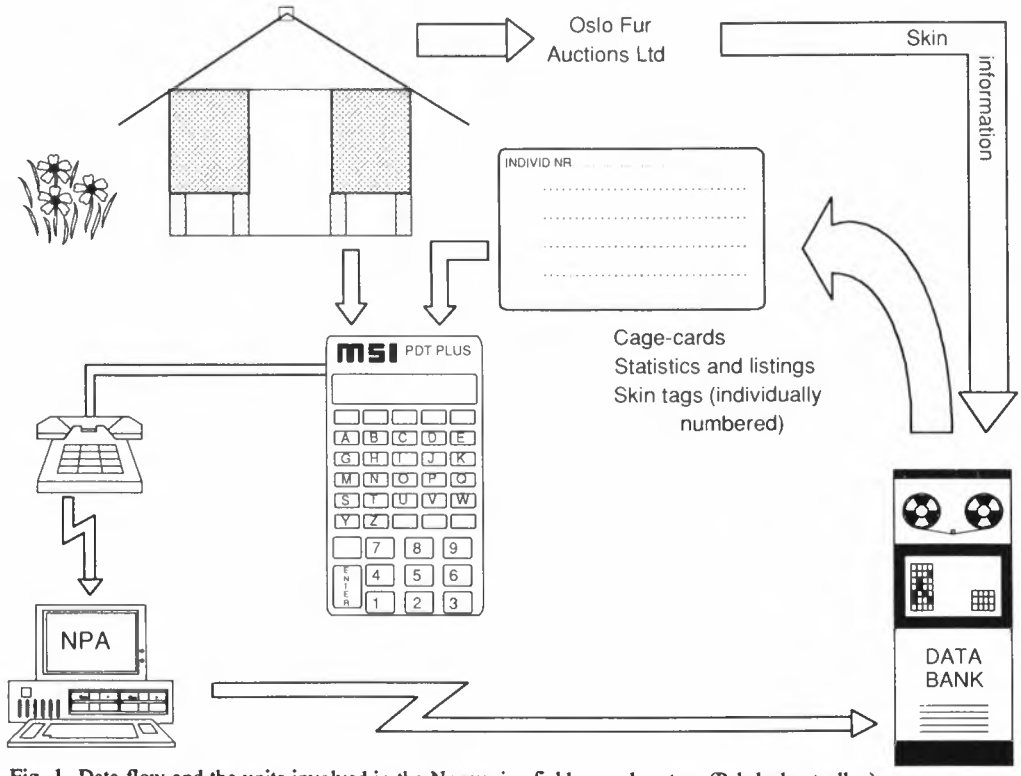

Fig. 1. Data flow and the units involved in the Norwegian field record system (Pelsdyrkontrollen)

Table 1. Members of the field recording system (PK) in Norway 1986-91

YEAR	TOTAL	PK	%
1986	3638	316	8.7
1987	3742	487	13.0
1988	3686	608	16.5
1989	3498	748	21.4
1990	3008	792	26.3
1991	2801	664	23.7

Total = member of the Norwegian Fur Breeders Association

EVALUATION OF THE RESULTS

The reproduction results from the field control and from the total population are presented in Table 3, as the number of kits per female three weeks post partum. In the field control per mated female and for the whole population per registered female at January 31. The results for mink in the field control have increased by one kit during the last five years, which is relatively stable per year. Expressed as a deviation from the whole population, the figures for mink are 0.8 kits in 1991, increasing from 0.5 in 1986 in favour of the field control.

Table 2. Breeding females of mink and foxes in Norway. Total number of breeding females in Norway, and of the members of the field recording system (PK) respectively

Year	Mink			Silver fox			Bluefox		
	Total	PK	%	Total	PK	%	Total	PK	%
1986	130337	14454	11.1	63345	8484	13.4	99209	9888	10.0
1987	117375	19315	16.5	89578	14982	16.7	104232	13847	13.3
1988	123631	209821	17.0	112648	20828	18.5	100201	17085	17.1
1989	111091	17314	15.6	104502	21458	20.5	64799	11843	18.3
1990	69597	10444	15.0	74058	16410	22.2	56728	11266	19.9
1991	58261	10394	17.8	48676	12836	26.4	40779	10863	26.6

The results for the foxes varied during the period 1986-91. For the silver fox an increase in 0.2 kits is observed in the field control, the same as for the whole population. The difference in 1991 was 0.5 kits in favour of the field control.

Table 3. Breeding results for mink and foxes in Norway. Members of the field recording system (PK): Kits per mated female at 3 weeks post partum. Total: Kits per registered female in the Norwegian Fur Breeders Insurance Association, at 3 weeks post partum

	1986	1987	1988	1989	1990	1991
Mink:						
PK	4.2	4.3	4.6	4.7	4.8	5.2
Total	3.7	3.9	3.9	3.9	3.9	4.4
Silverfox:						
PK	2.9	3.0	2.9	3.0	3.0	3.1
Total	2.4	2.6	2.5	2.5	2.5	2.6
Bluefox:						
PK(1)	5.4	5.8	5.6	5.6	5.4	5.4
Total(2)	4.2	4.3	4.2	4.3	3.9	4.4

(1) Pure bred only
(2) Incl. females mated with silverfox males

For the blue fox an increase in litter size was observed in the first years, but a decrease in 1991 to the 1986 level. The results for the whole population have fluctuated, with the largest deviation in the two last years.

It should be kept in mind that the mean of the whole population also includes the females in the field control, and therefore their results will affect this mean, often increasing it. The results, especially for mink, show the gain in litter size by using a selection index, as shown under experimental conditions (Einarsson 1987). It is difficult, however, to estimate the genetic changes under practical conditions.

In Table 4, skin size and skin quality are presented, as classified at the auction house. The classifications are discontinued and not normally distributed. It is therefore difficult to estimate the exact changes when changes occur only between classes. A more detailed

evaluation would require several graded classes.

Table 4. Development in skin quality and skin length for farms in the field recording system (PK) in Norway

	Mink			Silver fox			Bluefox		
	Qual.	Size	No. of skins	Qual. (OS)	Size (OS)	No. of skins	Qual. (OS)	Size (OS)	No. of skins
1987	6.96	6.27	16570	8.09 (6.85)	8.7 (8.01)	8181	8.11 (7.26)	8.1 (7.85)	28221
1988	7.16	6.36	22231	7.96 (6.68)	8.16 (8.03)	16744	8.11 (7.48)	8.21 (7.88)	32581
1989	7.14	6.21	14134	7.95 (6.94)	7.91 (7.87)	23521	8.12 (7.57)	7.97 (7.74)	40702
1990	6.98	6.25	31196	8.03 (6.93)	8.06 (7.93)	32261	8.23 (7.72)	8.2 (7.88)	31703
1991	7.41	6.24	10904	7.91 (6.86)	8.12 (7.94)	33638	8.22 (7.48)	8.21 (7.93)	33639

Qual. (quality) = 9 - 4 (9 = best)
Size = 9 - 4 (9 = longest)

Figures in ( ) are mean quality and size for all Norwegian skins sold through the auction houses
Number of skins originating from members of the field recording system

There are only minor differences in size classification for mink. The quality classification varies, however, and shows the highest value in the last year.

For foxes, the classifications for the two traits are also given for the whole population mean. The mean of classifications for skin quality from the field control is well above the total population mean. The size differences are smaller, but always in favour of the field control.

It is difficult to estimate the effect of the field control on skin size and skin quality, especially the genetic effects. The farms in the field control may also have a higher percentage of breeding animals sold, which would reduce the average classification of the quality and size of the skins from these farms. It is also possible that the farmers have held back skins in recent years because of the economical conditions.

The practical experiences from the field control are good, and the cage-card and statistics are helpful for the farmer. The selection index for litter size is very useful and easy to include in the breeding programme. The use of the skin quality index, however, is more complicated. The assumption that a representative number of skins are classified is often not fulfilled, especially in farms with large sales of breeding animals. For these farms the index may even be the opposite of the "true one". As the deviation from the average is from the whole population, the best farms can have more than 80% in only one class. This reflects an abnormal distribution and low variation. The use of skin quality classification should therefore not be used in an index in the field control, only in progeny

testing in the fox circles. It is then necessary to investigate for an optimum use of fur characteristics. However, this is connected with problems such as uncertain judgement by the farmer, short period of judging and calculating index for all relevant animals and the number of animals to be judged.

The breeding plan will be revised and changes proposed, as discussed. It is necessary to document for relevant fur characteristics that can be combined in an index.

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Correlation between the development of mink kits in the lactation and growth periods, correlations to fur properties and heritability estimations

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At birth distinct differences between sexes are found in both body weight and body length of mink kits. During the lactation period the body weight and body length at four weeks of age provide the best estimates of skin size. Much higher correlations are, however, found in September, October and at pelting. From the age of six weeks male kits show negative correlation between high body weight and fur quality of dried pelts. This correlation is increased from September onwards. The most pronounced negative correlation with fur quality is reflected in the condition at pelting, e.g. the relation between body weight and body length. For female kits the negative correlation is found only in December. At two and four weeks of age heritability for length is higher than that for weight. Maternal effects for both length and weight increase from two to four weeks of age.

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The nursing period is of vital importance to kit growth and thus also to pelt production. Therefore it is important to understand the relation between the early growth and the final skin size and pelt quality and the individual´s future reproduction capabilities.

Weight at pelting can be used as a measure for skin length, but high body weight at pelting has a negative relation to skin quality. In order to optimize breeding programmes it is important to investigate whether weight at pelting can be replaced or supplemented by other parameters measured at an earlier stage.

MATERIAL AND METHODS

During 1989-91 we recorded the weight and length development of kits from birth until pelting but with special focus on the nursing period. This formed part of a larger

investigation on the parameters connected with the lactation period and including selection for high respectively low growth from two to four weeks after birth. The investigation started in 1989 with 71 females and 21 males resulting in 351 kits, making by late June an average of 4.9 kits per fertile female. The corresponding numbers for the year 1990 were 79 females with 402 kits, 5.1 kits per litter, and for 1991, 91 females with 488 kits, 5.4 kits per litter.

The kits were weighed on electronic scales with an accuracy of one gram every two weeks during the nursing period and then once a month after weaning (Table 1). Body length was measured from the tip of the nose to tail root with 0.5 cm accuracy. During lactation the kits were measured on a horizontal surface. After weaning, the body length was measured during a brief period under ether anaesthesia. Further measurments were taken in September 1989, and in 1990-1991 in July and September. Each year pelted kits were measured and weighed immediately after euthanizing and those selected for breeders under anaesthesia in December.

Table 1. Schedule for recording body weight, body length and skin length

Time	Body weight	Body length	Average age (days)
At birth	WT0	L0	1
2 weeks	WT2	L2	14
4 weeks	WT4	L4	28
6 weeks	WT6	L6	42
Separation	WTJUN	-	59
29-06-90, 23-06-91			66
Separation 89 (5-7)	WTJUL89	-	
July (19-7)	WTJUL	LJUL	80
August (10-8)	WTAUG	-	101
September (15-9)	WTSEP	LSEP	119
October (26-10)	WTOCT	-	176
December (14-12)	WTDEC	LDEC90	224
Skin length		LSKINS	

In November all the kits were graded as live animals for size and fur quality (scores 1-5). Pelt information originates from 188 pelts from 1989 and 219 pelts from 1990. The pelt lenght were measured with an 0.5 cm level of accuracy and graded for quality using scoring from 1 to 12.

The results presented are averages and correlations calculated by means of the SAS statistical programmes. Heritabilities and common litter effects (maternal effects) are estimated for body weight and body length at two and four weeks of age. The model used for estimation of heritabilities is:

$$Y_{ijklm}=\mu+P_i+S_j+L_k+a_l+c_m+e_{ijklm}$$

P: periods i = 1, ...24
years = 1989, 1990 and 1991.
dates of birth (22-27), 28, 29, 30 April and
1, 2, (3-4), (5-12) of May.

S: sex j = 1, 2 1 = males, 2 = females.

L: litter size at birth k = 1, ...,5
size (1-3), (4-5), 6, (7-8), (9-11).

a: additive genetic effects l = 1,.....,1328. Random effect with expectation 0, and (co)variance matrix $A\sigma^2_a$, where A is the numerator relationship matrix.

c: (co)variance between full-sibs not due to additive genetic effects, that is maternal effects, non-additive genetic effects and common environmental effects. C is assumed to be identically and independently distributed (IID) $(0,\sigma^2_c)$
m = 1,.....234.

e: residual variation. IID $(0,\sigma^2_e)$.

Variance components are estimated using a derivative-free REML procedure (Meyer 1989).

RESULTS AND DISCUSSION

The development of body weight and body length for male and female kits in the nursing period and after separation is indicated in Table 2. In 1989 and 1990 the kits were weighed for the first time on the day following their birth and were marked individually at the age of two weeks. In 1991 the kits were marked individually at birth and weighed as close to birth as possible. For birth weights, therefore, only results from 1991 are included here. It can be seen from Table 2 that at birth there is a clear sex difference in both weight and body length. This difference becomes more marked after weaning, and in October the male average equals 1.8 times the female average.

The results of the development of male kits in relation to their later body weight, body length, live animal grading of size and fur quality, and the later skin length and quality are presented in Tables 3, 4 and 5. In all the tables only relations that show a significant correlation are included ($p < 0.05$).

When comparing body weight (Table 3), the highest correlation is found with September weight irrespective of time. Among the early kit weights, the highest correlation with September weight is found at the age of four weeks (0.55) and with December weight at the age of six weeks (0.37).

Correlations between body weight and body length are almost equal in September and December (Table 3). During lactation high correlations of weight with both September and December length are again found at the age of four weeks (0.65 and 0.60, respectively). September weight provides the best estimate for final body length (correlations 0.73/0.71).

Table 2. Development of body weight (g) and body length (cm) for male and female kits in the nursing period

Bocy weight	Males $\bar{x} \pm$ std	Females $\bar{x} \pm$ std	p-level
At birth	11.2 ± 2.0	10.5 ± 1.8	***
14 days	72.5 ± 12.8	66.3 ± 11.2	***
28 days	172.5 ± 29.4	156.0 ± 25.5	***
42 days	353.6 ± 74.7	308.0 ± 58.6	***
At separation	685.0 ± 129.3	534.3 ± 80.1	***
July	1125.0 ± 138.0	770.4 ± 73.6	***
August	1488.1 ± 181.0	920.1 ± 89.6	***
September	1833.5 ± 211.4	1028.7 ± 117.5	***
October	2164.0 ± 257.5	1206.6 ± 133.3	***
Body length			
At birth	7.6 ± 0.5	7.4 ± 0.4	*
14 days	13.9 ± 0.9	13.6 ± 0.8	***
28 days	19.4 ± 1.3	18.7 ± 1.2	***
42 days	25.8 ± 1.7	24.8 ± 1.6	***
September	47.3 ± 1.8	39.5 ± 1.4	***
December	47.7 ± 1.8	39.4 ± 1.2	***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 3. Correlation between body weight at different ages and later development of size and fur quality. Male kits. (Only significant correlation min. $p < 0.05$)

Body weight at age	Weight Sep.	Weight Dec.	Length Sep.	Length Dec.	Live animal Size	Live animal Qual.	Skins Size	Skins Qual.
At birth:								
individual	0.26	-	0.23	0.22	-	-	[1]	[1]
litter $\bar{x}$[2]	0.27	-	0.39	0.26	-	-	0.32	0.16
14 days	0.51	0.33	0.53	0.57	0.19	-	0.29	-
28 days	0.55	0.35	0.65	0.60	0.20	-	0.40	-
42 days	0.53	0.37	0.54	0.55	0.29	-	0.39	− 0.16
June	0.57	0.30	0.60	0.41	0.15	-	0.45	− 0.25
July	0.75	0.51	0.68	0.57	0.34	-	0.59	− 0.24
August	0.83	0.56	0.68	0.63	0.41	-	0.58	− 0.24
September	-	0.75	0.73	0.71	0.50	-	0.70	− 0.40
October	0.90	0.87	0.63	0.70	0.62	− 0.08	0.73	− 0.41
December	-	-	0.54	0.66	0.62	− 0.10	0.80	− 0.34

[1] skin gradings from 1991 not included, [2] litter average

Table 4. Correlation between body length at different ages and later development of size and fur quality. Male kits. (Only significant correlation min. p < 0.05)

Body length	Weight		Lengt		Live animal		Skins	
at age	Sep.	Dec.	Sep.	Dec.	Size	Qual.	Size	Qual.
At birth:								
individual	0.14	-	0.14	-	-	-	[1]	[1]
litter x[2]	0.30	0.17	0.33	0.36	-	-	0.32	-
14 days	0.44	0.30	0.51	0.54	0.16	-	0.27	− 0.13
28 days	0.48	0.32	0.60	0.54	0.20	-	0.47	-
42 days	0.52	0.36	0.58	0.59	0.28	-	0.39	-
July	0.65	0.41	0.69	0.63	0.30	-	0.47	− 0.24
September	0.73	0.54	-	0.77	0.35	-	0.72	− 0.18
December	0.71	0.66	0.77	-	0.55	-	0.74	− 0.21

[1] skin gradings from 1991 not included, [2] litter average

Table 5. Correlation between fatness (g/cm) at different ages and later development of size and fur quality. Male kits. (Only significant correlation min. p < 0.05)

Fatness	Weight		Length		Live animal		Skins	
at age	Sep.	Dec.	Sep.	Dec.	Size	Qual.	Size	Qual.
At bith:								
individual	0.26	-	0.22	0.21	-	-	[1]	[1]
litter x [2]	-	-	-	-	-	0.13	-	0.16
14 days	0.51	0.34	0.51	0.55	0.20	-	0.29	-
28 days	0.50	0.32	0.56	0.54	0.18	-	0.33	-
42 days	0.50	0.36	0.49	0.49	0.29	-	0.38	− 0.18
July	0.72	0.51	0.60	0.49	0.33	-	0.50	− 0.21
September	0.96	0.73	0.52	0.60	0.38	− 0.11	0.62	− 0.42
December	0.71	0.97	0.36	0.42	0.55	− 0.11	0.83	− 0.43

[1] skin gradings from 1991 not included, [2] litter average

Correlation with body size estimated at live animal grading increases evenly during the growth period. Correlations between early kit weights and live animal grading are generally low (0.16-0.20). but reach up to 0.62 in October-December. The early weights are unrelated to fur quality at live animal grading. Only animals with heavy body weights in October and December seem to have a slightly inferior fur quality at live animal grading.

The correlation between body weight and skin length increases during the growth period with the highest correlation at pelting (0.80). From the age of 42 days an increasing, negative correlation is found between body weight and pelt quality (at pelting -0.34). During the lactation period the highest correlation with skin length without a negative

correlation with quality is found at the age of four weeks (0.40).

The correlation between body length and later results can be seen in Table 4. The relation with September weight is stronger than that with December weight. For the early measures, the body length at six weeks has the highest correlation with September and December weights, 0.52 and 0.36, respectively. Correlations of early body length with length in September and December are equal. At the age of four and six weeks the correlations with both September and December lengths are high (0.60/0.58 and 0.54/0.59).

Correlations between measured body length and grading scores of size in November are in general low, but increase evenly during the growth period. The highest correlation among early body lengths is 0.28 at the age of six weeks. No correlation between body length and fur quality at live animal grading has been found.

Body lengths in September and December have an equal correlation with skin length compared with body weights at the corresponding times (length 0.72/0.74, weight 0.70/0.80). Among the early results the body length at four weeks provides the best estimate of skin length (correlation 0.47).

At the ages of four and six weeks no negative correlation was found between body length and pelt quality. However, from July onwards a negative correlation occurs but this is lower than the correlation with body weight.

The degree of fatness calculated as grams per cm body length is indicated in Table 5. The most marked differences compared with the results in Tables 3 and 4 are that in September the condition of the animal seems to have a slightly lower correlation with skin length (0.62) than the actual body weight (0.70) or length (0.72) in September. The fat condition in December results in large pelts (correlation 0.83) but increases the negative effect on quality (correlation 0.43).

The female kits show similar results to those of the male kits. However, a negative correlation with fur quality is found only for body weight and fatness in December (-0.18 and -0.36). The correlation between body weight at four weeks of age and skin length is higher than that in males (0.52).

Direct additive genetic effects (h^2) and maternal effects (c^2) are considerable (Table 6). Heritabilities for length are higher than those for weight. Maternal effects increase from two to four weeks, indicating that factors common for litters are very important.

Table 6. Heritabilities for early weight and body length

	Body length		Body weight	
	2 weeks	4 weeks	2 weeks	4 weeks
h^2	0.52	0.44	0.35	0.18
c^2	0.40	0.49	0.64	0.75

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Enhancing mink breeding in Holland

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The NFE introduced two new services to their members in the late 1980s: A technical economical administration program called TEAP to improve the production efficiency on the farms and the DanMink system to improve the selection work. The TEAP program was started in 1987 as a central system run at the NFE office, and it is used by 25-30% of the mink farms in Holland. The DanMink system,which was introduced in 1989,was the English version of the Danish PC system for minkbreeding. The system is used by 25 farmers,that is,10% of the Dutch mink farms.

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Although the fur industry in Holland started at the end of the 1920s,the main development has only taken place in the last 20 years.The value of the production has doubled during the last 10 years and reached 130 million dfl. in 1991. The Dutch proportion of the world fur production is approximately 5 %.

The number of mink farms in Holland from 1988 to 1991 is listed in Table 1.

Table 1. Number of mink farms in Holland

Year	Number of farms	Total number of female breeders	Average number of female per farm
1988	282	372.000	1340
1989	281	378.700	1348
1990	246	353.866	1438
1991	243	381.838	1571

The Dutch Fur Breeders' Association (NFE) was founded in 1928 as an umbrella organization of the local fur breeders' organizations.The activities of the NFE cover two main areas:

- policy of the industry
- providing members with support in farm management

In the late 1980s the NFE started up two new activities to improve the support for their members. These were the TEAP, a technical, economic administration to improve

production efficiency, and the DanMink(R) system to improve selection work.

MATERIALS AND METHODS

The TEAP was initiated in 1987 and comprises a central system run at the NFE office. Every month the farmers send in information about number of animals, mortality, feed consumption, feed prices, cost of vaccines, blood tests, etc. The mating- and whelping results are sent in after the two periods.

A monthly report is returned to the farmers giving the results of the feed consumption per month and accumulated for the total period. Mortality results are reported every second month, and mating- and whelping results are reported after those periods.
At the end of the year a total report is issued showing the main production results for the whole year.

The DanMink system, which was introduced in 1989, was the English version of the Danish PC system for minkbreeding. The program, which is used by the individual farmer on his own farm (Clausen 1990a, 1990b), is user friendly and includes the possibility of calculation of indices based on subjective gradings of the kits' pelt traits.
A total breeding value can be calculated on the basis of a litter size index and an index for various pelt traits. The total breeding value is used to select new breeding animals.

The litter size index is calculated on the counting of kits at three weeks of age.It is based on performance from related females for:

Males	Females
offspring	offspring
dam	dam
half sibs	half sibs
full sibs	full sibs
	own litters

The litter size of females with more than one litter is the average of all litters.The litter index for kits is calculated as the mean of the litter index for their sire and dam.

Family indices are calculated for the pelt traits as well as for the live body weight. They are based on the kits' own grading and the full and half sibling results. Both sexes are graded and family means are calculated after an adjustment for sex differences.

All subindices are adjusted to the same mean, 100, and the same standard deviation, 10. The kits' litter index and the indices from the grading time are combined into an overall breeding value.

$$BV = v1*I1 + \ldots\ldots\ldots + vn*In$$

The weight factors v1-vn are found in an iterative process where the farmer interacts with the DanMink system. An extensive support from the NFE office is available to all users.

RESULTS

The TEAP system is used in 25-30% of the mink farms in Holland.
The main results from the TEAP are listed in Table 2.

Table 2. Main results of the TEAP

	1988	1989	1990	1990
Average number of female	1412	1531	1416	1557
First year females (%)	56.4	54.1	45.1	52.8
Non-mated females (%)	2.2	2.7	2.0	3.3
Empty females (%)	7.0	7.0	6.2	7.2
Numer of kits on the 5 June per female on 1 March	5.04	5.05	5.26	5.25
Number of kits at the end of the year per female on 1 March	4.90	5.03	5.07	5.05
Feed consumption per kit from 1 January to 31 October (kg)	40.89	36.63	34.51	34.46

The DanMink system has been used by 25 farmers since the beginning of 1992, that is, 10% of the Dutch mink farms. Interest is growing but due to the low prices farmers are waiting to invest. Of the 25 users, 18 have their own computers and 7 are serviced by the NFE.

The use of LIVEGRADING in the DanMink program is a vast improvement in selection for pelt traits. The precondition for the calculation of subindices is that all the animals are graded in a certain line and that the gradings have a more or less normal distribution. The statistics presented in Tables 3 to 8 are based on the results of a Dutch DanMink-user. An example of this farmer's quality grading can be seen in Table 3.

The efficiency of the litter size index can be demonstrated by dividing the young females into groups according to the size of the litter they were born in or their own litter size index as kits. In Tables 4 and 5, 188 young females are divided into three groups as mentioned above.

The 62 one-year-old females from the largest litters had (5.19-5.04) 0.15 kits more than the average. The 62 one-year-old females with the highest litter size index as kits had (5.46-5.04) 0.42 kits more than the average.

The efficiency of the family index for quality can be demonstrated by dividing the graded kits in 1991 according to the grading of their parents in 1990 or the family index for quality, as shown in Tables 6 and 7.

The best third of the kits from parents with the highest grading was graded 0.26 and 0.25 above the average for males and females respectively. The best third of the kits from parents with the highest quality index was graded 0.56 and 0.37 above the average for males and females respectively.

Table 3. The distribution of scores for quality

scores for quality	Males		Females	
	Number	%	Number	%
1	39	7.99	32	6.15
2	146	29.92	153	29.42
3	154	31.56	191	36.73
4	105	21.52	108	20.77
5	42	8.61	34	6.54
6	2	0.41	2	0.38
Total	488	100.00	520	100.00

Table 4. Litter size of young females in 1991 according to mothers litter size in 1990

Mothers litter size	Number of females	Number of kits at second counting
High	62	5.19
Medium	63	5.14
Low	63	4.80
Total	188	5.04

Table 5. Litter size of young females in 1991 according to their own litter size index as kits in 1990

Females litter size index as kits	Number of females	Number of kits at second counting
High	62	5.46
Medium	63	5.22
Low	63	4.45
Total	188	5.04

Table 6. Kits average grading for quality in 1991 according to average grading of their parents in 1990

Average grading of parents 1990	Male		Female	
	Number	Average	Number	Average
High	56	3.32	50	3.28
Medium	57	3.25	51	3.14
Low	57	2.63	51	2.69
Total	170	3.06	152	3.03

Table 7. Kits average grading for quality in 1991 according to the average quality index of their parents in 1990

Average quality index of parents	Male		Female	
	Number	Average	Number	Average
High	56	3.62	50	3.40
Medium	57	2.88	51	2.94
Low	57	2.70	51	2.76
Total	170	3.06	152	3.03

An estimate of the genetic progress can be calculated by grouping the graded kits according to the birth year of their mothers. An example is given in Table 8.

Table 8. Grading of kits for pelt quality according to birth year of their mother. (Adjusted for birth year of the father and sex difference)

Birth year of the mother	Number of kits	Average grading
1987	33	2.22
1988	141	2.70
1989	295	2.69
1990	327	2.84

The Table indicates good progress in this line.

DISCUSSION

The TEAP produces reliable information about the average technical results of Dutch mink farms. This is of great value for the NFE in the two main areas of activities. The farmers can compare their own results with the average of all farms and the 25% best of the farms. Together with his own results from previous years it gives the farmer a picture of the efficiency on his farm.The reports have to be returned to the farmers before the results become outdated. As it is a central system the turnover is dependent on the most recent farm data.

The results derived from using the DanMink system indicate a potential for greater genetic progress. Using 2 litter size index as a basis for selection for litter size, the genetic progress can be improved,as shown in Tables 4 and 5.

Using livegrading,the genetic progress in pelt characteristics can be improved provided that there is a significant correlation between the farmers' grading of the live animal and the quality of the pelt.

The DanMink system calculates the family indices with standard heritabilities for all populations. The farmers' grading could have a different heritability, but extensive analyses show that the indices are very robust to differences in actual heritability.

ACKNOWLEDGEMENT

DanMink(R) is a registered trade mark of the Danish Fur Breeders'Association.

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Genetic variation in arctic foxes and in silver foxes from different farms

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Blood samples from 725 arctic foxes (*Alopex lagopus*) from eight farms, 698 silver foxes (*Vulpes vulpes*) from ten farms and 32 crossbreeds (*Alopex lagopus* x *Vulpes vulpes*) have been studied by means of agarose gelelectrophoresis, starch gelelectrophoresis, isoelectric focusing and two-dimensional electrophoresis. Seventeen electrophoretic markers have been revealed, nine of them being polymorphic in arctic foxes and nine in silver foxes, but five of them were mutual to both species. The observed genetic variations, among the farms for both species are pronounced and this might be due to different breeding regimes at the different farms and/or to random genetic drift. Allelic frequencies of the loci investigated are presented as well as the level of heterozygosity within the farms and species. Genetic divergence among the farms and between the species are also shown.

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The species arctic fox (*Alopex lagopus*) and silver fox (*Vulpes vulpes*) have both been used for fur breeding for more than seventy years. The public demand for quality and fashion has forced the breeders to exercise care over breeding regimes and control of the parentage. The use of genetic markers as blood groups, isozymes and RFLP is of great value for the controlling procedure. Recent studies on blood groups of arctic foxes have revealed nine polymorphic loci, and a similar study on silver foxes has revealed five loci (Larsen et al. in prep.). Previous studies by Serov et al. (1976) using starch gel electrophoresis have demonstrated a low level of variation in isozymes both within and between the species. Polyacrylamide electrophoresis has shown variation in several plasma proteins in arctic foxes but much less variation in silver foxes as described by Juneja et al. (1988). In addition two-dimensional electrophoresis has revealed two polymorphic loci in arctic foxes and only one in silver foxes, as described by Juneja et al. (1989). Andersen & Braend (1989) demonstrated variation in a locus determining esterase in plasma from both species by using isoelectric focusing in ultrathin polyacrylamide gels.

The aim of the present study was to investigate the genetic variations, in plasma proteins and isozymes of arctic and silver foxes from different farms by using different electrophoretic procedures.

MATERIAL AND METHODS

Blood samples were collected from 725 arctic and 698 silver foxes from eight farms and ten farms, in Denmark and Finland respectively (Table 1). When less than ten animals were obtained from a farm, the samples were excluded from the material used for statistic analyses. The blood samples were collected by the procedure described by Christiansen (1988), divided into blood cells and plasma, and stored at -80°C until use.

Table 1. List of farms and number of foxes, according to species

Farm	Alopex lagopus	Vulpes vulpes	Total
Aavaturkis	14	11	25
Brovst	12		12
Farm Syd		27	27
Foulum[1]	333	157	490
Hauta-Heikkilä		11	11
Juukaniitty		59	59
Lill[2]		101	101
Lyyski	139	24	163
Olgård		20	20
Oulu	60		60
Salla[3]	105	244	349
Salmijärvi		40	40
Samnii	34	2	36
Vammen[4]	28	2	30
Total	725	698	1423

[1] One fox is tested in both Foulum and in Salla, another fox in both Foulum and Vammen, and one fox is tested two times

[2] One fox is tested two times

[3] Seven foxes are tested two times, and one fox is tested in both Foulum and Salla.

[4] One fox is tested in both Foulum and Vammen.

The plasma proteins α_1B-glycoprotein (A1B), transferrin (TF), GC-protein (GC) and postalbumin (PO) were analysed by horizontal polyacrylamide gel electrophoresis, as described by Juneja et al. (1988). The plasma proteins prealbumin (PR), postalbumin 1 (PA1), pretransferrin 1 and 2 (PRT1 and PRT2), and protease inhibitor 1 (PI1) were analysed by two-dimensional electrophoresis, following the method by Juneja et al. (1989). The enzymes glucosephosphate isomerase (PHI) and phosphogluconate dehydrogenase (PGD) wee analysed by horizontal agarose gel electrophoresis and the enzymes adenosine deaminase (ADA), diaphorase 1 and 2 (DIA1 and DIA2), and mannose phosphate isomerase (MPI) by horizontal starch gel electrophoresis, as described by Simonsen et al. (1990, 1991). Esterase in plasma was analysed by isoelectric focusing in agarose gel, as described by Simonsen et al. (1992). For staining procedures, see Richardson et al. (1986).

RESULTS AND DISCUSSION

Eleven to sixteen loci were studied in blood from the two species. The inheritance of the loci was investigated by Juneja et al. (1988, 1989), by Simonsen et al. (1990, 1991), and by Andersen & Braend (1989). From some farms, the material represents a mixture of parents and offspring. Tests for homogeneity between the population of parents and the population of offspring were performed when possible but no significant test values were obtained. The allelic frequencies for the most common allele of the loci investigated in arctic fox and silver fox are listed in Tables 2a and 2b, respectively. Tests for fit to Hardy-Weinberg were carried out whenever possible. It was found that the distribution of excess/deficiency of heterozygotes is nearly equal, which means that there is no clear tendency in the data of the effect of inbreeding. The proportion of significant test values was found to be more than the expected 5% and this might reflect selection.

Table 2 a. Frequencies of the most common allele in 16 loci among arctic foxes from eight farms

Allele	Aavaturkis	Brovst	Foulum	Lyyski	Oulu	Salla	Sammi	Vammen
A1B-D	0.79	0.71	0.57	0.84	0.88	0.77	0.34	0.91
TF-F	0.89	0.38	0.68	0.57	0.66	0.79	0.82	0.80
GC-F	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
PO-F	0.96	1.00	1.00	0.94	0.93	0.98	1.00	0.95
PR-F	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00
PA1-B	0.79	0.67	0.59	0.60	0.93	0.73	0.52	0.65
PRT1-D	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00
PRT2-S	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00
PI1-F	1.00	1.00	0.99	1.00	1.00	0.97	1.00	0.98
PHI-B	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
PGD-F	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
ADA-2	0.61	0.42	0.58	0.76	-	0.72	0.80	0.67
DIA1-9	-	1.00	1.00	-	-	-	-	-
MPI-3	-	0.71	0.77	-	-	-	-	-
DIA2-1	-	1.00	1.00	-	-	-	-	-
EST-1	-	1.00	0.86	-	-	-	-	-

- not tested

The fraction of polymorphic loci at the 95% level ($P_{0.95}$) and the heterozygosity (H) of the species at each farm are presented in Table 3. The value of $P_{0.95}$ for both species is as expected, namely around 0.30, which is similar to the values given by Ferguson (1980). The level of heterozygosity for both species is low, but not exceptionally low, (see Ferguson 1980). The estimate of H for arctic fox is 0.14 and for silver fox 0.11. Previous studies on arctic and silver fox have revealed a rather low amount of genetic variation as described by Serov et al. (1976) and Simonsen (1982). It has to be stressed that this study was initiated with the aim of detecting polymorphic loci and hence the results might be biased by omitting monomorphic loci which have been revealed by the two authors mentioned above.

Table 2 b. Frequencies of the most common allele in 16 loci among silver foxes from ten farms

	Aava-turkis	Farm Syd	Foulum	Hauta-Heik-kilä	Juuka-niitty	Lill	Lyyski	Olgård	Salla	Salmi järvi
A1B-S	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
TF-D	1.00	0.98	0.98	1.00	0.92	0.94	0.98	1.00	0.97	1.00
GC-F	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
PO-D	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
PR-S	1.00	1.00	1.00	0.91	0.98	0.94	1.00	0.82	0.99	1.00
PA1-S	0.09	0.32	0.40	0.73	0.22	0.51	0.58	0.63	0.48	0.59
PRT1-F	0.77	0.91	0.45	0.68	0.67	0.60	0.75	0.53	0.77	0.91
PRT2-S	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
PI1-F	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00
PHI-B	0.96	0.91	0.98	1.00	0.92	0.92	1.00	0.66	0.95	0.80
PGD-F	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
ADA-2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00
MPI-3	-	0.96	0.79	-	-	-	-	-	-	-
DIA2-1	-	1.00	1.00	-	-	-	-	-	-	-
EST-A	-	-	0.90	-	-	-	-	-	-	-

-: not tested

Table 3. Level of poplymorphic loci at 95% level ($P_{0.95}$) and heterozygosity (H) in arctic and silver fox at farms studied, based on 11 -16 loci

Farm	Alopex lagopus			Vulpes vulpes		
	No. of loci	$P_{0.95}$	H	No. of loci	$P_{0.95}$	H
Aavaturkis	12	0.33	0.12	12	0.25	0.06
Brovst	16	0.31	0.15			
Farm Syd				15	0.27	0.11
Foulum	16	0.38	0.17	16	0.31	0.12
Hauta-Heikkilä				12	0.25	0.09
Juukanniity				12	0.33	0.12
Lill				12	0.42	0.13
Lyyski	12	0.42	0.15	12	0.17	0.09
Olgård				12	0.33	0.15
Oulu	11	0.36	0.09			
Salla	12	0.42	0.14	12	0.33	0.10
Salmijärvi				12	0.25	0.09
Sammi	12	0.33	0.14			
Vammen	12	0.42	0.13			

Tests for homogeneity in the allelic distribution among the farms reveal only 3 non-significant test values out of 28 pairwise comparisons for the arctic fox, the figures for the silver fox are 5 out of 45. This genetic divergence among the farms might be due to selection and/or genetic drift.

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Transponder used for identification in mink

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In May 1991, 15 mink kits were subcutaneously and aseptically implanted with a transponder. The transponders were easy to read and gave reliable imformation. In two cases the transponder was unreadable. X-ray pictures revealed that the transponders had disappeared from the animals, most likely because of incorrect implantation. At pelting time in December all 13 transponders were found subcutaneously where implanted. At that time it was found that the transponders were surrounded by some connective tissue, but neither the mink nor the skin were injured by the transponder.

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In a breeding programme with intact pedigrees of all animals it, is necessary for each animal to be marked unambiguously. The purpose of the marking is to facilitate the collection of all family information and to secure that all data are related to the appropriate animal.

An effective marking system is therefor a prerequisite for an effective breeding programme and the requirements of such a system are:

- it must be easy and safe to mark the animals
- the marks must not easily become lost
- the marks must be easy to read and must provide reliable information
- the system must be cheap to run

No direct identification system exists for mink. Therefore, until recently, the breeder and kit cards have been the only means of identification used in commercial breeding systems.

Lately, new techniques have been introduced for marking animals, and one of these is the use of transponders. Dutch pig breeders plan to use transponders to mark piglets so that they can be monitored right through to slaughtering. In Denmark, transponders are used for marking dogs and a dog register has been drawn up. As the use of transponders becomes more general the cost is expected to decrease to such a level that, in the near future, the use of transponders will become more realistic in mink breeding and research.

A transponder comprises a microchip inside a tubular glass enclosure partly covered by polypropylene. The microchip is preprogrammed during its manufacture with a unique 10-digit code which can be read by a scanner. The scanner transmits a low frequency radio signal which activates the transponder which then reflects its number to the scanner.

This means that the transponder is passive; without its own source of energy and can only

react when influenced by the scanner; that is how it derived its name, 'transponder' = transmitter + responder.

The purpose of this experiment was to investigate whether transponders could be used in mink breeding for identification of individuals.

MATERIALS AND METHODS

On 30 May 1991, 15 mink kits were injected with transponders, measuring 12 x 2 mm and delivered in a sterile-packed hypodermic needle. The transponders were of the trademark INDEXEL supplied by the RHONE MÉRIEUX NORDEN A/S.

The kits injected were born in April and included eight males and seven females from four litters. The neck area was disinfected with alcohol and the transponders were then injected dorsally in the neck between the cranium and scapula. All the transponders were readable after the injection. The kits were checked twice during the week after the injection and no harmful effects could be detected.

Two months later (29 July), the kits were scanned, but only 13 transponders could be read. The reading distance was 6-10 cm for kits held by hand or fixed in a grading trap. Unfixed mink in a grading trap made reading difficult.

The same results were obtained by scanning the kits on 25 September and 19 November.

At pelting time, the two mink with negative scanning results were X-rayed, as well as two mink with positive scanning results. All 15 mink were pelted and examined for tissue changes and pelt defects.

RESULTS

The injection of kits at four weeks of age was simple and caused no harmful effects. Kits can possibly be injected within the first week after birth. The purpose of this experiment was not to find the best injection site for keeping the transponder in the pelt, but only to check the system itself.

The readings in July, September and November were easy and simple to perform, but the design of the scanner is not optimal for use of the system on a commercial scale.

The X-ray results at pelting indicated that the two kits with negative scanning results had lost their transponders. At pelting time in December the other 13 transponders were found subcutaneously where implanted. At that time, it was observed that the transponders were surrounded by some connective tissue but neither the mink nor the skin suffered any injury.

DISCUSSION

This experiment demonstrates that transponders can be used for identification of mink before weaning, thus providing a useful technique in breeding and research work. Further

investigations will probably help to locate the best site for implanting the transponder so that it will remain in the pelt after pelting, which can be a great advantage in research work. Further observations and training will probably also make it possible to implant the transponder in a more permanent and secure way. It is our opinion that the missing transponders were lost as a result of incorrect injection or through the means of injection.

Since the transponder cannot be reused, the current prices of DKK 70 per transponder and DKK 5500 for the scanner can hardly make this a profitable enterprice. However, more general use of transponders and purchases of large quantities will probably help toward lowering the costs.

Embryonal development and embryo losses during the preimplantation period in the silver fox

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Embryonal development and embryo losses during the preimplantation period in the silver fox. The preimplantation period of 18 yearling silver fox females was studied on different days after artificial insemination. The mean number of corpora lutea was 5.9 per female and the number of eggs was 4.9 per female, ranging from 1 to 7. During the first two days only oviductal oocytes were present. After day 3 different stages of development from 2-cell (day 3) to hatched large blastocyst (day 14) could be seen. The oviductal passage took 4 - 6 days and the embryos entered the uterus at 4 - 8 cell or early morula stage. Degenerative changes occured in 27% of ova or embryos. The proportion of embryo degeneration was extremely high (54%) in three females inseminated with blue fox sperm compared to pure silver fox combination (29%). The relatively high preimplantational degeneration rate probably contributes to the low litter size of the silver fox.

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In Scandinavia silver fox production has gained great economic importance during the past ten years. The number of produced skins has increased from 68,000 in 1982 to over 400,000 skins in 1991. More than half of these originate from Finland, which accounts for more than 30% of the Finnish fox skin production. However, the breeding results in silver fox are not satisfactory. Pup production has varied between 2.61 and 3.02 per female in breeding during the last ten years, and with artificial insemination the result is 0.3 fewer pups. The corresponding figures for blue fox are 5.40-6.65 pups per female in pure breeding and even in crossbreeding with silver fox sperm the average result is 4.2-4.3 pups per blue fox female. The low breeding results of the silver fox raise the production costs of this species considerably, which of course makes farming economically unprofitable.

The breeding result is commonly used as a parameter in scientific studies and statistics on all domestic animals. However, in canine species relatively little is known about the physiological events which lead to the final litter size. Foxes have a typically canine pattern of reproduction. They are monoestrous seasonal breeders with spontaneous ovulation. The ova are immature at the time of ovulation and maturation must occur in the oviduct before fertilization (Farstad et al. 1991). The interest in studying and understanding these events in dogs and foxes has increased with the introduction of artificial insemination in these species. Several researchers (Møller & Frøysedal 1980; Jalkanen et al. 1988; van Beek et

al. 1988 among others) have studied hormonal status and heat detection in foxes, but the bulk of this work concerns blue fox. In addition ovulation and oocyte maturation (Farstad et al. 1989, 1991) and even embryo development in the blue fox (Valtonen et al. 1985) have been the subject of research, but information on the silver fox relating to these aspects is very limited (Johansson 1941). The aim of this study was to obtain information about the normal early embryonal development and the embryo losses in the silver fox after artificial insemination.

MATERIALS AND METHODS

The material included 20 one-year old silver fox females reared in normal farm conditions during the breeding season in 1991. The heat control was performed by measuring the electrical resistance of the vagina daily after the first swelling of the vulva up to two days after insemination or mating. The insemination was carried out in accordance with routine practice on the first day after the highest resistance value. All inseminations were made intrauterinely with fresh semen diluted in EDTA (Fougner et al. 1973). Two of the females were mated. One female showed no proper oestrus and was therefore excluded from the study. Three females were inseminated with blue fox sperm.

Eighteen of the females were sacrificed on days 1-12, day 14 and day 16 after insemination. Blood samples were collected for later hormone analyses twice a week from the beginning of the pro-oestrus to the day of sacrifice. The genitals were removed and their appearance and dimensions recorded. The number and the size of corpora lutea and unovulated follicles were registered.

The oviducts and uterine horns were prepared separately and flushed with modified PBS solution (phosphate buffered saline) onto separate dishes, where the ova or embryos were collected by means of a stereomicroscope. The eggs were then transferred to smaller dishes for microscopical examination and photography under a phase contrast microscope. The evaluation of the embryos was made according to their appearance and developmental stage.

The data on the genitals and the flushed eggs were arranged and analysed using the SPSS/PC+ program. Pearson's correlation calculation, chi-square and t-tests were performed.

RESULTS

Ovulations had occurred in each female at the time of sacrifice. A single unovulated, normal looking follicle with a diameter of 3-7 mm was present in three of the females (sacr. day 7, 10 and 16). The mean number of corpora lutea was 5.9 per female (Table 1), and the diameter varied from 5 to 9 mm with a mean of 6.5 mm. The ovulation sites could be easily detected on the first days after insemination. During the first week the corpora lutea had a dark, haemorrhagic appearance while growing in size up to days 7-9. Later they became lighter in colour, diminished in size and developed a more compact consistence.

The dimensions of the ova had a strong positive correlation with the number of

corpora lutea ($p < 0.001$). The mean diameter of the uterine horns was 6.6 mm and this did not change during the first 15 days after insemination. There were no differences in ovarian or uterine parameters between the left and the right side.

Table 1. The number of corpora lutea and ova or embryos in 18 silver fox females during the first 16 days after insemination

	Number	Mean per female	SD	Range
Corpora lutea	107	5.94	1.06	4 - 8
All eggs found	89	4.94	1.70	1 - 7
"viable"	65	3.61	2.00	0 - 7

Either oocytes or embryos were found in the oviducts or in the uterine horns of each female. The total number of eggs found was 89. The mean number was 4.9 per female, ranging from 1 to 7. The eggs were evaluated according to their appearance and developmental stage and classified into "viable" (73 %) and "degenerating" (27%) (Table 1).

The locations and the developmental stages of the eggs were recorded and related to the day of insemination. During the first two days only oviductal oocytes surrounded by a thick mass of cumulus cells were found. On the first day the cumulus cell mass was very compact but it became looser on day 2. Cumulus cells were not present after day 4 and certainly not in embryos of a stage later than the 4-cell stage. Three days after insemination 58% of the eggs were still in the oviduct, but on days 4-6 the oviductal passage of the embryos was complete with the embryos entering the uterus at the 4-8 cell or early morula stage. The first cell divisions could be seen on day 3. After that there was a relatively wide variance between and within the "litters" in the speed of development up to days 10-11. Most of the healthy embryos grew from morula to blastocyst stage during days 6-8 and then expanded with simultaneous thinning of the zona pellucida. The first "hatched" blastocysts released from the zona pellucida were found on day 11. After hatching, the blastocysts grew very fast before the implantation. On day 16 the embryos had a thin netlike structure and could not be handled by the flushing methods used.

A scheme of the normal embryo development in silver fox was drawn up on the basis of the developmental stage of embryos regarded as "viable" on different days after insemination (Table 2).

Marks of degeneration could be seen in 24 ova or embryos. Three of these were still in the 1-cell stage, two in the 2-cell stage, 15 had undergone a few cell divisions to the 4-16-cell stage and four were early morulas. Of the oviductal ova and early embryos, 96% had an undamaged appearance, but in the 15 females with uterine embryos 33% of these displayed signs of degeneration.

The rate of embryo degeneration was 54% in the three females inseminated with blue fox sperm. In the pure silver fox combination it was 29%. In the three females with an unovulated follicle the degeneration rate was 37%. No implantations could be observed in the females sacrificed up to day 16, only in the female sacrificed on day 16, were there signs of implantation.

Table 2. A scheme of embryo development in silver fox related to the day of insemination

Days after AI	Stage of embryo development		Remarks
1 - 2	1-cell		Maturation of ova Fertilization
3 - 4	2-cell early morula	(EM)	The last cumulus cells disappear Entry into the uterus
5 - 6	4-cell compact morula	(CM)	
7 - 8	morula blastocyst	(M) (B)	
9 - 10	expanding blastocyst	(XB)	
11 - 12	"hatched" blastocyst	(HB)	Release of zona pellucida
13 - 15	growing thin hatched blastocyst	(HB)	
16 - 17			Implantation

DISCUSSION

The principles of female reproduction in the silver fox follow the same pattern as that described in the dog and the blue fox (Holst & Phemister 1971; Farstad et al. 1989). Ovulation occurs after the LH peak and the final oocyte maturation takes place in the oviduct. In silver fox the reproductional functions during oestrus and at the beginning of embryo development seem to proceed faster than those in the blue fox. The best breeding results in silver fox insemination are obtained by insemination on the first day of the decrease in vaginal resistance, whereas in blue foxes insemination must be carried out on the second or third day after the highest value. (Fougner 1983; van Beek et al. 1988). This is in accordance with the hypothesis that the interval between the LH surge and fertilization is shorter in the silver fox than in the blue fox. It is still not clear, whether the difference in speed lies in both ovulation timing and the length of the period needed for maturation of the ova. The occurrence of silver fox embryos at the 2-8-cell stage three days after insemination supports the hypothesis of faster processes in silver foxes than in blue foxes,

whose fertilize dova are reported to be still in the 1-cell stage two to four days after mating (Valtonen et al. 1985). The tubal passage of the silver fox embryos from ovary to uterus is faster (4-6 days) compared with that of blue fox (7-8 days). Thus the silver fox embryos enter the uterus relatively young, at the 4-16-cell stage, whereas blue fox embryos grow up to the morula stage in the oviduct. Once in the uterus, the embryos migrate to randomly distributed locations in both uterine horns independently of the side of the ovary they originate from. The difference in the speed of the development seems to disappear during the expansion of the blastocysts, and the implantation occurs on days 16-17 in both species.

According to Venge (1959), the mean number of corpora lutea in the silver fox is 5.7, hich is in agreement with the results of this work. In the blue fox the number of corpora lutea is 12-18 and the number of embryos found during the preimplantation period is reported to be 24.5% lower than the number of corpora lutea (Valtonen et al. 1985). In this study the corresponding figure for silver fox was 17%. The difference in numbers can partly be explained by errors in the flushing techniques, but also other possibilities must be considered. Some of the ova may have been lost at the time of ovulation to the abdominal cavity. It is also possible that the preovulatory luteinization, which is a typically canine phenomenon (Mondain-Monval et al. 1984), sometimes leads to unovulatory corpora lutea.

The loss of embryos, during pregnancy is high in all canids. In blue fox the post-implantation mortality rate is almost 30% (Valtonen et al. 1985). In this study the rate of embryo degeneration was found to be relatively high during the preimplantation period in the silver fox, which may indicate smaller losses after implantation in this species. This could be explained by different litter sizes, as there is relatively more space in the uterus per silver fox embryo than per blue fox embryo for the successful formation and function of the placenta.

Among the "non-viable" embryos, the number of 1-cell ova was surprisingly low, considering these were supposed to be the only unfertilized ones. In the majority of the embryos the development had ceased by the 8-16-cell stage. Even ova which very likely were unfertilized because of unsuccessful heat detection cleaved to the 2-8-cell stage. This is in agreement with the observations of parthenogenetic cleavage in blue fox eggs (Farstad 1991, pers. comm.). The zona pellucida sheltered the dead embryos from resorption, as degenerated early embryos could still be found among the healthy blastocysts just before the implantation.

The rate of embryo degeneration was extremely high in the three females inseminated with blue fox sperm compared to pure silver fox combinations, which is in agreement with the very low breeding results achieved in trials to produce crossbreed offspring with silver fox females (Valtonen & Jalkanen 1988).

SUMMARY

The aim of this study was to examine the early embryonal development in the silver fox. A scheme of normal embryo development is presented and some aspects of the early embryo degeneration are discussed under the hypothesis that the relatively high preimplantation degeneration rate probably contributes to the low litter size of the silver fox.

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Species differences in fertility after artificial insemination with frozen semen in fox pure breeding

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Field trials with frozen silver fox semen using a programmable freezer and a new automatic freezing programme were initiated in 1988. Conception rates of 80% and mean litter size of 8 cubs resulted when frozen silver fox semen was used to inseminate blue fox vixens. Vixens were inseminated twice (24-h interval) and 100-150 million spermatozoa were deposited intrauterinely at each insemination. Also, silver fox females have been artificially inseminated intrauterinely with frozen silver fox semen (double insemination, 100 million spermatozoa per insemination) yielding an 81% conception rate and 3.6 cubs per litter at whelping (n = 21, 1991). These results are comparable with those obtained for artificial insemination with fresh semen. A gene bank has been established by freezing silver fox semen obtained from superior males, i.e., rare or otherwise valuable mutants. It was assumed that cryopreservation of blue fox semen would benefit from the improvement in post-thaw semen quality obtained by the new freezing technique developed for silver fox semen. However, artificial intrautrine insemination of 70 (1990) and 52 (1991) blue fox vixens with frozen blue fox semen (2 x 100 million spermatozoa) resulted in low (33%, 1990 and 48%, 1991) conception rates and mean litter sizes of only 2.3 and 5.8 cubs born per litter, respectively. Electron microscopical studies of post-thaw acrosome integrity of spermatozoa from blue and silver foxes did not reveal any differences between the two species in the severity of prevalence of acrosomal damage. Conclusively, differences are observed between the two fox species in fertility of semen frozen by the same freezing method, but studies of post-thaw acrosome integrity of the spermatozoa or other semen parameters studied by means of light- or electron microscopy could not explain the differences in fertility results.

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Preliminary experiments with freezing of blue fox semen were initiated in Norway as early as in 1970, using the simple technique of placing 0.5cc PVC medium straws on a rack over N_2 vapour in an open system as first described by Jondet(1984), Fougner et al. (1973) and

Aamdal et al. (1978). A motility of 70% was observed after thawing semen frozen by this method, and small field trials with frozen-thawed blue fox semen in 1973 and, later, in 1982 and 1983, yielded a 74% conception rate and 6.5 cubs born per litter (N=67) in 1973, and conception rates of 58-75% with 6-8 cubs born per litter in 1982 and 1983, respectively(Fougner et al.1973; Fougner 1982, 1983).

Freezing experiments with silver fox semen using an automatic programmable freezer and a new freezing programme were initiated in 1988 (Hofmo, 1988). The aim of using an automatic freezing programme was to reduce inter-batch variation in semen quality caused by different freezing conditions and also to increase freezing capacity, i.e. number of straws per freezing. Large-scale freezing would be necessary to obtain enough frozen semen for larger field trials.

The reasons for selecting silver fox semen for these experiments were mainly that freezing of semen could be initiated early in the reproductive season (February) and the semen could then be used for blue fox vixens coming on heat later in spring (March/April). Also, the hybrid offspring obtained by crossing the two different species blue and silver fox, the bluefrost, were still in high demand and the farmers were interested in using the frozen semen to obtain this hybrid for its valuable fur.

During the seasons 1989-90 frozen silver fox semen was tried out in large field trials with artificial insemination of blue fox vixens. The fertility results with double insemination with 100 million spermatozoa and intrauterine insemination were comparable to the results obtained by artificial insemination with fresh semen (Farstad et al. 1992). In the meantime, new interest had arisen in using frozen semen for pure breeding. Attention was particularly focused on using elite silver fox males in an artificial insemination programme involving elite silver fox females and, on a smaller scale, also using pure breeding by artificial insemination of blue fox females with frozen blue fox semen. It was therefore decided to carry out field trials with both frozen silver fox and frozen blue fox semen in pure breeding on a limited scale in 1989-91.

MATERIALS AND METHODS

Males and semen processing

In 1990 semen was collected from 102 mature silver fox males aged 2-8 years (mean $\pm$ SEM, 3.2 $\pm$ 0.2 years) and 12 blue fox males aged 3-4 years (mean 3.1 $\pm$ 0.1 years), and in 1991 from 55 silver foxes 2-7 years (4.1 $\pm$ 0.1 years) and 13 blue fox males aged 2-5 years (3.7 $\pm$ 0.3 years). Animal treatment and semen collection, processing and freezing /thawing procedures have been described previously (Farstad et al. 1992). The insemination doses contained 100 or 150 million spermatozoa in an insemination volume of 1.0 ml. Mean post-thaw motility for silver fox semen was 65 $\pm$ 0.5% (N = 304 frozen samples) in 1990 and 65 $\pm$ 0.4% (range 60-70%, N = 71 frozen samples) in 1991. For the frozen blue fox semen, the mean post-thaw motility was 59 $\pm$ 0.6% (range 55-65%) in 1990 and 65 $\pm$ 0.4%(range 60-75%) in 1991.

Electron microscopy

Semen from 8 male foxes, 4 silver foxes and 4 blue foxes were fixed and prepared for

transmission electron microscopy according to the procedure described previously (Hofmo & Andersen Berg 1989) to study the degree of acrosome damage after freezing and thawing. These semen samples were chosen randomly without taking any field fertilty or seminal characteristics into consideration.

Vixens

A total of 27 silver fox vixens from four different regions of Norway, aged 1-4 years (mean ± SEM, 1.7 ± 0.2), were artificially inseminated intrauterinely with 150 million spermatozoa per insemination dose in 1989. The vixens were inseminated twice. These vixens were females which would not allow natural mating, or which came in oestrus late in the silver fox season when semen collection from silver fox males was sometimes difficult. No special selection of males on the basis of their genetic value or previous fertility records was made for these vixens.

In 1991 21 silver fox vixens showing normal oestrus signs, aged 1-5 years (mean ± SEM 2.2 ± 0.4), from five different regions of the country were inseminated intrauterinely twice with 100 million spermatozoa per insemination dose. These silver fox vixens were elite females inseminated with frozen semen from elite silver fox males, i.e., males with a high genetic value based on index scores from pelt characteristics and field fertility records.

In 1990 and 1991 the blue fox vixens inseminated with frozen blue fox semen were provided by the farmers who had also participated in the hybrid crossing experiments in 1989 and 1990 and the treatment of the animals, heat detection routines and intrauterine insemination technique have therefore been reported previously (Farstad et al. 1992). In 1990 a total of 70 blue fox vixens were randomly selected to be inseminated twice with frozen blue fox semen containing 100 million spermatozoa per insemination dose. In 1991 a total of 52 blue fox vixens from the same two farms were inseminated twice with frozen semen doses containing 100 million spermatozoa. All blue fox vixens were inseminated by the same veterinarian.

RESULTS

Of the 27 silver fox vixens inseminated with silver fox semen in 1989, 16 whelped (60%), with a mean litter size ± SEM of 4.0 ± 0.3 cubs per litter at whelping. In 1991, 17 of the 21 females whelped (81%), with a mean litter size of 3.6 ± 0.2 cubs at whelping.

Of the 70 blue fox vixens inseminated with frozen blue fox semen in 1990, 33% whelped and the mean litter size born was 2.3 ± 0.7 cubs, litter size ranging from 1-13 cubs. In 1991, 48% of the 52 vixens whelped, and the mean litter size born was 5.9 ± 0.4 cubs (range 1-13). These results are shown in Table 1.

Although semen from each male was used on a small number of vixens, a large variation was observed in field fertility between males. This was particularly evident for the blue fox males. For the best two *Alopex* males, whelping rates of 85% and 100% were observed (6 of 7 vixens, and 4 vixens of 4, respectively). There were several males which did not produce any cubs (none out of 4 inseminated vixens conceived). In neither 1990 nor 1991 could any relationship be found between semen parameters studied in the phase

contrast microscope (post-thaw progressive motility or percentage of abnormal cells) and field fertility.

Table 1. Conception rates and mean litter size born after artificial insemination of *Vulpes vulpes* and *Alopex lagopus* vixens with frozen semen from males of the same breed

Year	Number of vixens	Breed ♀	Breed ♂	Number of sperma-tozoa million/ml	Number of insemin-ations per vixen	Whelping rate %	Litter size born $\bar{x} \pm$ SEM
1989	27	*Vulpes*	*Vulpes*	150	2	60	4.0 ± 0.3
1991	21	*Vulpes*	*Vulpes*	100	2	81	3.6 ± 0.2
1990	70	*Alopex*	*Alopex*	100	2	33	2.3 ± 0.7
1991	52	*Alopex*	*Alopex*	100	2	48	5.9 ± 0.4

Studies of the extent and degree of acrosome damage by means of transmission electron microscopy did not reveal any difference between blue and silver foxes or between fertile and non-fertile males. The degree of acrosomal damage observed was extensive (20-50%) for semen from males of both fox species compared with the post-thaw acrosomal damage observed in bovine or equine spermatozoa.

DISCUSSION

Fertility results for frozen silver fox semen were comparable with those obtained with artificial insemination of silver fox vixens with fresh semen in 1991, whereas the results from the 1989 insemination trials were somewhat lower. The explanation for the lower whelping rate in 1989 may be that this material consisted of vixens showing suboptimal oestrus, whereas the 1991 females were selected vixens in normal oestrus. Also, the males were selected, since evaluation of their previous field fertility was included in their index score. Between year differences may also have influenced the results. As far as sperm number per insemination dose is concerned, large field trials in hybrid crossing of *Alopex* females with frozen *Vulpes* semen have failed to show any differences in fertility results between 100 and 150 million spermatozoa (Farstad et al. 1992). It is therefore reasonable to assume that the quality of oestrus in the vixens combined with insemination with frozen semen from males with high proven field fertility contributed most to the increased whelping rate in 1991.

On the basis of the preliminary trials with frozen silver fox semen for pure breeding in 1989, it was decided to freeze semen from genetically valuable males to be used for elite matings in 1991 and also to freeze semen from rare mutants of *Vulpes vulpes*, such as, for instance, the burgundy, white face and pearl colour mutants, for long-term preservation in a semen bank. The fertility results from the elite inseminations in 1991 showed that frozen semen can be used in silver fox pure breeding and normal conception rate and litter size

can be expected. The establishment of a semen bank for silver foxes is therefore economically feasible and also offers the possibility of preserving valuable genes from this fox species. A gene bank for this purpose has now been established by The Norwegian Fur Breeders' Association.

The good fertility results obtained by using frozen silver fox semen to inseminate blue fox females obtained in the large field trials in 1989-90, together with the improvement in post-thaw quality and reduction in the extent of acrosome damage observed by the new freezing method compared with the simple styropore box method (Hofmo 1988), lead to the assumption that the new freezing technique would also be beneficial for blue fox semen. The poor results of only a 33% whelping rate for blue fox pure breeding compared with the 87% conception rate from hybrid crossing using randomly selected blue fox females from the same farms in both experiments, were totally unexpected. Especially because the initial field trials with blue fox semen using the styropore freezing method had resulted in quite satisfactory whelping rates in 1973, 1982 and 1983 (Fougner et al. 1973; Fougner 1982, 1983). Another difference was that with the new freezing method 6% (V/V) glycerol was used as cryoprotectant in the Tris- Egg- yolk buffer (Hofmo 1988; Farstad et al. 1992), whereas with the styropore system the glycerol content in the same buffer was 8% (V/V) (Fougner et al. 1973). The significance of the volume percentage of cryoprotectant in the Tris freezing extender on freezability has not been tested for blue fox semen.

Since we could not find an obvious explanation for the poor results, we tried to optimalize conditions by selecting males with very good initial semen quality and a known high fertility from AI with fresh semen for the 1991 trials, as was done for the silver fox males in 1991. The whelping rate and mean litter size increased (48% versus 33% whelping rate, 5.9 versus 2.3 cubs per litter at whelping). However, the results were not satisfactory compared with those from AI with fresh semen, where an 82% whelping rate and 8 cubs born per litter were obtained in 1991 (N=2318 vixens, 1910 litters registered (Fougner 1992).

We also observed considerable variation between the fertility of frozen /thawed semen between males, although subjective observation of seminal characteristics in the phase contrast microscope did not reveal any explanation as to why some males failed to produce cubs, whereas others would produce up to 13 in a litter and impregnate 6 out of 7 vixens.

However, on several occasions in the field we have observed, when comparing the motility of fresh silver fox and blue fox semen, that the progressive motility of the spermatozoa declined much faster in blue fox semen than in silver fox semen when the semen was left at room temperature. This phenomenon was observed whether the semen was undiluted or was diluted with the fresh semen extender containing EDTA, which is routinely used for diluting fresh fox semen for artificial inseminations in the field.

It was that the explanation could be that blue fox spermatozoa are more sensitive to unfavourable conditions than silver fox spermatozoa and that the freezing method or freezing extender with 6 (V/V)% glycerol represented unfavourable physical conditions for blue fox spermatozoa. It was reasonable to expect that such a difference between the species would be detected by studying post-thaw acrosomal damage to the sperm cells. However, although the material was small, we could not find a significantly higher number of damaged sperm cells or a more severe degree of acrosomal damage in the blue fox semen than in the silver fox semen. This is in accordance with previous findings of post-

thaw acrosome integrity of fox semen evaluated by light microscopy (Zalewski & Andersen Berg 1983). It was, however, unexpected that the degree of post-thaw acrosome damage did not seem to affect field fertility in silver foxes as much as for blue foxes. Therefore, the extent of post-thaw acrosome damage to sperm cells does not offer an obvious explanation for the difference in field fertility observed between the two fox species.

Selection of blue fox males with proven fertility for the production of frozen semen improved the results and may be a way to increase whelping rate after AI with frozen semen, but will most certainly not alone solve the problem of reduced fertility after the use of frozen blue fox semen. There are good grounds for further investigations into cryopreservation of blue fox spermatozoa including such factors as the composition of the freezing extenders, glycerol content, cooling and equilibration time and freezing curves followed by testing of the fertility of the frozen semen under laboratory and field conditions.

SUMMARY

A total of 27 silver fox vixens aged 1-4 years in 1989(two inseminations with 150 million spermatozoa per insemination) and 21 silver fox vixens aged 1-6 years in 1991(two inseminations, 100 million spermatozoa per insemination) from different regions of Norway were artificially inseminated with frozen silver fox semen. The animals inseminated in 1989 were vixens that would not mate naturally due to suboptimal oestrus, or vixens that came in oestrus so late in the breeding season that collection of fresh semen from the silver fox males was unsuccessful. The vixens inseminated in 1991 were elite females with normal oestrus behaviour and the reason for using frozen semen was to combine elite animals, i.e., animals of high genetic value. A conception rate of 60% (16 of 27) with a litter size at birth of 4.0 cubs was obtained in 1989 and, in 1991, 81% (17 of 21) conceived with a mean litter size of 3.6 cubs at whelping. Results were satisfactory, especially in 1991 and a gene bank has been established by freezing silver fox semen from genetically valuable animals.

It was assumed that cryopreservation of blue fox semen would benefit from the improvement of post-thaw semen quality obtained by the new freezing method developed for silver fox semen. However, artificial insemination of 70 blue fox vixens in 1990 with 100 million spermatozoa resulted in a whelping rate of only 33%. Mean litter size was only 2.3 cubs born per litter. With frozen semen selected from males with high fertility included as part of their breeding index, a 48% whelping rate and a mean litter size of 5.9 cubs born were obtained after artificial insemination of 52 blue fox vixens in 1991.

Although an improvement in fertility was observed when semen was selected, results were not comparable with those from fresh semen. Light- or electron microscopical studies of acrosome damage to frozen blue fox spermatozoa could not explain why frozen blue fox spermatozoa resulted in lower field fertility than frozen silver fox spermatozoa or fresh blue fox spermatozoa.

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Duration of spermatogenesis and spermatozoan transport in the mink (*Mustela vison*)

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The primary objective of this research was to determine the time required for development of spermatozoa from the primary spermatocyte to the differentiated spermatozoa and the epididymal transit time using ^{3}H- thymidine and autoradiographic techniques. Thirty-five days were required for the labeled sperm to appear in the head of the epididymides. Six additional days were required for the sperm to transit the epididymis and appear in the proximal end of the vas deferens. The time required for development of mature sperm from type A spermatogonia to the appearance of sperm in the proximal vas deferens was calculated as 58 days. Thus sperm used for inseminating females on 1 March would be type A spermatogonia on 31 December or 1 January (leap years) and mink with poor testicular development at this time would be infertile.

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The intensive inbreeding of animals having dark guard hairs with a sheen and a dark, dense underfur has co-selected for primary infertility in dark mink. Several types of primary infertility have now been identified - hypogonadotropic hypogonadism or true delayed puberty and constitutional delayed puberty (Ellis & Pace 1986). The latter results primarily from a slow-running biological clock so that reproduction is delayed with fertility developing after the normal breeding season, whereas the former results primarily from hypothalamic, pituitary and/or testicular lesions (see Alak et al. 1992, proceedings of this congress). As a result of the many different types of male infertility, the farmer and researcher really do not know when they should make testicular checks for ascertaining if an animal will be fertile at breeding time. It is generally concluded that testes do not produce mature sperm until they have reached 0.6 of their average testicular size.

This research was undertaken to ascertain how long it takes for spermatogenesis to occur and how long it takes for mature spermatozoa to migrate through the epididymis and the vas deferens. When the period of time required for this to occur is known, one can calculate back from the time of copulation to estimate when these spermatozoa were formed from stem cells.

MATERIALS AND METHODS

Ten dark mink, average weight 2.06 kg (ranging from 1.75 to 2.65 kg) provided by the Fur Breeders' Agricultural Cooperative in Sandy, Utah, were used in the study. The animals were maintained in the Utah State University Laboratory Animal Care Center with feed (provided by the Co-op), and water given *ad libitum*. All mink were anesthetized with Ketamine HCl/10% acepromazine (40 mg/1 mg per kg body weight) and injected with 1 mCi ^{3}H-thymidine in a saline solution via the sublingual vein. Starting on 23 January, a testis was removed from a given animal 0, 3, 7, 10, 14, 17, 21, 25, 28, 31, 35, 38, 42, 45, 49, 52, 56 or 78 days after injection while under anesthesia as described above with 15,000 units of penicillin G (Eli Lilly Co. Indianapolis, IN) given locally at the surgical site to protect against infection. Animals were alternated so that no animal had a second testis removed until all of the others had their first one removed. The testes, epididymides and vas deferens were prepared for autoradiographic localization (Franz 1989) of the ^{3}H label in the cells of the testes and epididymides. Five-millimeter sections of the epididymides and vas deferens were dissolved in soluene (Beckman Instruments Co., Dubugue, IA) and counted in a scintillation counter to ascertain when the pulse of radio labeled spermatozoa entered and passed through these two organs.

RESULTS

Three days after injection of the ^{3}H-thymidine, developing spermatocytes (stem cells) were seen lining the basement membrane of the seminiferous tubules, i.e., the spermatogonia and young spermatocytes were densely labeled. From days 3 to 31 the spermatocytes could be clearly observed in a step-by-step migration from the outer edges of the seminiferous tubules to the center of the lumen (Table 1). On day 35 postinjection there were still many labeled spermatocytes in the seminiferous tubules, but sparsely labeled sperm also began to appear in the caput epididymis. On day 38, densely labeled sperm were found in the caput epididymis. By day 42 the seminiferous tubules were virtually devoid of labeled spermatozoa, except for some residual labeled type A spermatogonia. At this time, the epididymis was saturated with labeled sperm. From days 45 to 52 the pulse labeled spermatozoa slowly moved through the epididymis followed by sperm cells with less and less radioactive label. On day 52, the terminal section of the vas deferens contained highly labeled spermatozoa.

Table 1. Summary of position of labeled sperm over time. Days Post-injection

	3	7	10	14	17	21	25	28	31	35	38	42	45	49	52	56
Seminiferous tubules	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XXX	XX	XX	X	X	X	X	X
Epididymis																
Head	0	0	0	0	0	0	0	0	0	X	XXX	XXX	X	0	0	0
Body	0	0	0	0	0	0	0	0	0	0	X	XXX	XX	XX	X	0
Tail	0	0	0	0	0	0	0	0	0	0	0	XXX	XXX	XXX	XX	0

XXX - Dense labeling of tissue (grains too numerous to count)
XX - Medium labeling of tissue (5-10 grains exposed)
X - Spares labeling of tissue (0-5 grains exposed)
0 - Tissue not labeled (no grains exposed)

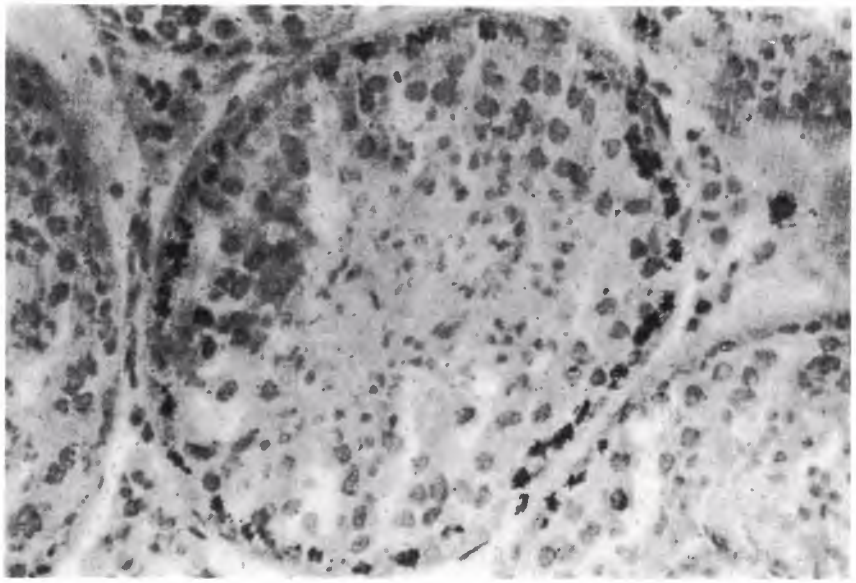

Fig. 1. Autoradiograph of testis taken three days post-injection showing radioactive labeled spermatogonia and spermatocytes around the basement membrane of the seminiferous tubule, center of figure. X175

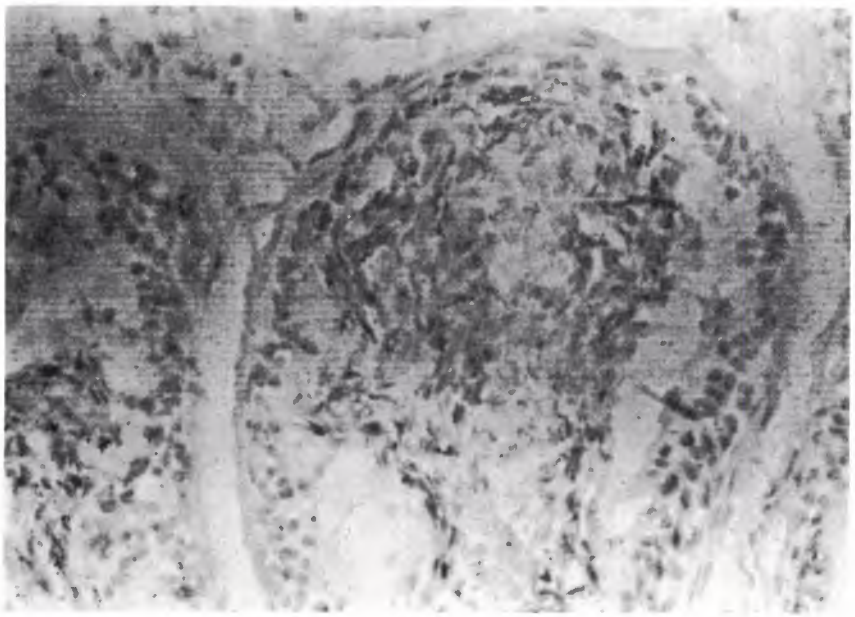

Fig. 2. Autoradiograph of testis taken forty-two days post-injection showing labeled spermatozoa in the cauda epididymis. X175

DISCUSSION

Sperm development in mink begins with the initiation of the active phase of spermatogenesis in late October. In late January the developed sperm are found in the lumen of the seminiferous tubules and all phases of the developing spermatogonia, spermatocytes and spermatozoa can be observed.

The most mature germ cells labeled when ^{3}H-thymidine was infused into the animals would be the primary spermatocytes that were preparing for the first meiotic division (preleptotene spermatocytes). Other germ cells in the process of dividing, such as type A and B spermatogonia, were also labeled. The labeled sperm first seen in the epididymis on day 35 were sparsely labeled (less than five grains of silver per cell, but above background). These sperm would have had to be in the final stages of DNA replication during the interphase, just prior to the first meiotic division of the primary spermatocytes.

The duration of the spermatogonial phase of spermatogenesis is not known for mink, but the duration of the meiotic phase and the spermatid phase can be estimated from this study to be 35 days. Pelletier (1986) divided the cycle of seminiferous epithelium into 12 stages for the mink and demonstrated that 4.58 cycles of the seminiferous epithelium were required to produce viable sperm from type A spermatogonia. With 35 days required for 3.1 cycles then 4.58 cycles would take 51.7 or approximately 52 days for the complete cycle. Epididymal transport of sperm required more than 3 days, but less than 7 days with the best estimate at 6 days. Thus, the time required for a type A spermatogonial cell to develop to a mature spermatozoan and to migrate to the juncture of the vas deferens would take 58 days and thus it was formed on 2 or 3 January (leap years). With several days required for the sperm to transit the vas deferens, sperm used for conception on 1 March would have been type A spermatogonia on 31 December. Therefore, the optimal time for testicular checking of dark male mink would be 31 December. Mink with testes smaller than 0.6 of their maximal size would not be producing any fully developed spermatozoa at this time. Animals with testes smaller than those of proven fertile males of other color phases should be rejected as breeders. Since only about eight days are required for spermatozoa to pass from the testes through the epididymis and vas deferens for breeding purposes, the 0.6 size rule would certainly apply at this later date. Those farmers who have rigorously selected for mink with large testicular size in January and who also carry out epididymal sperm counts in order to assess motility and lack of abnormal sperm forms as part of their breeder selection process have increased their fertility each year to a farm 7 average of 5.6 or higher.

This spermatogenic time course is consistent with that reported for other species: the rat 48 days (Clermont et al. 1959); the rabbit between 52 and 60 days (Swierstra & Foote 1963); the ram 49 days (Ortavant 1956); and man 64 days Heller & Clermont 1963).

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The effect of management system on the developmental changes in the testes and testosterone level in the blood sera of blue foxes (*Alopex lagopus* L.)

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Experiments on the effects of management systems on the morphotic and developmental changes occurring in the gonads of young and mature blue fox males were carried out on a total of 24 individuals under two management systems: (a) group light in detached cages with permanent access to light; and (b) group darkness - in roofed pavilions. The following parameters were determined histologically in the testes spermatogenic stages, presence and value of PAS (+) substance as well as the activities of Δ-5 3-β steroid dehydrogenase and diaphoresis. Changes in the spermatogenic activities of the experimental animals were observed in September and they indicated an accelerated spermatogenesis in group darkness. The highest activities of both enzymes (2.5-3.5) were observed in January-March. An increase in testosterone level from 0.7 to 2.0 mmol/l was observed in September in the blood sera of the darkness group males and those differences (statistically significant - $p < 0,05$) were observed throughout the experiment.

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According to Bielanska-Osuchowska (1983) and Rusen-Runge (1992), the process of spermatozoa formation, known as spermatogenesis, can proceed more or less intensively all the year round or, on the contrary, it can be reduced to a strictly defined season. Blue foxes (*Alopex lagopus* L.), like other representatives of the family Canidae, belong to the second group - monoestrous animals. Many studies indicate that the reproductive processes of carnivorous fur animals can be enhanced or inhibited, among other things, through controlling the length and intensity of lighting (Boisson-Agasse et al. 1982, 1988; Christiansen 1988; Kuzniecow 1981). So far, relatively few studies have been conducted on the effect of commonly used systems for farm management of carnivorous fur animals on the reproductive capacities of males or on the effects of these systems on the developmental and seasonal changes occurring in the gonads of male blue foxes.

The aim of this study was to determine spermatogenic and steroidogenic activities in the gonads of young and mature male blue foxes under two systems of management: (a) in cages, and (b) in pavilions.

MATERIAL AND METHODS

Studies were conducted from 1 August to 15 March on 24 males including:

a) 12 one-year-olds and older
b) 12 young foxes aged 3-10 months

Experimental males were divided into two equal groups. One group was kept in detached cages with a steady and direct access to light (light group), the other was kept in pavilions with limited access to light (darkness group).

At the beginning of every month experimental males were weighed and blood was collected from the forearm vein. Materials for studies (testes, epididymides, ductuli efferentes) were obtained through castration by a removal of both testes, which, after being measured and after their sections had been collected for histological examination, were placed in dry ice in order to inhibit an activity of tissue enzymes (at 76°C). The volume of testes was calculated based on the formula for counting the volume of elongated spheroid:

$$v = 1{,}33 \pi \times a \times b^2$$

where
v = volume of testes
a = length of right and left testes
b = width of right and left testes

The sections collected from both testes, 4-6 mm thick, were fixed in AFA or Cornoy's liquid and embedded in paraffin (Zawistowski 1983). The paraffin sections were stained by a routine method: haematoxylin-eosin and by PAS (Burck 1973).

The functional condition of the testes of the experimental foxes was estimated using: (1) a spermatogenic index determined on the basis of presence and amount of spermatogonia, spermatocytes, spermatids and spermatozoa in the wall and lumen of seminiferous tubules.
(2) measurements of the diameter of seminiferous tubules using micrometric and measuring eyepieces.

Steroidogenic activity was determined through:

- determination of activity Δ- 5 3-β of hydroxysteroid dehydrogenase by the Wattenburg method and of diaphorase by the Colombo method (Krygier-Stojanowska 1982).
- determination of testosterone level in the peripheral blood of experimental males by radioimmunoassay.

The results obtained were calculated statistically (Mean, SD). The significance of differences was evaluated by the Student's t-test (Ruszczyc 1981).

RESULTS

Body weight

Initial body weights were similar in both groups, averaging:

light group- 5,5 kg

darkness group- 5,85 kg

In the autumn period a gradual increase in body weight was observed, which was higher in the darkness group (7.1 kg) and was attained one month earlier compared with that found in the light group (Fig. 1). During conditioning in either group, a decrease in body weight by ca. 30% was recorded.

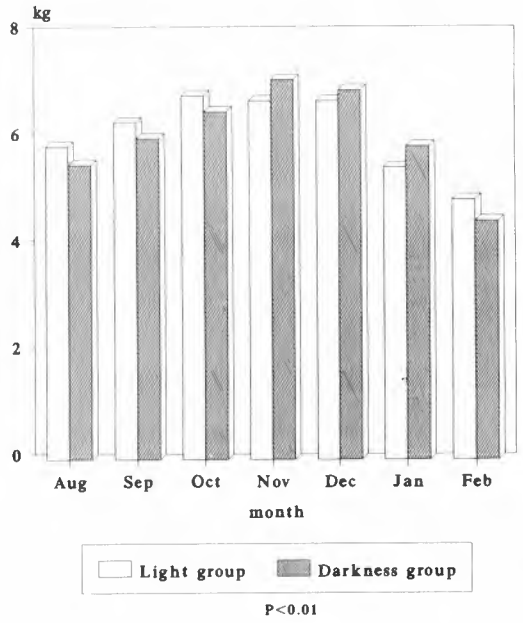

Fig. 1. Seasonal changes in body weight in experimental male blue fox

Size of testes

Beginning in September, a gradual increase in the size of testes was observed in parallel with the decreasing daylight (Fig. 2). A maximum size of testes was attained in both groups of animals in January, but this differed significantly between groups. In the darkness group the volume of testes was then 20.2 cm^3 and in the light group males 14.36 cm^3.

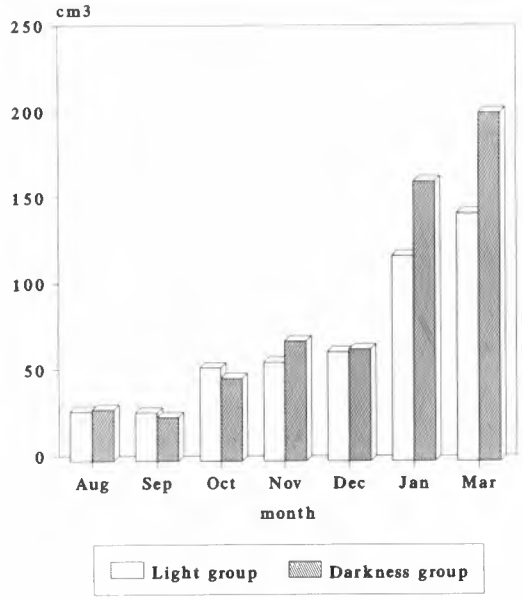

Fig. 2. Seasonal changes in volume of testes in experimental male blue fox

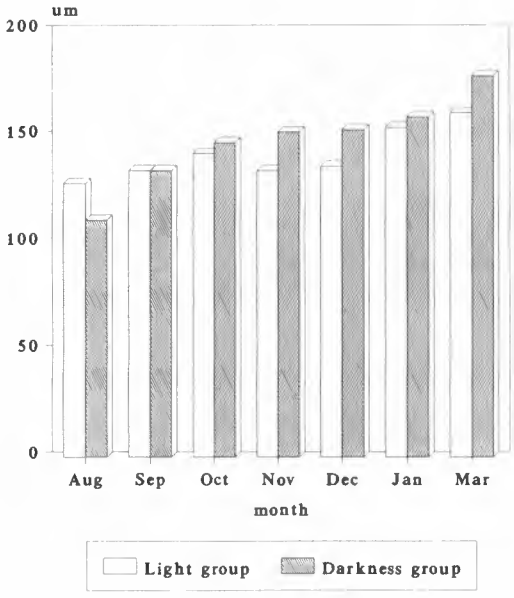

Fig. 3. Diameter of seminal canals in testes of experimental male blue fox

Diameter of seminiferous tubules

An initial small diameter (111 μ) of seminiferous tubules in the testes of the darkness group males since October began to increase very quickly, reaching a value of 178 μ by February. At the same time, the diameter of seminiferous tubules in the testes of the light group males was lower, reaching a value of 161 μ (Fig. 3).

Spermatogenesis

In males kept in pavilions (darkness group) an intensive process of spermatogenesis was observed as early as September and the division of spermatogonia A and B as well as primary spermatocytes could be seen. Whereas, in the light group, males spermatogenesis started one month later. Differences in spermatogenic activity between groups were maintained to December (Table 1).

Table 1. Differences in spermatogenic activity in testes of male foxes kept in cages and pavilions

Month	Spermatogonia		Spermatocytes		Spermatids	Spermatozoa
Group	A	B	I	II		
AUGUST						
Light	+	-	-	-	-	-
Darkness	+	-	-	-	-	-
SEPTEMBER						
Light	+	-	-	-	-	-
Darkness	+	+	-	-	-	-
OCTOBER						
Light	+	+	-	-	-	-
Darkness	+	+	+	-	-	-
NOVEMBER						
Light	+	+	+	+	-	-
Darkness	+	+	+	+	-	-
DECEMBER						
Light	+	+	+	+	+	+
Darkness	+	+	+	+	+	+
JANUARY/MARCH						
Light	+	+	+	+	+	+
Darkness	+	+	+	+	+	+

Testosterone level

The testosterone level in the blood sera of males also differed significantly between groups (Fig. 4). Starting in October, the testosterone level in the darkness group steadily increased from 0.7 mmol/l to 2.0 mmol/l. It was not until December that a considerable increase in the testosterone level was also found in the light group males. However, the level of this androgen was significantly higher in the darkness group (12 mmol/l).

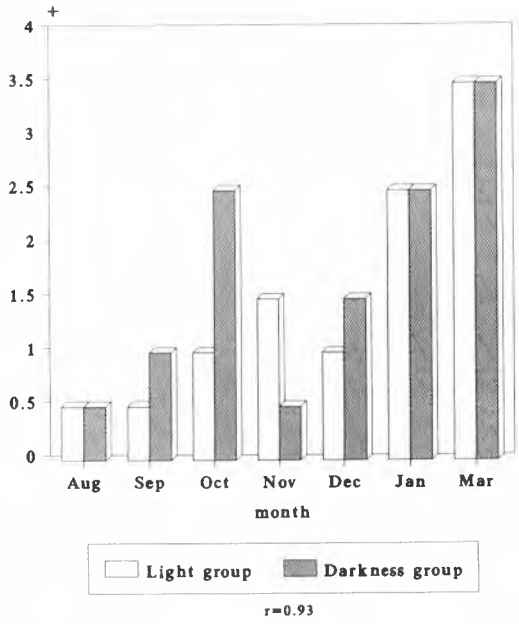

Fig. 4. Seasonal changes in blood testosterone level in experimental male blue fox

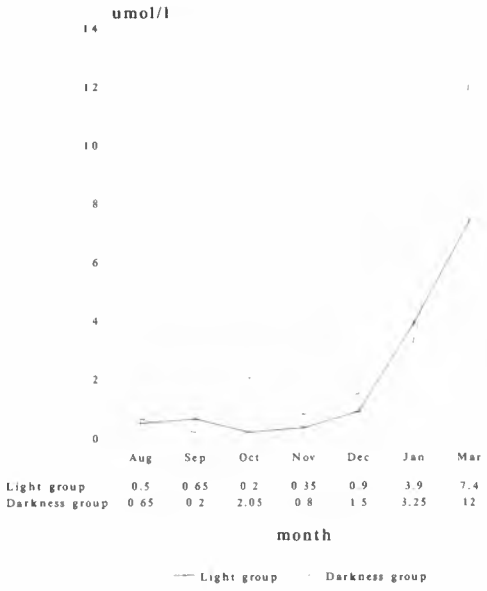

Fig. 5. HSD activity in testes of male blue fox

Activity of steroid dehydrogenase and diaphorase

Quality tests did not reveal any statistically significant differences in the activities of these enzymes in the testes of both groups of males (Fig. 5). During a sexually inactive period (July, August) small amounts of diaformazone in interstitial cells were observed, while the highest enzymatic activity was recorded from January to March.

DISCUSSION

The recommendation of the breeding regulations hitherto in force was that the management system for the basic stock of blue foxes should be that of detached cages. This advice was based on the rather common belief that these animals belong to the "long-day" type (Jochle & Lammond 1980; Hafez 1976). The studies of the past few years (Sundquist 1987; Boisson-Agasse 1982, 1988) clearly demonstrate that blue foxes, like other representatives of Carnivora, belong to the "short-day" animals and the processes initiating oogenesis and spermatogenesis start during the period of decreasing length of daylight, which was also confirmed in this study.The animals fed in the same manner in spite of being kept under various lighting regimes, have preserved their physiological property of depositing fat when approaching the winter solstice. Both the attainment of proper fitness in the autumn period and a subsequent defattening seem to have, according to some authors (Slawon 1987; Murohy et al. 1987; Mc Kibbina et al. 1984) a significant effect on the potency and fertility of fox and mink males.

As follows from this study a light factor seems to have a considerable effect on reproductive processes in blue fox males. In males kept from July to March in pavilions with limited access to light, both spermatogenic and steroidogenic activities are enhanced. A visible symptom of these activities was the rapid increase in the volume of testes and diameter of seminiferous tubules. In this group the volume increased 6.8 times in that period, whereas in foxes kept in cages with permanent and direct access to light the volume increased to a much lesser degree (fivefold). Also the diameter of the seminiferous tubules observed at the same time in the gonads of the darkness group males was considerably greater (178 μ) than that in the light group males (161 μ).

Using quality tests, it is impossible to state any statistically significant differences between the two groups in the spermatogenetic activity. Perhaps the differences might have been more apparent with by a cytometric method, as was used in the studies on melatonin utilization for reproduction of foxes by Smith et al.(1987) and by Sundquist et al. (1989) in their studies on mink reproduction.

In the first month of the experiments (August, September) the testosterone level in the blood sera of males of both groups was at the same low level (0.20 and 0.65 mmol/l). From December it started to increase in animals of both groups, attaining the highest level in March, being considerably higher (12 mmol/l) in the darkness group males than in the light group males (7.4 mmol/l).

The higher testosterone levels in the darkness group males are confirmed by the studies of activity of Δ 3 β steroid dehydrogenase (HSD). According to Turner (1987) there is a relationship between testosterone level and the condition of activity of sexual glands and ductuli efferentes. Each of the ways of steroid hormone biosynthesis is responsible for

transforming Δ 3-β of hydroxysteroids into Δ-4 of ketosteroids. The enzymes which catalyze these transformations are steroid dehydrogenases. Also, Mc Kern (1969) assumes Δ-5 3- β HSD activity as a creation of steroidogenic activity of the testes.

Since small amounts of androgens are produced in the suprarenal glands hence the blood serum testosterone level is indicative, according to Slebodzinska (1987) not only of steroidogenic activity of the testes but also of that of the suprarenal glands.

Based on the results obtained, it can be stated unequivocally that rearing the male blue foxes in pavilions with limited access to light does not decrease but has a beneficial effect on the dynamics of reproductive performance in males.

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The biological efficacy of pregnant mare serum gonadotropin (PMSG) to stimulate reproductive function in anestrous mink

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The number of viable offspring raised per breeding female is critical to the economic success of mink farming. A recurring problem during the breeding season is that 2 to 5% of the herd does not breed, due to persistent anestrus. Our objective was to investigate the biological efficacy of PMSG to stimulate reproductive function in anestrous mink. Anestrous mink (n=298) were identified by ranch management on nine different commercial mink ranches in Wisconsin and Utah between 15 and 22 March. On the day of identification and two days later each anestrous female was injected with 50 IU of PMSG or placebo. Treatment was administered in a blind research design fashion. Standard breeding practices were resumed four days after the second injection. There was a significant ($p < 0.05$) treatment effect of PMSG on the breeding success of these anestrous animals. PMSG-treated mink bred at a rate of 51.0%, compared with only 18.1% for the placebo-treated mink bred. The efficacy of treatment was further reflected in the number of breeding attempts following treatment. The PMSG-treated group averaged 3.23*0.11 attempts while in the placebo group they averaged 3.82*0.09. This significant decrease reflected the fact that PMSG-treated mink successfully bred. There was no difference in length of gestation between the two groups. The percentage of mink whelping was significantly higher in mink treated with PMSG (36.6%) versus those treated with the placebo (14.6%). Litter sizes in whelping females were similar in the two treatment groups but when averaged for all females in the study, the number of kits born was significantly higher in PMSG-treated animals (1.88*0.26) versus placebo-treated animals (0.68*0.16). These results demonstrate the efficacy of PMSG to induce estrus and successful breeding in non-breeding mink. The ability to induce over 50% of these females to breed will directly benefit the rancher economically.

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Mink have played a leading role as valuable fur-bearing animals since the turn of the century. Annual worldwide production is now approaching 30 million pelts per year. One of the most important factors regulating the economics of mink farming is the number of viable offspring obtained per female maintained in the breeding herd. In mink the ovulation is induced by copulation within 48 hours (Hanson 1947). Gestation is variable (40-60 days) since implantation is delayed in the mink (Hanson 1947) (Enders 1952). Litter size averages between four and six kits.

A recurring problem in mink husbandry is that 2 to 5% of the female breeding stock do not breed, due to persistent anestrus (Adams 1973). This loss in production represents millions of dollars lost in fur sales annually.

Fertility hormones, or, more specifically, gonadotropins, have been successfully applied to stimulate reproductive function in several species including the mink (Jarosz et al. 1987;Hammond 1952;Stout & Adair 1972). Follicle growth and egg development in mink can be stimulated with pregnant mare serum gonadotropin (PMSG) (Murphy et al. 1987;Wehrenberg et al. 1989). Ovulation has been induced in mink after injection of human chorionic gonadotropin (hCG) (Hattenhauer et al. 1984). These important observations demonstrate that mink respond to exogenous gonadotropins. Yet, limited research has been undertaken to apply this information on a practical basis. The research objective of this study was to investigate the biological efficacy of PMSG to stimulate reproductive function in anestrous mink and to demonstrate that this information can be applied on a practical basis to the mink industry.

MATERIALS AND METHODS

Testing Facilities

Fifteen commercial mink farms in Wisconsin and Utah, USA were solicited for participation in this study. All animals were maintained under standard farm conditions. While it is recognized that there were differences in farm operations and management which reflected the experiences and ideas of each proprietor, all farms were operated following the sound mink-husbandry procedures outlined in Mink Production (Jørgensen 1985). The care and use of all animals used in this study had been reviewed and approved by the University of Wisconsin-Milwaukee Animal Care and Use Committee.

Our objective in this study was to determine whether PMSG administration could improve reproductive performance of the breeding herd under field conditions. Therefore, farm management on each farm was responsible for numerous aspects of the study. To facilitate mink farmer participation, each farm was visited at least three times during the course of the study. A first visit occurred prior to the onset of the 1991 breeding season. During the first visit farmers were given a protocol explaining the procedures for reconstituting the PMSG or placebo, storage of the material, injection volume and route of administration. Mink farmers were instructed to select their own dates of hormone administration based on their experience of when they could identify successfully anestrous or non-breeding mink. Based on the fact that these were field studies, compliance matters were of primary concern.

Therefore a second visit was made to several of the farms on 18 March. Based on

these visits and direct telephone conversations with all of the participants, compliance with the experimental protocol was followed. In late March all farmers were again contacted and asked if there had been problems. All farmers reported that they had complied with the experimental protocol and had not encountered any problems during the course of the experiment. In June all of the testing facilities were visited by the investigators. All of the required information was transcribed from each animal's permanent breeding card to permanent laboratory files.

Animals

The farmers were instructed to select only anestrous mink at the end of the breeding season (approximately 16-20 March) which would, in their opinion, not breed. The ability of each farmer properly to select anestrous animals was determined by evaluation of the percentage of animals that bred following placebo treatment. It has previously been established that clear selection of anestrous mink can be accomplished on commercial mink farms (Wehrenberg et al. 1989; Wehrenberg et al. 1992). On six farms the percentage of placebo-treated mink which bred was greater than 40%. This indicated that these farmers were not successful in selecting only anestrous mink and therefore the results from these farms were deleted from the study.

Reagents and Supplies

PMSG (Folligon, lot #20032) and PMSG/placebo solvent were donated by Intervet America, Inc., Millsboro, Delaware, USA. Placebo consisted of 250 μg sodium phosphate, dibasic anhydrous (Sigma Chemical Co., St. Louis, MO), 250 μg sodium phosphate, monobasic anhydrous (Sigma Chemical Co.), 5 mg D-mannitol (Sigma Chemical Co.), and 500 μg methylparahydroxybenzoate (methyl paraben) (Sigma Chemical Co.). For each study site, the PMSG and placebo vials were similarly labeled with either an "A" or a "B". Different codes were assigned to each farm using a random number table. This procedure ensured that the principles of a blind study were met.

All materials were provided to the farmers during visits to each farm. Each farmer estimated the number of mink they would have for the study, and for each 50 mink, they received the following supplies: syringes for injection (1ml), syringes to reconstitute the lyophilized PMSG or placebo material (3ml), 1 vial of "A" hormone, 1 vial of "B" hormone, 1 vial of solvent marked "A", and 1 vial of solvent marked "B".

Table 1. Dosage and timing of injections

	Day 0	Day2
PMSG	50 IU in 0.25 ml	50 IU in 0.25 ml
Placebo	0.25 ml	0.25 ml

Placebo and PMSG were reconstituted in saline

Treatment Procedures

On the day of animal selection (day 0), the farmer injected each anestrous female with 0.25 ml of solution "A" or solution "B" (Table 1). Treatments rotated from "A" to "B" for every other mink. Since the animals were randomly located throughout the mink farm, randomization of treatment was ensured. Injections were IM and made in a hind leg of the animal. Two days later

(day 2) a second injection was administered. Animals were then left undisturbed for three days. Beginning on day 6, animals were again subjected to standard breeding practices. The interval between treatment and re-initiation of breeding was based on the fact that the mink estrous cycle is approximately seven days long.

Data Management and Statistical Analysis

Raw data were compiled and managed using the data management software program RS1 by BBN Software Products Corp.(Cambridge, MA, USA). All results were initially tabulated by farm. Approximately 3.9% of the animals studied were deleted due to incomplete records. These results were graphed and a preliminary analysis was done to determine whether only anestrous mink were chosen for the study.

A statistical analysis was performed on the data using SAS statistical software by SAS Institute Inc. (Carry, NC, USA). The data were classified according to hormone (treatment) and farm (replicates). Thus the analysis of variance consisted of a completely randomized block design (Snedecor & Cochran 1967). The main treatment effect of hormone treatment was evaluated using the hormone by farm interaction term for the hypothesis test. The data expressed as a percentage were analyzed by Chi-square using a 2 x 2 contingency table. These analyses were performed using SPSS/PC+ statistical software by SPSS, Inc. (Chicago, IL, USA).

RESULTS

Figure 1 illustrates the percentage of mink which bred following PMSG or placebo treatment at the nine individual farms where anestrous or non-breeding mink were selected successfully at the end of the breeding season. Data from these nine farms were combined and subjected to further analysis. This compiled information is presented in Table 2.

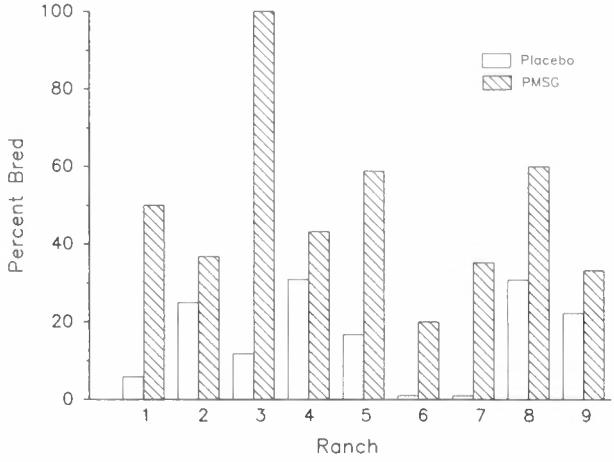

Fig. 1. The percentage of anestrous mink which bred following PMSG or placebo treatment at nine individual farms where anestrous or non-breeding mink were selected successfully at the end of the breeding season

Table 2. Reproductive performance parameters in anestrous mink

		Attempts to breed				Gestation	Kits born per female	
Treatment	N[1]	Before	After	% Bred	% Born	(days)	Pregnant	Total
Placebo	144	5.8±0.2	3.8±0.1	18.1	14.6	44±1	4.7*0.6	0.7±0.2
PMSG	153	5.9±0.1	3.2±0.1**	51.0**	36.6**	46±1	5.1±0.5	1.9±0.3**

[1]Discordance in numbers reflects the exclusion of some mink due to incomplete records
**$p < 0.01$ versus the corresponding placebo-treated group

There was no difference between the attempts to breed prior to treatment in the PMSG- and placebo-treated groups. This was expected,since animals were randomly assigned to each group. From a practical point of view the large numbers of attempts to breed reflect a considerable amount of work by the farmer with no productivity occurring. PMSG treatment was highly significant ($p < 0.01$) in reducing the number of breeding attempts following treatment. This significant decrease directly reflected the fact that PMSG-treated mink bred successfully.

There was a highly significant treatment effect of PMSG versus placebo injection ($p<0.01$) on the percentage of animals bred. The PMSG-treated animals bred at a rate of 51.0%, while only 18.1% of the placebo-treated mink bred. This significant treatment effect was further reflected in the percentage of animals giving birth. The differences between the treatment groups demonstrated the effectiveness of PMSG in improving reproductive performance.

The results show that there was no difference between the two treatment groups in the length of gestation. This is a critical observation showing that PMSG does not alter implantation. There was no effect of hormone treatment on the number of kits born per whelping female. This observation provides direct evidence that PMSG at the doses administered does not affect litter size. However, there was a significant difference ($p<0.01$) in the number of kits born when averaged over all PMSG-treated animals versus all placebo-treated animals. This observation represents clear evidence that PMSG treatment of anestrous mink can be used to increase mink production.

DISCUSSION

We have previously demonstrated that PMSG is efficacious in treating infertile female mink. Our initial study focused on animals rendered infertile due to alterations in photoperiod (Wehrenberg et al. 1989). More recently, we have investigated the use of PMSG in a group of animals identified simply as non-breeding animals at the end of the breeding season (Wehrenberg et al. 1992). In the later study we investigated the dose-response relationship between PMSG and reproductive performance and found that a 2 X 50 IU dose of PMSG yielded a significant treatment effect which was no different from that with the higher doses studied. Both of these earlier studies were conducted with direct investigator participation in all aspects of the study. The present study was designed to investigate whether PMSG treatment would also prove to be effective under standard farm

conditions. The significant decrease in the number of attempts to breed following PMSG treatment, the increase in number of mink breeding, and the increase in the number of kits born per all females treated demonstrate that PMSG can improve reproductive performance in those mink which do not breed during the normal breeding season. An earlier study by Murphy et al.(1987) has reported similar percentages in the number of anestrous mink breeding and giving birth following 100 IU of PMSG but litter size in this study only averaged two kits. This was markedly improved in the present study.

Reproductive failure in the female mink during the breeding season is not limited to those mink in the herd that will not breed. Another problem group is those animals which are "hard to breed". While not specifically defined, many farmers consider those mink not bred by approximately 12 March as "hard to breed". By this time, these females will have been handled and paired with males five to ten times with no successful matings. It is recognized that between 10 and 20 March the mink farmer must concentrate on second and third matings for the majority of the herd and can ill afford the time and effort required to breed the small population of the herd that is "hard to breed". Aware of this problem, we anticipated that some farmers may have selected both "hard to breed" and non-breeding mink for inclusion in this study. However, we have shown that under strict selection criteria less than 30% of the identified non-breeding mink treated with placebo finally breed (Wehrenberg et al. 1989,1992). Therefore, we established that for the results of a field site to be included in this study, no more than 40% of the placebo-treated mink could breed. As noted, six of the study sites were not included in this study since they failed to meet our selection criteria of non-breeding mink.

We evaluated whether the 2 X 50 IU dose of PMSG improved any of the breeding parameters measured in the "hard to breed" mink and noted no significant improvements. However, we have conducted a preliminary study on one farm where we administered a combination of PMSG and hCG to all mink which had not bred by the 15 March. In this study we used variable doses of PMSG (100 - 500 IU) and 250 IU of hCG. At the doses tested, we observed that between 89 and 100% of the females bred and between 60 and 82% of the females whelped (unpublished observations). We did not observe a dose-response relationship which suggests that lower doses may be efficacious. While these studies must be deemed preliminary since no placebo-treated animals were studied, they do suggest that higher doses of PMSG may be effective in treating "hard to breed" mink.

A common problem in treating female mammals with fertility hormones is superovulation (Hutz et al. 1984). It has been shown by dose titration studies, that there are significant between-subject differences within a species in the response to exogenous gonadotropins (Fleming & Yanagimachi 1980). Our results suggest that the 2X 50 IU dose of PMSG was appropriate in inducing ovarian function but insufficient to induce superovulation based on the fact that extremely large litters were not observed. The range observed in the present study was 1 to 13 kits. This is consistent with previously published values (Murphy et al. 1987). This is an important observation,since it would be of little value to increase the percentage of females breeding and whelping if this would result in the death of the offspring due to their small size or to the inability of the females to raise such a large litter.

The fact remains that approximately 50% of the PMSG-treated mink still fail to breed. Further investigations focusing on these animals are necessary to identify the cause of their

dysfunction. Likely causes include anatomic and physiologic dysfunction of the reproductive tract. Central nervous system involvement is not likely but cannot be ruled out since it is of critical importance in the behavioral aspects of the estrus.

SUMMARY

When the criteria of selecting only anestrous mink are met, the present study clearly demonstrates the efficacy of PMSG in enhancing the number of offspring born per female. The ability to induce over 50% of the anestrous females to breed (over twice the placebo group) will directly benefit the farmer economically. PMSG, at the dose used in this study (2 x 50 IU), exerted no adverse effects on productivity as demonstrated by the lack of a statistically significant difference between the number of kits born per whelping female in the two treatment groups and the lack of a difference in gestation length. PMSG did, however, double the percentage of mink giving birth and the number of kits born for all females. Indirect economic benefits of PMSG treatment will accrue as well. After treatment, PMSG-treated animals required less time for breeding as demonstrated by the significantly fewer attempts to breed. Because of the usual practice of "double breeding", this difference becomes even more time efficient. Collectively, these data support the use of PMSG for breeding enhancement in anestrous mink at the end of the breeding season.

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Effects of GnRH-analogue on fertility in mink

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Field trials were carried out over a three-year period to examine the effects of GnRH-analogue (fertirelin acetate) on fertility in female mink at a ranch (Zao Mink). In the first oestrus, all females were routinely mated and most mink with a second or third oestrus were later injected i.m. with 5 μg of the analogue immediately after mating. When all data were combined for the three-year period, the conception rate and mean litter size per female were 90.8% and 4.61% for the analogue group and 85.3% and 4.24% for the control group.

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In cows, the injection of GnRH og GnRH-analogue at the time of insemination has been used for improving fertility (Schels & Mostafawi 1978; Mori & Takahashi 1978; Nakao et al. 1983). In mink, injection of females with GnRH at the time of mating has also been carried out in an attempt to improve fertility (Myrphy 1976, 1979). However, GnRH or its analogue has never been applied to mink in Japan. Since only a small number of animals were used in earlier works, the effect of GnRH on the fertility of mink has not been clearly determined. A large-scale field trial may be neccesary to test the practical application of GnRH. At this congress, we present a report on how the effects of injecting an analogue of GnRH at mating can have an influence on the rate of conception in a large number of different varieties of mink over a period of three years.

MATERIALS AND METHODS

Female mink were used in field trials at the Zao Mink Ranch, Tohkatta, Miyagi Prefecture from 1989 to 1991. Females were mated with males routinely on the first day or on both the first and second days of the oestrus at the beginning of the breeding season (early March). Most of the females in whom the oestrus recurred 7-8 days later were injected with 5 μg of GnRH-analogue immediately after mating. The analogue used was fertirelin acetate (Takeda Chemical Industries C., Osaka, Japan), the dose being determined in light of the fact that this analogue possesses a biological potency 3-7 times stronger than that of natural GnRH (Umezu et al. 1974) and that the scale of injection (0.1 ml) is easier for ranch personnel to read. The analogue-treated group was termed [A]. If oestrus recurred on the following day or later, another injection of analogue was given.

The birth of pups was determined by vocalization of the kits. A few females were detected by sound as having had litters but were later found to have eaten their pups. These mothers were included in the calculations of reproductive success and of mean litter size.

In 1989 a total of 185 females of the Sapphire and Pastel varieties of mink were studied and the nubmer of matings, duration of pregnancy, conception rate and mean litter size/female were calculated. In 1990 and 1991, a total of 2987 females including Sapphire, Violet, White, Jet Dark and Dark were studied. Conception rate and mean litter size/female were recorded for each year. In the control group, female mink were injected with physiological saline in 1989; no treatment was administered in 1990 and 1991. The control group was termed [C]. The Chi-square test of significance was used to compare the difference in conception rate between [A] and [C].

RESULT

(1) 1989: The number of matings and duration of pregnancy tended to be slightly less in [A] (0.05 and 2.57 days in Sapphire; 0.17 and 1.09 days in Pastel). The conception rate of both [A] varieties tended to be higher than that of [C]. In Sapphire mink, the conception rate of [A] was 9.6% higher than that of [C] ($0.05 < p < 0.1$). Mean litter size was 5.50 in [A] vs 4.74 in [C] in Sapphire, and 4.48 in [A] vs 4.33 in [C] in Pastel (Table 1).

Table 1. Nuber of matings, duration of pregnancy, conception rate and mean litter size/female of minks treated with GnRH-analogue in 1989

Variety	Ana-logue (1)	N (2)	No Matings (Day) (3)	Duration of preg. (4)	Concept. rate (5)	Total no. pups (6)	Mean litter size/female (7)
Sapphire	-	43	3.23 +0.12	46.58 (+0.61)	38/43	204	4.74 +0.39
	+	50	3.18 +0.08	44.61 +0.73	49/50 (98.0%)	275	5.50 +0.33
Pastel	-	42	2.91 +0.05	45.69 +0.54	36/42 (85.7%)	182	4.33 +0.40
	+	50	2.74 +0.08	44.60 +0.36	45/50 (90.0%)	224	4.48 +0.37

(1) administration (+) or non-administration (-) GnRH-analogue
(2) number of minks used
(3) number of matings and (4) duration of pregnancy were calculated per pregnant female
(5) conception rate and (6) total number of pups in a group
(6) a total number of pups in a group
(7) mean + standard error (S.E.)

(2) 1990: The conception rate of [A] was significantly higher than that of [C] in Sapphire ($p < 0.05$). Mean litter size/female was slightly higher in [A] than in [C] in all varieties of mink (Table 2).

(3) 1991: The conception rate of [A] in Sapphire and White mink was significantly higher than that for the same varieties in the [C] group ($p < 0.05$). The rate of Violet mink tended to be higher in the [A] group than in the [C] group ($0.1 < p < 0.05$). The mean litter size increased by 20.8, 8.6 and 29.8% higher in Sapphire, White and Dark, respectively, as compared with the rates in [C] (Table 2).

(4) 1989-91: Records for the total three-year period were combined and arranged into two categories, namely, light and dark coloured mink varieties. In mink with light-coloured fur, the conception rate of [A] was remarkebly higher in Sapphire ($p < 0.001$) than that in [C] and the litter size was also higher. When the conception rates of the three varieties of light-coloured mink were combined, a significant difference was detected between [A] and [C] ($p < 0.001$). In mink with dark fur, there was no difference between [A] and [C] in any colour variety, and when records of the three varieties were combined, the mean litter size/female of [A] was seen to be 18.8% higher than that of [C]. When all data of the three-year period for all varieties were combined, the conception rate was found to be significantly different ($p < 0.001$) with 90.8% in [A] and 85.3% in [C], respectively. The mean litter size was 4.61 in [A] and 4.24 in [C], an increase of 8.7% with the analogue injection (Table 3). When mean litter size was calculated per pregnant female, it was found to be 5.08 and 4.96 in [A] and [C], respectively. The difference was very small.

Table 2. Conception rate and mean litter size per female mink treated with GnRH-analogue in 1990 and 1991

Year	Variety	Analo-gue (1)	N (2)	N concept. (3)	% consept. (4)	Total pups (5)	Mean litter size/female (6)
1990	Sapphire	-	196	179	91.3	870	4.44
		+	208	201	97.6*	942	4.53
	Violet	-	134	126	94.0	581	4.34
		+	142	129	90.8	622	4.38
	Jet Dark	-	117	107	91.5	493	4.21
		+	124	115	92.7	531	4.28
1991	Sapphire	-	806	681	84.5	3413	4.23
		+	154	140	90.9*	787	5.11
	Violet	-	339	289	85.3	1608	4.74
		+	81	75	92.5	342	4.22
	White	-	84	69	82.1	381	4.53
		+	71	66	93.0*	349	4.92
	Dark	-	363	288	79.3	1270	3.49
		+	168	132	78.6	761	4.53

(1) administration (+) or non-administration (-) GnRH-analogue
(2) number of minks used and (3) the number of minks which conceived
(4) percentage of conception rate
(5) total number of pups in a group
* Conception rate was significantly greater in the analogue-treated group than in the control group: $p < 0.05$

DISCUSSION

In this field trial, which involved a large number of mink over a three-year period, the effect of GnRH-analogue on conception rate was clearent and the mean litter size/female was found to be increased as a result, although the type of GnRH, the time of injection and the dose of hormone were different from those of earlier reports (Murphy 1976, 1979). In particular, the conception rate of the Sapphire variety with light-coloured fur was remarkably improved. Furthermore the rate increased in White but did not increase in Violet with light fur. The mean litter size but not the conception rate of mink with dark-coloured fur increased with the analogue injection, and the conception rate significantly increased when all data were combined, regardless of colour variety. The mechanism lead-

Table 3. Conception rate and mean litter size per female mink treated with GnRH-analogue for three years (1989-91)

Variety	Analogue (1)	N (2)	Conception rate	Mean litter size/female
Sapphire	-	1045	85.9%	4.29
Violet	-	473	87.7%	4.63
White	-	84	82.1%	4.53
Light fur	-	1602	86.3	4.41
Pastel	-	42	85.7%	4.33
Jet Dark	-	117	91.5%	4.21
Dark	-	363	79.3%	3.50
Dark fur	-	522	82.6%	3.73
Total	-	2124	85.3%	4.24
Sapphire	+	412	94.7%***	4.86
Violet	+	223	91.5%	4.32
White	+	71	93.0%*	4.92
Light fur	+	706	93.5%***	4.70
Pastel	+	50	90.0%	4.48
Jet Dark	+	124	92.7%	4.28
Dark	+	168	78.6%	4.53
Dark fur	+	342	85.4%	4.43
Total	+	1048	90.8%***	4.61

(1) administration (+) or non-administration (-) of GnRH-analogue

(2) number of minks used

Conception rate of the analogue-treated group was significantly higher than that of the control group: * $p < 0.05$, *** $p < 0.001$

ing to the improvement in conception rate in minks treated with the analogue is not fully understood. Nakao et al. (1983) reported that the same analogue administered at insemination was effective in improving the pregnancy rate in cows. It is known that exogenous GnRH or GnRH analogue facilitates LH release in cattle (Mori & Takahashi 1978) and also in mink (Murphy 1979). It is thought that the GnRH analogue stimulates the release of LH when the release is insufficient in females. Pituitary LH was fully released with administration of the analogue fertirelin acetate, as this agent is more biologically

potent than natural GnRH. This process may later lead to the elevation of conception rate and the increase of litter size in mink. It is unlikely that the number of ova increases with the analogue because there was little change in the number of pups per impregnanted female.Thus, the GnRH-analogue can possibly be used for field application from the standpoints of biological effects, safety, ease of administration and cost.

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Constantly pregnant... well almost. Reproductive hormone levels of the fisher (*Martes pennanti*), a delayed implanter

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Cherepak, R. B. & M.L. Connor 1992. Constantly pregnant... well almost. Reproductive hormone levels of the fisher (*Martes pennanti*), a delayed implanter. Norwegian Journal of Agricultural Sciences. Suppl. no. 9: 150-154. ISSN 0801-5341.

Female fisher exhibit an obligatory delayed implantation, with a total gestation length of 327-358 days. The cues which terminate the delay, and the length of active gestation are unknown. Breeding occurs in spring one to two weeks postpartum and ovulation is believed to be induced. Blood samples were collected from 14 females over a two-year period with progesterone and oestrogen levels determined by radio immunoassay (RIA). Progesterone profiles suggest that the lengthening days following the winter solstice stimulate corpora lutea activity capable of producing progesterone levels of over 40 ng/ml. Active gestation is approximately 50 days and spontaneous ovulations are likely to occur. Oestrogen profiles suggest that seasonal follicular activity begins in early January (annual range of 17ß-oestradiol: 5-80 pg/ml).

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The fisher (*Martes pennanti*) is a large North American member of the family *Mustelidae*, which has long been cherished for its luxurious and valuable pelt. High pelt values sparked interest in commercial fur-producing circles in the early 1900s, and also in recent years. While these commercial operations met with little or no success, several peculiarities of the fishers' reproductive physiology were discovered on these fur farms in the 1930s.

The fisher exhibits a postpartum oestrus and is pregnant approximately 50 weeks of the year (Hodgson 1937). While female fishers are sexually mature and mate at one year of age, first litters are not born until the female is two years old, after a total gestation of 327-358 days (Hall 1942; Eadie & Hamilton 1958; Wright & Coulter 1967). Oestrus occurs 1-2 weeks postpartum (Hall 1942) at which time ovulation is believed to be induced by mating (Ewer 1973). Carcass analysis confirmed the occurrence of delayed implantation in this species (Enders & Pearson 1943a).

Many unanswered questions regarding the reproductive physiology of the fisher remain. What are the environmental cues and physiological mechanisms involved in regulating the onset and termination of the delay in this species? What is the length of active gestation? Is the fisher indeed an induced ovulator?

This research was undertaken in an attempt to address som of these questions, specifically from an endocrinological perspective. Determination of the fishers progesterone

and oestrogen profiles over the long term may provide information on the cues regulating implantation (and the delay), active gestation length and whether or not ovulation is indeed induced in this species.

MATERIALS AND METHODS

Fourteen female fishers, ten wild-caught and four captive-born, were maintained in southern Manitoba (49° 53'N, 97° 09'W). When the research began, the animals were 5 (n=1), 17 (n=3) and 29-77 (n=10) months of age. A standard wet mink ration was provided, supplemented with additional red meat products in order to resemble more closely their predominantly meat diet in the wild.

Blood samples were collected monthly from September 1989 until January 1990, bi-weekly until March 1990, weekly until the end of May 1990 and infrequently until October 1990. In the second year of the study, samples were collected weekly from mid-December 1990 until mid-June 1991. Samples were collected by jugular venipuncture while the animals were under ketamine hydrochloride anaesthesia. In an attempt to avoid possible diurnal variation in hormone levels, the animals were sampled in the same order, and at approximately the same hour of each sampling day. Serum was removed the next morning and frozen at -20°C until analyzed for progesterone and 17ß-oestradiol by radioimmunoassay (RIA). All samples from individual animals were analysed in the same assay.

RESULTS

Serum hormone levels: progesterone profiles are presented in Fig. 1a (1st year), 1b, 1c and 1d (2nd year); and 17ß-oestradiol profiles are presented in Fig. 2a (1st year) and 2b (2nd year).

Serum progesterone profiles from the 1st year (Fig. 1a), fell into two categories. Ten of the females had no apparent changes in progesterone (1-2 ng/ml) during the study period, while the remaining four females had substantial increases (up to 45 ng/ml). In the latter group, progesterone started to increase in early January, peaked in late February and returned to low levels by the end of April. A second, smaller increase in progesterone ($\sim$5 ng/ml) was observed in mid-May. Second year profiles (Fig. 1b, 1c and 1d) resembled the first, but with an additional category, a group with only a moderate increase in progesterone (Fig. 1c).

Serum 17ß-oestradiol profiles of all animals combined from each year (Fig. 2a, b) show fairly constant levels (means= 20-35 pg/ml). There was considerable variability in 17ß-oestradiol levels between animals (s.e.m. values) and within animals (typical range within animals: 15-50 pg/ml (not shown here).

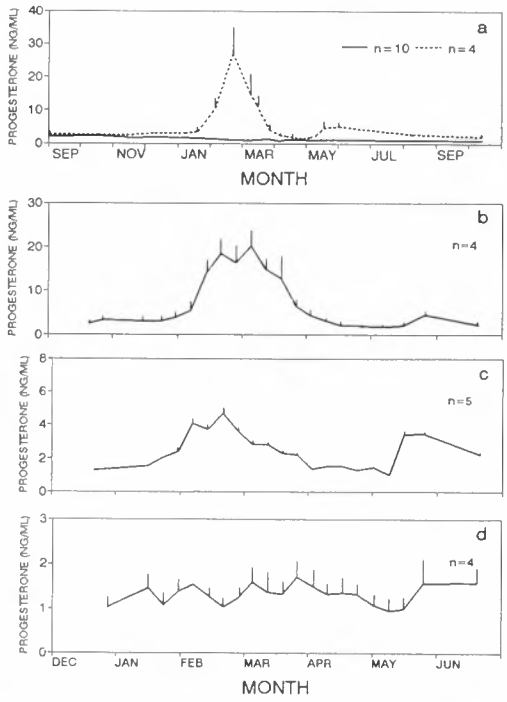

Fig. 1. Fisher progesterone profiles. a) 1st year, b) 2nd year-high levels, c) 2nd year-low levels and, d) 2nd year-low levels, (means + s.e.m.)

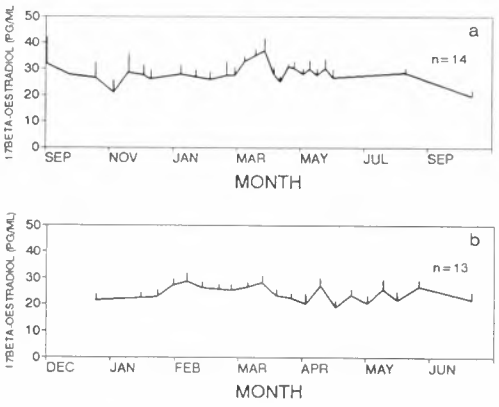

Fig. 2. Fisher 17β-oestradiol levels. a) 1st year, b) 2nd year, (means + s.e.m.)

DISCUSSION

Photoperiod is widely accepted as an environmental cue which is responsible for diurnal
and circadian rhythms in most vertebrate species. The reproductive cycles of many mustelids have been shown to be regulated by photoperiod, including: marten (*Martes sp.*) (Pearson & Enders 1943b), European badger (*Meles meles*) (Canivenc & Bonnin 1981) and skunk (*Spilogale putorius latifrons*) (Mead 1971). Sundqvist et al. (1989) reviewed the work of several authors which implicated photoperiod in control of the reproduction in the mink (*Mustela vison*), including the length of delayed implantation.

Circanual progesterone profiles shown for fishers are similar to those reported for pregnant badgers (Canivenc & Bonnin 1981) and spotted skunks (Mead 1971) under natural photoperiods. While the badger requires day shortening to initiate implantation, the skunk requires increasing daylength. Like the skunk, it appears that increasing daylength, in this case following the winter solstice, is the cue to reactivation of the corpora lutea, and subsequent implantation in the fisher. The small increases in progesterone seen in May occurred around oestrus and likely indicated that ovulation had occurred. As no litters were born during the period of study, no explanation for the variability of progesterone peaks is possible.

Active gestation length in the fisher is unknown but has been estimated at 30-60 days. If the fisher is like the badger (Canivenc & Bonnin 1981) and mink (Møller 1973) in that implantation occurs shortly before or at the

time of maximum progesterone production, it can be estimated that implantation in our fishers would have occurred in mid-late February. As mentioned, no litters were produced during the study period, although two of the females had whelped on 8 and 9 April in previous years. If these dates are representative, active gestation length could be estimated at approximately 50 days.

Most mustelids are believed to be induced ovulators (Ewer 1973). While this has been verified in many of these species, it remains speculative in the fisher. Several of our females showed an increase in progesterone although they had not been observed to mate the previous spring. This lends support to the hypothesis that spontaneous ovulations may occur in this species, as has been suggested to occur in the mink (Møller 1974) and skunk (Wade-Smith et al. 1980). Further evidence comes from an unbred female whose reproductive tract was flushed in August 1991, resulting in the recovery of an unfertilized egg.

The combined 17ß-oestradiol profiles shown here (Fig. 2a and b) do not show substantial changes from a baseline of approximately 25 pg/ml. However, the s.e.m. values indicate considerable variation between animals for sampling days. From individual profiles (not shown here) it can be ascertained that follicular activity increases in early January.

SUMMARY

These results from progesterone profiles suggest that photoperiodic cues are responsible for the termination of delayed implantation in the fisher, active gestation is approximately 50 days and ovulation may occur spontaneously. Oestrogen profiles suggest that follicular activity begins in early January.

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Fertility in mink unilaterally ovariectomized or ovariohysterectomized

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In order to obtain information on the potential fertility in female mink, 10 and 11 mink respectively were unilaterally ovariectomized and unilaterally ovariohysterectomized and compared to laparotomized mink in the control groups. The surgery took place in the period December-January. With the limited number of females, the results in the ovariectomized mink revealed no evident difference in fertility rate and number of kits per fertile female compared to the control group. In the ovariohysterectomized mink the number of kits per fertile female was 2.6 compared with 6.7 in the control group. With a modified ELISA technique the plasma progesterone levels were measured in the gestation period in the ovariohysterectomized mink and the control group and showed equal levels with a peak at approx. 56 ng/ml just before whelping time. The remaining ovary displayed a compensatory hypertrophy.

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In mink, selection for increased litter size (at birth and/or at weaning) has been effective, and heritability for litter size at weaning was calculated to be about 0.2, but varied between farms, estimation methods, age of the female and colour types (Einarsson 1981).

So far, there has been no selection for components of litter size (i.e.ovulation rate and embryonic survival) in mink but this has been successful in mice (Bradford 1969). Results from Huang et al. (1987) suggest that uterine length may be an important component of uterine capacity in swine. Litter size of unilaterally ovariohysterectomized gilts was proposed as a measure of uterine capacity.(Leymaster et al. 1986).

The objective of the present study was, in a pilot investigation, to determine the breeding results in mink unilaterally ovariectomized. The following year, the effect of unilateral ovariohysterectomy on mating performance, breeding results, kit and female weights and plasma-progesterone levels was investigated.

In this experiment, it is hypothesized that the ovulation rate in the remaining ovary after unilateral ovariectomy or ovariohysterectomy is compensated by ovarian hypertrophy and the production of ova approximately equivalent in number to that of the intact female (laparatomized). This compensatory ovarian hypertrophy and production has been found in swine (Brinkley et al. 1964). In the case of unilateral ovariectomy in mink there are no records about the transuterine migration of ova and/or embryos.

MATERIALS AND METHODS

On 4 and 19 December 1989, a total of 10 and 7 Pastel females were unilaterally ovariectomized (UO, group 1) or laparotomized (L, group 2), respectively. The females in groups 1 and 2 were randomly selected among females born in April-May 1988 and with normal litters in 1989.

On 19 and 22 January 1991, 11 sibling Scanglow pairs, i.e. 22 animals, were selected among females born in 1989 and with normal litters in 1990. Each of the two animals in a sister pair were randomly assigned to one of two procedures - unilateral ovariohysterectomy (UOH, group 3) or laparotomy (L, group 4).

Animals were anaesthetized by intramuscular injection using a combination of 2 mg propionylpromazinum (Combelen ^{R} Vet.)/kg body weight and then (about 15 min) later with 14 mg pentobarbitol/kg body weight intraperitoneally.

Using aseptic techniques, the operational procedures used followed normal surgical practice.

Feeding, housing and farm management were carried out according to normal farm practice. In 1990 the females in groups 1 and 2 were mated in the 1:9 system with randomly selected males. In 1991 both members of a sister pair (groups 3 and 4) were mated to the same male, also in the 1:9 system. In the 1:9 system up to two opportunities for remating were offered every female.

The matings started in both years on 4 March. In 1991 a complete record was taken of the dates from the first and second matings and unsuccessful matings.

In 1990 no hormone analysis was carried out. In 1991, blood samples for progesterone analysis were taken on 15 March,27 March, 19 April and the day after whelping. The blood samples were taken by puncture of the vena cephalica, approx. 2-3 cc in heparinized tubes. Plasma was stored at -20°C until progesterone analysis.

Progesterone was analysed by the ELISA method with a 1Q Serum Progesterone Test Kit (Equine) (Novo Bio Labs Ltd.). A spectrophotometer (Elizareader, Inter) was used for measuring the absorbance at 492 nm. A calibration curve was drawn using the mean absorbance values of the standards, versus progesterone concentration (in ng/ml). The mink plasma samples were diluted in gelding plasma to ensure that the progesterone levels were within the calibration curve area. Each plasma sample was analysed twice. Analysis of the same standard gave a variation coefficient of less than 10.

RESULTS

In 1990 all 17 females in groups 1 and 2 were mated and the breeding results are presented in Table 1.

Using a t-test procedure, no significant differences between the two groups were calculated in number of live pups/litter ($p < 0.40$), gestation length from the second mating ($p < 0.75$) and gestation length from the first mating ($p < 0.2$, not shown).

In 1991 all 11 sibling pairs in groups 3 and 4 were mated and gave birth.

The mating results are presented in Table 2.

The breeding results are presented in Table 3. Compared with ovariectomy, ovario-

hysterectomy caused a clear decrease in the number of kits born.

Table 1. Breeding results in unilaterally ovariectomized or laparotomized (groups 1 and 2) female mink

	Group 1, UO	Group 2, L
Number of female	10	7
Female mated once/twice	9/6	7/3
Number of births	9	7
Number of live kits/litter		
Min.	2	1
Max.	8	11
Average	5.6	6.9
Std.dev.	2.1	3.5
Dead kits/litter, average	0.33	0.57
Gestation length:		
Min.	40	39
Max.	44	46
Average	42.5	43.3
Std. dev.	1.6	3.8

Table 2. Mating results in unilaterally ovariohysterectomized or laparotomized female mink

	Group 1, UO	Group 2, L
Number of female	11	11
Females mated once/twice	11/7	11/10
Average date for 1st and 2nd mating	7/3 and 15/3	7/3 and 16/3
Number of unsuccessful matings total	12	7

The difference in gestation length between groups UOH and UO is calculated from the date of the second mating with a p-value < 0.16. The gestation length calculated from the first mating gave 57.6 and 54.1 days for UOH and UO, respectively, and a p-value < 0.09.

The female and kit weights are presented in Table 4.

Results from the progesterone analysis are shown in Table 5.

The results of a t-test did not reveal any difference in progesterone level between the two groups within the same time. The progesterone fluctuation in the two groups was equal, but at a level higher than that found by Clausen (1987) using an RIA method.

Table 3. Breeding results in unilaterally ovariohysterectomized or laparotomized female mink

	Group 3, UOH	Group 4, L
Number of females	11	11
Number of births	9	10
Number of live kits/litter		
Min.	1	1
Max.	4	10
Average	2.6 A	6.7 B
Std. Dev.	1.3	2.7
Gestation length		
Min.	44	42
Max.	59	50
Average	49.2	45.3
Std. Dev.	5.6	2.3

A and B in the same row: p-value < 0.001

Table 4. Weight of females at surgery in January 1991, day after birth, and kit weights (average/std. dev.)

	Weight of females, g		Kit weights, (g) days after birth	
	January 1991	Days after birth	Total litter	Average
Group 3, UOH	939/127	937/110	28.6/16	11.5/3.7
Group 4, L	937/103	973/84	71.8/27	10.8/2,0

Table 5. Plasma progesterone analysis (ng/ml), form 15 March, 27 March, 19 April and day after birth (average/std. dev.)

	Group 3, UOH	Group 4, L
15/3	4.0/1.9	3.8/1.6
27/3	29.9/29.4	23.6/11.9
19/4	54.8/22.3	58.5/18.7
Days after birth	4.1/1.0	4.5/1.3

DISCUSSION

The results from the unilaterally ovariectomized (UO, group 1) mink in Table 1 indicate that the reproduction traits are unaffected compared with those of intact laparotomized female mink. Although it has not been investigated, it is realistic to assume that embryos originating from the one ovary have achieved a transuterine migration owing to the fact that

one of the unilaterally ovariectomized mink gave birth to eight kits.

It is difficult to judge whether the unilateral ovariohysterectomy (UOH, group 3) harms the mating results, but there is a slight decrease in females mated twice and an increase in unsuccessful matings, compared to the laparotomized female mink, as seen in Table 2.

The breeding results in UOH mink are poorer than those in laparotomized mink (p-value < 0.001, see Table 3). There are no significant differences in gestation length, calculated from the first or second matings, but the gestation length in the laparotomized animals is shorter, probably due to a higher number of kits.

In Table 4, the UOH and laparotomized females had the same weight in January, but the laparotomized females had a higher weight on the day after birth, probably partly because of the intact organs.

Progesterone levels increased in this investigation from mid-March to the end of April and dropped at birth to levels equal to those in the untreated females found by Clausen & Therkildsen (1990) using the same analysis techniques. Surgical removal of both ovaries late in the gestation of mink demonstrated a rapid drop in the progesterone level and abortion, and the authors concluded that the ovaries are the main organs for progesterone production. In this investigation it is clearly demonstrated that one ovary is enough to produce a normal breeding result (Table 1) and that one ovary is enough to produce a normal level of progesterone in the gestation period (Table 5).

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Genetic and endocrine aspects in the regulation of the reproductive function in silver fox

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Selection for domestic behaviour is accompanied by a change in the endocrine regulation of the reproductive function. Domesticated females have a lower plasma progesterone level at the anoestrus than undomesticated ones, which is due to lower adrenal progesterone production. The increased plasma progesterone level in domesticated females in comparison with that of undomesticated ones at the oestrus and preimplantation period of pregnancy coincides with the higher ovulation rate in the former than in the latter. The plasma testosterone level in domesticated males is lower than that in undomesticated males in the breeding season, but this difference is eliminated during sexual activation.

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The selection of silver foxes for lack of aggression towards humans (domestic behaviour) leads to changes not only in the behaviour but in many physiological and morphological properties (Belyaev 1985; Trut 1988). It was assumed that selection for behavioural characteristics can change endocrine function connected with the behaviour through the nervous and neuroendocrine regulatory mechanisms. The present article describes some of the results of the research on hormonal activity of gonads in silver foxes after many years of selection for domestic behaviour.

MATERIALS AND METHODS

The work was carried out on sexually mature female and male silver foxes (*Vulpes vulpes*) aged 2-3 years, bred on the experimental animal farm of the Institute of Cytology and Genetics. The animals chosen for this study were from a population selected for domestic behaviour (here called "tame" or domesticated) and from a commercial population showing clearly aggressive behaviour towards humans (comparatively wild or undomesticated). Peripheral blood samples were taken from v. saphena in the same females (10-15 animals in the group) once or twice a month during the anoestrus, three times during pro-oestrus and once after the first mating. The onset of the pro-oestrus was determined by taking vaginal smears and by the external appearance of the genitalia, and that of the oestrus also

by vaginal smears and the readiness of the females to mate.

For estimation of the endocrine function of the testis under domestication we used the model of social interactions when males were presented with females. In September and during the mating season (January-February), a female was introduced for one hour to males of both behavioural groups. In September and January, anoestrus females, and in February females in oestrus, were introducted to males. Before the introduction and one hour after its start, blood was taken from the males, and the plasma testosterone level was measured.

In December several females were sacrificed in order to make an *in vitro* study of progesterone production by ovaries and adrenals. The glands were incubated in Krebs-Ringer bicarbonate buffer with a glucose content of 200 mg%, in an atmosphere of 95% oxygen, 5% carbon dioxide, at 37°C. In order to determine potential fertility of females, a special group of animals was sacrificed at different stages after the implantation and the number of corpora lutea and implantation sites were recorded.

Sex steroids in the plasma and gland incubates were estimated using a radioimmunological method with commercial kits (Cea-Ire-Sorin, France). The results were analysed using the Student's t-test.

RESULTS

It was found during the anoestrus, changes in plasma oestradiol are similar in tame and undomesticated animals (Fig. 1). The lowest concentration occurs in summer. In domesticated and undomesticated females, a significant increase in blood plasma oestradiol concentration is found in autumn. After that, its level declines and begins to increase again by the onset of the breeding season.

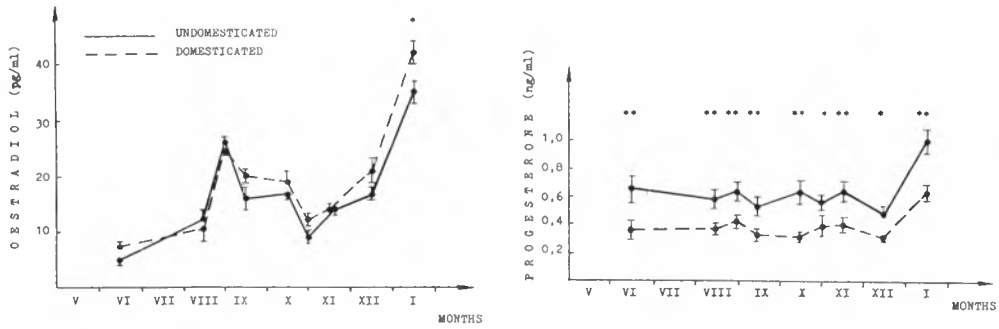

Fig. 1. Oestradiol and progesterone concentrations in the blood plasma of silver fox females during the anoestrous. The significance of differences between domesticated and undomesticated animals is marked by an asterisk in all figures

* $p < 0.05$ ** $p < 0.01$

For almost the whole anoestrus period the oestradiol concentration in tame and undomesticated females does not change significantly. However, in January the oestradiol concentration in domesticated animals significantly ($p < 0.05$) exceeds that in the undomesticated ones. The progesterone concentration throughout anoestrus fluctuates insignificantly, but increases by the beginning of the breeding season ($p < 0.05$) in both sets of animals. During almost the whole anoestrus period the blood concentration of progesterone in tame females is significantly lower than that in undomesticated ones (Fig. 1).

While the reproductive tract of females is functioning actively, characteristic changes are observed in blood sex hormone concentrations (Fig. 2). In both groups the oestradiol concentration rises during pro-oestrus to reach maximum concentrations before it ends. During the oestrus cycle the oestradiol level decreases in association with ovulation. During pro-oestrus the concentration of oestradiol does not differ significantly between the two groups of animals, but during the oestrus it is significantly lower in tame than in undomesticated foxes (Fig. 2). The progesterone level in the pro-oestrus period also rises significantly in both groups. During the oestrus it rises on average by 4-6 times (Fig. 2) owing to the development of the corpora lutea, which secrete progesterone and at oestrus the progesterone level in tame animals is significantly higher than that in domesticated animals ($p < 0.01$). Changes in blood progesterone concentration during pregnancy are identical in the two groups. The concentration significantly increases, reaching maximum value by the fifth to the tenth day of pregnancy, after which it gradually declines until the end of pregnancy. The concentration of oestradiol in blood kis comparatively stable throughout pregnancy. In the preimplantation period and during the last week of pregnancy the concentration of sex hormones in tame animals is significantly higher than that in relatively wild animals (Fig. 2).

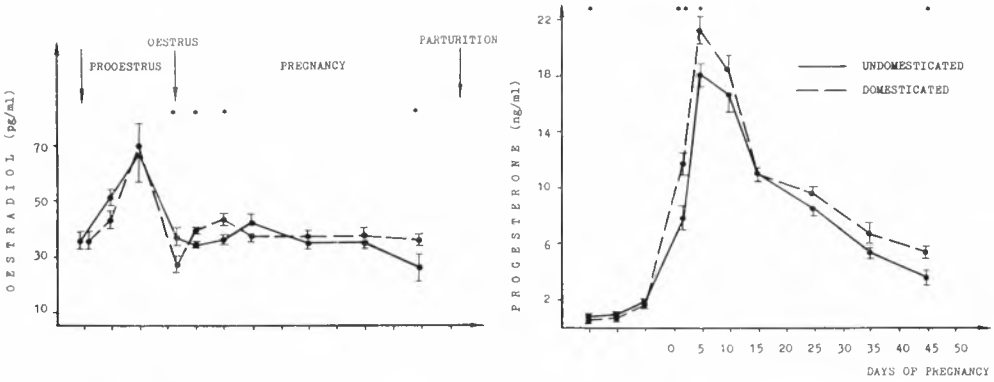

Fig. 2. Oestradiol and progesterone concentrations in the blood plasma of silver fox females during the pro-oestrus, oestrus and pregnancy

The results shown in Table 1 indicate that the progesterone production by silver fox adrenals *in vitro* is considerably higher than that by the ovaries (on average by 100 times) in both groups of animals. Progesterone production by the ovaries does not differ between domesticated and undomesticated silver foxes, but this hormone production by adrenals of undomesticated animals is higher than that by adrenals of domesticated animals. Plasma

progesterone level is also higher in the former than in the latter. It can be seen from a comparison of corpora lutea that their number in domesticated females exceeds that in relatively wild females (Table 2).

Table 1. Plasma levels of progesterone and its production by ovaries and adrenals *in vitro* in silver fox females at anoestrus

	Plasma level (ng/ml)	Production by ovaries (ng/both gl/hr)	Production by adrenals (ng/both gl/hr)
Undomesticated	0.67±0.04 * (10)	0.95±0.07 (10)	117.8±6.1 * (10)
Domesticated	0.29±0.03 (9)	1.03±0.07 (9)	84.3±10.9 (10)

No. of animals in parentheses
* Significant differences ($p < 0.05$) between domesticated and undomesticated animals

Table 2. Number of corpora lutea and implantation sites in silver fox females

	No. of corpora lutea	No. of implantation sites
Undomesticated	6.3±0.2 * (59)	4.6±0.3 (59)
Domesticated	7.3±0.2 (50)	5.2±0.3 (50)

* Footnote as in Table 1

The plasma testosterone level in both groups of males shows a characteristic seasonal variation, i.e. it increases by the mating season with the maximum concentration occurring in February, while a low level is found in September. Significant differences in basal (control) testosterone concentration have been found between the two groups of animals during the reproductive season (February) when the testosterone level is significantly lower in domesticated than in undomesticated animals (Table 3). The presence of females had no effect on the testicular hormone activity of the two groups of males in September. During the breeding season (February), receptive females are able to increase testosterone levels in domesticated males but not in undomesticated males (Table 3).

Table 3. Plasma levels of testosterone in silver fox males after introduction of females (NG/ML)

	September		January		February	
	control	Intr. of female	control	Intr. of female	control	Intr.of female
					*	*
Undomesti-cated	0.4±0.2 (6)	0.6±0.2 (6)	4.2±1.1 (6)	6.3±1.0 (6)	12.0±1.0 (6)	9.9±1.1 (6)
Domestica-ted	0.3±0.1 (19)	0.2±0.1 (19)	3.1±0.3 (19)	3.6±0.4 (19)	4.9±0.5 (19)	7.1±0.8 (19)

* Footnote as in Table 1

DISCUSSION

The prolonged period of sexual quiescence in the silver fox is characterized by very low concentrations of plasma oestradiol and progesterone in females in comparison with the breeding season, when the endocrine function of gonads rises to its highest activity and at the end of pro-oestrus the oestradiol level reaches its maximum value and the progesterone level comes to its maximum on the fifth to tenth days of pregnancy. Similar dynamics of sex hormone level in the blood in the reproductive cycle of red and blue fox were reported by Mondain-Monval et al. (1977), Bonnin et al. (1978), Møller et al. (1980) and Sirotkina et al. (1990).

It has already been noted that, being lower through most of the anoestrus, the progesterone level of tame foxes is significantly different from that of undomesticated ones. An obvious explanation might be that this difference is due to increased secretion of this hormone by the ovaries. However, *in vitro* progesterone production of ovaries during the anoestrus (Table 1) is not significantly different in the two groups. In addition to gonads, adrenals are able to synthesize and to secrete significant amounts of progesterone into general circulation in some rodents (Holzbauer & Godden 1974; Plas-Roser & Aron 1981). A comparison of the *in vitro* progesterone production by adrenals and ovaries in female silver fox shows that the adrenals may contribute substantially to the plasma progesterone pool in this species. Secondly, the progesterone production by adrenals of domesticated animals is lower than that by adrenals of undomesticated animals. It is possible that the difference in progesterone plasma level between domesticated and undomesticated females is caused by the difference in adrenal progesterone secretion.

A difference was also found between females of two behavioural types at oestrus and during the preimplantation stage of pregnancy (Fig. 2). One possible explanation for the observed difference is that tame foxes ovulate more egg cells and a larger number of corpora lutea are formed. Measurements of the number of corpora lutea in domesticated and undomesticated pregnant females confirm this supposition (Table 2).

During the breeding season, the introduction of fertile females to domesticated males resulted in a significant increase in blood testosterone, an effect not seen in undomesticated males (Table 3). It should be noted, however, that being significantly lower in the domesticated foxes the base testosterone level is different in the two groups. This difference is eliminated during sexual activation (Table 3). In males of several species in the presence of a receptive female, even when there is no direct contact, there is an activation of the pituitary-testis system, expressed as an increased level of luteinizing hormone and testosterone (Kamel et al. 1977; Johnston & Bronson 1982). These hormonal reactions are induced principally by female pheromones and their effects are mediated through the males' sense of smell (Wysocki et al. 1983). In the commercial rearing of foxes, in which animals are crowded together in a small space, the pheromone concentration from females in oestrus during the breeding season evidently reaches levels sufficient for stimulating a hormonal reaction. It is possible that the pheromone alone, with no direct contact of males with females, is enough to stimulate the males endocrine system in undomesticated males, but is insufficient for domesticated males. Thus, in the latter, direct contact stimulates an additional activation of testosterone secretion.

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Influence of exogenic Gn-RH applications on reproduction performance of standard mink females

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Once-mating systems involving ovulation induction (OI) and ovulation stimulation (OS) with single i.m. injections of 10 μg Gn-RH or 2 respective 3 μg Gonavet (D-Phe^6-LHRH) from the firm Berlin Chemie/Veyx, Germany, have been tested under commercial management conditions with regard to their action on the reproduction performances of standard mink females. The suitability of the OI and OS-sustained once-mating systems depends upon the age of the females. The best reproductive potencies were found with the OI+1 system in young females and OI+1 or 1+OS systems in old females. The reproduction performances were thereby enhanced up to or above the level of twice-mated females. The 1+OS mating systems in young females and the OI+8-9 system in young and old females were found to result in smaller litter sizes or mating rates and are therefore less suitable. A first experiment with mating ratios rising from 1 : 4 to 1 : 9 on the basis of OI-sustained once-mating, led to encouraging results with the number of born kit per male increasing to 2.6 without lowering the individual female performances. Also, the effectiveness of twice-mating systems could be elevated by exogenic Gonavet applications. Females 1+1+OS-mated showed higher litter size and number of corpora lutea than 1+1-mated females. The rate of oocyte degeneration and embryonic mortality remained unaffected by OS. In total, the emphasized once- and twice-mating systems with hormonal support are suitable to intensify the breeding process in mink. However, the scientific and ethical questions involved will have to be discussed before a general application can take place.

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In recent years different mating systems have been tested and applied in mink breeding with some success. According to results obtained by Adams (1981) and Bernatzkij et al. (1980) with exogenic gonadotropins or those obtained by Murphy (1976, 1979) and Hattenhauer & Pötschulat (1984) with gonadotropin-releasing factors, it seems viable to incorporate Gn-RH applications in mating systems with the aim of intensifying the mink breeding process.

In this connection the improvement in reproduction performance of once-mated fe-

males and the enhancement of litter size of twice-mated females by exogenic applications of Gn-RH are of some significance.

Experiments were therefore carried out to test biotechnically sustained once-mating methods with the aim of overcoming the well-known lowered reproduction performance of once-mated females in order to develop a method for increasing the mating ratio, and to test biotechnically sustained twice-mating methods enhancing the litter size of the mated females. In addition, the development of embryos from Gn-RH-treated mothers was investigated.

MATERIAL AND METHODS

With the exception of the last mentioned, all the other investigations were carried out in several mink breeding farms under practical management conditions. All the experiments were undertaken with standard mink. The number of females per group changed from experiment to experiment according to the facilities of the respective farms, and this is documented in the result tables. Gn-RH was applied as synthetic Gn-RH (Berlin Chemie/Veyx) in a single i.m. injection of 10 μg/female into the hind foot or as a single i.m. injection of 2 respective 3 μg Gonavet (Fa. Berlin Chemie/Veyx), a synthetic Gn-RH-analogue with the chemical configuration D-Phe^6-LHRH.

The experimental data were obtained as individual records of each female in each group and statistically analysed by an analysis of variance using a multiple t-test, in the case of portion figures by computing the confidence intervals.

The embryonic development investigations were undertaken at the Ambulatory and Obstetrical Clinic of the Veterinary Faculty at the University of Leipzig. The oocyte degeneration and embryonic mortality rates were ascertained by ovary and oviduct flushings of respective uterus openings from twice-mated females at different embryonic stages. The number of corpora lutea (c.l.) was counted histologically by serial cuts.

RESULTS

Biotechnical sustained once-mating methods

The results from ovulation-induced (OI) once-mated young females compared with those from untreated twice-mated young females in the control group are presented in Table 1. The females in group 2 were Gn-RH-treated on the same date the control females were mated for the first time, whose second mating date coincided with the first mating in group 2. The applied mating modus OI + 8-9 in this group led to a significantly lowered mating rate and significantly higher pregnancy rate and litter sizes compared with the twice-mated females. In another experiment the action of ovulation stimulation (OS) of once-mated young and old females was proved compared to that of twice-mated control females and young females treated with a modified ovulation induction (OI + 1). The OS-treated females received their Gn-RH injection immediately (up to 1 h) after mating, the OI-treated females about 24 h before the planned mating date.

Table 1. Results of ovulation induction (OI) of once-mated females

	Gr. 1 Control females twice-mated	Gr. 2 OI females once-mated
Females set	449	396
Mating rate (%)	100	92.6
Pregnancy rate (%)	81.7	88.6
Litter size	5.2	5.8
Kits per female set	4.2	4.7

Table 2 contains the main results. The mating and pregnancy rates between the directly comparable groups did not differ significantly from each other nor did the litter sizes, because of high variability of the latter traits in general. Nevertheless, it was found that there is a strong tendency towards higher litter sizes with the 1+OS mating system in old and the OI+1 system in young females compared to the corresponding control females, whereas the 1+OS system with young females led to a decreased litter size.

Table 2. Results of modified ovulation induction (OI) and ovulation stimulation (OS) of once-mated females

	Gr. 1 Control (1+8)		Gr. 2 OS (1+OS)		Gr. 3 OI (OI+1)
	Y[1]	O[2]	Y[1]	O[2]	Y[1]
Females set	204	204	183	184	40
1st matingrate (%)	93.4	100	94.35	97.9	95.0
Pregnancy rate (%)	87.6	86.7	79.2	87.9	84.2
Pregnancy duration (d)	45.6	44.5	48.7	47.9	47.8
Litter size	5.4	5.4	4.9	6.1	5.9
Litter size by weaning	4.9	4.7	4.3	5.3	5.2

[1] = females in the 1st breeding period
[2] = females in the 2nd breeding period

Biotechnical sustained once mating with increased mating ratio.

Since the final target of once-mating systems is virtually to increase the mating ratio from the usual 1:4.5 to at least more than double that rate, we planned a production experiment comparing the results of a usual 1 : 4 mating ratio with an increased 1 : 9 ratio using OI supported once mating.

The main results are listed in Table 3. Whereas the mating and pregnancy rates did not differ significantly between the groups, both litter size and number of kit per male were markedly enhanced in the OI supported, once-mating and increased mating ratio. In this group the average number of kit number per male surpassed the mean value in the twice-mated group with the usual mating ratio by 2.6.

Table 3. Results of OI-supported once-mating with increased mating ratio

	Control twice-mating usual ratio	OI+8 once-mating increased ratio
Females set	410	482
Mating ratio	1 : 4	1 : 9
Mating rate (%)	99.5	98.3
Pregnancy rate (%)	87.0	82.5
Litter size	5.3	6.4
Kits per female set	4.6	5.2
Kits per male	17.7	46.9

Gn-RH application in twice mating systems

With regard to intensifying the breeding process the question arose as to what extent Gn-RH applications can stabilize or improve the reproductive capacity of twice-mated females. So the effects of two different OS mating systems, with in each case 2 μg/female Gonavet (D-Phe^6-LHRH), on the reproduction performance of such females have been tested.

Table 4 gives the most important results. Apart from the second mating in groups 3 and 4, the mating ratios between corresponding groups did not differ significantly. Compared to the old females in all the other groups, the females in group 3 exhibited a significantly higher pregnancy rate and an insignificantly improved litter size. The mating and pregnancy rates as well as the litter size of young females in the control and 1+8+OS groups did not differ significantly. Whereas the OS system 1+1+OS revealed a tendency towards higher pregnancy rates and litter sizes, the OS system 1+8+OS decreased these performance traits in the case of old females. Moreover, all old females mated to the 1+8 systems (groups 1 and 2) displayed lower pregnancy rates and litter sizes than the females mated to the 1+1 systems (groups 3 and 4).

Table 4. Effects of ovulation stimulation with Gonavet upon the reproduction performance of twice-mated females

	Gr. 1 Control (1+8)		Gr. 2 OS (1+8+OS)		Gr. 3 OS (1+1+OS)	Gr. 4 Control (1+1)
	Y[1]	O[2]	Y[1]	O[2]	O[2]	O[2]
Females set	211	57	210	51	50	50
1st mating rate (%)	92.9	100	93.3	96.1	100	100
2nd mating rate (%)	63.8	56.1	64.3	53.1	100	90
Pregnancy rate (%)	83.7	80.7	84.7	85.7	98.0	90
Pregnancy duration (d)	45.1	45.6	44.8	45.1	45.7	46.8
Litter size	3.9	4.5	4.1	4.3	5.2	4.8

[1] = females in the 1st breeding period

[2] = females in the 2nd breeding period

Influence of Gn-RH on embryonic development

In supplemental experiments the actions of exogenic Gn-RH applications on embryonic development have been investigated. The number of c.l. formed, the degeneration rate of oocytes and the embryonic mortality rates during several development stages served as development criteria. The investigations were conducted with 16 twice-mated 1 + 1 untreated or with 3 μg/female Gonavet ovulation stimulated (1 + 1 + OS) young females for estimation of c.l. formation and with a total of 46 females for embryonic mortality estimations in both groups. The detailed results are published elsewhere.

A slightly higher number of c.l., a slightly lower pregnancy rate and duration and a slightly higher litter size by the OS females are recorded in Table 5, but all differences are insignificant according to the Mann Whitney N-test.

Table 6 also reveals small and insignificant differences in oocyte degeneration rates, embryonic degeneration rates during the different developmental stages and in the total between the females of the control and OS groups.

Table 5. Number of c.l., pregnancy rate and duration and litter size of twice-mated OS females

		Untreated controls	OS females
Number of c.l.	mean	15.0	16.8
	+ s	5.48	4.06
Pregnancy rate (%)		89	85
Pregnancy duration (d)	mean	47.0	46.4
	+ s	2.0	1.8
Litter size	mean	4.92	5.18
	+ s	2.41	2.36

Table 6. Degeneration rate of the oocytes and embryonic mortality of twice-mated OS females

		Untreated control	OS females
Degeneration rates (%)			
Pregnancy days	5-11	38.5	42.4
	12-16	31.3	22.6
	17-19	-	-
Embryonic mortality rates (%)			
preimplantive phase		57.3	55.4
postimplantive phase		23.4	30.7
Embryonic mortality rates in total (%)		67.3	69.0

DISCUSSION

The submitted results indicate that the well-known negative effects of once-mating on the

reproduction performance of females may be overcome by applying OI or OS to once-mated females. The OI+8-9 system increased litter size up to the level of and above the level of twice-mated females, but seems to depress mating rates to some extent (Table 1). Although this does not necessaryly lower the total number of kits born in a population, because in most cases this depressing effect will be compensated or overcompensated by increased litter sizes and/or pregnancy rates in the OI population, our results suggest that the OI+8-9 system is not to be recommended.

The OI+1 mating system applied to young females avoided mating rate depression and improved litter size in a similar manner to that of forementioned system (Table 2). Since the 1+OS system led to lowered litter size in young females (Table 2), the OI+1 system therefore represents the preferred once-mating system applied to young females.

In contrast to these results, both mating systems applied to once-mated old females gave almost the same good results with regard to mating or pregnancy rates and litter size (Table 2). Consequently, 1+OS and OI+1 are favourable mating systems for once-mated old females.

Because the said biotechnically supported once-mating systems equalize the performance deficits of once-mated females, they implicate their application for increasing mating ratios. As shown in Table 3, an increase in of the ratio from 1:4 to 1:9 was possible by OI-supported once-mating without any decrease in individual performance records. The markedly increased number of kits born per male in this group is reflected in the breeding possibilities involved in increased mating ratios with regard to the effective utilization of top breeder males.

The fact that also twice-mating systems can be more effective with Gn-RH support reveals the strong tendency to higher litter sizes in the 1+1+OS mating group (Table 4). The effectiveness of exogenic Gn-RH or one of its analogues with regard to the higher reproductive capacity of females is, after all, confirmed by the rise in the number of c.l. in the 1+1+OS mating system (Table 5), although the Gonavet injection took place only a few hours before ovulation occured. In this connection it should also be noted that the oocyte degeneration rates and the mortality rate were not enhanced by exogenic Gn-RH applications (Table 5).

Finally, it can be concluded that the emphasized once- and twice-mating systems are acceptable for intensifying the breeding process in mink. But, besides scientific problems, there are also ethical questions involved which have to be addressed before a general application can take place.

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Collection of ungelatinizable semen from nutria (*Myocastor coypus*)

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Studies on the semen collection by the EE method were conducted using: (a) deep anaesthesia (halothane or ether) or (b) premedication, using, initially, Rompum (Rometar 2% Leciva Praha) + Atropine sulphate, Combelen (Biowet) + Atropine sulphate and, later, Combelen alone, which gave the same effect. Combelen was given intramuscularly at a dose of 1-2 ml depending on body weight. Both methods provided stimulation of males with an electric current of 15-25 mA and a constant voltage of 4 mV. The semen obtained from all males subjected to deep anaesthesia (halotane, ether) became gelatinized after a few seconds, whereas the semen obtained from the males subjected only to premedication (Combelen) did not gelatinize even after 24-48 h storage at room temperature and at +4°C. The ability to collect ungelatinizable semen makes its direct assessment and utilization of AI possible.

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In most species of farm animals the methods for collecting semen and its assessment are worked out and utilized on a large scale in breeding practice. Little consideration has been given to nutria semen sampling. From the literature records it follows that, so far, only Jakubicka & Barta in Czechoslovakia and Helleman et al. in Chile have undertaken trials on the collection of semen from nutria males. However, the methods elaborated by these authors cannot be used for testing the reproductive performance of males since the semen obtained in this way became gelatinized.

The aim of this study was to work out a method for collecting ungelatinizable liquid semen.

MATERIALS AND METHODS

An experiment was carried out on 24 one-year-old and older male nutria of Greenland and standard varieties. The animals were kept individually under the usual management systems: in cages without bathing facilities or in boxes with access to bathing water.

Trials with semen collection were carried out in the periods of maximum sexual activity (autumn, spring).

Before the trials were carried out, the experimental males were placed in a specially constructed cage, which prevented the nutria from moving around, thus reducing to a minimum the possibility of injury or lesions. An additional advantage of this type of cage was the easy access to the animals' perineum and anus.

The experiment, the aim of which was to find a method for sampling semen from male nutria, was conducted in four stages differing in application of pharmacological anaesthetic and analgetic agents. The first trials were performed on animals subjected to ether anaesthesia. The ether was inhaled by the animals through a mask. The next trials were conducted on the nutria subjected to premedication using Rompun (Rometar 2% Leciva Praha) and Atropine (Injectio Atropini sulfus 0.6% Biowet Gorzow) at doses of 2 mg/kg b.w. and 0.05 mg/kg b.w., respectively. In the second stage, Rompun was replaced by the neurolept commonly used in veterinary practice - Combelen (Biowet Pulawy) - at a dose of 0.5 mg/kg b.w., which was administered in combination with atropine (0.5 mg/kg b.w.). In the third experiment, in order to eliminate any parasympaticolytic effect of atropine, Combelen was given alone.

About 10-15 minutes after medication was administered, the rectum was evacuated with a 3% NaCl solution and the penis, after being removed from the prepuce, was rinsed with a physiologic salt solution heated up to body temperature.

At all stages of the experiment semen was collected by the electroejaculation method (EE) i.e., applying electric current stimulation, using an electroejaculator of own production equipped with a bipolar electrode, comprising a handle and an electrode probe 12.8 mm long and 7.0 mm wide.

The electrode was inserted into the anus and electroejaculation was performed using electric current impulses of 14 mV voltage, 25 mA intensity and 50 Hz frequency. The duration of the impulses was 5 sec with 3-sec intervals. During electroejaculation the electrode was manipulated so as to adhere to the sacral section of the spine where the centres of erection and ejaculation are located.

Semen was collected in a glass container heated by a water jacket at 38°C. Directly after sampling, the semen was subjected to macro- and microscopic evaluation concerning colour, consistency, smell, pH, colume, motility and viability of spermatozoa, their concentration and morphology, according to the method described in an earlier paper (Jarosz & Szeleszczuk 1991).

RESULTS

Using an ether anaesthesia always resulted in the semen collected from the male nutria being of mucous consistency and becoming instantly gelatinized, forming a compact, elastic plug, which assumed the shape of the vessel, into which it was collected. The semen gelatinization made it impossible to perform a proper estimation of the semen, as well as its dilution and preparation of insemination.

On the other hand, all the ejaculates obtained from the males subjected to analgesia (premedication), irrespective of the pharmacological agent used, showed no trace of gelatinization, even after being stored at room temperature or 0°C for 24-48 h (Table 1). Per 38 trials of electroejaculation, 31 ejaculates were obtained, accounting for 81%.

Table 1. Results of collection of semen from nutria males

Lp.	Colour	Cons.	Smell	pH	Vol.	Motility	Density	Current	Time
ROMPUN		(XYLAZIN)	+	ATROPINE		SULPHATE			
1	W	M	S	6.0	1.0	80/+++	D	17	2
2	W	M	S	6.0	1.0	80/+++	D	13	3
3	W	M	S	6.0	1.3	80/+++	D	15	3
COMBELEN + ATROPINE SULPHATE									
4	-	-	-	-	-	-	-	10	10
5	-	-	-	-	-	-	-	12	10
6	W	W	S	7.0	2.0	20/++	R	13	13
7	-	-	-	-	-	-	-	15	15
8	W	M	S	7.0	1.0	80/+++	D	13	5
9	W	M	S	7.0	1.2	80/+++	D	15	5
COMBELEN									
10	W	W	S	6.8	2.0	80/+++	D	17	5
11	W	M	S	6.4	0.7	70/++	D	15	5
12	W	M	S	6.4	1.0	80/+++	D	10	5
13	W	W	S	7.0	0.4	30/++	R	17	7
14	W	W	S	7.0	0.1	10/+	R	18	7
15	W	M	S	6.6	0.1	90/+++	DD	10	5
16	Y	W	S	6.4	1.2	-	-	17	10
17	Y	W	MS	6.0	3.2	-	-	18	10
18	W	M	S	6.4	1.0	60/+++	D	10	7
19	W	M	S	6.0	1.1	60/++	D	10	8
20	W	WM	S	6.0	2.3	40/++	D	14	8
21	W	WM	S	6.4	1.0	-	-	17	8
22	W	M	MS	6.0	1.5	10/+	R	15	9
23	W	M	S	6.4	1.5	60/+++	D	13	9
24	W	M	S	6.0	1.4	30/+	D	12	7
25	-	-	-	-	-	-	-	18	10
26	W	W	S	6.4	2.5	-	-	18	15
27	Y	W	S	6.0	5.0	70/+++	R	11	8
28	W	W	S	6.0	7.0	80/+++	SD	15	7
29	W	M	S	6.0	3.0	30/+	SD	17	6
30	-	-	-	-	-	-	-	17	15
31	Y	M	S	7.0	2.8	80/+++	D	13	7
32	W	WM	S	6.0	1.5	80/+++	DD	12	8
33	UN	W	S	6.4	0.5	-	-	18	15
34	W	W	S	6.4	3.5	-	-	18	15
35	W	W	S	6.8	6.0	40/+	R	12	10
ETHER									
36	W	G	S	6.5	2.2	GELATINOUS	SEMEN		
37	W	G	S	6.5	2.8	GELATINOUS	SEMEN		
38	W	G	S	6.8	1.0	GELATINOUS	SEMEN		
39	W	G	S	6.8	3.5	GELATINOUS	SEMEN		
40	W	G	S	6.8	2.0	GELATINOUS	SEMEN		
41	W	G	S	6.8	0.8	GELATINOUS	SEMEN		
42	W	G	S	6.8	0.5	GELATINOUS	SEMEN		
43	UC	G	S	6.8	1.5	GELATINOUS	SEMEN		
44	UC	G	S	6.8	0.5	GELATINOUS	SEMEN		

W- White, M - Milky, W - Watery, S - Specific, G - Gelatinous

Semen was secreted in droplets in accordance with the rhythm of electric current stimulation and copulatory movements. During ejaculation, semen was secreted in three fractions (separation was not always possible), the first being colourless, the second milky but becoming colourless again at the end of the process. Fraction I, which was also obtained on partial stimulation of males (EE), did not contain spermatozoa. Fraction II was the proper one with spermatozoa, its volume being in the range from 0.1 to 1.1. cm^3. The

last fraction contained only a few spermatozoa, being a secretion from the accessory sexual glands.

Mean volume of the semen collected by means of these methods was 1.80 cm^3, ranging from 0.1 to 2.3, and even to 7.0 cm^3 (Table 2).

Table 2. Volume, motility, pH and concentration of spermatozoa in semen from nutria subjects to analgesia (Combelen and Rompun)

Specification	Mean	Variance	Standard deviation
Sperm volume (cm^3)	1.8051	2.297	0.7277
Motility (%)	59.1667	5.2446	25.6933
pH	6.4346	0.0764	0.3839
Concentration thous./mm	4.033	----	3.9516

A complex evaluation of the collected semen is presented in Tables 1-2 and in an earlier paper (Jarosz & Szeleszczuk 1991).

DISCUSSION

The reproductive tract of the nutria male compared with that of other fur animal males is of a slightly different structure (Lutnicki 1960), with particularly well developed accessory, bulbo-urethral, vesicular prostate and coagulative glands. As in other rodents (rat, mice, golden hamster, chinchilla, their discharge, directly after copulation, results in the formation of a vaginal plug, which makes an outflow of semen from the female reproductive tract impossible (Ewy, 1989). The formation of the vaginal plug is caused, according to Barta & Jakubicka (1983), by the presence of the coagulative gland, situated near the prostatic gland. While, according to Hafez (1970) the vaginal plug is formed from the coagulated masses of the vesical and prostatic gland secretions.

The formation of the vaginal plug made it difficult to obtain semen from nutria males. Combelen, applied prior to electroejaculation for analgesia in animals, inhibited semen coagulation in a manner not fully elucidated.

Because of a strong defence reaction and lack of erection, Helleman 1987 gave up collecting semen by the electroejaculation method. Whereas, based on the studies by Jakubicka & Barta (1984), only EE appeared to be an effective method of collecting semen from nutria males. However, here too, it was found that after only a relatively short time the semen started to coagulate.

The main differences between the method of semen collection used by the authors and that by Jakubicka and Barta were found in the application of different techniques of psychic tranquilization and in decreasing the reaction to pain.

Prior to EE, Jakubicka and Barta applied deep narcosis through an inhalation of a mixture of halothane and oxygen. Halothane (CF CHBrCl) as well as diethyl ether (ether pro narcosi) are widely used narcotics in surgery. Like all inhaled narcotics, they act on

cortical centres and after their application on animals, loss of consciousness (hypnosis) occurs as well as analgesia, areflexia and myorelaxatio.

In contrast, propionylpromazine (Combelen) exhibits an inhibitory action on subcortical centres, as a result of which only weak stimulating impulses are sent from those centres to the cortex. Thus it has only a tranquillizing effect, alleviating stress and aggression and relieving anxiety.

According to Garbulinski (1984), neurolept from a group of phenothiazine, to which Combelen belongs, inhibit the effect of noradrenaline, dopamine and serotonin in the central nervous system. And, as is known, these amines are neuromediators in the brain stem and particularly in the reticular system, thalamus and hypothalamus. A decrease is observed, especially at higher doses of phenothiazine, in the neurosecretory function of the hypothalamus followed by a decrease, according to this author, in the release of adrenal hormones, among others of FSH, LH and oxytocine. According to Podlewski & Chwalibogorska-Podlewska (1987), with higher doses of propionylpromazine even disturbances in the endocrine secretion can occur, resulting in disturbed menstruation.

However, it is possible that the inhibition of coagulation in nutria males was caused by hormonal disturbances, but could have resulted from a peripheral effect of Combelen, which, according to Trusiewicz et al. (1991), manifests itself by a spasmolytic action - relaxation of smooth muscles of which the sexual accessory glands are composed.

The ejaculates obtained through pharmacological blocking did not coagulate even after 24-48 h, which is of great significance for semen estimation and conservation and provides the possibility of its utilization in insemination of nutria females.

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Mouse monoclonal antibodies detect epitopes of immunoglobulin γ-, κ-, and λ-chains common to several fur animal species

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Diagnostics and investigation of diseases in fur animals calls for highly specific and standardized reagents to the Ig molecules of the various isotypes. A panel of 26 monoclonal antibodies specific to mink IgG was produced. Eleven antibodies reacted with the Fc-fragment of γ-heavy chains, 4 with conformational epitopes of Fab-fragment, 3 with λ-light chains and 4 with κ-light chains. In a double immunodiffusion, 23 of the 26 antibodies formed precipitin lines in the presence of polyethylene glycol. Antibodies against κ- and λ-chains, and a part of those against γ-chains cross reacted with IgG of other Mustelidae species as well as some other fur animal species remote from mink (fox, arctic fox).

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The study of the fur animal immune system is of great importance for better protection of populations against infectious agents. Diagnostics and investigation of diseases in these species call for highly specific and standardized reagents to the Ig molecules of the various isotypes. Monoclonal antibodies (mAbs) are instrumental in this respect because there are no limitations to their large-scale production, and they are more amenable to standardization than polyclonal antibodies.

In this paper we present a characterization of a panel of 26 mAbs reacting at high titers with the γ- heavy (H), λ- and κ-light (L) chains of mink IgG. Some of the mAbs recognize the epitopes present on the IgG molecules of other mammalian species (dog, horse, fox, arctic fox).

MATERIALS AND METHODS

BALB/c mice were immunized with mink IgG and spleen cells were fused with myeloma NS0/1 by the standard method (Kipps & Herzenberg 1986). Antibodies in the hybridoma supernatants were identified by ELISA in 96-well microtiter plates coated with mink IgG. Cloning was performed by limiting dilutions and hybridomas were grown as ascitic tumors in pristine-primed BALB/c mice.

mAbs were purified from ascitic fluids by affinity chromatography on Sepharose CL-4B coupled with mink IgG. Antibodies were biotinylated with N-hydroxysuccinimidobiotin.

IgG subpopulations carrying the putative isotypes of L chains (L1 and L2) were separated using mAbs G80 or G88 respectively coupled to Sepharose CL-4B. Content of L-chain subpopulations in mink sera was determined by ELISA using L1 and L2 as control samples.

Lymphocytes producing L1 or L2 isotypes were separated using Petri dishes precoated with the mAbs G80 or G88. The cell proportions were calculated after immunochemical staining by biotin-labeled mAbs.

Immunodiffusion assay (IDA) was performed in 1.2% agar gel prepared on PBS containing 3% PEG 6000. SDS-PAGE was performed according to Laemli (1970). Electrophoretic transfer to nitrocellulose was carried out following the procedure by Towbin & Gordon (1984). The nitrocellulose strips were incubated with ascitic fluids and then with peroxidase conjugated rabbit anti-mouse IgG.

Northern blot assay was performed according to Maniatis et al. (1983). A fragment derived from the mouse pSV2-gpt clone kindly provided by Dr H.G.Zachau (Institute of Physiologist Chemistry, Munich) was used as a C_{κ} probe. An 0.4 kb PstI-HindIII fragment derived from the mink pIGL2 clone was used as a Cλ probe (Nayakshin et al. 1990).

RESULTS AND DISCUSSION

We selected 26 clones specific to the isotypic determinants of γ-and L-chains of mink IgG. In IDA in the presence of 3% PEG, 23 of the 26 mAbs formed precipitin lines of different intensity with mink IgG (Fig.1).

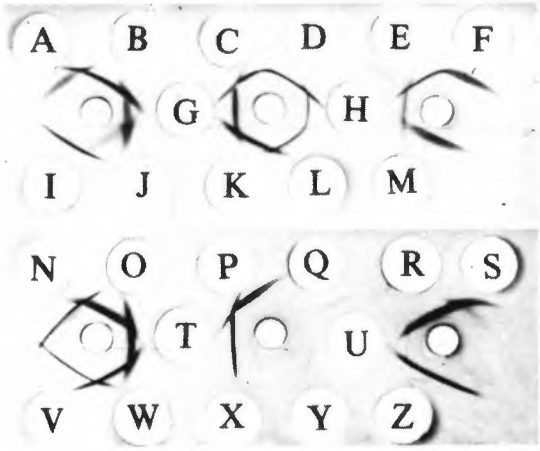

Fig. 1. Immunodiffusion tests. mAbs: G32 (A), G34 (B), G37 (C), G44 (D), G47 (E), G50 (F), G77 (G), G79 (H), G80 (I), G82 (J), G86 (K), G88 (L), G92 (M), G95 (N), G100 (O), G103 (P), G105 (Q), G107 (R), G113 (S), G114 (T), G116 (U), G120 (V), G121 (W), G122 (X), G123 (Y), G126 (Z). Each small inner well contains pooled mink IgG

Based on the patterns of precipitin lines and the inhibition tests in ELISA, the precipitating mAbs were divided into 10 groups tentatively referred to as "specificities". Within each mABs group recognized the same or overlapping epitopes, and mAbs from different groups recognized different epitopes.

The results of immunoblotting of reduced mink IgG (Fig. 2A) demonstrated that only mAbs of "specificity" 1 reacted with the reduced γ-chains and those of "specificities" 4, 6, 7 reacted with the L-chains. The mAbs of "specificities" 4 and 6 gave reactions with the L-chains differing somewhat in molecular weights.

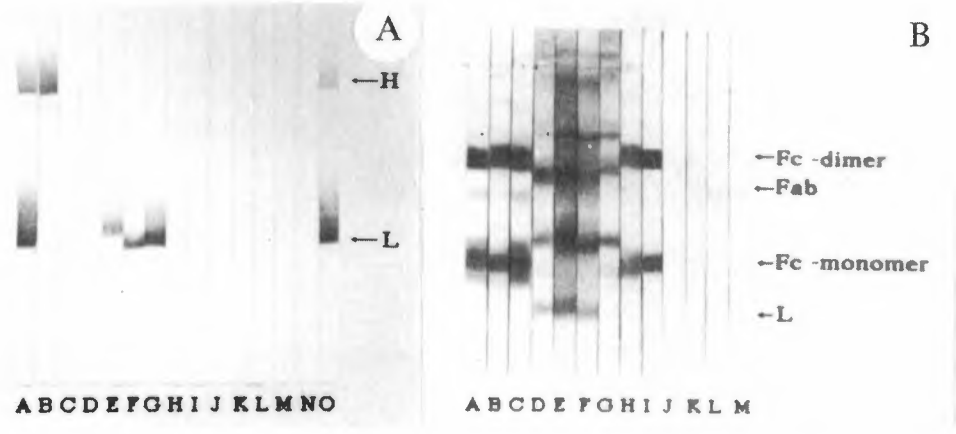

Fig. 2 A and B. Immunoblotting assay of mAbs. A. The mAbs used to develop the strips were G44 (B), G47 (C), G50 (D), G80 (E), G88 (F), G116 (G), G77 (H), G86 (I), G113 (J), G114 (K), G121 (L), G122 (M), G123 (N). Strips A and O were developed with the use of a mixture of all the tested antibodies. Arrows indicate the positions of the immunoglobulin H- and L-chains. B. The mAbs used to develop the strips were G44 (A), G47 (B), G50 (C), G80 (D), G88 (E), G116 (F), G77 (G), G86 (H), G114 (I), G105 (J), G113 (K), G122 (L), G123 (M). Arrows indicate the position of the protein bands used to determine the antibody specificities. The bands represent the following fragments: Fab, Fc-dimer, Fc-monomer, L-chain (Taranin 1988)

Immunoblotting of the non-reduced papain digest of mink IgG revealed clear-cut reactions of the precipitating mAbs with IgG fragments which have retained intra- and interchain disulfide bonds (Fig. 2B). It was found that mAbs of "specificities" 1, 2, 5 and 8 were directed against the epitopes of the Fc fragment of the γ-chain. The mAbs of "specificities" 3 and 9 reacted only with the Fab fragment, i.e. recognized the conformational determinants formed by both the γ-and L-chains. The mAbs of "specificities" 4, 6, and 7 reacted with all the fragments comprising the L-chain. Non-precipitating mAbs, as well as mAb G113 ("specificity" 10), gave no reaction with fragments of IgG in the immunoblotting assay.

By means of mAbs G80 and G88 coupled to Sepharose CL-4B we succeeded in isolating two IgG fractions slightly differing in molecular weights of their L-chains. These subpopulation variants will henceforth be referred to as L1 (bound by G80) and L2 (bound

by G88). It was found that L1 was precipitated by antiserum against human λ-chains, and L2 by antiserum against human κ-chains (Fig.3).

Fig. 3. Immunodiffusion assay of L1 (A) and L2 (B) fractions of mink IgG (C) with rabbit antisera against human κ- (D) and λ- (E) light chains, pooled human serum (F), pooled mink serum (G)

We measured the concentrations of isotypes L1 and L2 in the sera of 12 healthy mink. The average L1:L2 ratio was approximately 1.2:1, i.e., 54:46%. Almost the same proportion of L1+ and L2+ producing cells was determined by immunocytochemical analysis in mesenteric lymph nodes.

Table 1. Reactivity of anti-mink IgG mAbs with the IgG of some mammalian species

	mAbs[1]							
	44[2]	47	95	88	86	80	114	121
Mustelidae								
Marbled polecat (*Vormela peregusna*)	+[3]	+	+	+	+	+	+	+
Pine marten (*Martes martes*)	+	-	-	+	-	±	-	+
European mink (*Mustela lutreola*)	+	+	+	+	+	+	+	+
Stoat (*Mustela erminea*)	+	+	+	+	+	+	+	+
Sable (*Martes zibellina*)	+	+	±	+	-	+	+	+
Otter (*Lutra lutra*)	+	+	+	+	-	+	+	+
Polecat (*Mustela putorius*)	±	+	+	+	+	+	+	+
Sea otter (*Enhydra lutris*)	+	+	+	+	-	+	+	-
Non-Mustelidae								
Dog	+	+	-	±	-	±	-	+
Fox	+	±	+	±	-	+	-	±
Arctic fox	+	+	+	±	-	+	-	±
Horse	+	+	-	-	-	-	-	-
Pig	-	+	-	-	-	-	-	-
Rabbit	-	-	-	-	-	-	+	-

[1] Ascites were used in this assay

[2] 44 means mAb G44, 47 means mAb G47, 95 means mAb G95, etc.

[3] + indicates a sharp precipitin line, ± indicates a blurred precipitin line, - indicates no visible precipitin line

In view of the previous reports suggesting that mink IgG L chains are represented exclusively by λ-type (Hood et al. 1967), further support was needed for our finding that κ-chains compose about half the L chains of mink IgG. To confirm these data, Northern blot hybridization of Cλ and Cκ probes to total RNA from L1+ and L2+ cells was performed after their partial enrichment with the use of mAbs G80 and G88. Judging by the intensity of the hybridization signals, it is clear that the relative enrichment of a protein type (L1 or L2) proceeds in parallel with that in an mRNA type (λ or κ) (data not shown). Thus, the L1 isotype is the L-chain of λ-type and L2 -is of κ-type.

Finally, it was found that some mAbs formed sharp precipitin lines with the IgG of taxonomically distant species (Table 1.). These mAbs may serve as Ig-specific reagents for diagnostics of infectious disease and study of immune response of fur animal species.

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Hypothalamic immunocytochemical localization of proGnRH and GnRH and pituitary and testicular pathology in type I and type II, primary infertile, dark male mink

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Attempts have been made to ascertain the cause of primary male infertility using immunocytochemical localization of proGnRH and GnRH, *in situ* hybridization of mRNA for GnRH and pituitary and testicular histopathology. ProGnRH and GnRH immunoreactive neurons were observed in the preoptic basal hypothalamic to the caudal basal hypothalamic areas. Adjacent sections through the preoptic basal hypothalamus, when reacted with proGnRH and GnRH antisera, showed the same distribution indicating that the same cell reacted for both antisera. Two forms of infertility were observed: Type I had an apparently normal hypothalamic function, hypoplastic testes and microcytic degeneration of the pituitaries while Type II had degenerative testicular lesions and normal pituitaries, but insufficient GnRH secretion due either to a lack of stimulation or excessive inhibition of GnRH secretion.

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Dark or standard mink have resulted from intensive inbreeding for numerous but different genes that increase pigmentation in an additive manner (Cochrane & Shackelford, 1991). Over the last 15 years, the amount of melanin in the pelage has increased resulting in a more intense dark color with pigment now extending further down the hair shaft to just above the bulb region (Ellis, unpublished observations). Breeding mink for highly desirable traits also co-selects male primary infertility that affects 20-30% of breeding males (Tung et al. 1981, 1984), since the genes for reproduction, productivity, coat color and fur characteristics are all located on the same chromosome near the centromere (Ellis & Pace 1987). These animals lack luteinizing hormone (LH) and testosterone secretion, exhibit testicular hypoplasia, and respond non-uniformly to either exogenous hCG or GnRH therapy (Tung et al. 1984; Ellis & Pace 1987). To date, however, the etiopathogenesis of this condition in mink remains unclear.

The purpose of this investigation was to: (1) ascertain if any differences exist in the proGnRH and GnRH containing neurons in the preoptic (POA) and basal hypothalamus (BH) between fertile and infertile dark male mink, (2) use radioimmunoassays (RIA) to detect any changes in immunoreactive levels of proGnRH and GnRH in the POA and BH, and (3) evaluate the pituitary-testicular axis of infertile dark males for hypothalamic and gonadal morphologic abnormalities. In doing so, two distinct forms of primary infertility have been elucidated.

MATERIALS AND METHODS

All mink used in this study were obtained from the Utah Agricultural Fur Breeders' Co-operative from mid-February to March, the time of peak testicular development and fertility. It was their first breeding season, they were clinically healthy and had reached normal adult body proportions. Fertility status was assessed by testicular palpation and attempted or interrupted matings with a receptive female and by vaginal smears for sperm evaluation. Only males with small testicular size (1-5 on a scale of 10) would not breed or deposit sperm or deposited poorly motile or immotile sperm (score of 2 on a performance scale of 4 max.). Fertile animals had testicular scores of 7-10 and sperm performance scores of 4-5. All animals were maintained under natural photoperiod and given water and feed *ad libitum*.

While under deep sodium pentobarbital anesthesia, mink were perfused intracardially with phosphate buffered saline (pH 7.2). The fixed brains, pituitary glands and testes were immediately removed, weighed and processed for the evaluations described below. The mean total thickness of the germinal epithelium and the mean diameter of seminiferous tubules were determined after 50 such measurements on each animal's testes with a Zeiss IBAS 2000 image analyzer coupled with an Axioplan research microscope and IBM host computer. Morphometery was performed on a video-captured image processed up to the level of statistical graphing.

Immunocytochemical localization of proGnRH and GnRH was accomplished as described elsewhere (Ronnekleiv et al. 1989) for control and Type II infertile males utilizing specific antibodies against these two entities (ARK-2, 1:3000 & El-14,1:10,000 dilution, respectively). For tissue extraction and measurement of proGnRH and GnRH by RIA, brains were rapidly removed from the skulls of additional animals and dissected on ice to obtain the neural tissue surrounding the POA and BH. RIA of these two compounds was carried out as described elsewhere (Kelly et al. 1989).

RESULTS

Control animals had mean testicular weights of 7.03 $\pm$ 0.30 g, reproductive scores of 4+ and normal-appearing pituitary glands and testes. Type I infertile males had small testes (0.9 $\pm$ 0.29 g), zero reproductive scores, and abnormal pituitaries that displayed a cribriform pattern of optically clear spaces partitioned by thin fibrous interlocking septa, severe reduction in numbers of glandular epithelial cells, and hypoplastic testes (Fig. 1).

Type II infertile males had medium-sized testes (3.53 ± 0.54 g), a reproductive score of 1+, normal pituitary glands, but degenerative testes with more prominent tubular lumens than those of the controls and Type I infertile males, but with three to four times more giant spermatid giant cells and extensive spermatogonial cytoplasmic vacuolization, nuclear pyknosis of germinal cells (Fig. 1). ProGnRH and GnRH containing neurons and fibers were well defined and clearly visualized in the POA-BH in all three groups of animals. Sequential immunostaining of the same sections for proGnRH and GnRH with a double labeling technique showed that both the precursor and processed decapeptide coexisted in the same cell bodies.

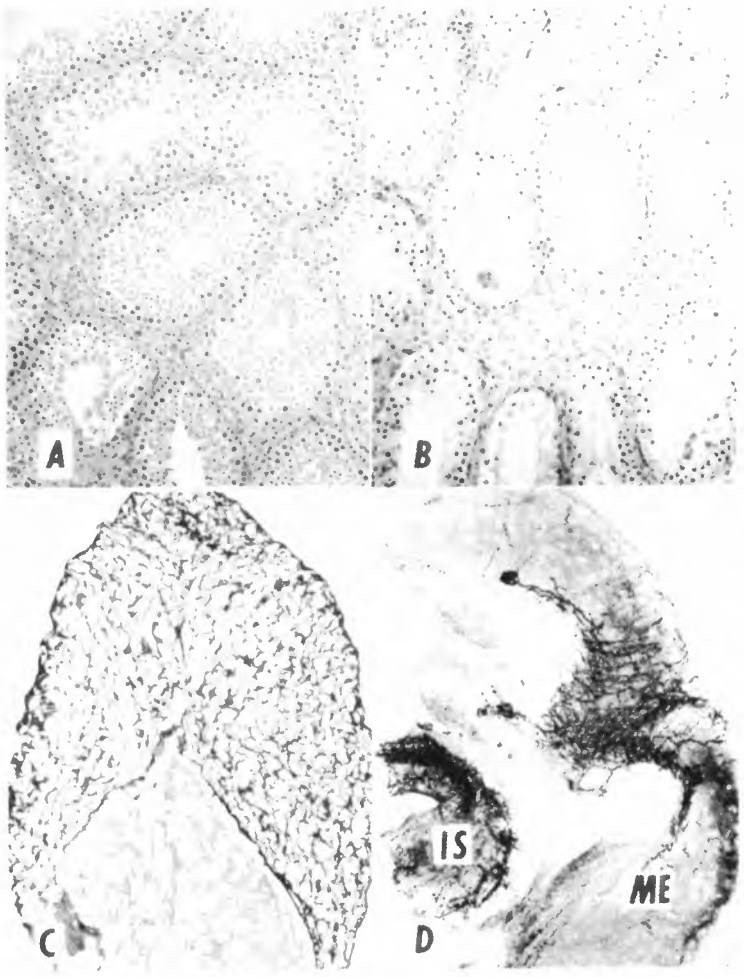

Fig. 1. Light micrographs of: A)normal testes; B) hypoplastic testes of Type II infertility showing prominent tubular lumens and thinned disorganized germinal epithelium with variable cytoplasmic, condensed nuclear chromatin and spermatid giant cells; C) pituitary gland of Type I infertility showing large cystic spaces lined by thin septal walls uniformly distributed throughout the anterior, intermediate and posterior lobes; and D) hypothalamic median eminence (ME) and infundibular stem (IS) illustrating immunoreactive GnRH neurons

There were no significant differences in the immunoreactive levels of proGnRH and GnRH in the POA and the BH between the controls and Type II infertile males. Higher levels of immunoreactive GnRH were, however, observed in the BH than in the POA for the control, compared to the infertile animals.

DISCUSSION

The morphologic lesions of the pituitary glands of Type I infertile mink with numerous prominent cystic spaces not lined with endothelial cells are suggestive of edema and cellular death resulting from spontaneous autocytotoxicity. In man, pituitary hypofunction does not occur until at least 75% of the anterior lobe is destroyed and then tropic deficiencies supervene in the following temporal order: gonadotropins, GH, TSH, and, lastly ACTH (Robins et al. 1986). The hypoplastic testes of this group are similar to those of sexually immature mink and are, in all probability, induced by the lack of gonadotropin stimulation. Similarly, the lesions of the testes of Type II infertility appear to be due to spontaneous autotoxicity, as is also the malfunctioning of the hypothalamus in this group of animals.

Infertile animals with severe testicular lesions would respond poorly to exogenous gonadotropins, whereas those with lesser lesions would respond much better, as we have previously reported. Those with the more severe pituitary lesions would respond very poorly to GnRH.

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Blood protein polymorphism in polish foxes

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Madeyska-Lewandowska, A., C. Magnac & T. Zdunkiewicz 1992. Blood protein polymorphism in polish foxes. Norwegian Journal of Agricultural Sciences. Suppl. no. 9: 190-195. ISSN 0801-5341.

We have studied the polymorphism of prealbumin, α_1B-glycoprotein, transferrin, two region of postalbumin, two system of activity the protease inhibitor and four region of pretransferrin - using the method PAGE and 2D-PAGE. These results suggest that the proteins polymorphism could be used for genetic control in fox families. These examinations offer high possibilities of conforming or excluding fatherhood which may improve the results of breeding.

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An investigation into the protein polymorphism in the blood serum of foxes was conducted on two species: *Vulpes vulpes* and *Alopex lagopus* using the method of one- or two-dimensional electrophoresis on polyacrylamide. Besides the proteins already described in the literature (prealbumins, Pr; transferrins, Tf; vitamin D binding proteins, Gc; α_1B-glycoproteins, α_1B; α_1-protease inhibitors, Pi1 and Pi2; postalbumins Pa1; pretransferrins, Prt1) new polymorphic systems were indentified: prealbumin Pr2 and pretransferrins Prt2, Prt3 and Prt4.

The two fox species belong to the dog family and are carnivorous digitigrades that inhabit all continents.

The first works on the polymorphism of blood proteins in foxes were published by Kaminski & Balbierz in 1965. The authors differentiated the proteins by means of electrophoresis on starch gel and the polymorphic forms observed were arranged into two systemes.

In 1966 Kaminski et al. identified eight different transferrin genotypes. Comprising four to five zones of varying staining intensity. The same authors demonstrated the presence of natural antibodies in the blood serum of foxes for both agglutinin and haemolysin.

Balbierz et al. (1977), demonstrated that it was possible to use the transferrin polymorphism for parentage determination in polar foxes. For the determination of Tf types, matings were conducted among three animals (1 male and 2 female) with a different

Tf genotype in such a way as to obtain a phenotypic differentiation in the progeny which would make the parentage obvious. For additional control, the phenotypes of acid blood corpuscle phosphatase were also determined. The authors demonstrated that the determination of the polymorphism of transferrins and of acid blood corpuscle phosphatase can be used in the exclusion of paternity in breeding work.

Serov & Khlebodarova (1973) and Serov at al. (1973b; 1975; 1976; 1976b) conducted an electrophoretic analysis of a series of proteins and enzymes in the blood serum and internal organs by means of electrophoresis on starch gel. They demonstrated various polymorphic forms of albumins, transferrins, haptoglobin, carbonate anhydrase, carboxylesterase, arylesterase, hexokinase, diaphorase, 6-phosphogluconate dehydrogenase.

Stanislawska (1981) demonstrated that both physiological and pathological changes must be taken into consideration when examining the activity of esterases. This is particularly important in the case of pregnant and lactating vixens.

Madeyska-Lewandowska & Brzozowski (1983) conducted investigations of the blood serum and semen proteins in foxes. The semen proteins displayed several polymorphic forms.

The use of one- and two-dimensional electrophoresis of polyacrylamide gel rendered possible the identification of new protein systems in the blood serum of foxes. Juneja et al. (1988; 1989) described a genetically conditioned polymorphism of prealbumin (Pr), α_1B-glycoprotein (α_1B), postalbumin (Pa1), pretransferrin (Prt1). They also identified the vitamin D binding protein (Gc).

Among the proteins hitherto described in the world literature, only the polymorphism of transferrins, carboxylesterases and acid blood corpuscle phosphatase was examined in the species of foxes bred in Poland. The investigations presented in this paper aimed at the identification of protein systems in the blood serum of foxes not yet examined in Poland.

MATERIAL AND METHODS

The material comprised blood samples drawn from two fox species: *Vulpes vulpes* (105 samples) and *Alopex lagopus* (195 samples) from four breeding farms (the Gdansk, Gliwice, Poznań and Warsaw districts).

The separation of blood serum proteins, using one- and two-dimensional electrophoresis on polyacrylamide gel, was conducted in accordance with the method of Juneja et al. (1988, 1989).

Specific staining according to Uriel & Berges (1968) was used for the identification of α-protease inhibitors.

RESULTS AND DISCUSSION

The electrophoretic separation of the blood protein serum in foxes by way of one-dimensional electrophoresis on polyacrylamide gel is presented in Fig. 1, while that obtained by way of a two-dimensional electrophoresis in the agarose-polyacrylamide gel arrangement is presented in Fig. 2. This figure indicates that transferrins (Tf) and one other

protein fraction, marked "A", migrate with the same electrophoretic speed. Thus reading Tf phenotypes only from a one-dimensional picture (Fig. 1) may, in some cases, prove incorrect.

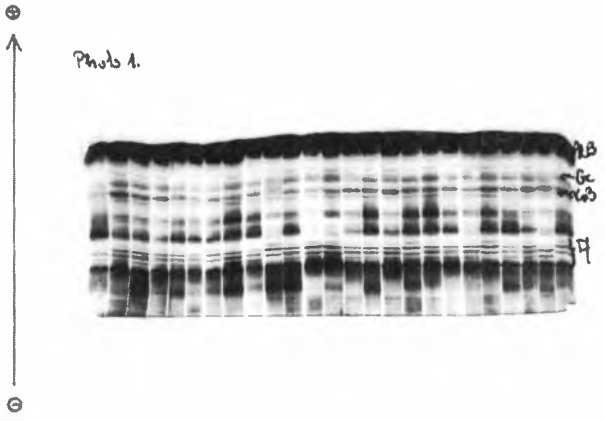

Fig. 1. Electrophoretic separation of proteins in the blood serum of foxes on polyacrylamide gel using the horizontal method. Protein symbols. Alb - albumin, Gc - vitamin D binding protein, α_1 - α_1-glycoprotein, Tf - transferring

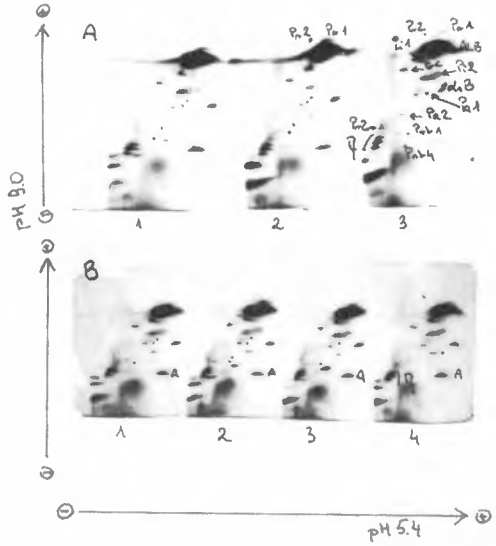

Fig. 2. Electrophoretic separation of proteins in the blood serum of foxes using two-dimensiona electrophoresis in the agarose-polyacrylamide gel arrangement (pH 5.4 and 9.0 respectively). Protein symbols: Prl prealbymin 1, ALB - albymin, Gc - vitamin D binding protein, Pi1 and Pi2 - α-protease inhibitors, α_1B - α_1B-glycoprotein, Pa1 postalbumin 1, Pa2 - postalbumin 2, Prt1 - pretransferrin 1, Prt2 - pretransferrin 2, Prt3 - pretransferring 3, Prt4 - pretransferring 4, Tf transferrin
Part A: protein phenotypes from 1 to 3: $Pa1^{DF}$, $Pa1^{DF}$, $Pa1^{F}$, α_1B - FF, BD, Prl - S, S, F, Prt1 - DS, DF, F
Part B: from 1 to 4: Prl - S, S, S, Pa1 - DF, DF, F, F, $_1$B - D, D, D, D Prt1 - F, DF, F, DF

The terminology for the polymorphic forms was accepted in accordance with Juneja et al (1988, 1989) for the systems described earlier and a new terminology was proposed for the new systems.

Within the region of prealbumin Pr1, three phenotypes were observed (F, FS and S) in the polar fox and two (F and FS) in the silver fox. In both species three phenotypes of postalbumin Pa1 were observed - D, DF and F. Five phenotypes of α_1B-glycoprotein (BF, D, F, FS and S) were indentified in the silver fox, while in the polar fox - six were observed (BF, BS, D, DF, F, FS). Moreover, 87% of the polar foxes were allele D homozygotes, while the silver foxes examined were among 50% of the D homozygotes.

Within the region of pretransferrin Prt1, three polymorphic forms were observed: D, F and S. In the silver fox two phenotypes were observed (D and DF) while in the polar fox four were observed - D, DF, DS and F. Within the transferrin system three phenotypes were observed: F, FS and S in the polar fox and two - D and DF - in the silver fox. The differences in phenotypic occurrence in relation to the individual polymorphic systems between the species examined are demonstrated by the frequency (Table 1).

Table 1. The frequency of alleles controlling the polymorphism of selected proteins in the blood serum of the silver and polar fox

Loci	Allele	*Vulpes vulpes* (Silver fox)	*Alopex lagopus* (Polar fox)
Pr	Pr^F	.8125	.5657
	Pr^S	.1874	.4342
A1B	$A1B^B$	.0681	.0151
	$A1B^D$	.5000	.8750
	$A1B^F$	.2954	.0701
	$A1B^S$	.1364	.0378
Pa1	$Pa1^D$	.4000	.2020
	$Pa1^F$	.6000	.7979
Prt1	$Prt1^D$	.7500	.5390
	$Prt1^F$	.2500	.4480
	$Prt1^S$	0	.0230
Tf	Tf^D	.9600	0
	Tf^F	.0400	.6730
	Tf^S	0	.3269

It was found that between the two species of foxes, not only were there differences in the frequency of alleles (Pr1 and Pa1), but also certain alleles occurred only in one species. For instance allele $Prt1^S$ occurred only in the polar fox. The Tf^D transferrin occurred only in the silver fox, while Tf^S occurred only in the polar fox.

A similar differentiation was observed by Juneja et al. (1988, 1989). Similarly to Juneja et al. (1989) it was found that there was no polymorphism of the vitamin D binding protein (Gc) or of the α-protease inhibitors Pi1 and Pi2.

Moreover, the present investigations demonstrated the existence of a hitherto undescribed polymorphism of four proteins in the blood serum of foxes: prealbumin Pr2 (three phenotypes A, AB and B) pretransferring Prt2 (two phenotypes A and 0) pretransferrin Prt3 (two phenotypes) pretransferrin Prt4 (two phenotypes).

The presence or absence of this last protein in the blood serum seems to depend on the degree of physiological development of the animal. The heritability model for these proteins is at present being investigated and will be presented in another paper, together with a complete analysis of the number of alleles conditioning the polymorphism of Pr2, Prt2, Prt3 and Prt4.

On the basis of the results from the present investigations, it can be concluded that individual alleles of systems of polymorphic proteins such as α_1B-glycoproteins, pretransferrins Prt1 and transferrins may be used as genetical markers for identifying the parentage in all types of crossing between the two fox species.

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Molecular cloning of mink immunoglobulin λ, κ and γ-chain cDNA

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Immunoglobulins are encoded by a gene complex comprised of three gene families for λ-light, κ-light and heavy chains. We cloned a number of cDNAs encoding mink immunoglobulin λ,κ, and γ-heavy chains. The sequences of two λ, one κ, and one γ cDNA clones were determined and analysed. Genomic blot hybridization revealed that the mink λ-locus contains multiple V- and C-gene segments. The isolated mink genes can be used as specific probes in further studies on the organization of Ig gene families and their expression in healthy and affected minks and related *Carnivora* species.

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Immunoglobulins (Ig) consist of two heavy (H) and two light (L) polypeptide chains. There are two types of L chains, λ and κ and five classes of heavy chains: μ, δ, γ, α and ε. The Ig chains are coded for at three separate loci, mapped to different chromosomes. L-chain families are comprised of groups of three distinct genetic elements: the variable (V) and joining (J) mini-gene encodes the light chain variable domain, and the constant domain is encoded by the C gene. A high degree of diversity of the V domain of H chains is provided, along with the V and J segments, by an additional group of diversity (D) gene segments. The organization and regulation of the expression of the Ig genes have been widely studied in humans, mouse, rat and rabbit. However, little is known about the Ig genes in representatives of the *Carnivora* order. Our previous studies of genetic polymorphism of Ig L- and γ chains in domestic mink have indicated that this species has several Cλ and Cγ genes (Belyaev et al. 1986; Volkova et al. 1989). The aim of the present work was to clone genes representing different Ig gene families and to analyse their genomic structure.

MATERIALS AND METHODS

DNA probes

The probes were: a) a 308-bp EcoRI-PvuII fragment from pIGL-2, henceforth referred to as the Vλ specific probe; b) a 300-bp PvuII-PvuII fragment from pIGL-2, henceforth referred to as the Cλ specific probe.

cDNA library

The cDNA was synthesized according to Gubler & Hoffman (1983) using mink spleen poly(A+)-RNA isolated as described by Aviv & Leder (1972). The cDNA was ligated into EcoRI-cut and dephosphorylated arms of λgt11 and λZAP II as described by Maniatis et al.(1983). The cDNA library was screened for antigen-producing clones by means of polyclonal and monoclonal antibodies against mink Ig γ, κ and λ chains. Sequence analysis was performed according to Maxam & Gilbert (1980). Ig sequences contained in the EMBL Data Library were used to calculate the degree of homology with the help of the sequence analysis programm MicroGenie.

Southern blot hybridization

High molecular weight DNA from cultured mink fibroblasts was digested with EcoRI. Resolved DNA was transferred to nylon membranes (Gene Screen Plus, NEN) using the vacuum blotting transfer techniques. Prehybridization and hybridization were performed following manufacturer's instructions.

RESULTS AND DISCUSSION

The libraries were screened for IgG cDNA expression with poly- and monoclonal antibodies against the L and H chains of mink IgG. Of the total number identified, 64 were λ, 60 were γ-, and 3 were κchain encoding clones. After insert size determination and restriction mapping, we chose the following clones; two λ (pIGL-2, 805 bp and pIGL-10, 705 bp), two γ- (pIGH-47,1.6 kb and pIGH-14, 1.5 kb) and one κ clone (pIGK-3, 800 bp). The clones were sequenced and their primary sequences were compared with their known counterparts in other species.

λ-chain cDNA

A sequence analysis of the λ clones revealed that they contain rearranged λ chain genes differing from each other in all the three composing segments (Fig. 1). Both clones are incomplete, pIGL-2 insert does not contain a part of the V segment which encodes the leader peptide and several amino acids of the FRI region, and pIGL-10 does not contain most of the V segment. The pIGL-2 sequence has a 636-bp open reading frame and a polyadenylation site 67 bp apart from the stop codon. An uncompensated deletion of a single nucleotide shifting an open reading frame and giving rise to an additional stop codon is observed at the start of the C1 region of pIGL-10. The corresponding gene is presumably a pseudogene. The sequences of pIGL-2 and pIGL-10 showed the highest homology degree with genes of the human λ chains, the homology being 68-75% for the V region and 75-81% for the C-region.

Fig. 1. Sequence comparison of cDNA clones encoding mink Ig λ chains. Asterisk indicates deletion introduced to maximize homology. Stop codon and poly (A) addition signal are underlined

Fig. 2. Sequence comparison of mink and human Ig γ- and κ chain V regions.

A. Mink cDNA clone pIGH-47 and human active γ-chain gene (Kudo et al. 1985).

B. Mink cDNA clone pIGK-3 and human active κ chain gene (Klobeck et al. 1984). Asterisks indicate deletions introduced to maximize homology.

γ- and κ chain cDNA

The insert of clone pIGH-47, 1,6 kb long, contains almost all the gene sequences encoding the γ chain of mink Ig. The homology between the V segments of this clone and that of the human γ chains ranges from 62% to 85% for different VH subgroups. The homology range for the J segment is 61-75%, for the CH3 domain from 72% to 74%. In contrast, the D segment shows little, if any, homology, when their counterparts in humans, rabbit, mouse and rat were analysed (Fig. 2a). Sequencing of pIGH-14 is incomplete, and its analysis is in progress.

The insert of clone pIGK-3 encoding the κ chain contains part of the V region, as well as J, C and 3'-untranslated sequences. Like the λ and γ clones, pIGK-3 show highest homology with the human genes in the V region: 65-80% for the V- and 76-95% for the J segments, respectively (Fig. 2b). In the C region, the homology with the counterparts of other mammals is relatively low, 56-69%. These significant differences may have been one reason why in their amino acid sequence analysis Hood et al. (1967) have not succeeded in identifying κ chains in mink.

Genomic structure of the λ locus

The number of V and C genes encoding the mink λ chains was evaluated by southern blot hybridization. Five hybridizing components were observed in an analysis of EcoR I digested DNA from mink fibroblasts when the C λ specific probe was used (Fig. 3a). These results, together with data in the literature on gene size and intergenic spacers in the families of the Ig λ genes in species other than mink, provide evidence for the presence of at least five Cλ genes in the mink haploid genome.

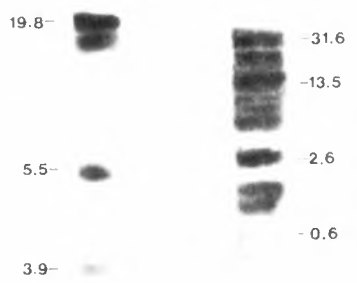

Fig. 3. Hybridization of mink genomic DNA to Cλ (A) and Vλ (B) probes. Sizes are shown in kb.

A λVspecific probe with mink DNA at high stringency conditions yielded at least 12 hybridization signals ranging from 0.5 to 31.6 kb. This pattern is the one expected for the V-loci comprised of a large number of V-genes. When taking into account the literature data on possible effects of polymorphism and overlapping of fragments, it may be suggested that the mink has 20-30 V genes detected by our probe. Consequently, the λ family of mink Ig genes has a complex organization like that of human λ genes (Hieter et al. 1981; Anderson et al. 1984).

At present we are extending our studies to include the mink γ and κ loci. It is hoped that more information will be gathered on how mink Ig genes are organized and how they function. Probes containing V-, D-, J- and C segments from three Ig gene families would be helpful in an analysis of the expression of immunoglobulin genes in healthy and diseased mink and also in identifying the immunoglobulin genes in other Carnivora fur animal species.

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Random amplified polymorphic DNA (RAPD) markers for paternity identification in multiple-sired mink litters

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An experiment was conducted to identify parentage in multiple-sired mink litters using random amplified polymorphic DNA (RAPD) analysis. Genomic DNAs were prepared from ear tissue collected from individuals in six multiple-sired mink litters. Several 10-mer arbitrary primers were screened for polymorphisms. RAPD products were separated and visualized through 1.5% agarose gels. Selected primers amplified reproducible polymorphic DNA markers that could be used to identify litters if the marker was present in one sire and absent in the dam and the other sire. The parentage of all progeny in multiple-sired litters was identified using these primers. In addition, a male-specific RAPD marker was found. Analysis of two or three RAPD markers can accurately and efficiently identify parentage in a multiple-sired litter in mink.

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Parental identification in domestic animals has been routinely carried out by means of blood type and other biochemical polymorphism analyses. These methods generally require large fresh blood samples and are often affected by the specific stages of animal development. DNA fingerprints are now routinely used for parentage identification in humans using hypervariable mini satellite DNA probes (Jeffreys et al. 1985). Soller & Beckmann (1983) demonstrated the use of restriction fragment length polymorphic (RFLP) markers for parental identification in domestic animals. Using DNA fingerprints, Kashi et al. (1990) reported parental identification in cattle.

Random amplified polymorphic DNA (RAPD) analysis was proven to be a reliable procedure for genome mapping and genetic resource characterization (Williams et al. 1990; Andersen & Fairbanks 1990). RAPD analysis does not require specific DNA sequence information on target DNA regions but uses arbitrary primers to generate random polymorphic DNA segments. Welsh & McClelland (1990) described the application of a similar technology, arbitrarily primed polymerase chain reaction (AR-PCR), and used it for strain identification in mice (Welsh et al. 1991a) and for parental determination in maize hybrids

(Welsh et al. 1991b). Using RAPD technology, Xiong et al. (1991) reported that polymorphisms could be found within multiple-sired mink litters.

The objective of this investigation was to screen several arbitrary primers to unambiguously identify the paternal parent of multiple-sired mink litters with two sires.

MATERIALS AND METHODS

DNA Extraction

About 0,5 cm^2 fresh ear tissue from individuals associated with six multiple-sired dark mink (*Mustela vison*) litters were collected. The tissue was cut into fine pieces and incubated in digestion buffer (100 mM NaCl, 10 mM Tris.Cl, pH 8.0, 25 mM EDTA, pH 8.0, and 0.5% sodium dodecyl sulfate and 0.1% proteinase K) at 50°C for 12-18 h. The samples were extracted twice with an equal volume of phenol/chloroform/isoamyl alcohol. The DNA was precipitated in 0.3 M sodium acetate (pH 5.4) and with 100% ethyl alcohol. The DNA pellet was rinsed with 70% ethyl alcohol and resuspended in about 1.0 ml of TE buffer (10 mM Tris.CL, 1 mM EDTA, pH 8.0).

RAPD analysis

Sixty oligodeoxynucleotide 10-mer primers of arbitrary sequence were purchased from Operon Technologies, Inc. Amplification reactions were performed in a 15 ul reaction mixture (Xiong et al. 1991). The thermal cycler was programmed for 45 cycles of 1 min at 92°C, 1 min at 50°C and 2.5 min at 72°C. After the cycling was completed, the entire 15 ul sample was loaded on a 1.5% agarose gel stained with ethidium bromide. Primers were screened using DNAs from two sires and a dam. Primers that provided reproducible polymorphic genetic markers were used to screen all of the potential parents of the mink litters. If the polymorphic marker was present in one sire and absent in the dam and the other sire in a litter, this primer could be used for progeny screening. Primers meeting these criteria were then used to amplify all progeny in the litter. Progeny that carried a particular marker could be positively identified as an offspring of the sire that carried the same marker.

RESULTS

Genetic Markers from Primer Screening

Among the 60 arbitrary primers, 15 primers produced polymorphic markers. The polymorphic markers generated with primer D-03 (5'-GTCGCCGTCA), F-06 (5'-GGGAATTCGG), G-17 (5'-ACGACCGACA), I-10 (5'-ACAACGCGAG), I-14 (5'-TGACGGCGGT) and I-16 (5'-TCTCCGCCCT) were reproducible and easily distinguished in gels (Fig. 1). Primer G-17 generated a polymorphic marker linked with the Y-chromosome (Fig. 1).

Parentage Identification

Parent screening of six litters with primer D-03 showed that only litter 5 had the required

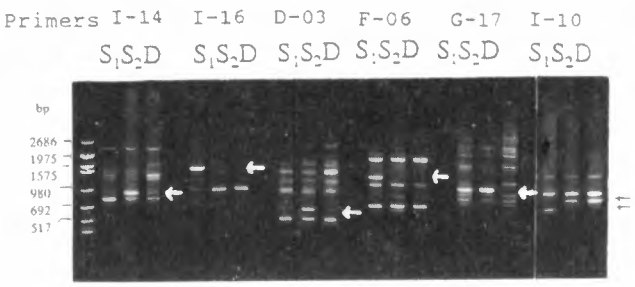

Fig. 1. Primer screening with mink DNAs. The polymorphic markers (arrow) were stably reproducible. Primer G-17 amplified a male-specific marker (arrow)

band patterns (Fig. 2). Progeny screening of litter 5 with primer D-03 revealed a poly-morphic marker that was pre-sent in progenies O_2, O_5 and O_6 (Fig. 2 & 3A). Thus, these progeny were all sired by sire S_2, the only parent that carried the marker. Accurate identifi-cation of the remaining pro-geny of litter 5 was acomp-lished using primer F-06 (Fig. 3B). A marker was present in progenies O_1, O_3, O_4, and sire S_1, indicated these three pro-geny were sired by sire S_1. Additional screening with primer I-16 (Fig. 3C) did not contradict paternity identifi-cation determined with pri-mers D-03 and F-06.

DISCUSSION

The use of multiple sires to increase conception is frequent in animal production. For the case with more than two sires, RAPD analysis may be used for paternity identification, although larger numbers of markers may be needed to screen the parents. A marker must be absent in the dam and present in only one of the multiple sires in the final screening. Paternity identification will be more efficient if the selected primer generates more than one marker. By combining two primers in one reaction, the number of potential markers may also be increased.

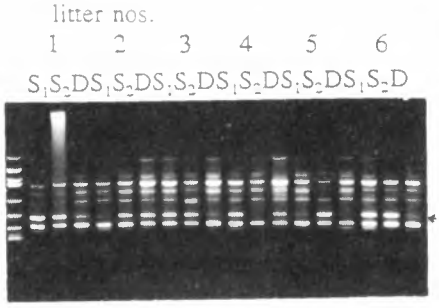

Fig. 2. Parent Screening With Primer D-03. The marker (arrow) was present in one sire (S_2) and absent in the dam (D9 and the other sire (S_1) in litter No. 5

Reproducible RAPD markers are critical. In this study, it was found that under the identical conditions, some primers generated unreproducible polymorphic bands or smears. This may be due to the DNA sequence of these primers. Only primers providing reproducible polymorphic bands were used as genetic markers.

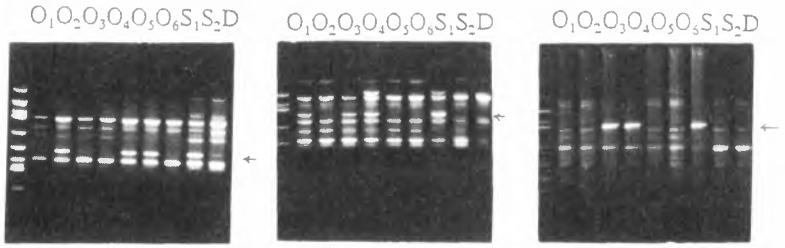

Fig. 3A Fig. 3B Fig. 3C

Fig. 3. Progeny screening in mink litter No. 5. A. With primer D-03. The marker (arrow) was present in progenies O_2, O_5, O_6. These progenies were sired by sire S_2. B. With primer F-06. The marker (arrow) was present in progenies O_1, O_3, O_4 and sire S_1. These progenies were sired by sire S_1. C. Withe primer I-16. The marker (arrow) was present in progenies O_3, O_4 and sire S_1, which verified that O_3 and O_4 were sired by sire S_1. The marker was absent in progeny O_1. The genotype of sire S_1 for this marker is heterozygous

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Immunohistochemical study of GH and PRL cells in the mink (*Mustela vison*) during its growth

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Vidal, S., P. Sánches, A. Román & L. Moya 1992. Immunohistochemical study of GH and PRL cells in the mink (*Mustela vison*) during its growth. Norwegian Journal of Agricultural Sciences. Suppl. no. 9: 206-212. ISSN 0801-5341.

No detailed studies have been performed on the histomorphological changes in GH and PRL cell populations during the growth of mink. We sought to determine these changes by means of immunohistochemistry Avidin-Biotin Complex (ABC). In our study we used 24 farm mink of the wild variety, including six 3-week-old mink (beginning of lactation), six 2-month-old (end of lactation), six 6-month-old (pre-puberty) and six 8-month-old mink (puberty), with 50% male and 50% female in each group. The hypophyses of these animals were perfused and fixed with 2% paraformaldehyde-0.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4-7.6) and then removed and postfixed by immersion in the same fixative, for two hours at 4°C. Only the right hemihypophysis was used, and this was embedded in paraplastin and then serially cut at 3μm. The sections obtained were labelled with either human anti-GH raised in rabbit (NIDDK-AFP-1613102481) 1:500 or human anti-PRL raised in rabbit (NIDDK-AFP-55781789) 1:1000. Pronounced age-related changes were observed for these morphometric parameters studied. The results of this study reveal the strong correlation between the observed changes and the growth and the onset of puberty.

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Although numerous reports have investigated and identified the cells of the adenohypophysis in different mammal species, e.g. in rat (Takahashi 1991; Nikitovitch-Winer et al. 1987; Kurosomi 1986), goat (Shirasawa et al. 1985), pigs (Dacheux 1980), and in musk shrew (Iwama et al. 1990), we still have little information concerning the morphology (Murphy & James 1976; Weman 1974) and histomorphometric changes that take place in the adenohypophysis cells of the mink over different periods of its life cycle.

Using immunohistochemical techniques, we conducted a study on the changes in GH and PRL cells during the growth of the mink by means of the changes in different morphometric parameters: surface area , volume density. The results are presented in this paper.

MATERIALS AND METHODS

Twenty-four farm minks of the wild variety were divided into four groups. The first two groups were sacrified during a positive photoperiod and the last two groups during a negative photoperiod: Group 1: six 3-week-old mink (beginning of lactation), Group 2: six 2-month-old mink (end of lactation), Group 3: six 6-month-old mink (prepuberty) and Group 4: six 8-month-old mink (puberty), with 50% male and 50% female in each case. The hypophyses of these animals were perfused and fixed with 2% paraformaldehyde - 0.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4-7.6) (Fumagalli & Zanini 1985) and then removed and postfixed by immersion in the same fixative for two hours at 4°C. Only the right hemihypophysis was used, and this was embedded in paraplastin and then serially cut at 3μm. The sections obtained were labelled with either human anti-GH raised in rabbit (NIDDK-AFP-1613102481) 1:500 or human anti-PRL raised in rabbit (NIDDK-AFP-55781789) 1:1000, visualized with peroxidase (ABC complex) and revealed with DAB (Hsu et al. 1981).

The following morphometric parameters were determined: mean area and volume density. A student's t-test was used to compare these parameters in female and male mink.

RESULTS

Variations in the distribution and volume density in GH and PRL cells during the growth of mink

GH cells are distributed mainly on the caudal area of the adenohypophysis, near the *pars intermedia*, where they form cords (Fig. 5). In males, a marked growth of volume density starting from Group 1 was observed, while a non-significant reduction in volume density of GH cells was observed in Group 1 and Group 2 females. The decrease in this parameter between Groups 2 and 3 is clearly illustrated in Figs. 3 and 4.

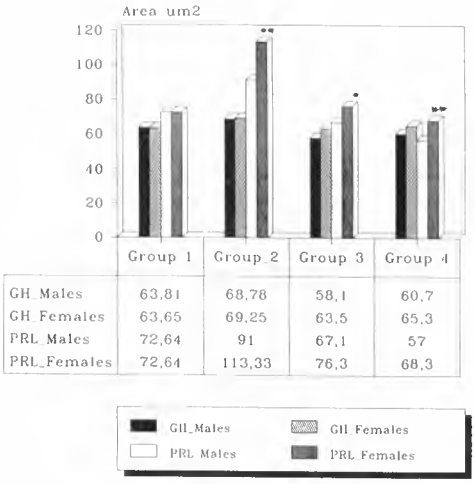
PRL cells are distributed throughout the gland, mainly located around blood vessels (Fig. 6). A decrease in volume density of PRL cells was observed in Groups 3 and 4 (these animals were sacrificed under environmental conditions of a negative photoperiod, contrasting with Group 2), which was stronger in females than in males.

It should be emphasized that a high value of volume density of PRL cells occurred in mink of Group 2 (Fig. 3). These animals were sacrified under the environmental conditions of long days, as compared with the other groups.

Variations in the morphology and surface area of GH cells and PRL cells during the growth of mink

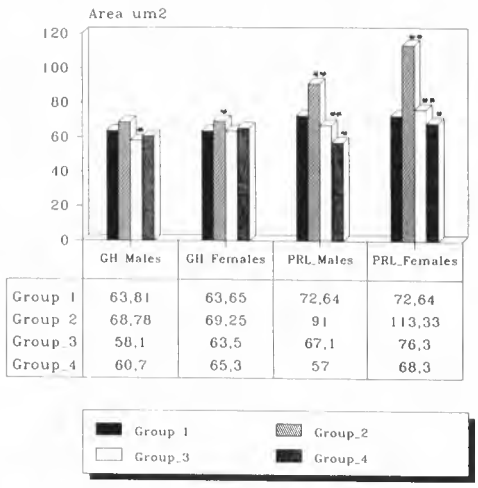
GH cells are oval or round in shape with a central nucleus (Fig. 5). The variation in the mean area of GH cells showed a similar line in both sexes. The biggest differences, which were found between Groups 1 and 2, were due to the animals belonging to Group 2 being almost double the size of those in Group 1 (Fig. 1 and 2).

PRL cells showed a marked dimorphism (Fig. 6). In nearly all the groups the value of the mean area of PRL cells was higher than that in GH cells. In both sexes the mean area was directly correlated with photoperiod, deriving the maximum value in Group 2 (longest days) and the minimun in Group 4 (shortest days) (Figs.1 and 2).



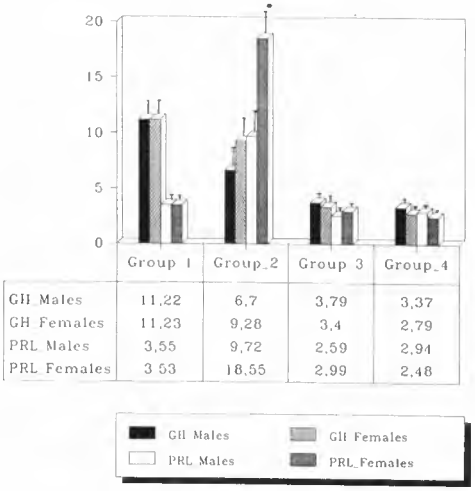
	Group 1	Group_2	Group 3	Group 4
GH_Males	63,81	68,78	58,1	60,7
GH_Females	63,65	69,25	63,5	65,3
PRL_Males	72,64	91	67,1	57
PRL_Females	72,64	113,33	76,3	68,3

Fig. 1. Changes in mean area in GH and PRL cells in Groups 1, 2, 3 and 4 of mink (*Mustela vison*). The values of SEM are only represented when they are greater than 10%



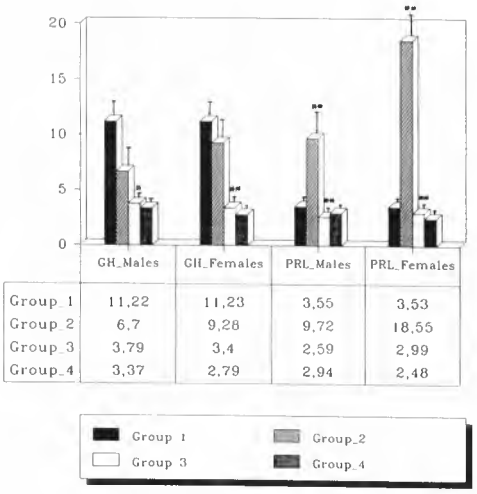
	GH_Males	GH_Females	PRL_Males	PRL_Females
Group 1	63,81	63,65	72,64	72,64
Group 2	68,78	69,25	91	113,33
Group 3	58,1	63,5	67,1	76,3
Group 4	60,7	65,3	57	68,3

Fig. 2. Changes in mean area in GH cells of males and females, and PRL cells of males and females during the growth of mink (*Mustela vison*). The values of SEM are only represented when they are greater than 10%



	Group 1	Group_2	Group 3	Group_4
GH_Males	11,22	6,7	3,79	3,37
GH_Females	11,23	9,28	3,4	2,79
PRL_Males	3,55	9,72	2,59	2,94
PRL_Females	3 53	18,55	2,99	2,48

Fig. 3. Changes in volum density in GH and PRL cells in Groups 1, 2, 3 and 4 of mink (*Mustela vison*)



	GH_Males	GH_Females	PRL_Males	PRL_Females
Group_1	11,22	11,23	3,55	3,53
Group_2	6,7	9,28	9,72	18,55
Group_3	3,79	3,4	2,59	2,99
Group_4	3,37	2,79	2,94	2,48

Fig. 4. Changes in volume density in cells of males and females GH, and PRL cells of males and females during the growth of mink (*Mustela vison*)

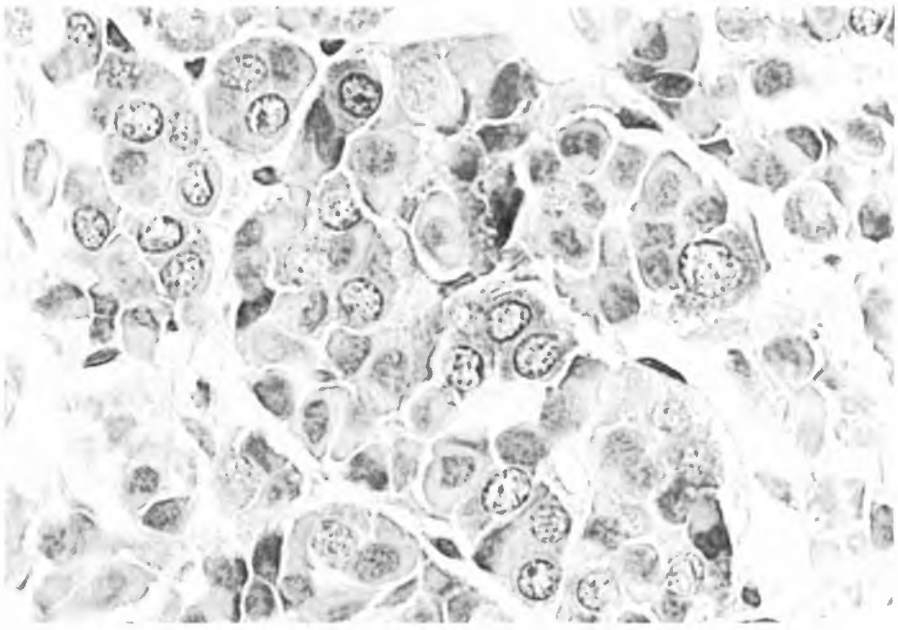


Fig. 5. Sections of mink pituitary immunohistochemically stained with anti-growth hormone. These are oval or round and located in groups (x 1040)

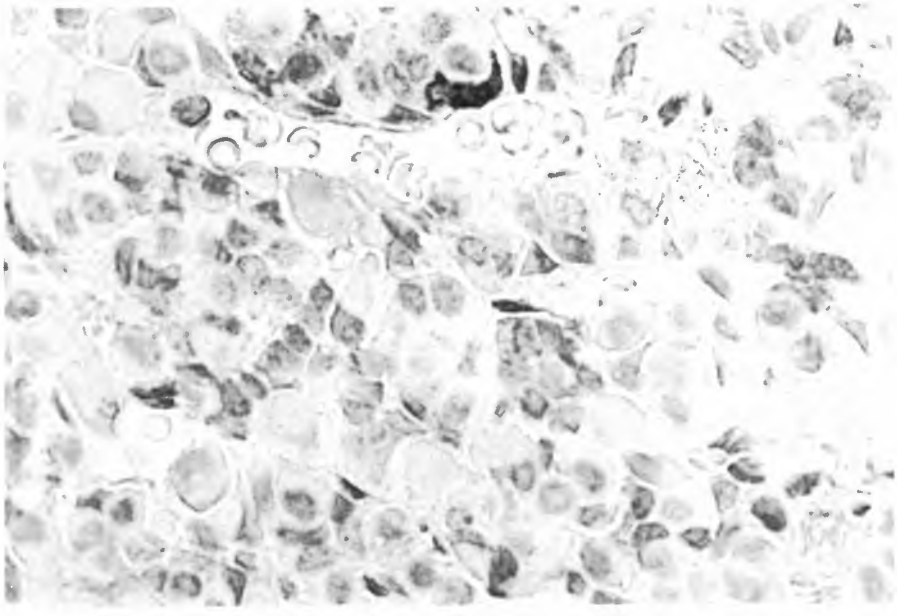


Fig. 6. Sections of mink pituitary immunohistochemically stained with anti-prolactin hormone. They are located around blood vessels (x 1040)

DISCUSION

It was observed that both somatotrophic and lactotrophic cell populations vary in females and males during the growth of mink.

GH cells are distributed throughout the adenohypophisis, but mainly on the caudal area of this gland, which indicates a rule of distribution similar to that of GH cells of the pituitary gland of the baboon (Herbert & Silverman 1983).

PRL cells of mink, just as in other species, e.g. the rat (Nogami & Yoshimula 1980), display a high dimorphism, distributed near the blood vessels, in close relation with hypophyseal irrigation (Sasaki & Iwana 1988). Sasaki pointed out that the distribution of PRL cells in the adenohypophysis may be related to the mechanism of regulation that manages oxytocin and oestrogens, carried in blood vessels, in PRL cells.

The volume density and the mean area of GH cells and PRL cells were considered to reflect the functional activity of those cells. Thus, it has been observed that the volume density of GH cells, in both female and male mink, decreases with age just as it does in many other species (Takahashi 1991).

The variation in mean area of GH cells in both sexes and in all of the groups studied, is less significant than the variation in PRL cells, according to the Smets's results in rat (Smets et al. 1987). These authors observed that in immature neonatal rats the GH cells were not differentiated from those of adult rats, suggesting that morphological maturation of these cells had been reached at birth.

The variations in volume density and mean area for both sexes substantiate the results obtained by other authors in many studies on the variation of plasma PRL levels in relation to photoperiod (Martinet et al. 1982,1984; Boissin-Agasse et al. 1983). The maximum value for these parameters was therefore found in Group 2, and the minimum value in Group 4. It was also observed that, as in the rat (Dada et al. 1984), the PRL cells were bigger in the female mink than in the male mink.

Future work will include studies on the ultrastructural relationships between different types of cells. A prerequisite of such studies, however, is a more precise knowledge of the morphological parameters of PRL and GH cells.

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Immunogold identification of the GH and PRL cells in the suckling mink (*Mustela vison*)

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PRL and GH cell types have been identified ultrastructurally in the adenohypophysis of many species on the bases of the size and shape of the secretory granules. However, more definitive identification of these cell types is required. Accordingly, we used an immunogold method. In our study 12 farm minks of the Wild variety (50% male and 50% female) were used, of which six were 3-week-old kits (beginning of lactation) and six were 2-month-old kits (end of lactation). The hypophyses of these animals were perfused and fixed with 2% paraformaldehyde-0.5 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4-7.6) and then removed and refixed in the same fixative, for two hours at 4°C. Only the left hemihypophyses were used and these were postfixed in O_4Os 1 h. at 4°C. The samples were embedded in Epon. The ultrathin sections were labelled by means of the immunogold method with human anti-rabbit GH (NIDDK-AFP-1613102481) and human anti-rabbit PRL (NIDDK-AFP-55781789). Our study demonstrated that the immunocytochemical localization of the different adenohypophysis hormones seemed the best way to make an accurate identification of the adenohypophyseal cells.

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Functional identification of cell types of the anterior pituitary gland, that is, which cells produce which hormones, has been a main subject of research among cytomorphological studies.

Nakane (1970) was the first to use the immunoperoxidase technique to localize pituitary hormones at the light and the electron microscopic level and, subsequently, from this many other studies have been carried out in order to identify by means of immunocytochemical techniques the ultrastructural differences between adenohypophyseal cell types in different species (Moriarty 1973; Kurosami 1986; Pelletier et al. 1978).

Nevertheless, very few ultrastructural studies of these cells have been conducted in mink (Murphy & James 1976).

We have attempted to establish a differential criteria for distinguishing the PRL and GH cells in suckling mink that have previously been identified immunocytochemically with antisera for prolactin and growth hormone.

MATERIAL AND METHODS

Of the 12 farm mink of the Wild variety (50% male and 50% female) used in the study, six were three-week-old kits (beginning of lactation), and six were two-month-old kits (end of lactation). The hypophyses of these animals were perfused and fixed with 2% paraformaldehyde-0.5% glutaraldehyde in 0.1M. cacodilate buffer (pH 7.4-7.6) and then removed and refixed in the same fixative for two hours at 4°C. Only the left hemihypophyses were used and these were postfixed in O_4Os 1 h. at 4°C. The hemihypophyses were each cut into four parts, two cranials and two caudals,(Sasaki & Sano 1986). The samples were dehydrated in a graded ethanol solution, including one bath of 70° ethanol with uranyl acetate, and then embedded in EPON 812. The sections were labelled according to the immunogold method (Roth 1983). First the sections were etched with a saturated aqueous solution of sodium metaperiodate. The grids were then floated in TBS with 0.5% BSA incubated with antiserum diluted in TBS with 0.5% BSA (Anti-human GH 1:800, Anti-human PRL 1:1000) at 4°C for 24 h. After several washings in TBS with 0.5% BSA, the grids were placed in the IgG-colloidal gold complex (20 nm) (Polysciences) diluted 1:10 in TBS with 0.5% BSA for 45 min at room temperature.

After rinsing in TBS with 0.5% BSA and distilled water, the grids were dried and stained in uranyl acetate and lead citrate before observation with a Jeol transmision electron microscopy.

The size of the secretory granules was determined at x 10000 with an Image Analysis program.

RESULTS

The GH cells were distributed mainly in the caudal area of the adenohypophysis, and normally formed groups. These cells displayed a central round nucleus (Fig.1).
The cytoplasm of the GH cells contained a small number of granules with a mean diameter of 296 nm. These granules, which were highly electron-dense, displayed a uniform size and absence of visible perigranular membrane (Fig.2). These cells also exhibited a high number of mitochondria and an endoplasmic reticulum well developed with thick stack all along the length.
The PRL cells (Fig. 3) had a marked polymorphism and were located mainly near blood vessels. A considerable exocytosis of the PRL granules towards the capillaries was observed. In the cytoplasm of the PRL cells some round secretory granules about 150 nm in mean diameter and a visible perigranular membrane were detected. These granules were less electron-dense than the GH cells granules. Endoplasmic reticulum and mitochondria were less well developed in these PRL cells than in the GH cells.

DISCUSSION

From our results we can conclude that the GH cells have a predominant synthetic activity over storage activity, as is substantiated by the great development of the endoplasmic ret-

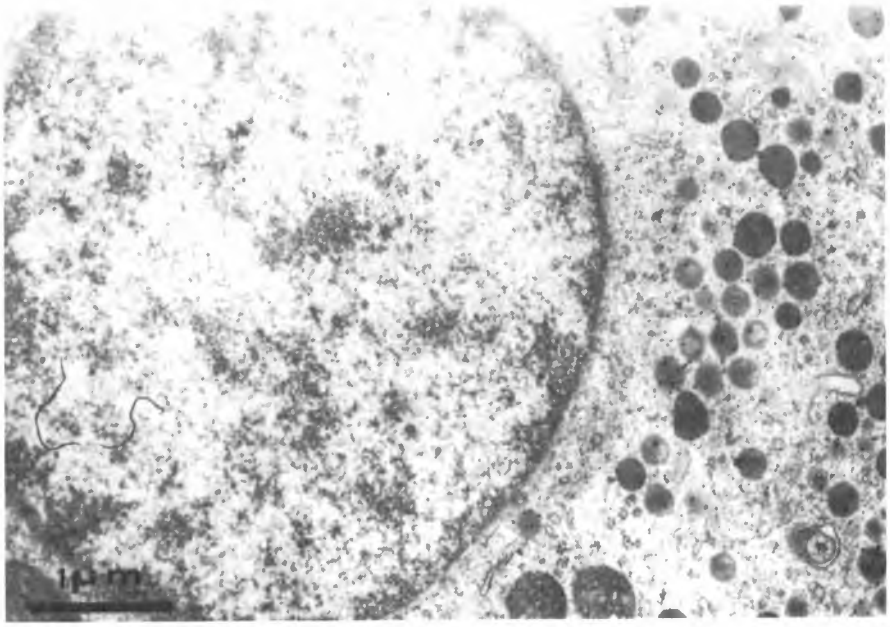

Fig. 1. GH cell. Immuno-labelling. Gold 20 nm. x 20.000

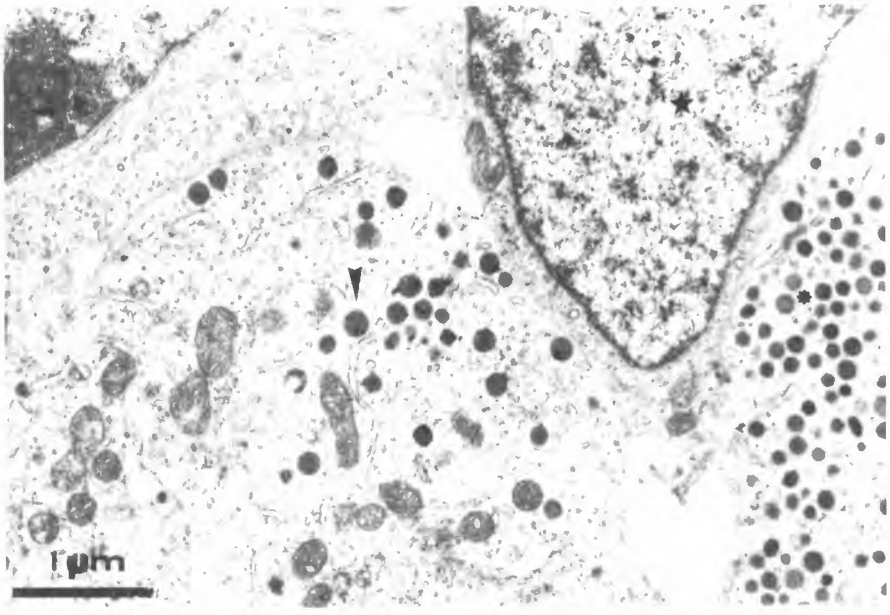

Fig. 2. GH cell. (▲) Immuno-labelling. Gold 20 nm. (★) Follicule-Stellate Cell (✱) Negative anti-hGH Cell

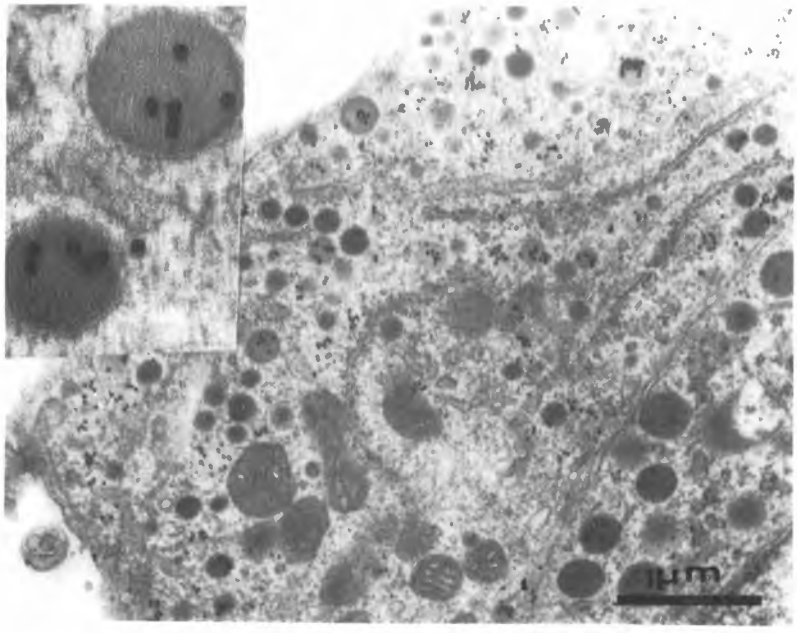

Fig. 3. GH cell. Immuno-labelling. Gold 20 nm. x 20.000. Inset PRl granules x 100.000

iculum. There are no differences between the ultrastructural characteristics observed by us and those observed by Murphy & James (1976) in adult animals. This suggests that morphologic maturation is already achieved at birth (Smets et al. 1987)

PRL cells displayed a similar morphology to that of type II PRL cells described first by Nogami & Yoshimura (1980). This cell type differs strongly from PRL cells observed by Murphy & James (1976) in adult animals which have a similar morphology to that of type I PRL cells (typical PRL cell). This result proves that granular polymorphism in mink is not an absolute criterion for identifying PRL cells (Nogami & Yoshimura 1982).

The size of granules in the PRL cells of suckling mink was 150 nm. This result is in agreement with that of Shiino & Yamauchi (1985) in rat. They demonstrated that PRL cell granules were smaller than those of GH cells during the postnatal period.

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Nutrition

Recent advances in the nutrition of fur animals

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During the ca. 100 years of fur animal domestication, there has been a line of continuity from the preliminary task of composing a usable feed ration via examination and description of the individual feedstuffs to the determination of standards for energy and nutrient supplies in the different stages of the production cycle of the animals. Research on the more basic subjects was not initiated until recently, which is reflected in the present limited knowledge compared with results available regarding other species of domestic animals. With reference to the nutritional subjects discussed at the IVth International Congress in Fur Animal Production held in Toronto (Murphy & Hunter 1988) and more recent publications, the aim of this paper is to give an overview of the ongoing research work and to suggest potential future experimental areas. The literature review should be seen in that light. A conventional approach to the subject is maintained by discussing separately the topics digestibility of nutrients, energy and nutrient supply and specific effects of amino acids, minerals and vitamins. The conventional division of the subjects into digestibility of the nutrients, their contribution to the energy requirement of the animals, and the specific effect they may also have, including being an amino-acid source, is maintained. Finally, an attempt will be made to identify future trends and areas within nutrient research which require further study.

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Digestibility is dependent on age and animal species. The ability of mink kits to digest the nutrients does not reach the level of mature animals until they are 10-12 weeks old (Elnif et al. 1988a; Tauson 1988a). Differences in nutrient digestibility between the fox and the mink (Skrede et al. 1978; Rouvinen et al. 1988) are mainly due to anatomical differences causing different rates of passage of time of the food through the alimentary tract (Enggaard Hansen 1978; Szymeczko & Skrede 1990; Faulkner & Anderson 1991).

For crude protein, the difference may amount to as much as 15 units (Skrede et al. 1980). A similar difference is found for crude fat mainly explained by a higher digestibility of saturated fatty acids in the fox (Jørgensen & Glem-Hansen 1973; Rouvinen et al. 1988; Rouvinen 1991). For carbohydrates, similar differences may be expected depending on origin and processing. The limited experimental data show that the difference between the two species increases with decreasing digestibility of the evaluated diets.

Although it may not be regarded as original research work, it is important to focus

on the lack of knowledge regarding the nutrient digestibility of the fox. The differences found in comparison with the mink show that there is a need for a feedstuff table based on digestibility trials with foxes.

The determination of digestible nutrients in the feedstuffs is based on the commonly used feedstuff tables, and although the individual feedstuffs differ somewhat geographically, the values will be of a more general nature. The latest compiled and revised feedstuff survey was published by the Scandinavian Association of Agricultural Scientists (NJF) in 1985. As some of the data are based on older experimental work, it ought to be pointed out that a recent series of digestibility trials on about 40 of the most commonly used feedstuffs for mink and foxes only shows minor deviations from the older data (Børsting 1992). However, these experiments demonstrated that different processing techniques may cause considerable changes in digestibility. So there is every reason to emphasize the importance of an accurate description of the feedstuff investigated as well as the treatment to which it has been subjected.

In recent years, the use of proteins of vegetable origin has increased, also for fur animals, and the evaluation of their digestibility also requires an accurate description of the processing technique (Skrede & Krogdahl 1985; Henriksen et al. 1987; Børsting 1992). Furthermore, the presence of inhibitors and other undesirable substances may also render calculation of digestibility coefficients difficult (Skrede & Krogdahl 1985; Sørensen 1990). Finally, the sensitivity to enzyme inhibitors specific to the species concerned should be indicated (Elnif et al. 1988b). Therefore, caution is recommended in the use of digestibility coefficients found in experiments with other animal species.

ENERGY SUPPLY

As in other species, the energy metabolism of mink and foxes depends on the life processes of the animals, including basal metabolism, deposition, i.e., growth, foetal development and milk production, muscular activity, and temperature regulation (Fig. 1).

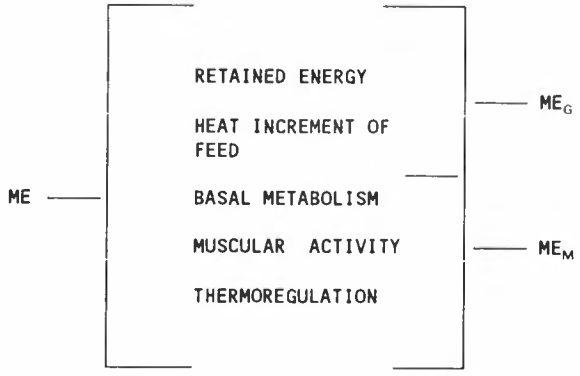

Fig. 1. Utilization of the metabolizable energy (ME) for maintenance (ME_m) and growth (ME_m)

In the determination of the energy value of feedstuffs the most important single factor is the digestibility of the energy (DE), which alone explains 60-70% of the difference between the feedstuffs. In fur animal nutrition, metabolizable energy (ME) is generally used as a basis for determination of the energy used by the animal for various production purposes and for determination of the energy value of feedstuffs and compound diets.

The efficiency of energy retention in mink and foxes is not yet known. However, there is a report on a series of trials on other animal species, carried out during the growth period (Müller & Kirchgessner 1979), from which it appears that the utilization of ME for fat deposition shows a very limited variation, approx. 1.3 kJ ME per kJ deposited, equalling a coefficient of utilization of about 80%. The results for protein show considerably greater variation, but it appears to be realistic to assume a coefficient of utilization of about 50%.

The energy concentration of the feed also exerts an influence on the total coefficient of utilization of ME, because increased energy density results in superior utilization defined as net energy available for life processes (Just Nielsen 1975). This effect has also been shown also for mink (Tauson 1988b), but not yet for foxes. It may be assumed that the energy concentration will be less import for both species, when high-energy density diets are used, expressed in terms of ME per kg dry matter (Jakobsen et al. 1960).

Table 1. Metabolizable energy from protein fat and carbohydrate in the feed for mink (%)

	December - whelping	Whelping - 30. June	1. July - 31. August	1. September - pelting
Protein, min.	35	40	28	3026
Fat	20 - 50	40 - 50	35 - 55	30 - 55
Carbohydrate, max.	25	20	30	30

Table 2. Metabolizable energy from protein fat and carbohydrate in the feed for foxes (%).

	December - whelping	Whelping - 15. July -	15. July 31. August	1. September - pelting
Protein, min.	35	37	28	26
Fat	20 - 45	35 - 50	35 - 55	35 - 55
Carbohydrate, max.	35	25	30	35

A detailed literature review and evaluation of the energy supply of mink and fox has recently been published (Enggaard Hansen et al. 1991). The authors recommend that a uniform basis for calculation of metabolizable energy values per gram digested nutrients should be agreed upon. It would be desirable if also the members of the International Fur Animal Scientific Association (IFASA) could agree on a universal basis for calculation of these values. In the Scandinavian countries, the following factors for calculation of ME from protein, fat and carbohydrates have been suggested:

Protein:	18.8 kJ (4.5 kcal) per gram digested
Fat:	39.8 kJ (9.5 kcal) per gram digested
Carbohydrate:	17.6 kJ (4.2 kcal) per gram digested

The percentage distribution of ME from protein, fat and carbohydrate has been retained as a basis for evaluation of the animals' energy supplies during various stages of their production cycle. Compared with previous Scandinavian recommendations, the most important difference is that the minimum recommended percentage of energy from protein has been reduced, whereas an increased amount of ME from fat was accepted. Tables 1 and 2 show the percentage standards for mink and foxes, respectively.

AMINO ACIDS AND PROTEIN

The extensive data available on protein and amino acid requirement and supply for mink have been reviewed and evaluated by Glem-Hansen (1990). Standards for amino acid supply were generally based on calculated dietary contents and only in a few cases on experimental work with one or more of the indispensable amino acids. The total body content of amino acids in growing mink increased similarly to that of the protein content. From the age of 20 weeks to pelting the deposition of cysteine increased relatively more than the protein content, which reflected the increased requirement for S-containing amino acids during the furring period (Glem-Hansen & Enggaard Hansen 1981). On the basis of a considerable number of experiments it was concluded that the content of S-containing amino acids (methionine + cysteine), equivalent to approx. 0.5 g/MJ (0.2 g/100 kcal) covers the requirement of mink during the growth period from weaning to August, whereas 0.7 g/MJ (0.3 g/100 kcal) should be anticipated during the furring period. Regarding the other indispensable amino acids, Glem-Hansen (1990) furthermore indicated the amounts required in the feed.

The evaluation in the review above was based on experiments with wet feedstuffs. Consequently, attention should be given to the risks associated with drying and heat treatment of the feed where both time and temperature are of importance. Most vulnerable are cysteine, lysine and arginine (Skrede & Krogdahl 1985) which may become unavailable to the animals.

In the pig, digestion has been thoroughly studied in the various parts of the alimentary tract by fistulation techniques (Just et al. 1985; Zebrowska et al. 1978). The results of these investigations showed that large quantities of amino acids were transported through the intestinal wall. In the anterior part of the duodenum, digestibility was 0 or even negative because of the endogenous excretion of mainly pancreatic juice. In the following sections of the duodenum digestibility increased due to the amino acid absorption. In colon/rectum the digestibility rate decreases, probably because of a slower decomposition of endogenous protein (enzymes and secreta), which may also result in a selective decrease in amino acid digestibility. Szymeczko & Skrede (1990) conducted a similar experiment with mink. Instead of being fistulated, the animals were euthanized, and the digestive tract was removed 1.5 and 3.5 hours, respectively, after ingestion of the experimental diet. The results followed the above-mentioned pattern but with a considerably more pronounced

negative digestibility in the first part of the small intestine than in other species. Furthermore, the digestibility of cysteine in fish protein was lower than the overall N-digestibility. This was even more pronounced for dried fish protein (fishmeal) in which also digestibility of aspartic acid was considerably reduced than for fresh fish protein.

The faecal digestibility might overestimate protein digestibility as a consequence of deamination of amino acids in the colon/rectum by microbial activity. However, this may be considered of minor importance in mink. Szymeczko & Skrede (1990) concluded that it would still be relevant to use faecal digestibility as a basis for future determinations of nutrient digestibility in mink.

MINERALS AND VITAMINS

Traditionally, research on mineral supply to mink and foxes has focused on deficiency problems such as anaemia and rachitis, and more specifically on the congenital screw-neck disease in pastel mink caused by manganese deficiency (Erway & Mitchell 1973). In 1982, NRC summarized the limited knowledge on the subject available at the time. The Scandinavian Association of Agricultural Scientists has resumed the subject and an updated review and evaluation of the relevant literature is now available (Tauson et al. 1991). This includes the first proper tabulation of the mineral contents of feedstuffs for fur animals, so in the future it will be possible to calculate the mineral contents of compounded diets.

For calcium and phosphorus, it was primarily a question of covering the requirements in the intensive growth period but more recent results show that the ratio between the two minerals is also of importance. The calcium-phosphorus ratio (recommended by NRC 1982) maximum is 2 but a ratio of this magnitude through the period from July to pelting resulted in a significantly lower body weight and a reduction in skin length of about 3 cm compared with ratios of approx. 0.9 and 1.4, respectively (Enggaard Hansen et al. 1991). Incidentally, these results are supported by results from experiments with ferrets (Edfors et al. 1990).

In recent years, the focus has been on sodium supply to mink during the lactation period on in relation to nursing sickness. Because of the short passage of time through the alimentary tract, the digestion of sodium is considerably lower in mink than in other monogastric species. Digestibility is only about 65 % of the amount supplied with the feed, and the addition of various water-absorbing substances may markedly reduce digestibility (Enggaard Hansen et al. 1985; Mäkelä et al. 1985). This is also a complication in the evaluation of older experiments in relation to nursing sickness, when various sodium concentrations are compared without a clear indication of whether water absorbents have been added to the actual experimental feed. Since Hartsough (1955) pointed out sodium deficiency as a cause of nursing sickness, no conclusive experiments have been carried out until recently, e.g. Clausen et al. (1992) and Wamberg et al. (1992). These authors describe the aetiology, pathology and pathophysiology of the disease, and they also point out important predisposing factors such as litter size and weight loss of the females, and that fluid therapy is essential in the treatment of the disease. They found a close relationship between the fluid metabolism and absorption. Factors affecting these have been investigated in a number of experiments and special reference can be given to reviews and original experiments by Neil (1992).

For many years iron deficiency anaemia has been known and described in relation to anaemiogenic fish species (*Gadidae*) (Ender et al. 1972; Työpönen & Lindberg 1988). One important factor for iron absorption in mink is cysteine as described by Skrede (1988), whose results were confirmed in a later study (Tauson & Neil 1992a & b). This study also shows that absorption may be affected by the dietary content of vitamin B_{12}, because oral supplementation increased iron absorption, whereas injection of the vitamin only causes an increase in the cobalt content of the liver reflecting an accumulation of the vitamin. Although the number of experimental animals was limited, the results were supported by different frequencies of white underfur for the two forms of application.

The available knowledge about vitamins for fur-bearing animals has been reviewed and evaluated (Juokslahti 1987) and since that time very little experimental data have been published, which must indicate that the vitamin supply for fur animals during the growth period covers their physiological requirement. The recommended standards for vitamin E (Juokslahti 1987), taking into account the varied dietary content of polyunsaturated fatty acids (PUFA), have been confirmed by mink experiments (Brandt et al. 1990; Rouvinen 1991), also during the reproduction period (Tauson & Neil 1991), and by experiments with fats of different origin in fox feeds (Rouvinen 1991).

PRODUCT QUALITY AND NUTRITION

The influence of nutrition on skin quality has predominantly been based on information on the raw, dried skin, sometimes supplemented with information about, e.g. colour, length of guard hair and underfur. Rouvinen's work (1991) on the influence of various fat sources on the fatty acid profile of the leather, as well as the importance of different storage conditions also related to the physical character of the leather, may be mentioned as an example of one of the few investigations within this field which also include the physical characteristics of the skin. A similar influence on the fatty acid profile of the leather of mink fed partly rancid marine oil (herring) during the growth period was demonstrated by Ulmanen et al. (1991), but the evaluation was only based on the weight of the animals and a conventional evaluation of the skin and not on the above-mentioned physical characteristics relating to leather quality.

Oxidation of fatty acids in the dried skins has also been mentioned as a problem. This is another field which ought to be elucidated under more well-defined conditions. Since the fatty acid profile may be affected, and because there are always considerable amounts of unsaturated fatty acids in the leather, there will be a latent risk of oxidation and despite the rate of the oxidation processes being temperature dependent, oxidation can be demonstrated even during freeze storage. Microbial activity with the consequent risk of enzyme production is also an important risk factor known from many practical storage situations, but in this connection it should be remembered that there are also chemical/biochemical methods and results (Michaelsen et al. 1991) which have demonstrated that the content of biogenic amines and certain fatty acids [palmitoleic acid (16:1(9c)) and vaccenic acid (18:1(11c))] produced by microbial activity increased when storage conditions were suboptimal. Such parameters will be useful tools in future investigations.

FUTURE RESEARCH ACTIVITIES IN THE FIELD OF NUTRITION

Generally, it should be borne in mind that because fur animals have so recently become domesticated the basic knowledge is inadequate in many respects, especially for the fox. However, in recent years the choice of research areas and tasks reflects some initiatives which, it is hoped, can be intensified.

The reproduction period is extremely important and seasonal activities regarding both dams and kits until an age of about 10 weeks must continue to have high priority. Energy and nutrient metabolism has certainly been the subject of many studies, but there is still a dearth of general basic knowledge on important points and hence of possibilities to explain the causal relations, with respect both to quality and especially to quantity.

Because of the reduction in the recommended percentage of ME from protein, there will naturally be a greater need for knowledge regarding amino acid requirement and regarding the content of accessible amino acids in the feed and hence the protein quality. The influence of the technical treatment of the individual feedstuffs will continue to be an important field of research, especially in relation the amino acid supply for fur animals. The development of the kits' production of digestive enzymes and the factors affecting it constitute an essential aspect of the research work carried out within the protein field.

The influence of nutrition on hair production and leather quality must be regarded as future fields of research activity, which ought to be developed in order to enable evaluation of optimal nutrition. Combined with the use of tools already established by extensive morphological studies of hair and leather, nutrition studies ought not to be limited to growth performance and conventional skin evaluation, but should also be related to effects of storage and preparation of the skin.

Finally, the economic difficulties of the fur trade ought also to be mentioned in this connection, because naturally they have had a hampering effect on research activities, especially on research work financed by commercial establishments. Unfortunately, in some countries public funds have also been reduced, with the result that the joint efforts in relation to nutrition and related research areas have diminished. For these reasons, and because of the complexity of future research work, it will be necessary to establish closer cooperation between individual research groups in order to continue to contribute the toward knowledge that also forms the foundation of the practical production.

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Use of ferret kits in the assessment of the biological value of protein in dehydrated mink feedstuffs

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Leoschke, W.L. 1992. Use of ferret kits in the assessment of the biologival value of protein in dehydrated mink feedstruffs. Norwegian Journal of Agricultural Sciences. Suppl. no. 9: 232-234. ISSN 0801-5341.

Practical mink ranch diets in North America provide the animals with about 32% of total metabolizable energy (M.E.) as digestible protein. Studies on the biological evaluation of protein quality in dehydrated mink feedstuffs with mink kits have used experimental diets with digestible protein quantity limited to 28% M.E. Research studies designed to employ ferret kits for the evaluation of protein quality in dehydrated mink feedstuffs indicate that digestible protein levels should be limited to 20% M.E.

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All factors considered, an examination of the true nutritional value of proteins present in dehydrated fur animal feedstuffs requires extended studies on biological evaluation throughout all phases of the ranch year - growth, fur development, and reproduction/lactation. Chemical evaluation including pepsin digestibility ratings, CS (Chemical Score), and EAA (Essential Amino Acid) indices are inadequate. Fur animal research facilities are limited in terms of number of animals available for experimental studies. It is simply impossible for fur animal nutritionists to conduct extended studies on each new dehydrated protein resource that appears on the world protein market. Hence, the absolute necessity for preliminary short-term experimental studies designed to act as a screening program with the potential to ascertain those dehydrated protein feedstuffs most deserving of long-term biological evaluation. These initial experiments conducted with weaning mink kits in June at the National Research Ranch are termed "Growth Promotion" (GP) studies. The potential for short-term GP studies significantly to expand the experimental work on the biological evaluation of protein resources is borne out by the fact that at the National Research Ranch we are able to conduct 12 to 16 GP trials each June but only six fur development and three reproduction/lactation experiments each year. Thirty years of GP studies at the National Research Ranch have shown that "All Fishmeals Are Not Created Equal", i.e., there is a very wide variation in the biological value of proteins present in fishmeals and other dehydrated protein feedstuffs available to the world fur industry. The GP studies have proven to be very useful as a screening mechanism in the selection of superior protein resources for additional long-range feeding trials. However, these GP studies with mink are limited to a period of one month each year, June, when weaning kits are available for experimental work. Thus, the decision to consider using weaning ferret kits which would be available a month later,July, as well as in other periods of the research year.

MATERIALS AND METHODS

The nutritional design and initial data on these GP studies were presented at the First International Congress on Fur Animal Production (Leoschke, 1976).

While most practical mink diets contain about 32% ME (metabolizable energy) as digestible protein, the GP studies with mink involved experimental diets with about 28% ME as digestible protein - the lower protein diets providing a degree of protein nutritional stress and thus an opportunity to assess the quality of proteins present in the new dehydrated protein resources.

The initial GP studies on ferrets with 28% ME as digestible protein yielded experimental data with the highest GP ratings ever recorded for dehydrated poultrymeals. These initial observations from GP studies on ferrets led to the experimental work being presented in this report. The study was designed to ascertain the level of protein nutritional stress (relatively low protein diets for rapidly growing kits) required with ferrets for the assessment of the quality of proteins present in dehydrated feedstuffs for modern mink nutrition.

The dietary design for the experiment presented in Table 1. Metabolizable energy calculations were based on the recommendations provided by the United States National Research Council Bulletin (NRC, 1982) - arbitrary digestibility and calorie values as fol-lows: protein 85 and 4.5, carbohydrates 75 and 4.0, and fats, 90 and 9.5.

Table 1. Composition of ferret experimental diets

Ingredients	% ME as digestible protein				
Grams	28	26	24	22	20
Quality white fishmeal	1520	1360	1210	1070	935
Pre-mix*	750	750	750	750	750
Rendered pork fat	950	950	950	950	950

*450 g potato flour, 240 g ground beet pulp, 30 g of a quality Vitamin Pre-mix and 30 g trace mineral salt

With weaning at six weeks, 20 litters of ferrets with five or more male kits were selected for the research work. the litters were divided by initial weights to provide five sets of twenty kits with ap-proximately equal average weights - about 500 g. The kits were paired in mink furring cages with a nest box - the toenail of one of the kits in each cage was clipped in order to facilitate identification. The experimental dietary mixture was provided twice a day for a period of two weeks with ample water provided by an automatic watering system.

RESULTS

Experimental data are presented in Table 2.

Table 2. GP studies - ferret kits - two-week gains

	Metabolizable energy as digestible protein				
%	28	26	24	22	20
grams	269 ± 46	271 ± 25	261 ± 43	255 ± 43	240 ± 52

DISCUSSION

It is apparent from the experimental data presented that GP studies with ferret kits for the biological evaluation of dehydrated protein feedstuffs for modern mink nutrition would require a maximum of 20% ME as digestible protein. It was found that the protein nutritional stress required for a valid assessment of the biological value of proteins in dehydrated mink feedstuffs was not provided by higher levels of protein content. This level of protein in GP diets is in sharp contrast to the 28% ME digestible protein currently utilized at the National Research Ranch for the biological evaluation of mink protein feedstuffs employing mink kits.

SUMMARY

In experimental studies on the evaluation of the biological value of dehydrated protein feedstuffs for modern mink nutrition, diets providing protein nutritional stress with a digestible protein level of 20% metabolizable energy should be used.

ACKNOWLEDGEMENTS

The experimental studies were conducted at the National Research Ranch Oshkosh, Wisconsin, with the support of the Milk Specialties Company, New Holstein, Wisconsin.

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Effect of flushing on LH release in mink

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LH release, oestradiol-17β, and number of corpora lutea (CL) were studied in two groups of 10 standard mink females after a single mating on 10 March and after the second of two matings on 9 and 17 March. The control group was kept in energy equilibrium, while in the flushing group, a two week period of 20% energy restriction was followed by *ad libitum* feeding from 5 March. Blood samplings, twice daily (07.00 h and 15.00 h), were started one day before and lasted until 3 days after matings. Generally, LH peaks were more distinct and more synchronized in the flush-fed females than in controls. Elevated concentrations of LH tended to be recorded later after the second matings than after the single matings. For females with distinct peaks, elevated LH concentrations were recorded during approximately 24 h.

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Flushing of mink, carried out by means of a two-week period of moderate restriction in energy allowance followed by *ad libitum* feeding from 3 to 5 days prior to the start of the mating season until matings were completed for the individual females, resulted in increased litter size, mainly in yearling females (Tauson 1985, 1988) but also in adults (Tauson 1988). The increase in litter size corresponded to an increased number of corpora lutea (CL) (Tauson et al. 1988; Tauson 1991). The positive effects of flushing have generally been recorded in females with a first mating early in the mating season and remated after 8-10 days, thus having two ovulations and two sets of CL. For females with a late first mating and remated on the following day, flushing did not improve reproductive performance (Tauson 1987). Plasma progesterone levels were not affected by flushing (Tauson et al. 1988; Tauson, 1991), but the interval between the first day of recorded increase in plasma progesterone over basal levels and the recorded day of peaking was reduced as an effect of flushing (Tauson et al. 1988). Apart from an increased number of CL, the increased number of growing follicles in the flush-fed females was indirectly demonstrated by increased levels of oestradiol-17β compared with the control animals (Tauson 1991). Moreover, flushing seems to enhance the early development of fertilized eggs in females mated early in the breeding season, whereas the difference between flush fed and control females declined as matings were delayed (unpublished observations).

The available information on the physiological effects of flushing in mink thus

indicates that flushing may enhance follicle maturation and increase ovulation rate. The present investigation was designed to evaluate effects of flushing on LH release, oestradiol-17β levels and number of CL in mink females mated once on 10 March or twice on 9 and 17 March , respectively.

MATERIALS AND METHODS

The experimental animals were female mink of the standard colour type aged 2-4 years. Weighing and grouping of the animals in a control and a flushing group, each including 10 females, was carried out on 12 February. Five females from each group were assigned for mating once, on 10 March, and the remaining females for mating twice, on 9 March and 17 March. The main experimental design is presented in Table 1. The animals were fed the standard ration of the farm, the percentage composition of which was as follows: cod offal 36%, Baltic herring and filleted scraps of the same 16%, slaughter house offal and poultry waste 20%, extruded cereal mixture, potato mash powder and steamed rolled oats 9-10%, wheat germ 1%, and wheat bran 1%. Vitamins were added according to standards (Juokslahti 1987). The percentage distribution of metabolizable energy (ME) was 47% from protein, 36% from fat and 16% from carbohydrates. The ME content of the diet was 16.9 MJ/kg dry matter (DM). Daily feed allowances are reported in Table 1.

Table 1. Experimental design and daily feed allowances in experiment regarding effect of flushing on LH release in mink

	Control		Flushing	
	Mating once	Mating twice	Mating once	Mating twice
No. of females	5	5	5	5
Period start for				
feed restriction	-	-	16 February	16 February
flushing	-	-	5 March	5 March
Daily feed allowance, (g)				
during restriction	190	190	150	150
during flushing	190	190	275	275
Date(s) of mating	10 March	9+17 March	10 March	9+17 March
Sampling period	9-13 March	16-20 March	9-13 March	16-20 March

The females were tried for mating and remating only on the planned dates. Blood samplings were performed by clipping the tip of a claw or by venopuncture in the ventral side of the tail twice a day starting at 07.00 h and 15.00 h the day before the single mating (9 March) or the second mating (16 March) and lasting until three days after matings (see Table 1). Blood was collected in heparinized tubes, and the separated plasma was stored at -20°C until assay. LH was analysed by radio immunoassay (RIA) according to the procedure described by Forsberg et al. (1989). Oestradiol-17β was analysed by enzyme immunoassay

(EIA) as described by Jones & Madej (1988). Severial dilutions of mink plasma produced inhibition curves parallel to the standard curves. The females were killed six days after the single or second mating. After fixation and embedding, the ovaries were serially sectioned in 10-μm-thick sections and every 20th section was stained with haemalum eosin and examined by light microscopy for number of CL.

Statistical analyses were carried out according to the GLM procedure in SAS (SAS Institute Inc., 1982). Animal live weight changes were analysed for effect of treatment. For the number of CL a model comprising effect of treatment group, mating system and interaction between treatment group and mating system was used. LH- and oestradiol-17β profiles were evaluated according to a split plot design model including fixed effects of treatment group, mating system, and time of sampling, effect of random female within treatment group and mating system, interaction effects between treatment group and mating system, treatment group and time of sampling, mating system and time of sampling, and, finally, treatment group mating system and time of sampling. Treatment group was tested against female effect, thus using female within treatment group and mating system as an error term when evaluating treatment effects. Results are given as means of least squares according to the described models.

RESULTS

One control female did not accept mating on 10 March, and two out of the five females tried for remating on 17 March did not remate. Moreover, endocrinological data of one control female indicated that she had ovulated as a result of handling. All flush-fed females mated as planned on 10 March, but one female did not remate on 17 March. Two other females mated on 10 March and one female mated on 9 and 17 March had to be excluded because of suspected ovulation as a result of handling. Hence, the results reported are based on data from three females per treatment, making å total of 12 females.

During the restriction period the flush-fed females had a moderate weight loss, whereas the control group females were maintained at close to energy equilibrium. During the flushing period both groups gained weight, the increase being greatest in the flushing group (Table 2).

Significant LH peaks were found in all treatments ($p<0.001$). Generally, the appearance of the individual LH release profiles indicated that flush-fed females had more pronounced and distinct peaks compared with control females. Furthermore, the peaks were more synchronized in females subjected to flushing, but treatment effects were non-significant ($p=0.29$). Elevated levels of LH were recorded at the first sampling, which is less than 6 h after mating for females with a single mating. Females performing two matings tended to have elevated concentrations of LH later than females mated only once. (Interaction effect between mating system and time of sampling $p=0.13$.) For females with distinct LH peaks, high concentrations of the hormone were recorded in peripheral circulation during approximately 24 h (Fig. 1). Plasma oestradiol-17β concentrations increased in all females from the start of sampling and after a peak following mating the levels declined, but treatment effects were non-significant. The number of CL was significantly affected by mating system, demonstrating that females mated twice had two

sets of CL. Flushing did not conclusively affect the number of CL (Table 3).

Table 2. Animal live weight changes (g) during restriction and during flushing (n=12)

	Treatment group		P-value; effect of treatment
	Control	Flushing	
Females mated once, on 10 March			
Weight change, g			
during restriction	-8	-71	0.25
during flushing	32	130	*0.04*[a]
Females mated twice, on 9 and 17 March			
Weight change, g			
during restriction	16	-44	0.06
during flushing	44	121	0.07

[a] Value in italics indicates a significant treatment effect

Table 3. Number of CL as an effect of treatment group and mating system (n=12)

	Control	Flushing	P-value;		
	Mating once	Mating twice	Mating once	Mating twice	effect of mating system[a]
No. of CL	9.3	17.0	10.7	14.7	*0.001*[b]

[a] Effect of treatment group and interaction between treatment group and mating system were also tested but found non-significant

[b] Value in italics indicates a significant effect of mating system.

DISCUSSION

LH release profiles after natural mating have not previously been reported for mink. Because of sampling frequency, the present data do not give any information on LH pulse frequency and amplitude, but clearly demonstrate that LH peaks occur within a few hours after mating and last for approximately 24 h. Elevated LH concentrations in peripheral circulation for a protracted period are not an unexpected finding considering the number

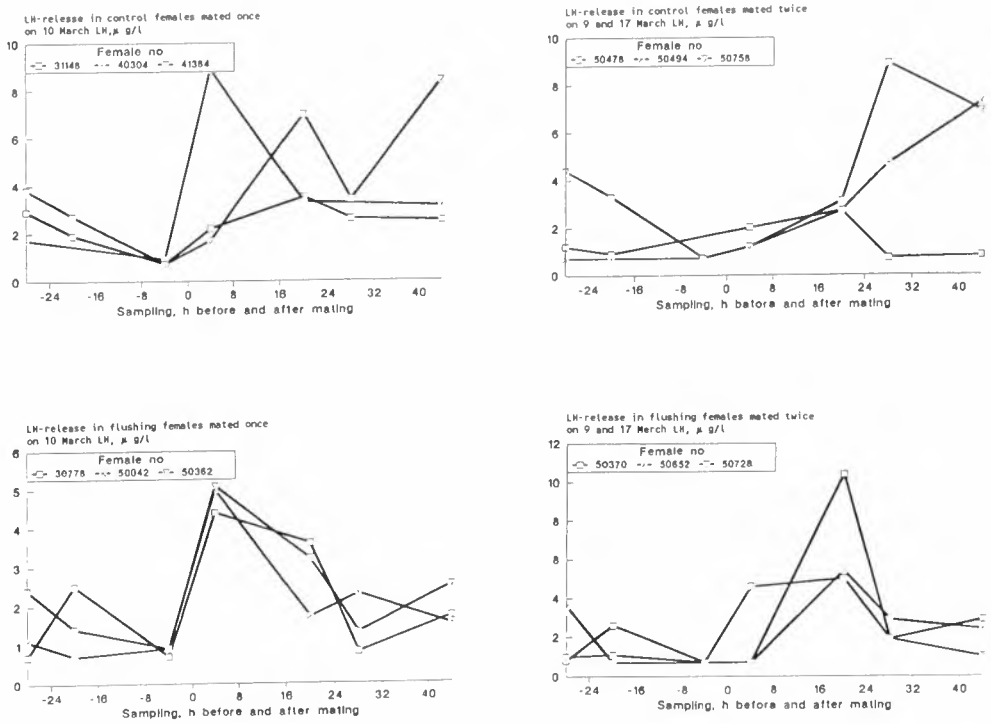

Fig. 1. Individual LH release profiles for control and flushing females after a single mating on 10 March and after the second of two matings on 9 and 17 March

of eggs ovulated in the mink. Similar patterns are seen in other polytocous species, for instance the bitch (Concannon et al. 1977) and the vixen (Farstad et al. 1989). The tendency for LH concentrations to increase later in females after the second mating could be a consequence of two ovulatory periods - an existing set of progesterone secreting CL from the first ovulation having an inhibitory effect on LH release. This theory is supported by the significantly increased number of CL in females mated twice.

The present results are in good agreement with previous findings which indicate that flushing of mink influences oestradiol-17β (Tauson 1991) and progesterone (Tauson et al. 1988) secretion from the ovaries and enhances early development of fertilized ova, especially in females mated early in the breeding season (unpublished observations). The flushing treatment, although non-significant, tended to give more distinct and synchronized LH peaks. In other species, LH pulse frequency increases shortly after realimentation of previously restricted animals (I'Anson et al. 1991). It is therefore possible that the suppressive effect of restricted feeding may be suspended by increased energy intake, thereby enabling other neural and hormonal signals to exert an influence on the hypothalamic-pituitary-gonadal axis.

Our conclusion is that a period of restricted energy allowance followed by *ad libitum* feeding before and during the breeding season may induce changes in endocrine parameters resulting in enhanced growth and maturation of follicles, and possibly also may improve viability of fertilized eggs in females mated early in the breeding season.

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Different dietary fat:carbohydrate ratios for blue fox in the reproduction period. Effects on reproduction, kit growth, milk composition and blood parameters

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The aim of this study was to examine the effect of different dietary fat:carbohydrate ratios on blue fox in the reproduction period. The fat:carbohydrate ratios in the experimental diets on a metabolizable energy basis were 55:1, 43:13 and 35:25. In an experiment, which included 39 blue fox females, blood analyses were carried out at eight stages from *ca.* 25 days prepartum to *ca.* 42 days postpartum. Plasma concentrations of triacylglycerols, free fatty acids, cholesterol, acetoacetate, glucose, albumin, total protein, urea, alanine aminotransferase, aspartate aminotransferase, calcium and phosphate revealed characteristic changes due to pregnancy and parturition. A high fat:carbohydrate ratio in the diet promoted a significantly higher plasma content of cholesterol and acetoacetate. The milk fat content tended to increase with increasing fat:carbohydrate ratio in the feed. The number of kits born was not affected by diet. The kit mortality was highest in the group receiving the highest fat:carbohydrate ratio. On the other hand, kit growth was reduced on the lowest dietary fat:carbohydrate ratio.

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The economic depression in fur animal production of recent years has intensfied efforts to reduce feed costs. One way of doing this has been to use fat more extensively as a main energy source. In Norway, fat energy is cheaper than carbohydrate energy, which implies that by increasingly replacing carbohydrates with fat, feed expenses will be reduced. The increase in use of fat has, however, coincided with a stagnation or even a decline in blue fox reproduction, mainly as a result of a higher kit mortality. It is likely that a high fat:carbohydrate ratio of the feed for blue fox females in the period from December to parturition is unfavourable. This is because a high fat:carbohydrate ratio gives a high-energy diet, which complicates the body weight reductions necessary before the reproduction season. On the other hand, after parturition, a higher dietary fat:carbohydrate ratio is desirable to cover the energy requirements for lactation and kit growth.

In this study, the influence of different dietary fat:carbohydrate ratios for blue fox females was observed during pregnancy and lactation in relation to reproduction, kit growth, milk composition and blood parameters.

MATERIALS AND METHODS

The experiment was carried out at the experimental farm of the Department of Animal Science at Ås. The experimental period was from 15 January to 3 August, 1990. At the end of the experiment, the last-born litter had reached 49 days of age. Each of the three experimental groups comprised 13 female blue foxes with an equivalent age distribution in each group. The animals were housed in ordinary double-spaced breeding cages equipped with wooden nestboxes. Single mating was performed and a heat detector was used to determine the optimum mating time.

The experimental feeds were made up of cod scrap, slaughterhouse offal, blood, fish meal, tallow, soybean oil, precooked wheat/oats, extruded corn (Suprex Corn), water and recommended amounts of vitamin mixture and iron supplement (Hemax). The calculated dietary fat:carbohydrate ratios on a metabolizable energy basis were: Group 1, 55:1; Group 2, 43:13; Group 3, 25:31.

The fat:carbohydrate ratios of the experimental feeds were obtained by replacing precooked wheat/oats and extruded corn with tallow and soybean oil. The animals were fed once a day. Restricted feeding was adopted prepartum but free access was allowed thereafter.

The body weights of females were recorded every four weeks before mating, at parturition and at 49 days postpartum. The litters were weighed at birth, at 21 and 49 days of age. Individual kit body weights were recorded at 49 days of age. Milk samples (2-5 ml) were taken at 7, 21 and 42 days postpartum. The samples were obtained by hand-milking after an injection of 0.2 ml oxytocin. Chemical analyses of the milk (dry matter, protein fat) were carried out at The Control Institute of Dairy Products, Oslo. Feed analyses were performed at the Norwegian Fur Breeders' Associaton, Oslo. Blood sampling was executed 25, 45 and 50 days after mating, and 1, 7, 14, 21 and 42 days postpartum. The sampling was carried out about 22 h after feeding. The blood samples (5 ml) were collected from a vein in the front leg. Plasma was obtained after 10 min centrifugation at 3000 rpm. The samples were stored at -70°C. Plasma concentrations of triacylglycerols, free fatty acids, cholesterol, acetoacetate, glucose, albumin, total protein, urea, alanine aminotransferase, aspartate aminotransferase, calcium and phosphate were determined on a multianalyser (Encore). Differences among means were tested statistically by analysis of variance.

RESULTS AND DISCUSSION

Chemical analysis of the experimental feeds indicated that the fat:carbohydrate ratios were close to the calculated values. The content of metabolisable energy (ME/kg) was 6.15, 5.65 and 4.10 MJ in Groups 1, 2 and 3, respectively. The differences in energy content were due to different fat:carbohydrate ratios and different amounts of water added to adjust the consistency of the feeds.

Thirteen females were not mated or were found to be barren (Table 1). Blood tests from these were excluded from the material which left 8, 9 and 9 females in Groups 1, 2 and 3, respectively.

Table 1. Reproduction, average kit weights and milk composition of blue fox fed different dietary fat:carbohydrate ratios

	Group 1	Group 2	Group 3
Fat:carbohydrate ratios	55 : 1	43 : 13	31 : 25
Number of mated females	10	10	10
Born litters	8	9	9
Kits pr. litter			
Born	11.8 (3.2)[1]	10.0 (3.0)	12.9 (1.9)
21 days postpartum	5.4 (5.7)	7.4 (3.3)	7.4 (5.5)
49 "	4.0 (5.0)	6.1 (4.3)	7.0 (5.1)
Kit weights, (g)			
Birth	68	76	71
21 days of age	408	377	277
49 "	1595a[2] (243)	1634a (180)	1351b (293)
Milk composition, (%)			
Dry matter	28.2 (7.0)	24.9 (5.2)	22.8 (5.2)
Fat	13.1 (5.0)	11.2 (4.0)	10.8 (4.7)
Protein	10.3 (3.6)	8.3 (2.5)	8.0 (2.2)

[1]Standard deviation

[2] Differences between means with different superscripts are significantly different ($p < 0.05$)

Pölönen (1990) demonstrated that the reproduction result of blue fox is negatively affected by the high body weight of the female at mating and by late parturition. In this experiment, the last weighing before mating (5 March) revealed these average body weights, 6.9, 6.7 and 6.7 kg in Groups 1, 2 and 3, respectively. The average body weight of the females at parturition was 5.3 kg in all the groups. Even the above-mentioned weights are not the actual ones at mating, it is likely that the average body weights at mating were above optimum for reproduction. The negative effects of the body weights should, however, be quite equally distributed among the experimental groups. The average time of parturtion was similar among the groups: for Groups 1 and 2, 2 June and for Group 3, 31 May.

The health conditions of the females were satisfactory, and no deaths occurred during the experimental period. The average litter size was normal in every group, but a considerable kit loss, including considerable individual variations, was observed. Most of the kit mortalities happened within a few days after parturition, declining to a low level at 21 days postpartum. Group 1 had the highest kit loss, but due to the limited material there were no statistically significant differences. In similar experiments Rimeslåtten (1976) and Fors et al. (1990) also found a tendency to a higher kit mortality in litters from females receiving a high dietary fat:carbohydrate ratio. Fors et al. (1990) found reduced liver glycogen stores in kits littered by females fed a high dietary fat:carbohydrate ratio one week prior to parturition, which should give the kits less chance to survive the first 24 hours. The liver glycogen stores of the kits were not determined in the present experiment, but a depleted glycogen store is a probable explanation for early kit mortalities. However,

the results in this experiment concerning kit mortalities varied considerably within the experimental groups, which indicates that the mortalities were not caused by dietary factors only.

The average kit growth, based on the body weights at 49 days of age, was significantly lower in the group (Group 3) receiving the lowest fat:carbohydrate ratio of the feed ($P < 0.05$). This difference is probably caused by a higher fat deposition in the groups receiving higher dietary fat:carbohydrate ratios, as Skrede (1981) found in mink. Furthermore, increasing litter size reduced the body weights significantly at 49 days of age ($P < 0.05$), producing even lower average body weights in Group 3.

The chemical composition of the milk varied independently of group and time of sampling, and no significant differences were detected among the groups for dry matter, fat and protein content of the milk. However, an increasing fat:carbohydrate ratio tended to increase both the fat and protein content of the milk (Table 1). This partly disagrees with results from Fors et al. (1990), although the results are not strictly comparable because of differences in the experimental design.

Generally, the blood parameters revealed characteristic changes due to pregnancy and parturition and confirm results from Näveri et al. (1988, 1989). It should also be pointed out that apart from dietary factors, the number of kits is a factor that could affect the blood parameters both prepartum and postpartum.

Among the dietary related blood parameters, the blood lipids content reflected the fat:carbohydrate ratio of the feed. But only cholesterol and acetoacetate showed significant differences between the diets (Fig. 1). Cholesterol levels 14 and 21 days after parturition, and acetoacetate levels 7 days after parturition, were higher in the group receiving the highest dietary fat:carbohydrate ratio ($P < 0.05$). Furthermore, the acetoacetate concentrations at 7 days and 2 days prepartum seemed to be in reverse ratio to the glucose level, though a considerable variation was observed.

Other blood lipids like triacylglycerols and free fatty acids tended to increase with a higher fat:carbohydrate ratio. These results are in accordance with those reported by Fors et al. (1990). Also, the albumin plasma concentration, which is involved in plasma lipid transport, tended to increase with increasing dietary fat:carbohydrate ratio and is partly the reason for the differences in total plasma protein (Fig. 2). Only insignificant differences were detected in plasma urea, ALAT, and ASAT (Fig. 2). The rise in these values at postpartum is probably due to lactational emancipation.

The plasma concentrations of calcium and phosphate showed changes mainly due to lactation and were not directly influenced by dietary factors.

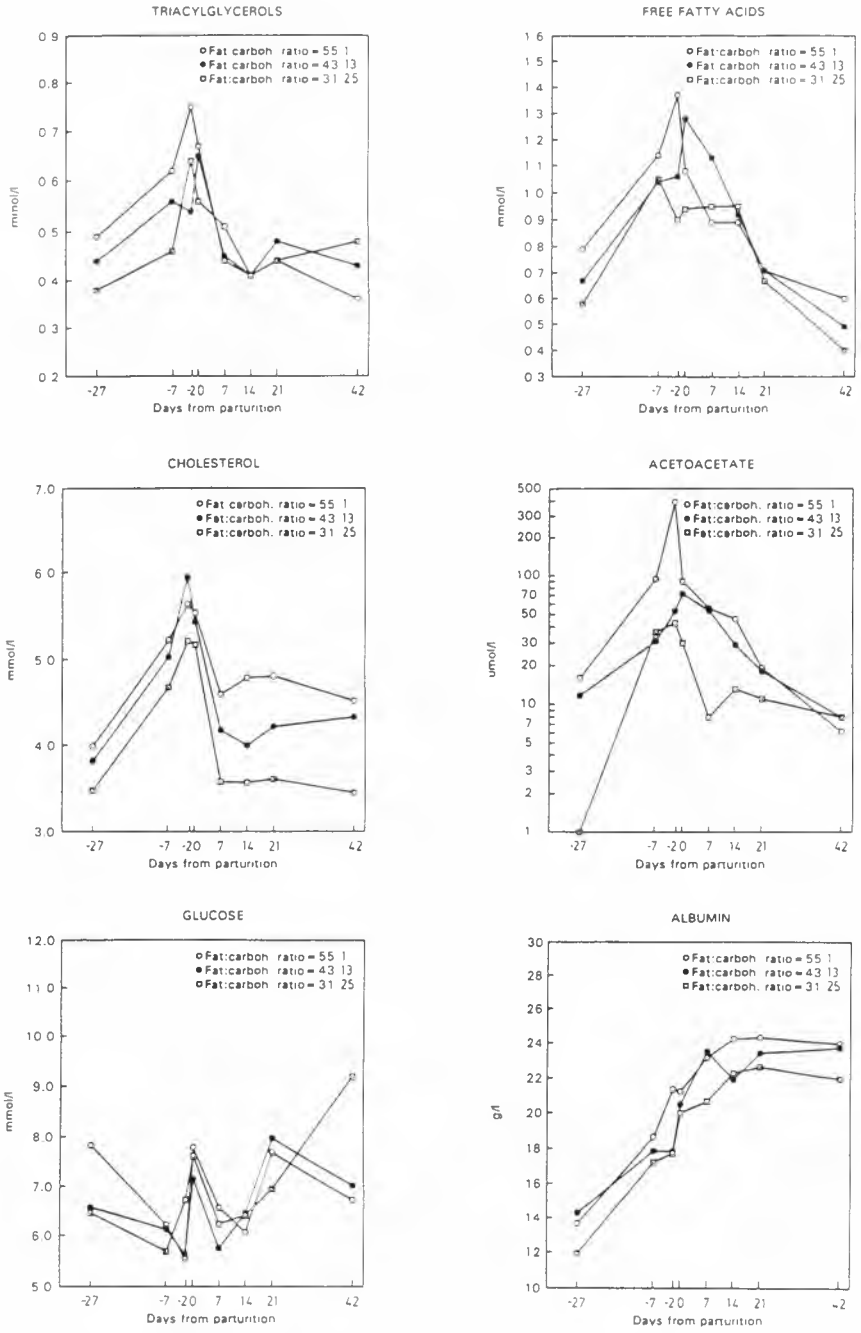

Fig. 1. Average plasma concentrations of triacylglycerols, FFA, cholesterol, acetoacetate, glucose and albumin in blue foxes fed different dietary fat:carbohydrate ratios

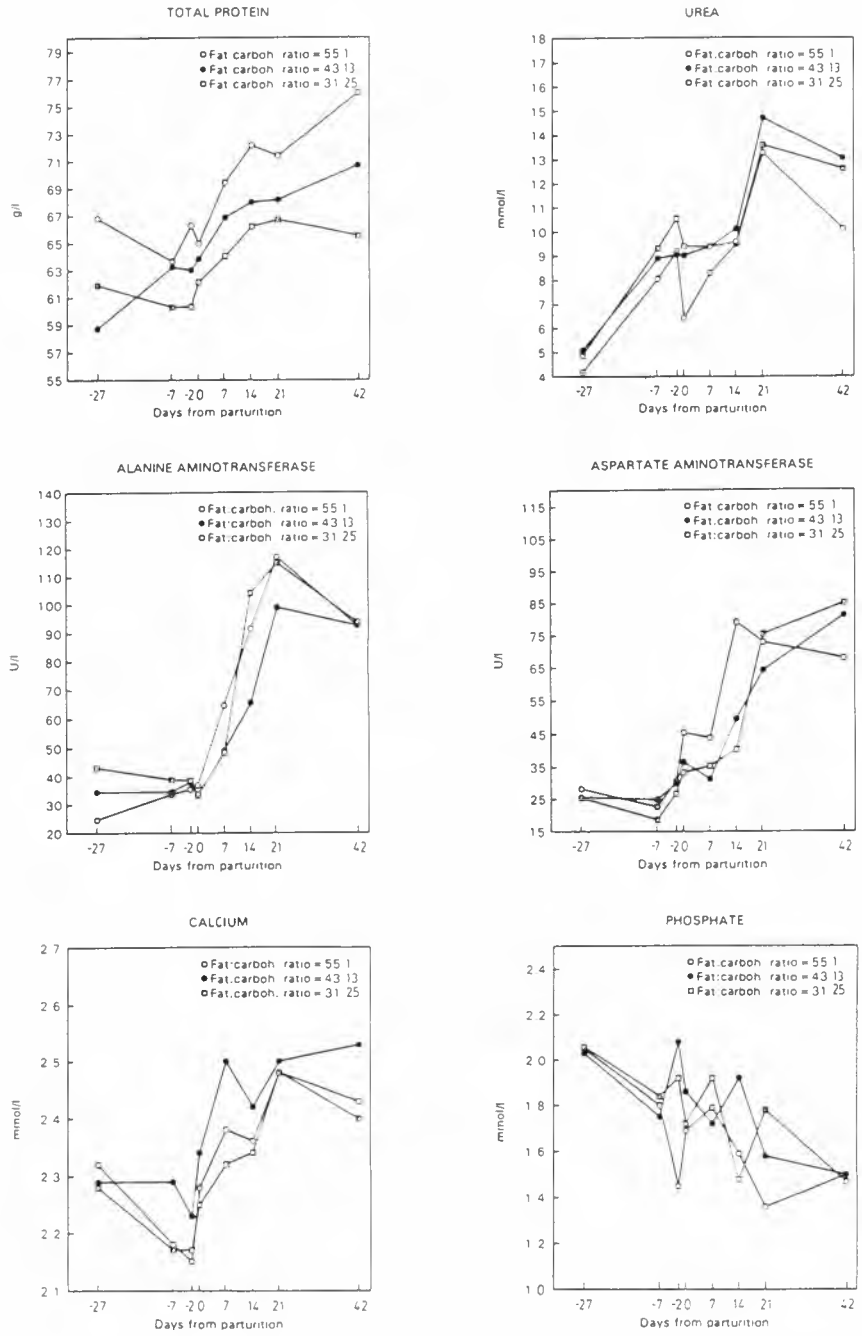

Fig. 2. Average plasma concentrations of total protein, urea, ALAT, ASAT, calcium and phosphate in blue foxes fed different dietary fat:carbohydrate ratios

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Effects of fish fat feeding on body fat composition of foxes

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Ranched blue and silver foxes were fed saturated fat (SF) and fish fat (FF) diets from weaning to pelting in order to clarify the effects of accumulation of omega-3 fatty acid in the body. Five males from each dietary group were sampled. The dietary background significantly influenced the fatty acid composition of all body fat depots in both fox species. The animals from the FF group had considerably more eicosapentaenoic (EPA), docosahexaenoic (DHA) and cetoleic acids in their tissues than the animals of the SF group. Moreover, silver foxes in the FF group had significantly higher levels of DHA in the liver (21.7%) compared to blue foxes (17.0%). In the SF diets, fat accumulated in the liver in large droplets, while in the FF diets it was present in small droplets. Furthermore, according to the histopathological evaluation, degenerative changes were more numerous and severe in the FF dietary group.

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The diet of farm-raised carnivorous fur-bearing animals is mainly compounded of fish and slaughterhouse offal, both of which are good sources of high quality protein and fat. Fat may also be supplemented to diets in order to maintain the optimal energy supply during different stages of the production year. The fat sources usually used are tallow, lard, destruction fat, vegetable oils, and fish oils. Typical of fish oils is their high content of the polyunsaturated omega-3 fatty acids: eicosapentaenoic (C20:5ω3 EPA) and docosahexaenoic (C22:6ω3 DHA) acids. Some fish oils also contain an isomer of the monoenoic erucic acid, namely cetoleic acid (C22:1ω11). Unlike mink in the wild, the feral silver and blue foxes consume fish only occasionally if ever (Linscombe et al. 1982; Samuel & Nelson 1982). During their evolution, the animals have adapted most efficiently to using the food sources available in their natural habitat (Nelson & Ackman 1988). It is possible that their intermediary metabolism cannot utilize dietary components which are not normally consumed. The aim of this study was to clarify the effects of animal fat versus fish fat feeding on blue and silver fox tissues.

MATERIALS AND METHODS

In the first experiment, blue and silver foxes were fed either a saturated fat (SF) or a fish fat (FF) diet. The SF diet was based on beef offal having tallow and fur animal carcasses

as the fat source, while the FF diet was based on fish offal having fish oil as the only fat source. In the second experiment, blue and silver foxes were fed diets supplemented with either lard (LD) or fish oil (FO), 5% in the diet. In the first trial, the fat level varied from 19-21% and in the second trial from 27-30% in dietary dry matter. The content of the typical fish fatty acids in the experimental diets is presented in Table 1. Both trials lasted from July until pelting in November or December, 1988. From each dietary group five males were sampled. Fatty acid composition of the liver, heart and subcutaneous fat was determined by gas chromatography (see Rouvinen 1991). Liver fat content was analysed as described by Maxwell et al. (1980). In experiment 1, the histopathology of the tissues was also examined. Statistical analysis was performed following the General Linear Models (GLM) procedure of the SAS, and the differentiation among the mean values by Duncan's multiple-range test (SAS Institute Inc. 1985).

Table 1. Content of cetoleic, eicosapentaenoic and docosahexaenoic acids in blue and silver fox diets in November 1988. Diets in experiment 1, saturated fat (SF), fish fat (FF); in experiment 2, lard (LD) and fish oil (FO)

Fatty acids	Expt. 1		Expt. 2	
% in fat	SF	FF	LD	FO
C22:1ω9+11	-	11.5	0.4	7.6
C20:5ω3	-	7.0	0.3	4.2
C22:6ω3	-	8.7	0.5	4.3

RESULTS AND DISCUS-SION

In experiment 1, the liver fat content of the blue foxes fed the FF diet was significantly higher than that in the blue and silver foxes fed the SF diet (Table 2). According to the histopathological examin-ation, the fat accumu-lation pattern differed be-tween the diets. In the SF diets fat accumulated in the liver in large droplets, while in the FF diets it was present in small droplets. Furthermore, degenerative changes were more numerous and severe in the FF dietary group (see Rouvinen 1991). In experiment 2, the blue foxes had a higher fat content in their liver on both diets than that in the silver foxes. No clear conclusions can thus be drawn from these results.

Dietary influences were clear in all body fat samples in both fox species (Table 2). The samples collected from the fish fat dietary groups (FF, FO) contained considerably more EPA, DHA and cetoleic acid than those from the tallow (SF) or lard (LD) dietary groups. The content of DHA was significantly higher in silver fox liver than in blue fox livers. Moreover, cetoleic acid was more prominent in silver fox heart and subcutaneous fat than in the corresponding tissues of the blue foxes.

Fish oil or high-erucic acid rapeseed oil have been shown to cause accumulation of fat accompanied by cell destruction, local inflammatory reactions and fibrous scar tissue growth in the heart muscle of the rat (Kinsella 1987). This is due to impaired fatty acid oxidation by the mitochondria. The 20 or 22 carbon atom-chained fatty acids must be shortened in the peroxisomes to 16 or 18 carbon atomfatty acids before their oxidation in

Table 2. Fatty acid composition of the tissue of blue and silver foxes. Diets in experiment 1, saturated fat (SF), fish fat (FF); in experiment 2, lard (LD) and fish oil (FO). Main effects included in the model are species (S), diet (D) and their interaction

Fatty acids % in fat	Blue fox		Silver fox		Significance		
Expt. 1	SF	FF	SF	FF	S	D	SxD
Liver							
fat %	3.7b	4.6a	3.8b	4.0ab	NS	<0.05	NS
C22:1ω9+11	-	0.4	-	0.4	NS	-	-
C20:5ω3	1.1b	9.0a	1.1b	8.7a	NS	<0.001	NS
C22:6ω3	7.6d	17.0b	10.8c	21.7a	<0.001	<0.001	NS
Heart							
C22:1ω9+11	0.2c	2.8b	0.2c	4.9a	<0.05	<0.001	<0.05
C20:5ω3	1.0c	7.4b	0.8c	8.7a	NS	<0.001	<0.05
C22:6ω3	3.0b	8.8a	3.1b	9.1a	NS	<0.001	NS
Subcut. fat							
C22:1ω9+11	0.2c	3.1b	0.2c	6.1a	<0.01	<0.001	<0.01
C20:5ω3	0.2b	2.0a	-	2.0a	NS	<0.01	-
C22:6ω3	0.4c	3.8b	0.4c	4.8a	NS	<0.001	NS
Expt. 2	LD	FO	LD	FO			
Liver							
fat %	4.9a	5.1a	4.1b	3.7b	<0.001	NS	NS
C22:1ω9+11	-	0.2	0.2	0.4	NS	NS	-
C20:5ω3	2.0d	6.2b	3.2c	7.1a	<0.01	<0.001	NS
C22:6ω3	10.2c	18.2b	19.9b	24.1a	<0.001	<0.001	<0.05
Heart							
C22:1ω9+11	0.2c	1.7b	0.5c	3.4a	<0.001	<0.001	<0.001
C20:5ω3	1.5d	6.8b	3.1c	8.3a	<0.001	<0.001	NS
C22:6ω3	3.8d	8.8b	6.2c	9.9a	<0.001	<0.001	<0.01
Subcut. fat							
C22:1ω9+11	0.2c	2.9b	0.2c	3.8a	<0.01	<0.001	<0.001
C20:5ω3	0.2b	1.8a	0.3b	1.8a	NS	<0.001	NS
C22:6ω3	0.6b	3.4a	1.0b	3.6a	NS	<0.001	NS

a-d: means within columns not sharing any common postscript are significantly different ($p < 0.05$)

the mitochondria can take place (Opstvedt 1984). It is unlikely that the longer feeding period explains the greater accumulation of these marine fatty acids in silver foxes, since their total feed consumption over time is lower than that of the blue foxes. There may, however, be a species difference in the ability to metabolize these polyunsaturated fatty acids. Heavy fish and fish fat feeding and cetoleic acid accumulation may thus be one reason for the so far unexplained cardiac failure observed in silver foxes in Finland (Smeds 1992). In addition, up to 25% of fur animal carcasses have been used in fox diets during the growing-furring period (Wallin 1991). Therefore, fur animal carcasses must also be considered as an additional source of the marine fatty acids, since they accumulate in high concentrations in all body tissues.

SUMMARY

Two feeding trials were performed with blue and silver foxes from weaning until pelting 1988. The foxes were fed either an animal fat (experiment 1, tallow; experiment 2, lard) or a fish oil supplemented diet. At pelting, liver, heart and subcutaneous fat samples were taken for fatty acid analysis from five males in each dietary group. Liver fat content was also analysed. In experiment 1, tissue samples were examined for histopathological changes. In both fox species, the tissue fatty acid composition strongly reflected that of the respective diets. In the fish fat dietary group, fat was accumulated in the liver in small droplets, while in the tallow diet it was present in large droplets. Degenerative changes were also more numerous and severe in the former group. The level of docosahexaenoic acid (C22:6ω3) was significantly higher in the silver fox livers than in the blue fox livers. Furthermore, the accumulation of cetoleic acid (C20:1ω11) was more prominent in the silver fox heart muscle and subcutaneous fat than in the corresponding tissues of the blue fox.

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Accuracy of nitrogen balance measurements in adult mink

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When comparing nitrogen balances measured in traditional balance trials with balances measured by slaughter techniques it is found that, the balance trials tend to overestimate the N retention. Some reasons for these differences might be an inappropriate collection of nitrogen excreted in the feces and urine and loss of nitrogen in the form of volatile ammonia. In 36 balance studies carried out in respiration chambers, the amount of ammonia released in 24 h accounted for 1.7-2.9% of the nitrogen found in urine. Data from method studies, in which mink urine with a known N concentration was used, showed that by applying different collecting and washing routines, 62 to 71% of the nitrogen excreted could be accounted for. Therefore, when performing nitrogen balance trials, it is recommended that the retrieval percentage of nitrogen for the routine applied be estimated.

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Nitrogen balance studies in farm animals are important in determining the protein requirement, evaluating the biological value of protein, or, in combination with respiration experiments, calculating the energy metabolism. The nitrogen balance can be measured by two different methods. One is the slaughter technique, by which nitrogen retention is measured as the difference in body N between animals slaughtered at the beginning and at the end of the experiment. The other is the balance technique, by which N retention is calculated as the difference between ingested nitrogen and nitrogen excreted in the feces and urine. The slaughter technique tends to underestimate the N retention because of difficulties in analyzing the total body N, but, on the other hand, the balance technique tends to overestimate the N retention primarily because of inadequate collection of feces and urine (Neergaard 1981; Just et al. 1982; Eggum 1989).

Studies comparing the slaughter technique and the balance technique have shown great discrepancies in nitrogen deposition between the two methods. In studies with rats and pigs the discrepancies vary from a few percent up to 72% (for a review see Just et al. 1982 and Eggum 1989). Enggaard Hansen et al. (1981) measured the N retention in growing mink, in the period from June to November, using the two techniques and found a discrepancy as high as 330%. A causative factor might be the high urea concentration in mink urine due to the high protein intake by this carnivore species. Overestimation of the nitrogen balance

in mink has been reported, e.g. by Charlet-Lery et al. (1980) who found a daily nitrogen deposition of 0.31 g on a yearly basis, corresponding to a gain of about 710 g protein/year, in adult mink that maintained their body weight throughout the experimental period.

The present study was carried out in order to optimize and standardize the procedures involved in measurement of the nitrogen balance of adult mink.

MATERIALS AND METHODS

Experiment I: The balance study was carried out using five groups (A-E) each comprising six adult mink males (pastel type) randomly selected from the breeding stock of the research farm. The animals were kept individually in metabolism cages (modified after original design by Jørgensen & Glem-Hansen 1973) in the laboratory. With the exception of one animal which was excluded from group A because of feed refusal, all the other animals behaved normally. The animals had free access to water and were fed *ad libitum* on a diet consisting of cod fillet, maize starch, lard, cellulose, soybean oil and mineral/vitamin mixture. The diets were compounded as shown in Table 1. The diets were mixed, individually weighed into metal cups and frozen before the experiments.

Table 1. Ingredients and chemical composition of diets in Experiment I

	Group				
	A	B	C	D	E
Ingredient (%):					
Cod fillet	50.6	72.2	82.0	88.0	94.0
Maize starch, extruded	19.2	14.0	8.0	4.0	0.8
Lard	16.0	8.8	5.0	3.0	0.4
Cellulose	2.0	2.0	2.0	2.0	2.0
Soybean oil	1.0	1.0	1.0	1.0	1.0
Mineral/vitamin mixture	2.0	2.0	2.0	2.0	2.0
Water	9.2	-	-	-	-
Dry matter	40.8	38.3	30.2	25.4	21.0
Chemical composition (% of DM):					
Protein	18.4	31.5	44.7	55.2	74.7
Fat	37.3	26.9	22.6	16.5	9.2
Sugar	14.3	13.6	13.2	10.3	3.5
Starch	26.8	22.6	11.7	6.1	3.4

Prior to the one-week balance period the animals became accustomed to the changed diet and environment during a two-week period. Feces and urine were quantitatively collected daily during the balance period and stored at -20°C until analyzed. Urine was collected in flasks containing 10 ml 5% (v/v) sulfuric acid. At the end of the balance period the

screen for collecting the feces and the funnel for urine collection were carefully washed with 100 ml 1% (v/v) citric acid, which was frozen pending further analysis. During the balance period each animal was placed in a respiration chamber for 24 h. The respiration chambers were constructed especially for mink and consisted of plexiglass boxes which allowed the animals maximum visual contact with these surroundings. The dimension of the chamber (volume=760 l) meant that the animal could be moved from the laboratory into the chamber while still inside its proper metabolism cage thus avoiding loss of feces and urine during transfer. This procedure also minimizes stress brought on by change of environment. The respiration plant is of the open-circuit indirect calorimetry type and the temperature and relative humidity are kept constant at 15-18°C and 65-75%, respectively. In order to measure volatile nitrogen loss from either the feces or urine in the form of ammonia, the exhaust air was passed through two gas washing flasks containing 10% (v/v) sulfuric acid which was subsequently analyzed for nitrogen content. The condensed water from the air-conditioning system was likewise collected and analyzed for nitrogen. Nitrogen was determined by the Kjeldahl procedure using the Tecator-Kjeltec system.

The amount of retained nitrogen (RN) was calculated as the amount of digested nitrogen (DN) minus the sum of nitrogen in the urine (UN), nitrogen collected from exhaust air (AN) and nitrogen in citric acid after wash (CN), [RN=DN-(UN+AN+CN)].

Experiment II: In order to quantify the amount of urine collected during the balance period as a ratio of the actual amount of urine produced by the animal, a set of experiments were carried out in an attempt to imitate the experimental conditions by simulated urination with precollected urine. Prior to the experiments mink urine was collected and frozen without additives. The nitrogen concentration in the urine varied from 4.36 to 4.78% N. At the beginning of the experiment six balance cages without animals were set up and three times a day for a five days period a total of 100 ml urine per cage and day was sprayed over the screens and funnels. The sprayed urine was collected and frozen as in Experiment I.

Four experiments were carried out in which different washing techniques were adopted. In *Experiment IIa* the screens for faeces collection and the urine funnels were sprayed with 100 ml 1% (v/v) citric acid at the end of the experiment. *Experiment IIb*: At the end of the experiment the equipment was carefully washed in 200 ml 1% (v/v) citric acid using a fine brush. *Experiment IIc*: The experimental installation was sprayed daily with 100 ml 1% (v/v) citric acid. *Experiment IId*: As for Experiment IIc but the citric acid was replaced with 5% (v/v) acetic acid. In all experiments the acid was collected in a plastic flask, weighed and frozen for later analysis.

By dividing the sum of nitrogen collected in the urine (UN) and nitrogen in the washing acid (CN) by the amount of nitrogen sprayed over the cage (SN) it was possible to calculate the percentage of nitrogen retrieved in the experiment, [%N retrieved=(U-N+CN)*100/SN].

RESULTS

The animals had a slight weight loss (4-9 g/day) during the experimental period (Table 2). The apparent digestibility of crude protein was high, 91.7, and did not differ between the

groups B to E, but was slightly lower for diet A (87.7), which had the lowest protein content. The increasing amount of DN from groups A - E was reflected in an increasing amount of nitrogen excreted in urine (UN) (Table 2).

Table 2. The average live weight (kg), weight change (g/day) and nitrogen metabolism (N g/day) for the animals in Experiment I

	Group				
	A	B	C	D	E
Animals per group (n)	5	6	6	6	6
Live weight, kg	1.90	1.85	1.75	1.84	1.98
	(.05)[1]	(.06)	(.05)	(.05)	(.02)
Weight change, g/day	-5	-6	-9	-6	-4
Digested nitrogen (DN)	0.69	1.65	2.86	4.23	4.77
	(.13)	(.11)	(.15)	(.19)	(.32)
Nitrogen, urine (UN)	0.54	1.01	1.92	2.88	3.47
	(.05)	(.13)	(.06)	(.17)	(.25)
Nitrogen, air (AN)	0.01	0.02	0.05	0.05	0.10
	(--)	(--)	(.01)	(.01)	(--)
Nitrogen, citric acid (CN)	0.02	0.06	0.07	0.10	0.07
	(--)	(.01)	(.01)	(.01)	(.02)
Nitrogen, retained (RN)	0.12	0.56	0.82	1.201.22	
[=DN-(UN+AN+CN)]	(.10)	(.07)	(.15)	(.06)	(.15)
Nitrogen, retained (CRN)[2]	-0.14	0.03	-0.16	-0.29	-0.59
[=DN-(UN+CN)*100/66]					

[1] ( ) = SEM, [2] Corrected in accordance with Table 3, Exp. IIa

Nitrogen loss in the form of volatile ammonia (AN) accounted for 1.2 to 2.1% of the DN and 1.7 to 2.9% of the nitrogen excreted in the urine (UN). The nitrogen collected by washing with citric acid (CN) amounted to 2.0 to 5.9% of UN, (Table 2).

As can be seen from Table 2 there was an increasingly positive nitrogen retention (RN) in groups A - E, and in the latter, the RN level was as high as 1.22 g N/day, corresponding to 7.6 g retained protein per day.

The agreement between the actual amount of nitrogen excreted in the urine and the amount collected in the balance experiment could be evaluated by simulated urination (Experiment II). In this experiment different washing techniques were adopted but there were no significant differences when the screens and funnels were washed daily (Experiment IIc) or at the end of the period (Experiments IIa & IIb) or when they were washed with citric acid (IIb) or acetic acid (IId). The amont of nitrogen recovered was only about two-thirds (Table 3). In Experiment IIa, where the washing procedure applied was the same as that in the balance study (Experiment I), the recovery of nitrogen equalled

65.8%. Recalculation of the amount of retained nitrogen in Experiment I by the correction for recovery of nitrogen found in Experiment IIa revealed corrected values for retained nitrogen (CRN) that were close to zero or slightly negative in the five groups (Table 2).

Table 3. Experiment II. Percentage of N retrieved by applying different washing techniques

	Experiment			
	IIa	IIb	IIc	IId
Wash[1] (s=spray,w=wash)	end,s	end,w	daily,s	daily,s
	Acid[1]	citric	citric	citricacetic
N retrieved %	65.8	62.2	66.6	70.7
(SEM), n=6	(1.6)	(1.6)	(1.0)	(4.6)

[1] See Materials and Methods

DISCUSSION

The many sources of error in nitrogen balance studies have been reviewed by van Es (1975), Just et al. (1982) and Eggum (1989) and the consensus is that the most critical step in measuring nitrogen retention is the determination of the amount of excreted nitrogen. Care should be taken in storing and analyzing feces and urine, but if the excreta are frozen immediately after collection, urine is acidified (pH < 5.0) and nitrogen is analyzed on a wet basis, only minor errors are likely to occur. The most important shortcomings are, however, the methods and care undertaken when collecting feces and urine. As noted by Eggum (1989) losses from urine are likely to be the most serious problem. The loss of nitrogen is mainly due to two factors, one of which is the escape of N in the form of volatile ammonia and the other is nitrogen stuck to the collecting screens and funnels. The volatile loss of N has, similar to the present experiment, been estimated by van Es (1975), who found, by determining the nitrogen in exhaust air from respiration experiments, that the amount of urinary and fecal N lost as ammonia was 0.5-1.5% of N intake for cattle, laying hens and chickens. For groups of pigs kept on slatted floors the ammonia loss was up to 5% of N intake. This is in good agreement with the results obtained in the present experiment where the nitrogen lost as ammonia was 1.2-2.1% of the digested nitrogen, corresponding to 1.0-1.9 % of N intake.

The N balances were, however, still higher than expected even after correcting the values from the balance study with the loss of volatile nitrogen . The N retention was positive in all the groups and in groups E and D as high as 1.20 and 1.22 g N/day, although the weight changes were negative in all groups.

Consideration is therefore given to determining the recovery percentage of nitrogen in urine by simulating the experimental conditions using precollected mink urine of a known nitrogen concentration. The recovery of nitrogen was rather low, varying from 62.2% to 70.7%. After correction of the values obtained in the balance study (Experiment I) by the N percentage recovered in Experiment IIa, where the procedure was the same as that used

in the balance study, the nitrogen retention (CRN, Table 2) was slightly negative or close to zero, which was in agreement with the weight changes of the animals during the experiment.

On using the same correction in a study of N retention in pregnant mink (Tauson et al. 1992) the N balances were found to be zero at the time of mating, rising to 0.5 g/day close to parturition. These balances seem to be in better agreement with the weight change in the pregnant mink in this period than those reported by Jarosz & Barabasz (1988) who found N retention in pregnant fitch to be above 1 g N/day in mid-pregnancy.

A correct determination of the amount of excreted nitrogen is of crucial importance especially when performing energy metabolism studies or measuring protein requirement, where a too low estimation of endogenous nitrogen in urine will lead to an overestimation of the protein requirement. This is, as pointed out by van Es (1975), particulary important when nitrogen deposition is low in relation to nitrogen intake, as is the case for carnivores and wild-reared animals.

It is concluded that by simulating the procedure for collection of urine in a balance study by using precollected urine, it is possible to determine the retrieval percentage of nitrogen and thereby calculate a more accurate value for the amount of excreted nitrogen, which leads to a more precise measurement of the N retention. It should be emphasized, however, that this in no way means that less care can be taken in collecting the excreta. On the contrary, it is most important that optimal care is taken in the balance experiment as well as in the N recovery experiment in order to secure the high degree of reproducibility necessary for the recommended correction.

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Energy metabolism and foetal growth in the pregnant mink (*Mustela vison*)

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Energy metabolism, weight of uteri, and selected blood parameters were studied in three groups of mink females, each comprising six animals, before mating, at the estimated time of completed implantation and close to estimated time of parturition. One-week balance periods included a 24 h measurement of heat production (HE) by means of indirect calorimetry in open-air circuit respiration chambers. At the end of each balance period, blood samples were taken and the animals were killed for collection of uteri. HE was not significantly affected by stage of gestation. Weight of uteri averaged 1.11 ± 0.16 g before mating, 3.29 ± 1.68 g in females that had recently become implanted, and 88.7 ± 53.1 g in pregnant females with 4-11 foetuses, indicating that the major part of energy retention in foetal tissues occurs close to parturition. Late gestation values for plasma protein, albumin, cholesterol, and creatinine differed significantly from preceding periods.

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The energy requirement for pregnancy in mink has not yet been determined and related to energy retention in uterine and foetal tissue. Determinations of metabolizable energy (ME) requirement for maintenance (ME_m) are further complicated by considerable variation in locomotor activity and by effects of ambient temperature. For adult mink in positive energy balance and in thermoneutral zone (20°C), ME_m was estimated as 527 $kJ/kg^{0.75}$ by indirect calorimetry (Chwalibog et al. 1980). In other species, heat production usually increases markedly during pregnancy, the increase being higher than the energy retention in foetal tissue as calculated for pigs (Moustgaard 1962), which is partly explained by an increase in thyroid activity. For mink, determination of energy requirement during different parts of gestation is also complicated by the delayed implantation and hence the difficulties in determining the exact stage of true gestation. During early pregnancy the energy retention in foetal tissue can be considered of minor importance and even during the late part of gestation, energy retention in mink foetuses is likely to account for only a limited part of the daily ME intake.

The present investigation was designed to describe energy metabolism during pregnancy in mink and relate it to foetal growth and energy retention in foetal tissues, as well as to describe changes in some blood parameters as affected by stage of gestation.

MATERIAL AND METHODS

The experiment was carried out with a total of 18 two-year-old mink females of the wild colour type. Balance experiments, measurement of heat production by indirect calorimetry and killing, were carried out before mating, at the estimated time of completed implantation (26 days after mating), and close to estimated time of parturition (47 days after mating) with six females on each occasion. The females were mated from 20 March and the matings were completed by 22 March for all females.

The animals were fed the same diet derived from a central feed kitchen. The feed for the total experimental period was weighed into daily portions and deep frozen until the day before feeding. The chemical composition of the diet was 29.9% dry matter, 3.6% ash, 16.0% crude protein, 4.5% fat, 2.0% total sugar and 2.0% starch. The animals were fed *ad libitum*.

The balance experiments were carried out with a seven-day adaptation period and a seven-day balance period, during which a 24-h measurement of heat production was carried out. The animals were kept in metabolism cages with devices for feeding, drinking water supply and equipped for quantitative collection of feed residues, faeces and urine. Urine was collected into a bottle containing 10 ml 5% H_2SO_4 and the cages were washed out with citric acid after completion of the balance periods. Quantitative collection of faeces and urine was carried out once a day and the total amount was stored deep frozen until analysis. Faeces were analysed for gross energy (GE), nitrogen, ash, crude fat, sugar and starch. Urine was analysed for GE and nitrogen. Because of inadequacies in the quantitative recovery of urinary nitrogen, data were corrected in accordance with the results of Elnif (1992).

Respiration experiments were carried out over a 24-h period for each female by means of indirect calorimetry in an open-air circulation chamber. The respiration chamber (760l) was designed so as to permit the direct insertion of a metabolism cage into the chamber. The principles for the gas exchange measurements and precision of gas analyses have been described by Chwalibog (1985, 1991).

Heat production (HE) was calculated from O_2 consumption and CO_2 production using the formula by Brouwer (1965) (HE, kJ=16.18 x O_2, l + 5.02 x CO_2, l - 5.99 x N in urine, g). The values for ME intake and retained energy (RE) were calculated from balances and chemical analyses. RE was calculated as RE=(ME-HE). Energy deposited in foetal tissues was calculated from uteri weights and data regarding chemical composition of newborn mink kits (Tauson & Englund 1989) (crude protein 11.9% x 23.86 kJ/g + fat 1.4% x 39.76 kJ/g + carbohydrates 0.6% x 17.56 kJ/g) and nutrients in foetal tissue were similarly calculated based on the data given above for chemical composition.

The animals were weighed at the start of the adaptation period, at the start of the balance period and at the end of the balance period. After termination of each balance period the animals were blood-sampled by puncture of V. cephalica antebrachii as described by Blixenkrone-Møller et al. (1987), and after anaesthesia with Ketalar® (1.5 ml) and Rompun® (1.0 ml) by cardiac puncture, after which the animals were immediately killed. Blood was collected into heparinized tubes and after separation the plasma was stored at -20 °C until assay. So far, blood samples from anaesthetized animals have been analysed for plasma creatinine, protein, albumin, cholesterol, triglycerides, Na, K and osmolality.

After tha animals were killed, the ovaries and uteri were collected and trimmed of fat and connective tissue. The uteri were weighed and deep frozen, and in ovaries from pregnant females the number of corpora lutea (CL) was estimated.

Statistical analyses regarding effect of stage of gestation were carried out according to the GLM procedure in SAS (SAS Institute Inc. 1982).

RESULTS

Among the females killed after an estimated completed implantation five out of six had actually implanted, and in the remaining female blastocysts were recovered. As can be seen from Table 1, the number of implantation sites and estimated number of CL were generally normal to high. Weight of uteri with content was not significantly different from that in the unmated females. One of the females killed 47 days after mating turned out to be empty, and was therefore excluded from the calculations. The rest of the females had several CL and normal to large litters. Foetal size differed from full term to rather small foetuses, the latter being estimated to have 7-10 days left to full term (Table 1).

Table 1. Weights of uteri, number of implantation sites and foetuses and estimated number of CL (mean ± SD)

Date of killing	Weight uteri + content (g)	Estimated no. of CL	Implantation sites/foetuses	Size of foetuses
25 March	1.11 ± 0.16			
15 April	3.29 ± 1.67	14.8 ± 4.1	9.0 ± 3.6	
6 May	88.7 ± 53.1	13.4 ± 2.8	7.2± 2.9	

Osmolality, Na, and K were independent of stage of gestation, whereas there were significant effects for plasma creatinine, protein, albumin and cholesterol. In all these parameters, levels found for females close to parturition differed significantly from those found for unmated females and for females which had recently implanted (Table 2). Plasma creatinine decreased in late gestation whereas protein, albumin and cholesterol increased. In addition, for triglycerides a non-significant increase was found in late gestation.

HE was independent on stage of gestation, but the ME intake was significantly increased for pregnant females. Because of the increase in ME intake there was a tendency ($p=0.07$) for increased RE with advancing stage of gestation. Nitrogen balances were close to zero for unmated females, slightly negative for females which had recently implanted and positive for females in late gestation (Table 3). The amount of nutrients and energy deposited in uteri and foetal tissue was very low in unmated females and females killed after implantation. For females killed close to parturition, the levels were significantly increased and showed a considerable variation between individuals (Table 3).

Table 2. Effect of stage of gestation on some blood parameters

	Unmated females	After implantation	Close to parturition	p-value
Osmolality, mOsm/l	328	322	325	0.39
Na, mmol/l	152.6	153.4	153.7	0.82
K, mmol/l	4.7	5.5	5.1	0.50
Creatinine, mmol/l	60.7[a]	67.2[a]	44.5[b]	0.02[c]
Protein, g/l	61.8[a]	58.7[a]	76.7[b]	<0.001
Albumin, g/l	26.2[a]	23.0[a]	31.2[b]	0.002
Cholesterol, mmol/l	4.3[a]	4.1[a]	5.9[b]	0.001
Triglycerides, mmol/l	0.90	0.96	1.08	0.41

[a,b] Values within row that share no common superscript differ significantly ($p < 0.05$)
[c] Values in italics indicate a significant effect of stage of gestation.

Table 3. HE, ME, RE, nitrogen balances and nutrients and energy in uteri and foetal tissue in relation to stage of gestation

	Unmated females	After implantation	Close to parturition	p-value
HE, kJ	581	628	580	0.71
ME, kJ	540[a]	760[b]	806[b]	0.02[c]
RE, kJ	-41	132	225	0.07
N-balance, g/day	0.07	-0.37	0.48	0.11
Nutrients and energy in uteri and foetal tissue				
Protein, g	0.13[a]	0.39[a]	10.51[b]	<0.001
Fat, g 0.02[a]	0.05[a]	1.21[b]	<0.001	
Carbohydrate, g	0.01[a]	0.02[a]	0.52[b]	<0.001
Energy, kJ	4[a]	11[a]	308[b]	<0.001
(range)	(3-5)	(6-21)	(69-506)	

[a,b] Values within row that share no common superscript differ significantly ($p < 0.05$)
[c] Values in italics indicate the level of significance of the effect of stage of gestation.

DISCUSSION

The present investigation is the first report on energy metabolism data for pregnant mink by means of indirect calorimetry. HE data indicate that the mink, unlike other species, does not have a considerable increase in HE as an effect of gestation. A possible reason for this may be that ME_m decreases with advancing stage of gestation due to decreased locomotor activity of the pregnant females. Earlier investigations have stressed the difficulties in determining ME_m values in adult (Chwalibog et al. 1980) and growing mink (Chwalibog et al. 1982) caused by great individual variation in activity. ME intake increased with advancing stage of gestation and as a result of this as well as the constant HE there was a tendency for RE to increase. For the unmated females, the ME intake was far below, and for the pregnant females slightly below, the average ME intakes of female mink reported by

Enggaard Hansen et al. (1991).

There is very little information available on the energetic costs of pregnancy for carnivorous species, but the present increase in ME intake in mink was of a similar magnitude or slightly below the levels reported for the dog (Holme 1982) and the cat (Loveridge & Rivers 1989). It is reasonable to assume that the main deposition of energy in foetuses occurs late in pregnancy and, even at a low efficiency of ME utilization for foetal growth, it amounts to only a limited proportion of the daily energy intake, which is supported by the calculated moderate energy content in uteri and foetal tissue also late in pregnancy in the present investigation. Furthermore, the calculated protein deposition in uteri and foetal tissue is in good agreement with Moustgaard & Riis (1957) at given uteri weights and comparable number of foetuses.

Calculations on energy costs for reproduction in wild and some domestic carnivorous species based on female weight, litter size and birth weight, and related to length of gestation (periods of delayed implantation excluded), have indicated that the daily deposition of energy in foetuses ranges from 0.4 to 18.5 $kJ/kg^{0.75}$ and day (Oftedal & Gittleman, 1989). For mustelids, the range was generally narrower and the values for weasel, European otter and Sable were 10.0, 11.3 and 13.8 kJ, respectively. A corresponding calculation based on the present results indicates a daily deposition of 10.3 $kJ/kg^{0.75}$ in foetal tissue in mink, which is in good agreement with Oftedal & Gittleman (1989) who, however, conclude that these values may underestimate the deposition in late pregnancy. From the calculated data, and with an assumed efficiency for energy deposition in foetuses of 0.11, which is used by Oftedal & Gittleman (1989) but is derived from cattle, and an ME_m requirement of 530 $kJ/kg^{0.75}$ (Chwalibog et al. 1980), the accumulated energy costs of pregnancy in mink would only amount to about five times ME_m.

Part of the increase in HE in other species is explained by increased thyroid activity. In the male mink, thyroxine has been shown to have a distinct biphasic seasonal change with an increase from March to May (Boissin-Agasse et al. 1981), but corresponding data for non-pregnant and pregnant female mink have not been reported. Data from lactating female mink indicated lower concentrations than for females prior to mating (own unpublished observations), so the impact of thyroid hormone concentrations on HE in pregnant mink females cannot be deduced at present, but T_3 and T_4 analyses will be carried out.

Data on effect of stage of gestation on blood parameters in mink are sparse. The present results indicate significant changes in late pregnancy for creatinine, protein, albumin and cholesterol, indicating changes in metabolic activity. Increasing levels of protein, albumin and cholesterol reflect true physiological changes, taking into account that plasma volume increases in late pregnancy. In the blue fox, plasma cholesterol increased significantly and albumin tended to increase, which is in agreement with our findings, whereas protein decreased from mid to late pregnancy (Näveri et al. 1988).

In conclusion, the present investigation indicates that there is no profound increment in heat production during pregnancy in mink. The energy costs for deposition in foetal tissue are limited, possibly with the exception of the last few days of pregnancy during which the main foetal growth occurs.

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Apparent and true digestibility of dry matter, crude protein and amino acids in diets for mature silver foxes

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The availability of amino acids to mature silver foxes and the influence of gut microbes on digestibility of nutrients are relatively unknown. Twelve adult male silver foxes were used to examine the utilization of dry matter (DM), crude protein (CP) and amino acids in a meat-type basal diet (A) when 15% was replaced by soybean meal (S), fishmeal (F) or meatmeal (M), or 25% replaced by wheat (W) or hulless oats (O). Half of the animals received weekly oral doses of the antibiotic Furazoladone (1 ml/5kg body weight) to estimate the effect of gut microbes on the availability of nutrients. True digestibility coefficients of CP and individual amino acids were found to be 3-8% units higher than those of apparent digestibility. Administration of antibiotics significantly improved the apparent digestibility of CP and ten individual amino acids by approximately 2% units. However, antibiotics significantly reduced the true digestibility of CP. It was concluded that the endogenous fraction in silver fox feces appears considerable; hence, true digestibility coefficients may be more valuable than those of apparent digestibility. The microbial population in the gut of silver foxes seems substantial and may be capable of reducing nutrient digestibility.

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Nutritionists are concerned with providing appropriate amounts of essential amino acids to maximize growth and performance of monogastric species. Amino acid composition of diets does not allow an accurate measurement of the useable nutrients. In order to estimate the amount of useable amino acids present, the availability or true digestibility coefficients must be known (Skrede 1979).

Estimation of apparent amino acids determined from fecal analysis is biased due to the amino acids present from endogenous sources and from microbial activity in the gastrointestinal tract. Calculation of true digestibility of amino acids to remove the influence of endogenous amino acids may involve the determination of these amino acids through feeding a protein free diet that is otherwise nutritionally complete. Fecal protein and amino acids from animals fed such a ration are then assumed to be of metabolic origin (Schneider & Flatt 1975).

Oral administration of antibiotics has been used to reduce the population of gut

microflora (Eggum *et al.* 1985; Skrede 1979). In monogastrics with complex gastrointestinal systems, the gut microbes are generally expected to influence digestibility. Eggum *et al.* (1985) found that the true digestibility of protein in skimmed milk powder and brown beans was improved when the antibiotic Nebacitin was administered to rats. Conversely, Knudsen *et al.* (1983) observed that the same antibiotic had no significant effect on the protein digestibility in rats. In monogastrics with a simple gastrointestinal tract, such as the mink, 48 ppm neomycin sulfate was found to show no significant effect on the true digestibility of protein or amino acids (Skrede 1979).

The purpose of this study was to compare apparent and true digestibility of amino acids in diets fed to mature silver foxes. An antibiotic was added to the diet to evaluate the influence of the gut flora on the utilization of amino acids from the gastrointestinal tract.

MATERIALS AND METHODS

Twelve mature male silver foxes (mean weight 6.9 ± 0.6kg) were individually housed in stainless steel metabolism cages (1.08m x 0.96m x 0.84m high) designed for separate collection of feces and urine. These cages were situated in a room in which the temperature was maintained at 16°C and light controlled to provide 12 h light and 12 h darkness. The foxes were randomly assigned to one of two treatment groups. Animals in group 1 received a weekly oral dose of the antibiotic Furazoladone ($1ml^{-1}5kg$ body weight) beginning one week prior to the experiment until the completion of the study. Foxes in group 2 were not given any antibiotics. All foxes were provided with a meat-based diet (basal diet A) over a one-week adjustment period. This diet, formulated to provide 3700kcal ME kg^{-1} (metabolizable energy) and 24.7% (crude protein), contained typical ingredients used for feeding ranched foxes (Table 1.). Chromic oxide was added at 0.5% of the diet dry matter (DM) as an indicator to measure DM digestibility. The foxes were weighed immediately prior to the experiment and weekly during the investigation.

Five test diets were prepared by substituting 15% of the basal diet DM for either soybean meal (S), fishmeal (F) or meatmeal (M), or replacing 25% of the basal diet DM by either wheat (W) or hulless oats (O). These six diets were fed to both groups of foxes in a 6 x 6 Latin square arrangement. Water was provided *ad libitum* from a metal nipple drinker equipped with a spill collection device. The animals were given 230 g feed d^{-1} in two equal portions at 08.30 h and 15.30 h. The foxes received each diet for a period of seven days. The first four days of each period was an adjustment phase followed by three days of total fecal and urine collection. During the seventh week, a protein free diet (Table 2.) was fed to all foxes. Data from those foxes readily consuming the diet were used to facilitate the measurement of endogenous crude protein and individual amino acids.

On collection days, representative samples of uncontaminated feces from each fox were individually placed in polyethylene Whirl-Pak bags, frozen at -20°C and maintained at this temperature until they were pooled for subsequent analysis. Fecal and diet samples were freeze-dried to determine DM. Samples were ground to pass through a 2 mm mesh screen. Crude protein (CP) was measured by the Dumas method (Ebling 1968) using a FP-228 Nitrogen Determinator (Leco Corp.). Amino acid analysis was performed using a Beckman System 6300 High-Performance Amino Acid Analyzer following acid hydrolysis

(Gehrke *et al.* 1985). Chromic oxide was determined following the method of Fenton & Fenton (1979).

Table 1. Apparent digestibility of dry matter, crude protein and amino acids in diets for mature silver foxes

	Supplemented Dietary Ingredients					
% added	Basal [1]	Soybean Meal 15.0	Fishmeal 15.0	Meatmeal 15.0	Wheat 25.0	Oats 25.0
DM	85.0a±1.0	84.0a±1.0	78.5b±1.0	85.3a±1.1	72.8c±1.0	71.6c±1.0
CP	88.3a±0.6	89.0a±0.6	84.6bc±0.6	84.1c±0.6	85.8b±0.6	85.6bc±0.6
Essential amino acids						
Thr	86.7a±0.9	87.4a±0.9	85.8a±0.9	86.7a±1.0	82.6b±0.9	80.8b±0.9
Val	91.6a±0.7	91.3a±0.7	88.3b±0.7	84.5c±0.8	86.4bc±0.7	88.1b±0.7
Met	93.4a±1.5	93.8a±1.5	93.9a±1.5	93.1a±1.6	81.4c±1.5	86.8b±1.5
Ile	88.8a±0.9	89.0a±0.9	86.7ab±0.9	81.9d±1.0	82.7cd±0.9	84.8bc±0.9
Leu	93.3a±0.8	88.7c±0.8	87.7c±0.8	89.4bc±0.9	91.3ab±0.8	91.0b±0.8
Phe	94.8a±0.7	94.6ab±0.7	92.5bc±0.7	90.7c±0.8	93.4ab±0.7	93.5ab±0.7
His	90.7a±1.0	91.1a±1.0	88.3ab±1.0	85.6bc±1.1	81.9d±1.0	84.9c±1.0
Lys	94.8ab±0.8	94.6ab±0.8	92.9bc±0.8	95.7a±0.9	91.0c±0.8	91.8c±0.8
Arg	93.0a±0.5	93.5a±0.5	92.1a±0.5	87.7c±0.6	89.5b±0.5	92.2a±0.5
Non-essential amino acids						
Asp	90.2a±1.0	79.1d±1.0	84.4c±1.0	88.3ab±1.1	85.4bc±1.0	86.3bc±1.0
Ser	85.9ab±1.0	87.5a±1.0	83.2bc±1.0	83.9bc±1.1	79.4d±1.0	81.1cd±1.0
Glu	91.2a±1.0	87.2b±1.0	83.8c±1.0	86.0bc±1.1	85.7bc±1.0	87.8b±1.0
Pro	90.3a±0.6	90.5a±0.6	89.7a±0.6	86.2b±0.7	87.6b±0.6	87.5b±0.6
Gly	88.2a±0.9	88.3a±0.9	85.7ab±0.9	85.0bc±1.0	87.2ab±0.9	82.5c±0.9
Ala	91.1a±0.9	89.6ab±0.9	85.1c±0.9	87.5bc±1.0	86.1c±0.9	87.4bc±0.9
Tyr	88.4a±1.2	88.3ab±1.2	85.0b±1.2	80.9c±1.3	85.7ab±1.2	85.6ab±1.2

a-d Means ± SE within the same row with different letters are significantly different ($p < 0.05$).

[1] DM comprised of: 50.1% beef by-product, 22.2% beef liver, 6.7% corn starch, 16.0% wheat starch, 2.2% corn oil, 2.0% cerelose, 0.3% vitamin/mineral premix (Faulkner & Anderson 1991), 0.5% chromic oxide

A general linear model program (Statistical Analysis System Institute, Inc. 1985) was used to analyze the data with the following model:

$$Y_{ijklm} = \mu + G_i + F_j(i) + D_k + GD_{ik} + P_l + GP_{il} + e_{ijklm}$$

where μ is the overall mean, G_i is the fixed effect of the antibiotic treatment group ($i = 1\text{-}2$), $F_j(i)$ is the random effect of fox ($j = 1\text{-}6$) within treatment groups, D_k is the fixed effect of diet ($k = 1\text{-}6$), GD_{ik} is the interaction of treatment group and diet, P_l is the fixed effect of test period ($l = 1\text{-}6$), GP_{il} is the interaction of treatment group and period, and e_{ijklm} represents the error term. One of the foxes receiving antibiotics while consuming diet M was excluded from the data set because of sampling complications. The PDIFF option was used to compare ls means when significant differences occurred ($P \leq 0.05$) in the analysis of variance.

Table 2. True digestibility of crude protein and amino acids in diets for mature silver foxes [1)]

	Supplemented Dietary Ingredients					
	Basal [2)]	Soybean Meal	Fishmeal	Meatmeal	Wheat	Oats
% added		15.0	15.0	15.0	25.0	25.0
CP	93.0ab±0.5	92.4b±0.5	89.3c±0.5	87.2d±0.5	94.9a±0.5	93.9a±0.5
Essential amino acids						
Thr	96.0bc±0.8	94.7cd±0.8	94.7cd±0.8	93.5d±0.9	102.6a±0.8	97.b±0.8
Val	95.4a±0.6	94.3a±0.6	92.2b±0.6	87.4c±0.7	95.5a±0.6	94.9a±0.6
Met	96.7a±1.5	96.7a±1.5	96.5a±1.5	95.7a±1.6	88.8b±1.5	93.0a±1.5
Ile	92.6a±0.8	91.6ab±0.8	90.0b±0.8	84.7c±0.9	90.8ab±0.8	91.5ab±0.8
Leu	96.9a±0.7	93.1b±0.7	92.4b±0.7	92.4b±0.8	97.7a±0.7	96.7a±0.7
Phe	97.6ab±0.7	96.5bc±0.7	95.3c±0.7	92.8d±0.8	99.2a±0.7	98.1ab±0.7
His	93.8a±1.0	93.5ab±1.0	91.6ab±1.0	88.2c±1.0	88.9c±1.0	90.8bc±1.0
Lys	97.8±0.7	97.2±0.7	96.4±0.7	98.0±0.8	97.8±0.7	97.4±0.7
Arg	96.4ab±0.5	95.9bc±0.5	94.9c±0.5	89.8d±0.6	97.7a±0.5	97.5a±0.5
Non-essential amino acids						
Asp	94.4ab±1.0	85.1d±1.0	90.0c±1.0	91.5bc±1.1	94.8a±1.0	93.4ab±1.0
Ser	92.7a±0.9	92.2a±0.9	89.4bc±0.9	88.2c±1.0	93.9a±0.9	91.4ab±0.9
Glu	95.1a±0.9	91.0bc±0.9	88.7c±0.9	89.5c±0.9	93.4ab±0.9	94.5a±0.9
Pro	94.1a±0.6	93.7a±0.6	93.3a±0.6	88.4b±0.6	94.8a±0.6	93.9a±0.6
Gly	92.3b±0.9	91.8b±0.9	90.0bc±0.9	88.0c±0.9	95.0a±0.9	90.3bc±0.9
Ala	94.8a±0.8	93.0a±0.8	89.7b±0.8	90.3b±0.8	94.9a±0.8	94.1a±0.8
Tyr	94.6b±1.2	92.1bc±1.2	90.3c±1.2	85.3d±1.2	98.5a±1.2	95.1b±1.2

a-d Means ± SE within the same row with different letters are significantly different ($p < 0.05$)

1) Calculated using protein free diet with DM comprised of: 22.4% corn starch, 54.4% wheat starch, 6.5% cerelose, 6.5% cellulose, 0.98% vitamin/mineral premix (Faulkner & Anderson 1991), 8.18% corn oil, 0.54% beef Oxo Cubes, 0.5% chromic oxide

2) Composition as described in Table 1.

RESULTS AND DISCUSSION

Apparent digestibility of DM was lowest in diets W and O (73.8% and 71.6% respectively) (Table 1.). Although significant variation in the percentage of apparent digestibility of CP existed among the six diets, all were highly digestible in the range of 84.1 to 89.0%. Apparent digestibilities of essential and non-essential amino acids were also variable (81.4% Ile to 95.7% Lys in diet M; and 79.1% Asp in diet S to 91.2% Glu in basal diet A). Apparent digestibility of DM, CP and individual amino acids in diet A were as high or higher than those of all the other diets. Diet S also exhibited equally high digestibilities except in the cases of Leu (88.7%), Asp (79.1%) and Glu (87.2%). Diets F and M showed considerable variation in apparent digestibility of amino acids. Similarly, Skrede (1979) found that the amino acids in meat-and-bone meal were not well utilized by mink presumably due to the meal composition and/or unfavorable processing conditions.

True digestibility of CP (Table 2.) was approximately 3-8% units higher than the

apparent digestibility of CP. The endogenous protein fraction in silver fox feces appears large. Skrede (1979) noted that the mink tends to excrete relatively large amounts of metabolic fecal nitrogen (MFN). True digestibilities ranged from 84.7% Ile in diet M to 102.6% Thr in diet W. The latter value may be attributed to synthesis of threonine by microbes in the gut.

Oral administration of the antibiotic significantly improved the apparent digestibility of CP and ten individual amino acids (Table 3.). The digestibility was generally increased by 2% units with the addition of the antibiotic. It is possible then that a substantial population of microbes may exist in the gastrointestinal tract of foxes and that this population is able to reduce the digestibility of CP and various individual amino acids by the fox. True digestibility of individual amino acids was not significantly affected while the true digestibility of CP was reduced with the addition of antibiotics (92.3% vs. 91.2%). These results with foxes appear different from those with mink where oral doses of neomycin sulfate had no effect on the apparent digestibility of fat, protein or carbohydrates, or the true digestibility of amino acids or nitrogen (Skrede 1979).

Table 3. Effect of antibiotic on digestibility of dry matter, crude protein and amino acids in diets for mature silver foxes

	% Apparent digestibility ± SE			% True digestibility ±SE		
	Antibiotic	No Antibiotic	Sig.[1)]	Antibiotic	No Antibiotic	Sig.[1)]
DM	80.2±1.1	78.9±1.1	NS			
CP	87.2±0.4	85.3±0.4	**	91.2±0.2	92.3±0.2	**
Essential amino acids						
THR	86.4±0.6	83.6±0.6	**	96.5±0.3	96.5±0.3	NS
VAL	89.3±0.8	87.4±0.7	NS	93.0±0.5	93.4±0.5	NS
MET	91.4±1.4	89.4±1.4	NS	94.4±1.3	94.7±1.2	NS
ILE	86.6±0.8	84.7±0.7	NS	90.0±0.6	90.4±0.5	NS
LEU	91.4±0.6	89.0±0.6	*	94.7±0.4	95.0±0.4	NS
PHE	94.2±0.5	92.2±0.5	*	96.7±0.4	96.5±0.4	NS
HIS	88.1±1.3	86.1±1.2	NS	91.2±1.1	91.1±1.0	NS
LYS	94.3±0.5	92.7±0.5	*	97.2±0.4	97.7±0.4	NS
ARG	92.0±0.4	90.7±0.4	*	95.1±0.3	95.6±0.3	NS
Non-essential amino acids						
ASP	86.7±0.8	84.5±0.8	NS	91.1±0.6	92.0±0.6	NS
SER	84.9±1.1	82.1±1.0	NS	91.1±0.7	91.5±0.7	NS
GLU	88.5±0.7	85.4±0.7	*	92.2±0.5	91.9±0.5	NS
PRO	89.6±0.6	87.7±0.6	*	92.8±0.4	93.2±0.4	NS
GLY	87.4±0.5	84.9±0.5	**	90.9±0.4	91.5±0.4	NS
ALA	88.8±0.6	86.7±0.6	*	92.5±0.4	93.1±0.4	NS
TYR	86.9±0.8	84.4±0.7	*	92.1±0.5	93.3±0.5	NS

1) Means ± SE within row for parameters are: NS not significantly different ($p<0.05$), * significantly different ($p<0.05$), ** significantly different ($p<0.01$)

SUMMARY

Mature silver foxes appear to excrete substantial amounts of endogenous protein, therefore true digestibility coefficients may be more meaningful than those of apparent digestibility. The true digestibility coefficiets of CP and individual amino acids were approximately 3-8% units higher than those of apparent digestibility. A population of microbes capable of reducing nutrient digestibility appears to exist in the gut of mature silver foxes. Oral doses of antibiotics increased the apparent digestibility of CP and ten individual amino acids. Antibiotic administration significantly reduced true digestibility of CP, but, this reduction was only 1% unit and therefor may not be of any great significance to the animal.

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The effect of protein source on digesta passage and nutrient digestibility in polar foxes

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A digestibility experiment with six male polar foxes was conducted with three different sources of dietary protein, which were supplied by cod fish, cod fish and fishmeal, or fishmeal, respectively. A significant effect of the source of protein on ileal and total digestibilities was found. The highest values for the apparent digestibility of nutrients (dry matter, nitrogen, and amino acids) were found in the experimental animals fed on the diet with cod fish. An increase in fishmeal content in the investigated diets caused faster digesta passage and a decrease in nutrient digestibilities. No statistically significant differences ($p > 0.05$) in apparent fat digestibility were noted.

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Different sources of protein can influence the passage time in mink (Szymeczko & Skrede 1990) and the digestibility of nutrients (Zebrowska et al. 1978; Skrede 1979; Low 1979; Asche et al. 1989). To estimate nutrient digestibility in different species of fur animals the faecal method has been mostly used (Bieguszewski & Lewicki 1969; Skrede 1979; Kiiskinen et al. 1985; Szymeczko & Skrede 1990, 1991). The digestibility estimated by this method gives, however, the final results of digestion but does not give any view on the changes in digestibility occurring under the influence of the intestinal microflora. Especially in the case of protein, it seems possible that the faecal digestibility method overestimates the amount of amino acids absorbed from the alimentary tract (Payne et al. 1968; Bayley et al. 1974; Holmes et al. 1974; Zebrowska et al. 1978; Szymeczko & Skrede 1990).

The objective of the study was to compare the time of digesta passage and apparent ileal and faecal digestibility of some nutrients in polar foxes fed diets with different sources of protein.

MATERIALS AND METHODS

The experiment was carried out at the Animal Physiology Dept., Academy of Technology and Agriculture in Bydgoszcz, Poland. The experimental animals were six 18-month-old polar fox males of average body weight (5.95 kg) kept individually in metabolic cages. Three of the animals were fitted with simple single cannulae placed at the end of the small intestine (Table 1) according to the method developed by Szymeczko (not published).

Table 1. Length measurements of the digestive tract of experimental foxes (cm)

Measurements	Mean ± SD
Total digestive tract	210.0 ± 8.4
Small intestine	176.5 ± 10.8
Distance of the cannula from the pylorus	134.0 ± 8.8

Ten days after the insertion of the cannulae, the foxes were in succession given diets containing different sources of protein. Proximate composition of experimental diets and their respective amino acid compositions are shown in Tables 2 and 3, respectively. In the first experiment an eviscerated cod fish (EC) diet was used. During the second experiment 50% of the cod fish protein was replaced with fishmeal (ECFM diet). In the third feeding experiment the foxes were fed a diet (FM) with fishmeal as the only source of protein. All diets contained 35, 35 and 30% of energy from protein, fat and carbohydrate, respectively (Slawón 1987). Each diet was fed to the animals for 12 days in one meal per day at 08.00. Water was supplied *ad libitum*.

Table 2. Composition of diets (%)

Diet	EC	ECFM	FM
Ingredients:			
Eviscerated cod	43.29	24.41	-
Fishmeal	-	7.88	17.64
Maize starch/precooked	7.33	7.99	8.64
Soybean oil	2.86	3.02	3.15
Cellulose powder	0.75	0.89	1.08
Vitamin-mineral mix.	0.20	0.21	0.22
Water	45.57	55.59	69.27
Proximate comp.:			
Dry matter (DM) %	20.45	24.43	28.12
Nitrogen (% of DM)	5.64	5.45	5.65
Fat (% of DM)	12.88	15.61	16.15

After four days of feeding each diet the digesta passage was measured according to the method described by Hansen (1978). During the subsequent experimental days, the foxes were given diets mixed with chromium oxide. Faeces was collected during the last four

days and digesta within the last two days of each feeding experiment and kept at -18°C. Pooled samples of digesta and faeces of each animal were freeze-dried, ground and sifted to remove hairs. Samples of experimental diets, digesta and faeces were analysed for dry matter, nitrogen, fat and chromium oxide content in the laboratory of the Agriculture Academy in Bydgoszcz. Analyses of amino acids were carried out at the Central Laboratory of the Danish National Institute of Animal Science in Foulum.

Table 3. Amino acid composition of experimental diets (g/16gN)

Amino acid	Diet		
	EC	ECFM	FM
ARG	6.34	6.30	6.68
PHE	3.93	4.09	4.44
HIS	2.37	2.38	2.41
ISO	4.64	4.67	4.87
LEU	7.72	7.68	7.76
LYS	7.62	7.77	7.86
MET	3.48	3.49	3.82
THR	4.26	4.43	4.60
VAL	5.17	5.32	5.52
ALA	6.29	6.17	6.18
ASP	10.05	9.94	10.18
CYS	1.02	1.01	1.04
GLU	14.61	14.21	14.26
GLY	6.33	6.38	6.54
PRO	3.96	4.36	4.55
SER	4.73	4.74	4.93
TYR	3.31	3.49	3.91

RESULTS AND DISCUSSION

The amino acid composition was determined for each experimental diet. As shown in Table 3, the varying sources of protein caused minor changes in the composition of individual amino acids. The different diets were very well accepted by the experimental foxes, and the entire daily portion was consumed in 10 - 15 min.

The time for digesta to pass through the digestive tract varied statistically between the control group of animals fed on the eviscerated cod diet (EC) and foxes receiving diets with an increasing amounts of fishmeal (ECFM and FM). The longest time of passage, 567.8 $\pm$ 58.3 min, and the lowest amount of faeces, 41.3 $\pm$ 5.9 g, were recorded in foxes after the EC diet. The replacement of raw cod fish meat with fishmeal accelerated digesta transit and faeces output (Table 4). The present data are in good agreement with the trend of changes of the feed passage rate measured by Szymeczko & Skrede (1990) in mink given diets with fishmeal.

Table 4. Digesta passage (min) and excretion of faeces (g)

Measurements	Diets EC	ECFM	FM
Digesta passage1	567.8	456.8[1)]	418.8[2)]
	58.3	86.6	60.9
Faeces excretion	41.3	57.2[2)]	75.8[3)]
	5.9	8.7	11.0

1) $p < 0.05$, 2) $p < 0.01$, 3) $p < 0.001$

Buraczewska et al. (1973) have shown a decrease in the digestibility of nitrogen and lysine in the distal section of the small intestine in pigs as a result of heating casein for five hours. Our studies with foxes fed a diet with 50% protein from fishmeal have demonstrated that heat-damaged products cause the decline of apparent digestibility of nitrogen and individual amino acids in the digesta collected from the terminal part of the small intestine (Table 5). Results obtained by Szymeczko & Skrede (1990) have also revealed that fish meal protein was less efficiently digested compared with whole fish meat protein in the last section of the small intestine in mink.

Table 5. Apparent ileal digestibility of nitrogen and amino acids (%)

Amino acids	Digesta after EC	ECFM
NITROGEN	87.93	68.67[2)]
ARG	84.90	72.01
PHE	70.51	69.52
HIS	82.27	70.61
ISO	83.33	69.05
LEU	84.11	72.57
LYS	85.67	74.95
MET	88.41	74.73
THR	75.74	65.35
VAL	80.16	68.14
ALA	82.91	68.10
ASP	78.34	64.14
CYS	56.20	51.13
GLU	82.35	68.66
GLY	76.79	58.88
PRO	73.04	62.19
SER	76.55	64.10
TYR	80.36	69.28

2) $P < 0.01$

Apparent faecal digestibilities of dry matter and fat are given in Table 6. Dry matter digestibility in the diets containing test fishmeal (ECFM and FM) was significantly lower than that of the eviscerated cod fish diet (EC). The average digestibility coefficients of fat were relatively high and there were no statistically significant differences between diets.

Table 6. Apparent faecal digestiblity of dry matter and fat (%)

Nutrients	Diet EC	ECFM	FM
Dry matter	70.09	62.48[2)]	61.12[2)]
	1.58	3.69	4.51
Fat	95.06	95.74	95.06
	1.67	1.10	0.46

[2)] $p < 0.01$

The results of the study with foxes and the data reported by other authors (Zebrowska et al. 1978; Szymeczko & Skrede 1990, 1991) have shown lower but more variable apparent digestibilities of nitrogen and individual amino acids in the small intestine than in the whole alimentary tract (Tables 5 and 7). The highest digestibility coefficients within the small intestine of polar foxes after EC and ECFM diets were for methionine, lysine and arginine, the lowest for cystine, proline, threonine and phenylalanine.

Table 7. Apparent faecal digestibility of nitrogen and amino acids (%)

Amino acids	Faeces after diets EC	ECFM	FM
NITROGEN	94.51	89.45[2)]	84.22[3)]
ARG	90.07	86.37	83.71
PHE	90.60	84.76	80.86
HIS	89.72	86.26	83.33
ISO	92.02	86.32	82.82
LEU	91.40	87.03	83.61
LYS	91.18	87.38	84.52
MET	93.48	88.23	83.70
THR	87.77	83.70	81.80
VAL	89.11	84.57	81.28
ALA	89.19	83.37	78.33
ASP	88.13	81.96	78.96
CYS	71.90	65.41	64.71
GLU	91.20	86.63	83.21
GLY	85.94	79.38	74.01
PRO	84.93	80.49	76.98
SER	87.57	82.21	79.45
TYR	88.52	85.84	83.65

[2)] $P < 0.01$, [3)] $P < 0.001$

The apparent faecal digestibilities of nitrogen and amino acids are presented in Table 7. In contrast to the control eviscerated cod-containing diet, fishmeal diets revealed poorer digestibilities of investigated nutrients. However, the differences were smaller than those for ileal digestibilities. Comparison of individual amino acids on all the experimental diets showed the highest apparent digestibility for methionine and the lowest for cystine, glycine and proline.

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Microbiological and proteolytic spoilage of fur animal feed

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A laboratory trial was conducted to investigate spoilage of fur animal feed on the wire of the pens. Three types of mink-feed formulae were tested: (1) broiler byproducts (pasteurized and fermented) mixed with raw fish; (2) broiler byproducts (pasteurized and fermented); and (3) control feed (raw broiler byproducts mixed with raw fish). The feed was stored in portions of 200 g at 20°C for 20 h. A rapid bacterial growth was observed in the control feed resulting in a pH drop from 6.2 to 4.9 during the 20 h of storage. During storage the TVN/total N increased from 1.3% to 1.9%. In feeds 1 and 2 no changes were observed in the number of bacteria, pH (5.2 and 4.4, respectively) and TVN/total N. It is concluded that fur animal feed without any preservative agent is spoiled within 20 h at ambient temperatures of 20°C or more. Consequently, unless fur animals are fed at least twice a day, their feed should include an effective preservative against both microbial and enzymatic spoilage.

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Fur animal feed is generally compounded of a variety of raw materials, the main components of which are raw animal byproducts, originating from poultry offal and fish processing. These raw materials are contaminated with large numbers of micro-organisms. Enzymes, of microbial origin, involved in the spoilage of protein are also present (Berg 1986; Jørgensen 1985; Juokslathi 1980; NRC 1982; Urlings et al. 1992). Feeding practices are important considerations for optimal performance of fur animals. Feed should be kept under conditions in which any kind of spoilage is reduced to the lowest possible level. In Denmark and The Netherlands fur animal feed is prepared in feed centres, where the quality of the raw materials and the feed is closely monitored after the standards described by Jørgensen (1985). To keep the feed at a high quality level: (1) the temperature of the feed is reduced to between -1 and +2°C on delivery to the farm; (2) the feed is delivered daily from the feed centres; and (3) preservatives such as acids or sodium metabisulphite are sometimes used. In this way the quality of the feed is ensured until it is delivered to the farm. After delivery, the feed is stored in insulated tanks in which there will be no marked temperature change during the first 24 h of storage. The last part of the feeding practice

is the most susceptible to environmental circumstances. The feed is placed in the pen, which is located outside and therefor subject to local environmental conditions. Effective temperature control is unrealistic when the feed is provided to the animals in this way. This paper gives an outline of the bacterial and proteolytic spoilage of ready-mixed mink feed at ambient summer temperatures, resembling the condition the fur animal feed is exposed to outside in the pen. Different feed formulae are tested.

MATERIALS AND METHODS

Poultry byproducts (entrails, feet, heads and blood) for the production of fermented poultry offal were obtained from seven- to eight-week-old broilers and processed at a broiler slaughterhouse as follows: Immediately after slaughter the byproducts were mixed and ground through a meat grinder equipped with a 5 mm plate, and mixed with 4% (w/w) dextrose. The mixture was heated for four minutes at a core temperature of 95°C in a continuous flow-term heating process. After heating, the product was cooled to 25°C and inoculated with *Lactobacillus plantarum* at a level of 10^6 colony-forming units per gram. The product was stored at a temperture of 20°C in plastic containers with screw caps (15 l) and left to ferment for 21 days.

Table 1. Feed composition

Raw materials	Feed 1 (%)	Feed 2 (%)	Feed 3 (%)
Frozen fish offal	53	--	53
Frozen poultry offal	--	--	36
Fermented poultry offal[a]	36	--	--
Fermented poultry offal with blood[b]	--	89	--
Cereal	5	5	5
Water	5	5	5
Vitamins and minerals	1	1	1

[a] 29.8% heads, 45.3% entrails and 4.0% dextrose
[b] 25.4% feet, 17.9% heads, 38.8% entrails, 13.9% blood and 4.0% dextrose

The three feed formulae were prepared with thawed frozen raw materials and cooled fermented materials. The temperature of the feed at the start of the experiment was 2°C. The feed was stored at 20°C in 200 g portions, thus corresponding to the feed ration given to the mink daily.

Microbiological and chemical analyses

These were carried out immediately after preparation of the feed (0 h) and after 5, 10 and 20 h storage at 20°C. Three samples per treatment were collected in sterile plastic bags and the pH of each specimen was measured using an electric pH probe. Twenty gram portions of each specimen were cooled to about 0-2°C pending analysis. The samples were subsequently diluted with 180 ml buffered peptone water (BPW: pH 7.2±0.1, peptone 1%, NaCl 0.5%, $Na_2HPO_4.2H_2O$ 0.9% and KH_2PO_4 0.15%) and macerated in a stomacher for

two minutes. Decimal dilutions were prepared using peptone (0.5%) saline (0.85%) solution. The following colony counts per gram product were assessed: Mesophilic aerobic flora was cultured on poured plates of tryptone glucose extract agar (TGEA) and colonies counted after three days' incubation at 30°C;

Enterobacteriaceae were cultured on poured plates of violet red bile glucose agar (VRBG) with a similar overlay and colonies were counted after 20-24 h incubation at 37°C (Mossel et al. 1979);

Enterococci were cultured on spread plates of kanamycin aesculin azide agar (KAA), and colonies were counted after 24 h incubation at 37°C (Mossel et al. 1978).

Lactobacilli were cultured on spread plates of the medium according to Rogosa, and colonies counted after three days', incubation at 30°C under reduced O_2 using the Gaspack system (Rogosa et al. 1951).

Sulphite-reducing *Clostridia* were cultured in poured plates of sulphite agar with D-cycloserine (400 $mg.kg^{-1}$) with a similar overlay. Colonies were counted after anaerobic incubation for 24 h at 37°C (Mossel & Pouw 1973).

Total nitrogen (analyses after Kjeldahl; Anonymous 1968) and total volatile nitrogen (TVN, by the Kjeldahl method; Lindgren & Pleje 1983) were analysed in the feed.

RESULTS

Feeds 1 and 2 were chemically and microbiologically fairly stable during the 20 h storage at 20°C (Figs. 1 and 2). The pH in feed 1 was 5.2 to 5.3 and 4,4 in feed 2 during storage. The bacterial counts remained quite stable, but the number of yeasts increased by 1 log unit over the 20 h in both feed formulae. It was found that the pH in feed 3 (control) had decreased from 6.2 to 4.9 after 20 h. TVN/total N levels changed markedly only in feed 3, from 1.3% at 0 h to 1.9% at 20 h storage. Microbial growth was mainly seen in feed 3, in which over the 20 h the mesophilic aerobic flora and the *Lactobacilli* count increased by 2 log units and the *Enterobacteriaceae* and the *Enterococci* counts increased by 1 log unit. Sulphite-reducing *Clostridia* increased in all feeds during storage, but was most pronounced in feed 3 to levels below 1.8 in $logN.g^{-1}$.

DISCUSSION AND CONCLUSIONS

As a result of the low and stable pH (4.4) and the high numbers of *Lactobacilli* in feed 2 almost no bacterial growth was observed, which in turn resulted in no amino acid breakdown, as measured in the TVN/total N.

Pasteurization of poultry byproducts can also reduce the initial amino acid breakdown in these products, as reported by Fransen (1990). In feed 1 the pH was decreased and stable at 5.2, while, as a result of the fermentation, the numbers of *Lactobacilli* were high.

Because of the use of raw fish byproducts in feed 1 some minor changes in the feed quality occurred during storage at 20°C. It has already been reported by others that acids can serve as effective preservatives in mink feed (Berg 1986; Jørgensen 1985; NRC 1982; Poulsen & Jørgensen 1977; Poulsen & Jørgensen 1986ab). It can be concluded that the use

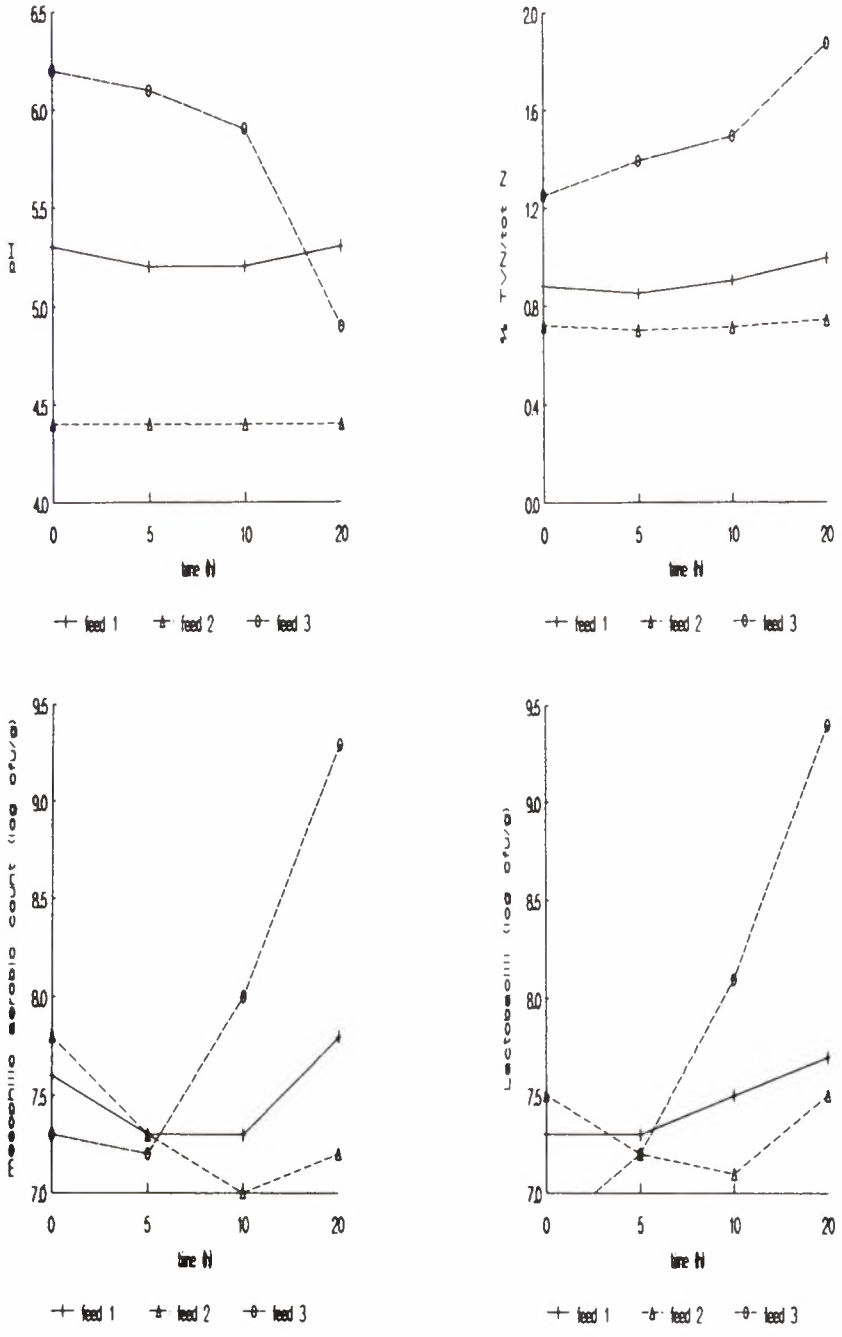

Fig. 1. pH, TVN/total N, mesophilic aerobic count and *Lactobacilli* count (in $\log N.g^{-1}$) in feed formulae 1, 2 and 3 during 20 h of storage at 20°C (see Table 1 for feed composition)

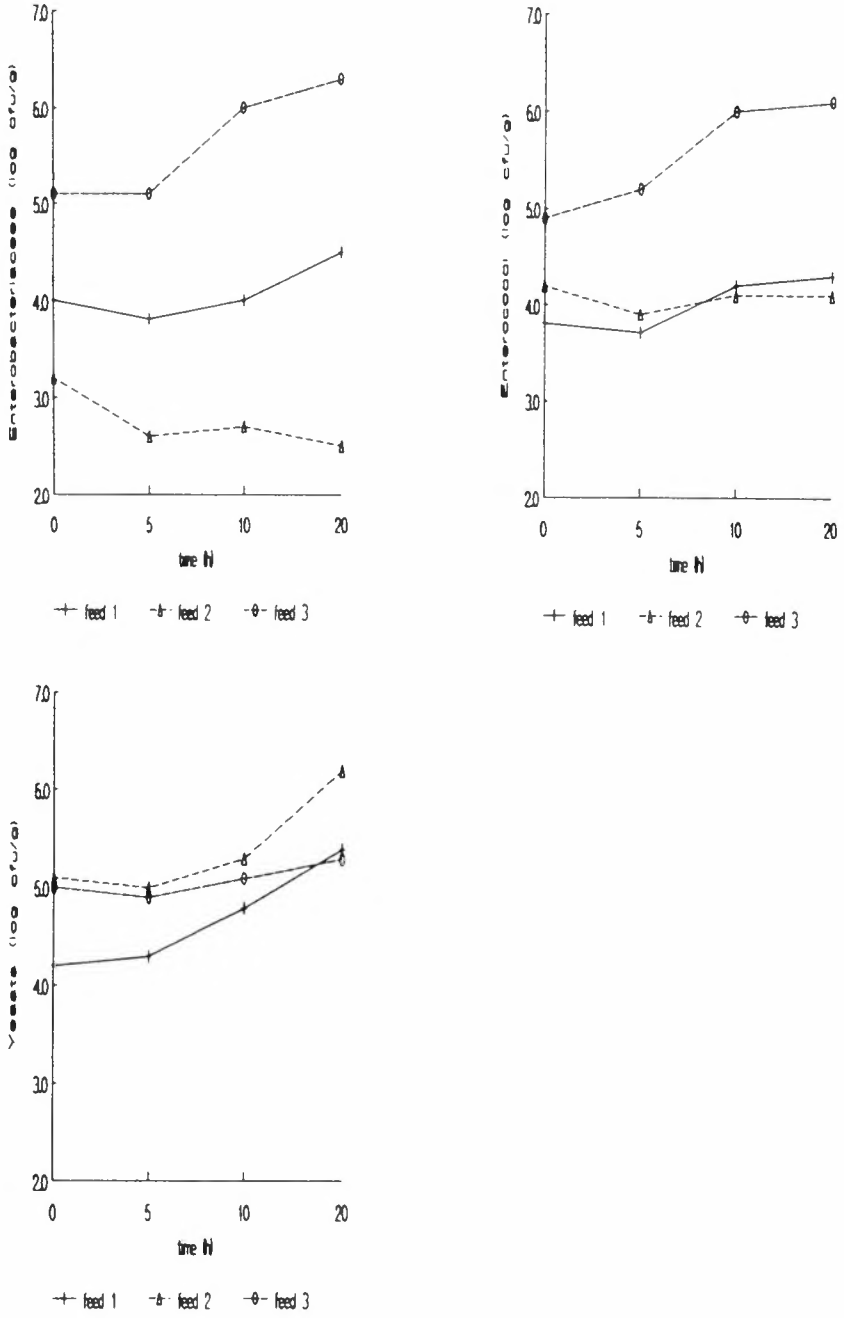

Fig. 2. *Enterobacteriaceae, Enterococci*, and yeast count (in $\log N.g^{-1}$) in feed formulae 1, 2 and 3 during 20 h storage at 20°C (see Table 1 for feed composition)

of pasteurized and fermented broiler byproducts in fur animal feed can delay the deterioration of this feed on the wire of the pen. In this way both microbial and enzymatic spoilage is delayed during the first 20 h of storage, even when substantial amounts of raw fish byproducts are present in the feed.

In feed 3 (control feed without any preservatives) the pH decreased rapidly during the 20 h storage because of the rapid bacterial growth which resulted in the formation of, for example, acids. This microbial spoilage also caused breakdown of amino acids, which was reflected in an unacceptable level of TVN, after 10 h storage, compared with the levels referred to in the literature (Jørgensen 1985).

Microbial and enzymatic spoilage can occur quickly in fur animal feed as a result of the use of raw animal byproducts in the feed. As long as fur animals are housed in open sheds in ambient climatic conditions, it is concluded that fur animal feed without any preservative agent will be spoiled within 20 h when ambient temperatures are at 20°C or more. As a result, fur animals need to be provided with feed containing an effective preservative against both microbial and enzymatic spoilage, or, alternatively the animals will have to be fed at least twice a day.

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The effects on some physiological and performance indices of adding formic acid-preserved feed to the meat ration of ferrets

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Forty young ferrets divided into two groups of 20 were used in the study. The control group was fed a diet containing 40% fresh meat offal, 10% fish offal, 25% cooked pearl barley, 10% wheat bran, 8% green forage and vegetable and 7% sour milk. The meat ration of the experimental group (E) consisted of 15% fresh meat offal and 25% meat offal preserved with formic acid (1.5 kg formic acid was added to 100 kg meat feed), the other ingredients of the diet were the same as those in the control group. The supplementation of ferret feed with formic acid-preserved feed had no effect on the acid-base parameters, the body weight and grading of live animals, but a decrease was observed in the digestibility of crude protein.

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In numerous experiments with chemically preserved feed for fur-bearing animals, organic acids such as lactic, formic, acetic and propionic acid as well as inorganic acids such as sulphuric, hydrochloric and phosphoric acid have been used.

An experiment carried out in 1983 (Pölönen et al. 1991) demonstrated how formic acid affects breeding results when added to the ready mixture. After five months it was found that some morphological indices of tested males were markedly lower than those in the control group. After seven months, however, there were no differences between the experimental and control groups. The use of formic acid did not affect whelping results in females when females in the experimental group were mated with males from the control group. The supplementation of mink feed with formic acid in experiments carried out by Bieguszewski (1991) and Bieguszewski et al. (1991) had no effect on most of the morphological and biochemical blood indices or on the acid-base balance.

The main objective of the present experiment was to study the effects of adding feed preserved with formic acid to the meat ration of ferrets on the digestibility of nutrients, acid-base parameters and weight gains as well as on the grading of live animals.

MATERIALS AND METHODS

Fourty young ferrets (males and females) were used in the study, and the investigations covered the period from August to 15 December 1988.

The animals were divided into two groups of 20 animals each. The control group (C) was fed a diet containing 40% fresh meat offal, 10% fish offal, 25% cooked pearl barley, 10% wheat bran, 8% green forage or vegetables, 7% sour milk and mineral-vitamin mixture (Polfamix N,2 g/1 kg feed). The meat ration of the experimental group (E) consisted of 15% fresh meat offal and 25% meat offal preserved with formic acid (1.5% formic acid was added to 100 kg meat feed), the other ingredients of the diet were the same as those in the control group.

The digestibility of nutrients was investigated in five females from each group from 21 September to 27 September. The animals were placed in cages equipped for quantitave feeding and faeces. Water was available ad libitum.

In blood samples collected from the animals before pelting (16 December) acid-base parameters were determined.

The weight gains were tested four times during the growing period. After the winter fur appeared (15 December), the grading of live animals was conducted.

RESULTS AND DISCUSSION

The digestibility coefficients of the nutrients are presented in Table 1. In the experimental group there was a reduction in the digestibility of all the tested nutrients. For crude protein digestibility the difference was statistically significant. This could have an effect on the digestibility of organic matter. Hansen & Glem-Hansen (1980) did not find any effect of the amount of sulphuric acid used to preserve fish on the digestibility of crude protein and crude fat in mink. A slightly positive influence on the digestibility of carbohydrates was observed after sulphuric acid preservation.

Table 1. Digestibility coefficients of the nutrients (%) Mean values (x) and standard deviations (SD)

Nutrients	Group of animals C		E	
	$\bar{x}$	SD	$\bar{x}$	SD
Dry matter	63.52	3.28	60.70	3.65
Organic matter	64.55	3.28	61.51*	3.43
Crude protein	90.21	1.53	88.24*	1.59
Crude fat	80.05	4.28	76.42	7.55

* = difference statistically significant ($p \leq 0.05$)

As can be seen in Table 2 the experimental feeding did not result in any unfavourable effects on the acid-base parameters of the blood. Providing acid-preserved feed to fur-bearing animals in accordance with experiments carried out with mink by other authors (Jørgensen et al. 1976; Poulsen & Jørgensen 1977), can cause metabolic acidosis. The

weight gains of animals are given in Table 3. Adding preserved feed to the ration did not have any statistically significant influence on body weight during the last stage of the experiment. According to Helgebostad & Svenkerud (1977) the feeding of hydrochloric and sulphuric acid-preserved fish had a negative influence on the weight gain of mink. The grading of ferrets is presented in Table 4. The animals from the experimental group had better scores than the controls (30-23 points). None of the control ferrets reached a score level of 30-29 points and 10% of them scored only 22 or 21 points.

Table 2. Acid-base parameters of ferrets' blood. Mean values ($\bar{x}$) and standard deviations (SD)

Parameters	Group of animals C		E	
	$\bar{x}$	SD	$\bar{x}$	SD
pH	7.29	0.03	7.30	0.03
p CO_2(mmHg)	42.67	2.77	43.00	2.51
p O_2(mmHg)	59.92	6.32	56.07	3.67
HCO^-_3(mmol/l)	21.14	2.02	21.30	2.26
Base excess(mmol/l)	-4.58	2.11	-4.45	2.49

Table 3. Body weight of ferrets (kg) Mean values ($\bar{x}$) and standard deviations (SD)

Age in weeks	Group of animals C		E	
	$\bar{x}$	SD	$\bar{x}$	SD
12	0.73	0.11	0.66	0.12
16	0.91	0.13	0.82*	0.06
20	0.99	0.13	0.97	0.10
27	1.02	0.17	0.97	0.08

* = difference statistically significant $p \leq 0.05$

Table 4. Grading of ferrets. Body size and coat quality (in points)

Group of animals	No. of tested animals	Points 30-29	28-26	25-23	22-21	20
C	20	-	9	9	2	-
E	20	3	8	9	-	-

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Morphological and biochemical indices of blood of mink fed with chemically preserved feed additives

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The experiment was carried out in two series with standard mink. In experimental group 1 of the first series, 50% of the frozen meat-fish fodder was replaced with by feed preserved with formic acid. In experimental group 2, 33% of the meat-fish was replaced by slaughter blood preserved with sulphuric acid and sodium benzoate. The meal ration of experimental group 1 in the second series was the same as that in the control group in the first series but 5 ml formic acid was added to 1 kg fodder each day. Mink of the second experimental group received the same diet as that in the second experimental group in the first series but only slaughter blood was preserved with sulphuric acid. No significant differences in the morphological and biochemical indices, acid-base balance parameters or the iron level and TIBC in mink fed the diet with formic acid were found. The addition of slaughter blood preserved with sulphuric acid and sodium benzoate or with sulphuric acid only to the meal ration of mink, had a slight effect on some indices of the red blood cell system. In experimental group 2, no change occurred in the biochemical indices, acid-base parameters, iron level of blood plasma or TIBC.

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The purpose of the experiments carried out by Pölönen et al. (1991) was to examine the possibility of using organic and non-organic-acid-preserved fish or meat as an ingredient of feed for mink. The authors observed that experimental feeding did not have any unfavorable effects on breeding results and pelt quality. Jørgensen et al. (1976) proved that long-term us of large quantities non-organic-acid-preserved fish for mink affected breeding results and acid-base balance. According to the results obtained by Poulsen & Jørgensen (1977) and Poulsen & Jørgensen (1986) a reduction in the pH value of the feed preserved with sulphuric acid below 5.5 may cause metabolic acidosis. The influence of herring preserved with sulpuric acid on the time it takes to pass through the gastrointestinal tract of mink was significant in Hansen's (1978) investigation. Hansen & Glem-Hansen (1980) also examined the effect of sulphuric acid on the digestibility of rations containing 0.20 and 40% acid-preserved fish in growing mink. The digestibility of crude protein and crude fat was unaffected. In our experiment, carried out earlier (Bieguszewski et al. 1991) we found an increase in the digestibility coefficient of crude protein in mink fed a diet in which 50%

of the meat feed was preserved with formic acid. There were no difference in nitrogen retention.

The main objective of the present experiment was to study the effects of adding feed preserved with formic acid as well as with sulphuric acid and sodium benzoate on some morphological and biochemical indices of mink blood.

MATERIAL AND METHODS

The experiments were carried out in two series, in the first of which 60 standard minks were divided into three groups. After separation from the females, the animals were fed different diets. The control group of mink was fed the diet containing: meat-fish fodder, fresh or frozen - 75%, lean milk powder 1%, cooked pearl barley or bruised wheat - 14%, wheat bran - 6%, green forage and vegetables - 4%. In the meal ration of the first experimental group, 50% of frozen meat-fish fodder was replaced by meat-fish fodder preserved with formic acid (1.5 kg formic acid was added to 100 kg meat-fish fodder). Preserved fodder was stored in barrels for 2 - 4 weeks. In the meal ration of the second experimental group, 33% of frozen meat-fish fodder was replaced by slaughter blood, preserved with sulphuric acid and sodium benzoate (0.7 kg sulphuric acid and 0.7 kg sodium benzoate was added to 100 kg blood). In blood samples collected from mink before pelting, morphological and biochemical indices as well as acid-base parameters were indicated. In the second series, 30 mink were divided into three groups. The control group received the same diet as that in the first series. The meal ration of the first experimental group was the same as that of the control group but 5 ml formic acid was added to 1 kg fodder each day. Mink from the second experimental group received the same diet as that of the second experimental group in the first series but slaughter blood was preserved only with sulphuric acid without sodium benzoate. In blood samples collected from the animals, before pelting, the same parameters as those in the first series were indicated as well as the content of iron in blood plasma and total binding capacity of iron blood plasma (TIBC).

RESULTS AND DISCUSSION

The addition of feed preserved with formic acid and sulphuric acid to the rations did not have any negative effects on the morphological indices of the blood erythroblastic system or on the number of white blood cells (Table 1). In the first series of experiments, the number of red blood cells, haemoglobin and haematocrit values were higher in mink fed with preserved slaughter blood. Only the difference in haemoglobin content between control and experimental group 2 was statistically significant. In the second series the haemoglobin value and the number of red blood cells were higher in the second experimental group but the difference was not statistically significant. Slaughter blood with a high content of iron could have a favourable effect on the erythropoietic progress in animals that had the lower iron level (Table 3) which could be related with higher utilization of blood plasma iron. No statistically significant changes in total content of protein and urea of blood plasma were noticed in either the first or second series of experiments (Table 1). As is well known the

liver is the organ in which urea and synthesis of most of the blood plasma proteins take place. On the grounds of these biochemical indices in this experiment we can assume that acid-preserved feed did not have a negative effect on liver function.

Table 1. Morphological and biochemical indices of mink blood

Indices	Group of animals					
	I series			II series		
	Control	Experimental		Control	Experimental	
		1	2		1	2
Number of red blood cells T/l	10.92 ±0.83	10.91 ±0.86	11.10 ±0.86	8.97 ±1.25	9.26 ±0.75	9.32 ±0.99
Haemoglobin content g/l	227.64 ±12.32	228.76 ±11.94	241.03x ±10.77	204.40 ±10.10	205.80 ±13.80	206.80 ±9.30
Haematocrit indices l/l	0.62 ±0.04	0.59 ±0.04	0.66 ±0.03	0.59 ±0.03	0.58 ±0.04	0.58 ±0.03
Number of white blood cells G/l	5.63 ±1.26	5.31 ±1.69	6.27 ±1.89	5.45 ±1.92	4.77 ±1.11	5.49 ±0.91
Total protein of blood plasma g/l	78.23 ±11.10	76.36 ±9.60	78.13 ±13.90	56.00 ±7.78	52.40 ±7.52	61.60 ±4.62
Urea of blood plasma μmol/l	7.22 ±2.60	6.30 ±2.10	6.44 ±2.70	6.10 ±1.10	6.60 ±0.56	6.60 ±0.97

x - differences statistically significant $p \leq 0.05$

The supplementation of mink fodder with formic and sulphuric-acid-preserved feed did not have any influence over hydrogen ion concentration (pH) and buffer systems (Table 2). The absolute values of pO_2 and pCO_2 as well as HCO_3^- and BE were different from those in experiments carried out by Poulsen & Jørgensen (1977) but these authors collected mink blood samples under anaesthesia.

The iron level and total binding capacity of iron in blood plasma (TIBC) are good indices of erythropoietic progress. The iron level in human blood plasma is about 20-32 μmol/l and TIBC 45-75 umol/l (Pawelski 1977). The iron level decreases and TIBC increases in some anaemia types caused by iron deficiency. In the experiment on foxes (Stanislawska et al. 1987) the authors demonstrated that a reduction in iron level occurred simultaneously with a TIBC increase in females during the periods of pregnancy and lactation. The corresponding results were obtained in mink in the present experiment (Table 3), The slight reduction in iron level in the blood plasma of the experimental groups appeared along with a statistically insignificant increase in TIBC.

Table 2. Acid-base balance parameters of mink blood

Parameters	Group of animal					
	I series			II series		
	Control	Experimental		Control	Experimental	
		1	2		1	2
pH	7.21 ±0.08	7.22 ±0.05	7.19 ±0.06	7.19 ±0.03	7.23 ±0.02	7.24 ±0.03
pO_2/mmHg/	63.10 ±7.45	63.43 ±15.61	67.78 ±7.78	72.70 ±10.46	67.56 ±8.96	70.14 ±13.16
pCO_2/mmHg/	45.00 ±5.50	49.25 ±8.55	47.89 ±7.41	42.56 ±7.14	44.50 ±12.60	47.91 ±11.10
HCO_3^-/μmol/l/	19.74 ±2.35	20.21 ±3.80	18.29 ±2.28	15.70 ±4.69	17.96 ±3.43	19.49 ±4.02
Base excess /μmol/l/	−7.78 ±1.50	−7.16 ±3.72	−9.39 ±2.59	−12.30 ±7.32	−9.20 ±3.90	−7.47 ±5.15

Table 3. The iron level and total binding capacity of iron in mink blood plasma (TIBC)

Indices	Control	II series Group of animals Experimental	
		1	2
The iron level (μol/l)	41.67 ±9.66	35.45 ±7.03	38.41 ±6.53
TIBC (μol/l)	60.00 ±7.46	66.48 ±15.20	65.12 ±6.90

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Taste appeal trials with poultry offal for mink

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The purpose of taste appeal trials is to achieve an expression of the effect of ingredients and feed composition on the feed consumption of the animals, primarily as a response to smell and taste. Trials are carried out either with lactating females or with growing kits. Each experiment lasts four weeks, and two groups of 10 females or two groups of 10 male kits and 10 female kits are used. In weeks 1 and 4 all the animals have the opportunity of choosing their feed, in weeks 2 and 3 the two groups are only offered one of two kinds of feed. Feed consumption and weight gain are registered. Trials where poultry offal is added to the feed have shown that lactating females, kits in the early growth period and kits in the late growth period all prefer feed containing poultry offal ($p < 0.0001$ in all trials).

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Very little literature has been published on the subject of palatability of mink feed. Several articles, however, have been written on taste appeal trials with pigs and especially piglets (Danielsen 1991; Gatel & Guion 1990 and others).

In the lactation period and the growth period there are sometimes problems in getting the mink to eat enough feed. Through taste appeal trials we hope to find ingredients, feed composition and technical treatments of the feed that appeal to the mink and thereby entice them to consume the desired quantities of feed.

In the long run, when we know the minks' preferences, aromatic compounds could be added to the feed to increase consumption in certain periods. But, to begin with, different ingredients have to be tested.

Poultry offal is an interesting ingredient because it has an acceptable nutritional value, is low-priced and is in plentiful supply.

In 1990 an 1991 three trials were carried out with poulty offal: one with lactating females (trial 1, 1991), one with kits in the early growth stage (trial 2, 1990) and one with kits in the late growth stage (trial 3, 1991) . In the following secxtion a general description of the experimental design and procedure as well as a specific description of the three trials are given.

MATERIALS AND METHODS

Housing

There were two groups in each trial and the experimental design comprised 2 x 10 m cages

specially designed for the purpose. Mink cages are usually provided with an extra cage placed just above the drinking valve. In the extra cage there is a double floor with enough distance to give room for two salvers in which feed waste is collected. The feed is placed in two plastic cups at one end of the extra cage.

The type of cage used for the taste appeal trials seen from the side is illustrated in Fig. 1 and seen from above in Fig. 2.

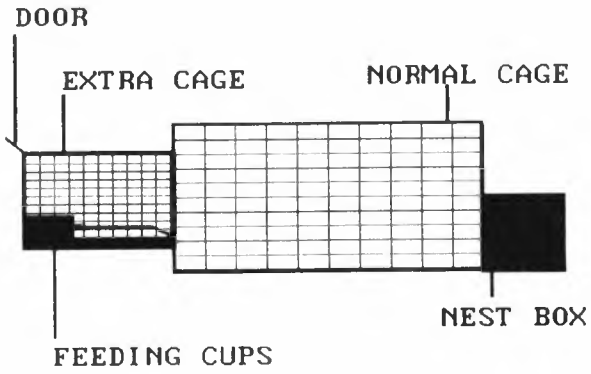

Fig. 1. Cage used for taste appeal trials with mink - seen from the side

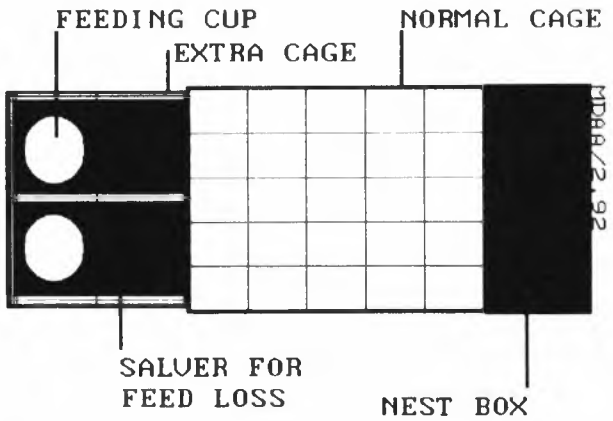

Fig. 2. Cage used for taste appeal trials with mink - seen from above

Animals

The trials were carried out with breeding animals, i.e. lactating females or growing kits. In this way the experimental design was as close as possible to the practical conditions.

Trials with lactating females included 20 females (1 per cage) and trials with growing kits included 20 male kits and 20 female kits (1 male and 1 female per cage). Ages and

weights were aimed at being equal in the two groups from the beginning of the trial.

Trial 1: 20 lactating females of the scanbrown type. Kits born within one week an on average 5 days old at the start of the trial.

Trial 2: 20 male and 20 female kits of the scanblack type. Kits born within one week and on average 10 weeks old at the start of the trial.

Trial 3: 20 male and 20 female kits of the scanbrown type. The trial started in the middle of September.

Feeding procedure

Each trial lasted four weeks and the two groups were fed according to the procedure set out in Table 1.

Table 1. Feeding procedure for taste appeal trials with mink

	Week 1	Week 2	Week 3	Week 4
Group 1	Diet 1 + Diet 2	Diet 1	Diet 1	Diet 1 + Diet 2
Group 2	Diet 1 + Diet 2	Diet 2	Diet 2	Diet 1 + Diet 2

Feed allocation

Week 1: All the animals were given both diets and were free to choose which one they liked best. In this way the animals' immediate response to the two diets were registered.

Week 2: The animals in group 1 were only given diet 1 and the animals in group 2 were only given diet 2. This revealed whether a possible aversion to one of the diets in week 1 was strong enough to induce the mink to eat less if they did not have the opportunity of choosing.

Week 3: Same procedure as week 2. The two weeks with no opportunity to choose a diet allowed the animals to get used to the two diets respectively.

Week 4: All the animals were again given both diets and were free to choose. This revealed whether the animals have become accustomed to the feed or i they returned to the feed preferences displayed in the first week.

Feed

The two diets were only produced once in sufficient quantities for all four weeks, whether or not the animals choose to cover their needs from one of the feed types only. Diets were planned to be aimed equal with regard to content of protein, fat, carbohydrates, energy and also energy distribution. The feed was frozen in portions corresponding to one day's consumption.

Feed composition is shown in Table 2.

Table 2. Feed composition in taste appeal trials 1, 2 and 3

	Trial 1		Trial 2		Trial 3	
Group	1	2	1	2	1	2
Ingredients	%	%	%	%	%	%
Poultry offal	-	18.9	-	20.3	-	19.4
Cod offal	41.0	35.5	21.2	14.6	20.4	14.6
Trawl fish	25.0	9.5	21.2	14.6	20.4	14.6
Fish silage	3.0	2.8	10.6	11.3	10.7	10.7
Fishmeal	6.0	5.7	3.2	3.0	4.9	2.9
Haemoglobin meal	1.0	0.9	0.5	0.6	1.0	1.0
Vitamin mixture	4.0	3.8	4.2	4.5	4.4	4.4
Barley	4.0	3.8	9.6	10.1	6.8	6.8
Wheat	-	-	3.3	3.5	6.8	6.8
Wheat germ	1.0	0.9	-	-	2.2	2.2
Skimmed milk powder	1.0	0.9	-	-	-	-
Corn gluten	-	-	2.2	2.3	-	-
Meat and bone meal	-	-	1.6	1.7	-	-
Lard	0.5	-	5.3	5.1	6.0	4.9
Soybean oil	1.5	0.5	3.7	2.8	3.9	3.1
Water	12.0	16.6	13.5	5.7	12.4	8.6
Digestible nutrients, %						
protein	14.9	14.9	12.8	13.7	13.8	14.1
fat	4.2	3.9	11.1	11.7	13.5	11.7
carbohydrates	3.1	3.0	6.9	7.8	7.2	7.8
Energy distrib., %:						
protein	56	57	30	30	28	31
fat	33	32	55	54	58	54
carbohydrates	11	11	15	16	14	16
Metabolizable energy:						
kcal/100 g	120	116	192	205	221	205

Registrations and tests

Feed rations were weighed out and leftovers and waste were weighed. Feed consumption was calculated on the basis of wet feed, dry matter and energy (kcal). In this paper only the energy consumption is presented.

The mink were weighed on the first day of each week and the day after the last allocation of feed. In trials with lactating females the mothers were weighed as already described and the kits were weighed at the beginning and at the end of the trial.

T-tests were used to test differences in feed consumption and weight.

RESULLTS AND DISCUSSION

Most of the taste appeal trials with pigs or piglets have been carried out either as

production experiments where pigs are divided into groups and each given a diet (Danielsen 1991; Gatel & Guion 1990), or as a free-choice exsperiment where all the pigs are free to choose between diets. In the latter type of experiment, performance on each diet cannot be measured. None of the two methods is sufficient to decide whether the addition of an ingredient or a flavour is desirable or not. Preference on a free-choice basis is not always for the diet which will give optimum performance (Aumaitre 1978; Wahlstrom et al. 1974), and when a single feed is imposed on the animals, they are capable of consuming unpalatable rations as measured by free-choice tests (Aumaitre 1978). It is therefore important that the experimental design takes both performance and free-choice into account, as was the case in these trials:

Trial 1 (lactating females)

Number of kits born per female is 6.6 in group 1 and 6.7 in group 2. Energy consumption is shown in Fig. 3. In both groups the females' immediate response (week 1) to the two diets was significantly in preference of the feed containing 19% poultry offal ($p < 0.001$). In weeks 2 and 3 there was a significant difference ($p < 0.05$) in consumed energy in the two groups. The higher consumption in group 2 may have been caused by the fact that the diet containing poultry offal had the better taste, or by the fact that there were somewhat fewer one-year-old females in this group. In week 4 sathe differences between the two diets were even more obvious than in week 1 ($p < 0.0001$, for both groups.

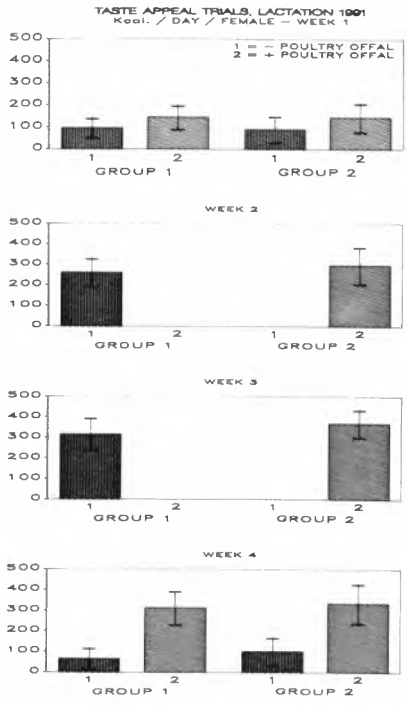

Fig. 3. Energy consumption (kcal/day/female), weeks 1-4, taste appeal trials, lactation 1991

The development of the weights of females and kits is shown in Figs. 4 and 5. There was no significant difference between the two groups with regard to development of female weight. There was no difference between kit weights (Fig. 5) at the start of the trial, but during the experimental period kits from group 2 gained significantly more weight than kits from group 1 ($p < 0.05$). This may have been due to the previously mentioned greater energy comsuption and/or the fact that there were fewer young females in group 2.

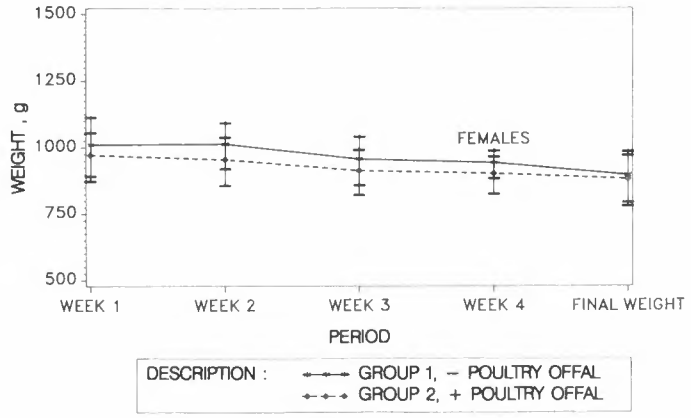

Fig. 4. Development of female weight, taste appeal trials, lactation 1991

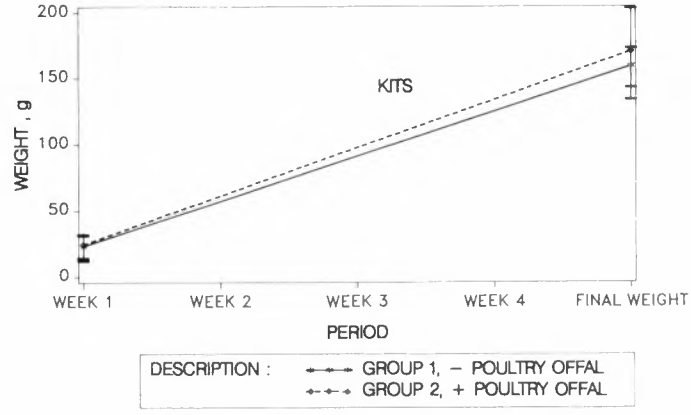

Fig. 5. Development of kit weight, taste appeal trials, lactation 1991

Trial 2 (kits, early growth)

Average daily energy consumption per pair (1 male + 1 female) is shown in Fig. 6. The immediate response to feed containing poultry offal was positive ($p < 0.0001$) in both groups (week 1), just like that of the females in trial 1. But it was found that if the kits were not given the opportunity of choosing, then the quantitites eaten were not different

(weeks 2 and 3). in week 4 - after two weeks with only one kind of feed - both groups still prefered feed containing poultry offal ($p < 0.0001$).

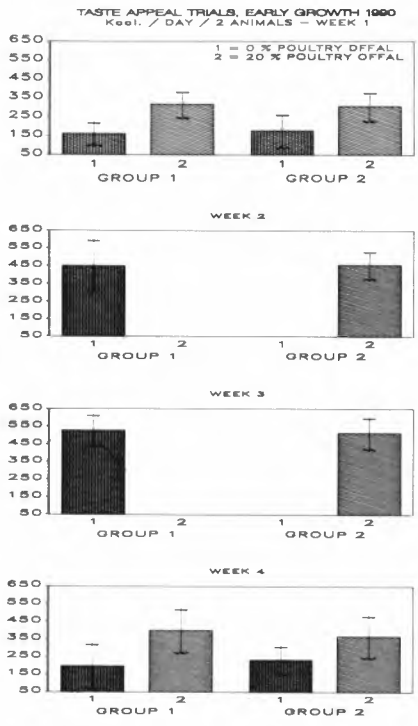

Fig. 6. Energy consumption (kcal/day/2 animals), weeks 1-4, taste appeal trials, early growth 1990

The development of kit weights is illustrated in Fig. 7. There was no significant difference in weight gain between the two groups, nor would a difference be expected, while the energy consumption did not differ between weeks 2 and 3.

Trial 3 (kits, late growth)

Average daily energy consumption per pair (1 male + 1 female) is presented in Fig. 8. The picture is exactly the same as that in trial 2, but even more stressed. The immediate response to feed containing poultry offal was positive ($p < 0.0001$) in both groups (week 1), but again, when the kits were not given the opportunity of choosing, no differences were observed between the quantities eaten (weeks 2 and 3). In week 4 both groups still prefered feed containing poultry offal ($p < 0.0001$).

Development of kit weights is illustrated in Fig. 9. There was no significant difference in average weight gain between the two groups.

The three trials seem to confirm what has already been observed in pigs, namely that the feed intake only in the second instance is linked to factors of palatability and appetence (Aumaitre 1978).

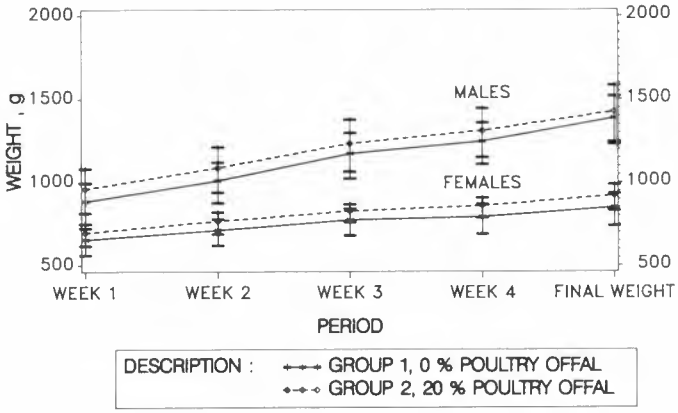

Fig. 7. Development of weight, taste appeal trials, early growth 1990

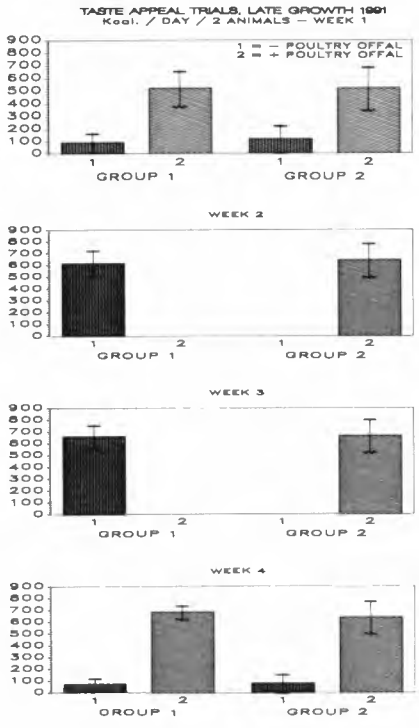

Fig. 8. Energy consumption (kcal/day/2 animals), weeks 1-4, taste appeal trials, late growth 1991

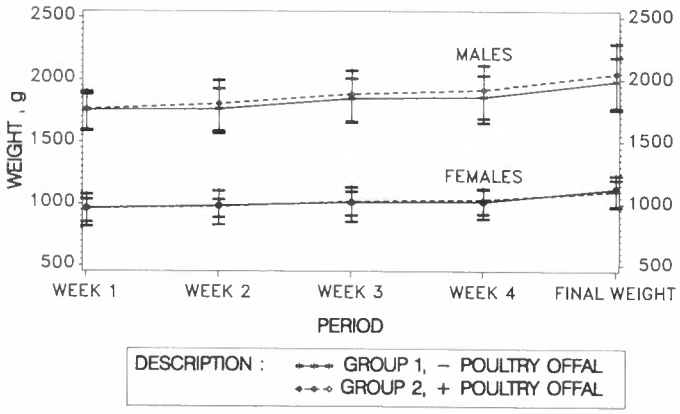

Fig. 9. Development of weight, taste appeal trials, late growth 1991

CONCLUSION

The tree trials concurrently demonstrate that poultry offal in an amount of about 20% of the feed has a positive effect on palatability of mink feed.

Total feed consumption and weight gain are not in general affected by the taste of the feed.

The positive effect of poultry offal on feed palatability did not induce the mink to eat any more of this feed than the feed without this positive effect. The physiological need for nutrients for lactation and growth seems to be so compelling that a suboptimal taste of the feed does not necessarily reduce feed consumption.

It is, however, most likely that a strong unpalatable taste could possibly reduce feed consumption, but the question remains whether a palatable taste could increase it. More trials are needed in order to answer this question.

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Compositional changes in mink (*Mustela vison*) milk during lactation

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Preliminary longitudinal studies on changes in mink milk composition were carried out. Milk samples were obtained in 34 apparently healthy Pastel mink throughout the normal six-week lactation period. A parallel and significant increase in milk fat and dry matter (DM) was observed during the entire lactation period. The protein content of mink milk varied within 8-10%, whereas lactose decreased significantly from the initial value of 2.5%. Moderate changes were observed in mink milk osmolality but significant changes were observed in several major electrolytes. Mean concentrations of calcium and phosphorus increased during lactation (from 22 to 40 mmol/l and from 35 to 48 mmol/l, respectively). Sodium and chloride varied considerably (range: 35-60 mmol/l). Magnesium remained fairly constant at 3 mmol/l, while potassium decreased from 33 to 26 mmol/l. A large individual variation was encountered in all variables tested. The results are discussed with respect to species differences and in relation to the estimated nutritional requirements of nursing kits.

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The current knowledge on basal physiology of fur-bearing animals in connection with lactation is very limited. Only sporadic investigations on longitudinal changes in the individual composition of mink milk, and factors affecting these, have been conducted. Obtaining individual milk samples of sufficient volume and quality, for a number of chemical analyses demands fine-tuned methods, technical skill and endurance.

During the first four weeks of lactation the female mink is the only source of nutrition for the kits. At top lactation (25 days post partum) the female sustains a litter biomass, sometimes exceeding her own. As a consequence of the high average litter size in farm-raised mink, an adequate nutrition covering the high demands of the lactating female is necessary for successful reproduction.

The main objectives of the present study were: to describe longitudinal changes and individual variations in gross chemical composition and mineral content of mink milk as a basis for understanding the nutritional needs of female and litter during lactation; and to investigate the influence of diet on milk composition.

MATERIALS AND METHODS

Animals, management and diets

A total of 692 Pastel females were included in the study. Group 1 comprised of 540 females which were part of a trial with different dietary fat and oil sources, determining the effect on lipid synthesis in mink (Wamberg et al. 1992a), but in this context the females were only used to determine the mineral content of milk. Groups 2 and 4, each comprising of 76 females, were fed the same stock components but in different amounts, resulting in different ratios of metabolizable energy in order to determine whether dietary composition influenced the composition of mink milk. In group 1, 24 females having given birth on 1 May and all having six kits were selected for milking. In groups 2 and 4 respectively, five females with the same specifications as those in group 1 were selected.

The housing, handling and management procedures were those previously described (Clausen et al. 1992) and in accordance with the guidelines of the Danish Fur Breeders' Association (Anonymous 1988).

The composition of the diets used throughout this study is set out in Table 1. All animals were treated equally and feed the same diet up until 15 April when the experimental feeds were supplied.

Table 1. Average composition of basic feed mixtures (%)

Component	Mineral trial	Gross composition trial	
	Mixture 1	Mixture 2	Mixture 4
Fish offal	43.0	48.2	45.0
Industrial fish	15.1	23.6	22.0
Low temp. fish meal	4.0	4.2	3.9
Whole blood meal	1.6	1.2	1.1
Potato protein	0.8	0.9	0.8
Potato pectin	2.0	0.9	0.8
Wheat germ	1.6	1.2	1.1
Wheat bran	2.0	-	-
Heat-treated wheat/barley mix	0.6	6.8	8.7
Fish oil/Rendered lard/soya oil	7.2	3.6	7.2
Vitamin/mineral premix[b]	0.2	0.2	0.2
Water added	21.9	9.2	9.2
Digestible protein[c] g/100 g[a]	12.1	15.2	14.2
Digestible fat[c], g/100 g	8.3	5.5	8.4
Digestible carbohydrate[c], g/100 g	1.7	4.0	4.9
Metabolizable energy[c] (ME)	5.85 MJ/kg[a]	5.75 MJ/kg	6.90 MJ/kg
ME distribution ratio (P:F:C)[d]	39:56:5	50:38:12	39:48:13

[a] wet feed mixture "as fed";

[b] VITAMIX (Vitfoss A/S, Graasten, Denmark), containing: **Vitamins (per kg)**: Vit. A, 3 500 000 IE; vit. D_3, 350 000 IE; vit. E, 30 000 mg; vit. B_1, 12 500 mg; vit. B_2, 6 000 mg; vit. B_6 4 000 mg; vit. B_{12}, 10 mg; cholin.HC1, 10 000 mg; niacin, 10 000 mg; d-pantothenic acid, 4 000 mg: para-amino benzoic acid, 1 000 mg; folic acid, 150 mg; biotin, 100 mg. **Minerals (per kg)**: Fe(II) fumarate, 75 g; Mn(II) O, 12.5 g; $Cu(II)SO_4.5H_2O$, 5 g; ZnO, 20 g; $Ca(IO_3)_2$, = .05 g:

[c] Calculated

[d] Protein : fat : carbohydrate

Sampling and sample preparation

Mink milk/mineral trial. For each of the four dietary groups with different fat/oil components, pooled milk samples from six apparently healthy dams were collected over three days (two dams per day) at five-day intervals.

Gross composition trial. In groups 2 and 4, individual samples of five apparently healthy dams were collected twice a week during the six-week lactation period.

In the morning the dam was separated from her kits for approximately one hour and, following a single intramuscular injection of 1 IU per dam of synthetic oxytocin (Oxytocin Vet.^{R}, Ciba-Geigy, 10 IU/ml), approximately one millilitre of mink milk was obtained using a modified human milking machine (Medela, type 015, Barr, Switzerland). The milk samples were stored at -20°C in 2 ml Bio-Freeze plastic vials with a rubber washer (Costar), containing three 2-3 mm glass beads, until analyzed. Before taking subsamples for different chemical analyses the samples were placed in a thermobath at 40°C for five minutes and subsequently homogenized on a laboratory shaker.

Chemical analyses

Analysis of the composition of the feed rations (DM), crude protein (CP), crude fat (CF) and ash, using standard methods as described in a previous publication (Wamberg et al. 1992a).

Milk protein was analyzed in subsamples of 0.5 ml using a Kjel-Foss device. The crematocrit value of milk samples was determined as described by Ganguli et al. (1969) and Lucas et al. (1978). In order to convert crematocrite values to fat percent following the regression equation $y=0.49X + 1.77$ ($N=17$ $p < 0.0001$) between crematocrite values and Roese Gottlieb, fat extractions on the same samples were used. For the analyses of lactose, the Boehringer Mannheim Lactose/D-galactose enzyme kit (kat.no. 176303) and Carrez-reagent was used. Dry matter was determined by drying 50μl capillary glass tubes with a milk sample for 24 h at 105°C. The analytical methods used for the determination of sodium, potassium, calcium, magnesium and (total) phosphorus were those given previously (Wamberg et al. 1992b).

All analyses were performed in duplicate, and the analytical accuracy was checked by means of analyzing aqueous reference solutions with each batch of samples as previously described (Wamberg et al. 1976, 1992a).

Statistics

The students t-test was used in comparing milk composition from different feed trials (SAS 1988).

RESULTS AND DISCUSSION

Although the chemical composition of mink milk has been investigated previously (Jørgensen 1960; Conant 1962; Kinsella 1971; Glem-Hansen et al. 1973; Glem-Hansen & Jørgensen 1975; Olesen 1988; Aulerich 1989) in general the data are available for only part of the lactation period and samples are pooled or statistical material is very limited. In this

study the purpose was to collect a sufficient number of samples on an individual basis from as many animals as possible during the whole lactation period.

A parallel and significant increase in milk fat and DM was observed during the entire lactation period (Table 2), with increasing fat increasing from initial values of 7.5% to about 20% and DM ranging from approximately 20% to 35%. This result is in agreement with the observations of Glem-Hansen & Jørgensen (1975) and has also been observed in the domestic ferret (Schoknecht et al. 1985). These chemical changes apply to changes in body composition, where most mammals show increased DM and fat content during early growth (Spray & Widdowson 1950). This pattern also seems valid for mink kits data available (Skrede 1981; Olesen unpublished). High milk fat is usually seen in animals that have a short nursing period and high postnatal growth rate (Oftedal 1984).

Table 2. Chemical composition and individual variations in mink milk during lactation (mean ± SD)

Days post partum	N	Protein (%)	Fat (%)	Lactose (%)	Dry matter (%)
3	8	7.4 ± 1.0	7.5 ± 1.1	2.2 ± 0.3	19.5 ± 2.2
8	5	7.2 ± 0.8	6.4 ± 1.1	1.7 ± 0.4	19.2 ± 1.2
11	8	7.1 ± 0.9	9.0 ± 1.5	1.5 ± 0.2	22.7 ± 2.6
16	9	8.0 ± 0.8	9.5 ± 2.3	1.3 ± 0.2	24.9 ± 4.3
18	9	7.4 ± 0.8	9.9 ± 2.5	1.2 ± 0.3	23.3 ± 3.0
22	9	7.7 ± 0.9	8.2 ± 2.2	1.1 ± 0.3	22.3 ± 3.0
25	9	7.6 ± 0.7	10.3 ± 3.3	1.0 ± 0.3	25.3 ± 4.2
29	9	8.2 ± 1.2	13.6 ± 1.4	0.9 ± 0.3	29.0 ± 2.8
32	9	8.8 ± 2.1	11.1 ± 4.0	0.9 ± 0.3	24.9 ± 6.9
36	6	10.3 ± 1.3	13.5 ± 3.1	0.4 ± 0.3	29.6 ± 5.5
39	9	9.7 ± 1.5	18.7 ± 1.9	0.1 ± 0.1	37.6 ± 3.6

There are significant individual variations in the fat content of mink milk as indicated by the relative standard deviation (RSD or CV-value) ranging between 10% and 36%. Student's t-tests on diet-imposed changes in milk fat revealed that only in two of the 11 days when milk was sampled did the high fat diet (group 4) show a significant ($N = 9$, $p < 0.05$) higher milk fat content than that found in the group on a low fat diet (group 2). Despite our belief that diet-induced changes in fat content of the milk is possible in the mink, we chose to pool milk data from the two diet groups (see Table 2).

The protein content of mink milk varied slightly within 8-10%, whereas lactose decreased significantly from the initial value of 2.5%, almost disappearing in late lactation (Table 2). The protein content of mink milk is approximately seven times higher than that in most primates, but values of 8-10% are in accordance with most other carnivores (Conant 1962; Oftedal 1984).

Statistical analysis revealed no significant diet-induced changes in protein content of the milk at any point during lactation. RSD values ranged between 10% and 23%. In this study the only carbohydrate analyzed was lactose. Conant (1962) claims that lactose is not the dominant carbohydrate in mink milk. In rat milk neuraminlactose is a well known carbohydrate component, but makes up only one-third of total carbohydrate (Kuhn 1972). In earlier data, where the carbohydrate fraction was calculated as a difference (NFE),

values for mink milk ranged from 6-8%, but a clear chemical profile of the carbohydrate fraction of mink milk has not yet been established. This leaves a very interesting field of research because of the close connection between carbohydrates in milk and milk yield (Holt 1983). In spite of this interaction, large species differences exist for the level of carbohydrate in milk (Oftedal 1984), and for most other animals, including those in this study, there is a strongly negative correlation between fat and lactose content.

Osmolality varied between individuals (RSD between 1% and 9%) but appeared rather constant during lactation (Table 3).

Table 3. Mineral content (mmol/l) and osmolality (mOsm/kg H_2O) in mink milk during lactation. Values ar mean ± SD

Days post partum	Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	Total P	OSM
1-3	49.3 ± 10.3	33.3 ± 4.9	21.7 ± 7.3	3.2 ± 0.3	48.8 ± 6.3	34.6 ± 4.5	423.8 ± 38.4
8.\-10	50.5 ± 3.5	33.3 ± 1.3	35.4 ± 9.6	3.1 ± 0.3	58.7 ± 4.9	43.9 ± 4.2	476.0 ± 5.3
15-17	44.0 ± 6.2	30.5 ± 3.0	31.1 ± 1.9	2.7 ± 0.1	47.8 ± 8.5	41.0 ± 1.7	461.3 ± 37.6
22-25	37.5 ± 1.5	27.8 ± 0.8	34.2 ± 1.2	2.7 ± 0.2	41.8 ± 2.9	43.3 ± 3.3	434.5 ± 25.9
29-31	39.0 ± 1.6	29.3 ± 1.3	40.1 ± 4.2	3.1 ± 0.1	43.8 ± 1.3	48.2 ± 4.6	466.3 ± 19.5
37-39	36.6 ± 3.0	26.2 ± 2.0	41.7 ± 5.1	2.8 ± 0.3	51.0 ± 4.4	44.1 ± 4.4	460.2 ± 12.5

Sample size: Every mean value is calculated on the basis of four samples each pooled from 12 individual samples

Mean concentrations of calcium and phosphorus increased during lactation from 22 to 40 mmol/l and from 35 to 48 mmol/l, respectively, satisfying the growing demands for these minerals in the kits. Sodium and chloride varied considerably (range: 35-60 mmol/l). Magnesium remained fairly constant at 3 mmol/l, while potassium decreased from 33 to 26 mmol/l. Except for potassium, such changes reflect normal changes in body composition found in many mammals (Spray & Widdowson 1950). Rather large individual variations were encountered in all minerals tested.

Interestingly, the concentrations of calcium and phosphorus in mink milk are approximately a factor of 10 lower than those found in basic mink feed, while the other minerals under consideration in this study were only approximately a factor of 2 lower than those in basic mink feed.

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Effects of dietary fluoride on growth, reproductive performance, and tissue fluoride levels of the fox (*Alopex lagopus*)[1]

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The effects of dietary F on fox growth, reproduction, and tissue F accumulation were evaluated by feeding a commercial pelleted diet (104 mg F/kg) supplemented with 0, 50, 150, or 250 mg/kg F (as NaF). Body weight gain of three-month-old blue fox pups was not affected by dietary F addition and plasma, urine, and femoral F concentrations increased linearly with increasing dietary F. No treatment effects were observed on fur density, color, or texture. Females from each group were maintained on their respective diets through three successive breeding cycles. Addition of 250 mg F/kg to the diet had no effect on litter size or pup body weight at four weeks of age. Pup survival rate at four weeks was adversely influenced by 250 mg F/kg only during the third litter. Growth rates of pups maintained on their dam's experimental diet were not affected by dietary F level. Skeletal or dental signs of F toxicosis were not observed at any time during these studies. During the third breeding season, vixens were switched from 50 mg/kg supplemental F to 600 mg/kg F. Pups whelped to these females had reduced survival rates, but litter size and lactation performance were not affected. Based on the results of these studies, fox are relatively tolerant of dietary F.

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Animals routinely ingest variable but low levels of fluoride (F), with no detrimental effects, but prolonged ingestion of F above the species' tolerance level may result in F toxicosis. Although the effects of excessive intake of F in many farm and laboratory animal species have been well documented (National Research Council 1974), relatively little information is available regarding the effects of this nutrient on fox. Eckerlin et al. (1986) have recently reported reproductive problems including agalactia and high kit mortality in fox fed a dry

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commercial fox food and have concluded that the F content of the diet (100-300 ppm F, dry matter basis) was responsible for the health effects observed.

In mink, diets containing up to 350 ppm F (as NaF) have been fed with no reduction in breeding or lactation performance, but with a negative effect on kit survivability (Aulerich et al. 1987). Reproductive problems related to dietary F were not observed in dogs fed a high F dog food (460 ppm F), although these dogs exhibited characteristic signs of F toxicosis including tooth mottling and exostoses (Main et al. 1987). Marks et al. (1984) fed rats this high F dog food diet through two generations with no adverse effect on pup survival. Currently, there is no accepted tolerance level for F in fox diets. The following studies were conducted to evaluate the effects of dietary F on the reproductive performance of fox over multiple generations.

MATERIALS AND METHODS

Arctic blue fox (*Alopex lagopus*) were used in three experiments conducted at a commercial fox ranch in Wisconsin, USA. All animals were individually housed outdoors under covered sheds in standard galvanized steel cages. The cages were equipped with an automatic watering system which allowed free access to fresh water. The basal diet, which contained 32% animal protein meals was adequate in all major and micronutrients, and by analysis contained: 10.8% moisture, 36.5% protein, 14.6% fat, 7.9% ash, and 104 ppm F. Additions of F were supplied as NaF at the expense of processed grain. Diets were pelleted and provided ad libitum from metal hoppers.

In Experiment 1, fox pups of approximately three months of age were allocated to four groups (8M and 8F) and fed diets containing 0, 50, 150, or 250 ppm supplemental F for approximately three months. They were weighed at the start and conclusion of the trial, were physically examined every two weeks for evidence of dental abnormalities, and blood, urine, and bone samples were obtained from 7M and 2F of each group at the end of the 98-day trial. Each pelt was evaluated for hair density, color, and texture. In Experiment 2, the 6F and 1M remaining in each group from Experiment 1 were maintained on their respective diets through the breeding, gestation, and pup-growth periods for two successive years. Females were mated according to customary commercial fox breeding procedures. Cages were equipped with a wood whelping box following confirmed matings, and the number of live and stillborn pups recorded at whelping. Pups were counted and weighed at four weeks of age to assess lactation performance of the females. Fox pups were weaned onto and fed the vixens' respective diets until pelting in late November of each year, at which time samples for analysis were obtained. During this time, oral examinations were conducted every two weeks to monitor for dental lesions. Pup body weights were recorded in late August and prior to pelting. In January of the third year, all remaining adult fox consuming the 50 ppm supplemental F diet were switched to a diet containing 600 ppm F (Experiment 3). All other adult fox remained on their respective diets (0, 150, or 250 ppm F). These four groups were fed through the subsequent breeding, gestation, and early pup growth periods.

The fox were anesthetized with i.m. (12 mg/kg) ketamine hydrochloride (Vetalar, Parke-Davis, Morris Plains, NJ) and 6 ml blood obtained via cardiac puncture into

heparinized syringes and plasma separated. Urine samples (10 ml) were collected by manual expression of the bladder and filtered through cheesecloth to remove sediment. Anesthetized fox were then euthanized and necropsied. The left femur was removed and stored at -20°C, along with plasma and urine samples, for future analysis. Fluoride content of plasma, urine, and femur samples were determined as previously described (Clay & Suttie, 1987).

RESULTS

Body weight gain of fox pups was unaffected by total dietary concentrations of F up to 350 ppm. The initial body weight of the four groups ranged from 3.49 to 4.10 kg (SEM 0.38). Individual daily food consumption was not recorded during the three-month growth period but was estimated at 350 g. No dental lesions or other physical signs of F toxicosis were observed during the experiment. Fur quality characteristics (hair density, color, texture) at pelting were not different among groups. Plasma, urine, and bone F concentrations all increased linearly with increasing dietary F (Table 1).

Table 1. Plasma, urinary, and femoral fluoride concentrations of pups fed supplemental fluoride for 98 days[1]

	Supplemental dietary fluoride (ppm)			
	0	50	150	250
Plasma F (μg/ml)	0.04 ± .02[a]	0.12 ± .07[a]	0.36 ± .13[b]	0.46 ± .19[b]
Urinary F (μg/ml)	3.6 ± .1[a]	9.8 ± 4.2[a]	23.2 ± 7.0[b]	34.1 ± 6.3[c]
Femur F (μg/g ash)	937 ± 226[d]	1760 ±375[e]	3349 ± 443[f]	5502 ± 619[g]

[1]Values are mean ± S.E. for 6-9 observations (plasma F), 4-7 observations (urinary F), and 9 observations (femur F). Means within a row with different superscripts (a-c) are significantly different at ($p < 0.01$) and (d-g) different at ($p < 0.001$)

Reproductive and lactation performance parameters over three successive breeding seasons are summarized in Table 2. As expected, breeding performance of female fox improved during their second and third reproductive seasons. In years 1 and 2, supplemental dietary F of up to 250 ppm had no apparent effect on litter size, pup survivability, or pup body weight at four weeks of age (Experiment 2). Pup survivability was markedly reduced when supplemental dietary F concentration was increased to 600 ppm during the third year. The survival rate of pups whelped to vixens fed 250 ppm supplemental F for three years was also reduced compared with pups from females fed the basal diet or the basal diet with 150 ppm F added. However, litter size and lactation performance were not adversely affected when the dietary F burden was increased to 600 ppm F during the third year.

Bi-weekly oral examinations of the adult fox and growing pups over the three-year study failed to indicate any adverse effects of prolonged F ingestion. Body weight gains of pups from vixen fed 0, 50, 150, or 250 ppm supplemental dietary F were not significantly

different. Likewise, no adverse effect of long-term F ingestion on fur characteristics was observed. Femur F levels of pups increased with increasing dietary F and were higher in pups whelped during the second year of the study than in the first year.

Table 2. Effect of supplemental dietary fluoride on reproduction and lactation performance of female fox and on survival of their pups over three generations[1]

Year	Item	Supplemental dietary fluoride (ppm)			
		0	50	150	250
One	No. ♀ whelped/No. ♀ mated	2/3	1/2	2/2	2/3
	Litter size (No.)	3.5 ± .5	7.0	13.0 ± 2.0	10.0 ± 2.0
	Pup body wt at 4 wks (g)	585 ± 86	-	605 ± 122	623 ± 98
	Pup survival to 4 wks (%)	100	0	92	100
		0	50	150	250
Two	No. ♀ whelped/No. ♀ mated	2/3	3/4	3/4	2/3
	Litter size (No.)	10.0 ± 1.0	7.0 ± 1.6	10.7 ± 1.9	11.5 ± 0.5
	Pup body wt at 4 wks (g)	592 ± 151	631 ± 118	624 ± 114	617 ± 112
	Pup survival to 4 wks (%)	90	95	94	96
		0	150	250	600
Three	No. ♀ whelped/No. ♀ mated	3/4	2/4	4/4	2/3
	Litter size (No.)	11.3 ± 2.0	11.5 ± 1.5	9.2 ± 2.3	12.5 ± 1.5
	Pup body wt at 4 wks (g)	589 ± 112	601 ± 112	637 ± 78	640 ± 57
	Pup survival to 4 wks (%)	91	91	57	16

[1]Values are mean ± S.E. for the number of litters or pups indicated

DISCUSSION

These data indicate that young fox fed a commercial pelleted diet supplemented with up to 250 ppm F were able to raise three normal litters of pups. The inclusion of 50, 150, or 250 ppm F in the diet had no influence on reproductive rate, litter size, or pup weight at four weeks of age. The pup survival to four weeks was adversely influenced by 250 ppm supplemental F, but only for the third litter. The supplementation of the diet with 600 ppm F during the period of the third litter only also severely influenced pup survival, but did not affect reproductive rate, litter size, or weight of the surviving pups. Increased dietary fluoride was associated with increased concentraton of plasma, urinary, and femoral F during a three-month period of growth in young fox, and the pups born to vixens consuming increased levels of dietary fluoride also demonstrated increased skeletal fluoride loads.

The basal diet utilized contained 104 ppm F by analysis. The roughly linear response of plasma, urine, and femur F to increasing fluoride intake (Table 1) allows the estimation that fluoride in the basal diet is from 25% (urine and plasma data) to 50% (femur data) as

bioavailable as NaF. Most of the fluoride in the basal diet would be in the form of bone present in the animal protein portion of the diet. The estimate of about 30% availability of this form of fluoride compared to NaF would be consistent with previous data from dog (Greenwood et al. 1946) or rat (Zipkin et al. 1970) studies.

These data suggest that the fox is rather resistant to adverse effects of fluoride ingestion, and are not consistent with the conclusion (Eckerlin et al. 1986) that reproductive problems, including agalactia and high pup mortality were the result of consumption of a diet containing 100-130 ppm F. This diet would be the equivalent of less than 50 ppm F as NaF, while the present study has clearly shown that a diet supplemented with 150 ppm F (as NaF) has no adverse effect over three litters. Limited reports in mink (Aulerich et al., 1987) and rats (Marks et al., 1984) would also suggest that rather high intakes of fluoride can be tolerated with no adverse effect on rate of reproduction, lactation, or survival and growth of young.

SUMMARY

The effects of dietary F on fox growth, reproduction, and tissue F accumulation were evaluated by feeding a commercial pelleted diet (104 mg F/kg) supplemented with 0, 50, 150, or 250 mg/kg F (as NaF). Body weight gain of three-month-old blue fox pups was not affected by dietary F addition and plasma, urine, and femoral F concentrations increased linearly with increasing dietary F. No treatment effects were observed on fur density, color, or texture. Females from each group were maintained on their respective diets through three successive breeding cycles. The addition of 250 mg F/kg to the diet had no effect on litter size or pup body weight at four weeks of age. Pup survival rate at four weeks was adversely influenced by 250 mg F/kg only during the third litter. Growth rates of pups maintained on their dam's experimental diet were not affected by dietary F level. Skeletal or dental signs of F toxicosis were not observed at any time during these studies. During the third breeding season, vixens were switched from 50 mg/kg supplemental F to 600 mg/kg F. Pups whelped to these females had reduced survival rates, but litter size and lactation performance were not affected. Based on the results of these studies, fox are relatively tolerant of dietary F.

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The influence of zinc supplementation on growth and reproduction of mink

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During a three-year period an investigation was carried out on the effects of zinc suplementation on growth and litter size in standard mink. The mean content of zinc in the analysed feed exceeded the recommended level, but the lower zinc intake was reflected in the zinc of hairs of growing mink. It was found that the daily zinc intake of less than 6 mg by female mink before and during the breeding season compared with an intake of more than 10 mg zinc tended to reduce the litter size. The high Ca-concentration of mink feed (C as a Zn antagonist) has to be considered in further investigations.

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Zinc and other trace elements are an essential part of mink feed. However, very little is known about the specific needs of the mink, Anke & Risch (1979) and Berestov et al. (1985) have shown that the different content of minerals in feed is reflected in the content of minerals in the hair. Lohi et al. (1991) found a clear correlation between the amount of Ca, Mg, Na and Se in feed and in the hair of mink, but the levels of Zn, Cu and P in feed were not reflected in the hair. Tjurnina & Tjutjunnik (1982) have published the limits for content in mink hair with 238 to 275 mg/kg dry matter. Zinc is included in the synthesis and release of plasma retinolbinding protein and is also an essential cofactor for many enzymes, including those that regulate vitamin A metabolism (Richter & Flachowsky (1989). In laboratory animals, zinc deficiency results in profound and long-lasting effects in the immune system (Chandra 1989). The objective of the following paper was to investigate the effect of zinc supplementation on growth and reproduction of mink and how the zinc content in feed is reflected in mink hair as well as in the internal organs.

MATERIAL AND METHODS

In mink feed the minerals derive partly from the different feed ingredients and partly from special mineral suplements. In our growth experiments a control group was provided with feed supplemented with "Edapan" containing vitamins and minerals (group I).

In the experimental group II, zinc was removed from the Edapan, such that in this group any intake of zinc was derived from the natural feedstuffs. In 1988, 1989 and 1990 these groups, each of which included 16 males and 16 females were compared from July

to November. From January to May the female breeder minks of the two groups were compared for reproduction rate. One group was fed with the basal mink diet for breeders and the other was fed with the same diet but without the additional zinc. The basal diets for breeders and growers are presented in Table 1. The mineral content of the feed samples, and hairs and organs such as liver, kidneys, ribs and brain were analysed in the Institute of Nutrition at the University of Jena. The hairs were cut directly over the skin. Fat was extracted by diethyl ether and drying was performed at a temperature of 105°C. Zinc was determined by means of atomic absorption spectroscopy.

Table 1. Composition of the basal mink diet

	Breeding diet (%)	Growing diet (%)
Pig, intestines	22.8	16.6
Cattle, intestines	16.2	11.6
Poultry offal	24.4	23.6
Fish offal	10.0	25.0
Whey cheese	3.0	5.0
Milk powder	2.5	2.5
Fishmeal	-	2.5
Bran	9.6	-
Yeast	-	3.0
Water	10.0	10.0
Edapan	1.5	1.0

RESULTS AND DISCUSSION

The analyses of the zinc content of the mink diet gave a result of 200 mg for growing mink and 157 mg for breeding mink based on 1 kg dry matter. In the diets without zinc supplements the content of this trace element was reduced to 124 mg and 80 mg per 1 kg dry matter, respectively. Both values exceed the recommended level of 60 mg/1 kg dry matter (Glem-Hansen 1984).

The reduction in the zinc content of the mink diet did not have a negative effect on growth, as can be seen in Table 2. In all the experiments there were no significant differences in body weight between the groups. The mortality in the experi-ments was very low.

The feed consumption was not influenced, the average feed consumption from July to November amounting to 34.9 to 35.6 kg of ready mixed feed.

The levels of zinc in hairs and internal organs of the different groups in 1989 are indicated in Tables 3 and 4. The group II mink, i.e. those without additional zinc, had a significantly lower content of zinc in the hairs. It was found, however, that the hair of females contained more zinc than the hair of males.

Because of the role of zinc in the synthesizing of keratin the zinc content of the hairs could be important for the fur quality. No significant differences were found in the zinc content of the liver, kidneys, ribs, brain and heart (Table 4).

In the breeding seasons of 1989, 1990 and 1991, females of both groups were compared with regard to less than 6 mg in experimental group II and more than 10 mg in control group I. The group with the lower zinc consumption had a significantly lower litter size (Table 5). There was also a tendency for females with a lower zinc consumption to have a longer duration of pregnancy and a higher proportion of barrenness. The content of zinc in the hairs of the female breeding minks differed significantly in 1989 but not in

1990. The results of 1991 are not availably (Table 6). Possibly the high Ca concentration of mink feed (25 to 30 mg/1 kg dry matter) is responsible for the negative effect of the lower zinc consumption on litter size, as the role of calcium as an antagonist of zinc has to be considered.

Table 2. The effects of different content of Zn in mink feed on growth (n = 16 ♂; 16♀). Values in g (mean ± s)

1988 age, wks.		10		26
Control	males	742 ± 104		1929 ± 262
	females	595 ± 59		1161 ± 123
No Zn-suppl.	males	749 ± 106		1954 ± 350
	females	596 ± 93		1179 ± 166
1989 age, wks.		8	18	28
Control	males	672 ± 138	1907 ± 171	2335 ± 236
	females	456 ± 69	1036 ± 116	1179 ± 105
No Zn-suppl.	males	594 ± 86	1842 ± 149	2340 ± 186
	females	482 ± 60	1041 ± 62	1260 ± 89
1990 ages, wks.		8	18	28
Control	males	621 ± 64	1828 ± 367	2313 ± 516
	females	466 ± 65	1113 ± 114	1325 ± 170
No Zn-suppl.	males	647 ± 80	1879 ± 320	2482 ± 419
	females	494 ± 71	1013 ± 118	1170 ± 131

Table 3. The effects of different content of zinc in mink feed on the zinc content in hair. Values in ppm (mean ± s)

		Hair
Group I		
Control	males	701 ± 276
	females	965 ± 516
Group II		
No zinc suppl.	males	590 ± 210
	females	790 ± 255
F-value	sex	9.7 +
	group	2.35 +

Table 4. The effects of different zinc content in mink feed on the zinc content of organs. Values in ppm

	Control			No zinc suppl.			t-value
	n	mean	s	n	mean	s	
Liver	9	169.6	38.3	17	213.3	88.2	1.75-
Kidneys	7	181.3	61.4	11	255.9	155.3	1.35-
Ribs	9	346.1	221.8	18	472.5	182.9	1.48-
Brain	7	142.7	47.5	17	165.4	98.2	0.76-
Heart	7	118.4	36.4	12	115.0	81.5	0.12-

Table. 5. The effect of zinc content in the mink diet on litter size

	Control	Without zinc-suppl.
n	33	61
Daily zinc intake, mg	10	6
Litter size, mean	5.38	4.42
s	2.04	2.36
t-value = 2.05 +		

Table 6. Content of zinc in hairs of female breeding mink. Values in ppm

Year		Control	Without zinc addition	
1989	n	15	5	
	mean	427	358	sign. (t = 2.18)
	s	104	36	
1990	n	19	19	
	mean	563	531	not sign.
	s	272	218	

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The effect of dietary acidifiers in diets of mature ranched foxes with a history of chronic urolithiasis

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In an eight weeks' trial 24 adult male silver foxes were studied, in a complete randomized block with fixed effects, in order to evaluate the effect of dietary acidifiers. The animals were fed a dry diet at 190 g/day or the same diet supplemented with acidifiers; ammonium chloride (0.99%), phosphoric acid (2.68%) or Alimet, a methionine hydroxy analogue (1.68%). Biweekly, three days' voided urine, sterile urine and blood were collected. Blood packed cell volume (PCV), blood creatinine, blood urea nitrogen (BUN), urine pH, urine culture and urine specific gravity were measured. No significant differences ($p < 0.05$) were observed among treatments for PCV (44.6-45.8 ml/100ml), BUN (6.11-6.81 mM/l), blood creatinine (74.2-84.7 mM/l) and urine specific gravity (1.036-1.043). However, a significant difference ($p < 0.05$) was evident for urinary pH between the control diet (7.5) and the treatment containing phosphoric acid (6.2), with the other acidifiers producing median values.

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Urolithiasis is a condition caused by the occurrence and effects of calculi or excessive quantities of crystals in the urinary tract, which may irritate the mucosal lining. The occurrence of urolithiasis in ranched foxes in Canada is considered a major area of concern (Ontario Animal Research and Services Committee 1992). Struvite is the most common substance in uroliths in male dogs over one year of age, with 50-75% of those affected having struvite uroliths (Lewis et al. 1989). These struvite uroliths are composed primarily or exclusively of magnesium-ammonium-phosphate hexahydrate crystals ($MgNH_4PO_4$). Struvites can also contain small amounts of calcium phosphate (apatite) and/or ammonium urate crystals.

Constant alkalinity of the urine is thought to predispose dogs to magnesium-ammonium-phosphate (MAP) urolith formation since struvite crystals are insoluble in alkaline urine (Klausner et al. 1980). Therefore, attempts to increase the solubility of crystals in the urine include dietary additions to alter urine pH. This process creates a less favourable environment for crystallization, as well as inducing a state of diuresis, which increases the volume of urine in which the crystals are dissolved or suspended (Osborne et al. 1989).

This study was conducted to evaluate the effect of dietary acidifiers in mature male ranched foxes with a history of chronic urolithiasis.

METHODS AND MATERIALS

Animals and Procedures

Twenty-four mature male foxes were randomly assigned to one of four dietary groups. Half the foxes were housed in individual stainless steel metabolism cages (1.08 m x 0.96 m x 0.84 m). These cages were located in a temperature and light controlled room, (16°C with 12 h light and 12 h darkness). The remaining animals were housed in individual pelter cages in a commercial type pelter shed under summer conditions.

Feed was given twice daily, at 0900 h and 1600 h, with each fox receiving 190 g feed/day. Water was provided *ad libitum* with the quantities consumed being monitored.

On a biweekly basis, three days' voided urine was collected. During these collection periods, urine pH was measured with a pH meter (calibrated with standard buffer) and urine specific gravity was measured with a refractometer within one to two hours of being voided.

Biweekly blood and sterile urine samples were collected. Blood was drawn from the cephalic vein and urine was collected via catheterization of the glans penis. In order to facilitate collection, 0.5 cc innovar-vet (Fentanyl droperidol) was used to sedate the animals whenever necessary. Sterile urine samples from catheterized animals were streaked on blood and MacKonkey agar plates to expose any urinary tract infection (UTI) and to identify the bacteria species involved. The remaining urine was measured for pH and specific gravity.

Packed cell volume (PCV) was determined by means of the microhaematocrit method; BUN, and creatinine levels of the plasma were also determined.

Diet Composition

Different dietary acidifiers were added to a common basal diet [non-acidified(NA)]. The levels of the different acidifiers were: ammonium chloride (AC) at 0.99%; phosphoric acid (PA) at 2.68%; or Alimet (a methionine hydroxy analogue from the Monsanto Chemical Co.) (AL) at 1.68%. In the basal diet the acidifiers were added in the equivalent amount as the basal diet (Table 1).

Statistics

A statistical analysis was conducted using the General Linear Model procedure. Where significant differences ($p < 0.05$) occurred, the Student-Newman-Keul's test was used to differentiate among treatment means (SAS 1985).

RESULTS

Urine specific gravity, PCV, BUN, and blood creatinine were not significantly different ($p < 0.05$) among any of the treatments (Table 2). Urine specific gravity values (1.036-

Table 1. Composition of the non-acidified diet offered to foxes with a history of chronic urolithiasis (g/100 g)

Ingredients	g/100 g
Corn gluten meal	10.0
Corn (extruded)	25.0
Wheat (extruded)	28.5
Meat meal	3.0
Fish meal	3.0
Poultry by-product meal	3.0
Whey (dried)	4.0
Soybean meal	7.8
Vitamin/Mineral permix*	0.5
Molasses	1.0
Antioxidant premix **	0.5
Tallow	13.7
TOTAL	100.0

* Vitamin - mineral premix contains (g/100 g of complete diet) : vit. A (500 * 10^6 IU/kg) 1 mg, vit. D premix (50 * 10^6 IU/kg) 1 mg, vit. E (50,000 IU/kg) 10 mg, riboflavin 0.3 mg, Dl-Ca pentothenate 1.4 mg, niacin 1.2 mg, vit. B 12 1.0 mg, folic acid 0.7 mg, biotin 0.5 mg, choline chloride 66.7 mg, thiamin 0.2 mg, pyridoxine 0.2 mg, manganese oxide 1.8 mg, zinc oxide 3.8 mg, copper sulphate 2.0 mg, cobalt carbonate 0.1 mg, vit. K 0.4 mg, selenium premix 33.0 mg, ferrous sulphate 37.0 mg, wheat middlings 262.7 mg, ground limestone 50.0 mg ethoxyquin 25.0 mg.

** antioxidant premix contains (g/100 g diet): ethoxyquin 15 mg, and wheat middlings 485 mg

1.043) were within the normal range for beagle dogs (Abdullahi et al. 1984). Mean values among treatments for PCV (44.6-45.8 ml/100 ml), BUN (6.1-6.81 mMol/l) and blood creatinine (74.2-84.7 mMol/l) were within the normal range for silver fox (Benn 1986). A significant difference ($p<0.05$) was observed for urine pH values, however. The non-acidified (NA) diet at pH 7.5 was higher than those diets acidified with phosphoric acid (PA) at pH 6.2 ($p<0.05$).

All feed was consumed by every fox and there was no decrease in intake due to acidification of the diets. Water intake varied greatly. Weekly consumption was 2002 g when no dietary acidifier was used. Water consumption tended to be lower on the acidified diets, 1582 g 1713 g 1305 g for the AC, PA and AL treatments respectively. Acidification of the diet appeared to reduce water consumption by 10 to 15%.

Some cultures of *Staphylococcus aureus* and *Staphylococcus epidermidis* were isolated from samples of sterile urine but no patterns were found to indicate that certain foxes had any UTI. Both species of staphylococcus are found as normal flora of the urine and are recognized as opportunistic pathogens, with *S. epidermidis* being described as a low virulent pathogen (Timoney et al. 1988).

DISCUSSION

Urine specific gravity, PCV, BUN, and blood creatinine in foxes fed the diets with different acidifiers added were similar to those fed the non-acidified diet.

The diets with additional phosphoric acid as the acidifying agent had lower urine pH than the non-acidified diet (6.2 vs 7.5) ($p<0.05$). The pH of urine when phosphoric acid was added to the diet (6.2) was not significantly different ($p<0.05$) compared to the other

dietary acidifiers (AC; 6.9 and AL; 6.7). Generally the urine pH was reduced with the addition of dietary acidifiers. If the other acidifiers had been used at slightly higher levels a greater reduction in pH might have been achieved. It is not known at this stage if increased acidifier of the AC or AL type would have caused reduced feed intake at higher levels.

Table 2. Blood and urine parameters from foxes provided with dietary acidifiers

	Dietary Acidifiers			
	Control (NA)	Ammonium chloride (AC)	Phosphoric acid (PA)	Alimet (AL)
Blod				
PCV (ml/100 ml)	44.6	45.6	45.8	45.6
BUN (mMol/l)	6.76	6.81	6.11	6.29
Creatinine (mMol/l)	74.2	81.3	84.7	76.8
Urine				
pH (mEq)	7.5 a	6.9 ab	6.2 b	6.7 ab
Specific gravity	1.036	1.042	1.036	1.043
Water consumption (ml/day)	280.0	220.0	240.0	180.0
Feed consumption (g/day)	190.0	190.0	190.0	190.0

* means within a row with different letter postscripts are significantly different ($p < 0.05$)

Osborne et al. (1986) emphasize the importance of this pH difference. A change in pH of only 0.6 units (e.g. 7.4 to 6.8) will result in a 75% increase in the apparent solubili-ty of struvite. Studies have shown that acidification of urine to a pH of approximately 6.0 has been effective in promoting struvite dissolution (Osborne et al. 1989). Edfors et al. (1989) state that feeding a diet that consistently maintains a urine pH of less than 6.4 will prevent, dissolve, and reduce recurrence of struvite uroliths.

Jørgensen (1985) found that mink fed phosphoric acid (at 0.6% of wet feed) tended to give an acidic pH of the urine which kept the minerals in solution so they passed out of the urinary tract instead of precipitating out and forming stones. Long (1984) used phosphoric acid as a dietary acidifier on a ranch of foxes where there was a problem with urolithiasis. The change in urine pH is consistent with the findings in this study.

CONCLUSION

Phosphoric acid at 2.86 % of the diet effectively reduced the urine pH. The dietary acidifiers tested did not cause any reduction in feed intake.

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Feeding devices reduce waste in mink feeding

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Wet feeding devices for mink were compared to the conventional feeding on cage wire. The treatments were 1) dry pellet feeder, 2) control, wet feed on wire, 3) spill tray, 4) slant tray, and 5) cup feeder. The first experiment was performed during the suckling period using five females with litters per group. The second experiment was run during the growing-furring period with two males and three females in each group. The feed consumption and the amount of feed wasted were measured on a dry matter (DM) basis. The influence of dietary DM on the amount of feed wasted was also clarified. During the last two weeks of the suckling period, the feeder groups wasted significantly less feed (week 1; 7-10 %, week 2; 5-12%) than than the control group (17%, 21%), respectively. During the growing-furring period, the average amount wasted in the feeder groups (1-3%) was also significantly lower than the control (6.5%). One percent decrease in the dietary DM increased the feed waste by 3.8%, when the DM of the diet declined from 33-25%.

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In conventional mink farming, the animals are fed on the cage wire. This requires, however, good consistency in the feed mixture and limited water content (Berg 1986; Jørgensen 1985). Furthermore, in this type of feeding some feed is wasted through the wire, which increases the feeding cost. By using feeding devices more water could be provided to the animals directly in the diet. During the first weeks of early growth, it is also important to provide the kits with easy access to feed. Furthermore, the devices would help reduce feed waste. The purpose of the present study was to develop and test different types of feeding devices for mink. Main emphasis was on decreasing the amount of feed wasted. The effect of the dietary water content on feed waste was also studied.

MATERIALS AND METHODS

The experiments were performed at the Fur Unit of the Nova Scotia Agricultural College, Truro. The animals were housed in 50.8 x 58.4 x 30.5 cm (w x l x h) cages provided with nest boxes. The cages were constructed of 2.5 x 2.5 cm wire mesh. The feeding devices on trial were as follows: (1) dry pellet feeder, (2) control, wet feed on the cage wire, (3) wet feed on wire with a spill tray (6 cm from the top of the cage), (4) wet feed in a slant

tray feeder (width 18 cm), and (5) wet feed in a cup feeder (diameter 16 cm). The animals in group 1 were fed with commercial dry feed. Groups 2-5 were fed with a wet feed mixture compounded of fish, beef and poultry offal, cereals and water. The dry matter (DM) content of the wet diet was about 30%. The experiments were run during the suckling period and during the growing-furring period.

In the suckling period experiment, 25 female mink with litters were used. There were five females per treatment. The average litter size was 4.92. The experiment was conducted during June 1991 in two consecutive seven-day periods with two days for adjustment. The kits were approximately 40 and 47 days old, respectively. A weighed amount of feed was given twice daily to each female and her litter according to their consumption. The dry feeders were kept full at all times. The feed wasted from the previous day was collected from a plastic sheet located below the cages, and the rejected feed was collected from the feeding devices. Moreover, the feed carried to the nest boxes was treated as rejected. The feed delivered and the feed collected as waste or rejected were weighed and analysed for their DM content. The growing-furring period experiment was performed during October 1991. Each experimental group included two males and three females, which were caged singly. The trial lasted for seven days with two days for adjustment. The animals were fed according to their appetite. The collection of the leftovers and waste, and the analysis and calculations were done as described above.

The amount of feed wasted at different dietary water contents was determined without animals. Three hundred (300) grams of feed was delivered on top of 10 cages (2.5 x 2.5 cm wire) at 9.00 a.m.. The feed that fell through the mesh was collected from a plastic sheet at 300 p.m.. The waste percentage was determined on a DM basis for five different dry matter contents ranging from 24.9-32.5%.

The differences between the experimental groups were tested by the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS Institute Inc. 1985). In the suckling period trial, the main effects tested were the experimental group and litter size. In the growing-furring period trial, the effects of the experimental group and sex of the animal were tested. When the effects were statistically significant, the differentiation among the mean values was calculated by the Student-Newman-Keul's test ($p<0.05$). The dependence of the feed waste on the DM content of the diet was calculated by linear regression (SAS Institute Inc. 1985).

RESULTS

In the suckling period trial (Table 1), during both weeks, feed consumption was equal in all groups when calculated on a dry matter basis. The amount of feed wasted through the wire was, however, significantly higher in the control group (2, wet feed on wire) than in the feeder groups (1, 3-5). During the second week the animals in the dry feeder group 1 wasted significantly less feed than those fed the wet diets (groups 2-5). In addition, the litter size was shown significantly to affect the feed consumption of the animals during both experimental weeks, litters of 5-7 kits consuming more than the litters of 3 and 4 kits. In the growing-furring period experiment, the feeding devices significantly decreased the amount of feed wasted. During this period, however, the amount of waste was considerably

less than in the corresponding device groups during the suckling period.
The amount of feed wasted (y) was shown to increase linearly, according to the following equation $y = 122.72 - 3.79x$ ($R^2 = 0.880$, $p < 0.001$), when the DM content of the diet (x) was decreased.

DISCUSSION

All feeding devices employed in this study significantly reduced the amount of feed wasted when compared to conventional feeding on the wire. The average waste percentage in conventional feeding during the suckling period was 17-21%, while in the wet feeding device groups (3-5) it varied from 7-12%. In addition, slightly less feed was wasted on a dry matter basis (5-7%), when dry pellets were used in comparison to wet feed. In the growing-furring period trial, 6.5% of the feed was wasted when fed on the wire. In the dry feeder (1) and wet feeding device groups (3-5) the waste ranged from 1-3%.

Table 1. Average daily feed consumption and waste percentage for mink dams and their litters during the suckling period and for singly caged males and females during the growing-furring period

	Group					Significance	
	1 Dry feeder	2 Cage wire	3 Spill tray	4 Slant tray	5 Cup feeder	G	LS/S
Suckling period[1)]							
Week 1							
Feed cons., g DM	172.5±54.0	134.0±26.0	141.3±18.0	157.1±28.3	167.7±17.9	NS	<0.001
Waste %	6.9± 5.5b	17.1± 6.7a	9.0± 2.9b	9.8± 2.8b	7.3± 3.0b	<0.05	NS
Week 2							
Feed cons., g DM	239.0±55.8	204.7±41.4	247.6±24.1	249.4±14.9	229.1±42.4	NS	<0.05
Waste %	5.0± 2.4c	21.3± 1.9a	11.6± 1.6b	8.7± 3.2b	10.8± 2.8b	<0.001	NS
Growing-furring period[2)]							
Feed cons., g DM	71.8±14.1	67.9±12.5	65.9±13.1	71.9±14.1	71.4± 9.8	NS	<0.001
Waste %	0.9± 0.5b	6.5± 3.2a	3.1± 2.1b	2.9± 2.2b	1.3± 1.2b	<0.01	NS

a-c: means±S.D. not sharing any common postscript are significantly different ($p < 0.05$)
1) the main effects tested were group (G) and litter size (LS), interaction was non-significant
2) the main effects tested were group (G) and sex (S), interaction was non-significant

In the conventional feeding on the wire, the amount of feed waste was shown to be significantly dependent on the amount of water in the diet. One percent decrease in the dietary DM increased the waste through the cage wire by nearly 4%. The equation, however, tends to overestimate the waste, since the animal would eat part of the feed that

falls through.
When low DM diets are used in order to provide the animals with more water directly in the feed, wet feeding devices minimize the amount of feed wasted. The devices are, however, an extra expense and add feeding and cleaning labour. Additional costs and labour could be justified during the suckling period, when the number of animal units to be fed and the number of feeders required is low.

SUMMARY

Mink are usually fed on the cage wire, which increases feed waste. In this study different feeding devices were compared with the conventional feeding method. The experimental groups were (1) dry pellet feeder, (2) control, wet feed on wire, (3) spill tray, (4) slant tray feeder, and (5) cup feeder. The first experiment was run during the suckling period for two consecutive weeks. There were five females with their litters per group. The second experiment, lasting seven days, was performed during the growing-pelting period. Each group included two males and three females. The feed consumption and the amount of feed wasted were measured on a dry matter (DM) basis. The influence of dietary DM on the amount of feed wasted was also clarified. During the suckling period, the animals in the feeder groups wasted significantly less feed (week 1 7-10%, week 2 5-12%) than the control group (17%, 21%, respectively). During the growing-furring period, the average amount wasted in the feeder groups (1-3%) was also significantly lower than that in the control group (6.5%). One percent decrease in the dietary DM increased the feed waste by 3.8%, when the DM content declined from 33 to 25%.

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Effect of storage time on the stability of ALAT, ASAT, CK, urea and creatinine in mink plasma

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In physiological investigations of mink blood it is often desirable to store plasma samples for some time prior to carrying out an analysis. The purpose of this study was to examine the stability of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), creatine kinase (CK), urea, and creatinine in mink plasma stored at -20°C. Blood samples were collected from 10 male mink. The plasma samples were immediately aliquoted and frozen at -20°C. One aliquot of each sample was analysed after 7, 14, 28, 42, 56, 86, and 168 days. Storage time had a strong effect on the stability of the enzymes CK, ALAT and ASAT. The effect was most pronounced for CK, less so for ALAT and least of all for ASAT. The stability was high for urea, and creatinine was stable.

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In physiological investigations of mink, blood samples are frequently collected over a short period at pelting time. In order to use laboratory capacity in the best possible way, it is often desirable to store plasma samples for some time prior to analysing for clinical-chemical variables. Furthermore, it is sometimes necessary to send samples for chemical analysis to a specialized laboratory.

The purpose of the present study was to examine the stability of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), creatine kinase (CK), urea and creatinine in plasma stored at -20°C.

MATERIALS AND METHODS

Collection of samples

In April 1989 after the mating period, blood samples were collected from 10 male mink used for mating. The animals were anaesthetized with Na-pentobarbital (30 mg per kg b.w., i.p.) and 20 ml blood samples were collected by heart puncture. Blood samples were stabilized with Na-heparin and centrifuged at 3000 RPM for 10 min. The plasma supernatant was aliquoted (0.5 ml) into 2.5 ml transparent polystyrene tubes with caps. All blood samples were centrifuged within one hour after collection, and the plasma samples

were frozen immediately at -20°C. One aliquot of each sample was analysed fresh within four hours after collection. The aliquots were stored at -20°C. After 7, 14, 28, 42, 56, 86, and 168 days one aliquot of each sample was analysed. Because of limited amounts of plasma, the analyses for urea were performed after storage for 28, 56, and 168 days and for creatinine after 28 and 168 days.

Assays

The activity of the enzymes alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), and creatine kinase (CK) was determined kinetically according to recommendations from the Scandinavian Committee on Enzymology with kits from Boehringer Mannheim GmbH Diagnostica. The amount of urea was determined by an enzymatic method, and the concentration of creatinine was determined by a colorometric method (Jaffé method) both with kits from Boehringer Mannheim GmbH Diagnostica.

Statistical analysis

The statistical analysis was carried out by means of SAS procedures (SAS Institute 1987). Average and standard deviations were determined by standard methods. Differences between the initial concentration and concentrations during the storage period were tested by a non parametric test (Wilcoxon Signed Rank test).

RESULTS

The number of specimens studied for each variable and the activities of enzymes, and concentrations of urea and creatinine in fresh plasma samples are presented in Table 1.

Table 1. Initial concentrations, mean value, and standard deviation (SD) of ALAT, ASAT, CK, urea and creatinine in fresh plasma samples

Variables	No. of specimens	mean	SD	Range of initial concentration
ALAT, μkat/l	10	2.4	0.89	1.3 - 4.0
ASAT, μkat/l	10	6.3	5.7	1.6 - 21
CK, μkat/l	10	10.2	130	15 - 438
Urea, mmol/l	10	7.6	1.8	4.4 - 9.6
Creatinine, μmol/l	10	83	17	65 - 123

The decreases in initial values of CK, ALAT, and ASAT in plasma are shown in figs. 1-3. In the figs. an average curve is inserted, and the standard deviations at each time are stated.

After storage for 14 days at -20°C the activity of CK was significantly different from the initial activity ($p<0.01$). After 28 days of storage the concentration of ALAT was significantly different from the initial concentration ($p<0.05$). The activity of ASAT was changed considerably after 84 days of storage ($p<0.06$), and after 168 days the decrease

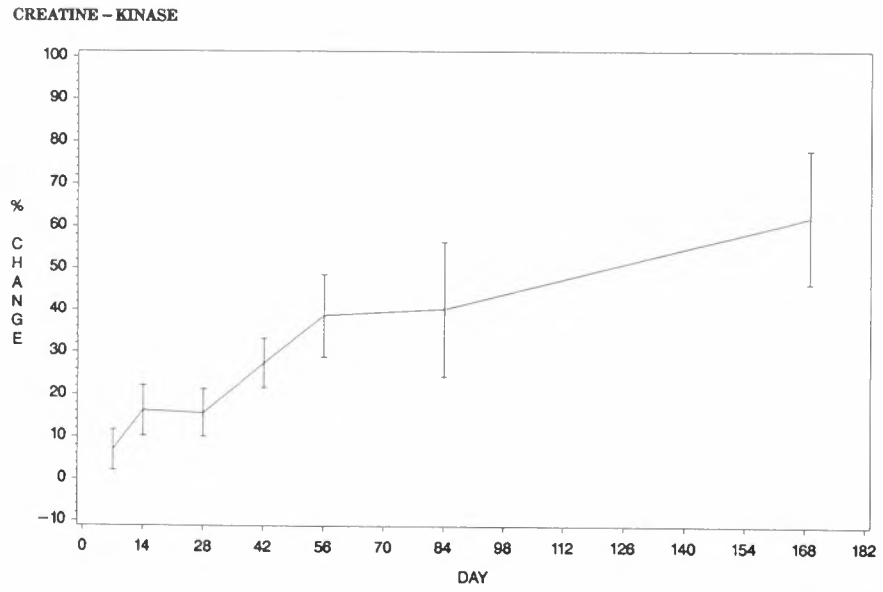

Fig. 1. Changes (%) in initial activity of creatine kinase (CK) in plasma during storage at -20°C. Mean values and standard deviation (± SD)

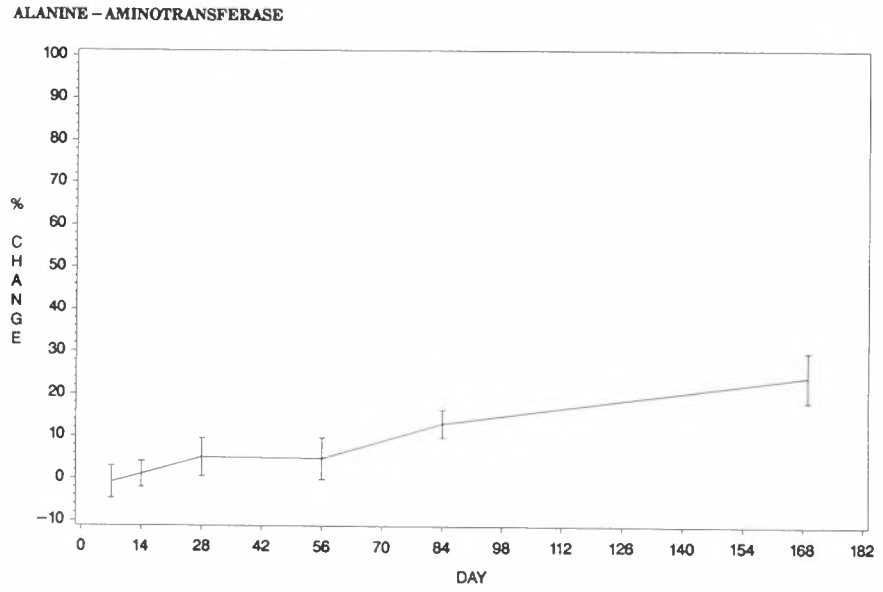

Fig. 2. Changes (%) in initial activity of alanine aminotransferanse (ALAT) in plasma during storage at -20°C. Mean values and standard deviation (± SD)

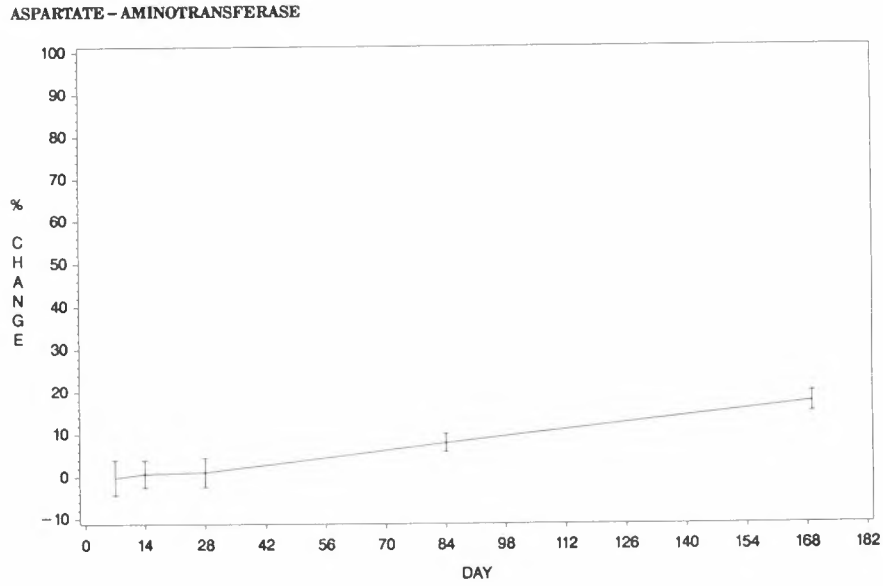

Fig. 3. Changes (%) in initial activity of aspartate aminotransferanse (ASAT) in plasma during storage at -20°C. Mean values and standard deviation (± SD)

Table 2. Changes in the mean concentration (±SD) of urea and creatinine during storage at -20°C

	n	Urea mmol/l	Creatinine μmol/l
Fresh samples	10	7.6 ± 1.8	83 ± 17
Day 28	10	7.6 ± 1.7	80 ± 15
Day 56	10	7.6 ± 1.7	-
Day 168	10	7.4 ± 1.7**	80 ± 12

** $p < 0.01$, compared with concentration in fresh samples by a Wilcoxon Signed Rank test.

was significant ($p < 0.01$). The concentration of urea (Table 2) remained almost unchanged after 56 days of storage and changed significantly after 168 days of storage ($p < 0.01$). The concentrationof creatinine remained almost unchanged after 184 days of storage.

DISCUSSION

The activity of CK is very sensitive to storage time at -20°C. After seven days the activity

on average decreased by 6.9% (±4.8%) ($p=0.08$) as compared with the initial activity, and after 14 days of storage the activity dropped significantly ($p<0.01$) by 16% (±16%). Investigations into the stability of CK in human serum have shown that by storage for one week the stability is higher at -20°C than at +4°C (Bunting et al. 1981). When stored for 24 hours in darkness, the stability was found to be identical at +4°C and -22°C (Perry et al. 1979), and found to be equally good when the samples were exposed to light.

When stored for 14 days the activity of ALAT was changed by 1.0% (±3.0%), While after 28 days of storage there was a significant change of 4.1% (±4.3%) ($p<0.05$). After 168 days of storage the activity decreased by 24% (±5.9%). Investigations have shown that both plasma and serum samples can be stored for up to 48 hours at 4°C without any significant change in the activity of ALAT (Hertenstein & Zelenski 1987). Furthermore, it was found that storage of serum samples for four weeks ought to take place at -70°C rather than at -30°C.

After 42 days the activity of ASAT decreased by 2.0% (±3.7%) and after 84 days by 8.0% (±2.2%) ($p=0.06$). After 168 days the change became significant ($p<0.01$), amounting to 17% (±2.4%). Previous investigations have shown that ASAT is stable for up to 48 hours at 4°C and 24°C (Ieki et al. 1985). A recent search of the literature did not reveal any information on the effect on ASAT of freezing.

After 56 days of storage the concentration of urea was the same as in fresh samples. After 168 days of storage the concentration changed significantly ($p<0.01$) from 7.6 mmol/l (±1.8 mmol/l) to 7.4 mmol/l (±1.7 mmol/l). Urea in urine has proved to be very stable when stored at -15°C, as the concentration was unchanged over a period of seven days (Soliman et al. 1986). After storage for 28 and 168 days, the creatinine concentration of 83 μmol/l in fresh samples changed to 80 μmol/l. In urine samples creatinine was found to be relatively stable over a period of seven days when stored at -15°C (Soliman et al. 1986).

The results of the investigation led to the conclusion that storage time at -20°C had a strong effect on the activitiy of the enzymes CK, ALAT, and ASAT in mink plasma. The activity of CK decreased by 7% after seven days, the activity of ALAT by 4% after 28 days, and the activity of ASAT by 8% after 84 days. The effect was less marked for urea, but still significant after 168 days. Creatinine was found to be stable during 168 days of storage. When evaluating and comparing clinical-chemical analyses, storage temperature and storage time between sampling and time of analysis must therefore be taken into account.

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Feeding breeding fitches with diets at various levels of energy from fat and carbohydrates

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The aim of these studies was to attempt to define the optimal fat:carbohydrate energy ratio in diets for fitches. Experiments were carried out in the years 1986-89 on a total of 120 mature females and 50 males as well as on 200-280 young fitches. The animals were divided into three experimental groups and fed diets with the same level of protein (30% of dietary ME) and various levels of fat (30-60% ME) to carbohydrate (10-40% ME) energy ratio. The results included reproduction indices for females and males, and growth rate and quality of winter fur coat in kits.

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Knowing the correct proportions of nutrients in feed rations for fitches will ensure the most practical feeding from both the physiological and economic points of view. Unfortunately, there is a paucity of information on this topic and there are too few literature records (Barabasz 1986, 1992) on the subject to provide us with unequivocal answers to the question of what is the most adequate ratio of fat to carbohydrates in the diet of this species. The interrelationship of these two components, according to Pierieldik et al. (1987) or Slawon (1987), affects the degree to which they are utilized by animal organisms and when inappropriate proportions of these two components are used, serious disturbances can occur in the metabolism of the fitches. One of the most common diseases is a fatty degeneration of liver and yellow fat disease in mink and fitches (Brooks et al. 1985, Slawon 1987), caused by an excess of fat and polyunsaturated fatty acids in the feed, also acidosis or ketosis, which results from feeding animals with unbalanced rations (Leoschke 1985, 1986). Very often the wrong dietary proportions are not so drastic as to cause instant disturbances in the organisms, but they can bring about a decrease in productivity, simultaneously leading to a deterioration of the economic effect of the breeding programme.

With a view to investigating the above-mentioned relationship and to defining the optimal fat:carbohydrate ratio in rations, experiments were carried out on feeding farm fitches with diets containing the same amount of protein but differing in fat:carbohydrate ratio.

MATERIALS AND METHODS

Studies were conducted in the years 1986-89. Every year a total of 120 mature females and 50 males of breeding stock as well as 200-280 young fitches kept in pairs in typical net cages 40 x 40 x 80 cm, were used.

The nutritive value of rations was estimated according to the percentage of energy from protein, fat and carbohydrates in the total metabolizable energy (ME) of the ration. Protein energy was maintained at the same level (30%) throughout the experiment while an experimental factor was expressed in the form of various fat:carbohydrate ratios, which, depending on breeding season, were in the wide range (from 1:1.5 to 1:0.15). The percentages of ME from fat and carbohydrates in diets for four breeding seasons are presented in Tables 1-4.

Table 1. Fitch female whelping and sexual activity of males fed diets with various percentages metabolizable energy of ME from fat and carbohydrates in the period January - 15 May

Group	% of ME		FEMALES				
	Fat	Carbo-hydrates	No. of animals	% of matings	Conception rate	Mean litter size	Mean litter weight g
I	30	40	32	96.8	93.6	9.77	115.7
II	35	35	64	100.0	93.0	9.04	117.8
III	40	30	58	96.7	75.0	7.41	81.4

Group	% of ME		MALES			
	Fat	Carbo-hydrates	No. of animals	No. of matings	No. of mated females	Length of sexual activity period (days)
I	30	40	13	8.1	3.0	9.9
II	35	35	27	12.3	4.2	17.4
III	40	30	37	4.6	1.6	7.2

Table 2. Kit growth up to weaning with diets with various percentages of ME from fat and carbohydrates, in the period 15 May to 15 July

Group	% of ME		No. of animals	Age 21 days		Milking capacity	Age 42 days	
	Fat	Carbo-hydrates		litter size	litter weight (g)		litter size	litter weight (g)
I	40	30	54	8.77	639	872	7.83	1706
II	50	20	85	7.25	605	1020	6.69	1488
III	60	10	146	6.75	554	963	5.37	1081

Table 3. Body weight of young fitches (g) fed diets with various percentages of ME from fat and carbohydrates during their fast growth: from 15 July to 15 September

Group	% of ME		No. of animals	15 July		15 August		15 September	
	Fat	Carbo-hydrates		males	females	males	females	males	females
I	35	35	83	-	-	487	421	767	586
II	45	25	188	228	199	476	466	876	736
III	55	15	337	264	246	532	447	933	674

Table 4. Body weight (g) and exterior estimations with various percentages of ME from fat and carbohydrates during the period of winter fur coat priming: from 15 September to November

Group	% of ME		No. of animals	BODY WEIGHT			
	Fat	Carbohydrates		15 October		15 November	
				males	females	males	females
I	40	30	230	1028	813	1296	943
II	45	25	104	1075	786	1395	948
III	50	20	210	1219	891	1414	927

Group	% of ME		No. of animals	EXTERIOR ESTIMATION				
	Fat	Carbo-hydrates		Clarity of fur coat	Density of fur coat	Length, resilience and silkiness of fur coat	Exterior	Total estimation
I	40	30	230	2.31	4.73	5.70	2.58	23.10
II	45	25	104	2.26	4.66	5.85	2.41	23.91
III	50	20	210	2.22	4.38	5.00	2.75	22.03

Rations were formulated based on the typical components generally used on the farm, such as beef with bones, fish offal and fishmeal, poultry byproducts, dairy products (milk, curds casein), eggs and boiled ground grain, wheat bran, yeast, vegetables and green fodder.

During the reproduction period the following observations were carried out : in males - number of matings and length of sexual activity period, while in females - percentage of mating, conception rate, mean litter size, litter size at 21 and 42 days, number of kits and weight of litter. Based on the difference between litter weight at 21 days and birth weight, multiplied by 2 (2 g milk per gram of weight gain), milking capacity of females was estimated. Also, growth index of kits up to 42 days (%), i.e. up to weaning, was calculated.

During the growth period the young were weighed every month (from July to November), and during the period of fully mature fur coat (November), their conformation was assessed according to the "Standards of estimating conformations of breeding fitches (1984)" in force in Poland. For analysis of the quality of the fur coat, following traits were considered:

1. Clarity of fur coat	max. 3 points
2. Density of fur coat	max. 6 points
3. Length, resilience and silkiness of hair	max. 6 points
4. Exterior	
5. Total estimation	max. 30 points

The results of measurements of the studied production traits were presented as arithmetical means and standard deviations; significance of differences between studied traits was calculated by a multifactorial analysis of variance and Duncan's test. For calculations, a statistical package Sp SS for PC computers was utilized.

RESULTS

Our findings concerning female deliveries and sexual activity of male fitches (Table 2) point to groups I and II as those displaying the most beneficial fat:carbohydrate ratios, viz., 30-35% of ME from fat and 35-40% of ME from carbohydrates. In animals fed such diets a high conception rate (93%) as well as high values for litter size and weight were recorded. An increase in energy from fat up to 40% with a simultaneous decrease in energy from carbohydrates to 30% (group III) significantly reduced the number of fertilized females (75%), mean litter size (7.41 kits per female), mean litter weight (81.4 g) and index of reproductive performance in males.

During the lactation period of female fitches and growth period of young kits till weaning the most effective results were obtained in group I, fed a diet with 40% energy deriving from fat and 30% from carbohydrates (Table 3). Litter size at 21 and 42 days of age was the highest in this group as well as the number of kits at weaning (83%). Relatively good results were also found in group II, fed a diet with ME from fat increased to 50% and ME from carbohydrates decreased to 20%.

Concerning rations for young fitches (from mid-July to mid-September), the best weight gains were recorded in males of group III, fed a diet with 55% of ME from fat and 15% of ME from carbohydrates, and in females of group II, fed a diet with 45% of ME from fat and 25% of ME from carbohydrates (Table 4).

Final body weight and estimates of fur coat quality are presented in Table 5. An analysis of these results indicated that more effective results were obtained in group II, fed the diet with ME from fat estimated at 45% and from carbohydrates at 25%. Final body weight of males in this group was 1395.6 g and of females 948.9 g; in addition, also the structure of the coat and final estimate were better (23.9 points) in this group.

DISCUSSION

The results obtained in our study indicate that the adult fitches in the reproduction period can be fed diets with a similar percentage of ME from fat and carbohydrates. As a desirable ratio, 30-35% ME from fat and 35-40% of ME from carbohydrates was considered. Feeding the basic stock fitches diets with such indices, had a satisfactory effect

on reproduction in females and males. However, comparative studies concerning feeding farm fitches in Poland (Barabasz et al. 1989) revealed that quite good results can be obtained in this period with rations containing 32% of ME from protein, 46% from fat and 19% from carbohydrates. Also during this period, Babunowa (1987) considered 20% of ME as an optimal level from carbohydrates, while Bednarz & Frindt (1991) accepted 20-30% of ME from fat and 20-40% from carbohydrates as an appropriate ratio. Pierieldik et al. (1987) recommended that during the reproduction period adult fitches should be given a slightly higher percentage of protein (to 45% of ME), 30% of ME from fat and 25% of ME from carbohydrates.

During the lactation period of females, it was found that it was necessary to increase the percentage of energy from fat and to decrease that from carbohydrates in comparison with the preceding period. As similar strategy was reported by Pierieldik et al. (1987), who explained it by stressing the need for keeping females in good condition and providing them with a sufficient amount of exogenous fatty acids. Bednarz & Frindt (1991) recommended feeding diets with 20-40% energy from fat and 20-30% from carbohydrates during the lactation period. All the authors agree that during this period it is advisable to increase considerably a percentage of protein in the feed, up to as much as 50% of ME, with a simultaneous high level of fat.

Our findings concerning the feeding of young animals after weaning suggest a slightly different demand of females and males for energy from fat and carbohydrates. Generally speaking, it can be said that during a period of fast growth the young fitches demand a relatively high level of energy from fat and a lower level from carbohydrates. According to Pierieldik et al. (1987) young fitches display the most intensive growth and attain high final body weight when fed a diet with a high content of fat (36-47% of ME) or even more. With a smaller amount of fat in the diet the growth of young fitches becomes considerably slower. Bednarz & Frindt (1991) recommended rations with 25-40% of ME from fat and 20-40% of ME from carbohydrates for this period. Babunowa (1987) found that during this period the highest weight gain in the young stock can be obtained by feeding the animals diets containing only 20% of energy from carbohydrates (grain feeds), which confirms the results obtained in this study. Moreover, an analysis of the feeding of young fitches in large farms in Poland (Barabasz et al. 1989) has shown that in this period they can be fed effectively with diets affording 30-40% of energy from fat and 20-30% from carbohydrates.

During the period of winter fur priming when fitches were fed diets with 30% energy from protein, better weight gains and fur coat quality were obtained in the group in which the amount of energy from fat was 45% and from carbohydrates 25%. A more detailed analysis of our findings indicates, however, a possibility of elevating the level of carbohydrates in the diet to 30% of ME at the cost of lowering the level of energy from fat, as follows from the results of group I. According to Leoschke (1986) an increased percentage of carbohydrates in the diet is fully justified in this period, since it improves the degree of animal fattening and affects the synthesis of fatty acids which, in turn, are responsible for the colour of the fur coat.

In summary, it can be stated that a reasonable use of fats and carbohydrates in feed for fitches makes it possible to economize on the more expensive components such as protein and improves the palatability and the productive properties of a feed.

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Effect of feeding intensity prior to parturition on postnatal growth in blue foxes (*Alopex lagopus*)

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The objective of this experiment was to investigate whether feeding intensity during gestation affects lactation rate and, hence, cub growth in blue fox. Ninety-three nulliparous blue fox females were divided into low (LE) and high energy (HE) groups which received 14.6 and 19.5 MJ of ME/kg dry matter, respectively, from gestation day 35 onwards. From day 3 postpartum both groups were fed HE diets. Energy change at the beginning of the third trimester resulted in more rapid growth of cubs during lactation. Cub weights at 14 and 21 days of age were 231 and 361 g with the LE diet and 189 and 336 g with the HE diet, respectively. Differences were significant at both stages. Preparing females for lactation during the third trimester proved to be beneficial and resulted in higher growth rates of cubs.

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During the last third of gestation the nutrient requirement of the female blue fox increases because of the rapid development of the fetus. According to Lewis et al. (1987) there is only a slight change in the vixen's body weight or nutritional needs during the first 5-6 weeks of gestation, when less than 30% of fetal growth occurs. Fetal size rapidly increases during the last 3-4 weeks of pregnancy and, as a result, the weight of the vixen is expected to increase by 15-25% by the time of whelping. Moreover, during the last weeks of pregnancy a female carrying a large litter may have such an enlarged abdomen that she can have difficulty in eating a sufficient amount of food. Usually either an increased number of meals per day or a higher feed density is recommended. The energy requirement remains high during lactation to support adequate milk production. During the first three weeks of life of blue fox cubs the mother's milk is the only source of nutrients. In dog pups, liver glycogen is rapidly depleted after the birth and the amount of subcutaneous fat is rather minimal (Lewis et al. 1987). Pups double their birth weight within one week. This means that they should gain 2-4 g/day/kg of expected adult weight (Lewis et al. 1987). The increased fat percent in the dry feed for cat (21% vs 12%) resulted in a 10% higher birth weight, a 40% greater kitten survival rate and a 30% higher weight gain (Lewis et al. 1987). Adding fat to sow diets during late gestation improved piglet survival (Moser & Lewis 1981; Pettigrew 1981), especially when herd survival rate was relatively low. However, there is no proof that supplemental fat increases piglet birth weight, but it reduces

sow weight loss during lactation and increases the weaning weight of the litter. Moreover, Kirkwood et al. (1988) reported that adding fat to the diet during four successive lactations resulted in larger litters (by 0.8 piglets) in the second and subsequent parities. In the data on blue foxes demonstrated by Fors et al. (1990), high energy content in the feed during pregnancy resulted in increased early pup mortality (30.8% vs 18.7%). The later growth of cubs was not influenced by the feed status of the females.

The present study was conducted to evaluate the influence of two dietary energy levels during gestation on survival and postnatal growth of blue fox cubs.

MATERIALS AND METHODS

This experiment was performed at the experimental fur farm of the Finnish Fur Breeders' Association at Maxmo. Ninety-three nulliparous blue fox females were housed in individual breeding cages and were randomly divided into two diets, low (LE) or high energy (HE) diets with metabolizable energy densities of 14.6 and 19.5 MJ/kg dry matter, respectively. The dietary and nutrient compositions of the experimental diets are presented in Table 1.

Table 1. Dietary composition (%) and nutrient composition of the experimental diets

Item	Diet	
	High Energy (HE)	Low Energy (LE)
Slaughter offal[1]	20	20
Baltic herring	15	15
Cod offal	25	25
Standard cereal[2]	13	13
Fish meal	1	1
Soybean meal	2	2
Vitamin-mineral feed	2	2
Fat	2.5-	
Water	19.5	22
	100	100
ME MJ/kg DM	19.5	14.6
Percentage from		
Protein	34	40
Fat	44	33
Carbohydrates	22	27

[1] tripe and lungs
[2] cooked whole barley and wheat 1:1
[3] 60 % animal fat, 40 % soybean oil

Both low and high energy groups were fed a LE diet from the beginning of the pregnancy. One group was changed to the HE diet 35 days after mating, while the other group continued on the LE diet until day 3 postpartum. All females were weighed at mating but only 17 were weighed in the HE group and 11 in the LE group on day 7, while 11 HE

females and 10 LE females were weighed on day 14, and 25 in the HE group and 22 in the LE group on day 21 after delivery. Cubs were weighed individually at 3, 7, 14 and 21 days of age, both sexes separately. Reproductive performances were recorded on the following postpartum days: 1, 3 and 21. The data were analyzed using analysis of variance and regression analysis in the statistical software, Statgraphics (1989).

RESULTS

The reproductive performances and female weights of the groups are shown in Table 2.

Table 2. Body weight and reproductive performance of the females

	Feeding Level During the Last Trimester					
	High Energy	SEM	Low Energy	SEM	P-value; effect of Litter Size	Group
Female weight:						
at mating	6906	140	6977	156	-	0.739
7 d postpartum	4798	162	4598	137	-	0.403
14 d postpartum	4771	202	4708	165	-	0.817
21 d postpartum	4912	116	4819	120	-	0.588
Litter size:						
at birth	8.98	0.51	9.71	0.57	-	0.351
at 3 d of age	6.65	0.52	6.62	0.71	-	0.971
at 21 d of age	5.60	0.56	5.41	0.64	-	0.950
Survival rate, (%):						
at 3 d of age	74.3	4.76	68.1	5.91	0.021[1]	0.250
at 21 d of age	62.1	5.41	57.2	6.00	0.064[1]	0.468
between 3-21 d	78.3	5.17	79.8	4.73	0.001[2]	0.698

[1] at birth
[2] at 3 d of age
Statistical significance; $p < 0.05$

The experimental diet resulted only in small weight differences between females. The high energy diet fed from the beginning of the third trimester did not cause any significant differences in the reproductive performances between the two groups, but it did result in a lower number of cubs born (HE 8.98; LE 9.71). However, the difference was statistically non-significant. The survival rate in the LE group was lower and litter size was the same in both groups three days after parturition (HE 6.65; LE 6.62). Livability of the cubs from day 3 to day 21 was not affected by the diets and resulted in litter sizes of 5.60 and 5.41 for the HE and LE energy groups, respectively.

When the cub losses at three weeks of age were plotted against the litter size at birth, a strong positive relationship was revealed between these two factors; litters of more than nine cubs in paticular had elevated mortality rates (Fig. 1).

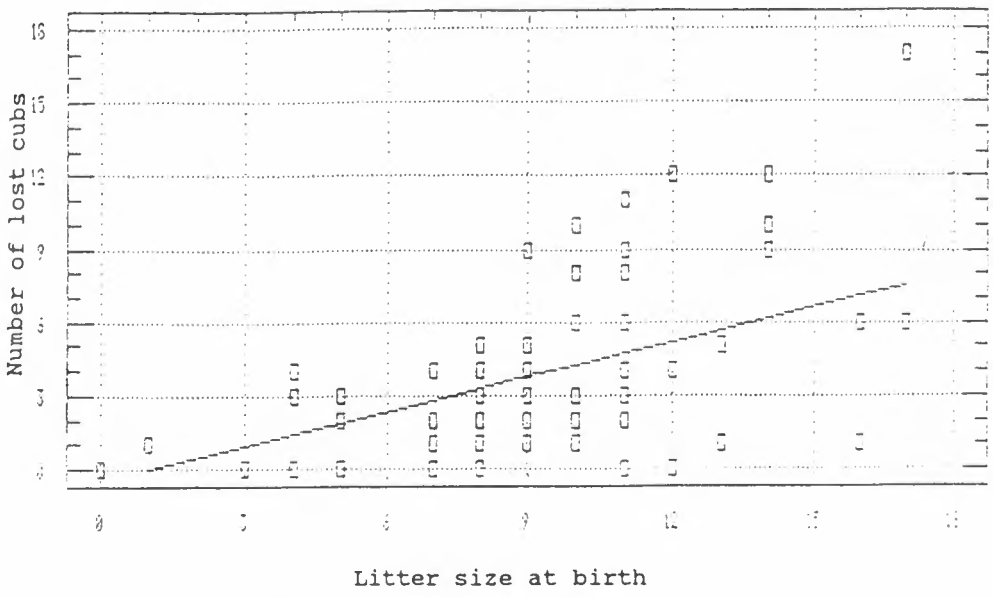

Fig. 1. Number of cubs lost during the first three weeks of life as a regression on litter size at birth (cubs lost = 0.58*litter size at birth - 1.58. $p < 0.001$, $R^2 = 24.1$ %)

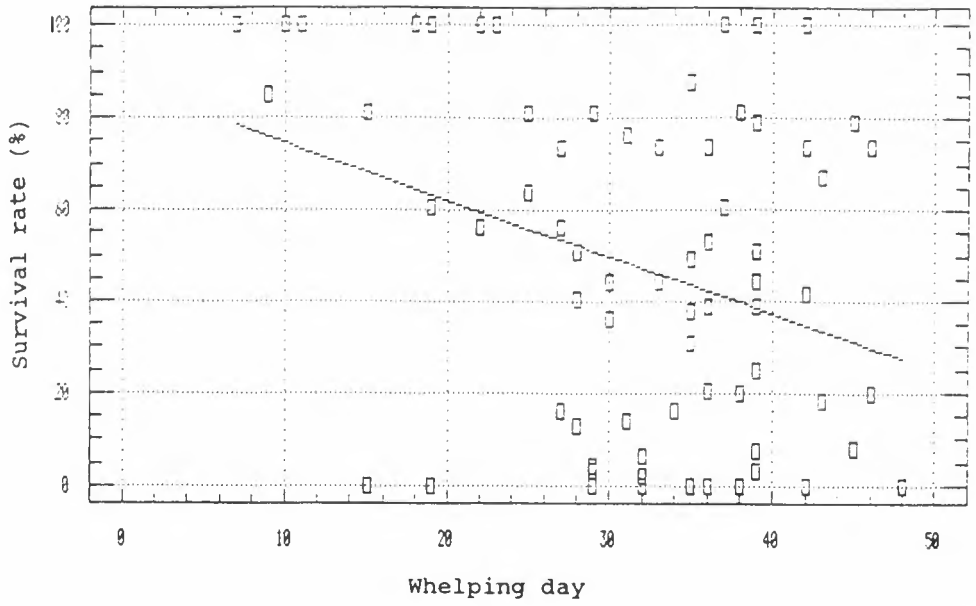

Fig. 2. Survival rates at three weeks of age as a regression on whelping day (day 1 = 1st May). Survival % = 89.5 -0.993 * whelping day ($R^2 = 6.41$ %, $p = 0.029$)

In addition, a negative relationship was found between cub survival rate and whelping date, but not between litter size and whelping date. Consequently, this resulted in a lower number of vital cubs per litter towards the end of the season, especially in late May and in June (Fig. 2.).

Table 3. Growth rate of cubs

	High Energy	SEM	Low Energy	SEM	P-value; effect of Litter Size	Sex	Group
Mean weight (g):							
at 3 d of age	98.5	2.41	101.8	2.45	<0.001	0.230	0.010
at 7 d of age	137	2.82	132	4.36	<0.001	0.202	0.223
at 14 d of age	231	5.30	189	4.68	<0.001	0.055	<0.001
at 21 d of age	361	6.37	336	7.96	<0.001	0.522	<0.001

Statistical significance; $p < 0.05$

It was found that the growth rates of cubs were significantly affected by the diet (Table 3). The HE feed promoted higher mean weights of cubs at the ages of 7, 14 and 21 days by 4%, 18% and 7%, respectively. There were no significant differences in weights between sexes.

DISCUSSION

In this study HE feeding during pregnancy did not cause fatness or result in increased cub mortality as was observed in the earlier study reported by Fors et al. (1990). Instead, the survival rate of cubs was lower in the LE group. The slightly bigger litters in this group seem to have contributed to the cub loss during the first days, since the relation of survival rate and litter size was significantly positive. According to Clark (1986), increased litter size increased the rate of stillborns and perinatal mortality in piglets.

It seems that nutrient supply for the growth of the fetus was sufficient and independent of the diet. The result of the study indicate that the weight of born cubs was not reduced by the LE diet. Fors et al.(1990) demonstrated that an LE diet with a high carbohydrate content resulted in higher liver glycogen levels in the newborn, which indicated a better neonatal survival of cubs. Furthermore, in the present experiment newborn cubs had a higher glycogen content (to be published elsewhere), but the survival rate was no better. Consequently, it is the female condition that is affected by the HE diet, not necessarily the fetus livability.

The effect of diet was more profound on the growth of cubs, especially during the second and the third week of life. Since the litter sizes were the same after the third day in both groups, better growth of cubs in the HE group must depend on differences in milk production. In sows, the addition of fat in the diet results in an increased milk yield and better conversion rations of milk into weight gain of piglets (Noblet & Etienne 1986). Restricted energy supplied increased mobilization of body fat resources and may reduce

body fat (Etienne & Noblet 1984). High energy fed in late pregnancy obviously conditioned blue fox females better for lactation and, hence, resulted in better early growth of cubs. High energy feeding during the last trimester of pregnancy did not increase early cub mortality or the number of weak cubs.

In our study as in the study of Pölönen (1990) cub mortality increased the later whelping took place during the season. It was found that the increase in the number of barren females was the reason for the decrease in reproductive performance late in the season, as was also reported in a study by Smeds & Ojala (1991). The problem of higher cub mortality rates in litters born late was not resolved by the feeding regimen conducted in this experiment and remains to be investigated at a later date.

SUMMARY

A high energy density of the feed fed during the last trimester of pregnancy compared with the high energy feeding which started three days after parturition resulted in a non-significantly lower number of cubs born per litter but a higher growth rate of cubs. Regardless of feeding regimen a negative relationship was revealed between survival rate at three weeks of age and the day of whelping. It seems that high energy feeding in late pregnancy conditions females for lactation, resulting in good early growth of cubs.

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Energy demand of the coypu from weaning to maturity

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Weight gain, feed intake and efficiency of gain were measured for five silver (S-group) and four beige (B-group) coypus from weaning until the age of 50 weeks. Both groups gained weight steadily until the age of 38 weeks, after which they began to lose weight. A plot of the average feed intake (y, g/day) on age (x, weeks) yielded the equations: $y = 33.84\ x^{0.50}$ ($r=0.96$) and $y = 25.83\ x^{0.49}$ ($r=0.78$) for the S- and B-groups, respectively. From the weights 1 kg to 4 kg the total gross energy (GE) demand was 231 MJ/animal for the B-group and 275 MJ/animal for the S-group. To reach 5 kg, an additional 154 and 142 MJ/animal, respectively, were needed. Low temperature resulted in increased feed intake, and slowed down weight gain. GE digestibilities in the S- and B-groups were 71.4 $\pm$ 5.8% ($\pm$sd) and 69.6 $\pm$ 5.7%, respectively. N digestibility was significantly better in the S-group (77.2 $\pm$ 4.8%) as compared with that in the B-group (74.4 $\pm$ 4.8%) ($p<0.001$).

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The coypu is an opportunistic vegetarian, which can survive on various diets and in different climatic conditions (Aliev 1967; Doncaster & Micol 1990; Gosling & Skinner 1984), but to breed coypu efficiently and economically much more information about feeding and basic metabolism is needed. Some research on coypu feeding during the growth period has been done (Di Marco & Garrido 1989, 1990; Di Marco & Ranea 1991; Kladovshchikov et al. 1986; Niedzwiadek et al. 1987) and a few feeding recommendations have been given (Kladovshikov 1982; Von Gert 1985). The aim of this experiment was to produce more information about energy demand, energy and nitrogen digestibilities of coypu during growth and maturity.

MATERIALS AND METHODS

Weight gain, feed intake, apparent digestibility of gross energy (GEDIG) and nitrogen (NDIG) were measured for male coypus from the age of 6 weeks to 50 weeks of age. Two experimental groups, S-group with 5 silver coypus and B-group with 4 beige coypus (one died before 30 weeks of age), were set up 1-2 weeks before the first digestibility trial.

The first four-day digestibility trial was made with six week-old animals and the others followed every fourth week. During the trial periods the animals were placed in metabolic cages but otherwise they were kept with their groupmates in wiremesh cages. Throughout this period the animals were fed *ad libitum* and were given tapwater from an automatic watering system. Feed (coypu pellets) contained gross energy 17.4 MJ/kg and protein 19.1% in dry weight, dry matter was 89%. Average daily temperatures were measured during the digestibility trials.

The digestibility trials were carried out using the total collection method. Feed consumption and faeces were measured daily. Faeces were stored at a temperature of +4°C until the end of each trial and then dried to constant weight at +100°C. Dry samples were ground in a Moulinette grinder (Moulinex, France) and subsamples taken randomly for analysis. Gloss energy (GE) content was measured with a gradient layer calorimeter (Calorimeter automatic MK 2000, FRG), nitrogen by the Kjeldahl method (Nordic Committee on Food Analysis 1970) using the Kjeltec System Apparatus (Tecator, Höganäs, Sweden).

The Student's t-test was used to investigate the differences between the two groups and Pearson's correlations to study the effect of age in digestibilities.

RESULTS

The animals gained weight steadily until the age of 38 weeks. The daily gain until the age of 22 weeks was 25 and 24 g/d in the S- and B-groups, respectively, and between 22 and 38 weeks 16 g/d in both groups. By the end of the experiment the animals had lost some weight. There were no differences in growth between the two groups (Fig. 1). The animals reached the weight of 4 kg in about 25 weeks and 5 kg in about 34 weeks.

Feed and energy intake increased in both groups until the age of 26 weeks and then decreased until the ages of 38 and 34 weeks in the S- and B-groups, respectively. After reaching these ages intake increased again (Figs. 1 and 2). The average energy intake calculated over all the trial periods was greater in the S-group ($p < 0.001$). This was mostly due to the B-group's lower intake at the beginning, at the end and at the age of 30 and 34 weeks (Fig. 1). Equations best describing the average wet feed intake during the experiment were: $y = 33.84\ x^{0.50}$ ($r = 0.96$) and $y = 25.83\ x^{0.49}$ ($r = 0.78$) for S- and B-groups, respectively, where y = feed consumption g/day and x = age in weeks (Fig. 2).

The decrease in energy intake in the middle of the growth period occurred simultaneously with the increase in the temperature on the farm during the summer (Fig. 3). In autumn the feed intake increased despite the arrested weight gain.

The energy demand required to grow a 1 kg animal up to 5 kg is presented in Fig 4. With up to 4 kg the total energy needed was 275 and 231 MJ/animal and with one additional Kilogram the total energy requirement increased up to 417 and 385 MJ/animal for the S- and B-groups, respectively.

The average GEDIG calculated from all trials was $71.4 \pm 5.8\%$ for the S-group and $69.6 \pm 5.7\%$ for the B-group. The NDIG value was better in the S-group ($77.2 \pm 4.8\%$) than that in the B-group ($74.4 \pm 4.8\%$) ($p < 0.001$). It seemed that the age of the animals affected the digestibility in B-group, but this was only for the first and last trials (Fig. 1).

When these values were ignored, the correlation was no longer significant.

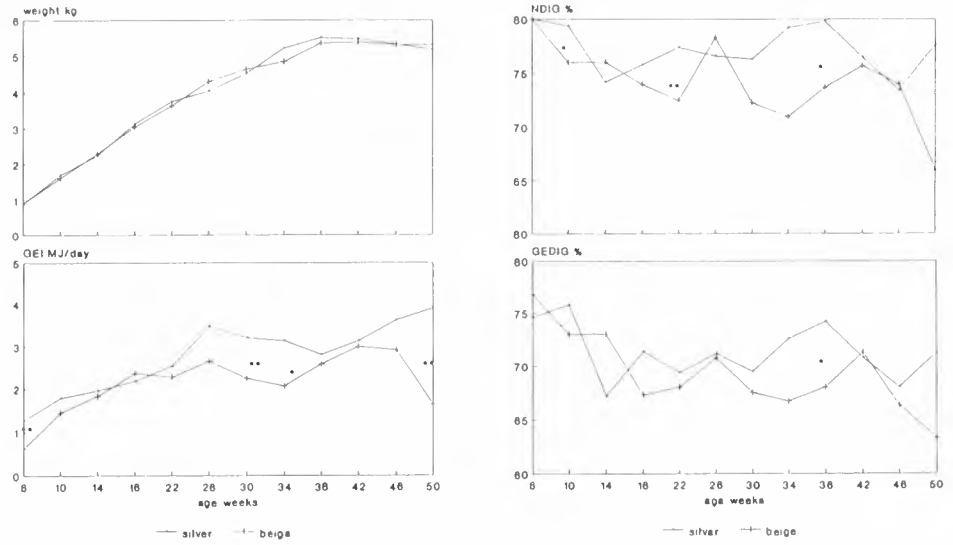

Fig. 1. Average weight gain, gross energy intake (GEI), nitrogen (NDIG) and gross energy (GEDIG) digestibility in S- (silver) and B- (beige) groups during the experiment. Significance between the groups: * $p < 0.05$, ** $p < 0{,}01$ (Student's t-test)

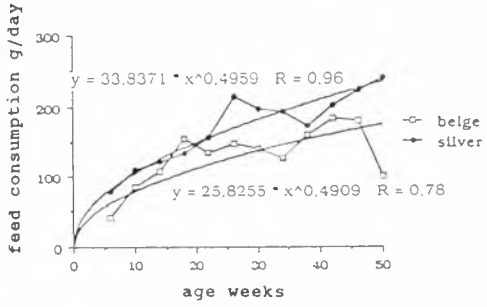

Fig. 2. The equations describing the increase of feed intake during the experiment in the S- (silver) and B- (beige) groups

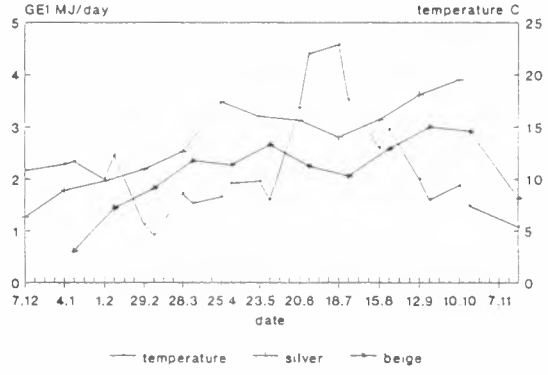

Fig. 3. Gross energy intake and temperature during each digestibility trial plotted against the date. The experiment started five weeks later with the B-group

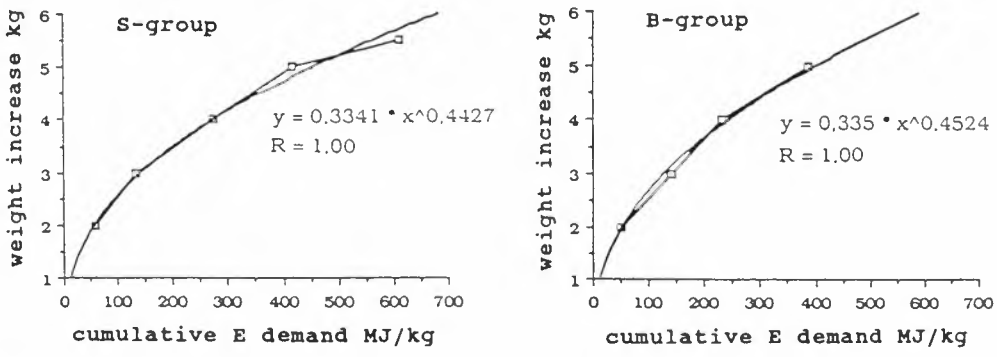

Fig. 4. Relationship between the weight of the coypu and energy demand in growing animals

DISCUSSION

The growth rate of the coypuy can vary considerably depending on the diet (Kuosmanen-Postila 1989), the feeding volume (De Marco & Ranea 1991), the breeding system, and the ambient temperature (Kuosmanen-Postila et al. 1990). In addition genetic factors may have an effect on the growth (Kuosmanen-Postila et al. unpublished data). Feeding recommendations are usually given according to the age of the coypu (Von Gert 1985; Kladovshchikov 1984). In this study the fastest growth period occurred before the age of five to six months. At this age the animals' weight reached 4 kg but the weight gain did not stop before they were nearly nine months old. Growth appeared to be faster and energy intake lower than in the recommendations given by Von Gert (1985), but weight gain was lower than that found by Di Marco & Ranea's (1991) in their experiment.

Two different strains of coypu were selected in order to compare the genetic differences in growth, as well as intake and utilization of nutrients. The results proved to be contradictory: the S-group consumed more energy and protein, NDIG was better, but even so, the growth of these animals was no better than that of the B-group animals. This may be due to a higher stress sensitivity of the B-group animals to the experimental situation; they ate less than the S-group animals during the digestibility trials but probably more between the trials. No age-related variation in digestibilities was observed.

When an animal is growing, the energy requirement per kg weight gain increases because the energy needeed for maintenance is also increasing. According to our estimation 35-50% more energy was needed to produce the fifth Kilogram compared to the three previous (1-4 kg) Kilograms. In rabbits higher slaughter weight also means a higher amount of fat in the carcasses (Rodriquez et al. 1982) and this is probably also the case with coypus. Because of this it might be economically advisable to slaughter the animals when their weight is nearer 4 kg than 5 kg.

In Finland coypus are raised indoors, but, despite that, seasonal changes in temperature cannot be avoided. A thermoneutral zone of +5° - +30°C has been measured for young (three to four months) wild coypu (Doncaster et al. 1990), while the lower critical temperature for farm-raised coypu of the same age seems to be higher e.g. +16°C

(Rouvinen 1985). Under these circumstances the metabolic rate of the coypu at 0°C is twice that measured at thermoneutral zone. In this study the lower energy intake of coypu in summer can be explained by a higher ambient temperature and slower weight gain, whereas the increased energy intake in autumn is mainly due to the lower ambient temperature. The exact influence of the temperature on the growth and feed consumption will be studied in further investigations.

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Pathology and diseases

Fur animal health: current status

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A survey is given of the most common traditional diseases in fur-bearing animals, together with a brief presentation of some recently described ailments. Most of the more or less specific contagious conditions for fur animals can be controlled by vaccination or hygienic measures in countries and areas in which a high standard is maintained in the raising of fur-bearing animals; an important exception is plasmacytosis in mink which still causes considerable losses despite the good results achieved with eradication programs, based on the counterimmunoelectrophoresis test. Feed-borne infections can be prevented or considerably limited by hygienic precautions or by boiling of suspect components in the feed. Some previously unrecognized conditions relating to nourishment have appeared during recent years; some of these conditions occur in rapidly growing individuals that are given special growth-promoting diets. It is suggested that these groups of conditions will be on the increase when fur animals are raised under otherwise optimal conditions. Genetically determined disorders may be excluded by selection, although there seem to be situations in which genetic factors play a contributory role in the development of diseases not considered as hereditary. Such multifactorial diseases are frequently difficult to explain fully, and their existence will probably represent a callenge to research on fur animal diseases in the future.

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The health situation of fur-bearing animals is influenced by local factors and probably rather different in the various countries in which fur animals are raised. It is thus difficult to obtain sufficient information to give an up-to-date review covering all parts of the world.

Experience demonstrates a considerable change in the profiles of diseases over the years. Some diseases disappear, and sometimes reappear after some years, and "new" disorders, never described, arise. This phenomenon is often difficult to explain, but must be seen in connection with the complexity of factors influencing sanitation and health: individual and genetic resistance and immune factors are important in infectious diseases; genetic selection, and dietary composition resulting in rapid growth and increased size, seems to bring about conditions which are unknown in unimproved animals. The nutritional state seems sometimes to play a decisive role even in infectious diseases and in some poisonings. The raising of a great number of animals within a small area facilitates the rapid spread of infections.

This presentation is predominantly based on information from Western Europe and

North America, and the blue fox (*Alopex lagopus)*, the silver fox *(Vulpes vulpes)* and the mink (*Mustela vison)* are the only species considered. Special attention is drawn to"new" diseases and conditions of comparative interest or of obscure etiology, while classical entities in which the etiological backgrounds are well elucidated, are given less attention. Although some aspects of research work are mentioned, a more comprehensive discussion of research related to fur animal diseases is beyond the scope of this survey.

HEREDITARY ABNORMALITIES

Ehlers-Danlos syndrome

This is a disease that produces reduced tensile strenght of the skin. It is known in mink in various areas in which mink are raised, but its scattered occurrence indicates that it is of limited importance, and families carrying the factor are usually destroyed. The diagnosis is based on electron microscopic studies. Blue fox with pelts having the appearance of this syndrome have been seen in Norway (unpublished).

Chediak Higashi syndrome

It has long been known that mink with the Aleutian gene are encumbered with the Chediak Higashi syndrome (CHS) which is inherited as an autosomal recessive trait (Leader et al. 1963). The syndrome is characterized by lysosomal accumulation of multilamellar bodies and lipofuscin pigments in various cell types, and affected animals are known to have reduced resistance to various infections. More recently, the Chediak Higashi syndrome has been described in foxes (Nes et al. 1985). As in other species the CHS animals are characterized by a tendency to bleeding. Platelet counts are normal, but aggregation of thrombocytes by adenosine diphosphate, serotonin, collagen and arachdionate is impaired (Sjaastad et al. 1990). The ultrastructure of abnormal granules in leukocytes and platelets is consistent with that of CHS individuals of other species; the granules seem to arise from fusion of pre-existing, normal-sized granules (Fagerland et al. 1987).

Hereditary spongy degeneration of white matter in silver foxes

Hereditary spongy degeneration of white matter is a newly recognized genetic disorder in silver foxes. The disease seems to represent an interesting model for the study of remyelination, and is presented in a separate report at this congress (Hagen & Bjerkås).

Hyperplastic gingivitis in silver foxes

A hyperplastic gingivitis, with advanced overgrowth of the gingiva, has been known in silver foxes in Scandinavia for some time now (Dyrendal & Henricson 1960); it represents a recessive inherited condition, which develops with increasing age. The disease is sometimes called oral papillomatosis, and although the lesions pathomorphologically correspond to papillomas, they should obviously be interpreted as hypertrophic alterations, and should not be compared with virus-induced papillomatosis in other animal species, such as dogs and cattle.

Amino-acid encephalopathy in mink
A complex amino-acid encephalopathy has recently been reported from Canada (Little et al. 1989); the condition is probably genetically transmitted as an autosomal recessive disease. Preliminary observations include marked elevation of certain amino acids in brain tissue. The pathomorphological characteristics are: reactive astrogliosis of the white matter, enlarged astrocytic nuclei and early gemistocytic change, vacuolation of the U-fiber layer beneath the cortex may be apparent. The changes bear some resemblance to TME microscopically, and the condition should not be confused with this latter disease.

Tyrosinemia (pseudodistemper) in mink
Tyrosinemia II is frequently referred to as "pseudodistemper" because of the clinical resemblance to distemper; the pathomorphological lesions are, however, very different from the latter disease, and consist of granulomatous inflammatory responses to the disposition of tyrosine crystals in various organs. A characteristic feature is the occurrence of bilateral cataracts (Sanford 1988). Its cause is a congenital deficiency of hepatic tyrosine aminotransferase. The condition is inherited as an autosomal recessive trait, and occurs in standard black mink which usually exhibit clinical signs when they are 5-6 weeks old. A more prolonged course may be seen, and is characterized by cutaneous bleeding.

Hyperchylomicronemia in mink
This condition has been observed in Norway and is suspected to be hereditary. It is characterized by a creamy appearance of the serum. At autopsy nodular masses are found in the stomach/pancreatic region (Nordstoga et al. 1991). The tissue is partly necrotic, with the appearance of a thick grey-white fluid on the cut surface. Microscopic examination reveals a lipogranulomatous tissue, alternating with spaces containing an amorphous, strongly sudanophilic substance; these spaces have probably developed from dilated lymph vessels. The lipogranulomatous tissue is rich in foamy macrophages and extends into the pancreatic parenchyma. The condition is assumed to be hereditary, as the corresponding condition in other species depends on a congenital absolute or relative lack of lipoprotein lipase, but enzymic determinations have so far not been carried out.

Hereditary muscular dystrophy in mink
A muscular dystrophy, comparable with the human amyotonic myopathy, has been described in mink (Hegreberg et al. 1976); genetic studies indicate an autosomal recessive mode of inheritance. Clinical signs are muscular weakness and atrophy, and elevation of muscle enzyme in serum. Microscopic examination revealed variable diameter of muscle fibers, scattered hyaline degeneration, fibrosis and regenerative response. Both type I and II fibers are affected.

NUTRITIONAL DISEASES

Anemia
Fish-induced anemia, frequently associated with reduced growth, achromotrichia and "cotton fur", caused severe losses in the Nordic countries some year ago, especially in

mink kits. The anemic condition developed when dietary protein consisted mainly of raw fish, in particular whiting (*Gadus merlangus*), coalfish (*Gadus virens*), and hake (*Merluccius vulgaris*), while anemia was averted when the same fish species were used after boiling. The most important anemiogenic factor in raw saltwater fish appeared to be trimethylamine oxide (Ender et al. 1972), and anemia has to a large extent been averted during recent years, mainly as a result of supplementation with effective organic iron compounds in the feed.

Vitamin B deficiencies

Deficiencies of vitamin B_1 (thiamine) have long been recognized in fur animal production, classically as Chastek paralysis. Most severe outbreaks have often been associated with thiaminases in fish used in the feed. A number of less well defined conditions have been connected with lack of B-vitamins in the Nordic countries, especially in foxes, and it is a common experience that extra supplementation of B-vitamins is useful in diseases of obscure etiology in adult animals, and in association with increased losses of young animals, especially fox cubs (personal observation; Smeds 1992, personal communication).

Conditions related to vitamin E/selenium deficiency

Vitamin E- deficiency in fur-bearing animals was first described as "yellow fat" disease, the description deriving from the appearance of the fatty tissue of dead animals. As a rule, the deaths occurred in fast-growing males early in the autumn. This condition is characterized by accumulation of ceroid in the adipose tissue, and is also sometimes accompanied by muscular degeneration. During recent years this disease has occured less frequently, but has been replaced by another condition considered to be related to vitamin E/selenium deficiency, which also occurs in rapidly growing males. The most important microscopic observations are microangiopathy and degenerative lesions in the myocardium and skeletal muscles. This variant seems to be most frequent in mink, but has also been observed in silver foxes (Albert & Wenzel 1989, Nordstoga 1986). It has also been noted that extra supplementation of vitamin E has been useful in pregnant blue fox vixens with severe fatty change of the liver (Smeds, personal communication).

Congestive heart failure in juvenile foxes

Congestive heart failure is a clinical situation caused by heart disease, and can affect either the left or right ventricle, or both. In cats there is documentation that shortage of the sulfonic aminoacid taurine may cause congestive cardiopathy (Pion et al. 1987). Congestive cardiomyopathy has been described in western Canada in young silver foxes fed slaughterhouse offal supplemented with a dry cereal-mineral and vitamin premix. There was no evidence of vitamin E/selenium deficiency, hereditary or infectious disease, and ultrastructural studies indicated lesions corresponding to those in other animal species with congestive heart failure (Onderka 1988). Similar cases have been observed in eastern-Canada; most cases were young male silver foxes, fed a commercial pelleted feed.Taurine deficiency was suspected (Ferns & Clark 1988). Sudden deaths in young silver foxes with enlarged hearts and pulmonary edema have also been observed in Finland recently; vitamin E supplementation did not seem to provide any protection (Smeds, personal information). Although not fully elucidated, these deaths in foxes most probably are associated with

nutritional factors, perhaps of multifactorial character. From Czekoslovakia outbreaks of cardiopulmonary insufficiency have been reported, in which foxes vaccinated against distemper seemed to be protected (Jasso 1991).

Rickets
Rapidly growing young mink and foxes may develop rickets when there is a shortage of vitamin D in the feed, or when the calcium: phosphorus ratio is inadequate. Once a serious problem, rickets is today a rare condition in most countries that raise mink and foxes.

INFECTIONS

Many of the infections that occur in mink and foxes are feed-borne. As slaughtherhouse offal from mammals and poultry is mostly used in its raw state, a number of infections from other domestic animals may be contracted through the feed; the health in fur-bearing animals thus reflects to a certain extent the zoo-sanitary situation in other domestic animals within an area (Løliger et al. 1988). Agents can multiply in stored feed or only be passively transported through the feed. In certain situations feed ingredients from slaughtered animals are cooked to prevent transmission of contagion, and with few exceptions (TME) this is an effective measure. Some infections are highly contagious and spread readily from animal to animal when introduced, while others require a continuous influx of agents to maintain the infection. Healthy carriers may exist and eradication may sometimes be difficult and time consuming.

BACTERIAL INFECTIONS

A number of microbes, frequently transmitted from feed or drinking water, may give sepsis or localized or generalized inflammatory changes resulting in single or multiple deaths. These include *Bacillus anthracis*, *Pasteurella multocida*, *Listeria monocytogenes*, *Brucella*, *Francisella tularensis*, hemolytic streptococci, *Klebsiella* and *Escherichia coli*. Some of the more common or newly recognized infections are listed below.

Pseudomonas infections
Outbreaks of hemorrhagic pneumonia occur in mink in most mink-producing countries, but may be quite effectively prevented by vaccination. A polyvalent vaccine is commonly used. As a rule, deaths are greatly reduced within a week after vaccination. The clinical disease seems to be an air-borne infection, but the initial means of introduction of the microbe to the farm can probably vary, and can often come from moist soil. There are great differences in the pathogenicity between the various strains; one possible factor responsible could be the elastase activity of the bacteria, as elastase has been found to be a virulence-enhancing factor in hemorrhagic pneumonia in mink (Elsheikh 1985). Animals often die very suddenly. Macroscopic lesions consist of consolidated, hemorhagic pulmonary tissue, in one or both lungs. Microscopic examination reveals that there is no evidence of an inflammatory response in peracute cases, when the bloody appearance is exclusively due

to hemorrhagic necrosis. In somewhat more prolonged cases there are obvious inflammatory changes, with myriads of bacteria, frequently localized perivascularly, or within necrotic arterial walls. Peculiar features are that the tunica elstica interna is spared, and that the necrotizing mural changes are not accompanied by thrombosis; these vascular alterations are considered as nearly pathognomonic for lesions provoked by *Pseudomonas* infections ("*Pseudomonas* vasculitis").

Pseudomonas aeruginosa sometimes occurs in blue fox vixens in farms using artificial insemination. Uterine infections sometimes progress to septicemia, but the most serious losses are often associated with abortion and increased mortality among young cubs.

Salmonellosis

Both mink and foxes are relatively resistant to *Salmonella* organisms, except during pregnancy when abortion is a frequent consequence, expecially after exposure to *S.dublin* and *S.typhimurium*.

In countries with widespread occurrence of *Salmonella* spp. these organisms are frequently blamed as a major cause of enteric disease, although the real importance is sometimes difficult to estimate.

Campylobacter infections

Campylobacter spp. (*C.jejuni, C.coli*) may colonize intestinal mucosa and are known to cause diarrhea and probably intestinal adenomatosis in blue foxes (Eriksen et al. 1990): *C. jenuni* has produced reproductive failure and abortion in experimentally infected mink (Bell & Manning 1990; Hunter et al. 1984, Hänninen et al. 1988). The importance of these agents in fur animal production is, however, still being debated.

Tuberculosis

Tuberculosis is well known both in mink and foxes, the route of infection almost always being the alimentary tract (Løliger 1970). The intenstine, mesenteric lymph nodes and the liver are primarily affected. Generalization may sometimes occur, especially when the infecting agent is *Mycobacterium bovis* which is more pathogenic both in mink and foxes than the avian type. Avian tuberculosis may occur as a dual infection with plasmacytosis in mink, with an autopsy picture very similar to that of plasmacytosis. Infection with *M.tuberculosis* is rarely reported in fur-bearing animals.

Septicemia in newborn animals

Although neonatal mortality seems to be considerable in all areas where fur animals are raised, the impression is that deaths are not always primarily associated with infections. Streptococci, staphylococci and *E.coli* are among the bacteria that are most commonly involved in postnatal septicemia. It is, however, probable that many of these newborn animals are weak when born and therefore unable to suck, or that milk production has failed in their dams.

VIRAL INFECTIONS

Infectious plasmacytosis (Aleutian disease)

Plasmacytosis is a persistent parvovirus infection in which the agent interferes with the immune system, with resulting elevation of the gammaglobulins; it is followed up by immune complex formation and fatal immune complex disease. The condition has for many years been a scourge for the rearing of mink throughout the world. Originally considered to affect mainly animals of the Aleutian type, it soon became evident that other color varieties could also be affected and the most serious losses in Norway have sometimes been seen in the black varieties. There has, however, been considerable variation in both the prevalence of the infection and the mortality rates, the severity of the disease being dependent on several factors, including nutritional and climatic conditions. Some observations indicate that introduction of a "new" strain of the agent may exacerbate the disease in a district in which the infection has stabilized over a number of years. Plasmacytosis is generally considered to impair reproduction, although information from some countries indicates that this varies between farms and areas.

Diagnostic tools were greatly improved by the introduction of the counter-immunoelectrophoretic (CIEP) test. There is a good correlation between this test and pathomorphological findings based on the examination of liver and kidney specimens, which are the organs most commonly screened. It should be borne in mind, however, that lesions first appear in the bone marrow, spleen and lymph nodes, and that hepatic and renal changes in some animals develop relatively late in the course of the disease. Hence, the CIEP test should be considered more sensitive than the histopathologic diagnosis. Diagnostic screening programs, aiming at eradication of the infection, have been carried out in several countries and have been succesful to a considerable extent. In Denmark, where the campaign against plasmacytosis, based on the CIEP test, has been carried out since 1976, good results have been attained, but total eradication has as yet failed; false positive as well as false negative reactors have been found, the latter representing a complicating factor in combating the infection (Hansen, personal communication).

In 1982 a new type of plasmacytosis was observed in Denmark, causing interstitial pneumonia in mink kits within the first two-and-a-half months after birth. Subsequent studies revealed that experimental infection of dams before pregnancy decreased the number of kits, and that infection in mid-pregnancy caused fetal deaths, resorption of fetuses or abortion (Alexandersen 1986).

Occular inflammatory lesions, mainly localized to the uvea, occur regularly in plasmacytotic animals, together with retinal degeneration (Hadlow 1982, 1984).

Distemper

Distemper is still a problem in some areas, both in mink and in foxes, although vaccination quite effectively prevents the infection in most cases. The severity may vary considerably from outbreak to outbreak and mortality is sometimes very low. Although a highly contagious infection, experience shows that frequently only one species is affected during outbreaks of distemper in mixed farms. A distemper-like virus which was isolated from seals during an epidemic in the sea waters of north-western Europe in 1988 has been transmitted to mink under experimental conditions, but it is still an open question whether

this phocine virus represents a source of infection for fur-bearing animals (Blixenkrone-Møller et al. 1990)

Aujeszky's disease

Morbus Aujeszky is well known both in mink and in foxes. It has been suggested that pathomorphological lesions in these animals are less specific than in other animal species, but Kimman & van Oirschot (1986) claim that widespread vascular lesions constisting of hyaline and fibrinoid degeneration are present in mink. The agent most commonly originates from swine wastes; thus, the infection was diagnosed in Korea in mink, two years after the first reported outbreak of the infection in pigs (Hwang et al. 1991). The most effective measure to prevent the infection is to exclude raw swine offal from fur animal feed, or to cook such components.

Newcastle disease in mink

A lethal condition, characterized by severe meningo-encephalitis, occurred in the Netherlands in the early 1970s. Detailed studies showed that the agent of Newcastle disease was responsible, and that the infection was transmitted by feeding offal from infected chickens (Haagsma et al. 1975).

Parvovirus infection

Intestinal parvovirus infection (*virus enteritis*) occurs in all countries where mink are reared. The infection may be very serious in young kits, but the losses may be quite effectively controlled by prophylatic vaccination. Clinical features and intestinal pathology are quite characteristic, and the diagnosis is in most cases simple; the infection should not be confused with other intestinal entities of more obscure etiology. Parvovirus infection is reported from Finland as an intestinal disease and causing reproductive disturbances in foxes.

Influenza-like diseases in mink

Interstitial pneumonia was recognized in association with an acute and contagious respiratory disease in mink in Sweden. An avian influenza A virus, belonging to serotype H10N4 was isolated and suspected to be of etiological importance (Englund et al. 1986).

Transmissible mink encephalopathy (TME)

This transmissible disease with a long incubation period was first reported by Hartsough & Burger (1965). Although rare, this condition is one of the infectious diseases in fur-bearing animals which have received most attention during recent years, mainly because of the pathologic similarities with scrapie in sheep and the dramatic occurrence of bovine spongiform encephalopathy in Great Britain. As experience indicated that a sheep origin was unlikely, another source of infection was discussed, and a possible unrecognized scrapie-like disease in cattle or in wild ruminants in the United States has been considered. Fatal spongiform encephalopathy has been induced in calves by intracerebral inoculation of infected mink brain material, and bovine brains passaged back to mink have been shown to be highly pathogenic (Marsh et al. 1991). The origin of TME is, however, still unknown.

Epizootic catarrhal gastroenteritis in mink

An apparent contagious gastroenteritis, different from parvovirus infection, has for some 20 years been known in North America, some of the Nordic countries, USSR and China. Clinical signs are similar to those of parvovirus infection, but the mortality is low. Adult dark mink seem most susceptible. The etiology is not known, but there seems to be a synergistic effect of some vira (rota, corona, parvo, calcini), and even bacterial infections may play a role (Gorham et al. 1990).

PARASITES

INTERNAL PARASITES

A numer of flukes, tapeworms and roundworms may infest various organs of fur animals with their occurrence depending on geographic and climatic conditions throughout the world. Parasitic disorders related to internal parasites are closely connected with sanitary performance. Modern management usually prevents completion of the life cycle of internal parasites, and such parasites are now of less importance than in the past, when great losses occurred, especially in foxes. Some parasites, such as trichinae, may be transmitted by feeding raw, contaminated meat, by cannibalism, or through rats frequenting the farms.

Protozoa

Although most protozoan infections are subclinical, several protozoa may produce clinical disease under certain conditions.

Coccidiosis

Intestinal coccidiosis, with clinical signs, and sometimes also considerable mortality, is well known both in mink and foxes, particularly in young animals. A variety of coccidial species belonging to the genera *Isospora* and *Eimeria* have been reported in the intestinal tract in diseased or healthy animals; they are sometimes believed to originate from rabbit or chicken components of the feed.

Toxoplasmosis

Toxoplasma gondii is recognized as a pathogen in both mink and foxes. In pregnant animals the placenta may be infected, with subsequent abortion or congenital infection of the fetuses. Its real importance is poorly evaluated, but preliminary investigations suggest a rather limited distribution in fur farms in the Nordic countries; the agent occurs sometimes in dual infections, combined with viral hepatitis or distemper. Pathomorphological lesions in the central nervous system in congenital toxoplasmosis in blue foxes have recently been described in detail by Bjerkås (1990).

Encephalitozoonosis

Encephalitozoon cuniculi (Nosema cuniculi) has been known as a pathogen in blue foxes in the Nordic countries since the middle of the 1960s. The infection is subclinical in adult

animals, but gives rise to considerable losses in fetuses and cubs. The dams are infected either before or during the gestation period, indicating a transplacental infection of fetuses (Mohn et al. 1982). Clinical signs of inappetence, reduced growth, ataxia and cataracts are common, and polyarteritis nodosa and renal lesions are constant evidenced at autopsy. Clinical encephalitozoonosis in blue foxes is accompanied by extensive plasma cell proliferation in various organs and significant elevation of gammaglobulin levels. In the acute cases of involvement, numerous parasites can be found in renal tubular cells, in blood vessels and in cataractous lenses. Detailed studies of host-parasite relationship and pathomorphological lesions in the central nervous system have been carried out by Bjerkås & Nesland (1987). Recently, the disease has also been reported in farmed blue foxes in Czechoslovakia (Persin & Dousek 1991).

In Norway overt encephalitozoonosis has also been observed in mink, with similar pathomorphological lesions as those found in blue foxes. In addition, multiple renal cysts are constant findings (Zhou et al. 1992); this latter finding remains unexplained, but it seems probable that renal involvement occurs very early in fetal life in mink. Multiple renal cysts are otherwise seldom seen in mink, although the condition has been described as a hereditary disease in related mink (Henriksen 1988a).

In addition to cataracts the most severely affected animals develop other ocular alterations, consisting of iridocyclitic lesions, including polyarteritis nodosa.

EXTERNAL PARASITES

Sarcoptic mange (*S.scabiei)* has during the last decades been widely distributed in wild red foxes (*Vulpes vulpes)* and has also been a problem in farmed foxes in the Nordic countries, some other european countries and has also been observed in Canada (Onderka, personal communication). If left untreated, this condition quite often progresses to a serious disease, with great pain for the animals, emaciation and death. Otodectic mange (*O.cynotis)* is still widely distributed in foxes. Although this parasite affects the wellbeing of the animals considerably, it is of minor importance as a pathogen. Fleas can also be troublesome; as bloodsuckers they may create an anemic condition, especially in young animals, and, in addition, they may spread a number of infectious diseases.

POISONINGS

As predators, wild mink and foxes are exposed to a number of toxins accumulated in the environment. Domestic fur animals are to a considerable extent protected against environmental pollution, although such intoxications sometimes occur. Small doses may influence reproduction, lactation, growth, or skin or fur quality, without any other notable damage. Actual toxins include rest concentrations of heavy metals (mercury, lead), pesticides, PBSs, PBBs, while mycotoxins and nitrosamines are formed during production and/or storage of feed ingredients. Species differences do occur; mink are thus highly sensitive to aflatoxin (Koppang & Helgebostad 1972).

Dimethylnitrosamine poisoning
A toxic principle was discovered in herring meal in Norway in the 1960s, when it caused great losses in fur animals as well as in other farmed animals. Its toxic effects were later intensively investigated, and some aspects of the consequence of application of nitrosamines to fur animals are dealt with in separate communications at this congress by Koppang & Helgebostad.

Botulism
It is well known that mink are highly susceptible to *Clostridium botulinum* type C toxin, and most outbreaks of botulism in mink have been provoked by type C toxin. It is difficult or even impossible to prevent the occurrence of this microbe in ingredients of fur animal feed. The feed should be stored at low temperature to prevent multiplication of the agent and toxin production. Prophylactic vaccination is, however, considered necessary by many farmers, and vaccination has been widely used during recent decades. In contrast to the highly susceptible mink, foxes are regarded as resistant to botulinum toxin. There seems, however, to be reason to believe that botulism also may occur in foxes under certain circumstances (Haagsma 1980, personal observation), and suspect feed should also be avoided even with foxes.

DISORDERS OF COMPLEX ETIOLOGY

Non-specific gastroenterits
Gastroenteritis, unassociated with any known infection is a frequent condition both in mink and foxes. The disease is often linked to sanitary or nutritional circumstances, and is frequently transient and of short duration, although some animals may develop a more chronic diarrhea, with reduced body weight, or even emaciation. Ulceration of gastric mucosa is a common complication.

Nursing disease in mink
This conditon strikes nursing females with large litters at about the time of weaning; the dams gradually loose appetite and weight, become dehydrated and succumb in an emaciated condition. At autopsy additional findings include fatty change of the liver and gastroenteritis, frequently with ulcerations of gastric mucosa. Several hypotheses, involving metabolic derangement, have been proposed as the causal background; Losses may be considerable in some farms. This ailment is discussed in a separate report at this congress (Nørgaard Clausen et al.)

Wet belly in mink
Affected animals are unable to control urination and the urine incontinence damages the pelt on the belly. The causes are still unexplained, but there are indications of a complex etiology, involving nutritional, genetic and environmental factors.

"Wet" mink kits
In the Nordic countries a diarrheic condition has been reported in preweaning mink kits,

accompanied by a greasy yellow-brown exudation in the skin, progressing to alopecia. The intestinal contents are found to be watery at autopsy; the entity is regarded as a multifactorial condition, with some relevance to feed composition and hygienic factors, possibly combined with some infectious agents, but without any proved connection with welldefined infections (Henriksen 1988 b). Preparations of lactic acid bacteria have been administered to attempt to stabilize intestinal flora (Pedersen & Jørgensen 1992).

Fatty change of the liver

Advanced fatty change of the liver is occasionally reported as a major problem both in mink and in foxes. A number of deficiencies and intoxications have been associated with excessive accumulation of lipids in the liver, and the condition is not a specific entity. Lack of factors known to induce hepatic lipoidosis in experimental situations (choline, essential fatty acids) is unlikely, and most cases are probably due to either excessive use of fat in the feed or starvation. Spoiled feed is sometimes incriminated because of its content of bacterial or mold toxins. Practical experience shows that extra supplementation of vitamins belonging to the B group is useful.

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Towards a more specific serological diagnosis of Aleutian disease

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Counterimmunoelectrophoresis (CIEP) is used worldwide to test mink blood for the presence of antibodies against the parvovirus infection ADV. This serological method is fast, inexpensive, sensitive and easy to perform, with a limited number of manipulations required. Currently more than 80% of the Danish mink farms have been tested and 67% of the farms are free from Aleutian disease. Since we sometimes test animals that are diagnosed as weakly positive, the need for a more specific serological diagnostic method arises. CIEP is still our method for routine diagnostics, but when occasional positive reactors appear in an otherwise negative population, we have several methods for re-evaluating the samples. The positive mink are killed and serum collected by heart puncture. Liver and kidney are formalin fixed. Our routine re-examination is by CIEP and Rocket line immuneelectrophoresis (RLIE), but an additive CIEP has been developed that can distinguish false positive reactions from a faint but true precipitin line. We have tried to use Western blotting and peroxidase labelling of virus in cells by means of the patient serum, but up until now it seems that this method is of rather low sensitivity.

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At the IFASA meeting in Toronto in 1988, we reported that only 42% of the Danish mink farms had Aleutian disease (AD) free mink (Hansen 1988). Now, four years later, the figures are as outlined in Table 1. It is obvious that the increase in AD-free farms derives from the untested farms as well as from AD-positive farms.

As the number of negative animals tested increases, an increase in the statistical number of false positive reactions also occurs. In 1990 mink from 380 farms were re-examined because of a positive reaction during the screening. In the 107 farms where one single animal had been examined it was found that upon the second testing there was no indication of a positive reaction. Thirty-eight farms were re-examined with 2-10 reactors and all proved to be negative upon re-examination. The remainder, 235 farms (62%), had at least one animal confirmed as positive. We still consider counterimmunoelectrophoresis (CIEP) (Cho & Ingram 1972) the best method of screening for antibodies against Aleutian disease virus (ADV), but we need other confirmatory methods of a high specificity as well as a good sensitivity. Recently, false positive reactions caused by vaccination (Munck 1990) or infectious diseases such as avian tuberculosis or erysipelothrix (Englund & Meyerland personal communication 88) have been reported.

This paper describes the methods we have chosen for re-examination of the occasional positive animal in supposed AD-negative population.

Table 1. Comparison of AD-status in Denmark in 1988 and 1992

Registration [1]	Beginning of 1988 No. of farms	%	Beginning of 1992 No. of farms	%
A-farms no positive reactions	1968	42	2488	67
B-farms 1 per mille positive	414	10	281	8
C-farms 2 per mille postitive	267	6	143	4
D-farms[2] <5% positive, pelted	271	6	67	2
G-farms[3] tested farms with AD+	566	12	320	9
0-farms not tested farms	1161	25	398	11
Total	4647		3697	

[1]The farms are registered according to how far they are in the AD eradication programme
[2]Farms with <5% positive animals for pelting were classified as D-farms in1988. In 1992, however, D-farms were only allowed to have up to 2% positive animals for pelting
[3]G-farms do not necessarily pelt the positive animals, but divide the farm into a positive and a negative section

MATERIAL AND METHODS

Sera

All tested sera are from mink that presented an unexpected positive reaction in the routine testing. The animals were anaesthetized and blood was taken by heart puncture. Within ten hours the blood is centrifuged and the serum separated and stored at -20°C until the tests are performed.

Rocket line immune electrophoresis (RLIE) is performed as described by Aasted et al. 1986. Briefly, an 0.7% HSA Agarose (Litex, Vallensbaek Strand, Denmark) in Gelman buffer (50 mM Tris-barbitone pH 8.6) with 10% glycerol is cast in a plastic mould. The line is made up of 200 µl DANAD antigen (The Antigen Laboratory, Glostrup, Denmark) titre 4 and 200 µl positive mink reference serum from United Vaccines, VI, USA. Ten microlitres of patient serum is applied in a well between the two troughs. If antibodies are present in the well they will break the line. After the electrophoresis the plate is washed in saline and deionized water. After total drying it is stained with Comassie Brilliant Blue and the plate is read.

Counterimmunoelectrophoresis (CIEP) and additive counterimmunoelectrophoresis (Uttenthal 1992) is performed on 10 x 10 cm glass plates in 1.8 mm high 0.7% HSA Agarose (Litex) in Gelman buffer. Ten microlitre wells are punched in three pairs in rows, the distance between the centre of the holes is 7 mm. In the first row serum is electrophoresed towards the commercially available virus antigen (DANAD), this first row constitutes the normal CIEP test. In row no. 2, serum is electrophoresed towards a special negative antigen containing no virus, but all the cellular debris usually present in the antigen. In the third row, 2 μl high titred anti-ADV mink serum is applied to the well before addition of 10 μl patient serum, this mixture is electrophoresed against the virus antigen. After 30 min at 5 V/cm the gels are washed and stained with Comassie Brilliant Blue R250 and the precipitation lines are read. The gel is outlined schematically in Fig. 1. A false positive reaction will give one band in rows 1 and 2 and a double band in row 3. If antibodies against both virus and cells are present, there will be double bands in both rows 1 and 3 and a single band in row 2. A true, but weak reaction will give one weak line in row 1, and this precipitate will be added to the one with the positive serum, giving *one* strong band in row 3. Row no 2 can be omitted if a negative antigen without virus is not available.

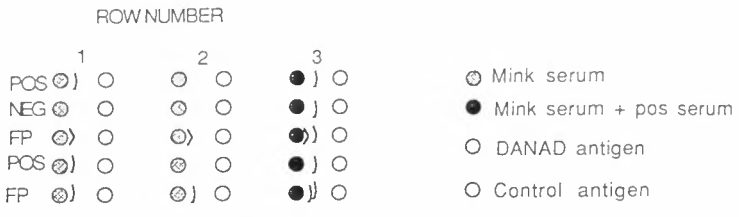

Fig. 1. Graphic presentation of additive counterimmunoelectrophoresis. Three pairs of wells are electrophoresed simultaneously. In no. 1 and 3 the antigen is made from cells with virus, whereas the antigen in row no. 2 is without virus. In rows 1 and 2, mink serum is applied to the left wells, in row 3 the mink serum is supplemented with 2 μ high titred anti-ADV mink serum. The outcome of the reactions is recorded on the left of the rows as positive (POS), negative (NEG) and false positive (FP)

RESULTS

The main part of the re-examined sera had a clear and consistent positive or negative serological reaction in both CIEP and RLIE. The autcome was 52 questionable sera that were retested by the additive CIEP. In 19 cases a double band was formed in the additive CIEP, i.e. the sample showed a false positive reaction and was consequently classified as negative. In the remaining 33 samples the weak antibody response was added to the line, i.e. the samples were positive. These 19 samples were selected among 1.2 x 10^6 serum samples from expected negative populations. They thus represent one sample in 63000 tested. The false positive reactions were usually negative in RLIE (16 out of 19 tested), but we have observed that RLIE is less specific and lipids or proteins can give non-specific

reactions.

DISCUSSION

Worldwide, millions of mink serum samples are screened for antibodies against ADV. The method used in routine diagnostic tests is CIEP as it is cheap and easy to perform. It is also a very specific test, with an estimated specificity of over 99.97% (Chriél 1990). In other words, less than three samples out of 10000 negative samles are wrongly judged as positive. Even so, we would like to make an even more specific retest on these samples.

False positive reactions, as assessed by the additive CIEP, are often found among sera from recently vaccinated animals. Further studies are in progress to determine which cellular antigens the mink react against.

In cases where the serological answers are inconsistent these are supplied with a histological examination of kidney and liver. But the histopathological changes usually appear after the seroconversion.

ACKNOWLEDGEMENTS

The technical skills of Ulla Christiansen are gratefully acknowledged. This study was supported by the Danish Fur Breeders' Association Research Foundation.

SUMMARY

The eradication of AD in Danish mink populations has now reached the stage where 75% of the mink farms are free or almost free of the disease. Routine diagnostic tests are still being carried out by counterimmunoelectrophoresis (CIEP), but here we report on the retesting of the animals that had a reaction in the routine testing.

The main part of the sera is consistently positive or negative in the serological testing by CIEP and Rocket line immunoelectrophoresis (RLIE) whereas some of the sera require a more specific test to distinguish whether the reaction is towards ADV antigens or cellular debris. To test the specificity of the antibodies, we have developed an additive CIEP. This system has been applied to 52 questionable sera, selected from among 1.2 million blood samples from negative animals. Nineteen revealed a false positive reaction and 33 indicated a weak but positive reaction. It is important *both* to find the weak positive reactors and to be able to point out the false positive samples.

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Early detection of Aleutian disease virus in mink by polymerase chain reaction

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Comparisons were made of counter-immunoelectrophoresis (CIEP) of serum samples with polymerase chain reaction (PCR) amplification of DNA extracted from peripheral blood cells for the diagnosis of Aleutian disease virus infection. Mink were infected with an intraperitoneal inoculation of 100 µl of a 10% spleen homogenate from an infected mink. At day 0 all mink were tested negative by PCR and CIEP. On day 3, two mink were positive by PCR. One of the mink was also tested positive by CIEP by day 3, but not by PCR. Seven days after inoculation, one mink was positive by PCR, but all five were tested negative by CIEP. At ten days, four mink were positive by PCR and all five were positive by CIEP. PCR was combined with Southern blot to increase the sensitivity of detection. This method was found to be too sensitive, in that nine out of ten mink found to be negative by CIEP were positive by PCR combined with Southern blot. Although the reagents used for the assay were not contaminated, the high numbers of false positives was probably due to contamination of samples. PCR may be combined with the CIEP test to allow a more sensitive detection of Aleutian disease virus in valuable breeding stock, but Southern blotting should not be combined with PCR because of the high number of false positive results.

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Aleutian disease in mink is caused by a parvovirus and results in persistent infection, a strong immune response to the virus, and deposition of immune complexes with resulting tissue destruction (Porter 1986; Aasted 1985). Diagnosis of the infection is generally made by counter-immunoelectrophoresis (CIEP) of serum and viral antigens (Cho & Ingram 1973). Aleutian disease continues to be a problem in many areas despite widespread use of CIEP for detection of carriers. It is possibile that mink can be infected with virus before an immune response occurs which could be detected by CIEP. To investigate this possibility, a comparison was made between CIEP and detection of viral DNA by polymerase chain reaction in experimentally infected mink.

MATERIALS AND METHODS

Animals

Five dark mink, tested negative by CIEP, were transferred to cages adjacent to experimentally infected mink on a commercial mink ranch. The experimental infection was achieved by intraperiteonal injection of 2 ml of a 10% spleen homogenate in phosphate-buffered saline from an infected mink. Blood samples for CIEP and PCR testing were obtained by toenail clip. Samples were taken one day before inoculation, and on days 3, 7 and 10 after inoculation.

For PCR combined with Southern blotting, 15 CIEP-negative mink from the Fur Breeders' Cooperative in Salt Lake City, Utah were used.

Preparation of samples

Two millilitres of blood was obtained in tubes containing heparin. White blood cells were purified from whole blood on Ficoll-Hypaque gradients (Sigma chemical company). The cells were washed twice with 5 ml phosphate buffered saline (PBS) after which 465 μl of digestion buffer (20 mM Tris HCl, 20 mM EDTA, 0.5% SDS, pH 8.0) was added to each sample. For positive control samples, feline Crandall kidney cells infected with Aleutian disease virus were grown in Dulbecco's modified Eagle's medium with 10% fetal calf serum in 150 cm^2 flasks at 30°C. The cells were separated from the flasks with trypsin, washed three times in phosphate-buffered saline, pH 7.2, and resuspended in 465 μl digestion buffer. Negative control samples were derived from feline Crandall kidney cells, treated similarly to the positive controls.

DNA extraction

To blood and tissue culture cells (in digestion buffer) 35 μl of a 10 mg/ml stock solution of proteinase K was added, and the samples digested overnight in a 55°C waterbath. Samples were extracted with phenol, then with 25:24:1 phenol: chloroform: isoamyl alcohol and, finally, with 25:1 chloroform:isoamyl alcohol. All extractions were carried out using aerosol-resistant pipette tips. Fifty microlitres of 0.5 M NaCl and one millilitre of 100% ethanol were added and the samples centrifuged at 12,000 g for 30 min at room temperature. Samples were washed once with 70% ethanol and suspended in 100 μl of 10 mM Tris-HCl, 1 mM EDTA, pH 8.0.

Primers used

Primers were selected from the 5' end of the viral genome, in a highly conserved non-translated region (Bloom et al. 1988). Nucleotide positions of the primers are given in parentheses.

5' primer: GATGAGCAGAGGAGACTGCA (224-243)
3' primer: AGTAACCTAAGCAACAGTGA (583-602)

Using these primers, the band expected should be 378 base pairs.

Polymerase chain reaction

Reactions consisted of the following components: 49.5 μl double distilled water, 0.25 μM concentration of each primer, 0.2 mM of all four deoxynucleotide triphosphates, 50 mM KCL, 10 mM Tris-HCl (pH 8.8) 1.5 mM MgCl2, 0.2% Triton X-100, 2.5 units Taq DNA polymerase (Promega) and 0.5-1 μg DNA samples (total volume of 100 μl). All reagents were added using aerosol-resistant pipette tips in a fume hood. The reactions were overlaid with 100 μl mineral oil. The reagent control contained autoclaved water in place of a DNA sample. For each gel run, there was a positive control, negative control and reagent control. PCR amplification was carried out using a thermal cycler in the following manner:

Denaturation:	95°C for 30 sec
Primer annealing:	55°C for 30 sec
Primer extension:	1 min 30 sec at 72°C

Thirty cycles were performed.

After amplification, samples were analyzed by electrophoresis on a 1.5% agarose gel in TAE buffer (Ausubel 1987). Bands were visualized with ultraviolet light and photographed using polaroid film.

Southern blot

DNA from agarose gels was transferred to nylon membranes using a pressure blotter apparatus. Hybridization with a probe internal to the primers used for PCR amplification was carried out as described previously (Ausubel et al. 1987). This probe was made by amplifying a 186 base-pair fragment internal to the primers used for PCR amplification of samples, from Aleutian virus DNA. The probe was labeled with alpha-32P dCTP by random-primed labeling.

Counter-immune electrophoresis

This was performed using commercially available reagents (United vaccines, Madison, Wisconsin) according to the manufacturer's instructions. The samples were run on an 80 x 100 mm agarose gel.

RESULTS

The results of the CIEP and PCR tests for experimentally inoculated mink are given in Table 1.

All five mink were negative before inoculation by CIEP. One tested positive on day 3, the others remained negative on that day. On day 7, all five were tested negative and on day 10 all five were tested were positive.

By PCR, all five mink were negative before inoculation, two tested positive on day 3, one tested positive on day 7 and four tested positive on day 10.

To confirm that the bands seen by means of ethidium bromide staining represented Aleutian disease sequences, Southern blot was done on PCR-amplified DNA from mink leukocytes of five mink naturally infected with Aleutian disease virus. Bands of the proper

size were produced in all of these samples, and all were hybridized with the Aleutian virus probe. Southern blot was also performed on samples taken at earlier times when mink were tested negative by CIEP. Although none of the mink were positive by PCR, all of the samples at day 0 exhibited bands of the proper size that hybridized with the probe when Southern blotting was performed. Other CIEP-negative mink were then examined for the presence of Aleutian DNA by PCR and Southern blot. Ethidium bromide staining of PCR products on agarose gels revealed no bands. However, Southern blotting revealed bands of specific size in 9 out of 10 CIEP-negative mink (data not shown).

Table 1. Comparison of CIEP and PCR tests for detection of Aleutian disease virus infection

		Day after infection			
		0	3	7	10
Mink No.					
8	PCR	-	+	-	+
	CIEP	-	-	-	+
10	PCR	-	-	-	+
	CIEP	-	-	-	+
11	PCR	-	-	+	+
	CIEP	-	-	-	+
13	PCR	-	+	-	+
	CIEP	-	-	-	+
14	PCR	-	-	-	-
	CIEP	-	+	-	+

DISCUSSION

The results with experimentally inoculated mink indicate that in the early stages of infection PCR testing results in earlier detection of Aleutian disease virus in mink than with CIEP testing. Under the conditions described here, the time between detection by PCR and detection by CIEP can be as long as 4-7 days. This is in contrast to infections with other agents that cause persistent infection, such as retroviruses, in which this period can be weeks or months. Under natural conditions, however, this interval may be longer.

The PCR results were not consistent in that mink tested positive on day 3 were not positive on day 7. This may have been due to the amount of DNA present in the samples. However, the optical density readings indicated that there were about 6-12 μg per sample, and the optical density reading for sample 11, which was positive, was less than that for some samples which were negative. Another possibility is that the kinetics of viral replication differed in the animals, and the amount of viral DNA present was below detectable levels at certain times.

Animal No. 14 was positive by CIEP on day 3, negative on day 7 and positive on day

10. The reasons for this are not clear, but this pattern is not unheard of (Herbert Kammer, United Vaccines, personal communication). This animal also was not positive by PCR on day 10, when all the other animals were positive. It was positive 20 days after infection (data not shown), possibly indicating a slower replication of the virus in this animal.

Combining Southern blot with PCR testing resulted in large numbers of false positives. Although the possibility cannot be ruled out that these mink had a low level of virus infection with no detectable antibody present, it is more likely that these results are due to contamination with viral DNA from environmental or other sources. However, we can rule out contamination of the reagents, since the reagent controls were negative by Southern blot.

The results indicate that mink can be infected with the virus for a period of time and not have any detectable antibody. With experimental inoculation, this time period is very short. This period may be longer under conditions of natural transmission. Although detection of infection by PCR quite often occurred before the appearance of antibody, this was not always the case.

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Enzyme immunoassay of antibodies against Aleutian disease virus

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Two highly specific and sensitive procedures of enzyme immunoassay were developed for detection of antibodies against Aleutian disease virus. The ELISA procedure on 96-well microplates was used for screening of mouse hybridomas; dot immunoassay was used for testing mink sera applied onto nitrocellulose. The ELISA and dot assay were shown to be 1000 and 100 times, more sensitive, respectively, than countercurrent electrophoresis. Dot assay revealed 19% more positive samples than counterimmunoelectrophoresis in comparative analysis of 1250 mink from farms affected by Aleutian disease. Dot immunoassay was found to be comparatively less time- and labor-consuming and may be regarded as a useful diagnostic test.

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Aleutian disease (AD) is a lymphoproliferative disease induced by the AD parvovirus (ADV) and causes decreased fertility in farm-bred mink. To study and diagnose AD, highly specific and sensitive tests detecting the antibodies to its viral agent are needed. The diagnostic methods developed for this purpose range from relatively sensitive counter-immunoelectrophoresis (CIEP) to the highly sensitive radioimmunoassay (Aasted & Bloom 1983; Cho & Ingram 1973). In recent years immunoenzyme assay has been widely used for detection of numerous antigens and antibodies. Our aim was to apply the enzyme-linked immunosorbent assay (ELISA) for screening mouse hybridomas that produce monoclonal antibodies (mAbs) against ADV. Another aim was to develop dot enzyme immunoassay on nitrocellulose sheets as an alternative to CIEP as a diagnostic tool of AD.

MATERIALS AND METHODS

Virus antigen

In vitro-produced antigen was prepared in cultures of a feline renal cell line infected with the ADV-G isolate of the Utah1 strain of ADV. The cell line and ADV-G were kindly provided by Prof. B. Aasted. The viral antigen was purified as previously described (Wright & Wilkie 1982; Aasted 1985). The concentration of standard preparation of ADV antigen (0.5 optical units/ml at 280 nm and 1:256 titer in CIEP) was arbitrarily taken as 0.5 mg/ml.

Hybridoma production

BALB/c mice were immunized with ADV antigen and the spleen cells were fused with myeloma NS0/1 as described by Kipps & Herzenberg (1986). Hybridoma supernatants were tested by ELISA and CIEP. Hybridoma clones were grown as ascitic tumors in pristine-primed BALB/c mice.

Elisa

96-well microtiter plates were coated by standard ADV antigen at a dilution of 1:500 in 0.1 M $NaHCO_3$ (100 l per well). After incubation overnight, the plates were washed in O.1 M $NaHCO_3$ containing 0.05 % Tween-20 (solution A). Hybridoma culture supernatants or sera in serial dilutions were added to the wells and incubated for 1 h, thereafter the plates were washed again in solution A. Rabbit anti-mouse IgG conjugated with horseradish peroxidase in solution A was used at the second stage. In the case of mink sera testing, peroxidase conjugated protein A was used. After 1 h incubation, the plates were washed anew, and each well was supplemented with a substrate solution containing 0.05% o-phenylenediamine. The reaction was stopped and optical density was read at 495 nm.

Dot immunoassay (DIA)

Mink serum samples were applied onto nitrocellulose sheets. After drying, the unbound surface of the nitrocellulose was blocked, and the membrane was incubated in 0.1 M $NaHCO_3$ containing 0.1% Triton X-100 (solution B) andperoxidase labeled ADV-G. After 1 h incubation, the nitrocellulose was washed in solution B and stained with a substrate buffer containing 3.3'-diaminobenzidine tetrahydrochloride.

Immunoblotting assay

SDS-polyacrylamide gel electrophoresis of crude preparation of ADV-G and electrotransfer onto nitrocellulose sheets were performed by standard techniques (Laemmli 1970; Towbin & Gordon 1984). After blocking with 20% fetal calf serum, the sheets were cut into strips which were then incubated for 1 h with hybridoma supernatants or with mink sera diluted in solution B. The strips were washed and incubated with peroxidase conjugated rabbit anti-mouse IgG (in the case of mAbs testing) or peroxidase conjugated protein A (in the case of mink sera testing). Enzyme activity was visualized as described above.

RESULTS AND DISCUSSION

The sensitivity of the screening method is of importance in raising mAbs to different antigens. The concentration of mAbs in hybridoma supernatants varies, as a rule, from 0.1 to 5 μkg/ml. We used the standard sandwich scheme of ELISA with peroxidase labeled secondary antibodies for screening the hybridomas producing mAbs to ADV. The serum of mouse immunized with ADV-G (the titer in CIEP - 1:32) was used for building the calibration curve (Fig. 1A). In ELISA, the minimum reliably detectable dilution of this serum was 1:64,000. In the first screening 20 hybrids were detected whose supernatants reacted positively in ELISA. Three hybrids were taken for subsequent cloning. The clones were propagated, and the antibodies they produced were assayed by immunoblotting. Fig.

2 shows that two mAbs (AD1 and AD10) recognize both viral proteins p75 and p85 from ADV-G.

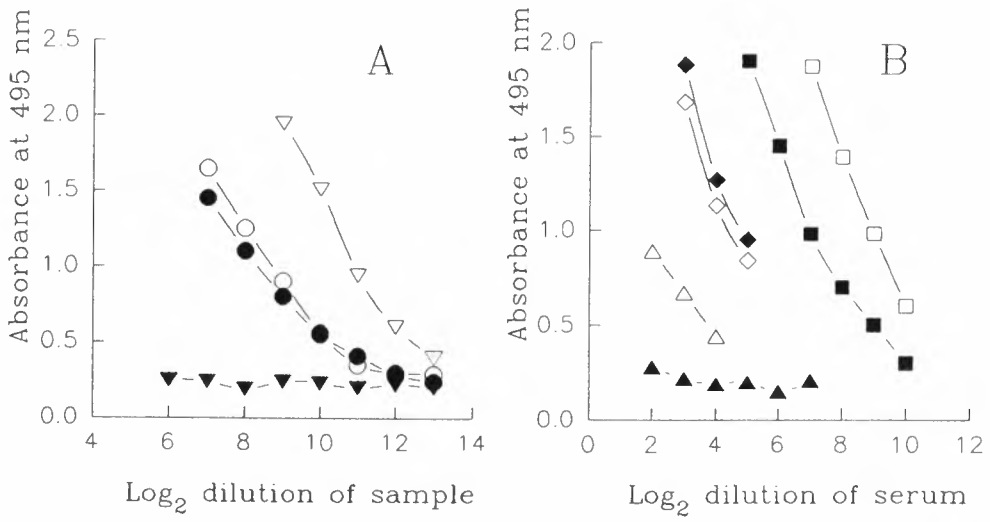

Fig. 1. ELISA binding curves of two hybridoma ascites: AD1 (○) and AD7 (●); prediluted (1:20) mouse immune serum (▽); mouse non-immune serum (▼); three mink sera negative in CIEP but positive in DIA (△, ◇, ◆); two mink sera positive in CIEP (titer 1:32 and 1:16 respectively) pre-diluted 1:20 in the given ELISA (□ and ■)

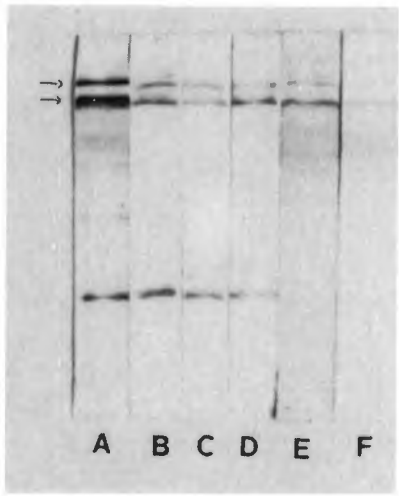

Fig. 2. Immunoblotting assay. The strips were developed with the strong AD-positive serum (diluted 20 times) (B, C, D); mAb AD1 (E); mAb AD10 (F). Arrows indicate the position of viral proteins p75 and p85

It should be noted that culture supernatants of the given clones did not react with ADV in CIEP. However, the ascitic fluids of clones AD1 and AD4 formed a precipitin line in this assay. Judging by the present results, ELISA is superior to CIEP in immunoscreening because it permits the chaice at the early stage of the cloning procedure of those hybridomas which eluded detection in CIEP.

The same ELISA scheme was used to detect AD-specific antibodies in the sera of infected minks and was proved to be 250-1000 times more sensitive than CIEP with different sera.

Dia

Twenty samples of mink AD-positive sera were titrated concomitantly by CIEP and the developed DIA procedure. The sensi-

tivity of DIA exceeded that of CIEP by 64-128 times with different sera.

Comparative tests of 1250 serum samples of mink from an AD-infected farm by were performed CIEP and DIA. The tests demonstrated that with the DIA procedure about 19% more infected individuals are detected than with CIEP (Table 1). Moreover, the DIA procedure was found to be comparatively less time- and labor-consuming. A fragment of the results for mink sera testing obtained with DIA can be seen in Fig. 3.

Table 1. Analysis of 1250 different mink serum samples in CIEP and DIA

Number of sera	DIA		CIEP		Positive in DIA but negative in CIEP	Positive in CIEP but negative in DIA
	Pos.	Neg.	Pos.	Neg.		
1250	100	1150	82	1168	20	2

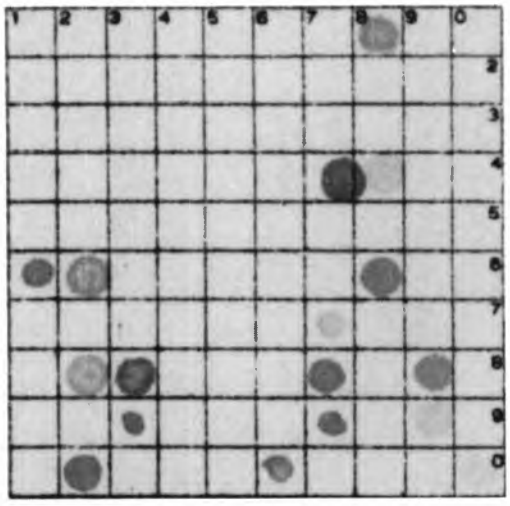

Fig. 3. Fragment of testing of mink sera using DIA; 100 samples were applied to nitrocellulose sheets and developed as described in Material and Methods. AD-positive sera are revealed as colored dots of different intensities

Several mink samples which were tested negative with CIEP and positive with DIA were retested by the ELISA procedure as well as by immunoblotting assay. The specificity of the positive reaction was confirmed in all the cases studied. With ELISA, these samples yielded titration curves with slopes similar to those of calibration curves (Fig. 1B). With immunoblotting of the ADV-G antigen, they revealed a typical pattern of virus proteins (Fig. 2).

Thus, being highly sensitive and specific, ELISA and DIA of antibodies against ADV can be utilized as discriminative tools in studies of the different aspects of AD. ELISA is preferred in choice of hybridoma production and in other cases demanding a very high level of sensitivity of analysis. The DIA method can be used for mass diagnostic purposes as an alternative to the widely used CIEP in eradication programs.

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Hereditary spongy degeneration of white matter in silver foxes

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Hereditary spongy degeneration of white matter in silver foxes is a rare genetic disorder of the central nervous system. We report on continued breeding studies of the disease and correlate pathomorphologic lesions in the CNS with clinical signs at different stages of the disease process. Fifty-three silver fox cubs were born in 14 litters, and 29 of these developed spongy degeneration of white matter. The distribution of affected cubs within litters strengthens our previous assumption of an autosomal recessive mode of transmission. Twelve affected cubs were allowed to live beyond the period of maximal disability, and they all showed marked clinical improvement after five months of age. Vacuolation of the myelin-forming oligodendrocyte is an important early lesion in this disease. The onset of clinical signs can be correlated with the development of marked vacuolation of myelin sheaths, expansion of extracellular spaces and demyelination, while the clinical improvement in older foxes coincided with resolution of the vacuolation and remyelination. In conclusion, the present condition in silver foxes provides a model for detailed investigation of a naturally occurring myelin disease.

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Spongy degeneration of white matter in silver foxes is a genetic disease of the central nervous system (CNS). The first cases were discovered in 1985 and, so far, the disease does not represent a significant problem for the Norwegian fur industry. The disease may, however, prove to be a useful comparative model of naturally occurring myelin disease.

After the first cases were observed on a farm in central Norway, a number of affected foxes and their littermates were transferred to the Research Farm for Furbearing Animals in Heggedal. These foxes were used to establish the mode of inheritance of spongy degeneration of white matter and also to provide material for pathomorphologic studies.

Spongy degeneration of white matter in silver foxes is characterized morphologically by a symmetrically distributed vacuolation and myelin loss in the deep white matter of the brain and in the subpial white matter of the spinal cord, particularly of the dorsal and ventral funiculi. Ultrastructurally, the disease shows vacuolation of the myelin-forming oligodendrocyte, vacuolation of myelin sheaths, extracellular expansion and demyelination (Hagen et al., 1990)

Clinical signs, mainly pelvic limb weakness and ataxia/paresis, occur between 9 and 15 weeks of age and progress over the following 4 weeks. After 5-6 months of age, marked clinical improvement has been observed (Hagen & Bjerkås 1991).

The aim of the present report is to present results of continued breeding studies of this disease (initial studies were presented by Hagen & Bjerkås 1991) and correlate the pathomorphologic lesions with clinical signs at different stages of the disease process.

MATERIALS AND METHODS

Breeding studies

Fifty-three live silver fox cubs from 14 litters were born in controlled breeding studies at the Research Farm for Furbearing Animals, Heggedal. Of these, 24 developed clinical signs of spongy degeneration of white matter (diagnosis confirmed histologically in 17), and 5 were killed prior to the onset of clinical signs and diagnosed histologically as having spongy degeneration of white matter. The litters were born from different combinations of normal foxes, proven or suspected carriers and clinically affected foxes. Twelve affected foxes were allowed to live beyond the period of maximal disability and they all showed marked clinical improvement.

Morphological studies

This study comprises material from 6 silver foxes: 3 homozygotes that were examined before the onset of clinical signs, and 3 clinically affected animals (Table 1). Foxes nos. 4-6 have been described as cases nos. 1, 3 and 4 in a previous publication (Hagen et al. 1990).

Table 1. Case material. Pathomorphologic studies

Fox No.	Age when killed	Clinical condition
1	23 days	Normal
2	41 days	Normal
3	64 days	Normal
4	98 days	Paraparesis of 1 week duration
5	7 1/2 months	Severe pelvic limb ataxia, onset at 11 weeks of age
6	18 months	Normal after 1 year of age, recovered

Foxes nos. 1, 2, 3 and 5 were perfused via the left ventricle with 4% glutaraldehyde in 0.1 M phosphate-buffer, pH 7.2. Foxes nos. 4 and 6 were perfused via the left ventricle with a modified Karnovsky fixative (0.9% glutaraldehyde, 0.7% paraformaldehyde in 0.14 M sodium cacodylate, pH 7.2).

Paraffin-embedded tissue from all levels of the CNS was sectioned and stained with haematoxylin and eosin (HE) and used to define histopathologic lesions. The electron microscopic studies were performed on spinal cord tissue. Quadrants of spinal cord tissue were cut from approximately 1 mm thick transverse slices of the upper cervical cord. The specimens were post-fixed in osmium tetroxide and embedded in Epon or Taab resin. Semi-thin sections (1μm thick) were stained with toluidine blue and used to define

histopathologic changes and also to select suitable areas for examination in the electron microscope. Ultra-thin sections from selected areas were stained with lead citrate and uranyl acetate, and examined in an electron microscope.

RESULTS

Breeding studies

The distribution of affected offspring from different parental combinations is summarized in Table 2. The mating of a proven carrier, i.e. had earlier given birth to cubs with spongy degeneration of white matter, to a normal unrelated male did not produce any cubs that developed the disease. However, the mating of a proven carrier to an affected male resulted in a varying proportion (total 19 of 32) of cubs developing spongy degeneration of white matter. When both parents had shown clinical signs, all offspring developed the disease, and in this study that was 7 cubs from 3 different litters. Both sexes were equally affected. The distribution of affected cubs is consistent with an autosomal recessive mode of transmission.

Table 2. Distribution of foxes with spongy degeneration in litters of different parental combinations

Parental combination (male - female)	No. of litters	No. of live cubs	No. of affected cubs
Affected - affected	3	7	7
Affected - proven carrier[a]	7	32	19
Affected - suspected carrier[b]	2	7	3
Suspected carrier- suspected carrier	1	4	0
Normal[c] - proven carrier	1	3	0

a Proven carrier= clinically normal vixens that had produced affected offspring before the breeding study.
b Suspected carrier= clinically normal foxes which had originated from affected litters.
c Normal= unrelated, clinically normal male fox.

Morphological studies

A clear progression of lesions in the white matter was detected with increasing age (Table 3). Fox no. 1 was considered normal in HE-stained sections and showed less than three vacuoles per high power view in semi-thin sections of the upper cervical cord. Vacuolation became increasingly prominent in foxes nos. 2-4. In foxes nos. 1 and 2, the vacuoles were almost exclusively located to the cytoplasm of oligodendrocytes, while in fox no. 3 some, and in fox no. 4 numerous vacuolated myelin sheaths were present together with vacuolated oligodendrocytes. Extracellular expansion was prominent, and evidence of partial as well as internodal demyelination was found in fox no. 4. Fox no. 5 showed less intense vacuolation, and in addition to demyelination, signs of remyelination were found. In fox no. 6, the vacuolation was largely resolved and numerous remyelinated axons were present in the subpial white matter.

Table 3. Patho-morphologic lesions in the ventral and dorsal funiculi of the upper cervical cord.

Fox No.	Vac. of oligodendr.	Vac. of myelin	Demyelination	Remyelination
1	Mild	N.D.	N.D.	N.D.
2	Mild	N.D.	N.D.	N.D.
3	Moderate	Mild	N.D.	N.D.
4	Severe	Severe	Yes	N.D.
5	Moderate	Moderate	Yes	Yes
6	N.D.	Mild	N.D.	Yes

N.D. = not detected.

The spinal cord lesions were always confined to the subpial white matter. Vacuoles were found with decreasing frequency in the funiculus cuneatus, the ventral funiculus and the funiculus gracilis. Vacuolation of lateral tracts was only seen in fox no. 4.

DISCUSSION

The present study has reinforced our previous assumption (Hagen & Bjerkås 1991) of an autosomal recessive transmission of spongy degeneration of white matter in silver foxes. With knowledge of the mode of inheritance, it should be possible to maintain a low incidence of this disease in the population of silver foxes.

Spongy degeneration of white matter in silver foxes probably has its greatest relevance as a natural model of myelin disease. In this context, the sequence of events in the disease process is intriguing. An early morphologic change was cytoplasmic vacuolation of the myelin-forming oligodendrocyte. Oligodendrocyte vacuolation preceded clinical signs by several weeks, and the onset of clinical signs could be correlated morphologically with the occurrence of marked vacuolation of myelin sheaths, expansion of extracellular spaces and demyelination. These findings suggest that an initial impairment of oligodendrocyte metabolism disturbs oligodendrocyte-myelin interaction. The necessity of survival of oligodendrocytes for maintenance of myelin sheaths is well established (Raine 1985). In the present disease, it would appear that myelin sheath vacuolation and eventual demyelination are consequences of suboptimal function of oligodendrocytes with loss of ability to maintain myelin sheath stability.

In contrast to most other naturally occurring diseases with myelin sheath vacuolation, which have been examined only at certain stages of the disease process or in a limited number of cases, the present disease model in foxes enables detailed studies of sequential events in the disease process. In other diseases, the relationship between myelin sheath vacuolation and possible glial cell involvement has largely remained obscure. The finding of early oligodendrocyte lesions in the present condition may therefore be of relevance to other natural myelin diseases. Furthermore, the present condition is a useful supplement to the experimental gliotoxic conditions (reviewed by Blakemore et al. 1983), where it has, in most instances, been an open question whether myelin sheath vacuolation resulted from oligodendrocyte damage, or whether the two lesions were generated independently.

The clinical improvement in older foxes could be correlated morphologically with resolution of the vacuolation and remyelination. Although remyelination is a normal consequence of demyelination that has been frequently documented in experimental situations, extensive remyelination and the restoration of normal clinical function appears to be a rarity in a naturally occurring disease.

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Distemper in mink in the NW of Spain

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An episode of distemper was diagnosed between July and December in a group of mink farms near one another in NW of Spain. The farms have a high level of Aleutian disease infection. Thirteen animals died, Wild and Standard varities, with a clinical history of acute distress, thickening of the foot pads and hyperkeratosis. The minks were autopsied, and samples from different tissues were collected according to the routine for histopathological studies. Interstitial pneumonia, hyperkeratosis and inclusion bodies in the epithelia and nervous tissue were the most important findings. An indirect immunoperoxidase technique using a monoclonal antibody against the nucleocápside of the distemper virus demonstrated the presence of a viric antigen, as described for the systemic phase of the disease. All animals were positive to the Aleutian disease CEIP test and two animals had lesions of progressive Aleutian disease.

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Distemper virus (DV) of the genus Morbillivirus is a highly contagious pathogen which causes acute systemic (Appel 1969) or chronic (Yamanouchi 1980) infections in numerous members of the Carnivora. DV infection in mink was first described in 1930 (Apple 1972) and later confirmed by many authors (Bindrich et al. 1959; Nicolas et al. 1989; Blixenkrone-Möller 1989).

The morbidity and mortality caused by DV infection in mink vary considerably depending on the age of the mink, with mortality droping significantly at around eight weeks of age (Hansen & Lund 1976); the variety, pastel minks are more susceptible to distemper; the severity of the attack of DV and the state of the immune system of the animals in relation to the Aleutian disease virus infection (Hansen & Lund 1976).

Vaccination against DV in mink is a common protection measure in farms. Kits born of unvaccinated mothers can be protected at the age of four weeks by vaccination, whereas kits born of vaccinated mothers must be treated at the age of 11 weeks because maternal antibodies can interfere with the vaccination (Hansen & Lund 1972).

The present report describes the pathological findings from an epizootic distemper outbreak in 1987 observed in mink farms in NW Spain.

MATERIAL AND METHODS

Animals

Thirteen wild mink, aged between five and six months were included in our diagnostic

study. All the animals came from the same farm, which was located in a high density area of small mink farms. The farm was correctly isolated and its animals vaccinated following a routine prophylaxis programme.

Pathological methods

A systemic necropsy was carried out in the animals, with samples taken of lung, trachea,third eyelid, stomach, intestine, spleen, lymph nodes, liver, kidney, urinary bladder, and brain. The samples were fixed in 10% formalin (24-48 h), embedded in paraplast following the usual standard histopathological techniques. Sections, about 5 μm thick, were stained with haematoxilin and eosin, Shorr-S3, and Giemsa methods.

Immunohistochemical methods

The antiserum used to label the virus was a monoclonal antiserum against the DV nucleocapsid (dilution 1/3200) (Orvell et al. 1985). The immunolabelling of the samples was made in 5 μm thick paraffin sections following the avidin-biotin-peroxidase-complex (ABC) technique. The reagents used in this technique were taken from a commercially available ABC kit. Tissue from normal mink was used as a negative control and tissue of foxes and dogs, previously diagnosed as having systemic distemper, was used as a positive control.

Samples of blood were taken from the dead animals in order to carry out the CEIP test against the Aleutian disease virus and to evaluate the level of tyrosine by means of the HPLC.

RESULTS

Clinical and epidemiological aspects

The disease began in July in farms where animals had not been vaccinated against DV in this area, and it rapidly spread to neighbouring farms (whether their animals had been vaccinated or not), because the enclosures were inadequately fenced-off and because of the interchanging of material amongst farmers (gloves, cages,etc.).

We identified the disease from July to December in the farms of this area, in which the levels of infection of the Aleutian disease virus were very high. The animals suffered severe respiratory distress, serious or mucopurulent nasal discharges, thickness and hyperkeratosis of the foot pads. No nervous or digestive signs of alteration could be observed. The levels of tyrosine in the blood were between 29 and 32 μmol/l. All the mink were found to be positive to the CEIP test against Aleutian disease.

Necropsy findings

The most important lesions observed at necropsy were identified as diffuse interstitial pneumonia with secondary emphysema of the lungs, hyperaemia and catarrhal swelling of the upper air tracts, as well as the hyperkeratosis referred to above.

Two animals presented small kidneys with whitish foci, and irregularities in the surface of the liver.

Histopathological findings

All the mink had similar microscopic lesions in the respiratory system. The alveolar septa of the lungs were thickened by infiltration of mononuclear cells, type II pneumocytes had proliferated and some alveolar lumina contained serofibrinous exudate. Infiltration of alveolar spaces and bronchi by neutrophiles was observed, too, in several cases. The epithelium of the trachea, bronchi, and bronchioles was necrotic and contained from scant to numerous acidophilic inclusions, generally in the cytoplasm and occasionally in the nucleus.

Lesions of moderate demyelinizing encephalitis were observed in the brain, especially around the fourth ventricule. Small acidophilic inclusions were present in the cytoplasm of glia cells and neurons. Ependymal cells were necrotic in some areas and contained acidophilic inclusions. No syncytral cells could be found.

Lymph nodes and spleen presented lesions of lymphocyte depletion. In many ephithelial cells, such as those of the urinary bladder, stomach and small intestine, acidophilic cytoplasmic or nuclear inclusions were identified.

Two animals had lesions of progressive Aleutian disease consistent with plasmocytosis in the spleen, lymph nodes, liver and kidney, bile duct proliferation and diffuse glomerulonephritis, principally.

Immunocytochemical findings

The ABC technique revealed DV antigen in lungs and respiratory tracts, spleen, lymph nodes, epithelia of the urinary bladder, renal pelvis (Fig. 1), third eyelid (Fig. 2.), gastric and small intestine mucosa, and brain as well as in tissue macrophages and monocytes. The positive reaction was characterized by a uniform brown granular cytoplasmic staining or a more intense staining of the inclusions. The intensity of staining varied from mild to intense.

The positive peroxidase staining in lungs was mainly found in macrophages, interstitial cells, and alveolar cells; bronchiolar and bronchuial epithelial cells were clearly positive to the technique. Alveolar or bronchial exudates stained positively, too.

In the brain, the DV antigen was demonstrated in astrocytes, microglia, neurons and epidymal cells. Granular cells in the cerebellar cortex were also positive.

DISCUSSION

An epizootic distemper in mink farms was diagnosed by histopathological and immunohistochemical methods. A differential diagnosis of tyrosinaemia was made by means of blood analysis. The disease was produced in DV-vaccinated animals that were CEIP-positive against Aleutian disease.

In several reports (Hansen 1971; Hansen & Lund 1972) the protecting capacity of DV vaccines in minks was studied. The protection is good when the vaccination is performed before exposure to DV and in kits at an age of 16-20 days from vaccinated females. The efficiency of DV vaccination is lower when the animals are infected by the Aleutian disease virus and the mortality rate is higher. The animals studied in this report came from a farm highly infected by the Aleutian disease virus with an usual programme of vaccination; it seems that in this case the potency of the vaccine was weaker than expected.

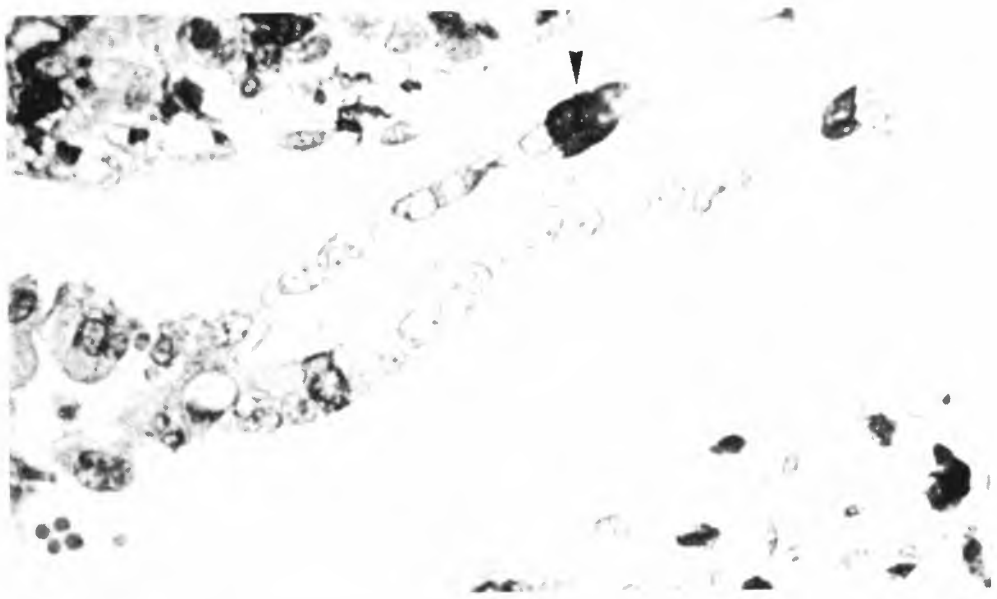

Fig. 1. Avidin-biotin peroxidase positive material in a cell of the renal pelvis epithemlium. Mink. 400X

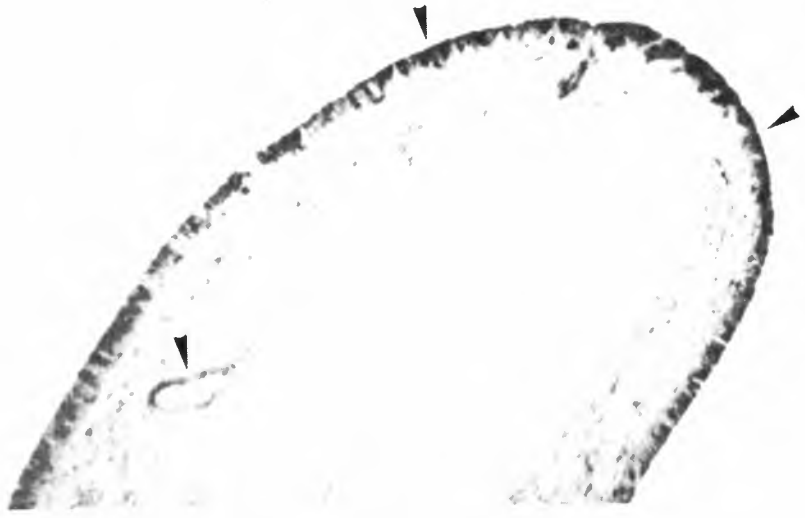

Fig. 2. Dense positive material to the avidin-biotin peroxidase immunolabelling technique in the epithelium of the third eyelid (▸) and in the endothelium of capillary vessels. Mink. 200X

Clinically, DV infections in minks could be confused with tyrosinaemia when the disease affects kits from five to six weeks of age. The differential diagnosis in the present dossier was made according to the age of the animals (between five and six months) and confirmed by the results of the blood analysis.

The lesions observed in this case were similar to those described in mink and other animals like dogs (Appel & Gillespie 1972) and foxes (Nieto et al. 1990). In the analyzed animals the most uniform lesion observed was interstitial pneumonia and the presence of inclusion bodies in epithelial cells and macrophages. Since it is usual for pneumonia to occur in systemic disease, it has been suggested that circulating antibodies do not prevent the replication of DV in the lungs. Secondary infections were frequently described with CD infections, especially those linked with other viral antigens such as adenovirus, para-influenza, herpes or reo-virus or bacterial infections such as *B. bronchiseptica*, the pathological findings in these cases being those of an alveolitis (adenovirus-DV pneumonia), or purulent bronchopneumonia (secondary bacterial infections, Miry et al. 1983) .

Non-suppurative encephalomyelitis is the most common brain lesion of DV infection in dogs (Vandelvelde et al. 1985; Palmer et al. 1990). Vandelvelde et al. (1981) classified the distemper lesions as acute, subacute or chronic; according that the lesions observed in our series of mink were acute, characterized by several degrees of demyelination without infiltration of inflammatory cells (in agreement with Summers et al. 1984ab).

Small or large acidophilic intracytoplasmic or nuclear inclusions constitute one of the most important lesions used to diagnose distemper infections in Carnivora and other species such as seals (Osterhaus 1988) or dolphins (Domingo et al. 1990) affected by Morbillivirus. Inclusions were found in dogs with acute and subacute distemper and a lower number in chronic lesions (Vandelvelde et al. 1981). Inclusion bodies were easily identified in our study on mink, whereas this observation is not so definitive that a sure diagnosis can be made. Different artefacts, phagocytosis images, necrosis of the epithelial cells or other viral infections, such as herpes virus or rabies could produce similar lesions (Wisnicky & Wipf 1942; Dagle et al. 1979), moreover when inclusion bodies are scarce, histological sections must be studied for a long time in order to identify them (Palmer et al. 1990).

The use of immunohistochemical techniques to identify the DV antigen in the cells has been demonstrated as a better method for distemper diagnosis than the identification of inclusion bodies (Ducatelle et al. 1980; Miry et al. 1983; Nieto et al. 1987; Nieto et al. 1990; Palmer et al. 1990). The detection of DV antigen in the cells and not of inclusion bodies could be explained by the time needed to develop them or by their disappearance in chronic lesions. In our series, the positive cases to DV antigen and those negative to inclusion bodies were classified as hyperacute/acute DV infection.

The use of immunohistochemical techniques has some limitations in the diagnosis of distemper in vaccinated animals, because there is a possibility of labelling the antigen used in the vaccine. Blixenkrone-Moller (1989) observed that in minks vaccinated with an attenuated vaccine strain of canine DV, the viral antigen was observed in the lymphoid system 6 to 12 days after vaccination and not in any other locations like the brain or epithelia. A gradual disappearance of the DV antigen has been reported in dogs with a history of illness of 60 days or more (Fairchild et al. 1971). In our experience all the animals were vaccinated but as the vaccination had been inoculated some time previously, there was no problem.

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Maternal immunity in mink kits to mink virus enteritis and distemper

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Studies were carried out to investigate mink kits from females which were revaccinated with a distemper and MEV-containing vaccine just prior to breeding and/or experienced a mink virus entritis (MEV) field exposure. Animals from two different farms were tested. The focus on the first was MEV and on the second, distemper. Serological data were collected in addition to challenge data. For both agents, based on challenge data, a successful vaccination could be given when the kits were 10 weeks of age. One hundred percent of the unvaccinated kits were susceptible to distemper infection at 16 weeks and to MEV infection at 12 weeks of age.

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Immediately after birth, mink kits receive a full complement of their mother's antibodies in the colostrum. These antibodies protect them from disease before their own immune system is competent. The antibodies are cleared as any other foreign protein. The rate of clearance is relatively constant and the duration is contingent upon the initial quantity of antibody received from the mother.

Ideally, the kit should receive enough maternal antibody to protect it from specific infections until its own immune system is fully competent, at which time a vaccination should be given. If a vaccination is given while the kit is plotected by maternal antibodies, these may react with the vaccine antigen. This will reduce the effective amount of antigen available to the kit, potentially below the dose needed to stimulate protective immunity.

This phenomenon has been documented for various agents in other species. (See Pollock 1982; Acree 1983; Macartney 1988) Studies have been conducted on maternal antibodies to mink enteritis virus (MEV) by Gorham et al.(1987). The present investigations repeat some of these studies and include comparable information on distemper.

PURPOSE

The aims of the studies on kits whose mothers experienced a field exposure and/or were revaccinated just prior to breeding were:

1. To determine the period during which the kit is protected by maternal antibody from

infection by endemic MEV or distemper virus (The Challenge Study).

2. To determine the age at which the kit may be successfully vaccinated against MEV and distemper (The Vaccination Study).

DESIGN

The field work was completed during 1989 and 1990. Two sets of kits were investigated. The source of animals was a farm that had a confirmed disease problem (MEV or distemper) the previous season and/or had been revaccinated just prior to breeding. Siblings were divided into groups that were used in either the Challenge or Vaccination Study.In the Challenge Study, different groups of unvaccinated kits were challenged with MEV (1989) or distemper (1990) at 6, 8, 10, 12, 14, or 16 weeks of age. In the Vaccination Study, different groups of unvaccinated kits were given a single vaccination with a 4-way vaccine from United Vaccines, Inc. (Madison, WI) that included MEV (KV), *Clostridium botulinum* Type C toxoid, *Pseudomonas aeruginosa* bacterin and distemper (MLV) at 6, 8, 10, 12, 14 or 16 weeks of age. These six groups were challenged on the same day with either MEV (1989) or distemper (1990) at least four weeks after the last group was vaccinated. Blood samples were taken from all kits for antibody determination.

RESULTS OF THE 1989 STUDY

The 1989 study utilized kits from a farm that had a confirmed Type 1 MEV (See Parrish et al. 1982, 1988) outbreak during the summer of 1988. The effect of maternal immunity to MEV was investigated.

Clinical symptoms for MEV include anorexia and passage of mucoid stools that can be white or pink or contain some blood. Fecal samples were extracted with chloroform and tested for the presence of virus by hemagglutinating activity (HA) using rhesus monkey erythrocytes (pH 6.2). The HA titer is defined as the inverse of the first dilution which demonstrates less than 100% agglutination. This is expressed as HA units per 50 μm (HAU/50μL).

Antibody levels to MEV were assessed by hemagglutination inhibition (HI). The HI titer is the inverse of the serum dilution which no longer inhibits the agglutinating effect of 8 HAU of MEV. This is expressed as HI units per 50 μm (HIU/50 μL).

MEV challenge studies - unvaccinated kits

Unvaccinated mink kits were challenged at the designated ages with Type 1 MEV and scored by clinical symptoms for a period of 12 days. Challenge and serological results are listed in Table 1.

The vaccination studies

Kits were assigned to groups based on birth date. As the kits were not all born on the same day, the stated age is an average. The first groups were vaccinated on 30 June 1989 with

1 μL of the 4-way vaccine previously described (United Vaccines, Inc.). All vaccinees and a group of unvaccinated controls were challenged with Type 1 MEV on 26 October 1989 and scored for the standard 12-day period by clinical signs of MEV and the presence/absence of virus shed in the feces. The challenge and serological results are listed in Table 2.

Table 1. MEV challenge/serology results of univaccinated kits

Challenge Age (wks)	Health #Protected/#Chall.	% Protected	Serology HI Titer*
6	6/9	67	Not Done
8	5/11	45	16.0
10	5/11	45	13.8
12	0/8	0	14.0
14	1/9	11	17.6
16	1/10	10	16.0

* HI Units/50 μL

Table 2. Distemper challenge/MEV (HI) serology of unvaccinated kits

Challenge Age (wks)	Health #Chall.	#Protected	% Protected	Serology (MEV) HI Titer*
6	10	10	100.0	277.0
8	9	9	100.0	160.5
10	10	6	60.0	16.2
12	9	6	66.7	5.2
14	10	3	30.0	3.9
16	8	2	25.0	0.0

*HI Units/50 μL

RESULTS OF THE 1990 STUDY

The 1990 study was carried out on kits from a farm that had an MEV outbreak just prior to breeding. The females were vaccinated at that time with a 4-way vaccine (previously described). This study investigated the protective effects of maternal immunity to the distemper fraction of the pre-breeding vaccination. Clinical symptoms of distemper (catarrhal form) include light sensitivity (eyes), anorexia, inflammation of the mucous membranes, dermatitis, especially of the nose and paws.

Maternal antibody to MEV was monitored by serology

Distemper challenge studies - unvaccinated kits

Unvaccinated animals were challenged with distemper at the age listed and scored by clinical symptoms for a period of 21 days. Distemper challenge results and MEV (HI) serology results are listed in Table 3.

Table 3. Distemper challenge/MEV (HI) serology of unvaccinated kits

Challenge Age (wks)	Health #Chall.	#Protected	% Protected	Serology (MEV) HI Titer*
6	10	10	100.0	277.0
8	9	9	10.0	160.5
10	10	6	60.0	16.2
12	9	6	66.7	5.2
14	10	3	30.0	3.9
16	8	2	25.0	0.0

* HI Units/50 μL

The vaccination study

The first groups were vaccinated on 19 June 1990 with 1 μL of the 4-way vaccine previously described (United Vaccines, Inc.). All vaccinees and a group of unvaccinated controls were challenged with distemper on 31 October 1990 and scored for 21 days by clinical symptoms of distemper (catarrhal form). Distemper challenge results and MEV (HI) serology results are listed in Table 4.

Table 4. Distemper challenge/MEV (HI) serology of vaccinated kits

Age (wks)	Health #Chall.	#Protected	% Protected	Serology (MEV) PreVac. HI Titer*	PreChall. HI Titer*
6	28	14	50.0	270.0	4.0
8	30	11	36.7	165.0	4.0
10	29	26	89.7	24.4	4.8
12	32	27	84.4	5.7	4.2
14	32	28	87.5	4.0	14.0
16	30	23	76.7	0.0	15.6
Controls	13	2	15.4	NA	4.0

* HI Units/50 μL

Comments on the serological data

As observed in Fig. 1 (data listed in Table 1), the HI titers (MEV) of unvaccinated kits in the 1989 study remained approximately the same, regardless of their age or the protection observed.

However, when prechallenge HI titers of the vaccinated kits are plotted against the age at which they were vaccinated, the titers are clearly highest in the mink that were vaccinated at 14 weeks of age.(Fig. 2, data listed in Table 2)

Figs. 3 and 4 (data in Tables 3 and 4) illustrate the HI (MEV) titers of the kits used in the 1990 studies. There is an enormous difference between this data and the HI serology from 1989 (Figs. 1 and 2).

Blood samples were not available from the old females in the 1989 study. It is possible that they were in a section of the farm that received a minimal exposure to the

MEV outbreak. If so, then the level of maternal immunity passed to the kits would not have been of the same magnitude as that observed in the 1990 study.

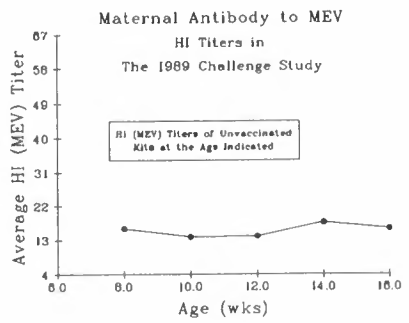

Fig. 1.

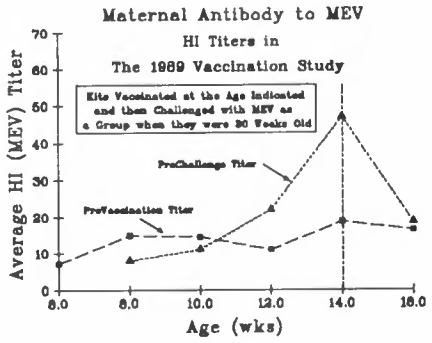

Fig. 2.

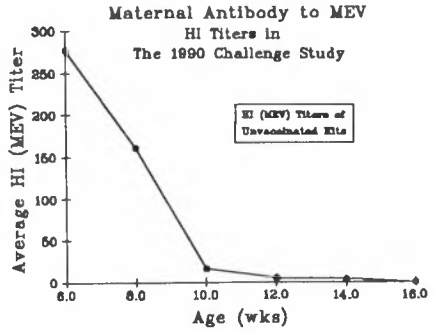

Fig. 3.

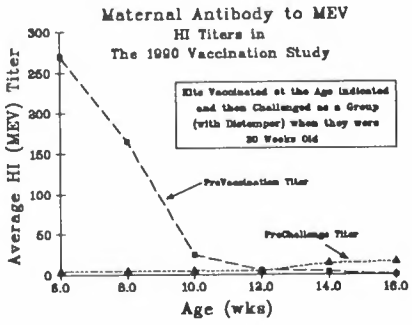

Fig. 4.

Table 5. HI (MEV) titer correlation of the old females and their kits

O.F. Titer*	Frequency	% of total	K.Av.Titer*
16.0	1	1.8	4.0
32.0	2	3.6	4.0
256.0	1	1.8	4.0
1024.0	8	14.3	43.6
2048.0	17	30.4	90.7
4096.0	14	25.0	185.1
8192.0	13	23.2	359.5

hi uNITS/50 μl

It would be very helpful to a rancher designing a vaccination program if kit antibody titers (and appropriate vaccination timing) could be predicted from those of their mothers. In the 1990 study, blood samples were taken from both the kits and their mothers (the "Old Females", 1+ years of age) when the kits were about 7.5 weeks of age. The HI (MEV) results are listed in Table 5.

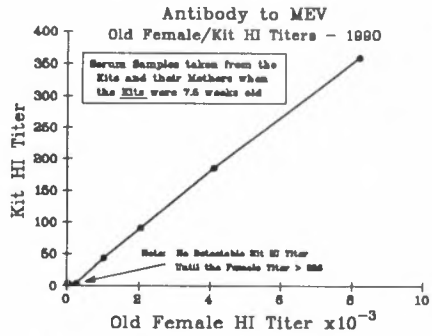

Fig. 5.

This relationship is illustrated graphically in Fig. 5.

Based on this data, when the kits were 7.5 weeks old, they did not have appreciable antibody to MEV (measured by HI) unless they were born to mothers with titers higher than 256.

CONCLUSIONS

Part 1 of the purpose was to determine at what age the kits become susceptible to infection with endemic virus which could remain on the farm after an outbreak. The challenge data (Tables 1 and 3) indicate that:

1. Some protective antibody to MEV is present for at least 12 weeks after birth.

2. Some protective antibody to distemper is present for up to 16 weeks after birth.

This data is illustrated graphically in Figs. 6 and 7.

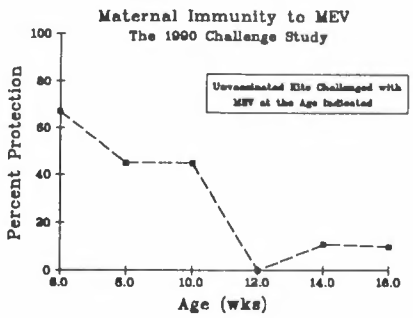

Fig. 6.

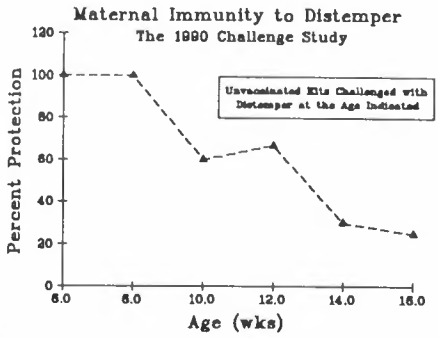

Fig. 7.

Part 2 of the purpose was to determine when the kits from such a farm could be successfully vaccinated.

The vaccination/challenge data (Tables 2 and 4) indicate that:

3. Vaccination with a 4-way vaccine containing MEV(KV) can provide protection when given to kits from hyperimmunized females at least 10 weeks after birth.

4. Vaccination with a 4-way vaccine containing distemper (MLV) can provide protection when given to kits from boosted females at least 10 weeks after birth.

These data are illustrated graphically in Figs. 8 and 9.

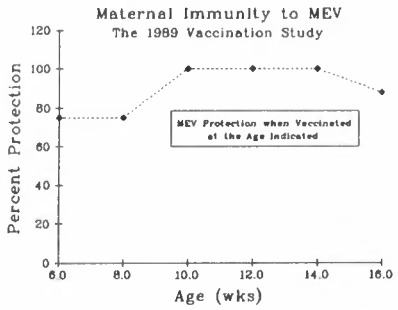

Fig. 8.

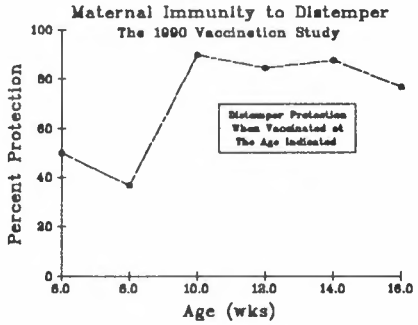

Fig. 9.

DISCUSSION

An effective vaccination program should permit the maximum effect to be achieved by each antigenic fraction and place the animal at risk of infection for the shortest possible time. This can only be achieved in mink that are fully immunocompetent and whose levels of maternal antibody are below certain thresholds.

These studies investigated the effects of MEV maternal antibody in kits from an outbreak farm and the effects of distemper maternal antibody in kits from females vaccinated in February prior to breeding. In both cases, there is a period of time when some of the kits are susceptible to disease but before they can be successfully vaccinated. This "window of vulnerability" is different for the two agents (see Figs.10 & 11).
Previous investigators (Gorham et al. 1987) have demonstrated that maternal antibody to MEV stimulated by a "silent infection" can delay a successful vaccination until the kits are at least 13 weeks of age. The current studies indicate that a delay in vaccination is necessary, although only until the kits are 10 weeks old. The variability between the 1989 and 1990 MEV antibody levels suggests that 13-week vaccination timing may not be unreasonable, depending on the specific situation.

A combination of good farm management and appropriately timed vaccinations is necessary to minimize the risk associated with the decline of variable maternal antibody levels and the "window of vulnerability" where a vaccination may not prevent infection.

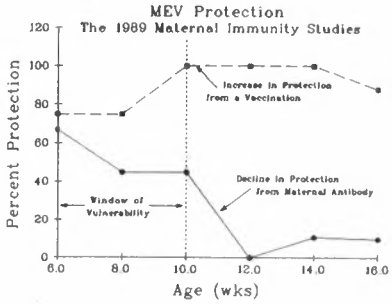

Fig. 10.

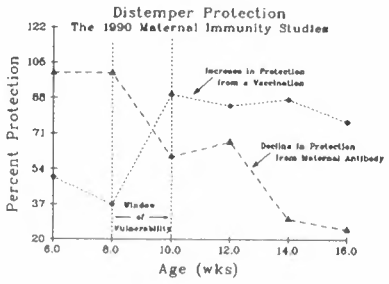

Fig. 11.

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Parvovirus infection and reproduction in blue fox vixens in Norway

Field studies and experimental infections

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Indrebø, A. & B. Hyllseth 1992. Parvovirus infection and reproduction in blue fox vixens in Norway. Norwegian Journal of Agricultural Sciences. Suppl. no. 9: 413-419. ISSN 0801-5341.

Field studies: The antibody titre against parvovirus using the haemagglutination inhibition test (HIT) was studied in blood samples from 585 blue fox vixens in 37 farms in 9 counties in Norway. Seropositive animals were found in 15 farms. There was no statistical significant difference in litter size in seronegative and seropositive farms. Experimental infections: 16 seronegative vixens were inoculated with cell culture grown blue fox parvovirus on day 17 or 18 after the last mating. A similar group received harvest from non-infected cell culture, and a third group received no inoculum. The uterus was examined for placenta zones at the time of pelting. Clinical, reproductive or serological data did not indicate an association between parvovirus infection and foetal losses in blue fox vixens. Based on these studies vaccination of blue foxes against parvovirus is not recommended in Norway.

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Reports from Finland indicate that parvovirus infection could be the cause of the poor reproduction results and high neonatal mortality in farmed blue foxes (*Alopex lagopus*) in Norway (Veijalainen 1987).

Antibodies to parvovirus in blue foxes were detected in Norway in 1980 (Indrebø & Krogsrud, pers. comm.) and in Finland in 1981 (Veijalainen 1986). Virus was first isolated in Finland in 1983 (Veijalainen 1987) and characterized serologically as parvovirus (Veijalainen 1988). Both antibodies and virus have been reported later in a large number of farms in Finland. Antibodies were found in farms with reproduction problems (Veijalainen 1986, 1987, 1988; Neuvonen et al. 1982). Limited experimental infections were carried out in 1985 and 1986, and the results seemed to support the observations made in the field (Veijalainen 1987). Based on conclusions drawn from these investigations, about 250000 blue fox vixens were vaccinated in Finland yearly until 1989 (Kangas 1988). In 1990 only a small number of blue foxes was vaccinated (Laine, pers. comm.).

Field studies and experimental infections of pregnant blue fox vixens were carried out in Norway in 1986-88 in order to examine whether there was an association between parvovirus infection and poor reproduction results in Norway.

MATERIALS AND METHODS

Field studies

Blood samples were collected in 1986-88 from 585 vixens in 37 farms situated in nine counties. The selected vixens were animals that had been mated, but were either empty, had small litters or had lost their cubs the previous season. In each farm 5-40 vixens were tested. The total number was 13% of all the vixens in the investigated farms. The total number of vixens in each farm varied from 20 to 380.

Farms with poor reproduction results were assigned to group 1 and those with good results to group 2. This grouping was based on the average litter sizes in the previous season being at least 0.2 cubs less or more, respectively, than the average for Norway based on data from the Fur Breeders' Insurance Association.

Blood samples were collected during the pelting season (October and November) or prior to the breeding season (February and March). The samples were examined for antibodies to parvovirus using the haemagglutination-inhibition test (HIT) as described by Veijalainen (1987) with some modifications. Racoon dog (*Nyctereutes procyonoides*) faecal virus was used as antigen. The diluent used was PBS (0.01 M phosphate, 0.15 M NaCl) with 0.1% bovine serum albumin added and pH adjusted to 7.0. Erythrocytes (1%) from green monkey (*Cercotithecus*) were used instead of pig erythrocytes. Erythrocyte-absorbed and kaolin-treated sera were mixed with virus antigen and incubated for 45 min at room temperature and then for 15 min on ice. A cooled suspension of erythrocytes was added, this was followed by incubation for one hour on ice before reading the results. Antibody titres ≥ 40 were considered positive.

Experimental infections

The experiments were carried out in 1987 and 1988. A total of 46 seronegative vixens were divided into three groups (groups A, B and C). Three animals were later excluded for reasons mentioned below. The animals were mainly one-year-old vixens. Seven animals in group A had litter mates in one or both the control groups (groups B and C).

On day 17 or 18 after the last mating, group A animals were inoculated both intravenously and per os with cell-culture-grown blue fox parvovirus. A Finnish strain of parvovirus isolated from fox and grown in cell culture from cat lung was used for the inoculation. Group B was inoculated in the same manner with harvest from the non-infected cell culture. Group C received no inoculum.

The animals were examined clinically every day during the first two weeks and, later, once a week. One vixen in group C was euthanized 33 days after mating because of uterine inflammation (*Echerichia coli*), and another vixen in this group was euthanized because of gangrenous mastitis and metritis shortly after giving birth to four dead cubs. These two animals were excluded from further investigation. One vixen in group A was excluded from the calculation of foetal loss as all the cubs were killed and partly eaten at the time of partus.

Blood samples were collected (*Vena cephalica*) from all groups at the time of inoculation and twice weekly in the first two weeks, and once a week in the subsequent two months, and by the time of pelting. The sera were examined for antibodies to parvovirus using HIT as described above.

The blood samples from six animals in each group were examined for white blood cells, haematocrit, haemoglobin and for 25 biochemical parameters (enzymes, proteins, minerals and ions as well as glucose, creatinine, cholesterol, bilirubin and urea nitrogen) at the Department of Biochemistry of the Norwegian College of Veterinary Medicine.

The animals in group A were euthanized in November, and the number of placenta-zones in the uterus was examined. A laparotomy was carried out on the animals in groups B and C in order to inspect the number of placenta-zones without having to open the uterus. Foetal losses in this study are calculated as the numeric difference between the placenta-zones and cubs born per pregnant vixen.

The results are analysed statistically following the Statistical Analysis System for Personal Computers (SAS Institute Inc. 1985). An analysis of variance was employed for statistical calculations. The standard error is given as SEM.

RESULTS

Field studies

In group 1 the average number of vixens in the farms were 125 (± 19.0) and in group 2 127 (± 18.6). The investigation revealed that 15 farms (41%) were seropositive to parvovirus, and 13% of the 585 vixens tested were positive. In the 15 seropositive farms 28% of the 268 vixens were seropositive.

The average litter size per mated vixen in the investigated farms was 4.40 (± 0.28) and the overall average for Norway for the same period was 4.3 (Table 1). There were no statistical significant differences in litter size in seropositive and seronegative farms within each group. In group 1 the average litter sizes for negative and positive farms were 3.17 (± 0.19) and 3.19 (± 0.22), respectively. In group 2 the corresponding figures were 6.01 (± 0.33) and 6.45 (± 0.36).

Table 1. The average number of weaned cubs per mated vixen in 22 seronegative and 15 seropositive (HIT) farms (n) in Norway. Titres ≥ 40 are considered positive

	Group 1			Group 2			Total		
HIT	n	$\bar{x}$	SEM	n	$\bar{x}$	SEM	n	$\bar{x}$	SEM
Negative	13	3.17	± 0.19	9	6.01	± 0.33	22	4.33	± 0.35
Positive	9	3.19	± 0.22	6	6.45	± 0.36	15	4.49	± 0.47
Total	22	3.18	± 0.14	15	6.19	± 0.24	37	4.40	± 0.28

Group 1: Average litter size minimum 0.2 less than the Norwegian average
Group 2: Average litter size minimum 0.2 more than the Norwegian average

Experimental infections

Antibodies to parvovirus were detected in animals in group A about three days after the inoculation. The average titre increased from <20 to about 10,000 within two weeks, and then declined slowly to about 2,500 at the time of pelting (about five months later). Groups B and C remained seronegative.

The animals in group A showed no clinical signs of illness which could be related to parvovirus infection. No statistical differences were found between the groups in body weight, rectal temperature or pulse rate during pregnancy or lactation. The difference in the number of leucocytes in group A compared with groups B and C was stastically significant on days 7, 10 and 15 after inoculation.

There was no statistical significant difference between the groups in haematocrit, haemoglobin or in any of the biochemical parameter values.

Two of the vixens in group A had no cubs at the time of partus, and none of these had placenta zones. In group B 8 vixens had no cubs, and 7 of these had no placenta zones, while one vixen had 10 zones. In group C one vixen had no cubs, but had one placenta zone.

In group A the average foetal loss was 2.2 (± 0.54) (24.2%) per pregnant vixen, and the corresponding figures in groups B and C were 2.9 (± 1.32) (23.0%) and 2.9 (± 0.74) (25.6%) (Table 2). There was no statistical significant difference in foetal losses in group A compared with those in the two control groups.

Table 2. The reproduction results in 43 blue fox vixens. Group A (n=15) was inoculated with cell culture grown parvovirus on day 17 or 18 after the last mating. Group B (n=14) was inoculated in the same manner with harvest from non-infected cell culture. Group C (n=14) received no inoculum

Group	Pregnant vixens (a)		Non-pregnant vixens (b)	Cubs born	Placenta zones	Average foetal loss per pregnant vixen	
	Gave birth	Empty - with placenta					
	n	n	n	n	n	$\bar{x}$	SEM
A	13	0	2	91	120	2.23	± 0.54
B	6	1	7	67	87	2.86	± 1.32
C	13	1	0	116	156	2.86	± 0.74

a: Vixens with placenta zones
b: Vixens with no placenta zones
c: Disrupted pregnancy

DISCUSSION

Field studies

The field data showed that antibodies to parvovirus are present in a large number of the tested farms (41%). In Finland blue foxes from more than 750 farms were tested in 1983-87. Antibodies to parvovirus were found in about 50% of the farms (Veijalainen 1986, Kangas 1988).

In our study 10-15% of the animals in each farm were tested, and the study includes only animals that were mated in the previous season, but were empty, had small litters or

had lost their cubs. This selection was made on the basis of the Finnish data in order to increase the possibility of seropositive animals. As opposed to the Finnish results, our study did not show fewer cubs born per vixen in the seropositive farms when compared with the seronegative farms. There were no statistical significant differences within each group when compared with seronegative farms in either group 1 or group 2 (Table 1).

In this study there was no definite evidence in the serological or reproduction data to indicate an association between parvovirus infection and poor reproduction results in blue foxes in Norway.

Experimental infections

The growth of the placenta leads to macroscopic changes in the uterus from day 17 in pregnancy. These changes are visible during the anoestrus period (Fougner 1972). Data from pig indicate that parvovirus infection in the first half of gestation results in foetal losses. Infections later in gestation does not cause death, but the foetuses develop antibodies against the virus and survive (Bachmann et al. 1975). The incubation time in blue foxes is 3-7 days (Veijalainen 1987). We chose to inoculate the vixens 17-18 days after the last mating. If the parvovirus infection increased the foetal losses, we expected to find a significantly higher foetal loss in group A compared with that in the control groups B and C.

The data showed no statistical significant difference in foetal losses between group A and the control groups. In group A the foetal loss per pregnant vixen was 2.2, and in both the control groups 2.9. In group A there was an overall loss of 24.2% and for the control groups together 24.7%. This is in agreement with Fougner (1972) who found a foetal loss of 22.5% in blue foxes (Fougner 1972). Our study did not confirm the conclusions from Finland that parvovirus infection is a cause of foetal losses in blue foxes.

In Finland 15 vixens were inoculated at different times during gestation (14-33 days) (Veijalainen 1987). The control group had 15 vixens that were mock infected with phosphate buffered saline at the same stages of gestation. The number of cubs born was compared in the two groups, the results indicated that there were more cubs in the control group than in the inoculated group. Similar infection studies were carried out on vaccinated and unvaccinated animals (15 in each group), and the results showed that a higher number of cubs were born in the vaccinated group than in the unvaccinated group. The Finnish studies estimated foetal losses by counting the cubs born, and did not account for the number of foetuses that were present at the time of infection as was the case in our study. This is believed to be an important reason for the different results and conclusions.

The number of cubs born varies considerably in blue foxes, and factors like age and genetic variation may play an important role. In our investigation we took care to make the groups similar concerning these factors.

The present study did not confirm the results from Finland that parvovirus infection during gestation is a probable cause of empty vixens. With only 15 vixens in each group, the data seem inadequate to draw such a conclusion when it was not known whether the vixens were pregnant at the time of the infection. The number of placenta zones indicates the number of foetuses at the time of infection (after day 17 of pregnancy). By comparing the numbers of cubs born with the count of placenta zones we concluded that parvovirus infection in pregnant blue fox vixens does not result in more empty vixens.

The inoculation resulted in a detectable antibodies after about 3 days. The average titre rose quickly and was nearly 10,000 at about two weeks. This indicates that the inoculation was successful.

The study of 25 biochemical parameters is in accordance with the clinical data that the experimental parvovirus infection did not influence the health of the animals. The only haematological parameter that revealed a significant difference was the number of leucocytes. In group A the average count was significantly lower than that in groups B and C during the first two weeks following infection. In groups B and C there were rises in the count of blood leucocytes from the time the experiment started until the middle of pregnancy, after which there was a decline until partus. This is in accordance with the study by Näveri et al. (1988) on blood leucocytes in pregnant vixens. No rise was found in group A in the present experiment. A reduction in the number of leucocytes in blood has been reported in parvovirus infection in the dog (Pollock 1982).

Observations from field studies and the experimental infections gave no evidence in the serological, reproductive or clinical data to indicate an association between parvovirus infection and poor reproductive results in blue fox vixens.

ACKNOWLEDGEMENT

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Fleas and farmed mink

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Several flea species are present on farmed mink. The squirrel fleas *Monopsyllus sciurorum* and *M. vison* are the most common. When present in large numbers, fleas seem able to cause anaemia and poor growth of the mink. Furthermore, the fur may be damaged. The fleas are also potential vectors of the Aleutian disease virus. Severe flea control problems are reported, probably due to insecticide resistance. Fleas can be very detrimental to the health of mink and control failures are a serious problem at present. More information is needed on the biology of the important flea species and on the efficacy of insecticides used to obtain a more efficient control.

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In recent years a growing number of inquiries have been directed to the Danish Pest Infestation Laboratory concerning the problems of flea control on mink farms in Denmark. This has lead to an investigation into the possibility of recommending methods for a more effective control, an issue which has rapidly raised a lot of questions. A number of different flea species on farmed mink have been observed; however, information about the geographical distribution, the abundance, and the biology of some of these species is scarce. In addition, there is a need for information on the efficacy of most of the insecticides used for control of the fleas. Furthermore, it would seem that no research has been carried out to ascertain the theoretical statements and practical experience of the farmers that fleas can cause, e.g. anaemia, stress, and damage to the pelt.

The purpose of this paper is to compile and comment on the available information about fleas on farmed mink, their veterinary importance, and the control of the fleas.

FLEA SPECIES ON FARMED MINK

The flea species *Nearctopsylla brooksi* has the native mink as a true host (Holland 1984); however, this North American flea species has never been reported as a pest on farmed mink. The ecological interactions between minks and fleas are obviously affected radically, when minks are farmed, and other flea species are known to take over (Table 1).

Table 1. The flea species reported on farmed mink and their principal hosts

Species	Hosts
Monopsyllus vison (the mink flea)	Squirrels (*Tamiasciurus hudsonicus*) and *Mustelidae*
Monopsyllus sciurorum (the squirrel flea)	Squirrels (*Sciurus vulgaris*), *Gliridae* and *Mustelidae*
Ceratophyllus gallinae (the hen flea)	*Passeriformes*
Ctenocephalides canis (the dog flea)	*Canidae* and *Felidae*
Ctenocephalides felis (the cat flea)	*Canidae* and *Felidae*
Pulex irritans (the human flea)	*Canidae* (*Vulpes vulpes*) and *Meles meles*
Nosopsyllus fasciatus (the rat flea)	*Muridae* (Rattus spp.)

(Compiled from Smit 1954, Peus 1972; Holland 1984; Traub 1985; Wenzel 1987)

A variety of flea species can be introduced into farms by their original or accidental hosts (e.g. foxes, birds and rodents) or originate from man or his domestic animals (dogs, cats, farmed foxes, pigs and fowl) (Winding 1969; Gorham et al. 1972; Larsen 1992b). Furthermore, the trade in mink between farms creates the possibility of a transmission of fleas between the farms (Suciu et al. 1984).

When introduced into the farm, the success of a flea species depends on its ability to adapt to the mink and its environment. Two flea species in particular are common: *Monopsyllus vison* in North America (Gorham et al. 1972) and *Monopsyllus sciurorum* in Europe (Winding 1969; Jurik & Kukla 1974; de Jonge 1979; Suciu et al. 1984; Mehl 1989; Larsen 1992b, 1992c). On farmed mink the *M. vison* and *M. sciurorum* obviously find another suitable *Mustelidae* host and a nest comparable to that of the original host.

Ceratophyllus gallinae is another flea species that has been reported several times on farmed mink in Europe (Peus 1968; Haagsma 1969; Winding 1969; Jurik & Kukla 1974), but the infestations seem only to be transient. Jurik & Kukla (1974) found that *C. gallinae* infestations, although extensive, usually disappear within the same year, to be followed the next year by an *M. sciurorum* infestation.

The other species reported are *Pulex irritans*, *Nosopsyllus fasciatus*, *Ctenocephalides canis* and *Ct. felis* (Gorham et al. 1972; Wenzel 1987; Mehl 1989), but they seem only to occur occasionally on farmed minks. Although they are all more or less indiscriminate in their choice of hosts, these fleas do not seem to be well adapted to the actual conditions. Gorham et al. (1972) mention that *Ct. canis* may appear occasionally on mink, when foxes and mink are kept in the same enclosure.

THE EFFECTS OF FLEAS ON MINK

A generally held view is that fleas can be detrimental to the general well-being of the infested mink (e.g. Gorham et al., 1972; Jurik & Kukla 1974; Suciu et al. 1984). It is mentioned that fleas, when present in large numbers, seem able to cause anaemia (Winding 1970; Hansen 1987; Englund 1989; Munck 1991) and even the death of the mink kits (de Jonge 1979). Furthermore, the female mink can become troubled (Winding 1969; Wenzel 1987) and often leave the nest boxes, thereby causing poor care of the kits (Hansen 1987; Englund 1989; Munck 1991). Also the pelt may be damaged when the mink react to the

fleas by scratching and biting (Winding 1969; Gorham et al. 1972; Wenzel & Berestov 1986; Wenzel 1987). Despite the numerous articles mentioning the effects of fleas on mink to the author's knowledge, no attempts have been made either to determine or to quantify these effects.

On farmed mink another important role of fleas is that of being potential vectors of pathogenic organisms.The fleas are mentioned as possible vectors of the distemper virus (Winding 1969) and of the Aleutian disease virus (AD) (Anon. 1986). Haagsma (1969) was unable to prove the existence of the AD agent in fleas (*C. gallinae*) found on an AD-positive mink. However, further research is needed before fleas can be excluded as a factor in the transmission. Assuming that the fleas are vectors, there seem to be several possible ways of transmission of the AD virus. The transmission could occur by simple mechanical transport of the virus on the mouth parts of the fleas, by fleas eaten by the mink while grooming itself and by flea faeces (semi-digested blood from the mink host) when licked up or otherwise ending up in the mucous membranes of the mink.

The flea species on the mink might also be a reservoir of the AD virus, because of their relatively long life span. One of the more common flea species, *M. sciurorum*, is known to feed repeatedly for several months and is able to hibernate (K.S. Larsen unpubl.). Moreover, if the fleas are vectors of the AD virus, a dissemination of the virus between farms is possible via an exchange of fleas on mobile hosts like rodents or birds.

The finding of the AD agent in a mosquito, *Aedes fitchii*, by Shen et al. (1973) indicates that other haematophagous arthropods should also be given some attention.

FLEA CONTROL ON MINK FARMS

As stated above, the success of the fleas is based on the fact that mink are suitable hosts in a suitable environment. The ultimate control strategy should therefore be to make the environment as unsuitable as possible for the fleas, e.g. by changing the farm management methods, and only using insecticides as a supplement.

Fleas are insects with a complete metamorphosis, i.e. from egg via larval and pupal stages to adult. This development takes place mainly in the nest box material, but sometimes also in the pen or on the ground if litter is present (Winding 1970). When an extensive flea infestation occurs, the juvenile stages of the fleas can be destroyed by removing all the nest box material and the litter. The material should immediately be removed from the farm and burned or ploughed in. It is important to maintain a good hygiene in the nest boxes. In particular, nest box material contaminated with mink faeces and urine, or with food leavings, seems to provide the fleas with good reproduction conditions (high humidity) through the dry summer period (Jurik & Kukla 1974). Such conditions should be avoided since they become a source of infestation to the rest of the farm.

Treatment of the mink, the nest box material and/ or the ground underneath the pens with insecticides is often used in flea control (e.g. Gorham et al. 1972; Wenzel 1987; Englund 1989), but unfortunately this methode is not particularly efficient.

In a survey in Denmark, Larsen (1992a) found that nearly all mink farmers control fleas routinely with a preventive insecticide treatment of the nest boxes in the spring,

supplemented with one or more further treatments over the summer and autumn, whenever fleas were found. In spite of this more than 40% of the farms were found to have flea infestations every year. In the same survey more than 30% of the farmers reported unsatisfactory flea control although insecticides were used.

Control failure has been experienced appears on mink farms throughout the Nordic countries even though a variety of different insecticides are used. The insecticides in question are malathion and lindane in Denmark, malathion in Sweden and Finland, and methoxychlor in Norway (Larsen 1991). The reduced efficacy could be due to insecticide resistance, but no investigations have been carried out to confirm this. If there is indeed an extensive resistance to malathion, Swedish mink farmers in particular will have a problem since malathion is the only insecticide approved for flea control on farmed mink in Sweden (Larsen 1992c).

DISCUSSION AND CONCLUSION

Although the two *Monopsyllus* species, *M. vison* and *M. sciurorum* seem to be the most important flea species on farmed mink, more information is needed. Because of the widespread geographical distribution of mink farming and the various farm management methods used, other flea species may also be of importance locally, and faunistic flea surveys should be carried out to clear this up. Since very little is known on the ecological relationships between the various flea species and the mink, it is difficult to focus about the essential conditions that enable the fleas to evolve into a pest on the farmed mink. Ongoing investigations at the Danish Pest Infestation Laboratory (DPIL) on the biology of *M. sciurorum* related to its presence on farmed mink may shed some light on these problems. The aim is to be able to recommend modifications to the current farm management practice and form a strategy for preventing the development of flea problems.

Concerning the veterinary importance of the fleas, a number of questions are still unadressed, especially those on the potential role of fleas as vectors of the Aleutian disease virus. Without further research, it is not possible to determine whether the reported effects on flea-infested mink occur only because of the fleas or as a result of a combination of the fleas and other factors; indeed, the effects may have nothing to do with the fleas at all. This year the DPIL is going to investigate some of the problems often mentioned in the literature, such as the effects of flea infestations, e.g. anaemia, damaged pelt, and changes in mink behaviour.

Further research is also needed in the area of flea control. Today considerable amounts of insecticides are used, e.g. in Denmark in routine preventive treatments for flea control, irrespective of the actual presence of fleas. In spite of this control failures are common and extensive, which could be due to insecticide resistance. Before using other insecticides instead of the ones commonly used to overcome control failure problems, the existing resistance pattern should be dertermined and described in order to obtain more efficient control of the insecticides used.

SUMMARY

In this review of the literature on fleas on farmed mink, information about the flea species present, their veterinary importance, and the control of the fleas is compiled and discussed.

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Nitrosamine-induced vascular and carcinogenic changes in mink (*Mustela vison*)

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In a lifetime experiment, 48 mink were given herring meal containing NDMA equivalent to a daily dose of 0.4 - 0.025 mg NDMA/kg bw. At a dose level of 0.2 mg NDMA/kg bw/day, liver cell necrosis was unusual. However, occlusive changes in the efferent hepatic vein, promoting distended blood-filled sinusoids, and precancerous liver changes developing into liver haemangioendothelioma, were common features. Daily exposure to 0.2 mg NDMA caused liver tumours in 100% of the mink. At reduced NDMA levels some mink succumbed to intercurrent diseases before the additive NDMA level for tumour development had been reached, no synergistic effects of the three different nitrosamines were observed. However, this was not unexpected since the NDMA levels were 200 - 1000 times higher than the NDPA and NPYR levels.

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After an epidemiologic examination on 21 December 1961 on a farm where two cows had died of toxic liver injury, it was concluded that the new disease was caused by toxic herring meal (Koppang 1963, 1964). In the subsequent three months a number of cows suffering from herring meal intoxication were examined, feeding experiments with sheep, cows and fur animals were started, and the two suspected factories in northern Norway, x and y, inspected. The diseased cows displayed the same symptoms as those described by Ulvesli (1955). The cows receiving the highest daily portion of herring meal had a sudden drop in milk yield, they reduced appetite and refused concentrates, symptoms explained as acetonaemi, commonly seen in cows with toxic hepatosis. Comparing these facts with information about excessive use of nitrites in factory x led us to our conclusion about the aetiology, which we expressed to the owner of factory x as: "Unlawful use of sodium nitrite".

The production of five different test meals of the same batch of raw, frozen herring was followed up in Øksfjord, and the experiment to prove our theory was initiated on 9 March 1962 with 12 ten-month-old sheep. The results were clear by April 1962 and revealed that a toxic compound was synthesized during the processing of nitrite-preserved fish to meal (Koppang et al. 1964). The fact that nitrite was commonly used in the fishmeal industry and in salt in the meat industry made this evidence a matter of world-wide concern affecting both people and animals (Koppang & Slagsvold 1964).

The conclusions drawn after the extensive clinical feeding experiment in 1952-54 (Breirem & Flatla 1955) gave the laboratories and manufacturers a false sense of security, assuming that the preservation of the raw material with nitrites could not cause poisoning. The danger of spoilage of large quantities of raw material led certain manufacturers to use nitrites in amounts which by far exceeded the regulation limits (Koppang 1964).

After reasonable analytical methods for measuring NDMA were worked out in the late 1960s, a NDMA control of the herring meal was established in Norway. Unfortunately, NDMA was often recorded in spite of the new restricted use of nitrite.

Amines identified as di- and trimethylamines were the "mother substance" for N-nitrosodimethylamine (NMDA) (Ender et al. 1964). This is only half the truth, as the real "mother substance" is trimethylamine-oxide (TMAO). The amount of TMAO in fresh saltwater fish varies with season and species, and generally increases with increasing fish size (Shewan 1961). During natural spoilage, TMAO decreases gradually and the possibility of NDMA formation is commensurately reduced (Koppang 1974c). Fishmeal produced from fresh unspoiled saltwater fish without the use of nitrite as a preservative can, in factories with directfired rotary dryers, contain NDMA. The N-nitrosification product may then come from the combustion of oil or from the heated air (Koppang 1974b). This fact made it highly desirable to elucidate the possible toxic and carcinogenic effects of small daily exposure to NDMA.

The present study was designed to investigate the effect of lifetime exposure to different levels of nitrosamines in order to elucidate whether there is a threshold value below which NDMA does not have any toxic and carcinogenic effects.

MATERIALS AND METHODS

The experiment was carried out at the Research Farm for Fur-bearing Animals, Heggedal, using 38 standard mink of the farm's own breeding stock. The control group comprised of 20 adult females.

The 58 mink were housed and fed as reported earlier (Koppang & Helgebostad 1987). The only variable was the amount of herring meal provided by the Herring Oil- and Herring Meal Industry's Research Institute. The meal was incorporated into the basic diet of the mink and contained approximately 20 ppm NDMA, less than 100 ppb di-N-propylnitrosamine (NDPA) and less than 20 ppb of nitroso-pyrrolidine (NPYR), (see Table 1 for a list of the different daily doses used). Each bag of herring meal was analysed for nitrosamine prior to use.

All mink were autopsied, and liver, spleen, heart, kidney, lung, small intestine and brain samples were fixed in 10% neutral buffered formalin, paraffin embedded and stained as reported previously (Koppang et al. 1964).

RESULTS (see Table 1)

Group 1. 0.4 mg NDMA/kg of body weight (bw) per day (five mink)
After two-and-a-half months of feeding, all the mink began to reject a portion of their daily feed, and only about two-thirds of the fed was consumed.

Table 1. Pathomorphological liver changes in mink fed herring meal containing NDMA

Group	No. of mink	Exposure Daily exposure	Days, Range Average	Total exposure Range, Average	Pathomorphological changes (no. of mink with the changes in parentheses)
1	5	0.4	115-195 (140)	35-56 (41)	Liver haemangioendothelioma& abdominal haemorrhage (2); periacinar haemorrhagic liver cell necrosis zone 3, ascites & cirrhosis (3)
2	5	0.2	344-445 (389)	68-89 (77)	Liver haemangioendothelioma & cholangioma (5); abdominal haemorrhage (4); hepatocellular carcinoma (1)
3	10	0.1	545-1549 (804)	54-154 (88)	Liver haemangioendothelioma& abdominal haemorrhage (6) & omental metastasis (5); precancerous liver changes (2); streptococcus sepsis (1); cachexia (1)
4	14	0.05	763-1483 (1134)	38-74 (56)	Liver haemangioendothelioma metastasis, omental & abdominal haemorrhage (10); precancerous liver changes, plasmacytosis & cachexia (2); hepatocellular carcinoma, pulmonary & CNS metastasis (1); myodeg. cordiso. edema pulmonum & cachexia (1)
5	14	0.025	526-1842 (1282)	13-46 (32)	Liver haemangioendothelioma, metastasis, omental & abdominal haemorrhage (2); hepatocellular carcinoma (1); jejunal sarcoma metastasis, omental & diaphragmatic (1): precancerous liver change & plasmacytosis (2); metritis & septicaemia (1); cholangioma & plasmacytosis (5); sacrificed at 536 days normal (2)

Three mink showed some areas of liver necrosis of zone 3, partial necrosis of the wall of the efferent hepatic vessels, regenerative changes with fibrosis of other acini and thickening of some hepatic vein branches. In the acini, with partially occluded hepatic veins, dilatation of the sinusoids to blood-filled spaces with nuclear enlargement of endothelial cells, precancerous changes occurred. Two mink died suddenly from abdominal bleeding caused by rupture of one of the multiple hepatic haemangioendotheliomas.

Group 2. 0.2 mg NDMA/kg bw/day (five mink)
All five animals displayed multiple haemangioendotheliomas throughout the liver. In addition, one mink developed hepatocellular carcinoma. Liver necrosis of zone 3 did not occur, but occlusive changes in the efferent hepatic vein system were a common feature.

Group 3. 0.1 mg NDMA/kg bw/day (10 mink)
Six mink displayed widespread multiple haemangioendotheliomas, omental metastases, tumour ruptures and abdominal haemorrhage.

Group 4. 0.05 mg NDMA/kg bw/day (14 mink)
Ten mink displayed the typical picture of haemangioendotheliomas with metastasis to the mesenterium and abdominal haemorrhage. All 14 displayed some occlusive changes in the hepatic veins.

Group 5. 0.025 mg NDMA/kg bw/day (14 mink)
Only two mink developed typical haemangioendotheliomas. One mink had jejunal sarcoma with metastases to the omentum, liver and diaphragm. Another displayed multiple cancer metastases which originated from a liver carcinoma. Two mink *showed precancerous liver changes* and plasmacytosis. Six mink died from other diseases. Two mink were the offspring of a female in this group, who had been exposed to NDMA during pregnancy and lactation. The two were included from time of weaning and had been fed nitrosamines for 526 days, making a total dose of 13 mg NDMA/kg bw. They were healthy when sacrificed and no occlusive changes in the efferent hepatic veins were observed.

Control groups
Occlusive changes in the efferent hepatic veins, periacinar liver necrosis and liver tumours did not occur, but some plasma cell infiltration has observed in liver and kidneys.

DISCUSSION

As observed earlier, when the daily dose of NDMA in the feed was 0.2 mg/kg bw/day or less, periacinar haemorrhagic liver cell necrosis in zone 3 did not occur. But NDMA caused occlusive changes in the branches of the efferent hepatic vein and subsequent development of vascular liver tumours. These occlusive changes first occurred when the NDMA dose had reached a certain level. The two mink in group 5 exposed to 13 mg NDMA/kg did not display any occlusive changes. It seemed that a threshold level of NDMA had to be exceeded before these vessels changes occurred.

The occlusive changes of the efferent hepatic vein would subsequently cause dilatation of the sinusoids draining to the occluded branches. The endothelial cells covering the dilated sinusoid bloodrooms appeared to have an inconsistent nuclear enlargement. These changes occurred prior to the development of haemangioendotheliomas and must be regarded as the initial stages of tumour formation, - a precancerous stage. (Wayss et al. 1970). Six mink from groups 3, 4 and 5 (two per group) who died from plasmacytosis or other non-specific diseases, displayed these precancerous changes. The development of the

precancerous stage into haemangioendothelioma seemed to be a time dependent process which may be independent of further NDMA exposure. In cattle, the development of haemangioendotheliomas was demonstrated along with changes in the hepatic vessels utilizing repeated liver biopsis to define the hepatic changes that occurred during and after continued NDMA exposure had stopped (Koppang 1974b).

In mink exposed to NDMA, the haemangioendotheliomas, which are usually multiple and occur to some extent in each liver lobe, may protrude to the surface of the liver, rupture and cause sudden death by abdominal haemorrhage. With a longer survival time, some of the haemangioendotheliomas presented a clearly malignant picture with slight metastasis to the mesenterium and the mesenteric lymph nodes. Macroscopic metastases in lungs and kidneys are not registered in the present NDMA experiment or in our diagnostic work with mink exposed to NDMA (Koppang 1966, 1980, 1981).

In our previous papers we discussed whether there is a threshold value below which an accumulative effect of NDMA exposure does not occur (Koppang 1974 a,b,c 1981). Cattle and sheep do not seem to be less susceptible to the toxic effects of NDMA than mink. Cattle, exposed to NDMA have recovered, the liver regenerated and new vessels developed close to the occluded ones. However, years later, haemangioendotheliomas developed from the changed vessels (Koppang 1981).

This experiment confirmed our earlier results that mink exposed to 0.15-0.2 mg NDMA/kg bw/day developed liver tumours in 100 % of the cases (Koppang & Rimeslåtten 1976). Lower doses of NDMA resulted in some mink succumbing to non-specific diseases before being exposed to sufficient levels of NDMA to cause liver tumours, as seen in groups 3, 4 and 5.

If the precancerous stage were included, the frequencies of carcinogenic changes caused by NDMA in this experiment are high: 40%, 100%, 80%, 93%, and 43% in groups 1, 2, 3, 4 and 5, respectively. The total NDMA exposure was 41, 77, 88, 56 and 32 mg NDMA/kg bw, in the respective groups. The daily exposure in group 5 was so low that it was difficult to reach a high enough total exposure of NDMA to cause liver tumours within the minks' lifetime. These results do not support the existence of any threshold value for NDMA effects.

The small amounts of NDPA and NPYR were respectively 1/200 and 1/1000 of the NDMA content. A synergistic effect of the three nitrosamines would therefore be difficult to detect. The pathomorphological changes observed in the mink correlated with those seen in an experiment involving the exposure of mink to commercial NDMA.

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N-nitrosodimethylamine (NDMA) induced toxic-, vascular- and carcinogenic changes in mink (*Mustela vison*)

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Treating 31 standard mink with single doses of 6-13 mg NDMA/kg bw resulted in a LD_{50} of 7 mg/kg bw. Forty-seven adult mink were repeatedly exposed to NDMA, daily or at longer intervals. In all mink exposed to NDMA, changes were seen in the efferent hepatic vein system. With daily exposure of less than 0.15 mg NDMA/kg bw, liver necrosis did not usually occur and the first changes observed were in the hepatic vein system after a total uptake of about two times the LD_{50}. The clinical and pathomorphological changes revealed in these NDMA experiments were found to be the same as those observed in malignant liver disease in mink caused by nitrosamines in the fish meal.

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In 1957-62, a new unknown malignant liver disease suddenly occurred in fur farms scattered throughout Norway. Some of the farmers suspected that herring meal was the cause of the disease. Thus, during the years 1958-61, a number of herring meal samples from disease-affected farms were used for experimental feeding, but without reproducing the malignant liver disease. Bøhler (1958, 1963), in his short description of the new disease, stated: "...that all feeding experiments, bacteriological and toxicological examinations had been negative". The oetiology of the malignant liver disease was completely unknown (Bøhler 1963; Rochmann 1960).

In the spring of 1962 our experimental feeding studies clearly indicated that the malignant liver disease in ruminants and fur animals was caused by toxic herring meal, and that it was the sodium nitrate used as a preservative that rendered the herring meal toxic (Koppang 1963, 1964, 1966, 1970; Koppang & Helgebostad 1962, 1966; Koppang et al. 1964).

The toxic factor N-nitrosodimethylamine (NDMA) was first identified two years later. In May 1964, Aldal measured the NDMA to be from 10 to 250 ppm in 24 different herring meal samples (Aldal & Koppang 1964). This was the first proven measurement of NDMA in food. Sakshaug at the same time independently confirmed the presence of 30-100 ppm NDMA in six other herring meal samples (Sakshaug et al. 1965).

Koppang has underlined the changes in the efferent hepatic veins in fur animals and

ruminants by herring meal intoxication (Koppang 1963, 1964, 1966, 1974a,b,c, 1980 and 1981). In the case of NDMA, the general consensus is that the primary lesion is parenchymal and that venous lesions, when present, are secondary (McLean et al. 1965; Carter et al. 1969).

It has been well documented that nitrosamines are powerful carcinogens (Magee & Barnes 1956, 1967; Druckrey et al. 1967). It is therefore of great importance for human and veterinary medicine to establish whether continuous exposure to low levels of NDMA can initiate pathological changes and tumour development.

The present communication considers the effects of pure NDMA on mink:

1. Single dose exposure and LD_{50} of NDMA
2. Long-term exposure
3. The effects of NDMA on breeding performance and offspring

MATERIAL AND METHODS

A series of experiments were carried out at the Research Farm for Furbearing Animals of the Norwegian College of Veterinary Medicine, which included 78 mink exposed to NDMA, and a control group of 20 adult females.

The mink were fed and housed as reported earlier (Koppang & Helgebostad 1987), with the only variable being the amount of commercial NDMA (The British Drug House Ltd.) given in a 0,35% or 1% solution in saline or in arachis oil to each animal. Care was taken to prevent vomiting in those mink which received NDMA by stomach tube. The subcutaneous injections were given behind the elbow. In long-term feeding trials, the NDMA was carefully mixed in the food and given three times weekly in experiment 2 or daily in experiment 3.

All the mink were autopsied and the heart, liver, kidney, lung and brain samples fixed in 10% formalin, paraffin embedded and stained as reported earlier (Koppang et al. 1964).

RESULTS

1. Single dose of NDMA. Experiment 1 and Table 1

(a) Clinical signs

An exact measurement of NDMA intake cannot be determined by mixing NDMA in the food because mink ususally take several hours to eat their daily ration, which means that some food will always become lost and some NDMA will evaporate or be degraded by ultraviolet light. Administration by stomach tube was often followed by vomiting. Consequently, exact dosing of NDMA could only be obtained by subcutaneous injection.

The first sign of toxicity, with nausea and vomiting, occurred within one hour after a high dose of NDMA. The animals became weak, drowsy, had difficulty in standing up, showed a loss of appetite and were polydipsic. Most of the mink were jaundiced before death. All mink injected subcutaneously with a dose higher than 8 mg NDMA/kg body weight (bw), died within three days. Of the mink exposed to 7 mg NDMA/kg bw, four died 4 1/2, 4 1/2, 18 and 232 days respectively after exposure, the remaining two being

Table1. Experiment 1: Mink exposed to a single dose NDMA, application method; number of mink that died, survival time in days; number of mink killed after exposure, pathological changes and the number of mink with these changes in parenteses

Mg NDMA/ kg b.w.	No of mink	Application		Mink died Survival days			Mink sacrified Survival days			Pathological changes
		Sub cut	Oral	No	X	Range	No	X	Range	
6	2			0			2	234		Precancerous liver changes (2)
7	6			4	65	4-232	2	132	30-234	Periacinar, haemorrhagic liver necrosis (3) Occlusive changes v hepatica (6) Kidney infarcts (1) Precancerous liver changes (2) Haemangioendothelioma (1)
8	3	3		3	3	3				Periacinar, haemorrhagic liver necrosis (3) Occlusive changes v hepatica (3)
9	10	3	7	7	5	2-14	3*	38	37-38	Periacinar, haemorrhagic liver necrosis (7) Occlusive changes v hepatica (10)
10	6	4	2	6	3	2-5				Periacianar, haemorrhagic liver necrosis Occlusive changes v hepatica (6)
11	1		1	1	32					Occlusive changes v. hepatica (6) Vessel changes heart and kidney (6) Scarcirrhosis (1)
12	3	3		3	2					Periacinar, haemorrhagic liver necrosis Acute changes v hepatica (3)

Number of mink 31

*Oral application of NDMA, dose uncertain

sacrificed 30 and 234 days after NDMA treatment. Both mink exposed to 6 mg NDMA/kg bw survived. The LD_{50} for mink thus appeared to be 7 mg NDMA/kg bw.

(b) Pathomorphological changes

All the animals that died within a week after administration of NDMA, exhibited haemorrhagic diathesis with abdominal and gastrointestinal haemorrhages. The heart was enlarged and flabby, the kidneys and liver swollen and often yellow-brown in colour as a result of diffuse fatty infiltration. There was acute, periacinar haemorrhagic hepatocellular necrosis in zone 3, and necrosis of the endothelium in the smaller branches of the efferent hepatic veins. With lower doses of NDMA and increasing survival time, livers tended to be larger and began to display a nutmeg-like appearance. Fatty infiltration in the liver cells and inflammatory changes were observed on the edge of the necrotic area. The first signs of regeneration were usually recognizable after 3-4 days, providing the changes in the efferent branches of the hepatic vein had not caused too many occlusions and acute thromboses, thus decreasing the blood flow and preventing regeneration. Usually within a week regenerative processes, with removal of most of the necrotic liver cells, were well established. Then a regeneration and organization of the liver cells occurred, partial obliteration of sublobular veins due to proliferation of fibroblasts and smooth muscle cells under the damaged endothelium and a moderate bile duct proliferation in the portal triads could be seen.

The chronic occlusive changes in the efferent hepatic veins took some time to develop. One mink exposed to 11 mg NDMA/kg bw in the food died 32 days later with pronounced vessel changes. One mink given 7 mg NDMA/kg bw recovered and looked healthy, but died suddenly 232 days later of a ruptured liver haemangioendothelioma. The liver tissue surrounding the tumours was almost normal microscopically except for occlusive changes in some of the efferent hepatic veins, and the corresponding sinusoids were dilated and congested, forming blood-filled spaces. Some of these areas were located immediately under the capsule. Dilatation of the sinusoids, with irregular nuclear enlargement of the endothelium, is the first precancerous stage in the development of haemangioendothelioma. Most of the liver tumours had the morphological appearance of a benign haemangioendothelioma. Since they are situated close to the liver capsule, they can easily rupture and the animal can die from abdominal haemorrhages.

In the three animals sacrificed 234 days after the subcutaneous injection of 6 or 7 mg NDMA/kg bw, occlusive changes in some of the efferent hepatic veins and dilations of adjacent sinusoids were prominent; all three mink showed the early precancerous changes associated with haemangioendotheliomas.

Mink that died during the first week after exposure to NDMA showed acute renal changes with necroses of tubular epithelial cells in the proximal convoluted tubules and some congestion of the glomerular loops, while some mink also had renal infarcts. Endothelial and/or smooth muscle fibre changes were observed in some of the smaller arteries and veins in organs other than the liver, especially in the gastric and duodenal wall in connection with gastrointestinal haemorrhages.

2. Repeated subcutaneous doses of NDMA. Experiment 2

(a) Clinical signs

Three mink received a total dose of 22-23 mg NDMA/kg bw, each injection being 4.4-7.3 mg NDMA/kg bw, with 12 or 15 days between each administration. Shortly before death, the animals lost their appetite, developed ascites, and became lethargic. The faeces appeared thin and tar-like, and one mink was jaundiced.

(b) Pathomorphological changes

The animals were all thin, the peritoneal cavity contained about 100-200 ml bloodstained, ascetic fluid. The abdominal vena cava, splanchnic and portal veins were congested. The liver was slightly enlarged with a nodular surface, the colour varying from yellow-grey-green to blue-red. The spleen was greatly enlarged, red-blue and weighed 7-9 g. Portal and mesenteric lymph nodes were enlarged and congested. The kidneys tended to be slightly enlarged, and two animals displayed bilateral kidney infarcts. The stomach and intestinal tracts were nearly empty, but contained some mucus mixed with blood, giving a tar-like appearance to the contents of the terminal colon. Hearts were enlarged and flabby. The lungs were small and showed petechiae. More chronic occlusive changes in the efferent hepatic veins were seen in all three mink. The pathoanatomical changes in all three mink were the same as those seen in malignant hepatosis (Koppang 1966, 1980).

3. Three-weekly treatment with NDMA mixed in the feed. Experiment 3 and Table 2

(a) Clinical signs

The mink in groups 1 and 2 ate well in the first 3-6 weeks, but then began gradually to refuse some of the fed. The mink were almost cachectic at death. In group 3 the mink ate well until one or two weeks before death; one female looked healthy when sacrificed.

Group 4(P), group 5(F_1) and group 6(F_2) (0.05 mg NDMA/kg bw/day)

Group 4 (P) was the parent generation and comprised two females and one male. Each of the two females had two litters during the experimental period, eight and four cubs respectively, in the first year. Ten of the 12 cubs born during the first year were immediately exposed to NDMA in the feed, group 5(F_1). Their mothers had been exposed during gestation and lactation. From the cubs produced by group 5(F_1), 15 were taken to represent a third generation of exposure and were immediately placed on the same lowdose NDMA diet, group 6(F_2), as their parents. In group 5(F_1), four mink without any clinical changes were sacrificed, two after 618 days and two after 1272 days of the trial. In group 6(F_2), eight mink were sacrificed when clinically healthy, three after 253 and five after 908 days of NDMA exposure.

At this low dosage, the mink ate well, and were in general good health, but a few were found dead in their cages without having shown any signs of illness. These mink showed no decline in fertility, their litters were of the same size as those of the control mink.

Table 2. Experiment 3: Number of mink in groups exposed to NDMA mg/kg b.w. three times weekly. Total NDMA uptake, days in experiment and pathological changes (number of animals with these changes in parentenses)

Group	No of mink	NDMA mg/kg b.w.						Pathological changes
		Three-weekly dose in	Estimated daily dose	Total uptake		Days in experimental feeding		
				X	Range	X	Range	
1	2	2.3	1.0	60		91	78-104	Liver haemoangioendothelioma (2) Myocardial infarcts (1)
2	3	1.17	0.5	60		190	151-246	Liver haemangioendothelioma (3) Myocardial infarcts (1) Glomerulinephritis (1)
3	5	0.35	0.15	48	15-74	322	106-498	Plasmacytosis, nephrosis and encephalitis (1) Liver hemangioendothelioma (4) Myocardial-, kidney- and liver infarcts (1)
4 (P)	3	0.117	0.05	48	43-56	982	874-1134	Liver haemangioendothelioma (3)
5 F (1)	10	0.117	0.05	8		162		Gastroenteritis cath. Dehyd. (1)
				47	31-63	945	618-1272	Sacrified (4). Occlusive changes vs hepatica (4) Precancerous liver changes (1)
				48	42-62	968	844-1250	Liver haemangioendothelioma (5)
6 F (2)	15	0.117	0.05	13		253		Sacrified (3). Precancerous liver changes (1) Occlusive changes vs hepatica (3)
				45		908		Sacrified (5). Liver haemangioendothelioma (5) Occlusive changes vs hepatica (5)
				37	32-42	757	656-850	Liver haemangiendothelioma (7). Occlusive changes vs hepatica (7)

38 mink

(b) Pathomorphological changes
One mink in group 3 died of plasmacytosis. In group 5 (F_1) one died of gastroenteritis and dehydration. All the other mink dislayed occlusive changes in some parts of the efferent hepatic veins. These changes were also seen in the three mink in group 6 (F_2) sacrificed after 253 days of trial and a total uptake of 13 mg NDMA/kg bw; one of these had already developed precancerous liver changes. The common tumours in these mink were of the haemangioendotheliom type, appearing in different sizes and numbers. Very few had metastasized to the portal and cranial mesenteric lymph nodes.

4. Daily exposure in the feed to 0,15 mg NDMA/kg bw, six adult male mink
Three males died after 178, 188 and 204 days, respectively.The other three were sacrificed after 210 days of the trial. All showed swollen livers with occlusive changes in the efferent hepatic veins and liver haemangioendotheliomas. Total NDMA uptake was 25-28 mg/kg bw.

5. Control group
Without any occlusive changes in the vein system or tumours.

DISCUSSION

Some scientists have claimed that NDMA is a selective liver cell toxin (Madden et al. 1970; Testas et al. 1978). It was found that all the mink that died within a week in our single dose experiment had periacinar haemorrhagic liver cell necrosis; usually only a small rim of liver cells around the triads, representing zones 1 and 2, was intact. The haemorrhage covered the necrotic endothelium of the sinusoids and the central veins. The vessel changes due to NDMA have frequently been overlooked or interpreted as secondary events (McLean et al. 1965; Carter et al. 1969).

Liver cell necroses have not been observed in mink exposed to a dose lower than 0,15 mg NDMA/kg bw per day. In these mink the first recognized changes occurred in the efferent hepatic veins as thickening of the wall, leading to occlusion of some affected parts of the vein, followed by dilatation of the corresponding sinusoids with irregular nuclear enlargement of the endothelium. Like Wayss et al. (1979), we consider these changes to be precancerous and the first step in the development of a haemangioendothelioma.

The changes observed in the mink exposed to NDMA in this experiment represent the same pathomorphological changes as those found in mink exposed to toxic herring meal (Koppang 1966, 1980, 1981; Koppang & Helgebostad 1966; Koppang & Rimeslåtten 1976).

The LD_{50}s for blue foxes and mink are 10 and 7 mg NDMA/kg bw, respectively.

Fertility of the mink in group 4(P), 5(F_1) and 6(F_2) was not reduced by exposure to NDMA, nor did the mink in groups 5(F_1) and 6(F_2) appear to be more susceptible to NDMA than those in group 4. Transplacental effects of NDMA reported in the syrian hamster (Althoff et al. 1977) with increased tumour incidence in the offspring, are not registered in these experiments.

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An ultrastructural study of inclusion bodies in a systemic distemper virus infection in foxes (*Vulpes vulpes*)

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López, M., F. Guerrero, L. Moya, M.I. Quiroga, R.F. Antonio & J.M. Nieto 1992. An ultrastructural study of inclusion bodies in a systemic distemper virus infection in foxes (*Vulpes vulpes*). Norwegian Journal of Agricultural Sciences. Suppl. no. 9: 444-450. ISSN 0801-5341.

A systemic distemper infection was diagnosed in a farm of fur foxes (*Vulpes vulpes*) by histopathological, ultrastructural and immunohistochemical examinations. Samples from eight farmed foxes were taken in 10% buffered formalin and processed, and 25-30 μm thick sections cut according to the paraffin routine methods were deparaffinated and processed for an electron microscopy study following the Reynold's method. Cytoplasmic and nuclear inclusions were identified by light microscopy and immunolabelling methods. Ultrastructurally, inclusions were characterized as a dense mass of tubular aggregates. This procedure is presented as a system for providing differential identification between inclusions due to distemper and other infections or artefacts.

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Canine distemper virus (DV) infection is reported to be one of the most important prevalent diseases in Spain. The previous principal hosts of DV were dogs and wild foxes, but the increase in fur production, especially in the NW of our country, has enhanced the number of hosts. Other virus infections caused by antigenically similar viruses of DV have recently been reported in Spain in dolphins (Domingo et al. 1990).

Distemper virus infections in foxes were described initially by Green in 1926 (cited by Appel & Gillespie 1972). Recently in Spain the disease has been diagnosed in wild foxes (Badiola, oral communication) and farmed foxes (Nieto et al. 1990) both cases being identified as systemic distemper.

Inclusions in cells have been described in many virus diseases as an important refereny in the pathological diagnosis. Inclusion bodies in distemper (DIB) were reported by Lentz (cited by Goss et al. 1948) and later recognized by numerous authors (Ducatelle et al. 1980; Vandelvelde et al. 1981) as cytoplasmic or nuclear acidophilic amorphous masses surrounded by a clear refringent area. Distemper inclusion bodies are located principally in the epithelia, neurons, and macrophages (Palmer 1990; Nieto et al. 1987), there are considerable variations in size and number of the inclusions (Ducatelle et al. 1980). The persistence of DIB in cells has been described as variable, but always over 1-2

weeks. Inclusions have been found in dogs with acute or subacute distemper, but seldom in the chronic form of the disease. The DV antigen in DIB has been identified by means of immunohistochemical techniques (Ducatelle et al. 1980; Miry et al. 1983; Nieto et al. 1987; Nieto et al. 1991; Palmer et al. 1990).

In the diagnosis, the observation of inclusions in different locations must be interpreted with caution because many other diseases or degenerative lesions in cells could produce similar lesions (Wisnicky & Wipf 1942; Dagle et al. 1979).

The general aspects of the DV-infection in foxes, the histopathological findings and the identification of DV-antigen in foxes were studied previously (Nieto et al. 1990).

The aim of this report is to study the morphology of DIB in foxes in order to facilitate in a differential diagnosis of the disease. The material was obtained from a spontaneous distemper episode in foxes (Nieto et al. 1990).

MATERIAL AND METHODS

Eight six month-old foxes (*Vulpes vulpes*), were received for diagnosis at our Department. The necropsy was performed in all animals and samples were routinely taken for histological and immunohistochemical methods: fixation in 10% buffered formalin, inclusion in paraffin, 5 μm thick cuts, and hematoxilin and eosin and Shorr-S3 stains.

Some cuts were processed according to the indirect immunoperoxidase method using a monoclonal antiserum against the DV nucleocapsid (Orvell et al. 1985).

For the electron microscopy studies we used material from the samples taken previously in 10% buffered formalin. In these cases we obtained 25-30 μm thick cuts which were deparaffinated, and refixed in1% osmium tetroxide in a buffer of 0.05 mol cacodylate. The specimens were then dehydrated and embedded in epoxy resins, 0.5-1 mμ. These sections were obtained and stained with uranyl acetate and lead citrate (Reynolds 1963).

RESULTS

Ligtht Microscopy

Identification of DIB was possible in eight foxes by means of histopathological and the histochemical techniques (Table 1). In two foxes, the epithelia were positive to the IPI but did not contain inclusion bodies, one fox was classified as doubtful in the identification of inclusions but was positive to the immunolabelling.

Small or large cytoplasmic and intranuclear eosinophilic inclusions were identified in the respiratory epithelium (Fig. 1) of the trachea, bronchi and bronchioles and in alveolar and septal macrophages (five foxes); in the epithelium of the urinary bladder (four foxes), renal pelvis (two foxes) and tubules of the nephron (one fox); in the bile ducts of the liver(two foxes)and in the lymphoid cells (one case). In all cases DIB were circular or ovoid with an unstained halo limiting them.

The DIBs, which were brown inside and dark brown in the periphery of the inclusions, were positively identified using the IPI technique.

Table 1. Identification of inclusions and DV antigen in different systems

Location/Case	1	2	3	4	5	6	7	8	
Respiratory	DIB	+	+	+	*	-	+	-	-
system	IPI	+	+	+	+	+	+	+	+
Lymphoid	DIB	+	0	0	0	0	0	0	-
system	IPI	+	0	0	0	0	0	0	+
Urinary	DIB	-	+	+	-	-	*	*	-
system	IPI	+	+	+	-	+	+	+	+
Nervous	DIB	-	-	-	-	-	-	-	-
system	IPI	+	+	+	+	+	+	+	+

+ positive to DIB or antigen identification; - DBI or antigen not identified;* doubtful observation of DIB; 0 without samples;IPI immunoperoxidase technique; DIB distemper inclusion bodies

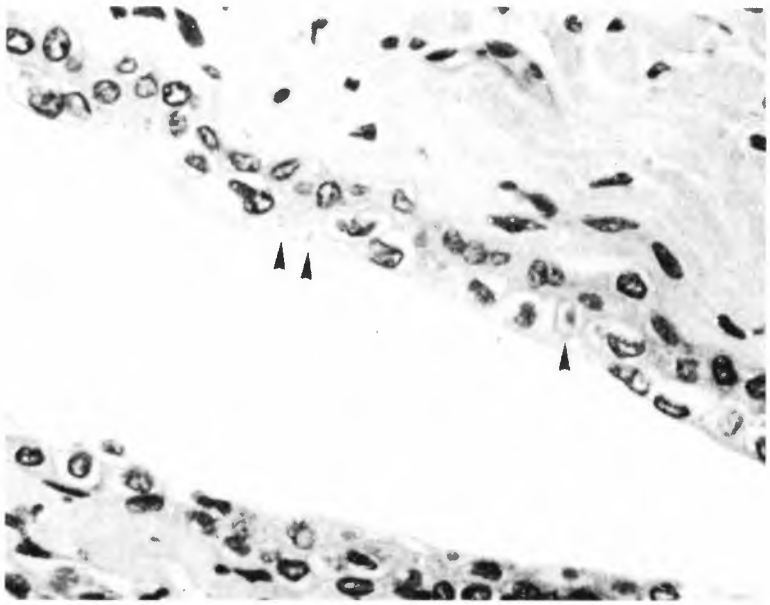

Fig. 1. DIB in transitional epithelium of the urinary bladder. H-E, 400X

Electron Microscopy

By means of ultrastructural examinations it was possible to confirm the presence of inclusions in both nuclear (Fig. 2)and cytoplasmic locations in the same cells as those identified by light microscopy.

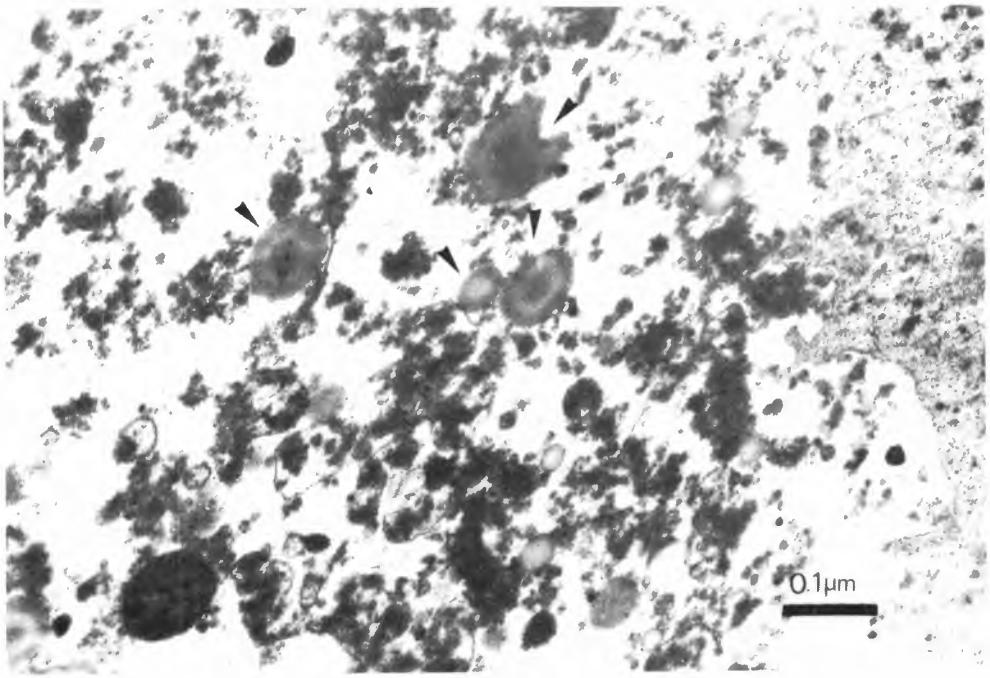

Fig. 2. Intranuclear inclusion (►) in a macrophage infiltrating the interalveolar wall

DIBs were recognized as a dense mass composed of tubular aggregates with a clear limit but never surrounded by a limiting membrane (Fig 3). The pattern of these tubules was variable, some of them having a high electron dense core whereas in most tubules the inner core was less electron dense than the outer rim (Fig. 4). The tubules had a diameter of 15-17 μm.

DISCUSSION

The presence of the filamentous material composing the inclusions is described confirming the diagnosis of DV infections in foxes. Our report indicates the good preservation of the ultrastructure of DIB, although the fixation procedure routine in light microscopy is not the best one for ultrastructural studies. This procedure facilitates the pathologists in their diagnosis of the disease, especially when the results of the immunolabelling are not clear or antibodies against DV are not available.

DIBs are found principally in acute and subacute distemper in dogs (Ducatelle et al. 1980; Vandelvelde et al. 1981), minks (Nieto et al. 1991) and in foxes (Nieto et al. 1990). Inclusions have been one of the most important findings in distemper diagnosis but in some cases the identification of these lesions could be mistaken (Dagle et al. 1979), this was not the case with the immunohistochemical and ultrastructural studies, however.

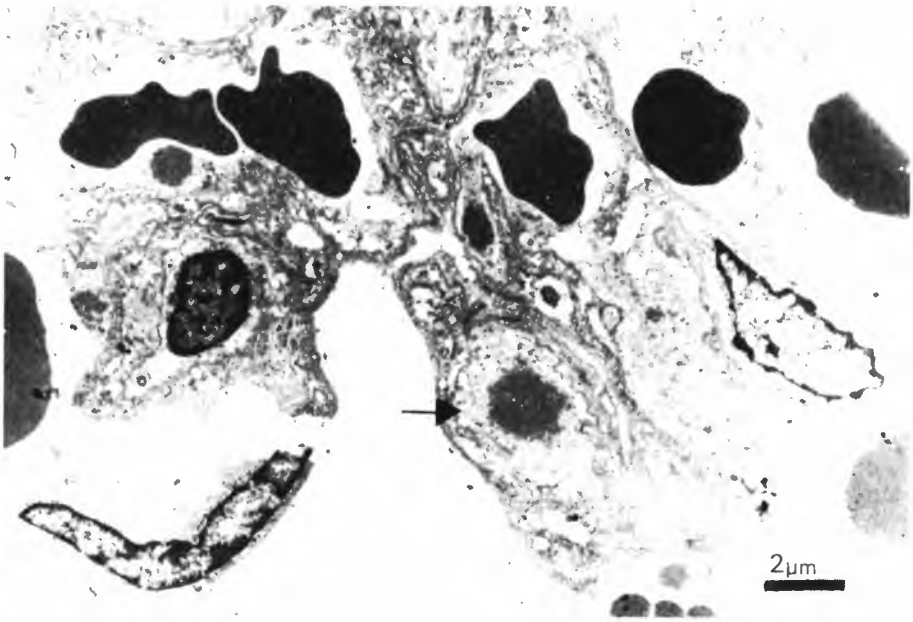

Fig. 3. Intracytoplasmic inclusion (→) in an alveolar cell

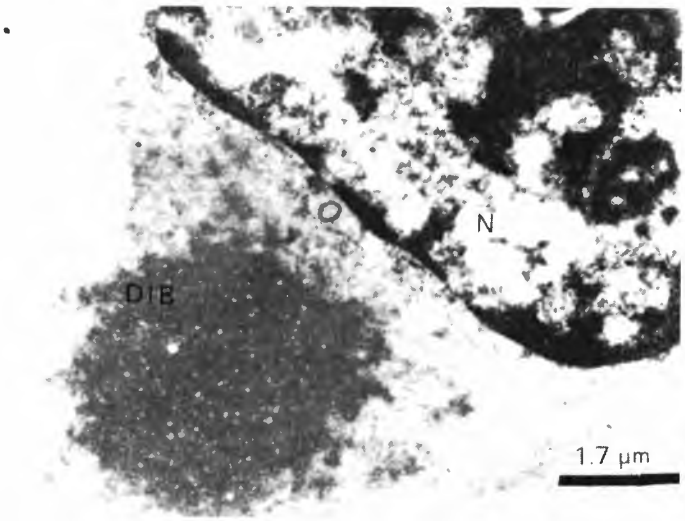

Fig. 4. Tubular aggregate in the cytoplasm of an alveolar cell. DIB distemper inclusion, N nucleus

The presence of filamentous fibres in DV-infected cells was described in several locations such as the epithelium of the urinary bladder in ferrets (Tarawa et al. 1961) or lymphoid cells in mink (Tajima et al. 1971). Koestner & Long (1970) confirmed similar findings in tissue cultures of canine cerebellum describing three phases in regard to location of viral particles. The first phase 7th to 14th post infection days (p.i.d.) was characterized by intracytoplasmic inclusions, the second phase -14th to 21st p.i.d.-, by intranuclear aggregates; and the viral budding along the cellular membranes was the most common feature during the third phase after the 21st p.i.d..

The diameter of the tubules in our material was similar to that described for isolated measles virus (Nakai & Imagawa 1969) and to that described in previous studies (Tajima et al. 1971; Koestner & Long 1970). Two types of tubular strands were also identified and interpreted as incomplete viral capsids in the case of tubules with a low density core and possibly represented the viral coat protein without the nucleic acid core. The number of this type of tubule was higher than that of dense tubules.

The presence of inclusions in the cytoplasm and in the nucleus represents the cellular sites of viral assembly. The maduration of nuclear tubules seems to be impossible, but it has been proposed that intranuclear bodies could be a form of long-term persistence, according to the findings observed in measles virus infection a similar virus disease in humans (Koestner & Long 1970).

ACKNOWLEDGEMENT

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Nursing sickness in female mink (*Mustela vison*)

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Epidemiological and pathophysiological studies on nursing sickness in mink were carried out at Fur Research Farm West. During the breeding seasons of 1989-90 the mean overall incidence rate of nursing sickness amounted to 12.8% with a 7.2% mortality loss. Sick dams raised significantly larger litters and suffered heavier weight losses than apparently healthy females. Postmortem severe dehydration and emaciation were found. Blood sampling in severely affected females disclosed: azotemic acidosis, low base excess, aldosteronism, hyperglycemia and hyperinsulinemia. Plasma concentrations of sodium and chloride were extremely low, while those of potassium, magnesium and phosphate were high. Urinary osmolality and solute concentrations were remarkably low due to impairment of the concentrating ability of the kidneys. In summary, nursing sickness is characterized by severe electrolyte and volume depletion, metabolic derangements and malfunction of several organs, presumably caused by the combined effects of genetic predisposition, inadequate or ceased dietary nutrient supplies during heavy lactation, and environmental stress.

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Nursing sickness is a widespread disease in mink which occur in the latter part of lactation or shortly after weaning (Hartsough 1955; Hunter & Schneider 1991). In Danish mink farms the morbidity and mortality rates of nursing sickness vary considerably between localities and breeding seasons (Henriksen 1985), and the incidence risk is particularly high among multiparous females raising larger litters (Clausen et al. 1992).

In the literature, however, available information on the nature of nursing sickness is scarce (Henriksen 1985; Clausen et al. 1992; Wamberg et al. 1992a) and further studies are greatly needed in order to identify the main factors responsible for the onset and development of the disease in nursing mink.

The present study deals with a retrospective survey of the epidemiological and pathophysiological characteristics of nursing sickness in mink during two successive breeding seasons at the Fur Research Farm West, Holstebro, Denmark. In addition to the main objective, this study provides a series of reference values pertaining to blood and urine of the normal healthy female mink in full lactation.

MATERIALS AND METHODS

Animals

The present study included a total of 3617 lactating mink of the Standard Black and Pastel color mutants of the 1989 and 1990 breeding stocks raised at the Fur Research Farm West (Table 1).

Table 1. Number of dams studied, average litter size and total litter biomass at weaning (day 43)

Type	Standard		Pastel		Average litter size (day 43)		Total litter biomass (day 43)	
	N	%	N	%	Standard	Pastel	Standard	Pastel
Healthy dams	1698	85.2	1457	89.7	4.8 ± 2.0[a]	4.8 ± 2.1	1448 ± 624	1448 ± 588
Sick dams, total	294	14.8	168	10.3	5.4 ± 1.8	5.3 ± 2.0	1635 ± 577	1568 ± 556
Sic, recovered	110	5.5	93	5.7	4.9 ± 1.7	4.8 ± 2.1	1608 ± 608	1429 ± 585
Sic, dead	184	9.3	75	4.6	5.7 ± 1.7	6.0 ± 1.6	1652 ± 559	1731 ± 472
Total	1992		1625					

Sampling of blood and urine

Blood samples were obtained by heart puncture during light sodium pentobarbital anesthesia (Wamberg et al. 1992a) in sick and healthy females, and following an overdose of the anesthetic a number of severely affected dams were subjected to post mortem examination. During the handling of the animals, samples of spot urine were collected and frozen for subsequent analysis. For further details on the analytical methods employed the reader is referred to previous publications (Clausen et al. 1992; Wamberg et al. 1992a).

RESULTS AND DISCUSSION

Epidemiology

According to the data listed in Table 1, the mean overall incidence rate of nursing sickness in lactating mink during the two breeding seasons was calculated to be 14.8% and 10.3% in the Standard Black and Pastel color types, respectively. Similarly, the mean overall mortality rates obtained in this period amounted to 9.2 and 4.6% in the two color mutants, respectively. Sick dams weaned larger litters, on average 0.5 kits per litter more than apparently healthy females (Table 1); and at increasing age nursing females weaned larger litters with a corresponding larger biomass and therefore faced an increasing incidence risk of nursing sickness (Clausen et al. 1992). During the final two weeks of lactation apparently healthy dams and sick dams suffered weight losses of about 14% and 31%, respectively in comparison to the 33% weight loss observed in sick dams that succumbed within the period of study (Table 2). Similar weight losses were observed in the study reported by Hunter & Schneider (1991).

Table 2. Changes in body weight of female Standard Black and Pastel color mutants during lactation

	Standard Black			Pastel		
	Healthy dams	Sick dams		Healthy dams	Sick dams	
		recovered	dead		recovered	dead
No of dams	1698	110	184	1457	93	75
Weight at birth	1150 ± 109[a]	1152 ± 107	1162 ± 117	1079 ± 112	1099 ± 120	1087 ± 124
Weight at day 29[b]	1102 ± 117	1134 ± 120	1104 ± 123	1030 ± 119	1062 ± 122	1036 ± 112
Weight at day 43[b]	963 ± 135	860 ± 176	742 ± 126	869 ± 137	836 ± 162	710 ± 121
% Weight loss day 29 to 43	13%	24%	33%	16%	21%	31%

[a] Values are Mean ± SD

[b] Post partum

Pathology

Clinically, the females suffering from nursing sickness were identified by the following signs: loss of appetite, severe dehydration and emaciation, rapidly increasing weakness, staggering gait, ataxia, lethargy and, in the final state, coma and death. The major postmortem findings included severe dehydration and emaciation, gastrointestinal hemorrhages and melena. On microscopic examination vacuolization of hepatocytes and of renal epithelial cells was found. These findings are in accordance with observations reported previously (Hartsough 1955, Henriksen 1985; Clausen et al. 1992).

Blood and urine

The blood acid-base data listed in Table 3 reflect the development of various degrees of metabolic acidosis and moderate respiratory depression due to the anesthetic procedure.

Table 3. Mean concentrations of whole blood and plasma constituents in sick and healthy nursing mink

	Sick Dams	Healthy Dams	P-value
	n = 31	n = 17	
pH	7.248 ± 0.094[a]	7.312 ± 0.028	NS[a]
PO_2, kPa	7.1 ± 3.7	5.6 ± 1.8	NS
PCO_2, kPa	6.5 ± 2.0	7.5 ± 0.7	NS
BE, mmol/l	-7.0 ± 5.3	0.3 ± 0.4	< 0.01
Sodium, mmol/l	120 ± 12	152 ± 6	< 0.01
Potassium, mmol/l	6.8 ± 1.6	3.5 ± 0.6	< 0.01
Calcium, mmol/l	1.90 ± 0.26	2.32 ± 0.14	< 0.01
Magnesium, mmol/l	2.58 ± 0.57	0.92 ± 0.07	< 0.01
Chloride, mmol/l	81 ± 9	113 ± 4	< 0.01
Phosphorus, mmol/l	9.7 ± 2.3	6.7 ± 0.9	< 0.01
Glucose, mmol/l	23.4 ± 15.5	6.5 ± 1.6	< 0.01
Urea, mmol/l	68.3 ± 33.0	6.0 ± 1.2	< 0.01
Protein, g/l	69.7 ± 15.2	62.6 ± 3.8	< 0.01
Creatinine, µmol/l	122 ± 49	70 ± 8	< 0.01
Insulin, µIU/ml	124 ± 23	21.5 ± 13.4	< 0.01
Aldosterone, pg/ml	1938 ± 925	169 ± 114	< 0.01
Osmolality, mOsm/kg	368 ± 37	319 ± 12	< 0.01

[a] Values are mean ± SD

[b] NS, not significant (> 0.05)

Plasma concentrations of sodium and chloride were extremely low and associated with a tenfold increase in plasma aldosterone. By contrast, the extracellular concentrations of potassium, magnesium and (acid) phosphate were remarkably high. The increase in plasma osmolality was accounted for by the elevated concentrations of urea and glucose and therefor, the effective plasma osmolality turned out to be slightly lower than normal.

The presence of hyperglycemia and a fivefold increase in plasma insulin indicated severe disturbances of carbohydrate metabolism (Wamberg et al. 1992b) along with increased tissue catabolism and malfunction of the liver, the kidneys and neuromuscular tissues.

The urine was almost devoid of sodium and chloride and urinary concentrations of (total) electrolytes and urea were markedly reduced. Accordingly, the concentration capability of the kidneys were reduced to approximately 40% that found in normal healthy mink (Clausen & Hansen 1989; Wamberg et al. 1992a 1992c).

Table 4. Mean concentrations of osmotic active constituents in spot urine obtained from sick and healthy nursing mink

Constituent	Sick Dams	Healthy Dams	P-value
	n = 15	n = 17	
Sodium, mmol/l	2 ± 1[a]	21 ± 9	< 0.01
Potassium, mmol/l	59 ± 19	116 ± 24	< 0.01
Chloride, mmol/l	3 ± 2	80 ± 19	< 0.01
Urea, mmol/l	578 ±116	1281 ±236	< 0.01
Osmolality, mOsm/kg	869 ±172	2116 ±209	< 0.01

[a] Values are mean ± SD

SUMMARY

In summary, nursing sickness is characterized by a poor nutritional status with severe extracellular volume depletion and profound metabolic disturbances, presumably resulting from the effects of inanition and/or, inadequate dietary and water supplies of the nursing female during the latter part of the lactation period (Hartsough 1955; Clausen et al. 1992 Wamberg et al. 1992a; 1992c). In this situation, any additional threat such as acute stress or adverse changes in environmental conditions may be of crucial importance for the development of nursing sickness.

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Metabolic and karyologic analysis of mink lymphocytes

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Samples of total blood from eight standard mink females were used in this experiment. These animals were specifically tested for plasmacytosis. Three of them were found to be negative and antibodies were detected in the other five. The animals were 18 months old, and their weight was 910 ± 125 g at the time of sampling. The aim of this work was to investigate the changes that occurred in oxygen consumption and in the karyotypes of mink infected with the plasmacytosis virus. It was foumd that the curve of maximum O_2 consumption of one cell cycle *in vitro* in standard cultivation conditions peaked within 46 - 50 h after the start of cultivation. The significantly higher values of O_2 in the group of animals with a diagnosis of virus plasmacytosis can be linked with the existence of reactive forms of the lymphocytes which are created as a reaction of the immunity system to the infection and which presumably represent transition cells developing into plasmacytes. The observed lymphocytes did not have any visual structural anomalies of chromosomes in their karyotypes and from this point of view they can be characterized as normal.

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Since the role of lymphocytes in the immunity processes of animals is vitally important, biologists always tend to focus on this reaction of leucocytes. An important factor which is an advantage in work with lymphocytes is that not only are they of biological significance but they are also easily attainable. Vital techniques can be used, and *in vitro* testing is also easily carried out.

When the inner balance of the organisms is impaired by the effect of environmental factors, it is possible to discover changes which are in progress on a subcellular and cellular level at the preclinical stage, e.g. the discovery of infectious mink plasmacytosis. Immunoelectrophoresis is used with some success in the detection of disease. The existence of antibodies in the serum of infected animals can be detected by specific immuno-electrophoretic testing. However, the dynamics of metabolism of competent cells in the immunity system has not been sufficiently investigated.

The aim of this work was to investigate the changes in the oxygen consumption and karyotypes of mink infected with the plasmacytosis virus.

MATERIALS AND METHODS

Samples of total blood from eight standard mink females were used in this experiment. These animals were specifically tested for plasmacytosis. Three of them proved to be negative and antibodies were found in the other five. The animals were 18 months old, and their weight was 910 ± 125 g at the time of sampling.

Blood was sampled under sterile conditions in plastic syringes along with an anticoagulant additive (Heparin Spofa), at a concentration of 1:100. The heparinized blood was left for two hours at room temperature, then 0.3 ml was taken from each sample and placed in a Warburg's test-tube with 2.7 ml of Ml99 medium. The concentration of hydrogen ions in the medium was assessed as pH=7.4. When carrying out direct measurements of 0_2 consumption it is necessary to ensure the absorption of CO_2 released when the physiological concentration of CO_2 in the gaseous phase of the measured volume is preserved. In order to do this a "CO_2-buffer" (0.5 ml 6N HCl +1ml diethylamine + 0.3 g KHCO3) was used for calibration of the CO_2 concentration.

Proper measurements were carried out in sterile conditions at a temperature of 37°C and at intervals of 28, 44, 48 and 70 h after the start of cultivation. The absolute values of oxygen consumption thus obtained (in mm^3) were calculated to the concentration of total proteins in the observed intervals. Lymphocytes were treated to permanent preparations after the cultivation finished and the mitosis was blocked at the metaphase stage. From these preparations the structural changes in chromosomes were analysed visually.

RESULTS AND DISSCUSION

Values of oxygen consumption were obtained during the cultivation periods and these are listed in Table 1. The highest O_2 consumption was in the 48th hour of cultivation,within all observed periods, the activity decreased in 70 h to the level of metabolic activity which

Table 1. Oxygen consumption means of lymphocytes from mink blood (mm^3. g^{-1}prot.)

Animal	Test	Hours of cultivation 28	44	48	70
1	-	51.06	98.34	143.20	49.00
2	-	50.96	85.99	125.12	47.31
3	-	50.22	87.40	136.25	51.28
4	+	55.50	125.94	212.43	60.26
5	+	62.43	136.14	198.54	58.94
6	+	67.28	142.73	226.86	65.26
7	+	70.96	148.24	242.85	69.38
8	+	67.90	146.36	238.37	65.58

+ ≤ 0.01

was reached in the 28th hour of cultivation. The activity of lymphocyte aspiration of the group of animals tested as negative to the presence of antibodies against the plasmacytosis virus (group 1) was compared with that of the group of animals tested as positive (group

2) and we can state that there are statistically significant differences in all observed periods (Table 2). On the basis of earlier methodological data, it is assumed that 48h after the start of cultivation the mitosis of lymphocytes is in progress, and it is also assumed that in connection with this the energey system of the cell is active and therefore the O_2 consumption is increased.

Table 2. Statistical significance of mean differences between infected and non-infected groups of animals

Group	Hours of cultivation 28	44	48	70-77
1(1+2+3)	50.74 ± 0.27	90.58 ± 3.90	134.86 ± 5.26	49.20 ± 1.15
2(4+5+6+7+8)	64.81 ± 2.70	134.88 ± 4.05	223.81 ± 8.22	63.88 ± 1.91
t-test	5.88++	8.76++	9.11++	6.60++

++ ≤ 0.05

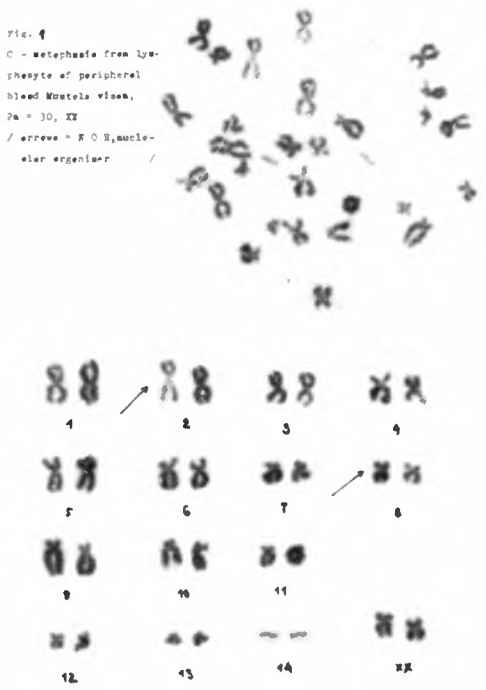

Fig. 1. C - metaphasis from lymphocyte of peripheral blood *Mustela vison*, 2n = 30, XX/arrows = N O R, nucleelar erganiser

On the basis of previous measurements of oxygen consumption by leucocytes of other species (ferret, rabbit) it is assumed that the curve of maximum O_2 consumption of one cell cycle *in vitro* under standard cultivation conditions peaks within 46-50 h after cultivation begins. The significantly higher values of O_2 in the group of animals with a diagnostic of virus plasmacytosis are perhaps connected with the occurrence of reactive forms of lymphocytes which are created as a reaction of the immunity system to the infection and which presumably represent transition cells developing into plasmacytes.

After karyological analysis (Fig. 1) of the metaphase it was stated that the observed lymphocytes did not have any visual structural anomalies of the chromosomes in their karyotypes and from this point of view they can be characterized as normal. Fig. 1 illustrate the metaphase and the karyotype of female 2n=20, XX. The karyotype contains 14 pairs of autosomes and one pair of gonosomes, X-chromosomes. Chromosomes Nos.1-4 are metacentric, Nos.5-8 are submetacentric, Nos.9-11 are subtelocentric and homologue No.14 is acrocentric. Autosomes Nos.2 and 8 have the nucleolar organizer - NOR.

Different pathogenicities of two Aleutian disease virus (ADV) strains in Norway

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Pathogenic variation of Aleutian disease virus (ADV) was suspected in Norwegian (N) mink farms on clinical and epidemiological grounds. An experiment was carried out in standard (St) and sapphire (Sa) (Aleutian) mink (M) to elucidate this suspicion. Virus material from one typical high-virulence farm (NADV1) and from one typical low-virulence farm (NADV2) were passaged once in StM before being inoculated into groups of StM and SaM in a 12-week experiment. Sequential blood samples were analyzed for concentrations of γ-globulin (%) and antibody measured in a counter immuno-electrophoresis (CIEP) test. In both StM and SaM there were significant differences (Student's t-test) in γ-globulins at weeks 4, 6, 8, 10 and 12; NADV1 had the highest values throughout. In SaM, γ-globulin concentrations were higher than those in StM for both NADV1 and NADV2 and the differences were less marked. There were significant differences between NADV1 and NADV2 CIEP titers for StM at weeks 1, 2, 10 and 12; for SaM at weeks 2, 3, 10 and 12. There were significant differences (analysis of variance) between NADV1 and NADV2 in body weight changes, kidney weight and platelet counts at euthanasia in week 12. It was concluded that NADV1 is a high-virulence strain and NADV2 is a low-virulence strain. The standard mink appears to be a better type than the sapphire mink for differentiating pathogenicities of ADV strains.

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Aleutian disease (AD) of mink, originally seen in 1946 and first described by Hartsough & Gorham (1956), is a persistent virus induced immune complex disease of considerable economic importance in commercial mink farming. In Denmark the loss in mink production ascribed to AD was estimated at $10 million per year (Aasted 1985). The causative agent has been characterized as a parvovirus (Shahrabadi et al. 1977; Bloom et al. 1980; Aasted 1980) and accepted as a member of *Parvoviridae* (Matthews 1982).

The name Aleutian disease was used since it caused severe disease in farms raising Aleutian mink. Many color phases of mink can be infected with the AD virus (ADV),

which produces varying degrees of disease, immune response and γ-globulin concentrations depending on viral strain and type of mink. Natural AD infections have only been observed in mink and ferrets, but antibodies have been detected in other species including wild skunk, wild fox, wild racoon and in man (for references see Aasted 1985). Experimental infections of dogs and cats have induced antibody responses (Gorham et al. 1976). In Danish transmission studies, organ materials from eight different species were taken four weeks after inoculation. Test inoculation in ADV negative mink resulting in seroconversion showed that mink, ferrets, Finn racoons, dogs, cats and mice had infectious virus, but not blue foxes or rabbits (Alexandersen et al. 1985).

Pathogenic variation of ADV was suspected in Norwegian mink farms, based upon clinical and epidemiologic grounds. It was important for the industry to find out whether production variations on some farms were due to differences in husbandry management, stress factors (An et al. 1978) and environmental conditions or due to variations in virulence of field strains of ADV (Gorham et al. 1976). Virus material was obtained from one farm with suspected "high virulence" virus and from one farm with suspected "low virulence" virus. The two materials were tested in standard and sapphire mink. Antibodies to ADV and γ-globulin concentrations were measured sequentially and various blood and organ criteria were examined at euthanasia 12 weeks after inoculation.

MATERIALS AND METHODS

Viruses & inocula preparation

Virus material were obtained from farms with high percentages of counter immuno electrophoresis (CIEP) seropositive mink.

Norwegian Aleutian disease virus 1 (NADV1), suspected to be a "high virulence" strain, was obtained from farm A, which had about 2,500 females farmed under an extensive type of management and consequently a low labor cost per animal. Reproduction had been poor, about three kits per female, and about 90% were seropositive. There had been a relatively high culling rate due to ADV infection-related diseases, and such animals displayed histopathologic changes that were characteristic for AD.

Norwegian Aleutian disease virus 2 (NADV2) suspected to be a "low virulence" strain, was obtained from farm B, which had about 400 females and a labor-intensive management system. These mink were also about 90% seropositive. Earlier reproduction had been poor, but had later improved significantly. There had been only a few AD mink in recent years, which could be partly explained through culling of iodine agglutination positive animals.

From each of farms A and B, pooled spleen, liver and kidney inocula from three seropositive mink were prepared for test inoculation. Twenty percent organ suspensions were made in PBS with 200IU penicillin, 200 μg streptomycin and 2.5 μg amphothericin B/ml, gauze filtered and centrifuged at 10,000 rpm for 2 h. Supernatants were used to inoculate three mink per pool. Mink inoculated with material from farm A (NADV 1) were sacrificed on day 10 postinoculation (p.i.) and pooled inocula were prepared as described above. Mink inoculated with material from farm B (NADV 2) were sacrificed on day 13 p.i. and prepared in the same way. These six mink had positive CIEP reactions, although

the day 10 samples were weak. Early histopathologic AD changes were seen in lymphnodes (mink sacrificed on days 10 and 13), in spleen and liver (mostly in those sacrificed on day 13), while changes in kidneys were seen only in animals sacrificed on day 13.

Mink

Female standard (St) and sapphire (Sa) mink (M), aged about 10-11 months, were obtained from two farms known to be free from AD for at least 15 years and all animals in both farms had negative CIEP tests. They were vaccinated against distemper, mink enteritis and botulism type C.

Counter immuno electrophoresis (CIEP)

CIEP was performed largely as described by Cho & Ingram (1972) and Bloom et al. (1975) with 0.7% high-m‚agarose (Bio-Rad) in high resolution buffer pH 8.8 with 0.01% sodium azide. Antigen was kindly supplied by Dr. Kammer, Invenex Veterinary Laboratories, Wisconsin, USA. Slides were electrophoresed for 55 min at 4 volts/cm and then viewed under indirect illumination. Results were read separately by two observers.

Serum γ-globulin

Concentrations of serum γ-globulin were determined by electrophoresis on cellulose acetate with a high resolution buffer. Recorded densitometer scannings were read as described by Tabel & Ingram (1970) and evaluated as described by An & Ingram (1977).

Blood and organ examinations at euthanasia

Body weights were recorded at the start and at the end of the experiment. For evaluation of weight changes during the experimental period, only those animals that survived were included. Organ weights were recorded at euthanasia. Platelets (Pl) and white blood cell (WBC) counts were made in an electronic cell counter. Packed cell volumes were determined with a microhematocrit centrifuge. Differential WBC counts were done on stained (Wright-Leishman) bloodsmears according to Schalm et al. (1981).

Experiment design

The experiment was conducted over 12 weeks beginning in mid-February. The mink were divided into four experimental groups of 5, sapphire and standard mink were infected with NADV1 or NADV2 and kept in individual cages in separate rooms. Each mink was inoculated intraperitoneally with 2 ml virus suspension. They were fed a standard ranch mink diet with free access to water. Blood for CIEP and γ-globulin assays were obtained by toenail capillary tube bleeding preinoculation and at 1, 2, 3, 4, 6, 8 10 and 12 weeks p.i.. Mink were weighed 2-4 days before inoculation and at euthanasia 12 weeks later.

An overdose of barbiturate intraperitoneally was used for euthanasia. Animals that died during the experiment were autopsied and microscopic sections from various lymphnodes, spleen, kidney, liver, bone marrow, heart, lung, brain, nasal area and adrenenal gland were examined.

Statistical evaluation

Student's t-test was used to analyze differences between means of the various groups

(Fig.1). Analysis of variance with the Duncan's multiple range test in the GLM procedure in SAS (SAS Institute Inc. Cary, NC, USA) was used as indicated (Table 1).

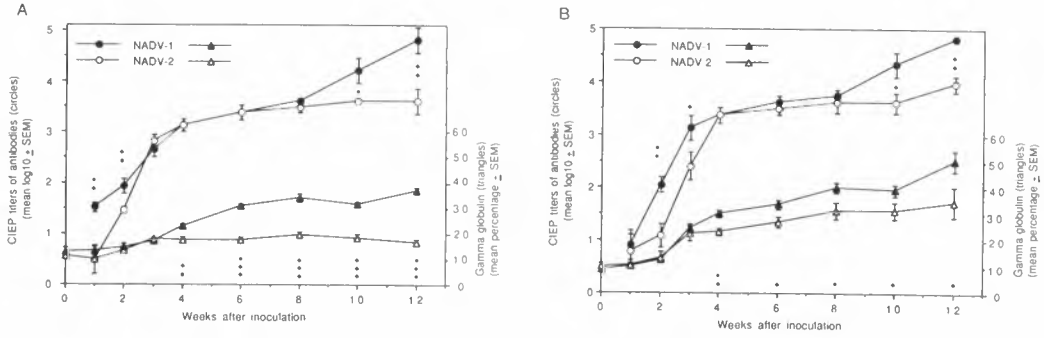

Fig. 1. Standard mink (A) and sapphire mink (B) infected experimentally with two Norwegian strains of ADV, NADV1 or NADV2 (-●- or -○- for CIEP antibody titers and -▲- or -△- for γ-globulins). Preinoculation CIEP readings are not included as the mink had no detectable antibody. Significant differences between pairs of antibody titers and γ-globulins (Student's t-test) are indicated as *, ** and *** for $p<0.05$, $p<0.01$ and $p<0.001$, respectively. Vertical bars indicate ± standard error of mean (±SEM)

Table 1. Standard and sapphire mink infected experimentally with two Norwegian strains of ADV, NADV1 or NADV2. Statistical comparison of various mean values of groups at euthanasia 12 weeks p.i.

Group No	ADV strain	Mink type	Body weight change[5] %[7]	Organ weight (g) Liver	Spleen[6]	Kidney[5]	Counts/mm³ Pl[1,5]	WBC[2]	Lc[3] %	PCV[4] %
1 (n=4)	NADV-1	Standard	-8.6	54.3	9.0	7.7	382.8	5,500	51.8	39.0
2 (n=4)	NADV-2	Standard	+7.7	62.3	7.4	7.5	1005.0	5,975	43.5	42.0
3 (n=4)	NADV-1	Sapphire	-21.3	56.9	20.5	10.0	450.5	7,150	34.0	32.0
4 (n=5)	NADV-2	Sapphire	+6.7	60.6	16.8	7.2	512.2	7,460	35.0	36.8

1) Platelets x1,000; 2) White blood cells; 3) Lymphocytes; 4) Packed cell volume (hematocrit)
5) Significant virus strain difference ($p<0.05$) according to analysis of variance,
6) Significant mink type difference ($p<0.01$) according to analysis of variance,
7) Calculated on those animals that were killed at 12 weeks

RESULTS

Antibodies to ADV by CIEP tests

Using Student's t-test, significant differences were determined at the beginning and at the end of the experiment. For StM the significances were $p < 0.01$ for weeks 1, 2 and 12, and $p < 0.05$ for week 10 (Fig. 1A). For SaM the significances were $p < 0.01$ for weeks 2 and 12, and $p < 0.05$ for weeks 3 and 10 (Fig. 1B). Titer values for NADV1 were higher than those for NADV2 for both StM and SaM. StM and SaM infected with NADV1 had the same high titers at 12 weeks, i.e., mean 65,536 ($\log_{10}$ 4.8165). Mink infected with NADV2 had different titers at 12 weeks, StM were lower with a mean of 4,096 ($\log_{10}$ 3.6124) than SaM with a mean of 9,410 ($\log_{10}$ 3.9736).

Serum γ-globulin

Percentages of serum γ-globulin showed more consistent and clear-cut significant differences in StM than in SaM, values for NADV1 were higher than for NADV2 for both mink types (Table 1A and B). For StM the significances were $p < 0.01$ for week 4, and $p < 0.001$ for weeks 6, 8, 10 and 12. For SaM the significances were $p < 0.01$ for week 4, and $p < 0.05$ for weeks 6, 8, 10 and 12. StM infected with NADV1 exceeded the 21% limit considered as hypergammaglobulinemic (An & Ingram 1977) on sera obtained at week 4 with a mean of 23.9%, StM infected with NADV2 never exceeded this limit, the highest value being 19.6% at week 8. SaM exceeded the 21% limit at week 3 for both NADV1 and NADV2 infections with means of 24.3% and 22.4%, respectively.

Blood and organ examinations

Various measurements taken mainly at euthanasia are recorded in Table 1. Significant viral strain differences ($p < 0.05$) were observed in body weight changes, renal weights and platelet counts. A significant mink type difference was noted for spleen weights ($p < 0.01$).

ADV associated disease

Submandibular abscesses were seen in each of two SaM in group 3. Three mink died during the study (weeks 7, 9 and 11 from groups 1, 2 and 3, respectively), and each animal had histologic evidence of terminal bacteremia. One died from acute gastric hemorrhage, one had severe suppurative rhinitis and cellulitis, and the third mink had widely disseminated bacterial embolism and infection secondary to bacterial endocarditis. The three mink had average or higher antibody titers and average γ-globulin concentrations the last of the preceding tests, and lesions were consistent with AD for two of the animals (died weeks 9 and 11).

DISCUSSION

Variations in ADV strain properties and pathogenesis (Karstad & Pridham 1962; Gorham et al. 1964; Eklund et al. 1968; Porter et al. 1969; Porter et al. 1977; Porter et al. 1980; Bloom et al. 1980; Porter & Cho 1980; Aasted 1980, Aasted et al. 1984a; Hadlow et al.

1983; Hadlow et al. 1984; Lochelt et al. 1987) and in mink breed susceptibility (Gorham et al. 1965; Eklund et al. 1968; Padgett 1969; Porter et al. 1973; Johnson et al. 1975) have been reported. In the present work two clinically and epidemiologically different Norwegian ADV strains, NADV1 and NADV2, were examined in a 12-week experiment with standard (St) and sapphire (Sa) mink (M).

SaM infected clinically with NADV1 became gradually emaciated and two SaM developed abscesses, and three animals were found dead towards the end of the experiment. Based on histological and other evidence it was considered that these animals died from conditions associated with primary ADV infection. Few symptoms and low mortality are not unusual even for the more virulent virus strains up to 12 weeks p.i.. Similar clinical observations have been recorded on a sequential basis by other authors (Eklund et al. 1968; Bloom et al. 1975); Hadlow et al. (1983) emphasized in their work, which is similar to ours, the need to distinguish between infection and disease when mink are exposed to ADV.

γ-globulin and antibody determinations are together probably the most important measurable disease indications available for the live animal. Clinical disease is associated with increasing amounts of serum γ-globulin. Mink γ-globulin levels above 21% [representing the mean of normal sera of 13.2% + 3xSD of 2.6% = 21% have been considered as hypergammaglobulinemic (An & Ingram 1977). It is, however, not clear how this is related to the terms "inapparent" or "non-progressive AD" that have often been used (An & Ingram 1978; An et al. 1978).

Aasted (1985) has used the following virulence definition based on various authors: 1) Highly virulent means that the isolate causes AD in mink regardless of genotype; 2) low virulent means that the isolate causes AD in Aleutian mink (SaM), but not in non-Aleutian mink (StM). NADV1 is considered a highly virulent strain based on this definition and on the data from the four experimental groups. There were a steady rise in γ-globulins up to week 12 for both types of mink, reaching nearly 40% in StM and over 50% in SaM (Fig. 1A & B). On the same basis NADV is characterized as a low virulent strain. γ-globulin increased steadily to about 35% in SaM, whereas in StM it rose transiently to nearly 20% and then declined at week 12 to below 17%. Other authers have reported similar transient rises in serum γ-globulin (Padgett 1969; Hadlow et al. 1984).

It should be noted that the mean preinoculation γ-globulin was actually lower for the ten SaM in groups 3 and 4 [9.52% ± 0.64% (SEM)] than for the 10 StM in groups 1 and 2 [12.13%±0.95%]. Furthermore, a non-infected SaM group tested in parallel with the four experimental groups showed variations in γ-globulin concentrations during the 12-week period from 8.70% ± 1.04% to 16.30% ± 1.25%; similar variations have been reported by other authers (An & Ingram 1977). Considerable individual variations were seen in our work and have been reported by others (Hadlow et al. 1984). It would be reasonable to conclude that the majority of group 2 StM had non-progressive infections, as only one of these mink had γ-globulin percentages above 21% at any stage during the 12 weeks, i.e., the mink that died in week 9 had 25% γ-globulin in week 8.

Antibody response to ADV and to other antigens have been reported by Porter & Cho (1980) and several other authors (for references see Aasted 1985). The correlation between the amount of specific antibodies and γ-globulin concentration is difficult because different units are used. Porter et al. (1984) found that γ-globulin concentrations of over 80% constituted a specific antibody to ADV. Aasted et al. (1984b) on the other hand, found

values varying from 4% to 57%. Thus, determination of a specific antibody is a less reliable indicator of AD than γ-globulin measurements. In the present work Student's t-test was used to compare CIEP test titers of the two ADV strains and it was found that antibody titers were significantly different at the beginning and at the end of the test period for both mink types. CIEP test results support the γ-globulin data.

At the end of the experiment 12 weeks p.i., the mink were sacrificed and various data were recorded and statistically analyzed (Table 1). In general, the results must be considered to support the above described evidence that the two strains examined were pathogenetically different. Significant viral strain differences were seen for body weight change, kidney weights and platelet counts. Eklund et al. (1968) showed initial platelet counts of 6-9 x $10^8 mm^3$ that dropped over a period of 12 weeks. In the present experiment there was a remarkable difference in that platelet counts were high for low virulent virus (NADV2) in StM. PCV data did not show virus strain difference, but it may be noted that, for instance the lowest value in group 3 at 32% (Table 1) was considerably below the 45.3% that was recorded in a non-infected SaM group tested in parallel. Similar observations have been reported by Eklund et al. (1968), and this is an indication of anemia. WBC values for normal female mink aged about 8 months old are given as 10.14 $\pm$ 2.79(SD)x10^3 by Berestov & Brandt (1989), but the type of mink is not stated. All our groups of infected mink had lower values and there was no significant viral strain difference.

CONCLUSIONS

Standard and sapphire mink kept under the same environmental and husbandry conditions responded to infection with two ADV strains in a way that satisfactorily proves different pathogenicities, i.e., NADV1 is a high-virulence strain and NADV2 is a low-virulence strain. The standard mink appears to be a better type than the sapphire mink for differentiating pathogenicities of virus strains.

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Marginal effects of levamisole and isoprinosine on pathogenesis of Aleutian disease virus infection in sapphire mink

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Levamisole and isoprinosine have been reported as having an influence on the pathogenesis of some virus infections in animals. An experiment lasting 12 weeks was carried out to examine whether these two drugs (two dosage levels each) separately or in combination could influence the pathogenesis of infection with Aleutian disease virus (ADV) in sapphire (Aleutian-*aa*) mink. With few exceptions, there were no significant differences at the various counter immunoelectrophores testing points (CIEP) in antibody titers, g-globulin concentrations and lymphocyte proliferations (Con-A) when both infected and non-infected drug-treated groups were compared with corresponding untreated groups. Isoprinosine and combination groups had lower CIEP and γ-globulin values at some early and late testing points. At six weeks levamisole appeared partly to restore suppressed Con-A-induced proliferation of lymphocytes caused by ADV infection. At euthanasia, no major differences in blood and organ values were found within infected or non-infected groups. The high dose levamisole treated group in each of these categories had significantly higher platelet counts than the corresponding untreated groups. Levamisole, isoprinosine or a combination of the two, had only a marginal influence on the pathogenesis of highly virulent Aleutian disease virus infection in sapphire mink.

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Aleutian disease (AD) of mink, originally seen in 1946 and first described by Hartsough & Gorham (1956), is a persistent virus-induced immune complex disease of considerable economic importance in commercial mink farming. Variations in virulence of virus strains and type of mink can influence the pathogenesis of the infection, giving various degrees of disease as well as inapparent infection (for references see Hyllseth et al. 1992). Efforts to find suitable control measures, inclusive experimental vaccination (Russel 1962; Karstad et al. 1963; Porter et al. 1972) and immunosuppression (Cheema et al. 1972) have generally been unsuccessful. Eradication programs have been developed and have proved very successful (Aasted 1985).

The findings of Kenyon (1978) that treatment of naturally infected mink with the immune modulator levamisole improved health status, reduced γ-globulins and had a positive effect on body weight, indicated that this drug merited further investigation. Reviews (Janssen 1976; Symoens & Rosenthal 1977; Symoens et al. 1979; Russel 1980; Renoux et al. 1980) and other reports pointed to levamisole as a drug with potential influence on the pathogenesis of AD, a disease which Porter & Cho (1980) had termed "A model for persistent infection". Levamisole had been tested mostly with herpes virus infections, either suppressing (Irwin et al. 1976) or enhancing immune responses (Wiesener 1978; Babiuk & Misra 1982), and had shown promising results in treating acute viral hepatitis (Par et al. (1977).

Isoprinosine had shown various effects on different virus infections. The drug decreased morbidity and mortality in animals infected with herpes- and influenza virus (Chang & Weinstein 1973), but had no significant effects against human influenza (Khakoo et al. 1981) and human hepatitis A virus infection (Lam et al. 1978). Several reviews had reported on the antiviral, immunologic and clinical effects of isoprinosine on virus diseases (Glasky et al. 1975; Chang & Heel 1981; Wybran et al. 1982).

The present experiment was designed to investigate the possible influence of levamisole and isoprinosine treatment on the pathogenesis of infection with a highly virulent ADV strain in sapphire (Aleutian-*aa*) mink.

MATERIALS AND METHODS

Virus

Norwegian Aleutian disease virus 1 (NADV1), a highly virulent ADV strain, was prepared and tested as described by Hyllseth et al. (1992).

Mink

Sapphire mink, about 10 months old, were obtained from a farm known to be free from AD for at least 15 years and all animals in the farm had shown negative counter immunoelectrophoresis (CIEP) tests. They were vaccinated against distemper, mink enteritis and botulism type C

Drugs (single or in combination) given intraperitoneally three times per week

Levamisole phosphate, 13.65% injectable solution ("Levasole", Pitman-Moore), was diluted in Hanks' balanced salt solution (HBSS) to give 5 mg/kg to mink in groups 2 and 8, and 1mg/kg to mink in group 3 (see Table 2). Isoprinosine, supplied by Newport Pharmaceuticals International, Inc. (Newport Beach, Ca., USA) was diluted in HBSS to give 200 mg/kg to mink in groups 4 and 9, and 40 mg/kg to mink in group 5. A combination of 1mg/kg levamisole and 40 mg/kg isoprinosine (low doses) was given to group 6 mink.

Lymphocyte transformation test (LTT)

All handled and treated of blood samples were kept on ice at 4°C. Ten millilitres heparinized blood (30 units/ml) diluted to 28 ml with diluent [CMF-Hanks' containing

antibiotics (100 IU/ml penicillin and 100μg streptomycin) and 10 units/ml of heparin] was underlayed with 22 ml Ficol-Hypaque, 1077 g/ml (Pharmacia), and centrifuged (4°C) for 30 min at 750G. The leukocytes were collected and suspended in diluent to 50 ml and centrifuged at 200G for 10 min to sediment the cells into a pellet. The supernatant was discarded, and this constituted the first wash. Hypotonic lysis was used for 1 min to remove the remaining RBC in the cell pellet and a second wash without heparin was performed.

Pelleted cells were suspended in 2 ml cell culture medium RPMI 1640 (Gibco) with antibiotics and 1% inactivated (56°C 30 min) normal mink serum. Samples were then examined for viability with trypan blue (mostly >95% viable), total and differential cell counts and then diluted in medium to contain ~250.000 mononuclear cells in 200 μl medium suitable for each well in a 96-well microplate.

Two triplicate microcultures (@ 200μl) were prepared for each mink. Twenty microlitres of RPMI was added to each of three control cultures, and 20 μl RPMI containing concanavalin A (Con A) (Sigma) to give a final concentration of 7 μg/ml was added to each of three mitogen cultures. The cultures were incubated for two days at 37°C in a humidified atmosphere containing 5% CO_2. Twenty microlitres RPMI containing 1 μCi of ^{3}H-thymidine (specific activity 6.7 Ci/mM, New England Nuclear, Boston) was added to each well. The cultures were incubated for an additional 20 h before standard harvesting and scintillation counting of radioactivity were carried out. Means of triplicate counts per minute (CPM) for each animal in a group were used for statistical evaluation. The stimulation index (SI) was calculated as follows:

SI = mean cpm in cultures with Con-A/mean cpm in cultures without Con-A.
These values were then treated as described under statistical evaluation.

Counter immuno electrophoresis (CIEP), serum γ-globulin, blood and organ examinations at euthanasia

CIEP tests, γ-globulin determinations as well as body weights, blood and organ examinations were carried out as described by Hyllseth et al. (1992).

Experimental design

The experiment was carried out over a period of 12 weeks beginning in mid-February. The mink were divided into experimental groups of 5, with the exception of group 7, which had 4 animals (Table 2). They were kept in individual cages in two separate rooms, one for the infected groups 1-6, the other for the three non-infected groups 7-9.

Each mink in the infected groups 1-6 was inoculated with 2 ml NADV1 virus supension intraperitoneally. Drugs were administered three times a week (Monday, Wednesday and Friday). The animals were fed a standard ranch mink diet with free access to water. Blood for CIEP and g-globulin assays was obtained by toenail capillary tube bleeding before inoculation and at 1, 2, 3, 4, 6, 8 10 and 12 weeks postinoculation (p.i.).

Heparinized blood for LTT was taken by cardiac puncture following sedation/analgesia at weeks 6 and 12 p.i.. A mixture of xylazine and ketamine hydrochloride (8 mg and 40 mg/ml, respectively) was given intramuscularly at 0.5 ml/kg live weight. Induction time was about 3-5 min and average duration was 30-45 min.

The mink were weighed 2-4 days before inoculation and at euthanasia. An overdose

of barbiturate was used for euthanasia. Animals that died during the experiment were autopsied and microscopic sections from various lymph nodes, spleen, kidney, liver, bone marrow, heart, lung, brain, nasal area and adrenenal glands were examined.

Statistical evaluation

Student's t-test was used to analyze differences between means of the various groups (Figs.1 & 2 and Table 1), and an analysis of variance with the Duncan's multiple range test in the GLM procedure in SAS (SAS Institute Inc. Cary, NC, USA) was used to compare blood and organ values (Table 2).

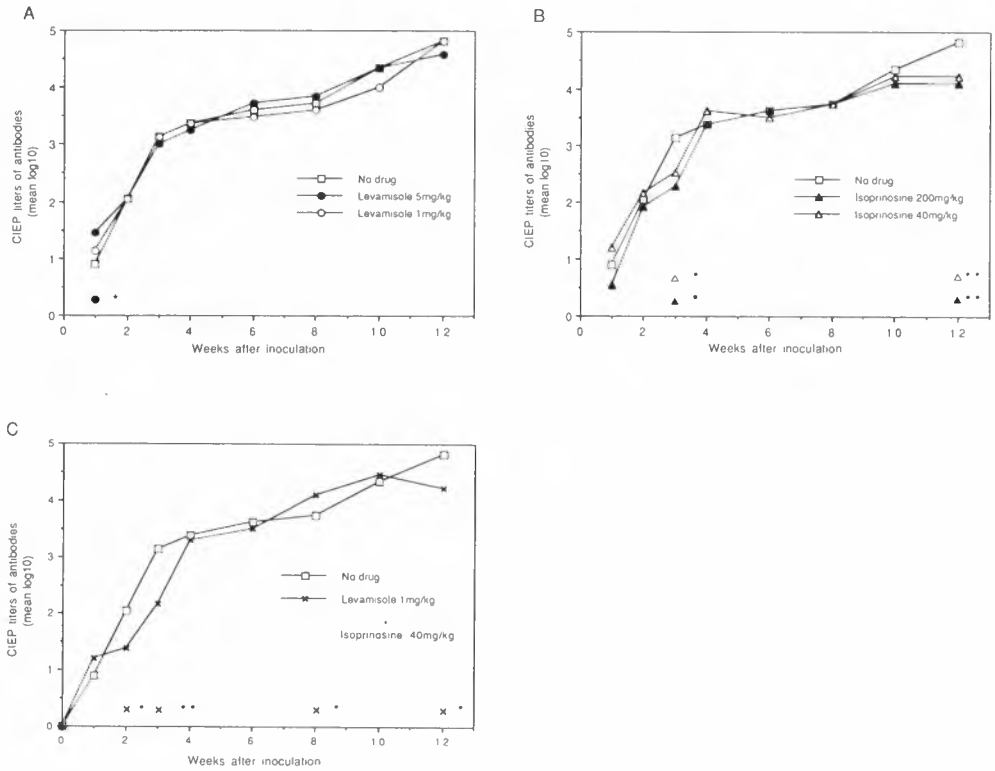

Fig. 1. Sapphire mink infected experimentally with a highly virulent strain of ADV (NADV1) showing means of CIEP antibody titers for untreated (control/no drug) group (- □ -) that are common for A, B and C. High and low doses of levamisole are shown in A (- ● - or - ○ -), high and low doses of isoprinosine in B (- ▲ - or - △ -), and a combination of low doses of levamisole and isoprinosine in C (- × -). Preinoculation CIEP readings are not included as the mink had no detectable antibodies. Significant differences between mean antibody titers of treated and control groups (Student's t-test) are indicated as * and ** for $p < 0.05$ and $p < 0.01$, respectively

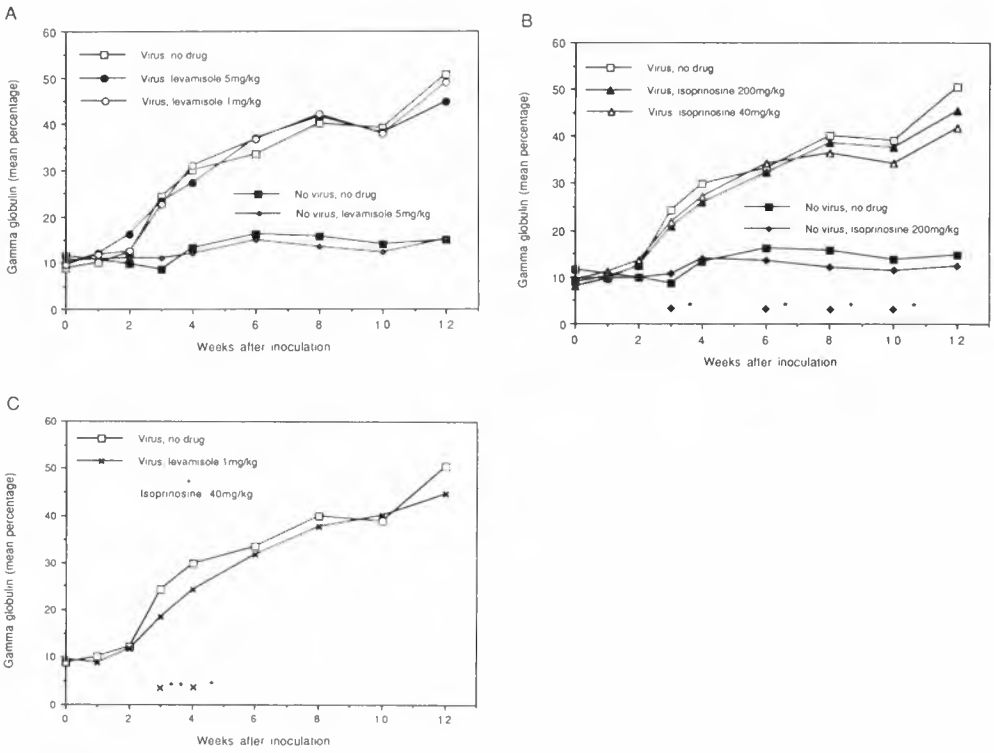

Fig. 2. Sapphire mink infected experimentally with a highly virulent strain of ADV (NADV1) showing means of g-globulins for untreated (control/no drug) group (- -) that are common for A, B and C. High and low doses of levamisole are shown in A (- - or - -), high and low doses of isoprinosine in B (- - or - -), and a combination of low doses of levamisole and isoprinosine in C (- -). Three non-infected groups are presented in A and B. Untreated group (- -) is common in both A and B. High dose levamisole is presented in A (- -), and high dose isoprinosine is presented in B (- -). Significant differences between mean g-globulin percentages of treated and control groups (Student's t-test) are indicated as * and ** for $p < 0.05$ and $p < 0.01$, respectively

RESULTS

Antibodies to ADV by CIEP tests

Specific antibody responses in experimentally infected mink are shown in Fig. 1A, B and C. Only small variations in antibody titers were observed. Significant differences were seen between untreated infected groups and both high and low dose isoprinosine groups (B) at weeks 3 and 12. The combination group (C) was lower at weeks 2 and 3, higher at week

8, and lower at the end of the experiment. Levamisole had little influence on specific antibody responses, the only significant difference was for the high dose group on samples obtained in week 1 (A). At weeks 10 and 12 low and high doses, respectively, of levamisole were below the untreated group, but the differences were not significant.

Table 1. Sapphire mink inoculated with a virulent Norwegian strain of ADV (NADV-1) and treated 3 x weekly with levamisole (Lev) or isoprinosine (Iso). Concanavalin-A-induced proliferation of peripheral blood lymphocytes in LTT at 6 and 12 weeks[1]

Group No.	Virus/ non-infected	Treatment	6 weeks after inoculation CPM[2]		SI[3]		12 weeks after inoculation CPM		SI	
1. (n=5)	Vi	No drug	470	A B[4]	2.24	A	8,233	A B	20.9	A
2. (n=5)	Vi	Lev 5 mg/kg	7,606	A	16.6	A B C D	8,061	A	18.8	A
4. (n=5)	Vi	Iso 200 mg/kg	381	A	0.43	B	9,653	A	14.2	A
7. (n=4)	Non-infected	No drug	39,700	A C	69.5	CE	54,568	C	242.1	B
8. (n=5)	Non-infected	Lev 5 mg/kg	40,980	C	92.0	E	42,453	BC	96.7	A C
9. (n=5)	Non-infected	Iso 200 mg/kg	38,509	BC	43.6	DE	72,714	C	153	BC

1) LTT on 20 sapphire mink prior to virus inoculation and drug treatment showed mean CPM 55,895 ± 5,480 and SI 75.8.2 ± 11.05
2) Mean response in counts per minute
3) Stimulation index
4) Student's t-test comparing groups, treatments with letter(s) in common are not significantly different at 5%

Serum γ-globulin

The mean γ-globulin preinoculation percentage for the 44 mink in the experiment was 9.65 ± 0.31% (SEM). Before the experiment the means of the nine groups varied between 8.0% and 11.6%. Concentrations of γ-globulin during the experimental period are indicated in Fig. 2 A, B and C.

Treatments of infected mink with high or low doses of isoprinosine appeared to have some reducing effect on γ-globulins at weeks 3, 4, 8, 10 and 12, but the differences were not significant, being $p < 0.1$ or $p < 0.2$ (B). The group treated with a combination of low doses of isoprinosine and levamisole showed a similar response, but the differences were more marked, with significances of $p < 0.01$ and $p < 0.05$ at weeks 3 and 4, respectively (C). For weeks 6, 8 and 12, the apparent differences were not significant ($p < 0.25$). Levamisole had a marginal influence on g-globulins, the only significant differences noted were for the high dose group on samples obtained at weeks 2 and 4, which were above ($p < 0.05$) and below ($p < 0.05$) the untreated group, respectively (A).

Table 2. Sapphire mink inoculated with a virulent Norwegian strain of ADV (NADV-1) and treated 3 x weekly with levamisole (Lev) and/or isoprinosine (Iso). Various mean values of groups at euthanasia 12 weeks p.i. compared statistically

Group[5] No.	Virus/ Non-infected	Treat-ment	Body weight change %[6]	Organ weight (g) Liver	Spleen	Kidneys	Conts/mm³ PI[1]	WBC[2]	Lc[3] %	PCV[4] %
1. (n=4)	Vi	No drug	-21.3 B[7]	56.9 AB	20.5 A	10.0 AB	450.5 CD	7.150 BC	34.0 A	32.0 CDE
2. (n=5)	Vi	Lev 5 mg/kg	-13.4 B	49.9 AB	18.9A	8.4 BC	946.0 AB	7.300 BC	46.6 A	30.8 DE
3. (n=3)	Vi	Lev 1 mg/kg	-30.7 B	64.5 A	16.6 AB	8.7 BC	395.0 CD	4.433 C	40.5 A	37.0 BC
4. (n=5)	Vi	Iso 200 mg/kg	-18.4 B	46.7 AB	15.3 ABC	9.4 ABC	331.6 CD	5.360 C	41.2 A	29.8 E
5. (n=5)	Vi	Iso 40 mg/kg	-16.7 B	65.2 A	20.8 A	11.2 AB	241.8 D	6.120 C	33.4 A	33.0 CDE
6. (n=4)	Vi	Lev. 1 mg Iso 40 mg	-15.0 B	54.5 AB	17.6 AB	11.9 A	324.0 CD	3.450 C	40.3 A	36.0 BCD
7. (n=4)	Non-infected	No drug	+2.6 A	42.6 B	6.5 D	6.2 C	416.7 CD	13.550 A	41.5 A	45.3 A
8. (n=5)	Non-infected	Lev 5 mg/kg	+28.1 A	49.4 AB	8.1 CD	6.7 C	1,116.0 A	9.580 ABC	50.6 A	34.8 BCDE
9. (n=5)	Non-infected	Iso 200 mg/kg	+19.9 A	46.4 AB	10.1 CD	6.4 C	711.2 BC	11.800 AB	47.8 A	39.6 B

1) Platelets x1,000; 2) White blood cells; 3) Lymphocytes; 4) Packed cell volume (hematocrit) 5) Experiment started with groups of 5 animals, except group 7, which had 4. 6) Calculated on those animals that were killed at 12 weeks 7) Treatments with letter(s) in common are not significantly different at 5% using Duncan's (1955) multiple range procedure

Apparent higher values at week 6 and lower values at week 12 were not significantly different from those of the untreated group ($p < 0.1$ and $p < 0.15$, respectively).

Differences were found between the non-infected untreated group and the high dose isoprinosine group at week 3 with values above those of the untreated group, and at weeks 6, 8 and 10 with values below those of the untreated group, significances were $p < 0.05$ (B). Smaller and non-significant differences were noted for the levamisole-treated non-infected group, concentrations being above those of the untreated group at weeks 2 and 3 and slightly below at weeks 6, 8 and 10 (A).

Lymphocyte transformation test (LTT)

Twenty female sapphire mink were tested in LTT with Con-A as the mitogen before the beginning of the experiment. Mean CPM was 55,895 ± 5,480 (SEM), and mean SI was 75.8 ± 11.05. Testing at 6 and 12 weeks was chosen in order to indicate any possible *in vivo* effect of the two drugs on lymphocyte activity. For this purpose only combinations of the infected/non-infected and the high dose drug treated/untreated (control) groups were selected (Table 1).

Con-A-induced proliferation was generally lower in the infected groups than in the non-infected groups, both at 6 and at 122 weeks. Within infected groups all except one were significantly different in CPM or SI at 6 or 12 weeks, the exception was SI of group

4, week 6. The suppression was most marked in groups 1 and 4 in week 6 samples. The individual variations within groups were generally rather large, and this explains why some unexpected results show no significance in Table 1. The following three CPM values are examples that had a significance level of $p < 0.1$ resulting in same letter: (a) Group 1 in relation to groups 7 and 9 at week 6, (b) group 2 in relation to groups 1 and 4 at week 6, (c) group 8 in relation to infected groups at week 12. The lack of significance was due to rather large individual variations in the infected groups.

Blood and organ examinations

The non-infected groups had increased their body weights during the experiment; the levamisole group increased the most and the untreated group the least (Table 2). The six infected groups had lost weight to variable degrees, the high dose levamisole group had lost the least and the low dose levamisole group had lost the most, even more than the control group. The highest liver weights were seen for both of the low dose drug-treated infected groups. Spleen weights in the untreated infected group were about three times higher than those in the non-infected untreated group. The high dose isoprinosine-treated mink had the lowest spleen mean weight within the infected groups. The mean kidney weight of the untreated infected group was about 50 % higher than that of the untreated non-infected group. Infected groups treated with high and low doses of levamisole and high doses of isoprinosine had values that were not significantly different from the non-infected groups. The low dose isoprinosine group and the combination group were 10-20 % higher than the untreated infected group.

Platelet counts were generally similar for the infected and the non-infected groups. The high dose levamisole-treated infected group had, however, significantly higher (2 times) mean counts than those of the control group. The corresponding non-infected group had mean counts nearly 3 times higher than those of the control group, but the difference was not significant. White blood cell (WBC) counts varied considerably between 3,500/mm^3 and 13,500/mm^3 and only the non-infected untreated group was significantly different from all the infected groups. The treated groups had variably reduced WBC counts, from none for the high dose levamisole group to the most marked reduction seen for the combined drug group. There were no significant differences found for lymphocyte percentages, either within or between the infected and non-infected groups. Both levamisole and isoprinosine-treated non-infected groups had higher counts than those of the untreated group. Within the infected groups, all but one of the treated groups, the low dose isoprinosine group, showed increased counts when compared with the control group; the high dose levamisole group had a higher mean count than the non-infected control group.

Packed cell volumes (PCV) (hematocrit) showed considerable variations within both infected and non-infected groups. The infected control group was significantly lower than the non-infected control group. Both infected and non-infected groups that were treated with high dose levamisole or high dose isoprinosine had lower PCV values than the relevant control groups, but the differences were significant only for the non-infected groups.

ADV associated disease

Submandibular abscesses were seen in two SaM in infected non-treated group 1. Four infected mink died during the study (week 11 in group 1, weeks 7 and 8 in group 3, week

11 in group 6), two animals had histologic evidence of terminal bacteremia and specific cause of death was not determined for another two. The four mink had average antibody titers and average γ-globulin concentrations in the last of the preceding tests, and histologic lesions were consistent with AD for each.

DISCUSSION

In the present work the immune modulating drugs levamisole and isoprinosine were tested in a 12-week experiment on sapphire mink infected with a highly virulent Norwegian strain of ADV. An overall look at the present experimental results for the infected groups indicates that the two tested drugs had only a marginal influence on g-globulins and antibody titers and in ameliorating characteristic body, organ and blood changes associated with ADV infection.

Since AD is an immune complex disease with exceptional hypergammaglobulinemia, it seems reasonable to consider γ-globulin values alone as a good disease indicator for infected mink. At the end of the experiment the low dose isoprinosine group had the lowest γ-globulin value (41.9%) and low dose levamisole had the highest value (48.9%) as compared with the control group, which had 50.6% (Fig. 1A & B). The other three infected groups had values between 44.8% and 45.4%. Increases in antibody titers followed in general the increasing γ-globulin values in infected mink. Isoprinosine in high doses and low doses in combination with levamisole is the drug that has given the more consistent differences for these two parameters when evaluated together. Both in the early stages and at the end of the experiment, the mean antibody titers for both isoprinosine groups and the combination group were significantly lower (Fig. 1B & C). γ-globulins also showed lower values for the same groups and at about the same stages, but the significances were less marked (Fig. 2B & C).

Other authors have reported various effects of the two drugs on some virus infections. Beneficial effects attributable to immunostimulation have been reported for levamisole treatment of parainfluenza virus infections (Ogunbiyi 1987; Mohan et al. 1987) and for acute viral hepatitis (Par et al. 1977). Early findings of positive effects of isoprinosine on herpes virus infections (Chang & Weinstein 1973) have not been supported in some later experiments. For instance isoprinosine was found to be ineffective against bovine herpes virus infection (Blecha et al. 1987) or to cause aggravation of pseudorabies infection (Flaming et al. 1989). The isoprinosine effects may be related to reduced immune responses. Beneficial effects reported for chronic active hepatitis B virus infections (Cianciara et al. 1990; Boron-Kaczmarska et al. 1990), which is also characterized as an immune complex disease, may also be due to reduced immune responses. Our results indicating no effect or only a slight enhancing effect of levamisole and a slight inhibiting effect of isoprinosine on immune responses appear to be similar to results by other workers.

In the present study, clinical observations did not indicate any special differences between treated and untreated infected mink, apart from some animals that displayed obvious emaciation and a somewhat lean appearance. All of the four mink that died towards the end of the experiment had lesions consistent with AD and had average antibody titers and γ-globulin concentrations. Other workers have reported such AD-associated deaths (Eklund

et al. 1968; Bloom et al. 1975; Hadlow et al. 1983).

The ADV infection induced complete suppression of the *in vitro* lymphocyte proliferative response to Con-A six weeks after infection (Table 1). This suppression was only partly restituted at 12 weeks after infection. Levamisole treatment resulted in only a moderate restitution of the response at six weeks, but this effect was not significant. Suppressed responses to mitogens inclusive of Con-A have been shown earlier by An & Wilkie (1981) and by Perryman et al. (1975), the latter also reporting that the reduction in Con-A response increased progressively with time after infection. In our work the suppression was more severe and the response improved from 6 to 12 weeks.

In our experiment there was no difference in platelet counts between untreated infected and non-infected groups. Eklund et al. (1968) reported quite a wide normal range of ca. 6-9 x $10^5/mm^3$ and showed a clear reduction in platelet counts in ADV infected sapphire mink during a 12 week period. In our study high dose levamisole showed an enhancing effect on platelets, the counts of the infected group being about 2 times higher and those of the non-infected group about 3 times higher than the counts of the respective control groups. Since platelet functions are closely associated with blood coagulation, and Aleutian-*aa* mink with the Chediak-Higashi type of immune deficiency also have a prolonged bleeding time, perhaps levamisole could be considered beneficial for ADV infection in this type of mink. Levamisole has been shown to reduce thrombocytopenia in distemper-vaccinated dogs (Pineau et al. 1980), but in their experiment a single injection of 2.2 mg/kg levamisole was used and can thus only indicate a levamisole effect on platelets.

CONCLUSIONS

Isoprinosine and levamisole showed only marginal effects on the pathogenesis of ADV in sapphire mink. Isoprinosine treated groups had significantly lower antibody titers and lower γ-globulin concentrations at some testing points. Levamisole enhanced Con-A-induced lymphocyte proliferation in infected mink six weeks after infection. This drug also had a significant enhancing effect on platelet counts in both infected and non-infected mink.

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Mink breeding hygiene in hot climatic conditions

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There are some difficulties in breeding mink in hot climatic conditions (Central Asia), because of high temperatures and low humidity in summer, the long light period in spring and autumn, and high sun activity. However, by means of special hygienic breeding procedures it is possible to obtain normal mink reproductivity (4-6 kits per female) and a high quality of fur. We recommend that animals be kept in two-rowed sheds, where the distance between the roof and the top of the cage is 120-150 cm. The air temperature in sheds of this kind is lower than that in the standard shed (by 7°C). The ordinary procedure for feed preparation and distribution must be changed. It was established that it is better to feed the animals twice a day - at 07.00 h and 20.00 h and the feed must be cooled. This procedure ensures that there is a decrease in the amount of bacteria and an increase in the amount of eaten feed by 5-11%. In addition, there must be a plentiful supply of fresh water. It was established that a high resistance to infection and high reproductivity of mink are possible under such conditions (certainly lower than that in the northern animals).

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Fur-farming is developing successfully now in Central Asia despite the specific climatic conditions of this region. But fur-farming under such hot continental climatic conditions needs some specific breeding technology.

In July the average daytime temperature is about 40°C with a maximum temperature of up to 50°C. There are usually considerable temperature differences between years, wide daily temperature fluctations, a small level of precipitation (less then 200 mm in a year, a long daylight period and dry air (in summer, humidity is only 20-25%. The common causes of losses in fur farms are cattle-plague of animals as a result of heat-stroke, reduced reproduction and high mortality in young animals.

In spite of the conditions, the mink have normal reproductivity (4-6 kits per female) and a high quality of fur.

RESULTS AND DISCUSSION

The results of our investigations suggest that in hot climatic conditions it is recommended

to keep the animals in two-rowed sheds, where the distance between the roof and the top of the cage is 120-150 cm (high shed). The air temperature in this type of shed is 3-7°C lower than that in the standard type shed. The air circulation rate in the high shed is 2-2.5 times greater than that in the standard shed, which is very important, especially in summer. The content of carbon dioxide and ammonia in the standard shed is 2-4 times higher than that in the high shed, and the amount of bacteria 1.5-2.5 times higher.

A minimum level of corticosteroids was found in the plasma of young animals (15% less than that found in the control group). The quality of fur of these mink was also higher and the skins were of larger size.

In hot climatic conditions, it is very important to give due care to hygiene in feeding mink. Mink feed is a very good breeding-ground for bacteria, and so the usual procedure for feed preparation and distribution in hot conditions must be changed.

Our investigations established that it is better to give the mink feed that has been cooled to +8-+10°C twice a day at 07.00 h and 20.00 h.

The control animals received feed at 09.00 h and 16.00 h. The amount of bacteria in the feed in the control group increased in the course of three hours on 5.7 mln/g, and in the experimental group on 1.1 mln/g.

There was 20-26% more uneaten feed in the control group than in the experimental group. The body mass of mink up to the time of slaughter (6 months) in the experimental group was 1952 ± 28 g (male) and 1293 ± 15 g (female), and in the control group 1637 ± 26 (male) and 1059 ± 14 (female). The length of body in the experimental group was 41.8 ± 0.2 cm (male) and 36.4 ± 0.3 cm (female), but in the control group it was 39.3 ± 0.3 cm (male) and 35.1 ± 0.2 cm (female).

Biochemical analysis indicates that the mink in the experimental group had an increased hydrocarbon metabolism. At the age of six months the glucose level in the experimental group was 175 ± 8 mg%, and in the control group 206 ± 7 mg%.

The number of sick animals (with indigestion) in the control group was 2.3 times greater than that in the experimental group. Lack of water on hot days leads to heat-stroke, as well as to poor quality of fur. It was established that mink need 300-800 ml of water a day. Animals that had sufficient amounts of water were bigger than the others. Sick rate and mortality rate in the experimental group (where a plentiful supply of fresh water was available) were 8.2% and 3.6%, while in the control group (with the usual water ration) the rates were 12.7% and 5.9%.

Our investigations indicated that adaptation of animals imported from northern regions is generally reached by the third year of acclimatization, and the period of adaptation of mutant animals (blue and palomino) is shorter than that of animals of the standard genotype (brown). In the first year of acclimatization the body mass of six-month old mink reproduced from imported animals was 20-50 g less than that of native animals, but by the third year of acclimatization there was no longer any difference.

It was found that the main differences between native and imported animals take place in summer in the first year. During this period the content of serum protein of imported mink is 5-8% less than that of native animals.

There is a considerable difference in content of corticosteroids between native and imported animals in the first year of acclimatization, but by the third year of acclimatization, the difference is absent. The fertility rate of acclimatized animals is 5-12%

greater than that of non-acclimatized animals and the mortality rate of non-acclimatizated animals is twice as high as that of acclimatized mink.
The prolonged influence of high temperature (35-40°C) leads to functional changes in organisms.

It was established, that the physiological standard of animals from the south is quite different from that of animals from the north. It is therefore necessary to take this fact into account when carrying out any veterinary procedures with various kinds of animals in hot climatic conditions.

Behaviour and welfare

Progress in the ethology of foxes and mink

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This is a review of the progress that has been made in the behavioural biology of farmed foxes and mink during 1988-92. This scientific area has undergone rapid growth, which is evidenced by the 71 reviewed publications of 52 different authors in seven countries. The review covers the effects of selection for domestication on behavioural ontogeny, hormonal aspects of behavioural ontogeny, early handling of cubs, rearing conditions and management related to behaviour, circannual and circadian rhythms of behaviour, stereotypies and stress, maternal behaviour, social behaviour, and behaviour related to resting platforms and nest boxes. Some ideas for further research are given.

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This paper reviews the progress that has been made in the behavioural biology of farmed foxes and mink since the last congress in fur-animal science in 1988 (Murphy & Hunter 1988). The last four years have shown an enormous increase in the scientific activity in this area. This review covers 71 publications with 52 different authors in seven different countries published during 1988-92. It is, however, something of an enigma that no article in this area has emerged from North America since 1988. There have been more studies on silver foxes than on blue foxes, probably as a result of the assumption that there are greater problems with reproduction in silver foxes, which may not be true. Behavioural studies on mink have covered broader topics than earlier.

A number of scientists in Novosibirsk are continuing the long-term study of the effects on various aspects of behaviour, genetics, endocrinology and morphology of selecting silver foxes for either domesticated or aggressive behaviour. Recent progress in these areas is presented in a booklet containing 14 papers, in Russian with English summaries (Trut et al. 1991). Two other collections of interesting work should be mentioned. Kaleta (1991) reviews his 10-year study in Poland on agonistic and other behaviour of silver fox males and vixens. Hansen (1991a) gives a review of recent studies on behaviour of mink related to their environment at The National Institute of Animal Science in Denmark.

DOMESTICATION AND BEHAVIOURAL ONTOGENY

Plyusnina (1991a) studied the effect on behavioural ontogeny of cubs 20-60 days of age of cross-mating between a domestic (genetically tame) and an aggressive parent. Cubs with

an aggressive mother and a domestic father more frequently became aggressive than cubs with a domestic mother and an aggressive father. All cubs with a rather tame phenotype had a period of primary socialization which exceeded 60 days of age, like that generally found in the domestic selection line. However, those tame cubs which had an aggressive mother showed a decrease in motor activity similar to that of cubs with both parents aggressive. Aggressive cubs of aggressive mothers exhibited a socialization period which ended by 40 days of age, similar to cubs with two aggressive parents. Aggressive cubs which had a domestic mother showed a decrease in motor activity at 50 days of age. The results reveal a maternal effect on both motor activity and duration of the socialization period, in addition to the previously known effect of domesticated genotype (Belyaev et al. 1985).

In a study of learning ability, domestic one-year-old silver foxes of both sexes showed a greater ability to be habituated to humans than undomesticated foxes (Vasilyeva 1991a). This test was performed much later than the end of the socialization period. When tested in a "shuttle box" with two compartments, domestic two-year-old vixens were more capable than undomesticated vixens of forming the conditioned response active avoidance (Vasilyeva 1991a).

Another selection experiment on temperament of silver foxes was started in Denmark in 1988 (Jeppesen & Pedersen 1990). The animals were selected in three lines, for aggression, timidity or inquisitiveness, based on their reactions in a test in which a human established eye contact with the animal close to the cage (Pedersen & Jeppesen 1990). Aggressiveness was easily selected for. Selection for inquisitiveness, presumably approximating the Russian selection for domestication, also resulted in more aggressive but less timid cubs. This indicates that there is a common factor among aggressive and inquisitive foxes. It could perhaps be called high self-confidence. Foxes which are aggressive towards humans (exhibiting defensive threat) can often be good reproducers (Braastad 1988).

Risopatron (1990) examined the heritability of fear responses of 563 silver fox cubs towards an unfamiliar human or an unfamiliar object suddenly falling onto the cage floor. The tests were made in nine farms with mutual use of the sires. The ownership significantly affected the positioning of the animals in the cage. For different behaviour parameters the estimated heritability (h^2) varied between 0.01 and 0.17 (distance moved in object test), based on paternal half-siblings without disturbing influence from the maternal effect. Fear responses based only on these tests were considered insufficient for effective selection in private farms.

Since the behaviour of an animal includes so many aspects, genetic selection for domestication could take many different directions. In a methodological study, Vasilyeva & Chepkasov (1991) analysed the validity of different selection criteria, as indicated by their capacity to adequately reflect a pattern of genotype-environment interaction, the structure of correlation ratios for different behavioural categories and the coefficient of relationship between individuals. The best criterion was found to be a domestication index, a linear function of behaviour reactions towards man determined by the principal components method.

Selection for domestication in mink

Selection for domestication of mink has started both in Russia (Trapesov 1991) and in Denmark (Hansen 1991b). During selection for domesticated or aggressive behaviour in mink, Trapesov (1991) found an increase in the variation of piebald spotting in both lines, with the highest variation in domestic animals. Kits with domestic mothers had a shorter flight distance towards humans, and a lower cortisol content in the blood, when tested at 2.5-3 months of age (Kharlamova & Gulevitch 1991). Whereas unselected kits displayed a marked and irreversible decrease in motor activity towards the end of a six-minute open-field test, neither tame nor aggressive kits showed this.

The Danish selection experiment was based on the reaction of the mink towards an unfamiliar object placed onto the cage door (Hansen 1991b). Mink were selected along three lines, for increased exploratory behaviour, for timid behaviour or for aggressive behaviour. Some changes in these behaviour categories occurred during the first three generations. Cross-fostering of kits revealed that kits developed the behaviour of their biological mother. Both mink of the aggressive and of the exploratory lines were more active in an open-field test than mink of the fear-motivated line (Houbak 1991).

HORMONAL ASPECTS OF BEHAVIOURAL ONTOGENY

Domestication is associated with changes in the pituitary-adrenal system. By the end of the prenatal period the cortisol content in foetal gonads was lower in domestic silver foxes than in undomesticated foxes (Schurkalova & Osadchuk 1991). No difference was found in testosterone level, indicating equal possibilities of steroid biosynthesis. At onset of puberty, marked differences were found in the corticosteroid function between domestic and undomesticated foxes (Oskina 1991a). Vasilyeva et al. (1991) studied the inheritance of cortisol concentration in blood in silver foxes. The results indicated that a major recessive gene controls this trait, and that selection for domestication is a selection for animals homozygous for this gene. In domestic foxes basal levels of corticosteroids showed hereditary variation shortly after birth (Oskina 1991a). Hereditary variation in undomesticated animals was found only during stress, and not before four months of age. Foxes selected for domestication were earlier shown to have a higher level of serotonin in the brain than unselected aggressive foxes (Popova 1988). Brain serotonin is known to play an important part in the mechanisms of aggression (Miczek & Donat 1989; referred by Plyusnina et al. 1991a).

When fox cubs selected for enhanced aggressiveness were tested in an unfamiliar situation in a test cage at different ages, they showed a decrease in exploratory behaviour and a rise in cortisol level at 45 days compared to at 30 days (Plyusnina et al. 1991a). Domestic foxes gradually increased their activity in this test between 30 and 60 days of age without variation in cortisol level. After injection with clodithane at 38-52 days of age, which reduced the cortisol level, aggressive foxes showed less fear and increased exploratory behaviour in the test. Clodithan did not affect the frequency of aggression. Aggression was decreased, however, and exploratory behaviour was increased in the foxes selected for aggression after treatment with l-tryptophan, a precursor of serotonin, at 45-55 days of age. These animals appeared to show less fear and less aggression even at seven months of age.

This study demonstrated that cortisol level and fear are related. Reducing fear with clodithan in this experiment did not extend the sensitive period of primary socialization, as earlier suggested (Belyaev et al. 1985). However, reducing both fear and aggression with l-tryptophan succeeded in prolonging the sensitive period. This suggests that also development of aggression contributes to ending the sensitive period.

Mink

Significant changes in the reproductive system were also found in mink during selection for domesticated behaviour. Domestic mink had earlier onset of oestrus and higher fertility compared with aggressive mink (Klotchkov & Trapesov 1991). In domestic mink a correlation was found between early oestrus and high fertility. No such relation existed in aggressive mink. When these aggressive mink were subjected to photoperiodic imitation of early autumn, folliculogenesis was stimulated and a larger proportion of them came into oestrus.

Injection of 5-hydroxytryptophan, a precursor of serotonin, considerably inhibited predatory attacks of mink on rats (Nikulina & Popova 1988). Abundant intake of tryptophan through the natural diet also delayed the attacks and killing of rats.

Since tryptophan deficiency in the diet is suspected of being related to aggression in general, even in humans, it would be interesting to investigate whether increased levels of tryptophan in the diet of foxes and mink could reduce aggressiveness. As indicated by Plyusnina et al. (1991a), increased tryptophan level around weaning might be sufficient.

EARLY HANDLING OF CUBS

Several studies have been made on the effect of early handling of fox cubs. Pedersen & Jeppesen (1990) handled cubs from 2 to 8 weeks of age for five minutes twice a day. Between 12 and 20 weeks of age the animals were subjected to three different tests on fear of humans and unfamiliar stimuli. The results showed clearly that handled foxes were less fearful than control animals. They also showed more aggression than controls, indicating a higher self-confidence. The latter is also indicated by more exploratory behaviour in an open-field test at 24 weeks, confirming the earlier results of Tennessen (1988). Cortisol and behavioural records in connection with the open-field test indicated that handled cubs had a lower stress sensitivity.

Braastad et al. (1989) showed that cubs subjected to early handling grew faster than control animals. Cubs handled from 4 weeks of age to 2 weeks after weaning were less fearful than cubs handled only until weaning. Recently, Pedersen (1992) has shown that handling during and shortly after the weaning period can be more effective than earlier handling, and it is indicated that a shorter handling period is needed. The same has also been shown for other species.

Vasilyeva (1991b) found that early and late handling affected the behaviour in the same direction. However, whereas early handling was most efficient in reducing flight distance and increasing contactability in female cubs, later handling or taming had a greater effect on male cubs. This was independent of the degree of domestication.

REARING CONDITIONS AND MANAGEMENT RELATED TO BEHAVIOUR

As an alternative to early handling, Pedersen (1991) gave silver and blue fox cubs visual contact with humans and the farm environment at 2-8 weeks of age. This was accomplished by opening a door, leaving only wire netting, in the top-mounted nest box facing the aisle. Tests of the reaction towards a human at 12-16 and 23-28 weeks of age showed that fear was reduced compared with the reaction in control animals. Hence, early visual experience with the farm environment enhances adaptation to captivity in foxes.

In Poland Kaleta & Stosznajder (1990) studied agonistic behaviour towards an approaching man in 912 6-8-week-old silver fox cubs under two different rearing conditions. Foxes in pavilions (sheds) more often tended to withdraw and displayed a cut-off response, whereas foxes in free-standing cages most often tended toward flight and more or less displayed panic. The latter also responded more quickly than the former. The conclusion is that cubs in pavilions were more habituated to humans passing close by. In other studies, it was shown that the flight distance of 2-month-old cubs and adult males was shorter the narrower the aisle was in the pavilions (Kaleta & Plochocka 1990; Kaleta 1991).

In a survey of the attitude of Norwegian fox farmers towards the use of neck-tongs for moving animals, 90% used neck-tong for moving blue foxes and 95% for silver foxes (Røhme et al. 1992). About 90% of the farmers regarded silver foxes to be more difficult to handle than blue foxes. When an experienced caretaker removed silver foxes from the cage by hand, the heart-rate was lower than when a neck-tong was used (Olsrød et al. 1992). The animals were also calmer when taken out by hand. However, taking out a fox by hand required more time than with a neck-tong (32 s vs. 19 s). Five percent of the foxes were too aggressive to be taken out by hand without risk of injury to the caretaker.

CIRCANNUAL AND CIRCADIAN RHYTHMS OF BEHAVIOUR

Domestication seems to modify both circannual and circadian rhythms of photosensitivity and behaviour. Increasing the day length to 20 h led to morphological changes in the adrenal cortex which were different in domesticated and undomesticated foxes (Lutsenko & Trut 1988). Whereas domestication enhances the influence of the photoperiod on the ontogeny of the pituitary-adrenal system, it seems to weaken the influence of the photoperiod on the pituitary-gonadal system. Both morphological and functional changes in the pineal organ have occurred during domestication (Kolesnikova 1991). Through melatonin this organ regulates both fur maturation and the reproductive function. Extra light during night was found to initiate both two moults and two reproductive cycles per year in some domestic vixens (Prasolova 1991).

In a study by Plyusnina et al. (1991b) none of the domestic silver foxes showed the sharp nocturnal activity phase which was common in aggressive and unselected foxes. The domestic foxes tended more often to fail to show clear periodicity in locomotor activity. If a weak nocturnal activity phase existed in domestic foxes, it started earlier in the evening and was more prolonged.

STEREOTYPIES AND STRESS

Mink

Studies on stereotypies and stress are far more numerous in mink than in foxes. Jeppesen (1991) has reviewed his own recent studies on stereotypies in mink. The frequency of stereotypies seems to be genetically related (Jeppesen et al. 1990), as earlier suggested by de Jonge et al. (1986). Kits from large litters developed more stereotypies than kits from smaller litters (Jeppesen 1991). Also, the mothers of large litters became more stereotyped in the following autumn than mothers of small litters. The latter are instead reported to display more of the least-stereotyped "restless" behaviour (Mason 1991a). Environmental disturbances such as human proximity, transfer to another farm section or delivery had strong inhibiting effects on the performance of stereotypies (Bildsøe et al. 1990a). A gradual increase in stereotypies was recorded between June and November, with a temporary decline in October, in both kits and adult mink (Bildsøe et al. 1990b). Mink kits kept in single cages developed stereotypies earlier in the autumn and with a higher frequency than kits kept in pairs (Jeppesen et al. 1990). Records of diurnal rhythms taken in a hot July showed a marked peak in stereotypies from after midnight until the morning (Bildsøe et al. 1990b).

Mason (1991b) reported more stereotypies in 6-month-old mink kits born late in the breeding season, probably because they were weaned from their mother earlier. Heavier kits with multiparous mothers developed more stereotypies consisting of head movements than lighter kits. This may be related to the observation that such mothers restrict their milk supply to heavy kits earlier than to light kits. Mason (1991b) suggests that this early frustration results in stereotypies that resemble nipple-searching movements.

Bildsøe et al. (1991) tested female mink with two types of stressors; forced immobility for one hour per day or 33% reduction in food. The immediate effect of the fear-related immobility test was to decrease the occurrence of activity and stereotypies. In contrast, the frustration-related food restriction led initially to an increase in stereotypies. Both stressors led to a higher frequency of stereotypies after the tests. Studies on the cortisol response indicated that high stereotyping females were less stressed than low stereotyping females. As is found in other species, an individual showing high frequency of stereotypies may not necessarily have poorer well-being. However, the very existence of stereotypies may indicate certain environmental inadequacies, which can cause stress particularly to those individuals that are unable to cope with this problem.

A stick test indicating the temperament of mink was found useful for identification of mink which displayed fear towards humans (Hansen 1991a). When mink females were kept in groups of 3 males and 3 females, they had a higher cortisol level than females kept singly (Hansen & Damgaard 1991a). They also received more bite damage. No differences between single and group-kept males were found. In general, females were more stress sensitive than males. This feature was also found by Jeppesen & Falkenberg (1990). They examined the effects of providing mink with play balls in an attempt to reduce stress and pelt biting. Although the mink lost interest in the balls after a month, the general activity and curiosity were still greater compared with the reactions of controls. However, no effect on pelt biting or stress was found.

It was not possible to demonstrate improvement in well-being by providing mink

females and their kits with a water tray for bathing (Hansen 1990), although the water trays served as an occupation. Giving lactating mink a refuge away from their kits reduced the frequency of stereotypies (Hansen 1990).

Foxes

Studies on stress in foxes are mainly related to various cage environments (Jeppesen & Pedersen 1991, 1992, see below). Stereotypies in foxes are not studied systematically. All silver fox vixens persistently perform digging movements on the wooden floor of the breeding box prior to delivery (Braastad, submitted). During the last pre-parturient day, vixens were observed to "excavate" on average for a total of 102 min, in long bouts of several minutes. The behaviour was repeated in an individual manner, without any immediate function, and could fit the definition of a stereotypy. It is suggested that it represents a motivation for making a shallow depression in the floor before delivery. Some silver fox vixens also perform stereotyped digging on the feed plate, perhaps motivated by a search of food.

MATERNAL BEHAVIOUR

In order to fully understand the reproductive behaviour of foxes, it is necessary to have more details of their maternal behaviour. The use of an infrared-sensitive video camera inside the breeding box has made this possible (Braastad 1988). In a paper describing the periparturient behaviour of successfully reproducing silver fox vixens (Braastad, submitted), a uniform distribution of births around the clock was found. Most deliveries were quite easy. The time-budget of behaviour showed a pronounced individual variation, but there were few differences in behaviour between primiparous and multiparous vixens. Vixens rested or slept for about 70% of the time during the first three postparturient days, but on average for only 11 min per bout. About 20% of the time was spent with grooming and inspecting the cubs. This was completely independent of the litter size. Hence, individual cubs from a small litter received more attention from their mother than cubs from a large litter.

Another paper compares the maternal behaviour of infanticidal and successfully reproducing vixens (Braastad & Bakken, submitted). About 70-80% of the cub mortality with infanticidal vixens seemed to be caused by bites from the mother. In half of the cases, the vixen bit the tail off the offspring prior to killing them. In some cases only a part of the litter was killed. Dead cubs were almost always treated as prey and later eaten. Sometimes they were "buried" under wool tangles for some time. Cub-killers did not show less cub-grooming than other vixens, but they were more often standing while grooming and appeared more restless. They also spent less time resting inside the breeding box. Primiparous vixens killed cubs earlier after birth (usually within four hours) than experienced vixens (often 1-2 days after birth). Cubs were not killed more frequently during daytime, and there was nothing to indicate that disturbances from humans could provoke cub-killing. Environmental disturbance seems unlikely as a mechanism initiating infanticide in our research farm.

Plyusnina (1991b) recorded automatically the time silver fox vixens spent in the cage

and in the breeding box during different periods around parturition. She reported infanticide as occurring mainly during the first day or on days 5-7 after birth. Abnormal and normal vixens differed in the distribution of time spent in the cage or the nest. Oskina (1991b) analysed hormonal differences between normal and infanticidal vixens. The results indicated disturbances in infanticidal vixens in the dynamics of the secretion of progesterone during pregnancy, but also prolactin level seemed to differ from normal.

Kaleta (1991) reported that infanticidal mothers themselves were born later during the reproduction season than good mothers. Differentiating between the various types of agonistic behaviour was most efficiently done with either a dummy (attrapp) emitting a sound or an approaching man. In such tests infanticidal vixens more often showed hesitation, immobilization or evasive behaviour. Kaleta (1991) recommends that future tests aimed at predicting propensity for infanticide should include two different objects, since it is more difficult reliably to reveal differences in agonistic behaviour with only a single object.

INTRASPECIFIC SOCIAL BEHAVIOUR

Because of an earlier suggestion that visual isolation of vixens during pregnancy and lactation could reduce social stress and improve reproduction, Jeppesen & Pedersen (1988) isolated silver fox vixens with cardboard partitions. They found no effect of visual isolation on the litter size at birth or weaning, or on cub mortality. The same result was obtained earlier with mink (republished by Jeppesen & Pedersen 1988). However, in both studies the control groups showed excellent reproduction. I would suggest that the effects of improving the social or physical environment are likely to occur more readily in low and moderately reproducing populations. This points to the methodological difficulty of solving problems found in private farms by scientific studies in an almost problem-free research station.

Silver foxes

Everyone who has been working with silver foxes has experienced their pronounced individual variation in temperament and behaviour. In order to fully understand their behaviour, this individuality has to be considered. Individuals may have different needs for the physical and the social environment. Hence, analysing data on population level may preclude important factors.

Bakken has studied the social behaviour of silver fox vixens with a number of tests on each individual over a period of several years on our research farm. After confirming earlier studies that revealed a relation between fear and poor reproduction (Kaleta & Lewandowska 1987; Braastad 1988; Kristensen 1988), he showed that timid vixens had a lower social status among their conspecifics than aggressive or inquisitive vixens (Bakken 1988). Moreover, based on tests of dyadic fighting ability, vixens of lower status had less successful reproduction, similar to wild-living red foxes (Macdonald 1980). High-status vixens weaned more cubs if their neighbours were of low social status than if they were of high status (Bakken, submitted b). Moreover, the litters of high-status vixens with low-status neigbours had a significantly higher proportion of males than litters of high-status

vixens with high-status neigbours (Bakken, in prep.). Low-status vixens did not wean unharmed cubs at all if their neighbours were of higher status than themselves, but some cubs were weaned if the neighbours were of equal status (Bakken, submitted b). When low-status vixens were spatially and visually isolated from their neighbours, they were able to reproduce better the following season (Bakken, submitted a). These results are interpreted as supporting the parental manipulation hypothesis of infanticide (Bakken, submitted a; Hrdy 1979).

Because of the correlation between social status, reproduction and fear, a fear test on female cubs might be an indicator of future reproduction in silver foxes. Bakken (1989, 1992) showed that high activity in an open-field test at 30 days of age both correlated with high social status at seven months and high reproduction in the first season. Thus, vixens with an offensive behavioural strategy show signs of this very early. The fear test, or a test on coping strategies, still needs to be optimalized before being put into practice.

Blue foxes

Korhonen & Alasuutari have studied the social behaviour of blue foxes kept in small groups of 2-4 males and 2-4 females in a large ground enclosure. A social hierarchy developed within a few days after formation of the group (Korhonen & Alasuutari 1991). Social rank and feeding order seemed to be the same. Males usually dominated females. The hierarchical development seemed to start at 3.5 months of age (Korhonen & Alasuutari 1992).

A study of the effect of group size in caged blue fox cubs showed that foxes kept individually were highly aggressive, ate less and had a slower rate of growth than foxes kept in groups of 2-4 (Zon et al. 1987).

Mink

Wild mink are considered to be solitary and territorial animals. Houbak (1990) investigated whether mink kept in groups of 3 males and 3 females could form a social hierarchy. She found some indication of a rank order in the majority of the groups, but this was seldom manifested as a feeding order. A sex-specific hierarchy was not found. Mink kept in groups did not show the increase in activity prior to feeding time which was found in mink kept singly (and described earlier: de Jonge et al. 1986).

BEHAVIOUR RELATED TO RESTING PLATFORMS AND BREEDING BOXES

Platforms in fox cages

For some years, Finnish and Danish scientists have disagreed over the need for resting platforms (shelves) for foxes. Danish foxes seemed to use the platform more often than Finnish foxes. Blue foxes in cages usually made brief visits to the platform, and more often during working hours than at other times of the day (Harri et al. 1991). Open platforms were preferred to platforms with walls. Platforms were more often used when temperatures were above 0 °C than at lower temperatures. Silver foxes decreased their use of the platforms during autumn and early winter (Harri et al. 1992). Wind did not increase the use (Harri et al. 1991). In a later study blue foxes utilized wooden and wire-mesh shelves

with about the same frequency (Korhonen et al. 1991). Hence, it does not seem that the platform functions as a shelter against bad weather. It is used because it is available as an observation place. However, there is a wide individual variation in its use as well as variation between silver and blue foxes (Harri et al. 1991, 1992). More research is needed in order to investigate the reason for this variation. Is there any correlation between fear or social status and the use of platforms? Disturbances in the farm seemed to promote the use of platforms (Harri et al. 1992). A small-scale experiment in pens found no significant correlation with social status (Korhonen et al. 1991).

Nest boxes for foxes

Since some silver fox vixens seem to be restless inside a traditional breeding box (Braastad 1988), various improvements to the boxes have been tried out. In a breeding box equipped with a narrow entrance tunnel, a smaller proportion of the vixens committed infanticide and the average litter size at weaning was 0.5 cubs higher than that in the control boxes (Braastad 1990, 1991). This result was obtained in nine private farms with a moderate reproduction. Video records in our research farm confirmed that vixens in tunnel boxes showed a more relaxed behaviour, without the increased activity during working hours which was found for vixens in traditional boxes (Braastad, in prep.).

Jeppesen & Pedersen (1990) tested the preferences of vixens for three different nest boxes provided during the whole year. Silver fox vixens preferred a box mounted on top of the cage, with entrance via a shelf. At the end of the experiment, foxes having continuous access to nest boxes had a lower base level of cortisol than vixens without nest boxes (Jeppesen & Pedersen 1991, 1992). Vixens with nest boxes displayed less fear towards humans and were more exploratory in an open-field test. This indicates that a whole-year nest box could reduce stress in silver foxes.

Harri et al. (1992) report that after an initial low use, silver fox cubs showed poor interest in a nest box during autumn. Instead, they preferred to lie on the box roof. This was also found in a study with eight blue foxes kept in a large enclosure (Alasuutari & Korhonen 1992). An experiment with a top-mounted box confirmed that this was used as a hiding place (Harri et al. 1992).

Nest box and cage size for mink

If mink females were kept in cages without a nest box, they showed a higher cortisol level and a lower eosinophil leucocyte level than in mink with a nest box (Hansen & Damgaard 1991b). This stress response was equal to that of daily immobilization for 30 min. Varying the cage size between 0.1 and 1.05 m^2 (standard is 0.27 m^2 in Denmark) did not affect stress physiological parameters (Hansen 1991a; Hansen et al. 1992). An increase in stereotypies in the largest cage was indicated.

THE NEED FOR FURTHER RESEARCH

This section deals with some ideas about future research in fur animal ethology. Other scientists may have different views from those of the author.

More focus is needed on individual variation in behaviour and needs, e.g. dependent

on fear level and social status. It is not *a priori* true that all animals in a farm population should be given the same treatment and environment. More direct studies on coping strategies and their consequences for farming of fur animals would be most welcome. This might include optimalizing the social structure among neighbouring animals, in terms of, e.g. social status, age and sex. Since there seem to be pronounced differences in the behaviour of mink and foxes between different farms, more studies from individual farms should be encouraged. This, however, raises some methodological problems.

Behavioural endocrinology needs further development. However, the focus on cortisol should be reduced, as this, according to newer theories, does not necessarily correlate with stress. Other relevant hormones related to coping strategies, social behaviour and reproduction which could be considered include prolactin, LH and testosterone. The possibility of reducing aggressivity with a tryptophan-enriched diet could be investigated.

In Europe, more projects should be aimed at solving those questions and problems raised in the Recommendations on fur animals presented by the Standing Committee of the European Convention on the Protection of Animals Kept for Farming Purposes. For foxes, it is necessary to study carefully the effect of increasing the cage size. This is of limited scientific interest, but of great public interest. Other ways of increasing the quality of the cage environment should also be sought. One last point: do not forget the males when studying fur animal behaviour.

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DA = in Danish, NO = in Norwegian, PO = in Polish with English summary, RU = in Russian with English summary.

Correlation between levels of cortisol, behaviour and nest box use in silver fox vixens

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Jeppesen, L.L. & V. Pedersen 1992. Correlation between levels of cortisol, behaviour and nest box use in silver fox vixens. Norwegian Journal of Agricultural Sciences. Suppl. no. 9: 505-511. ISSN 0801-5341.

A study was carried out on the correlation between levels of cortisol, behaviour and nest box use in 49 silver fox vixens kept for a period of two years in cages provided with different types of nest boxes. The results indicated that nest box use was a rather stable individual character. Base levels of cortisol were shown to correlate positively with defensiveness, exploration, and levels of cortisol following 20 min acute stress. Base levels of cortisol correlated negatively with nest box use and with relative increases in levels of cortisol following 20 min acute stress.

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Recently it was demonstrated that silver fox vixens used whole-year nest boxes for accommodation, when they were given the opportunity, and that they preferred boxes placed high up in the cage. It was also shown that silver fox vixens living in cages with nest boxes had considerably lower levels of cortisol compared with the cortisol levels of animals living without nest boxes (Jeppesen & Pedersen 1990, 1991; Pedersen & Jeppesen in prep.). The vixens living with nest boxes displayed some variation in base levels of cortisol. The purpose of the present study was to investigate whether this variation showed any correlation with a number of behavioural variables and previous frequency of nest box use.

MATERIALS AND METHODS

Forty-nine silver fox vixens born in 1986 or 1987 to 49 different mothers served as subjects. In the autumn of 1987 they were housed at random in 2 m^2 cages provided with four different types of shelters: an open box placed in the cage, a side-mounted box placed at the back of the cage, a top box mounted at the top of the cage, and a shelf mounted just beneath the entrance to the top box (Pedersen & Jeppesen in prep.; Jeppesen & Pedersen 1990). All tests referred to in this paper were performed in the autumn of 1989, thus reflecting the effect of two years' housing with nest boxes in the cages. During this two-year period the animals were cared for as normal breeding animals. Their use of the shelters was recorded in two 10-week periods in the autumn of 1988: period 1 from August

to October and period 2 from October to December. Each period included 25 scanning observations which lasted for 2 h and comprised 12 recordings of the position of each individual, that is, each individual was observed for up to a total of 300 times in each period. Following the weaning in July 1989, the vixens were kept separately in their cages and disturbed as little as possible until they were tested in October and November.

The testing proceeded according to the following schedule: First, the animals were exposed to some behavioural tests while occupying their cages. They were exposed to a human being, to a glove and being struck in order to assess their degree of fear, curiosity and defensiveness (human test, glove test and strike test, see Pedersen & Jeppesen 1990). Two weeks later, each animal was exposed to an open field test. The animals were caught in their home cage, their reactions on being captured were recorded, and a blood sample (sample A) was drawn to yield base levels of cortisol and circulating leucocytes. Then, the animals were transferred to an open field runway, measuring 1.2 m x 8.0 m x 0.8 m. The animals entered one end of the runway through a habituation room called 'field 0'; an unfamiliar object was placed at the other end of the runway. The runway was divided into eight fields, field 1 being nearest to the entrance and field 8 nearest to the unfamiliar object. Latencies to move, to reach each field and to reach the object, and the number of field crossings and contacts with the said object were recorded during the ten minutes following a five-minute period in the habituation room. Re-entering the habituation room was possible during the test. Afterwards the animals were caught in the runway to be brought back to the home cage. Reactions to capture were recorded, and a second blood sample (sample B) was drawn exactly 20 min after the first one. Two weeks after the open field test the animals were once again exposed to a human test, to a glove test and to a strike test.

Blood samples were analysed for eosinophil concentration (total count), for relative numbers of all leucocytes (differential count) and for cortisol concentration by a competitive immunoassay technique (Amerlite Cortisol Assay by Amersham). The time lapse between onset of catching a fox until blood sampling was completed never exceeded one minute. Pilot studies revealed that the effect of an ongoing sampling procedure on the level of cortisol in the sample was perceptible 2.5 min at the earliest, after the onset of catching. Blood samples were taken over a period of five days. Cortisol levels were found to be unaffected by day of sampling and by sampling order within each day.

RESULTS

Vixens were seen most frequently on the shelf and in the top box, and they used the shelters more frequently in period 1 (Table 1). There was a marked positive correlation between the individual use of the shelters in periods 1 and 2. For this reason, only the combined measure for nest box use in both periods and all shelters is used in the following presentation of the results. Tests have shown that all conclusions reached by means of this measure can in fact also be reached by using the ungrouped measures of the percentage of observations on the shelf in periods 1 and 2 and this percentage of observations in the top box in periods 1 and 2.

The mean levels of cortisol measured in the A and B samples differed markedly

(Table 2; $P < 0.01$, Mann-Whitney U test, two-tailed). The levels of leucocytes did not

Table 1. Mean percentage of observations of vixens in various shelters in periods 1 and 2, sum of observations in shelters and means of observations in periods 1 and 2 and individual correlation of use of shelters in periods 1 and 2

	Open Box	Side Box	Shelf	Top Box	Shelf + Top	All
Period 1	1.5	0.1	19.8	7.8	27.6	29.2
Period 2	1.8	0.0	9.7	1.9	11.6	13.4
Mean	1.6	0.1	14.7	4.9	19.6	21.3
Correlation	0.21	0.03	0.37	0.40	0.48	0.57
P	0.14	0.83	0.01	< .005	< .001	< .001

Table 2. Mean levels and correlations of cortisol and leucocytes (eosinophis, neutrophils, lymphocytes, monocytes)

	Mean levels	Spearman Rank correlation coefficients						
		Cortisol			Leoucocytes			
		A[2)]	B	B/A	eos	neu	lym	mon
Cortisol	(nmol/[1)])							
A	27.1	1.00	0.31	-0.81	0.02	0.06	-0.08	0.22
B	190.0		1.00	0.23	-0.28	-0.20	0.31	0.05
B/A				1.00	-0.22	-0.12	0.20	-0.18
Leucocytes	%							
eos	10.5				1.00	0.10	-0.36	0.14
neu	35.8					1.00	-0.88	0.48
lym	49.3						1.00	-0.47
mon	3.7							1.00

[1)]) Significant correlations bold-faced; coefficients > 0.28 are significant at 5% level, > 0.37 at 1% level
[2)]) A = sample before test, B = sample after test

vary between A and B samples, and the individual correlations between A and B measures were high and positive for all cell types. The two different methods used for assessing eosinophil changes did not yield substantially different results. For these reasons the leucocyte measures are represented here as means of the levels in the A and B samples and only the results obtained in the differential count are included (Table 2). The A levels of cortisol were positively correlated with the B levels and negatively correlated with the increase in cortisol from A and B, the B/A ratio. The A levels were not correlated with any of the leucocyte levels, while on the other hand, the B levels of cortisol were negatively correlated with the levels of eosinophils and neutrophils and positively correlated with the levels of lymphocytes. The levels of lymphocytes were negatively correlated with the levels of the rest of the cell types.

The A levels of cortisol were negatively correlated with the nest box use, and the nest box use was positively correlated with the B/A ratio. (Table 3). Fig. 1 shows the individual

variation in A levels of cortisol and in nest box use, and the correlation between these measures.

Table 3. Spearman Rank Correlation Coefficients between cortisol and nest box use and between cortisol and frequency of stays in the various fields of the open field runway. Mean frequencies of stays shown at the bottom

	Nest Box	Stays in Open Field Runway								
		F0	F1	F2	F3	F4	F5	F6	F7	F8
Cortisol										
A[1)]	-0.34[2)]	0.27	0.18	0.13	0.09	0.06	0.06	0.13	0.27	0.28
B	0.14	0.23	0.38	0.45	0.45	0.44	0.52	0.53	0.46	0.21
B/A	0.36	-0.10	0.07	0.15	0.18	0.20	0.23	0.16	-0.01	-0.18
Frequency of stays		3.5	11.9	16.1	18.1	18.0	16.2	13.5	8.3	3.0

[1)] A = sample before test, B = sample after test

[2)] Significant correlations bold-faced; coefficients > 0.28 are significant at 5% level, > 0.37 at 1% level

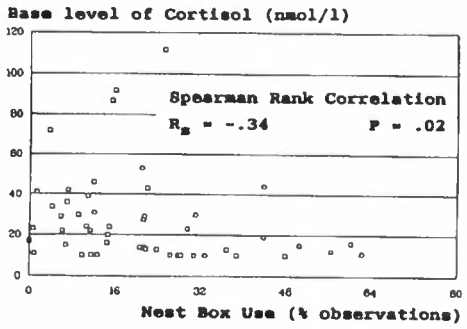

Fig. 1. Correlation between nest box use (percentage of observations in all shelters in both periods) and base levels of cortisol

Most of the animals moved actively around in the open field run-way and generally posi-tioned themselves in the central fields (Table 3). There was a marked difference between the correlation of the two cortisol measures and the frequency of stays in the various fields in the open field runway. The A levels were positively correlated mainly with the frequency of stays in the outer ends of the runway, the B levels were positively correlated most conspicuously with the frequency of stays in the central parts of the runway. The B levels of cortisol were positively correlated with total number of field crossings for each animal (r_s = 0.5; P = 0.0012). The latencies to reach each and every field were significantly negatively correlated with the B levels of cortisol and uncorrelated with the A levels.

The results of the glove tests and the strike tests did not indicate any significant correlations with the other results obtained. The results of the human tests indicated that most individuals could be classified as either defensive or curious (Table 4). The very few individuals that were classified as fearful were excluded from the analysis and presentation. Although differences were small, both tests showed that curious animals had the lowest A levels of cortisol. In test 1 they also had the lowest B levels, and in test 2 the greatest B/A ratio. The nest box use showed no significant correlation with the results of the human

tests. On the other hand, the animals scored as most defensive when captured were those that used their nest boxes less. The same tendency towards lower nest box use in defensive animals was seen in the human tests. The animals that made contact with the unfamiliar object in the open field test clearly were those with the highest base level of cortisol and the lowest B/A ratio.

Table 4. Relation between behavioural scores and levels of cortisol and nest box use [1)]

	Cortisol levels		ratio	Nest box use
	A (nmol/l)	B (nmol/l)	B/A	% obs.
Human test 1				
Defensive (n = 18)	30.1	210.3	8.7	19.0
Curious (n = 25)	26.6	166.4	10.6	23.6
P	*	*		
Human test 2				
Defensive (n = 25)	31.0	190.4	8.1	18.9
Curious (n = 21)	23.8	180.9	12.0	25.3
P	*		0.06	
Capture reaction				
Defensive (n =37)	27.3	183.5	9.2	18.7
Passive (n = 12)	27.8	212.4	12.3	28.8
P				*
OF-test, unfamiliar object				
- contact (n = 32)	20.1	184.0	11.1	21.8
+ contact (n = 17)	41.3	201.4	7.7	20.0
P	**		*	

[1)] * $P < 0.05$, ** $P < 0.01$, Mann-Whitney U-test

DISCUSSION

The cortisol levels measured in the A samples were not affected by day of sampling, by sampling order or by duration of ongoing sampling procedure. They should, therefore, represent base levels of cortisol in the vixens. The levels of cortisol in the B samples are roughly seven times greater than the base levels. They represent the vixens' reaction to the acute stress of being caught, blood-sampled and exposed to the open field test. Results show that vixens with low base levels of cortisol react faster or to a greater extent to acute stress (strong negative correlation between A and B/A). However, they do not reach any higher levels of cortisol in 20 min than do vixens with high base levels (weaker positive correlation between A and B). Recently, this relationship between base level of cortisol and adrenal reactivity was also found in mink (Bildsøe et al. 1991). The marked correlation between B levels of cortisol and leucocytes is difficult to interpret. There was no correlation

between A levels of cortisol and leucocytes, and the leucocyte levels were the same in both A and B samples. Therefore the result suggests that the relative frequency of leucocytes before stress is somehow related to the speed or magnitude of the adrenocortical reaction to acute stress.

The positive correlation between use of the shelters in periods 1 and 2 in the autumn of 1988 suggests that the individual tendency to use the shelters is rather stable. For that reason one might have expected the individuals to use shelters with the same relative frequency in autumn 1989 as they did in the previous autumn. The demonstrated negative correlation between nest box use and base level of cortisol may suggest, then, that ongoing use of nest boxes and base levels of cortisol are also correlated. Since it was shown previously (Jeppesen & Pedersen 1990) that foxes living without nest boxes had really high base levels of cortisol, it is tempting to suggest that the differential use of nest boxes in the present study was the reason for the variation in cortisol. The demonstration in the present study of the correlation between high base levels of cortisol and greater defensiveness and exploration do, however, also offer another possible explanation: The negative correlation between baseline cortisol and the percentage of nest box observation may reflect, quite simply, that highly defensive and exploratory animals (with high base levels of cortisol) spent more time out of the box, especially when observed by a human observer, as was the case in this study.

The marked positive correlation between B levels of cortisol and all expressions of activity in the open field runway may reflect the effect of an activity-induced increase in cortisol. The coincidence in distribution of mean number of stays in the various fields and the degrees of correlation support this conclusion. The pattern of correlations between stays in the fields of the open field runway and the base level of cortisol suggests that individuals with high base levels of cortisol were the most explorative, proceeding most frequently to the farthest ends of the runway. This interpretation is supported by the fact that those individuals that made contact with the unfamiliar object had higher base levels of cortisol. Thus, the correlation between base levels of cortisol and behaviour in human tests and open field tests indicates that within the range of present study the individuals with the highest base levels of cortisol were also the most defensive and explorative.

Generally, high base levels of cortisol induced by long-term exposure to unavoidable stressors do induce fear and reduce defensiveness and exploration (e.g. Henry 1976). Since the vixens of the present study showed an opposite correlation of cortisol and behaviour it is suggested that the present variation in base levels of cortisol do not include levels which threaten the well-being of the animals.

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Whole-year nest boxes and resting platforms for foxes

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Whether whole-year nest boxes and/or resting platforms are a necesssity for foxes or for man is still a matter for conjecture. This is due to the fact that there are considerable differences in preferences between silver foxes and blue foxes, between and within individuals, and between nest boxes and platforms. It seems obvious that the function of the platform is that of an observation place while the nest box serves as a hiding place. Since the duration of use of both is short term, it seems probable that foxes do not prefer a solid floor to a mesh floor, nor do they use boxes or platforms as a shelter against cold. Furthermore, the most interesting question of whether the presence of platforms and/or nest box affects the temperament of the foxes or vice versa is still controversial.

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Whether farm bred foxes benefit from, and accordingly, should have whole-year nest boxes is an age-old question. When asked about this almost every farmer can confirm having sometimes tried to offer nest boxes or solid resting platforms to his animals. The experiences have been variable, but the negative ones predominate. Recently, mainly in response to public opinion, the Standing Committee of the European Convention on the Protection of Animals Kept for Farming Purposes has recommended that each weaned animal (fox) shall have available a nest box or a platform and preferably both. This is the recommendation and the farmers have to live with it. However, there is no one particular accepted or even recommended construction model for putting this recommendation into practice. We have recorded the reactions of farm bred silver and blue foxes to a variety of nest box and platform designs in an attempt to discover the factors and situations which promote their use and to map and resolve some of the negative effects associated with many of the constructions.

MATERIAL AND METHODS

The use of platforms or nest boxes was monitored by direct visual observation, by means of an automatic recording aided by a thermometer (Harri et al. 1991) and video-recorder, and always over a 24-h period at intervals indicated in the figures. The experiments were

carried out in the Juankoski research fur farm of the University of Kuopio (blue foxes, red foxes) and at the Fur Farming Research Station at Kannus (silver foxes).

RESULTS

Blue fox

Most of the results on the use of resting platforms by farm bred blue foxes have been published previously (Harri et al. 1988, 1991; Korhonen 1987). New data have been collected but not yet analysed. In short, the platforms placed onto the cage floor very soon became very dirty, since the animals defecated and urinated onto them. When raised to about 20 cm below the cage ceiling, the platforms generally remained clean. There were individuals who lay on the platforms for hours while a majority of the animals used the platforms for only short periods of time. Thus the median better describes the amount of use than the mean value. Platforms without walls were used significantly more (median 78 min/d, mean 150 min/d) than those with walls (median and mean 7 and 19 min/d, respectively, $p<0.001$) (Fig 1).

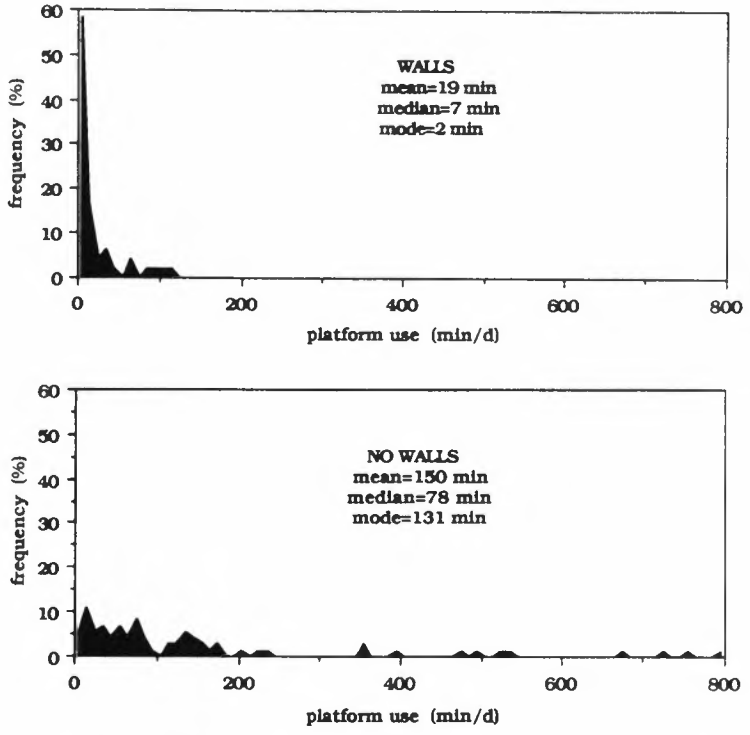

Fig. 1. Use of resting platforms without or with walls by a total of 47 farm bred blue foxes. Each individual fox was observed from one to seven times

After some time, even the animals that to begin with had used the platforms began to lose interest. Since the platforms were provided at the beginning of autumn, we do not know whether decreasing temperature or habituation with the platforms was the reason for the decrease in use. Any extraneous disturbances on the farm promoted the use, however.

The contribution of individual differences was the most important predictor, explaining 59% of the variance of platform use altogether. This was followed by the type of platform present (17%) and the temperature (5%). Disturbances explained a significant proportion (4%) of the variance in platform use during the working day only. The effect of sex or orientation of the platform with respect to sun and wind was not significant. A detailed observation on the behaviour of the animals revealed that it was always the same individuals that jumped onto the platform when a visitor approached them, while most of the other animals did not.

Silver foxes

Young silver foxes were provided with platforms in August. With time, they too lost interest in platform use (Fig. 2). Since recording of use did not take place before September, we do not know to what extent the animals used the platforms before that time. Neither do we know whether the decrease in use was asoociated with the gradually decreasing autumn temperature.

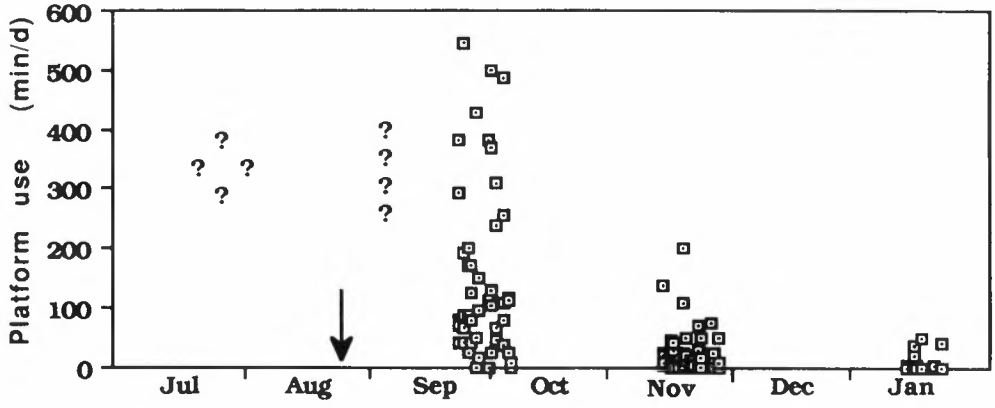

Fig. 2. Use of resting platforms by a total of 20 silver fox cubs during autumn and winter. Half of the animals were provided with platforms in August (arrow), while the other half were born into cages already supplied with platforms

Unexpectedly, the familiarity with the platform in the cage environment was not a significant factor affecting its use. On the average, animals born into cages with platforms used the platforms for 77 mins/d, while animals that had been provided with platforms no earlier than August used them for 63 mins/d (N.S.). Silver foxes preferred platforms from which they could observe the shed-house door (101 mins/d) to platforms facing in the opposite direction (38 mins/d) ($p < 0.01$). During the weekend the platforms were used less frequently than during the working days.

Pairs of weaned silver fox cubs were placed into empty wire mesh cages (105 x 120 cm, control animals), into cages equipped with a large nest box (43 x 45 x 40 cm, length x width x height, internal diameters) or a smaller one (round internal room, diameter 38 cm, height 32 cm). In these constructions the animals could use the nest box roof in addition to its internal compartment. Careful video-recording of two pairs revealed that after an initial slight interest, even this minimal use of the nest box interior gradually began to decline. Instead, the animals spent a major part of their daily time on the roof (Fig. 3).

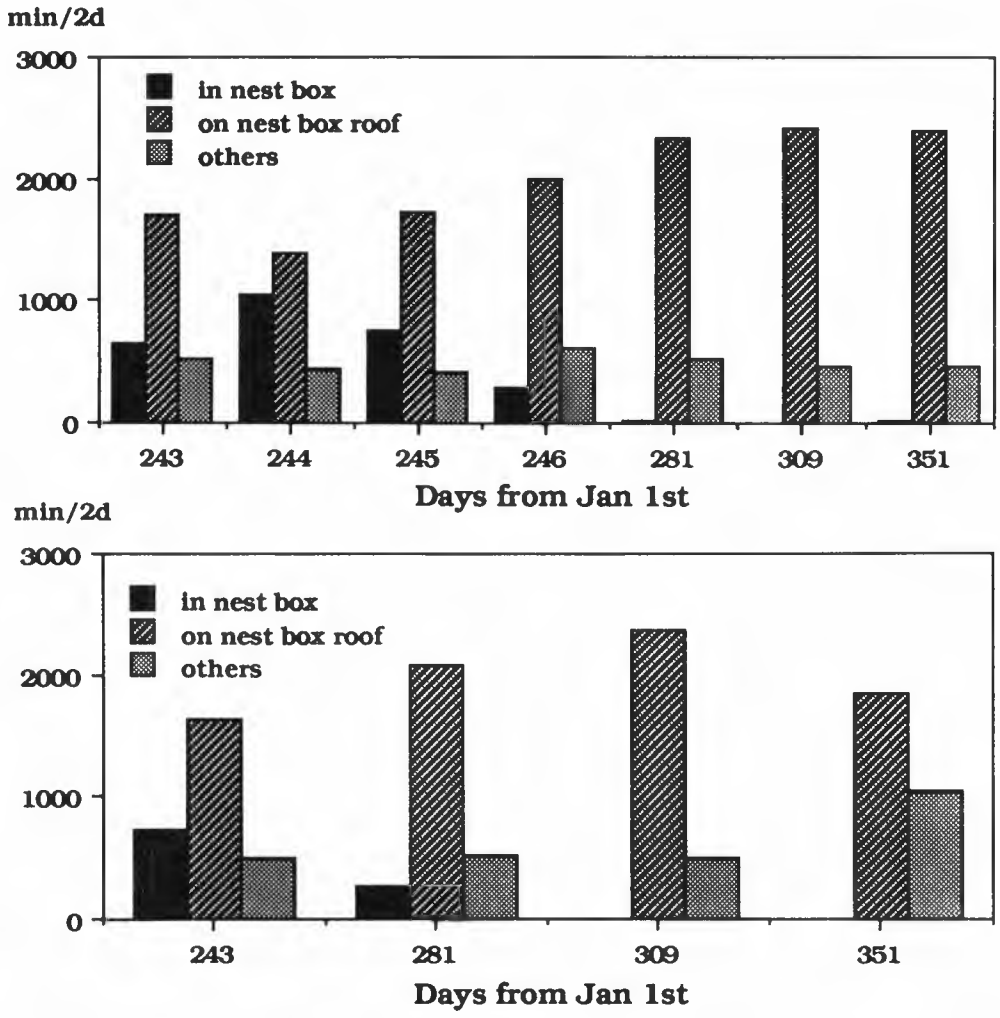

Fig. 3. Use of nest box and nest box roof by two silver fox pairs as a function of time the boxes were available for use

Some pairs were rather messy (Fig. 4). They defecated and urinated into the boxes. In most cases, however, only occasional pieces of faeces were found in the boxes.

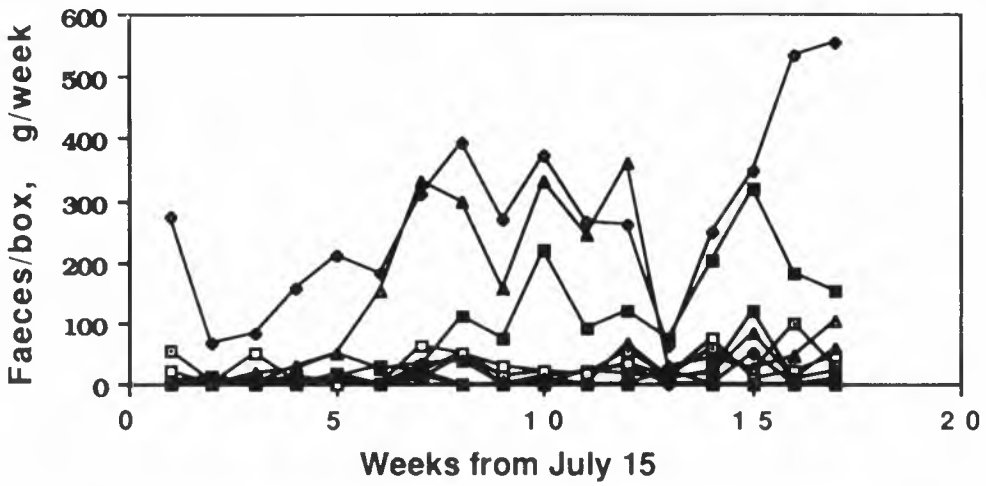

Fig. 4. Weekly amount of faeces found in nest boxes. Each nest box was placed into a standard cage 120 x 105 cm, each of which was occupied by two young silver foxes. The curves represent different nest boxes

It seems obvious that the defecation pattern typical of each pair remained constant for the whole observation period, i.e. once messy - always messy. This feature, again, was independent of the nest box size. As a result of this, some foxes became more or less dirty (Fig. 5).

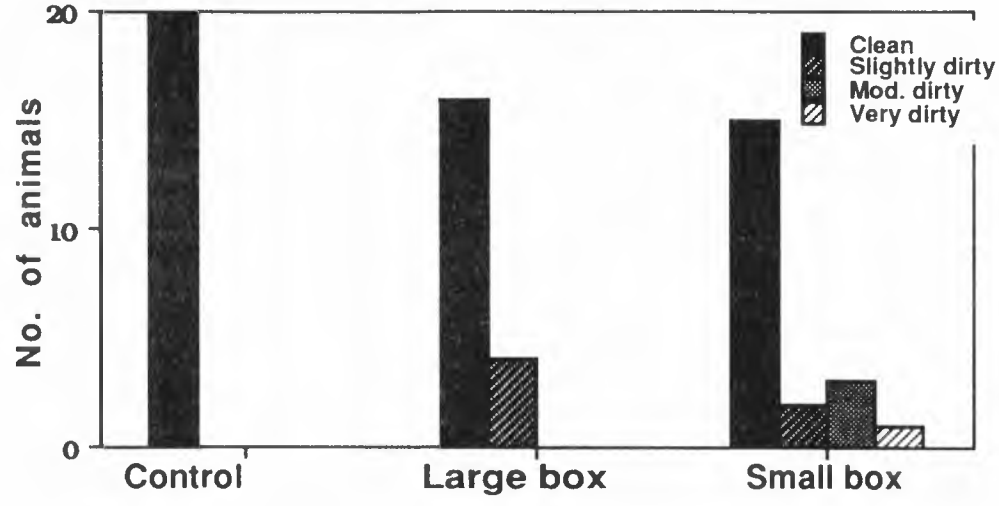

Fig. 5. Dirtiness of young silver foxes in cages with or without nest boxes

In December the animals were pelted and their pelts were scored on a scale from 1 to 10 by professional personnel. The colour purity of the skins of the animals with access to boxes was poorer than that of the controls (Fig. 6). This was mainly due to a yellow coloration of the belly region of the skins. In addition, the scores generally were more uniform for the animals without access to boxes, while typically the whole scale from 1 to

10 was used for nest box groups. Because of this wide variance and the rather small number of animals in each group, there were no other significant differences between the groups. The size of skins was similar for all groups.

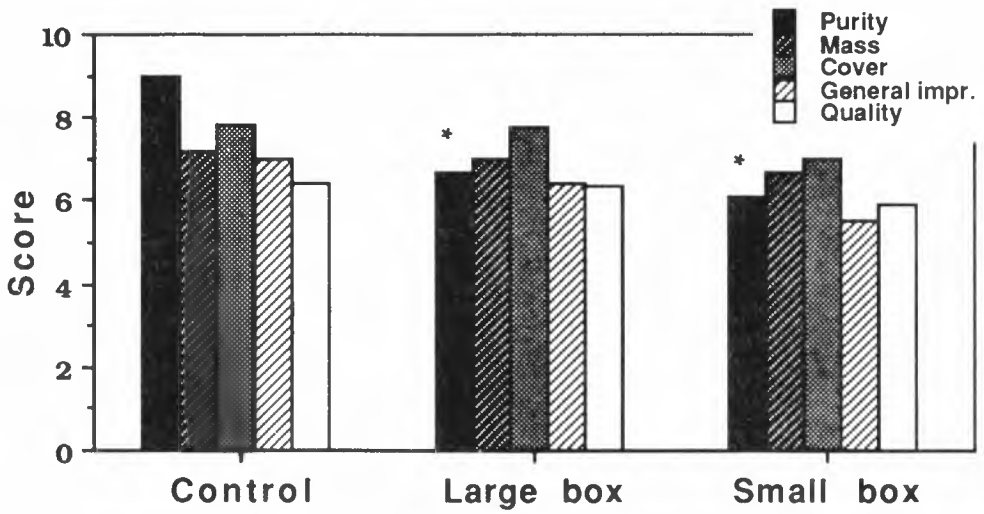

Fig. 6. Skin quality characteristics for control of silver foxes and for foxes with access to large or small nest boxes. For each parameter the score was ranked from 1 (poorest) to 10 (best). *-significantly different from control group ($p < 0.01$, Tukey's test)

A top-mounted nest box was offered to some red foxes in late autumn. The animals had access to the box through a platform which was mounted just below the entrance tunnel of the box. From visual observation it was found that the animals usually slipped into the box when a visitor was approaching them and, again, out of the box as soon as the visitor was some metres away. Video-recording revealed that each individual animal adopted a typical pattern in nest box and platform use (Fig. 7), which was not influenced by either temperature or any other weather conditions.

DISCUSSION

The present results raise more questions than they give answers to. It is obvious that the animals do not perceive the nest box or platform as a protection against the weather. On the contrary, the use of these shelters decreases rather than increases with decreasing temperature. Furthermore, the observation that platforms without walls or with a good view towards the shed-house door were clearly used more frequently than those without observational possibilities supports the concept that the animals regard the open platform as an observation place. It is also obvious that access to nest boxes results in poor pelt purity resulting from contacts with faeces and urine in the boxes. Fortunately, only a minority of animals are messy so this trait can easily be culled out from the population.

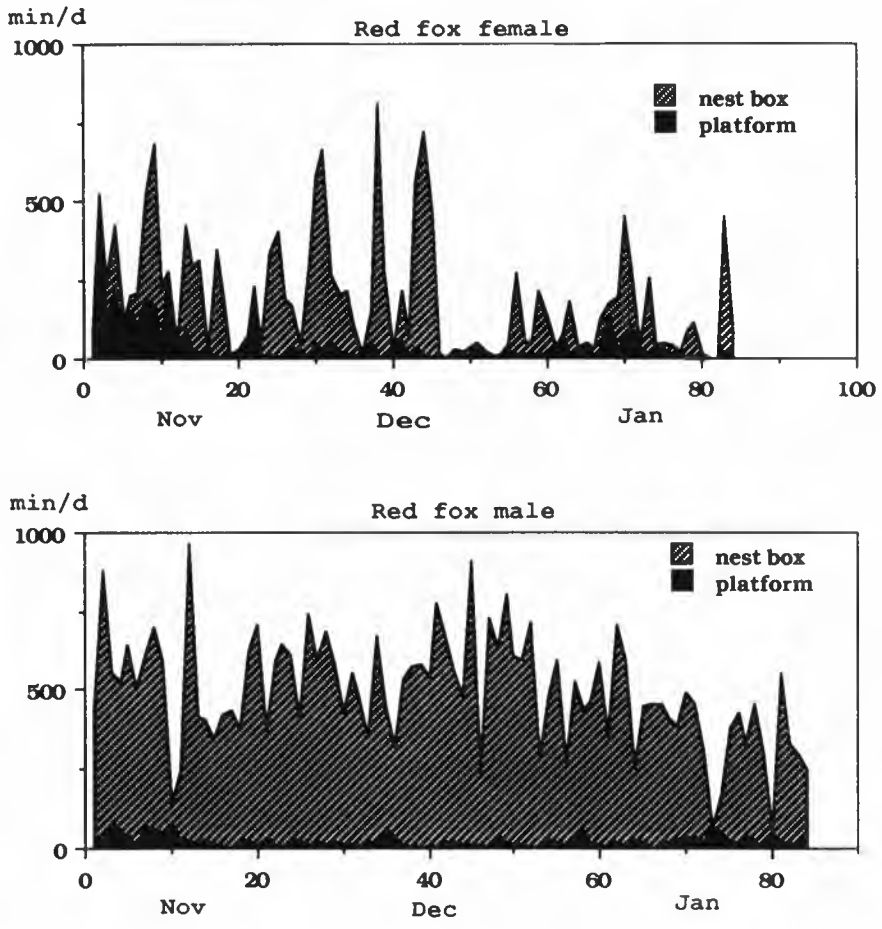

Fig. 7. Two individual patterns on use of a top-mounted nest box and a platform which served as an entrance to the nest box by farm-bred red foxes

The conclusions have been confounded by the evidence that the different nest box and platform designs used seem to have different functions. The top-mounted box seemed to serve as a hiding place for the red foxes, while for the silver foxes the usual nest box placed on the cage floor served as a defecation place only.

The platform on the cage floor was the defecation place for both species, whereas the same platform with walls raised slightly higher served as a place of escape, but only for some individuals. Platforms without walls were used slightly more and mainly as an observation post. However, the nest box roof, which by man's reckoning should have served the same function as the open platform, had, in the way it was perceived by the silver foxes, some quite different features because they spent a major part of their daily time on it. It is reasonable to point out that despite some significant factors affecting the

amount of use of platforms or boxes, the individual differences always accounted for a major part of the variance. These individual preferences again changed with time, species and the different constructions used.

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The relationship between open field activity, competition capacity and first year reproductive success among farmed silver fox cubs (*Vulpes vulpes*)

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Silver fox cubs between 4 weeks and 7 months of age, were subjected to various behaviour tests in non-social and social situations. The relationship between the test scores for both male and female cubs and the ability to predict reproductive performance among the female cubs under commercial farm conditions, from the behavioural test score, were assessed. The results indicated that female cubs with a high and those with a low competition capacity score when seven months old differed both in their activity scores in an open field test at 30 days of age and in reproductive performance under their first reproduction. Females exhibiting a defensive behavioural strategy (inactive females in the open field test with low competition capacity) weaned fewer cubs under their first reproduction than vixens with an offensive behavioural strategy (active females in the open field with a high competition capacity). These results indicate that it is possible, to some degree, to predict a female cub's future reproduction potential from knowledge about her behaviour in social and non-social situations.

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Previous investigations indicate that among farmed silver fox vixens the most aggressive vixens in the farm may be among the best reproducers (Bakken 1988; Braastad 1988; Jeppesen & Pedersen 1990) and that fearful vixens on average wean fewer cubs than less timid vixens (Kaleta & Lewandowska 1987; Bakken 1988; Braastad 1988; Kristensen 1988). It is also shown that timid, poor reproducers on average have a lower competition capacity than the good reproducers (Bakken 1988). These results indicate that differences exist between vixens with high and with low competition capacity in the ways they cope with their environment in social and non-social situations. Vixens with a high competition capacity were less infanticidal under standard farming conditions and, on average, displayed a more offensive behaviour strategy than vixens with low competition capacity (Bakken 1988).

The aims of the present study were (1) to investigate the relationship between early

fear reactions among silver fox cubs and their later competition capacity and (2) for the female cubs, to relate these results to their first year reproductive success. It was thought that, both from an animal welfare and an economical point of view it would be beneficial if the behavioural tests could be used to reject non-successful breeding females at an early age. Whether or not this can be done depends on the correlations between adult reproductive success and the females' behaviour when young.

MATERIAL AND METHODS

Animal material and timing of tests

A human reaction test and an open field test were carried out on 244 cubs (128 males and 116 females) from 57 different litters when they were exactly 30 days of age. Immediately after the tests, the cubs were individually marked. The human reaction test was repeated when the cubs were 49 days old, which was the day of weaning. The dominance ranking within the litters was observed during two tests when the cubs were from 120 to 150 days of age. The litter were kept together as a group from birth to the day of the last dominance ranking test and split up thereafter. Of these cubs, 120 (80 female cubs and 40 male cubs) were tested for competition capacity when they were between five and seven months old. In 1988, 80 cubs (40 female and 40 male) were tested, and 40 cubs (all female) were tested in 1989.

Forty-six of the female cubs tested for competition capacity were selected as breeding vixens and the results of their first reproduction were recorded. These vixens were a representative sample of the cubs tested in the behavioural tests. During reproduction the vixens were placed in traditional breeding cages (2.4 x 0.9 x 0.7 m.) with standard breeding boxes (0.79 x 0.47 x 0.37 m.). The vixens were separated by wire netting only. Their cubs were counted as soon as possible after delivery and the litter was checked once a day thereafter.

Human test

The cubs were taken away from their mother a few minutes before the human test, placed in a dark box and transported about 50 m away to the test room. The cubs' reactions towards the experimenter were recorded during the initial 20 sec after they were taken out of the transport cage and held (B30). Their reactions were assessed in terms of three different behavioural activity categories: (1) the cub did not react at all and remained calm for 20 sec (score=1); (2) the cub reacted but became calm within 20 sec (score=2); (3) the cub reacted and did not calm down during the 20-sec period (score=3). The recorded behaviours included all kinds of activity, e.g. crawling, yelping and biting. The test was repeated at 49 days of age (B 49), following the same procedure.

Open field test

In the open field test, the cubs were introduced to a uniform, unfamiliar environment. The square "open field" area comprised a wooden floor subdivided by white lines to form 25 squares, each of 23 x 23 cm. (Fig.1). After placing the cub in the starting position (middle square, row one), its pattern of activity expressed as the number of lines crossed and the

number of different squares entered was recorded for three minutes. Grid crossing was considered to have occurred when a minimum of at least half the cub (forelimbs and breast) had entered the next square. During the experiments, the observer was positioned behind a screen (Fig.1).

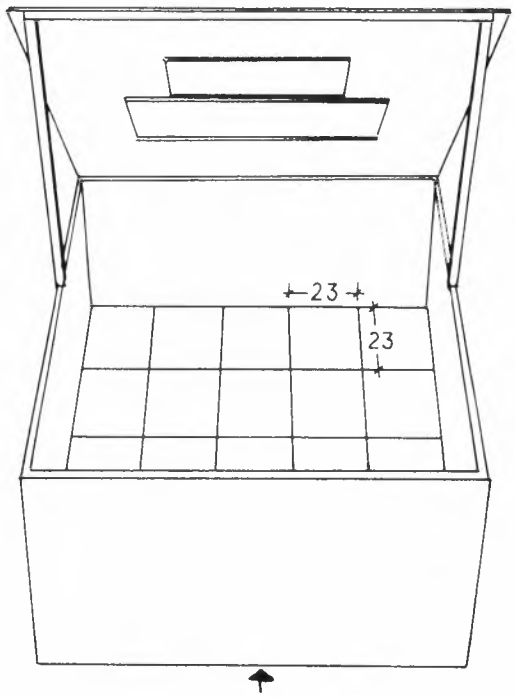

Figure 1. The open field. Square dimensions in cm are shown

Dominance ranking within the litter

The cubs were starved for one day before the test and food was offered during the test. The cub that first defended the food supply and ate, was assigned the highest dominance ranking (n) in the litter and was then taken out of the cage (n = number of cubs in the litter). The next cub to defend the food supply was assigned dominance ranking n-1, and then taken out. This procedure was continued until all the cubs had been ranked. The dominance ranking test was repeated one week later. The correlation between the two test scores was 0.66 (Spearman rank correlation). Adjusted dominance ranking was the average of the two dominance ranking scores from tests 1 and 2, adjusted to a litter size of four and expressed on a scale from 1 (lowest rank) to 4 (highest rank).

Tests of competiton capacity

Cubs were chosen for the competition capacity tests according to their previous activity scores in the open field test. The 20 most active and 20 least active female cubs in 1988

and 1989 and the 20 most active and 20 least active male cubs in 1988 were selected. In the competition capacity tests, high-activity cubs were tested against low-activity cubs of the same sex. The experiment was designed in such a way that the age difference within the pairs were minimized. Each cub was tested against three different opponents in pair interactions, each time in a new cage. Each pair was kept together for eight days and tested twice; on the fourth and the eighth day. The cubs were starved one day before each test. Shortly before the test they were given food and most of the cubs fought for the food during the test. The cubs were observed for ten minutes. The winner of each test was set to be the cub that ate the food when the other cub displayed subordinate behaviour (Fox 1970). The winner of each test was assigned a competition capacity score of one and the defeated cub scored zero. If the cubs neither fought nor displayed any subordinate behaviour, they were scored equal (0.5) in that test. The total competition capacity score of an individual cub was half the sum of its competition capacity scores in the six tests, i.e., zero was the lowest possible total competition capacity score and three was the highest.

Statistical analyses

Statistical analyses were made with the SAS system (Statistical Analysis Systems Institute Inc., 1986). The descriptive statistics are presented as means ± SD, frequencies and Spearman's Rho correlations. Comparisons between male and female cubs were made with two-tailed t-tests or Chi-Square tests. A maximum-likelihood factor analysis with varimax rotation was performed to determine the degree to which variability in the behavioural test results could be explained by common underlying factors.

RESULTS

Sex

There were no significant differences between the sexes in the activity results in the open-field test at 30 days of age (Table 1), nor in the animals' reactions towards humans at 30 or 49 days of age (B 30: $\chi^2=0,27$, NS, B 49: $\chi^2=2,72$, NS, Table 2). At 49 days of age a higher proportion of both females and males reacted in the human test than at 30 days of age ($\chi^2=30.7$, $p < 0.0001$, Table 2). On average, the males exhibited higher adjusted dominance rankings within the litters than did the female cubs ($p < 0.03$, Table 1).

Correlations between the cubs' behavioural score in the different tests

The number of squares entered and the number of lines crossed in the open field were very highly correlated (Table 3). These parameters also correlated with the subsequent total competition capacity score, for both the female and the male cubs (Table 3). The number of squares entered was correlated with the subsequent dominance ranking within the litter for the female cubs, but no significant correlation was found for the male cubs (Table 3). The activity score in the human test at 30 days of age correlated negatively with the number of lines crossed in the open field but not with number of squares entered (Table 3). Adjusted dominance ranking in the litter was not significantly correlated with the subsequent competition capacity score (Table 3).

Table 1. The test scores for male and female cubs

	MALES			FEMALES			
	N	Mean	SD	N	Mean	SD	p
Open field test							
Number of lines crossed:	128	39.8	24.7	116	41.9	24.4	NS
Number of squares entered:	128	16.1	6.2	116	16.6	6.5	NS
Adjusted dominance rank in the litter:	95	2.6	1.1	100	2.3	1.1	*
Competition capacity score:	40	1.5	0.8	80	1.5	0.9	

*: $p < 0.05$

Table 2. Proportion of male and female cubs in the different behaviour categories in the human tests[1]

The cubs' age when tested	MALES				FEMALES			
	N	Cat. 1	Cat. 2	Cat.3	N	Cat. 1	Cat. 2	Cat. 3
30 days	125	63.2%	30.4%	6.4%	116	65.8%	29.1%	5.1%
49 days	113	53.9%	19.5%	26.6%	116	45.7%	28.4%	25.9%

[1] Cat. 1: proportion of cubs that did not react in the test. Cat. 2: proportion of cubs that reacted but calmed down during 20 sec Cat. 3: proportion of cubs that reacted and did not calm down during 20 sec

Factor analysis

To summarize the whole correlation matrix, a factorial analysis was made. Two factors provided significant contributions. The first factor (F1) accounted for 72% of the overall variation. The second factor (F2) accounted for 30% of the overall variation. The two factors were rotated using the varimax criterion to render the dimensions of the space more recognizable. The correlations between the rotated factors and the different variables are shown in Table 4. Factor 1 had a high, significant, positive correlation with the cubs' activity scores in the open field test. Factor 1 also had a significant, positive correlation with the cubs' competition capacity score and their adjusted dominance ranking in the litter, but was not correlated with their test scores in the human reaction tests. Factor 2 had a high, significant, positive correlation with the cubs' reactions in the human tests, both at 30 and 49 days of age. This factor correlated negatively with the open field scores, but showed no significant correlation with the social competition scores.

Table 3. The Spearman rank correlation coefficients between the cubs' activity at 30 days of age in the open field test, their reactions towards humans and their subsequent social scores

	NSE[1]	CC	ADR	B30	B49
NLC, TOTAL:	0.87***	0.54***	0.07	-0.23***	-0.10
Males:	0.84***	0.44**	0.05	-0.25**	-0.07
Females:	0.90***	0.59**	0.13	-0.19*	-0.12
NSE, TOTAL:		0.51***	0.15*	-0.13*	-0.09
Males:		0.41**	0.14	-0.12	-0.06
Females:		0.53***	0.21*	-0.12	-0.12
CC, TOTAL:			0.15	0.04	0.09
Males:			0.26	0.03	-0.08
Females:			0.08	0.05	0.17
ADR, TOTAL:				0.01	-0.04
Males:				0.04	0.04
Females:				-0.04	-0.12
B 30, TOTAL:					0.44***
Males:					0.50***
Females:					0.39***

[1] NSE: number of squares entered in the open field. NLC: number of lines crossed in the open field. B 30, B49: the cubs' reactions at 30 and at 49 days of age towards humans. ADR: adjusted dominance ranking in the litter. CC: competition capacity score. TOTAL: overall totals for both sexes combined. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.

Table 4. The correlation coefficients between the behavioural test scores and the rotated factors F1 and F2 (N=110)

Behaviour test	Factors F1	F2
Open field[1]		
NLC	0.93***	-0.47***
NSE	0.93***	-0.41***
Human tests[2]		
B 30	-0.03	0.84***
B 49	0.01	0.79***
Social tests[3]		
ADR	0.26**	0.10
CC	0.68***	0.14

[1] number of lines crossed (NLC) and number of squares entered (NSE) in the open field.
[2] the cubs' reactions at 30 (B 30) and 49 (B 49) days of age, towards humans.
[3] adjusted dominance ranking in the litter (ADR) and competition capacity score (CC). *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.

Number of cubs born and weaned unharmed

Forty-nine of the female cubs tested were kept at the farm for reproduction. These vixens bore on average 3.22 (SD±2.11) cubs during their first reproduction and, on average, weaned 1.65 (SD±2.26) cubs unharmed. The only significant predictor of litter size at birth was the vixens' competition capacity score as cubs (Table 5). Both the activity scores in the open field at 30 days of age and the vixens' competition capacity scores when seven months old correlated significantly with the number of cubs weaned unharmed (Table 5). The best predictor of the number of cubs weaned unharmed was the competition capacity score of the vixen (Table 5), but F1 showed an almost equally high correlation (r = 0.48) with number of cubs weaned unharmed. Factor 2, however, was not significantly correlated with any of the reproductive parameters (Table 5).

Table 5. The correlation coefficients (R_s) between the vixens' reproductive results, their behaviour as cubs and Factors 1 (F1) and 2 (F2)

	NLC	NSE	B30	B49	CC	ADR	F1	F2
Number of cubs born	0.21	0.13	0.13	0.06	0.31*	-0.12	0.26	0.20
Number of cubs weaned	0.39**	0.35*	0.07	-0.01	0.50***	0.06	0.49***	0.06

1 NLC: number of lines crossed in the open field (N=49), NSE: number of squares entered in the open field (N=49). B 30, B49: The cubs' reactions, at 30 and at 49 days of age, towards humans (N=49). ADR: adjusted dominance ranking in the litter (N=46). CC: competition capacity score (N=46). *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.

DISCUSSION

The present study indicates that individual variation in the cubs' behaviour, in social and non-social situations, can be expressed by two independent factors. The first factor was positively correlated to the cubs' activity score in the open field and to the cubs' test scores in the social tests. This indicates a relationship between the cubs' exploratory activity at 30 days of age, in the early stages of their primary socialization (Belyaev et al. 1985), and their later ability to compete for, and win resources.

The second factor was positively correlated to the cubs' test scores in the human tests and negatively correlated to their open field activity.

Among dogs, two common responses to fear-provoking stimuli are escape and freezing (Mahut 1958; Murphree & Dykman 1965; Scott & Fuller 1965). This implies that timidness can be associated with either high or low activity. This complex relationship between fear and activity may also explain the negative correlation between F2 and the silver fox cubs' activity in the open field (an inhibitation of exploration activity) and the positive correlation between F2 and the cubs' activity in the human tests (an active escape response).

Both the active cubs in the open field test and the cubs of high competition capacity

were characterized as having high loadings of F1, but they differed in relation to F2. The most active cubs were characterized as having low loadings of F2, but there was no significant relationship between the cubs' F2 scores and their competition capacity score. A better division of the cubs' behavioural responses in the human tests might perhaps have given another results, because the data indicate that some positive relationship existed between the cubs' later competition capacity score and category 2 in the human reaction tests.

During the vixen's first reproduction the best indicator of reproductive performance, was her competition capacity score as a seven-month-old cub. Vixens with a high competition capacity score when cubs bore and also weaned more cubs than those with low competition capacity scores, although the F1 score was almost as good as a reproductive indicator. These results indicate that, under commercial farming conditions, a difference exists in reproductive potential between cubs exhibiting a defensive behavioural strategy (inactive cubs with low competition capacity) and those showing an offensive behavioural strategy (active cubs with high competition capacity) and that some indication of these differences is apparent from early infancy. These results are supported by earlier findings that the infanticidal vixens show defensive (Bakken 1988) hesitation, immobilization or evasive behaviour (Kaleta 1991) more often than non-infanticidal vixens, and vixens that display offensive aggression against humans (Bakken 1988; Braastad 1988; Jeppesen & Pedersen 1990) can be among the best reproducers in the farm. This can indicate that the good reproducers generally do have higher self-confidence than the poor reproducers and that F1 can be interpreted as an self-confidence indicator.

Data for rodents indicate that differences in behavioural strategies reflect fundamentally different strategies for coping with environmental conditions (Benus et al. 1989, 1990, 1991). These data also suggest that individual behavioural strategies, in interaction with the social environment, determine the individual's susceptibility to disease and its well-being (Koolhaas et al. 1988).

These results may demonstrate that more knowledge is needed on the behavioural strategies of female silver foxes, factors of importance for determining behavioural strategies, their ontogeny and how different individuals cope under different environmental conditions, both social and non-social, before cub tests can be optimalized and put into practice.

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Handling of silver foxes at different ages pre-weaning and post-weaning and effects on later behaviour and stress-sensitivity

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The effects of handling were studied in 344 silver foxes randomly assigned to eight groups. Seven groups were handled at different ages pre- and post-weaning and one group received no handling at all. Behavioural tests were carried out at the cub ages of 18, 24, 30 and 32 weeks and bleeding procedures at the age of 26 weeks. The results indicated that handling during weaning or post-weaning reduced the later fear responses of the foxes towards humans ($0.011 > p > 0.0001$, χ^2-test) at all ages tested, with the exception of at 30 weeks. No significant differences in fear responses were found between handled and non-handled groups or within the handled groups at that age. It was suggestsed that the general increase in fear responses in all groups at 30 weeks of age was caused by the bleeding procedure performed at 26 weeks of age. It was also suggested that handling during weaning and post-weaning reduced the fear responses of foxes towards humans when performed for three weeks or more, whereas handling pre-weaning had to be performed for six consecutive weeks before reductions in fearfulness could be found. The effects of handling were thought to be permanent because of low levels of fear among handled individuals at the age of 32 weeks. Levels of cortisol in the blood were concluded not to represent true base levels, and no conclusion could be drawn on the stress sensitivity of handled/non-handled foxes.

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The behavioural and physiological effects of early handling have been studied in various species such as dogs (Fox & Stelzner 1966), chickens (Jones & Faure 1981), sheep (Hargreaves & Hutson 1990) and pigs (Hemsworth et al. 1986a, 1986b). The objective of most of the studies was to find a method which resulted in more docile animals and thus in an improved adaptation to husbandry routines in the species concerned. Non-aversive handling procedures have resulted in less fearful, more explorative and more stress-resistent animals, whereas aversive handling procedures have resulted in fearful, passive and, in some studies, chronically stressed animals (Hemsworth et al. 1986b).

Foxes have been kept on farms for more than a 100 years but they are still not quite accepted as husbandry animals. This is partly because of the foxes' fear responses when approached by strangers and the necessity of using gloves or neck-tongs during routine management practices to protect the farmer from injuries.

A previous study of Pedersen & Jeppesen (1990) showed that handling of silver fox cubs at the age of two to eight weeks reduced their later fear responses towards humans as well as towards unfamiliar stimuli, and reduced the stress sensitivity in adult foxes. These results were in accordance with studies on handling effects on other husbandry animals. In the study of Pedersen & Jeppesen (1990) handling took place for six consecutive weeks pre-weaning. The objective of this study was to examine the effects of handling carried out for 3, 6 or 12 consecutive weeks at various ages pre-weaning, during weaning and post-weaning on the later behaviour and stress-sensitivity of foxes.

MATERIALS AND METHODS

Subjects and housing

From 102 litters of primiparous silver fox vixens 344 male and female cubs were randomly distributed in eight groups. The foxes were kept in four-row fox houses with double standard fox cages measuring 1.95 x 1.20 x 0.95 m. The cubs were weaned at the age of eight weeks by removal of the vixen, and siblings stayed together until aged ten weeks. Then the cubs formed pairs of female and male siblings in single standard fox cages. At 16 weeks of age all the male cubs were moved to their own cage and the foxes were kept separately also from that age.

Handling

The cubs in the eight experimental groups were handled at eight different age intervals according to the following setup:

DURATION: 3 WEEKS	6 WEEKS	12 WEEKS
GRP 1: 0-3 (N=25)		
	GRP 5: 0-6 (N=24)	
GRP 2: 3-6 (N=22)		
		GRP 7: 0-12 (N=27)
GRP 3: 6-9 (N=25)		
	GRP 6: 6-12 (N=26)	
GRP 4: 9-12 (N=23)		

GRP 8: NO HANDLING (N=172)

Handling was performed twice a day for five minutes, five days a week. The cubs were talked to, fondled along the back and behind the ears in their nest boxes or in the cage. The handling procedure was slightly changed after weaning with the handler gently talking to the foxes through the cage door. She would reach out and touch the foxes, if possible. If the foxes responded with defensive aggression or fear reactions she would slowly withdraw her hand.

Behavioural tests
At the ages of 18, 24 and 30 weeks all cubs were subjected to two tests. An experimenter, unfamiliar to the foxes, tested the foxes aged 32 weeks in groups 1-7. In the two different tests the foxes' reactions to a human being were registered (human and confrontation tests). The human test was carried as described in Pedersen & Jeppesen (1990). The confrontation test was carried out just after the human test: the handler opened the cage door and reached out for the fox. The fox's reactions within the space of 15 sec were recorded as displaying either defensive aggression, curiosity or fear, according to its body posture, facial expression, ear positions and vocalization (Fox 1970).

Blood sampling
Blood samples were collected from foxes aged 26 weeks. They were caught with a pair of neck-tongs, and a small area of their right front leg was shaved. Blood samples were collected in EDTA-treated tubes and rotated continuously. The blood was centrifuged at 3000 rpm for 10 min, and the supernatant was frozen and analysed for cortisol content using a competitive binding technique.

RESULTS

Behaviour
When the eight experimental groups were compared, significant differences were revealed in both behavioural tests at the age of 18 weeks ($p < 0.0001$, χ^2-test) and 24 weeks ($p < 0.0001$, χ^2-test).

A comparison of the responses of the seven handled groups revealed significant differences at the age of 18 weeks in the two behavioural tests (human test, $p < 0.01$, confrontation test, $p < 0.02$, , χ^2-test) and at 24 weeks of age in the human test ($p < 0.003$, χ^2-test) and confrontation test ($p < 0.001$, χ^2-test).

When differences were revealed among the seven handled groups each of the groups was compared with the control group (group 8): in the human test the foxes in groups 3-7 responded less fearfully at the ages of 18 weeks and 24 weeks (Fig. 1) compared with the responses of individuals in group 8. Individuals in groups 2-7 reacted with less fear in the confrontation test (Fig. 2) at the age of 18 weeks. Foxes in groups 3, 5, 6, and 7 also displayed less fear at the age of 24 weeks compared with the responses of foxes in group 8. At 24 weeks of age individuals in group 1 reacted more fearfully in the confrontation test compared with those in group 8.

At the age of 32 weeks only the confrontation test (Fig. 3) revealed significant differences in responses, with foxes in groups 1 and 2 exhibiting more fear in comparison with those in the other groups.

No significant differences were found between the eight groups or between the seven handled groups at the age of 30 weeks in either the human (Fig. 1) or the confrontation test (Fig. 2).

When responses within each group were compared at 18, 24, 30 and 32 weeks of age the levels of fear in the human test at age 30 weeks was found to be significantly higher in groups 3 ($p < 0.0001$, χ^2-test) and 5 ($p < 0.05$, χ^2-test). In groups 6 and 7 the same

differences were found, but they were not significant (group 6, $p < 0.09$, group 7, $p < 0.06$, χ^2-test). In the confrontation test the responses at 30 weeks of age also differed from those at ages 18, 24 and 32 weeks within these groups: No. 3 ($p < 0.009$), No. 5 ($p < 0.02$), No. 6 ($p < 0.002$), and No. 7 ($p < 0.071$), χ^2-test, with higher levels of fear at 30 weeks of age. In group 8 no differences were found according to age (18, 24 and 30 weeks) in either the human test or the confrontation test.

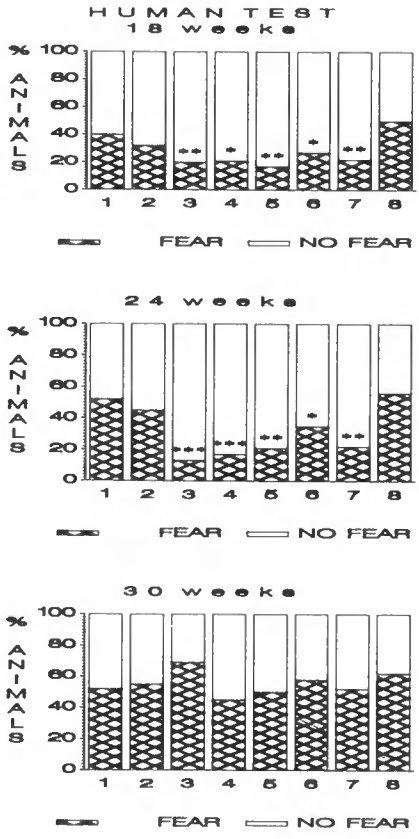

Fig. 1. Results from the human test performed at three different ages for each of the eight groups. Data are illustrated as percentage of animals reacting with fear (double-hatched) and without fear (unhatched). The levels of significance (χ^2-test, two-tailed) describe differences between the asterisked group and group 8 (control animals)
(* = $0.04 > p > 0.03$, ** = $0.007 > p > 0.001$, *** = $p < 0.0001$)

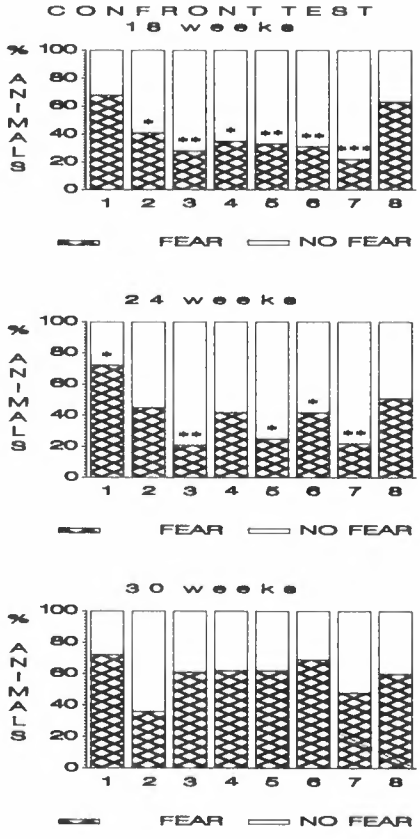

Fig. 2. Results from the confrontation test performed at three different ages for each of the eight groups. Data are illustrated as percentof animals reacting with fear (double-hatched) andwithout fear (unhatched). The levels of significance (χ^2-test, Two-tailed) describe differences between the asterisked group and groupe 8 (control animals)
(* = $0.051 > p > 0.01$, ** = $0.06 > p > 0.001$, *** = $p < 0.0001$)

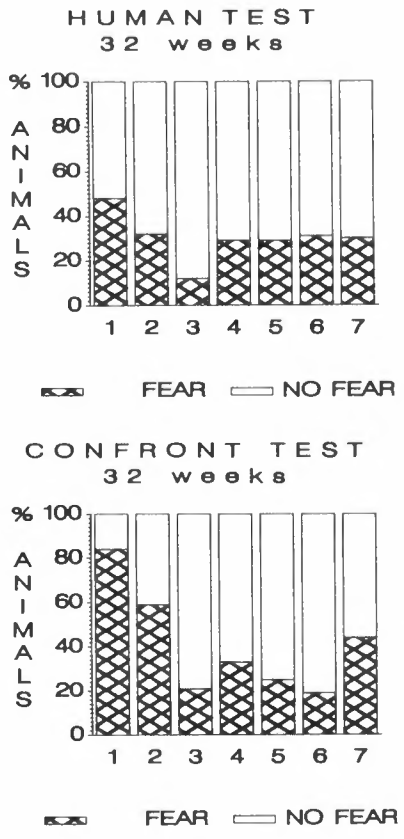

Fig. 3. Results from the human test and confrontation test performed at 32 weeks of age for each of the seven handled group. Data are illustrated as percentage of animals reacting with fear (double-hatched) and without fear (unhatched). differences are significant in the confrontation test, $p < 0.001$, x^2-test)

Cortisol concentrations

Concentrations of plasma cortisol were clearly affected by the sampling order. Earlier studies (Pedersen & Jeppesen 1990; Jeppesen & Pedersen 1991) revealed base levels of cortisol in silver foxes at 27-87 nmol/l. The lowest mean level in the present study was 139 nmol/liter (group 4). Levels of cortisol in the present study were found not to reflect true base levels and data on cortisol were therefore excluded from this presentation.

DISCUSSION

The results of the behavioural tests at 18, 24 and 32 weeks of age indicated that handling of fox cubs during weaning and postweaning for three consecutive weeks or more reduced their later fear responses towards humans. Since cubs tested at 32 weeks of age by an unfamiliar handler displayed similar responses to those evoked when they were tested at 18 and 24 weeks of age, with low levels of fear, it was indicated that the reduction of fear responses towards humans was general and not specifically towards people known by the foxes. Unfortunately the new experimenter could not find time to test the non-handled individuals. Therefore we do not know whether the control animals would have behaved differently towards an unfamiliar person than towards a familiar handler, but this seems unlikely.

At the age of 30 weeks handled animals behaved similarly to control animals, with a high number of animals showing fear responses. This high level of fear was most probably caused by the "aversive" experience the foxes were exposed to at the age of 26 weeks, with capture by neck tongs for the first time, shaving of front leg, and blood-collecting. Adult sheep handled gently for five weeks showed reduced flight distance and reduced heart rate responses to an approaching human but the effect of handling was obscured by the aversion tests of shearing (Hargreaves & Hutson 1990). The high mean levels of cortisol in all groups in the

present study indicated that the ongoing activity during the bleeding procedure was perceived by the foxes as stressful.

If the bleeding procedures had been postponed until behavioural testing had finished, and both a known and an unknown experimenter had tested all groups of animals at the age of 32 weeks firmer conclusions could have been drawn on the permanence of the effects of handling. But, as the responses of the handled animals at 32 weeks of age did not differ from their responses at 18 and 24 weeks of age, it seems reasonable to suggest that the handling effects were longer lasting.

Effects of handling were not measurable in the group handled pre-weaning at 0-3 weeks. Responses in this group did not differ from those of control animals in most tests, or they may even have been more fearful compared with the response of control animals, as at the age of 24 weeks in the confrontation test. The lack of effects of handling at the age of 0-3 weeks could be related to the sensory-locomotor development of the foxes. Reactions to sound occur at the age of two weeks, and opening of the eyes occurs at three weeks of age (Belyaev et al. 1985). At that early stage in their development fox cubs may not be able to perceive that they are being exposed to handling. Another explanation could be that they perceived the handling as being aversive. The touch of the human hand could not be avoided by the cubs, whereas cubs handled at all other age intervals were only touched if it was possible without causing fear or defensive aggression. Pigs handled aversively produced marked avoidance of humans and high base levels of cortisol indicating a chronic stress response (Hemsworth et al. 1986b).

The age of 3-6 weeks is supposed to be the sensitive period for socialization in silver foxes (Belyaev et al. 1985) and thus we should expect to find significant effects of handling at that age, but we only found effects of handling in the confrontation test at 18 weeks of age. Obviously, further research is needed to determine the boundaries of the sensitive period of socialization in foxes. However, the handling procedure revealed a pronounced effect on the reduction of fear responses when carried out from the age of 0-6 weeks, and thus the duration of the handling procedure seems to be important in achieving less fearful animals by handling pre-weaning, as was also found by Pedersen & Jeppesen (1990).

Cubs handled during weaning (6-9 weeks, 6-12 weeks) or post-weaning (9-12 weeks) displayed the most pronounced reduction in fear responses as did the group handled for 12 consecutive weeks. These results indicate that the frequent and repeated contact with humans perceived during weaning and post-weaning caused a habituation of the foxes to the presence of humans. Similar results were achieved with rabbits (Kersten et al. 1989; Podberscek et al. 1991), sheep (Hargreaves & Hutson 1990) and pigs (Hemsworth et al. 1986b.) handled post-weaning.

CONCLUSION

Handling of fox cubs during weaning and post-weaning reduced the fear responses towards humans in general, and at that time handling for three consecutive weeks was sufficient. Handling pre-weaning only reduced fearfulness when carried out for six consecutive weeks. No effects of handling could be found on physiological stress parameters, but this was attributed to the bleeding procedure. Frequent and repeated contact with humans could be

a way of achieving less fearful foxes on a more permanent basis and thus foxes which are more adapted to the farm environment.

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The effects of cage environment on the welfare of mink

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Sixty female male pairs of mink were placed in cages of three different sizes, i.e. 1.0 m^2, 0.27 m^2, and 0.1 m^2. In half of the cages of each cage size the animals were prevented from using a nest box. Based on behavioural observations of the females and stress physiological measurements of males and females, the significance of cage size and nest boxes to the welfare of farm mink was demonstrated. Furthermore, feed intake under these experimental conditions was recorded. In agreement with our previous investigations the results indicated that increasing the cage area, within the cage sizes tested, does not increase the welfare of farm mink. On the contrary some of the variables indicated reduced welfare for mink in large cages. Based on the behavioural and physiological variables used, we could, however, demonstrate an increase in: stereotypic behaviour, the heterophil/lymphocyte ratio, and the plasma cortisol concentration as well as a decrease in the number of eosinophil leucocytes in mink without nest boxes which is an indication of reduced welfare.

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The recent efforts to harmonize EEC legislation within the field of livestock production have also brought fur animal production into focus. For instance, the Commission of the European Communities has prepared a report: "Study into the legal, technical and animal welfare aspects of fur farming" (CEC Report 1991). The report calls for further investigations into the effect of cage size on the welfare of farm mink and into the possibility of enriching the environment. In comparison with the environmental design within other species of domestic animals, the design of cage environments in fur animal production has not changed very much over the years. There has, however, in fur animal production as well as for other species of domestic animals, been a reduction in the production area per animal. Within the past 20 years the dimensions of a typical Danish production cage have been constant (90 cm x 30 cm x 45 cm).

Previous investigations of cage sizes and nest boxes

At the IVth Congress in 1988 we presented an investigation of the effect of cage size and nest boxes on the behaviour, physiology and fur quality of farm mink (Hansen 1988).

Supplementary results from these investigations have been published since then (Hansen & Brandt 1989).

The results were based on behavioural observations recorded during the daytime periods from 07.00 to 09.30 and from 11.00 to 14.00. All the animals were fed according to normal farm routine.

The experiment demonstrated that there were no behavioural differences between mink in cages with an area of 1.0 m^2, 0.27 m^2 and 0.1 m^2, respectively. Neither could any differences be proved in the stress physiological variables: eosinophil leucocytes and differential leucocyte count.

It was found that in mink without nest boxes there was a higher activity level than that in mink with nest boxes. Mink without nest boxes also differed stress physiologically from mink with nest boxes in a lower level of eosinophil leucocytes and a higher heterophil/lymphocyte ratio.

Continued investigations of cage sizes and nest boxes

Partly to verify the results obtained on the effect of cage size and nest box on the behaviour and stress physiological status of farm mink, and partly to evaluate the validity of number of eosinophil leucocytes as a parameter of stress, the experiment was carried out with 132 mink.

MATERIAL AND METHODS

Cage environment

Immediately after weaning the animals were placed in male/female pairs in cages of varying sizes with and without nest boxes. An attempt was made to distribute each litter in each of the following types of cages:

Type (1) large cages	(area = 1.05 m^2)	+	nest box (10 cages)
Type (2) large cages	(area = 1.05 m^2)	-	nest box (10 cages)
Type (3) standard cages	(area = 0.27 m^2)	+	nest box (12 cages)
Type (4) standard cages	(area = 0.27 m^2)	-	nest box (12 cages)
Type (5) small cages	(area = 0.10 m^2)	+	nest box (11 cages)
Type (6) small cages	(area = 0.10 m^2)	-	nest box (11 cages)

Recording of behaviour

In this experiment the behaviour of the animals was recorded in September and October on video over a 24 h period. The frequency and duration of the behaviour of the females were recorded continuously in the first and third 15-min period of each hour.

Blood sampling

In September and October blood samples were taken for determination of number of eosinophil leucocytes and differential leucocyte count. In April the plasma cortisol concentration was determined in females.

Feed intake

To examine the feed intake during these experimental conditions, the animals were - unlike in our previous experiment - fed *ad libitum* throughout the experiment. Feed consumption was recorded as the difference between feed given and feed left over on the following morning. The animals were fed approximately at 13.00.

RESULTS AND DISCUSSION

Behaviour

The investigation revealed that the total activity level of farm mink was influenced neither by cage size nor by the presence/lack of nest boxes (Fig. 1A). Mink were active approximately 30% of the time. This variation from the previously found higher activity in mink without nest boxes compared to mink with nest boxes is probably due to the fact that in the previous experiment the mink were fed restrictively, and that observations of behaviour took place around feeding time in the morning and feeding around noon. Cage size did not have any effect on the passive position of farm mink (Fig. 1B). In general, mink kept in pairs chose to lie together, but if they did not have access to a nest box, they tended to lie together in pairs more than when they had access to a nest box ($p < 0.001$, GLM test).

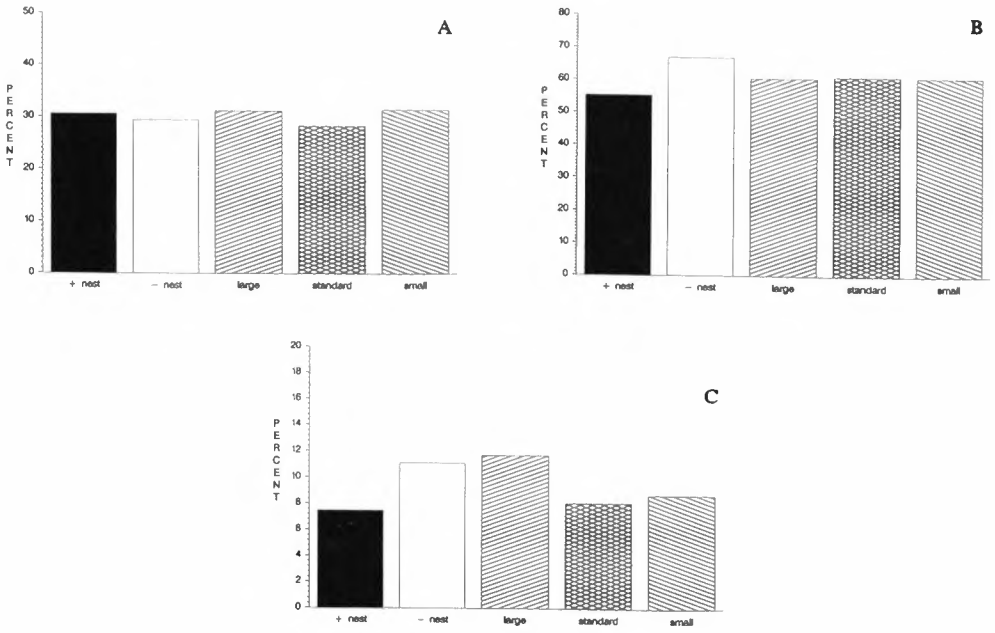

Fig. 1. The duration of behavioural elements as a percentage of observation time (beh.(min)/obs.(min) x 100) distributed on ± nest box and on cage size (large, standard, small)
A: total activity B: lying together C: stereotypies

Stereotypic behaviour occurred in 9.3% of the 24 h, corresponding to 31% of their active time. Mink in large cages performed more stereotypic behaviour than mink in standard and small cages ($p < 0.05$, GLM test). Likewise, we found a more stereotypic behaviour in mink without nest boxes than in those with nest boxes ($p < 0.001$, GLM test) (Fig. 1C). A significant diurnal rhythm was found in stereotypic behaviour ($p < 0.001$, GLM test) (Fig. 2). Stereotypic behaviour occurred most frequently in the morning from 04.00 to 10.00. The increase in stereotypic activity coincided with the increase in normal activity. The correlation between stereotypic and general activity was previously shown (Hansen 1990; Bildsøe et al. 1990). That the highest activity occurred in the morning hours and not just before feeding time is probably due to the fact that the animals were fed *ad libitum* and not restrictively, as is normal practice.

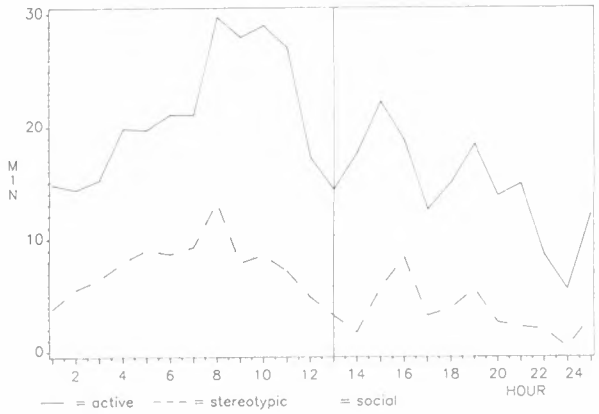

Fig. 2. Diurnal variation in total activity, stereotypic activity and social interactions in minutes/hour for the entire population of mink females

Stereotypic behaviour can develop from different behaviour patterns which are classically associated with frustration, thwarting or conflict of motivations (Mason 1991). Intention movement of escape or thwarted intention movements of approach to conspecifics or prey are likely causes for the development of stereotypies in farm mink. Factors other than the original causal stimuli can trigger an established stereotype, for instance hunger (Bildsøe et al. 1991), but also outer stimuli from cage mate or farmer may release stereotypic behaviour. A possible explanation to the increase in stereotypic activity in mink without nest boxes and in mink in large cages may be an increased input of releasing stimuli (movements, noises, etc.) from the neighbouring cages as compared to mink with nest boxes and mink in smaller cages. Stereotypies seem to be restricted to captive animals (Mason 1991) and can be viewed as a pathological consequence of disregulation of dopaminergic activity (Kuczenski 1983). In this respect they are an indication of reduced welfare. Alternatively, stereotyped behaviour may be an adaptive mechanism in response to behavioural arousal (Bildsøe et al. 1991; Hansen & Damgaard 1992 A-B; Schouten & Wiepkema 1991).

If stereotypic behaviour expresses an adaptive strategy, individuals performing

stereotypies in a given environment cannot be characterized as being more stressed than individuals not performing stereotypic behaviour, as the performance of stereotypic behaviour may reduce stress as indicated in the above-mentioned references. The occurrence of stereotypies is, however, an indication of an inadequate environment.

Physiology

Cage size did not affect the heterophil/lymphocyte ratio or plasma cortisol concentration of farm mink, but mink in large cages had a lower eosinophil leucocyte level than mink in standard or small cages ($p < 0.05$, Kruskal-Wallis test) (Fig. 3A). Mink in cages without nest boxes had a lower eosinophil level ($p < 0.05$, t-test) and a higher heterophil/lymphocyte ratio ($p < 0.05$, t-test) than mink with nest boxes (Fig. 3B) which confirms our previous results. As a further indication of increased stress, females without nest boxes had a higher plasma cortisol concentration than females with nest boxes ($p < 0.05$, t-test) (Fig. 4). Evaluated on the basis of population averages, all stress physiological variables show that mink without nest boxes are more subject to stress than those with nest boxes. The effect of cage size on the stress level of mink is less obvious, as only a lower level of eosinophil leucocytes indicates increased stress in mink in large cages.

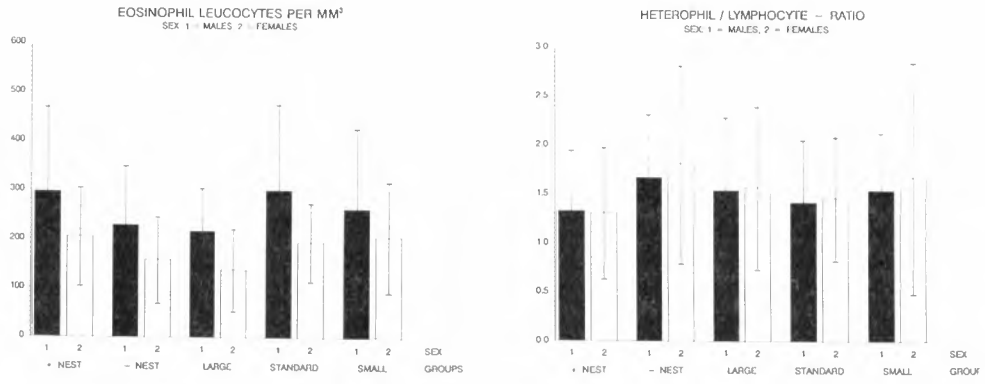

Fig. 3. Physiological variables measured. Values are mean ± SD. A: Number of eosinophil leucocytes, B: Heterophil/lymphocyte ratio, in male and female mink distributed on ± nest box and on cage size (large, standard, small)

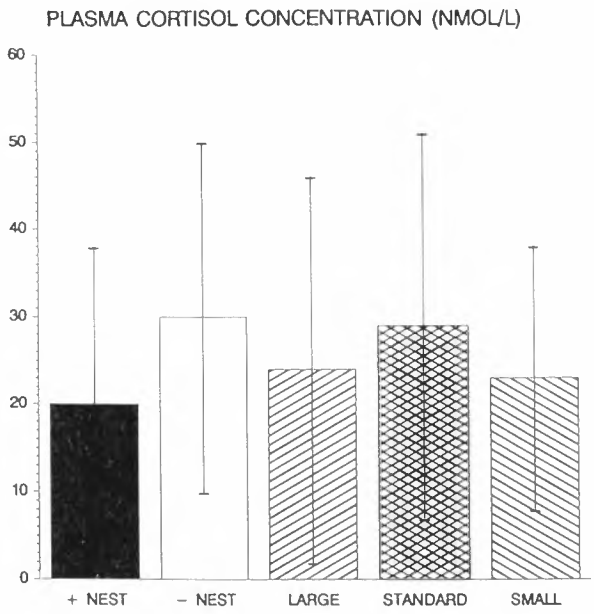

Fig. 4. Plasma cortisol concentration (mean ± SD) in mink females distributed on ± nest box and on cage size (large, standard, small)

The use of number of eosinophil leucocytes as an indicator of long-term stress has given contradictory results. Immobilization of male mink in a mink trap for one hour per day for 17 days is shown to increase the eosinophil level (Heller & Jeppesen 1985). In contrast, 30 min. immobilization of female mink for 10 days has proved to reduce the eosinophil level (Hansen & Damgaard 1991). The decrease found in the number of eosinophil leucocytes in mink without nest boxes supports the latter experimental immobilization experiment. Likewise, the increase found in the heterophil/leucocyte ratio, which has on several occasions been taken as an indicator of stress (Gross & Siegel 1983, Beuving et al. 1989), indicates that the stress level is higher in mink without nest boxes than in those with nest boxes.

Feed intake

The access of mink to a nest box has a significant influence on the feed intake of mink. In the experimental period (11 July to 15 November) mink without nest boxes consumed more feed than mink with nest boxes ($p < 0.001$, GLM test) (Fig. 5A). At the same time growth was higher in mink with nest boxes ($p < 0.05$, GLM test). The difference in feed intake was probably caused by a higher metabolism. From the middle of September (week 37), after the kits stop growing with regard to length and any further weight gain will be conditioned by fat deposits (Hansen & Damgaard 1991), the difference in feed intake between mink in the two types of cages became more marked. The time when the difference in feed intake between mink in the two cage systems increased, was found to coincide with the time when stereotypic behaviour developed in mink kits (Dodd 1985). The

increased feed intake in mink without nest boxes may partly be due to an increased need of energy for thermo regulation and partly to an increased need of energy for stereotypic behaviour patterns. In mink in small cages, feed intake fell after week 37 as compared to mink in standard and large cages ($p < 0.001$, GLM test) (Fig. 5B), but no difference in weight development between mink in the three cage types was recorded. A possible explanation may be the limited freedom of movement in the small cages not allowing very energy-consuming patterns of movement.

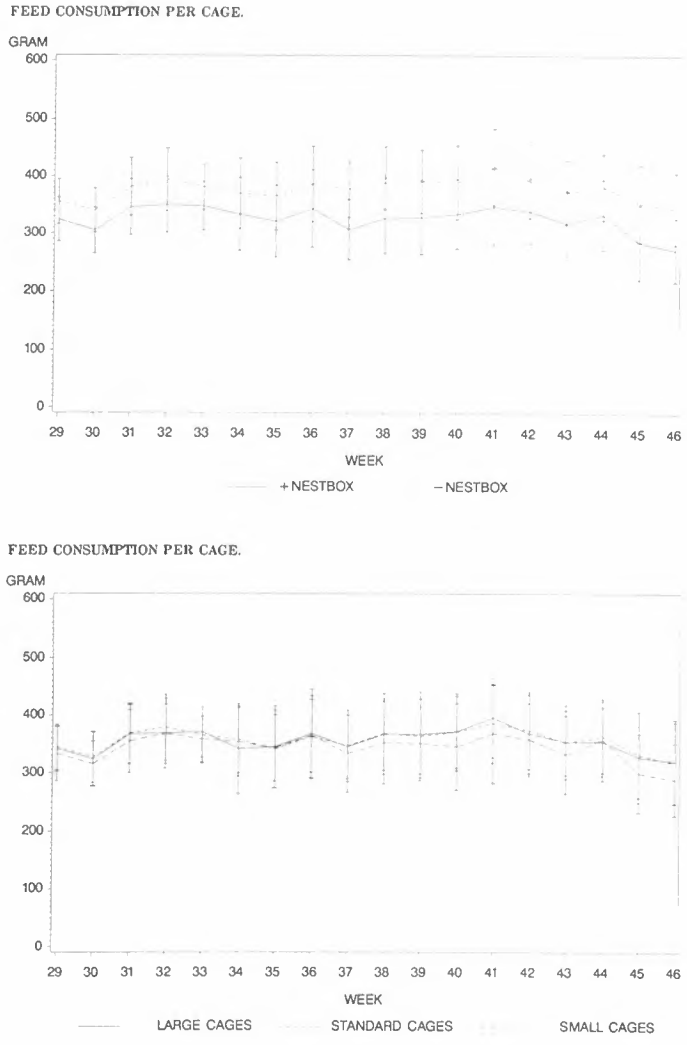

Fig. 5. Feed intake (grams per male + female). Values are mean ± SD. A distributed on ± nest box and B distributed on cage size (large, standard, small)

CONCLUSION

Based on the results obtained it can be concluded that even if the standard cage area is increased up to four times, this does not enhance the welfare of farm mink. Simply increasing the area without at the same time enriching the cage environment seems, on the contrary, to reduce the welfare measured as a decrease in number of eosinophil leucocytes and an increase of stereotypic activity.

The nest box, which is standard equipment in the production system, has a positive effect on the physiological stress level of farm mink, as mink with nest boxes have a higher level of eosinophil leucocytes, a lower heterophil/lymphocyte ratio, and a lower plasma cortisol level than mink without nest boxes. As regards behaviour, the lack of nest boxes results in an increase in stereotypic behaviour, indicating an inadequate production environment. As a direct effect of the missing nest boxes, feed intake increases, probably due to an increased need for energy for heat production. Indirectly, feed intake rises as a result of an increase in stereotypic behaviour in mink without nest boxes. The increase in feed intake does not have any positive effect on the growth of mink. In the small cages, feed intake is reduced, probably because of limited movement.

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Sociability and dominance relationships in farmed blue foxes

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The aim of the present work was to study the social behaviour of a group of blue foxes (four males, four females) housed in a large ground-floored enclosure. Behavioural patterns were monitored by video recordings and direct visual observations. The results indicated that blue foxes are social animals whose hierarchical development properly begins at the age of 3.5 months, and reaches maturity in mid-winter. Males are generally dominant over females, although body weight and dominance rank do not necessarily have a significant correlation. Social ranking order is most pronounced during feeding times. It can be concluded that sociability has a marked importance also for foxes in captivity.

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The arctic fox and its farmed colour mutation, the blue fox, have been previously considered to have solitary living habits (Fox 1969; Banfield 1977). In recent years, however, real evidence of their sociability and communal living in the wild has come out (Hersteinsson & MacDonald 1982; Eberhardt et al. 1983; Garrott et al. 1984). In addition, studies in captivity support the conclusion that the arctic blue fox develops a social organization and hierarchies when raised in captive groups (Wakely & Mallory 1988; Korhonen & Alasuutari 1991).

Recent studies on farmed foxes have revealed that individuals of higher and lower status can be found in cages (Bakken 1990). It thus seems obvious that the social status of the animals can have an influence on their reproductive performance, kit losses, etc. The aim of the present study was to further clarify the social behaviour and dominance patterns in farm bred blue foxes. Our ultimate purpose was to determine the factors which regulate the formation and development of social relationships among this species.

MATERIALS AND METHODS

The experiments were carried out at the Muddusjärvi Experimental Farm in Finnish Lapland. The subjects were farm bred blue foxes (four males, four females) originally from

the same litter, except for one female (F-4). The litter was born on 19 May, 1991 (female F-4 on 18 May, 1991). On 14 August, all of the animals were transferred into a large ground-floored enclosure (surface area 224 m^2) containing five wooden nest boxes (measuring 70 cm x 40 cm x 40 cm).

The behaviour of the animals was monitored with a video-camera (Panasonic NV-G1) 1-2 days weekly throughout the experiment. In addition, direct visual observations, each lasting 24 h, were made monthly.

The most common behavioural patterns, including numbers of social contacts, aggressions and dominance relationships were used for the determination of hierarchical order and social status. The hierarchical order of individuals as well as the number and outcome of challenges made by the other foxes were recorded (Korhonen & Alasuutari 1991). Feeding order was determined on the basis of aggressive encounters and visual status signals over feed items. All agonistic behaviours were pooled to determine dominance ranks. Dominance values were calculated as the arc sine of proportion of wins (Beilharz & Zeeb 1982).

The basal data were statistically treated by analysis of variance and regression analysis. Spearman's rank correlation was applied to test for the correlation between ranked dominance values and other traits.

RESULTS

The basal data are summarized in Tables 1 and 2. During the first period of the study (15 Aug. - 30 Sep.) the foxes were still growing and their hierarchical order was more or less indeterminate and playful in character. Therefore, Table 1 excludes the values for other contacts and contacts with the most dominant male. Nevertheless, male M-2 was already now ranked as the most dominant individual whereas females F-3 and F-4 were found to be clearly the least dominant ones. The relationships between the other foxes were still more or less variable. No significant relationship between sex and dominance rank was found. During the first study period, body weight, activity, use of nest boxes, lying on roofs and stones or urination frequency were not significantly correlated with dominance rank (Table 2). However, the number of feeding contacts and tameness value showed a significant dependence on dominance rank.

By the second study period (1 Oct. - 30 Nov.) a more stable situation had presented itselv. Dominance rank, as based on dominance values, was now somewhat different from the ranking of the first period. The males now assumed a significant dominance over the females. Male M-2 was clearly still the most dominant individual, arrogantly ruling the others, especially at feeding times. Females F-3 and F-4 continued to be the least dominant animals in the pack (Table 1). Body weight, number of contacts with the most dominant animal (M-2), tameness value, activity, urination frequency or lying on roofs or stones did not markedly correlate with dominance rank. The numbers of feeding contacts and other contacts (i.e. contacts outside of feeding times) had a highly positive correlation with dominance rank (Table 2). The use of nest boxes was most common ($p < 0.05$) in animals of lower domnance rank, i.e. in females.

The third study period (1 Dec. - 31 Jan.) showed a trend rather similar to that of the

second period. Male M-2 was still the dominant individual, and the other males were also generally dominant over the females (Table 1). The number of feeding contacts also significantly correlated with dominance rank. No correlation between dominance rank and other traits was found (Table 2).

Table 1. Summarized data for the periods I (15 Aug. - 30 Sep.), II (1 Oct. - 30 Nov.) and III (1 Dec. - 31 Jan.). Weighings (kg) and tameness estimations for the periods I, II and III were carried out on 13. Sep. - 14 Nov. and xx Jan., respectively. Number of contacts at feeding time, number of contacts outside feeding time (other contacts) and number of contacts with the most dominant individual, M-2 are expressed as percentages. Time spent inside nest boxes, time spent lying on roofs of nest boxes and time spent on lying on stones are given as percentages per 24 h (M = male, F = female)

Variable	M-1	M-2	M-3	M-4	F-1	F-2	F-3	F-4
Period I								
Body weight	4.7	4.5	3.8	4.6	4.7	3.8	4.5	3.9
Dominance value	57.2	90.0	19.3	52.7	54.6	48.9	2.8	1.8
Feeding contacts	9.4	39.1	3.5	10.9	9.1	12.0	8.7	7.2
Locomotor activity	36.1	35.3	38.1	38.6	34.2	36.7	38.9	32.5
Tameness value	4.0	3.0	3.0	3.3	2.3	2.3	2.0	1.3
Inside nest boxes	0	0.3	0.2	0	0	0.3	0.4	0.2
Lying on roofs	0.1	0.3	2.7	9.4	5.8	3.5	0.8	0.1
Lying on stones	10.8	6.7	33.3	17.8	33.8	16.4	20.8	31.6
Periode II								
Body wight	7.5	7.7	6.3	6.1	6.8	5.5	6.7	6.5
Dominance value	61.1	90.0	43.4	65.9	41.8	32.1	19.9	9.0
Feeding contacts	13.1	36.3	7.6	13.7	6.1	8.3	9.8	5.4
Other contacts	11.7	21.8	10.9	14.5	16.2	9.8	10.7	4.3
Contacts with M-2	15.4	-	16.7	12.8	23.1	17.9	9.0	5.1
Locomotor activity	22.6	27.2	30.6	31.7	26.3	32.6	31.1	23.8
Tameness value	2.0	3.5	4.0	4.0	3.0	3.5	4.0	3.5
Inside nest boxes	0	0.1	0.1	0	2.9	17.1	16.5	15.7
Lying on roofs	12.5	0.1	35.3	11.7	27.2	4.7	7.4	20.7
Lying on stones	0	1.7	0	0	0	9.2	2.6	19.2
Periode III								
Body weight	8.0	7.7	67	6.5	6.7	5.4	6.9	6.9
Dominance value	64.3	90.0	54.0	42.4	43.7	41.8	20.6	3.9
Feeding contacts	14.5	25.4	7.1	13.4	10.6	12.0	9.5	7.5
Other contacts	17.0	19.9	9.1	10.8	16.6	11.0	11.9	8.1
Contacts with M-2	34.5	-	13.1	14.8	12.5	17.9	12.2	10.5
Locomotor activity	26.3	23.7	24.1	24.1	26.7	24.0	13.8	21.5
Tamness value	2.3	2.7	2.0	3.0	2.0	1.7	2.0	1.7
Inside nest boxes	0.3	0.5	0.3	0.1	0.1	0.1	0.7	0.1
Lying on roofs	2.4	2.6	0.3	12.9	8.6	1.0	0.1	4.5
Lying on stones	0	0	0	0	0	1.3	0	7.7

Table 2. Correlation coefficients (Spearman's) between dominance rank and other traits

	Dominance rank		
	Period I	II	III
Body weight	0.70	0.33	0.39
Sex	0.54	0.87**	0.76*
Feeding contacts	0.72*	0.79*	0.71*
Other contacts	-	0.83**	0.67
Contacts with M-2	-	0.31	0.12
Tameness value	0.71*	0.05	0.64
Activity	0.19	0.03	0.70
Use of nest boxes	-0.35	-0.79*	0.36
Lying on roofs	0.07	0.24	0.05
Lying on stones	-0.55	-0.66	-0.65
Urination frequency	-0.47	0.24	0.03

DISCUSSION

The present results confirm the previous observations of Wakely & Mallory (1988) that juvenile hierarchies in arctic blue foxes are non-linear and associated with neither a particular sex nor weight class. After the animals reach their adult size (after the autumn equinox), however, the hierarchies seem to become more linear. Our results, on the other hand, are in contrast with the conclusion of Wakely & Mallory (1988) that in adult foxes the dominant individuals are the heaviest. Our previous paper (Korhonen & Alasuutari 1988) also showed a similar contrast. Forthermore, studies on the wolf have indicated that the size of the animal does not appear to be an essential criterion of dominance. Rather, some as yet poorly understood personality traits could have greater sway.

The dominance hierarchies observed were most pronounced during feeding times. Thus, access to limited resources such as food is evidently one of the major factors inducing the formation of social differences. Social hierarchies probably have evolved as a social interactive strategy in an environment where the abundance of food resources is both variable and unpredictable. The arctic blue fox is known to live in such extreme circumstances.

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Use of chronic jugular catheterization for repeated blood sampling to measure diurnal variations in blood parameters in mink

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A surgical procedure to insert chronic jugular catheters in mink has been adapted. To take repeated blood samples, a tethering system was used where the catheter was protected by a spiral spring. Swivels at both ends of the spring allowed the mink to move freely. Diurnal variations in concentrations of glucose, lactate, urea, total lipid, triglycerides, cortisol and insulin were measured. Glucose, lactate and cortisol levels were rather constant, indicating that blood sampling did not stress the mink, and therefore the method is very useful in studies where repeated blood sampling is needed. Significantly ($p < 0.01$) positive correlations were found between the plasma concentrations of glucose, lactate and cortisol, as well as between triglycerides, total lipids and urea.

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Until recently there was no recommended method for multiple daily blood samplings in studies with mink because both repeated handling and anaesthesia can be detrimental to the health of mink. A suitable tethering system for repeated blood samplings has now been modified from a system developed by Bonnefond et al. (1988). The background for our interest in repeated blood sampling is the lack of metabolic studies in mink on nutrients, their metabolites and regulating hormones. The main objective of our studies is to examine the relation between amino acid and glucose metabolism especially in lactating females with a high demand for *de novo* glucose synthesis.

MATERIALS AND METHODS

Surgery

Surgery was performed 4-5 days prior to blood sampling on four adult male and two female pastel mink with three kits each. The surgical procedure followed that of Bonnefond et al. (1988). For anaesthesia we used 0.2 ml 4% Pentobarbital-Na and 2.0 ml Saffan® (0.9%

α-xalone acetate + 0.3% α-dolone acetate) per kg IP. A Silastic catheter (1.4 mm o.d.; 0.6 mm i.d., Dow Corning) was extended subcutaneously from the scapulae to the ventral portion of the neck by use of a trocar. The left jugular vein was dissected free and the catheter was introduced about 8 cm towards the heart. The emerging part of the catheter was anchored in the muscular layer to keep it in position.

Tethering system

The tethering system (Fig. 1) used to protect the catheter was a modification from Bonnefond et al. (1988). The system consists of a metal spiral spring with swivels at both ends fixed to the back of the mink by an adhesive elastic band and to the wire cage, respectively, allowing the mink to move freely in the cage. The catheter inside the spring was of transparent polyethylene tubing (1.3 mm o.d.; 0.85 mm i.d., Medox Surgimed A/S, Denmark). The tethering system was fixed on the mink the day before blood sampling during anaesthesia with Ketalar® (40 mg ketamine chloride/kg IM.).

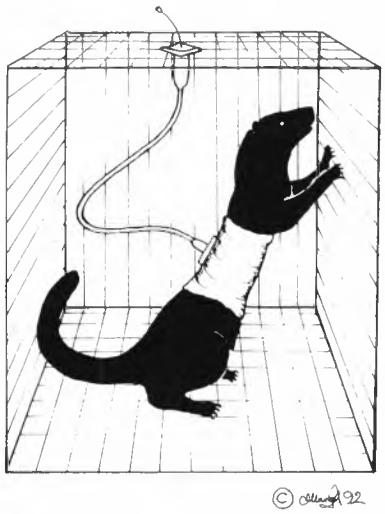

Fig. 1. Mink with tethering system for remote blood sampling

Feeding

All the mink were fed 240 g daily of a standard wet mink feed containing 118 kcal/100 g with the ratio 50:37:13 between metabolizable energy (ME) from protein, fat and carbohydrates. From the day before and during blood sampling the feed was offered in four portions of 60 g immediately after each blood sampling. Therefore variations in blood values represent diurnal variations independent of time from feeding.

Blood sampling

Blood sampling was performed as indicated in Table 1. At each sampling 2 ml (female 1.5 ml) blood was drawn followed by injection 2 ml saline and of 0.5 ml heparinized saline solution (250 IU/ml). Each blood sample was divided into two tubes coated with Na-heparin. NaF was added to one of the tubes to stop enzymatic activity in plasma for glucose and lactate analysis.

Blood analysis

The plasma content of glucose, lactate, urea, total lipids, and triglycerides was determined with kits from Boehringer Mannheim GmbH Diagnostica. Cortisol concentrations were determined by a competitive immunoassay technique (Amerlite Cortisol Assay, Amersham) and insulin concentrations by 125-I Radio immunoassay Kit (Interchemie).

Table 1. Hours of sampling during four successive days

Period	1		2	
Day	1	2	3	4
Hours	08.00	11.00	08.00	11.00
	14.00	17.00	14.00	17.00
	20.00	23.00	20.00	23.00
	02.00	05.00	02.00	05.00

Statistical analysis

All blood parameters were analysed with the following model by the GLM procedure in SAS (1987).

$$Y_{ijkl} = \mu + a_i + P_j + H_k + e_{ijkl} \quad (1)$$

where

Y_{ijkl} are the blood values measured
μ is the overall mean
a_i is a random effect of animal i; i = 1,..,4
P_j is a fixed effect of period j; j = 1, 2
H_k is a fixed effect of sampling hour k; k = 08:00, 14:00, 20:00, 02:00, 11:00, 17:00, 23:00, 05:00
e_{ijkl} are the random residuals

There were no significant interactions between the fixed effects of period and sampling hour for any of the blood parameters. Correlations between all blood parameters and between blood parameters and feed intake in the six hours preceding each sampling hour were analysed by the CORR procedure in SAS (1987). Before values for cortisol and insulin were used in the correlation analysis, these data were transformed by the natural logarithm.

RESULTS

The chronic catheterization method and the tethering system were well tolerated without disturbance of normal behaviour of the mink, which exhibited a normal pattern of sleeping, eating, drinking, and exploration as was also found by Bonnefond et al. (1988), and by Jackson et al. (1988) who used a rather similar system for ferrets. One of the nursing females died of unknown cause just before blood sampling began, and one of the males was euthanized after the first 13 samples because of bleeding. Blood samplings were easily managed, and all samples except one were obtained without clotting.

Mean concentrations in plasma of metabolites and hormones are presented in Table 2, whereas data for blood analyses and feed intake are plotted for each of the four animals in Fig. 2 (No. 2330 is the female). Correlation coefficients between the seven blood parameters and between these parameters and feed intake are given in Table 3.

Table 2. Mean concentrations in plasma of metabolites and hormones for three males and one nursing female. Standard deviation (SD = root MSE) and p-values for effect of animal, sampling hour and period are from model 1

	Mean	SD	P[1)]		
			Animal	Hour	Period
Glucose mmol/l	6.0	0.9	0.0001	NS	NS
Lactate mmol/l	1.7	0.7	NS	0.02	NS
Urea mmol/l	10.9	2.6	0.0001	0.03	NS
Total lipid g/l	9.4	0.6	0.0001	NS	NS
Triglycerides mmol/l	1.5	0.3	0.0001	NS	NS
Cortisol nmol/l	48.7	51.1	0.006	NS	NS
Insulin μU/ml	33.4	18.8	NS	NS	NS

[1)]No interaction ($p > 0.05$) was found between hour and period. NS: not significant ($p > 0.05$)

Table 3. Correlation coefficients between seven blood parameters and between these parameters and feed intake. P-values in parentheses

	Feed int.	Total lipid	Urea	Glucose	Cortisol	Lactate	Triglyc.	Insulin
Feed intake	1.0							
	(0.0)							
Total lipid	0.01	1.0						
	(0.96)	(0.0)						
Urea	0.02	0.72	1.0					
	(0.90)	(0.0001)	(0.0)					
Glucose	-0.32	0.09	-0.05	1.0				
	(0.01)	(0.50)	(0.70)	(0.0)				
Cortisol[1)]	-0.34	0.16	-0.03	0.63	1.0			
	(0.007)	(0.20)	(0.83)	(0.0001)	(0.0)			
Lactate	-0.14	0.11	0.21	0.55	0.38	1.0		
	(0.26)	(0.37)	(0.10)	(0.0001)	(0.002)	(0.0)		
Triglycerides	0.11	0.82	0.65	-0.07	0.00	0.01	1.0	
	(0.38)	(0.0001)	(0.0001)	(0.61)	(0.99)	(0.91)	(0.0)	
Insulin[1)]	-0.06	-0.18	(-0.05)	(-0.07)	-0.19	-0.12	0.05	1.0
	(0.65)	(0.16)	(0.71)	(0.59)	(0.15)	(0.37)	(0.72)	(0.0)

[1)] Values were transformed by the natural logarithm before correlation analysis

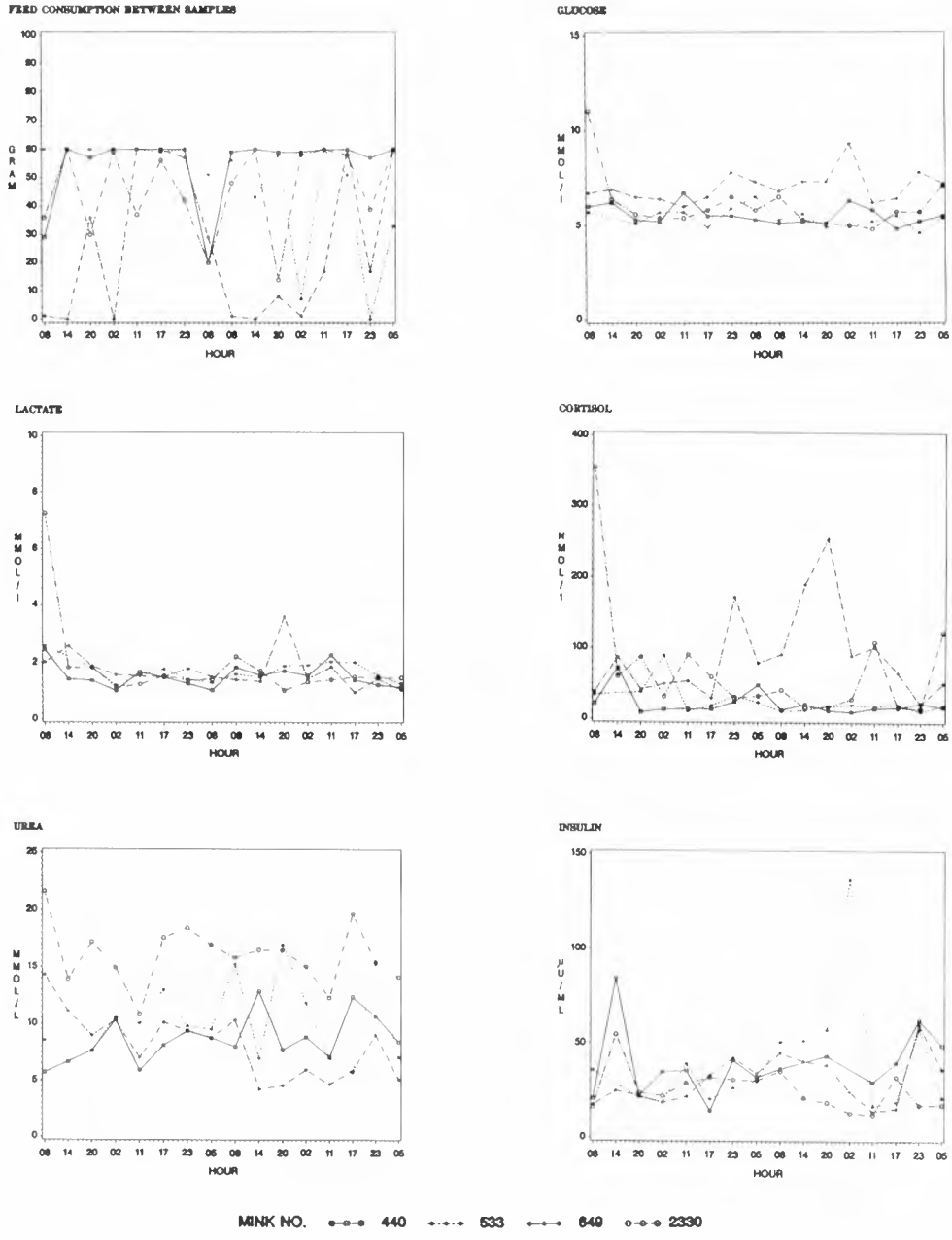

Fig. 2. Feed consumption between samples (g) and plasma concentrations of glucose (mmol/l), lactate (mmol/l), cortisol (nmol/l), insulin (μU/l), and urea (mmol/l) for three male mink (No. 440, 533, 649) and one nursing female (No. 2330). On the x-axis the sampling hours are shown chronologically through the sampling period of four days

DISCUSSION

Feed intake was not as constant as planned, and diurnal variations in blood parameters may therefore be partly dependent on the diurnal pattern in feed intake. Hence, there was a significant, negative correlation between glucose and feed intake as well as between cortisol and feed intake. When interpreting values of blood parameters, it must be taken into account that No. 649 at 20.00 on day 3 was anaesthetized with Ketalar®. This caused high values of cortisol and lactate, whereas glucose concentration was high in the sample taken six hours later. The significant effect of animal for urea, total lipids and triglycerides was due to higher values for the nursing female. In general, in accordance with the rather constant values for all blood parameters, there was very little effect of sampling hour and period, since the only slightly significant effects were found for lactate and urea.

Mean concentration and variation of the stress hormone cortisol were similar to values obtained in mink sampled by toe nail cutting immediately after being caught in the cage (Hansen & Damgaard 1991ab). In the latter study the mean cortisol concentration in 12 individually kept males varied between 26 and 42 nmol/l during four samplings from August to November, whereas the standard deviations (SD) varied between 18 and 37 nmol/l. In the other study (Hansen & Damgaard 1991a) the effect of immobilization was studied in 10 females. Before the first day of immobilization for five minutes in a mink trap, the mean cortisol concentration was 26 nmol/l ($\pm$ 18 nmol/l), whereas it was 313-414 nmol/l ($\pm$ 109-225 nmol/l) after repeated immobilization over a period of 10 days.

It is evident that the 49 nmol/l ($\pm$ 51 nmol/l) obtained in the present study is of the same order as the standard values obtained in the other two studies, and much lower than that obtained under the immobilization, which indicates that this method can be applied for repeated blood sampling without causing stress to the mink.

In a study by Jepsen et al. (1981) it was shown that most sedatives and analgetica severely altered plasma concentrations of glucose and lactate. Mean concentrations of glucose were 6.0-23.5 mmol/l ($\pm$ 1.1-10.6 mmol/l), and for lactate they were 0.4-2.3 mmol/l ($\pm$ 0.2-1.5 mmol/l). A previous trial in our laboratory (Børsting & Damgaard, unpublished data) has shown that sedation with Combelen® (2 mg propionylpromazium/kg IM) followed by anaesthesia with Saffan® (3 ml/kg IP) caused either increasing glucose concentration up to 16 mmol/l or a slight decrease in glucose concentration. Furthermore, Pentobarbital-Na (35 mg/kg IP) caused an increase in glucose concentration up to about 35 mmol/l after two hours. Glucose data from the present experiment show that this method of blood sampling is outstanding compared to anaesthesia during repeated blood sampling.

The positive correlations between cortisol, lactate and glucose are in good accordance with rat data (Gärtner et al. 1980) which indicate that stress can induce a 2-3-fold increase in lactate concentration within 1-3 min and a significant increase in blood glucose within 3-8 min. The highly significant positive correlations between urea, total lipids and triglycerides probably emerge from the fact that the concentrations of all these metabolites are dependent on the time from feeding. Despite the regular feeding regime, the eating pattern of the individual animals during the periods between samplings is unknown. However, also for these three parameters, values were rather constant, but for most purposes they should be measured in fasted animals.

In conclusion, the catherization method and tethering system described allow repeated

blood sampling at a low stress level and prevent the detrimental effects of anaesthesia and handling on concentrations of nutrients, metabolites and hormones in the blood.

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The number and activity of nipples in two-year-old females of arctic fox (*Alopex lagopus* L.) and their effect on rearing performance

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The aim of this study was to determine the number of active nipples in a population of foxes comprising 91 two-years-old females and to compare this with the number of nipples in primiparae. The number of reared cubs per female was also determined and compared with the number of nipples. The relationship between the number of nipples and the number of reared cubs was analysed and also the correlation between the number of born and reared cubs. The effect of litter size on the number of reared young foxes was ascertained.

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The reproduction of carnivorous animals appears to be one of the basic factors in their breeding. However, maintaining of these animals under farm conditions can raise some problems. For example, foxes, may display some undesirable reproduction traits such as weak libido, miscarriage, a low level of maternal instinct, infanticide and insufficient lactation.

The above-mentioned traits, with insufficient lactation or agalactia constituting the most important, result in a high cub mortality rate, particularly during the first period of rearing (Zwierzchowski 1984; Spisak 1986; Stępień 1988).

In the current literature there is a lack of information on the topography, morphology and the number of nipples in foxes. Therefore, the aim of this study was to compare the total number of nipples and the number of active nipples, taking into account the nipple activity in the first year of reproduction. Correlations were then assessed between the total number of nipples and the number of cubs born as well as between the number of active nipples and the results of rearing. Since these correlations were found to be significant, they were used as selection indicators in much the same way as they are used in swine (Pomykał & Chojnowski 1976).

MATERIAL AND METHODS

The investigations were carried out at the arctic fox farm in Brominy (part of the Siedlce Industry "Las"). The animals were kept and fed according to the usual practice in Central Poland. The investigated population included 91 blue or white arctic fox females, aged two-years. The reproduction indicators of these females for the previous year (the first year of reproduction) were known. In all vixens the total number of nipples and the number of active nipples (at 6 weeks of cubs age) were determined. The percentage of inactive nipples and the number of born and reared cubs were also recorded. The indexes of fertility and rearing were calculated according to the formula:

Index of fertility = number of born cubs in the second year of reproduction/number of born cubs in the first year of reproduction

Index of rearing = number of reared cubs in the second year of reproduction/number of reared cubs in the first year of reproduction

In both years of reproduction the number of active nipples was related to the number of born cubs. The correlation coefficients were calculated between the total number of active nipples and the results of rearing. In order to test the differences between active nipples (born and reared cubs in both years) the correlated observation sequences were made.

RESULTS

The results are presented in Tables 1, 2 and 3.

Table 1. The main reproduction indicators in two-years-old arctic fox females

Indicator	Colour type: Blue, Season of reprod. First	Blue, Second	White, First	White, Second	Females together, First	Females together, Second
No. of females	66	66	25	25	91	91
No. of nipples, total	12.74	12.77	12.56	12.56	12.69	12.71
No. of active nipples	11.88	12.71	12.24	12.54	11.98	12.66
Non-active nipples (%)	6.78	0.40	2.55	0.39	5.63	0.43
No. of cubs born	8.41	9.77	6.56	7.44	7.90	9.13
Index of fertility (%)	116.17		113.42		115.58	
No. of reared cubs	7.53	8.42	6.44	6.96	7.23	8.02
Index of rearing (%)	111.80		108.08		110.94	
Cub mortality (%)	10.45	13.80	1.86	6.45	8.48	12.15

Table 2. The results of rearing cubs of 91 arctic fox females

No. of nipples and no. of cubs		Season of reproduction			
		First		Second	
		No. cubs	%	No. cubs	%
More cubs than nipples	one cub more	1	1.10	0	0
	over one cub more	0	0	0	0
No. of cubs and no. of nipples are equal		1	1.10	5	5.49
More nipples than cubs	one nipple more	8	8.78	7	7.79
	two nipples more	4	4.40	9	9.89
	over two nipples more	77	84.62	70	76.92
No. of females together		89	97.81	86	94.51

Table 3. Correlation coefficients for arctic fox females

Correlation between:	Season of reproduction	Colour type		Females together
		Blue	White	
No. of nipples and no. of cubs born	first	0.213	0.233	0.197
	second	0.103	-0.046	0.102
No. of active nipples	first	0.562[x]	0.268	0.486[x]
	second	0.103	0.341	0.133

DISCUSSION

The mean number of cubs born in the second year of reproduction was 9.13 (Table 1), which was similar to the result obtained by Frindt et al. (1987). However, this indicator was higher in Spisak,s (1986) investigations. During the second year of reproduction the fertility and rearing indexes clearly increased (115.58% and 110.94% respectively). A similar improvement in reproduction indicators was observed by Bernacka et al. (1981). According to Kubacki (1987) the highest fertility level is obtained by three-year-old arctic fox females. The cause of the mortality rate (1.86-13.8) was probably environmental factors, particularly the climatic effect.

The comparison between the number of active nipples and the results of rearing (Table 2) indicated that in primiparae nearly all females (97.8%) reared fewer cubs than the number of nipples they possessed. In the second year of reproduction this number slightly decreased (94.5%). These data confirm the results of the study by Hernesniemi (1980) on mink which suggested that during the rearing period there should be a minimum of one nipple per cub.

The calculated correlation coefficients (Table 3) were low. They were found to be significant only in the case of the first year of reproduction. The coefficients obtained by the other students fluctuated between 0.280 (Bednarz et al. 1986) and 0.887 (Spisak 1986).

The negative correlation found in the case of white arctic foxes was rather unusual and was probably attributable to the small number of animals in this group.

The effects of the number of nipples and the number of born cubs on rearing in the case of two-year-old females were tested using regression coefficients. The coefficients of partial regression were 0.143 and 0.604 (highly significant) respectively. Thus, the correlation coefficient was low and non-significant in this case.

SUMMARY

1. Nearly all nipples in arctic fox females were active the investigated population.

2. The fertility and rearing indexes were higher in two-year-old females than in primiparae.

3. In both groups females reared fewer cubs than the number of nipples.

4. The number of born cubs had a more clear-cut effect on the results of rearing than the number of nipples.

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Production systems and management in the danish mink production

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Large variations in production systems and management were found between farms, but an effect on production results could rarely be demonstrated. The basic needs for housing of mink can be met in many ways, and different management systems may work perfectly. It is more important that the farmer is skilled at what he is doing, and that he is confident with his production and management practices. Production systems and management often differed between FK, indicating a regional or advisory effect. Thus, a variation in production results between FK may be due to differences in production systems or management. Whelping results were better in double than in multi row sheds and on farms where the breeding animals were mixed than where males and females were placed in separate groups. The growth functions give good descriptions of the weight development and can be used as standard curves for scanblack male mink kits. A positive correlation was found between average skin length and quality on Danish farms. Correlations between August and October weight and skin length and quality indicated a difference in body length between mink strains.

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The purpose of this project was to identify the variations between production systems and management on mink farms and feed kitchens (FK) in Denmark, and to evaluate the importance of these differences to the production results.

MATERIALS AND METHODS

From each of five FK, representing various regions of Denmark, 4-5 mink farms were chosen to represent differences as regards environment, management, quality, etc. The production systems and management in all production periods were described. Information regarding number of animals, feed intake, fur qualities, breeding results, etc. was collected from the various data bases of the Danish Fur Breeders' Association. The weight development of the animals was followed by means of a comprehensive weighing programme. All information pertains to scanblack mink.

RESULTS AND DISCUSSION

Production systems

The size of the project farms varied from 125 to 2800 females, while manpower varied from 0.5 to 4. The first man tended approx. 400 females, while each extra man tended approx. 650 females. This increase in efficiency with number of females was due to the extent of the operation, rather than to degree of mechanization.

Mink were housed in double or multirow sheds, almost exclusively built in wood and with asbestos roofing. Whelping results were better in the double than in the multirow sheds, as can be seen in Table 1.

Table 1. Reproduction in double and multirow sheds of mink of the scanblack type in 1987

Rows	N	% barren females	Kits per mated female at birth	Kits per mated female at 3 weeks	% lost kits
2	12	8.9± 2.9	5.1±0.4	4.9±0.3	3.6±3.1
>2	10	12.8±6.5	4.6±0.5	4.3±0.5	5.2±2.
		p=0.10	p<0.05	p<0.01	p=0.21

For all the reproduction parameters the best result were achieved in the double row sheds. The difference in kits per mated female was significant. Light conditions were also better in the double row sheds. As the reproductive cycle of the mink is governed by daylight, this may explain the difference in whelping results. Nine out of 10 multirow sheds used for whelping were closed. These sheds are warmer and less draughty than the open sheds. This may have an influence on the body weight of the females, and thereby the whelping result.

The design of mink cages was limited to a few standard types. All the pens were built in sections of 2 m. Sections with 6 pens of 2700 cm^2 each were used for breeding, birth, lactation and growth. Sections with 8 pens of 1800 cm^2 each were used for breeding animals kept individually. A third type with the nester placed in the upper part of the cage was used in one part of the country. In agreement with previous results (Aldèn & Tauson 1979; Jonasen 1987; Hansen 1988), the type and size of cages did not influence the production results.

The design of nest boxes and watering systems, which may be of great importance to the production result (Møller 1990, 1991), was very varied. The drinking water was kept at medium temperature throughout the year by circulation or electrical heating of the water or by insulation or screening of the water hose. The variations represented different ways of obtaining the same effect and had no influence on the production results.

The large variation in production systems indicates that there are many different ways of meeting the basic needs for housing mink.

Top nester cages and Forelco's circulation watering system were widely distributed in southern Jutland. Breeding boxes with a U-cylinder were common in northern Jutland, whereas the rest of Jutland used rectangular breeding boxes with drop-in bottoms and screens. Such geographic differences were a result of the location of the equipment producers as well as of a regional or advisory effect. These differences indicate that a

variation in production results between FK may be due to differences in production systems.

Management and husbandry

Management on mink farms is divided into various production periods throughout the year.

Because of delayed implantation of fertilized eggs, the mink can be mated several times during the same mating season. All the farmers aimed to mate the mink twice, at eight-day intervals, in order to bring about two ovulations.

Whelping results were better on farms where the breeding animals were mixed than where males and females were placed in separate groups, as shown in Table 2.

Table 2. Reproduction results of scanblack mink in 1987 for breeding animals placed in groups by sex or mixed

Placement	n	% barren females	Kits per litter at birth	Kits per litter at 3 weeks	% lost kits
Groups	11	12,5±4,9	5,2±0,3	5,0±0,3	5,8±2,7
Mixed	11	10,1±4,4	5,6±0,3	5,4±0,3	3,8±2,4
		p=0,25	p<0,05	p<0,05	p=0,11

It was found that for all the reproduction parameters the best results were achieved when breeding animals were mixed. The difference in litter size was significant. A similar effect was found by Enders (1952) in an investigation of visual isolation of females. A logical explanation would be olfactory stimulation from the males, but neither urine nor anal glands from males have proven to be effective (Møller 1991; Therkildsen 1991). As the placing of animals was related to FK, other factors may influence the results.

Management during pregnancy, birth, lactation and weaning differed only in detail and no significant effects were found. Most of the kits were placed in pairs of one male and one female after weaning. An old female paired with a male kit was also common.

Each farm had a certain level of pelt bites for males as well as females, but the level varied from year to year. This indicates a farm effect, but also a common environmental effect within years. Farms with large skins of good quality and farms with a poor whelping result had most pelt bites. A low temperature in November 1985 coincided with fewer pelt bites.

Only minor differences, often connected with FK, were found between farms in the selection of breeding animals. The most important dfferences seemed to be in how competently the pelt grading was carried out.

It can be concluded that management on the project farms revealed wide variations between farms. The differences were often connected with the feed kitchens, indicating a regional or advisory effect. However, an effect on production results could rarely be demonstrated. The main impression from the project was that many different systems may work perfectly. The important thing is that the farmer is skilled at what he is doing and that he has confidence in his management practices.

Weighing of kits in the nursing period

Ten to 20 litters of 4-9 kits born on the two most frequent days were weighed every 10 days during the lactation period on each farm in 1986 and 1987. The weight development is illustrated in Fig. 1 for both sexes and years.

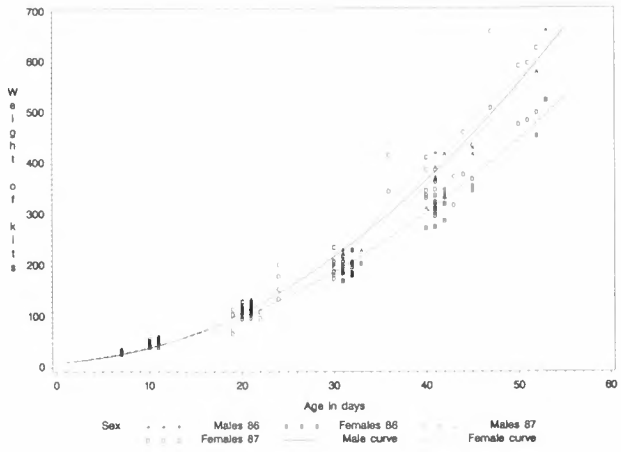

Fig. 1. Development of body weight for scanblack kits during lactation in 1986 and 1987. Growth functions are also illustrated.

The birth weight was set at 10 g for males and 9 g for females, and the following functions were fitted for mink kit growth:

$$\text{Male kit weight} = 10.0\ g + 0.95 * \text{age in days} + 0.20 * \text{age}^2$$
$$\pm 0.7 \quad \pm 0.41 \quad \pm 0.01 \quad R^2 = 0.98$$

$$\text{Female kit weight} = 9.0\ g + 1.80 * \text{age in days} + 0.14 * \text{age}^2$$
$$\pm 0.5 \quad \pm 0.28 \quad \pm 0.01 \quad R^2 = 0.99$$

The growth functions, illustrated in Fig. 1, give a good description of the growth from birth to weaning and can be used as standard curves for scanblack mink kits until weaning.

The weight of kits at the age of 31 days depended on litter size as well as on farm conditions, though litter size was restricted. It is therefore important to select homogeneous groups for weighing and to weigh at a certain age if the results are to be immediately comparable. A difference between years indicates a common environmental effect.

The early development of the kits provides a good indication of their final weight and of the skin size and therefore a high weight at weaning is desirable in practice. However, a considerable proportion of the growth potential is linked to litter size (Venge 1960; Einarsson 1980). The effect of kits' growth during the lactation period seems to be related to protein deposition (Glem-Hansen 1980). Other reasons for variations in weight at weaning can often be compensated for after weaning (Barabasz & Jarosz 1978; Hansen 1989; Kjær 1990). It is therefore more important to meet the protein requirement of the animals than to feed for maximum weight at weaning.

Weighing of weaned kits

On each farm 25 pairs of mink kits were weighed every two weeks from weaning till pelting in 1985, 1986 and 1987. The weight development of males is illustrated in Fig. 2. The following growth functions were fitted from weaning until pelting:

$$\text{Male kit weight} = \underset{\pm 60}{-701\ g} + \underset{\pm 1}{30} * \text{age in days} - \underset{\pm 0.004}{0.077} * \text{age}^2 \qquad R^2 = 0.95$$

$$\text{Female kit weight} = \underset{\pm 33}{103\ g} + \underset{\pm 0.6}{11} * \text{age in days} - \underset{\pm 0.002}{0.025} * \text{age}^2 \qquad R^2 = 0.95$$

The function for males, illustrated in Fig. 2, gives a good description of the weight development and can be used as a standard curve for scanblack male mink kits after weaning.

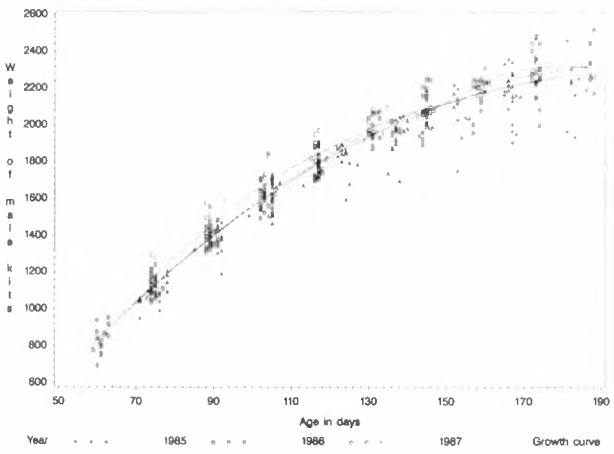

Fig. 2. Development of body weight for scanblack kits after weaning in 1985 to 1987. Growth functions are also illustrated.

Concerning weight development, skin length, skin quality and feed intake, differences were found between farms and, with the exception of feed intake, also between years. The difference between years indicates a common environmental effect, e.g. the weather. Some farms had almost the same growth curve each year, while others had large fluctuations between years.

On all farms and at all times of weighing, the variation amounted to approx. 10% of the weight. There was a close relation between the weight of males and females. The relation was constant over years, indicating that only one sex needs to be weighed when looking at the general weight development.

The amount of energy fed varied with up to 43 kcal/animal/day, corresponding to 4-5 kg of feed per skin produced.

A positive correlation between skin length and skin quality was found for the average

skin production of all Danish farms. A grouping of the project farms according to skin length and quality showed no difference in weight nor in daily gain. Feed intake was correlated to skin length and quality. The weight in August and in October was correlated to skin length but only the weight in October had a negative influence on pelt quality. The reason may be a difference in body length resulting in less fat animals and thus a better quality at the same weight.

The differences between the mink strains on the farms must be a result of different selection according to size and quality. An ideal weight development curve must take into consideration the body length and the quality of the animals on the individual farms as well as an effect of year.

Weighing of breeding animals

The breeding animals lost weight quickly just after the time of pelting in November, irrespective of the farmer's strategy regarding weight development. There was a negative correlation between the mean temperature in January to March and mortality on a national basis. As the standard deviation was 10% of the mean, thin animals will survive at any average weight. No fixed weight limits can be given, but a weight reduction of more than 30%, or to less than an average of 900 g, seemed unfavourable. As first-year females are especially sensitive, whereas the weight of the males is of minor importance, it would be more effective to weigh first-year females.

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Environmental enrichment in relation to behaviour in farmbred blue foxes

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The purpose of the present study was to clarify to what extent environmental enrichment (nest boxes, stones, large enclosure) affects behaviour in farmed blue foxes. A group of eight foxes was placed into a large L-shaped ground-floored enclosure (surface area 224 m^2) Use of the extra equipment and surface area was monitored by video recordings and by direct visual observations. The utilization of the enclosure surface area was not evenly distributed, but there were certain subareas which the animals obviously preferred. In general the use of nest boxes was minimal, but the roofs were somewhat more favoured. It was observed that the foxes clearly preferred to lie on the stones only as cubs.

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Traditionally, blue foxes have been housed in small wiremesh-floored cages. Many decades of farming experience have shown that this type of housing guarantees animals of good pelt quality and large body size. Moreover, it guarantees their health and welfare. This conventional farming system is therefore well suited to the needs of commercial fur production.

However, individuals concerned about the welfare of fur-bearing animals not only oppose the use of animals in general, but also the conditions in which these animals are housed in particular. Demands for larger cages and enclosures are especially common. Recently, the question of environmental enrichment has also been introduced (Nicol 1991). However, owing to the lack of sufficient data on the raising of farmed fur animals in larger enriched housing conditions, the animal welfare discussion has become a difficult and emotive one (Korhonen et al. 1991). The present work aims to provide behavioural data on blue foxes housed in enriched enclosure conditions.

MATERIALS AND METHODS

The experiments were undertaken at the Muddusjärvi Experimental Farm of the University of Helsinki in Finnish Lapland. The animals (four male and four female blue foxes) were housed in a large ground-floored enclosure (surface area 224 m^2). The enclosure contained five wooden nest boxes (70 cm x 40 cm x 40 cm) and two large stones (see Fig.1).

Behavioural patterns, including spatial use of the enclosure area and its nest boxes, as well as lying on roof and stones, were monitored with a Panasonic NV-G1 video-camera (1-2 times weekly) and by direct visual observations daily (c.f. Korhonen & Alasuutari 1992).

The data were statistically treated by analysis of variance, by Spearman's rank correlation and by Pearson's product moment correlation.

RESULTS

The basic data for the use of nest boxes, lying on the roofs of the nest boxes and lying on the stones are presented in Table 1 (see also Fig.1). The data were divided into three time periods, I: 15 Aug. - 30 Sep. (the growth period), II: 1 Oct. - 30 Nov. (the autumn period) and III: 1 Dec. - 31 Jan. (the winter period). Since behavioural observations and dominance rank estimations showed the dominance of males over females, the present data have been treated on the basis of sex.

Use of the nest boxes was generally minimal. Usunally the animals of both sexes went inside them only occasionally. The only exception was in period II when the females preferred to stay inside nest box n:o 5 (on an average 188 + 58 min/24 h).

Lying on the roofs of the nest boxes was a rather common habit (Table 1). However, the roofs of boxes no:1 and n:o 3 were seldom used. No statistical difference in nest box use between sexes was found.

Table 1. Time (min/24 h) spent inside nest boxes [1]), lying on roofs of boxes and lying on stones during the three periods

	Period I [2]		Period II		Period III	
	M [3]	F	M	F	M	F
Inside box no:1	0	0	0	0	0	0
Inside box no:2	1	1	1	0	1	0
Inside box no:3	2	1	0	0	2	1
Inside box no:4	0	1	0	0	0	1
Inside box no:5	0	1	0	188	2	0
Total inside boxes	3	4	1	188	5	2
On roof of box no:1	1	0	9	0	0	0
On roof of box no:2	27	36	30	12	4	0
On roof of box no:3	16	1	0	3	1	2
On roof of box no:4	0	0	2	15	59	3
On roof of box no:5	1	1	174	186	2	68
Total on roofs	45	38	215	216	66	73
Lying on stones	247	370	6	112	0	42

1) For the location of nest boxes and stones see Fig. 1
2) Periods: i (15 Aug. - 30 Sep.), II (1 Oct. - 30 Nov.), III (1 Dec. - 30 Jan.)
3) M = males (N = 4), F = females (N = 4)

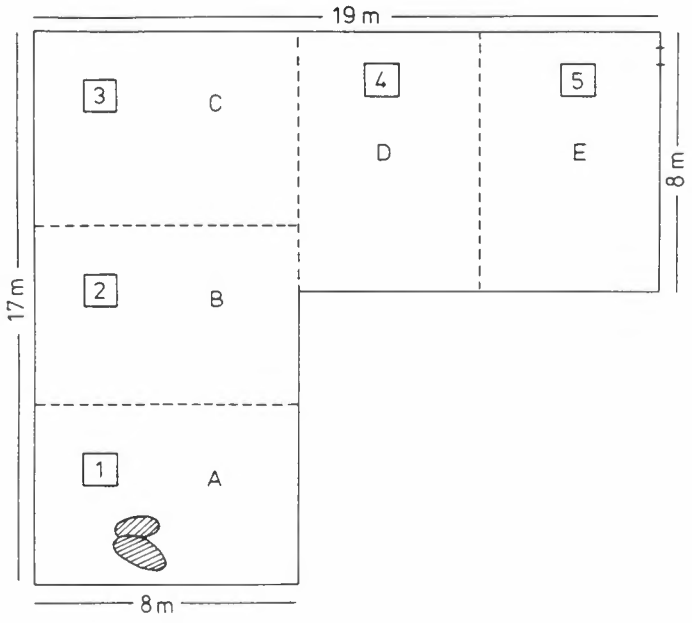

Fig. 1. Schematic illustration of the experimental arrangements. The enclosure area was divided into five different subareas marked A-E. Numbers 1-5 indicate the nest boxes. Two large stones are seen in subarea A (shaded areas)

Lying on the stones took place mostly during the first period, but dramatically declined after that (Table 1).

During the first period, the hierarchies were still generally unestablished and not of a serious nature. However, during the second and third periods clear hierarchies were already present and the animals were ranked according to their dominance status. High social status also means high rank (c.f. Korhonen & Alasuutari 1992). During period II, social status correlated negatively with nest box use ($p < 0.05$) and lying on stones ($p < 0.05$). During period III, however, no significant correlation was found between dominance and nest box use, lying on roofs or lying on stones ($p > 0.05$).

The spatial use of the enclosure surface area is presented in Table 2 (see also Fig.1). Use of the enclosure area was not evenly distributed, but the animals did prefer certain areas more than others. The least used area was C, whereas the most commonly used areas were A and E in general. No marked correlation was found between dominance rank and spatial use of subareas ($p > 0.05$). In addition, the use of nest boxes and the use of subareas did not show any significant correlation ($p > 0.05$).

DISCUSSION

The present results are in good agreement with our previous findings (Korhonen et al. 1991) that the use of nest boxes by blue foxes in large enclosures is minimal during the autumn and winter periods. In addition, both an earlier study and the present one have demonstrated that the foxes prefer to lie on roofs to some extent. Thus, it is obvious that the animals do not perceive the nest boxes as a shelter against the weather and cold. Since

they have a thick fur coat they can manage very well without any extra protection throughout the winter. They even seem to prefer lying and sleeping on the ground than inside the nest boxes (Korhonen et al. 1991). The fact that foxes prefer to locate themselves on the roofs of nest boxes is, in part, due to the fact that they generally prefer higher places in order to survey the environment. Another reason could be that the foxes used them simply because they were available and thereby enriched their behavioural scope.

Table 2. Spatial utilisation of enclosure area (%/24 h) during three periods

	M-1[1)]	M-2	M-3	M-4	F-1	F-2	F-33	F-4
Period I[2)]								
A[3)]	76.2	75.5	65.4	60.5	68.3	62.1	63.1	76.4
B	6.6	5,8	9.5	17.6	4.5	12.4	13.9	4.9
C	9.1	4.7	8.3	11.2	6.3	10.2	7.5	5.7
D	2.3	3.5	2.9	2.1	2.5	1.3	2.0	1.2
E	5.8	10.5	13.9	8.6	18.4	14.0	13.5	11.8
Period II								
A	16.2	20.7	17.3	18.0	9.4	21.6	23.4	45.2
B	12.6	7.9	6.6	6.1	7.3	11.8	19.4	6.1
C	5.9	4.8	5.2	7.1	5.9	8.4	6.8	3.6
D	42.6	55.2	36.4	23.0	7.8	21.2	11.7	6.8
E	22.7	11.4	34.5	45.8	69.6	37.0	38.7	38.3
Periode III								
A	55.9	28.3	56.0	52.7	60.0	75.3	68.3	58.8
B	8.7	37.1	6.7	22.1	10.8	11.0	6.8	5.3
C	4.9	5.9	4.7	5.1	6.6	5.6	4.6	4.7
D	13.8	5.8	15.5	3.0	4.8	2.2	11.9	3.3
E	16.7	22.9	17.1	17.1	17.8	5.9	8.4	27.9

1) M = males, F = females
2) Periods, see Table 1
3) A-E indicate different subareas presented in Fig. 1

The large stones were placed inside the enclosure for environmental enrichment. As cubs, the foxes preferred to lie on them but, later, their use declined dramatically. One explanation could be that when the weather grew colder, the stones also became cold and therefore uncomfortable to lie on.

The results indicated that the most dominant animals did not prefer higher places. Thus, the use of higher places cannot be considered as an indication of an animal's social status or dominance. Our previous paper supports the same conclusion (Korhonen et al. 1991).

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Fur properties

Pelage growth and structure in fur animals

Why is pelt research necessary?

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High quality fur production requires an understanding of normal hair growth and pelt structure. Comparison to normal pelt is vital in pelt defect research. Fur animal research is diversifying with new scientific methods applicable also in the every day life of the farmer. Hair cycles, moulting and proper pelting age are being clarified for most farmed fur animal species. Effects of factors such as hormones and feed on hair growth and fur quality have been considered. Anatomical and biochemical skin composition in relation to the age of the animal and to fur defects are of present interest.

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A pelt of good quality is a major aim in fur animal production. It is therefore important to clarify the mechanisms of pelage development, moulting and maturation and the factors affecting these. Any pelt defect lowers the commercial value and limits the use of a fur skin, and consequently lowers the income of a farmer.

The period of fur development is sensitive to disturbances, which may later manifest as pelt defects of various degrees. An illness, feed problems or some inherited factors can affect hair growth unfavourably.

Different species, or even colour types within a species seem to have specific fur defects. In dark mink, the most common pelt defect is the metallic defect with typical curved guard hairs. Brown mink have 'hippers', an incomplete fur growth on the hips. Woolliness with shortened guard hairs, is a common defect of blue fox fur. Silver fox pelts with curly hair usually have no or low commercial value. This defect was first noticed in Finland in the latter half of the 1980s and is now found also in other countries.

Research concerning hair growth and pelt structure is usually triggered by the problems that crop up and consequently studies are carried out on the most common pelt defects. The ultimate reasons for these are still unknown. A pelt problem most certainly also concerns the skin surrounding the defective hair. Today little is known about the chemical composition of normal and blemished fur animal skin.

In the attempts to solve problems relating to hair growth, normal pelt development has gained little attention. However, knowledge about normal fur growth is vitally important if we are to understand the character of pelt defects and make efforts to prevent them. A wider understanding of factors affecting hair growth and identification of mature

pelt structure will help a farmer in everyday practice in decisions concerning choice of breeding stock and timing of pelting. Too early pelting is carried out at the expense of skin maturity and leather quality. Leather properties affect further processes like dressing and finally the making of fur garments to meet the requirements of consumers and to guarantee a certain standard of a product.

APPROACHES OF PELT RESEARCH

Phenomena concerning hair growth have interested researchers for almost as long as there has been fur farming activity. The connection between light and fur growth was recognized early in this century, and species like ferret with two moultings a year provided interesting research material (Bissonnette 1935). The importance of studies on hair growth to fur farming was soon understood.

How to recognize the primeness of a fur is one at the basic questions. In early studies the hair length, the fur density and the skin pigmentation in mink and silver fox were the indicators of the priming of fur (Bassett & Llewellyn 1948; 1949, Skårman 1950a, b). These studies gave a clear picture of the fur structure and growth process. The method is still a useful one provided no great accuracy is required. Modern systems for studying fur morphology include light microscopy, digital systems and computerized picture analyses (Kondo et al. 1991; Rasmussen 1991).

The most reliable and illustrative method in studies on fur growth is that of histology. This method shows exactly the state and grouping of hairs in the skin during pelagial growth (Dolnick 1961; Dolnick et al. 1960). In creating a picture of the different phases of fur growth, successive skin biopsies are of great help. By observing the growing and the mature hairs in hair bundles, precise information is obtained about the development and the structure of the pelage (Blomstedt & Lohi 1980).

Systematic studies on fur development started in Finland in the second half of the 1970s when the mink 'metallic' pelt defect was the focus of interest. In this connection a gap in the knowledge concerning especially the first-year hair growth of fur animals other than mink was evident. This research field is now established all over the world, e.g. in Denmark, Japan, France, the USA and Canada.

WHAT HAS RESEARCH CLARIFIED?

Fur animals typically renew their hair cover once or twice a year, depending on the animal species. The summer fur of mink, ferret and blue fox is different from the winter fur, while silver fox and racoon dog, which have considerably longer hairs, change fur only once a year. A fur is composed mainly of two hair types: longer and coarser cover or guard hairs of varying lengths and clearly shorter and finer underfur or down hairs of relatively even length. In general, the growth cycle of an individual hair is similar in all haired animals. Active (anagen) phases of a hair follicle with hair production interchange with quiescent (telogen) periods of arrested hair growth. The length of these periods determines the pattern of fur development, maturation and moulting. Though most fur production comprises first

season skins, surprisingly little has been published about first year fur development. This phenomenon is best studied in mink.

Pelage development in young animals

The very first hair cover, the puppy or the whelp coat, is different from that grown later on. The whelp coat of mink from two to six weeks of age is made up of a uniform hair type thicker than down but thinner than guard hairs (Kondo et al. 1990). The normal two-layer structure of guard hair and underfur can be seen from the age of eight weeks onward. In ferret this structure can be distinguished at four weeks (30 days) of age (Galatik et al. 1988). Blue fox puppies moult their whelp coat well before the age of eight weeks (Blomstedt 1987). The mechanism that causes hair follicles first to grow whelp hair, then later to produce guard hairs is not known (Butcher 1951).

Down hairs grow in bundles with or without a guard hair. Both bundle types are found in mink pelage (Blomstedt & Lohi 1980; Kondo et al. 1989). A bundle emerges through a pore to the skin surface. The number and the growth phase of hairs in a bundle vary with the age of an animal and the season of the year. At eight weeks of age a young mink's second fur, the summer coat, grows distinctive guard hairs and underfur. The hair bundles are small, include approximately one mature and six growing down hairs. More than 80% of the guard hairs can be seen as growing (Blomstedt unpublished), indicating recent moulting of the whelp coat. The first summer coat in a female mink is mature for just a short time at the age of 17 weeks, winter hairs start to grow in the first half of September (Blomstedt 1989). In a young ferret couple it was observed that the summer coat reached maturity at 14 weeks of age, with no growing winter hairs observed during the subsequent two weeks (Blomstedt unpublished).

Moulting progresses in waves across the body, in the summer from head to tail and in the winter from tail to head (Bassett & Llewellyn 1949). Even different hair types have varying moulting patterns, ensuring that the animal has some kind of hair cover at all times. In a young mink it was observed that the summer guard hairs moulted before the down hairs (Blomstedt 1989). In the developing winter fur of mink, hair density per hair follicle grows in a similar manner in both sexes (Kondo & Nishiumi 1991). A dissimilarity was observed in summer fur moulting in females at 23 weeks of age in mid-October, and some weeks later in males. The winter fur of both sexes was in prime condition by the end of November. The winter fur in a female mink matured in 14 weeks in the hip, which meant a somewhat longer maturation time for the whole pelage (Blomstedt 1987, 1989). Priming of the winter fur takes 12 weeks in young ferrets, maturity being reached in early December (Blomstedt unpublished).

Unlike mink and ferret, in blue fox the first-year summer coat never reaches complete maturity before the winter hairs start to grow (Blomstedt 1987). The development of blue fox fur is more like the fur growth of silver fox and racoon dog (Blomstedt 1991a), which have puppy hair and adult fur during the first year. No mature hair cover was observed in young racoon dogs at 11-12 weeks of age as reported by Zon & Niedzwiadek (1991). Hair growth in blue silver fox, a sterile crossing of blue fox and silver fox, apparently resembles that of silver fox (Blomstedt 1987). 'Blue frost' is the commercial name of these animals, which are produced by artificial insemination and kept only for one season.

The maximum number of winter down hairs in the anagen period was observed in

October, at 22 weeks of age in blue fox (Joutsenlahti et al. 1988), at 21 weeks in silver fox and at 20 weeks in blue silver fox, while racoon dog reached this stage much later, 26 weeks. In mink, silver fox, blue fox and blue silver fox it was found that the guard hairs began to grow and reached maturity earlier than the down hairs (Blomstedt 1987).

The first adult fur of racoon dog becomes mature at 35 weeks of age, but priming has also been reported several weeks earlier (Reijonen & Joutsenlahti 1987). A group of blue foxes was pelted at 30 weeks of age, some weeks after normal pelting time, but 10% of the down hairs in the hip were still in the growth phase. In a silver fox cub it was observed that the maturation of the pelage indicated sex dependence and individual variation (Blomstedt 1986). Still at the age of 32 weeks the quota of growing down hairs in the hip varied from 20% in the females up to 33% in the males. The recommended pelting age is much lower in Denmark (Lyngs 1991), i.e. 25 weeks (176 days) for blue fox and 30 weeks (210 days) for silver fox, possibly depending on variations in the genetics of animal populations and on external factors. The pelting age of 29 weeks for blue silver fox appeared to be too low as the number of growing down hairs was 25% or more in the hip (Blomstedt 1987). Winter fur growth of foxes and racoon dog is presented in Table 1.

Table 1. Winter fur development in young foxes and recoon dog

State of fur development	Age in weeks			
	Blue fox[1]	Silver fox	Blue silver fox	Racoon dog
Whelp/summer guard hair moulting	12-15	16-19	13-19	-17
Whelp/summer underfur moulting	12-15	18-21	17-20	-
Growing underfur max/pore	22	21	20	26
Winter guard hair mature	28	29	27	31
Winter fur mature	>29	>32	>29	35

[1]Summer fur

Adult hair cover

Fur animals aquire a thinner hair coat for summer by moulting in the spring. The main features of the moulting pattern in ferret, mink, silver fox and blue fox have been known for many years (Bissonnette 1935; Bassett & Llewellyn 1949, 1948; Sokolov 1982), but more exact information is still needed. In blue fox moulting begins at the end of February, mink and ferret moult several weeks later to grow a true summer fur. Mink hair bundles contain almost 30% fewer hairs in the summer fur than in the winter fur (Blomstedt 1990).

Silver fox and racoon dog are long-haired animals with a biphasic moulting, but only one complete change of hair cover yearly. Moulting in the adult racoon dog, is more

distinct than that in the silver fox. Hair cover in summer, especially in racoon dog, includes old guard hairs about to be shed and the upper parts of new guard hairs of different types but practically of no underfur (Blomstedt 1991a). One may easily gain a false impression of a true summer fur. The terminology concerning a true summer fur should be clearly distinguished from hair cover composed of old winter hairs in summer.

A series of skin biopsies from the shoulder and the hip of an adult silver fox, taken between March and December, shows how the pelage in the back is renewed from head to tail (Blomstedt unpublished). This is in agreement with the results of Maurel et al. (1986), but not with those of Bassett & Llewellyn (1948).

The connection between light and fur maturation was observed many years ago (Bissonnette 1935). Now we know that the melatonin hormone, when administred in the summer, ripens the winter fur several weeks ahead (Allain & Rougeot 1980; Valtonen et al. 1988) Individual hairs grow equally well in natural and in melatonin-treated pelages, but in the latter more hairs grow simultaneously in the growth phase (Valtonen & Blomstedt 1988). Melatonin treatment is recommended for adult animals that are about to be pelted and is now in routine use in several countries.

Pelage composition

The overall impression of a fur is the sum of many factors, one of the most important being hair density. In general this is affected by factors such as animal species, age, sex, body part, feeding, season, etc. Recent studies have revealed that fur density in mink is controlled by the number of hairs per skin follicle (Kondo et al. 1989; Rasmussen 1991). The follicle number per area does not vary significantly between pelts with normal and poor hair density, nor with short nap skins with high hair density. The neck area is an exception with fewer follicles. The hair density in a dressed mink pelt of good quality is around 24,000 hairs per cm^2 in the mid-back section.

Even the thickness of a guard hair can affect the number of down hairs per follicle. In mink, the hair density of bundles with a long, thick guard hair is 14; in those with only thin down hairs it is significantly higher, 22 hairs in a prime female pelt (Blomstedt 1989). Furthermore, local fur development, moulting and priming tend to vary somewhat between these bundle types. The guard hair effect has been observed also in all fox types (Blomstedt unpublished). Blue fox hair bundles were large, with up to 60 hairs without a guard hair, but the mean number was 35 hairs per follicle. In silver fox it was around 21 and in blue silver fox 26 in samples from the hip (Blomstedt 1986, 1987). All hair bundles of racoon dog (Blomstedt 1991a) and ferret, according to preliminary studies (Blomstedt unpublished), include a guard hair. No guard hair effect was observed, and the hair density per follicle was 16 in ferret and 30 per follicle in racoon dog. In subsequent years the hair density of an animal can vary, as shown in the racoon dog. But then the reason for this must be feeding or other external factors.

In some pelt disorders the hair density differs from normal. Woolliness is a common defect in blue fox manifesting as a shallow area on the back. Follicles in the blemished area contain more (42-56) hairs, that are significantly more mature than those in the equivalent area in normal skin (36-51). Furthermore, guard hairs are abnormally short (Blomstedt & Joutsenlahti 1987). The metallic defect in mink is usually manifest as typically curved guard hairs, but even the hair density per follicle grows with an increasing effect of this defect.

It has been found that the bundle size is significantly bigger in young kits of 'metallic' parents than in kits of normal parents (Blomstedt 1991b).

Skin composition

A new field of research concerning hair and skin has opened up during recent years: the biochemistry of skin related to the hair growth cycle, age, feeding and fur quality of an animal. Mink is in this respect the most intensively studied farmed fur animal.

Hair is produced by epidermal follicles and surrounded by dermis, the major part of the skin, so that skin and hair make up a functional unity. In a prime mink pelage, hair follicles are short and the dermis is at its thinnest (0.6-0.7 mm). The dermal thickness is about twice that when follicles are growing hair (Blumenkrantz & Blomstedt 1987; Nishiumi et al. 1989; Kondo & Nishiumi 1991).

An important aspect of skin and hair growth concerns the essential properties of dermal connective tissue. This is comprised of a protein net-work of fibrous collagen and non-fibrous elastin, embedded in a gel-like matrix which includes thread-like proteoglycan macromolecules that cling to bigger units by glycosaminoglycans (GAG). In growing mink, the skin collagen content increases while the content of elastin concentrated especially around hair follicles (Dolnick 1965), and that of uronic acid, an index of proteoglycans, decrease (Nishiumi et al. 1991). Even the amino acid content varies significantly between a young and a mature mink skin. In the anagen phase the skin containes twice as much cystine residue and clearly less glycine, proline and hydroxylysine residue than in the telogen. With the exception of cystine these amino acids, are components of collagen. Mink skin reaches the adult type by the age of 22 weeks, which corresponds to the beginning of October (Nishiumi et al. 1991). Abnormal relations of mink skin amino acids have been found in the pelt disorder 'hipper' or 'flat-hipped' pelt with incomplete fur growth on the hips (Michaelsen et al. 1991). Especially hydroxylysine, lycine and glysine concentrations were found to be lower than normal. Collagen is rich in glycine, proline folds the collagen chains, and hydroxylysine and lysine stabilize the side-by-side arrangement of collagen chains to generate a stronger fibre (Darnell et al. 1990). Gram-for-gram, collagen is stronger than steel.

For proper formation of connective tissue, copper (Cu), zink (Zn) and iron (Fe) are essential metals. Certain copper enzymes are involved in collagen cross-link formation and in hair-colour generation. Artificially lowered concentrations of these metals caused distortions during different phases of hair growth. It has been demonstrated that in the treated areas black mink grew white hairs, there were abnormal pigment coagulations in hair follicles, and the skin became hyperelastic (Blumenkrantz & Blomstedt 1987). The younger the animal the stronger was the effect of the treatment.

SUMMARY

Studies on hair growth and pelt structure have presented a diversity even between species as similar in appearance as mink and ferret. In farm practice knowledge of the fur maturation process can be an advantage to breeders in early priming of fur and in choosing the right moment for pelting. The fur priming process can also be speeded up by controlled

light arrangements or by simulating the darkening days of the autumn by administration of melatonin.

There is a close relationship between skin and hair. Skin and hair constituents are defined quantitatively and qualitatively by histochemical and biochemical methods. Biochemical studies on the skin in relation to the hair cycle will help in giving us some idea of the factors affecting fur development and pelt quality. The first studies have already revealed differences in skin constituents between various pelts, but systematic research is still needed in order to establish the parameters of normal skin. Comparison between skins of different qualities might expose essential skin elements that affect fur quality. This information is necessary if improvement in pelt quality is to be achieved by genetics, nutrition or the environment of an animal. One day a fur farmer might be able to run tests during the growing period of the animal, and make the necessary corrections when the pelage can still be saved.

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Circannual melatonin rhythm in mink and its significance in fur growth and reproduction

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Quantitative collection of night-time urine was used to elucidate the temporal relationship between the total amounts of nocturnal melatonin production and furring and breeding cycles in mink. Under natural light conditions the production of melatonin increased in late summer in both male and female mink coinciding with the rest phase of the summer fur coat and was followed by the autumn moult. A decline of melatonin excretion occurred in males in late autumn at the time of testicular recrudescence. In spring time high melatonin secretion in January dropped to low levels in both sexes in March, the recognized time of nidation in females and observed testicular involution in males as well as initiation of spring moult. It appears that in mink the total amounts of nocturnal melatonin secretion do not change in direct relation to the duration of the daily dark period. Instead, significant seasonal increases or decreases were seen in association with moulting periods and reproductive changes.

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The pineal hormone melatonin is regarded as the mediator of environmental light information to the organism. The production of melatonin within the pineal gland is rhythmic in all mammals in which it has been studied. The pineal gland with hypothalamic structures such as the suprachiasmatic nucleus forms a circadian pacemaker system regulating hormonal and neuronal responses. Under diurnal photoperiodic conditions melatonin secretion occurs during the hours of darkness. Because daylength changes during the year, there are seasonal effects on the melatonin rhythm. The duration of the circadian nocturnal peak of pineal melatonin is considered the most important parameter for transmitting daylength information and for the regulation of photoperiodic responses. However, not only the duration of secretion but also the maximal concentrations and the total amounts of melatonin secreted daily may change according to the season, and these are assumed to have physiological significance.

The pattern of nocturnal rise in melatonin varies among species and within the same species in different photoperiods. In some species the night-time melatonin rise occurs as a rather discrete, short-term peak the duration of which appears not always to be quite equivalent to the duration of the dark period (Reiter, 1986). In another pattern of nocturnal melatonin production darkness is associated with a rapid rise in pineal melatonin. The levels

then reach a plateau at a high level and remain elevated for virtually the duration of the dark period.

To date, most melatonin cycles have been described in rodents (Reiter 1986) or ungulates (Kennaway 1984) maintained under controlled conditions in the laboratory or in natural light conditions but during only a few different photoperiods. Very few studies have dealt with the melatonin cycle in animals maintained under natural photoperiods throughout the year. Moreover, in mink the nocturnal pattern of melatonin increase has been studied in experimental conditions, and it is stated that in mink the duration of elevated levels of melatonin is proportional to the length of the dark phase, whatever the photoperiod (Ravault et al. 1986). However, the level of melatonin concentrations may be different in different photoperiods.

Melatonin and its metabolites are excreted in the urine. The excretion follows the rhythm of melatonin production and secretion from the pineal gland (Fellenberg et al. 1981). Quantitative collection of urine is a convenient way of following the long-term changes in melatonin secretion in animals in which repeated blood collection is difficult. In this study, excretion of melatonin in urine was monitored under natural conditions in order to elucidate the temporal relationship between the amounts of melatonin secreted and the main, known photoperiodic responses in mink, i.e. the annual moulting periods and breeding cycle.

MATERIALS AND METHODS

Ten standard dark adult mins (five males and five females) were housed outdoors under normal conditions and natural photoperiod and were fed ad *libitum*. A quantitative collection of night-time urine was made between 1500 and 0900 h at two-week to two-month intervals over a period of one year. A representative sample of each collection was stored frozen until analysed.

Urine melatonin was determined radioimmunologically as previously described (Vakkuri et al. 1984). Briefly, after centrifugation of mink urine (10 min at 2000 xg), samples of 1 ml were extracted with chloroform. The chloroform phase was washed with distilled water (2 ml) and evaporated in a vacuum. The residue, reconstituted in RIA buffer, was analysed for melatonin using a-MT-K1 antiserum and 1251-melatonin in RIA.

The onset and progression of the moult were observed by checking the colour of the skin. Pigment is produced in the growing hair, giving a dark colour to the skin, whereas in mature fur, the hair roots are unpigmented. The spring moult progresses from head to tail and the autumn moult from tail to head. The fur was considered to be prime when the skin was non-peeling and had a light colour. In the female mink the progress of winter fur growth and the different phases of fur development were also evaluated histologically twice a month in accordance with the method developed by Blomstedt (1988). The onset of testicular recrudescence was determined by weekly palpations of the testicles, and the breeding season was followed in accordance with normal farm routines.

The amounts of melatonin excreted in night-time urine are expressed as means $\pm$ SEM in picograms per hour. The analysis of variance for repeated measurements was used to assess the seasonal differences in males and females.

RESULTS

The mean nocturnal melatonin excretions in the urine of male and female mink during the decreasing photoperiod with increasing length of nights in late summer, autumn and early winter are presented in Figs. 1 and 3. Respective melatonin excretions during the increasing photoperiod with shortening nights are presented in Figs. 2 and 4. In males the nocturnal melatonin excretion varied between 335 $\pm$ 81 pg/h in January and 51 $\pm$ 14 pg/h in early July and in females between 168 $\pm$ 68 and 40 $\pm$ 4 pg/h respectively. The excretion of melatonin in the urine was higher in males than in females, obviously because of the greater size of the males. Under a natural photoperiod the production of melatonin increased sharply and significantly ($p < 0.01$) until August. Thereafter, it began to decline and in males it was significantly ($p < 0.01$) lower in October than at the beginning of August. In females the decrease was not obvious before November ($p < 0.05$). There was a sharp drop ($p < 0.01$) from January to April in males and from February to May ($p < 0.05$) in females. Thereafter, the excretion increased again but was low at the beginning of July.

The autumn moult started in the tail in the second half of August and was complete in both male and female mink by mid-October. The winter fur coat was mature, with no growing hair remaining at the end of November. The spring moult began from the head by mid-April and progressed over the whole body, ending at the tail in late June. The summer coat was completely mature in all animals by mid-July.

Testicular recrudescence was first observed at the end of November and became obvious in December, reaching the maximum in February. Regression of testicles began during March and was complete by June. Alle females exhibited signs of heat at the end of February and were mated twice between 5 and 15 March. They all conceived and whelpings occurred during the first week of May. The males were also used to service other females besides the five included in the present experiment, and all five males proved fertile.

A distinct increase in melatonin secretion in late summer in both sexes coincided with the rest period (telogen) of hairs of the summer coat. In males the total amounts of melatonin secreted then decreased until the beginning of November when testicular regression was first observed. The high values of melatonin secretion observed in January dropped to relatively low levels in both sexes in March which is the known time of nidation in females and observed testicular involution in males as well as initiation of spring moult.

DISCUSSION

It is generally assumed that the quantity of nocturnal melatonin secretion is directly related to the duration of the daily dark period so that the quantity continuously increases during the autumn until the winter solstice. Longer nights are associated with more prolonged periods of high melatonin secretion. The amplitude may, however, be lowered and hence the total amount of melatonin secretion remains at the same level or even decreases. This seems be the case in mink, in which, despite the increasing night length in autumn, the total amount of melatonin secretion decreased. In spring the decrease in melatonin secretion coincided with the decreasing night length and then levelled off in May. The annual

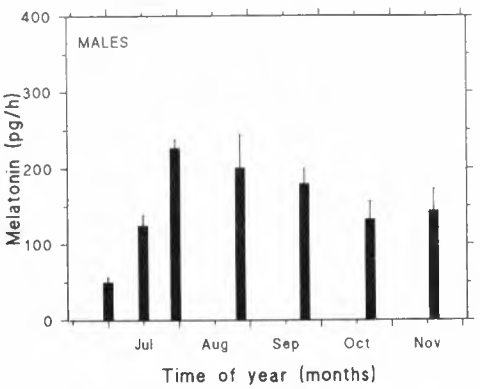

Fig. 1. Autumnal melatonin excretion (mean ± SEM) in five adult male minks, collection of night-time urine

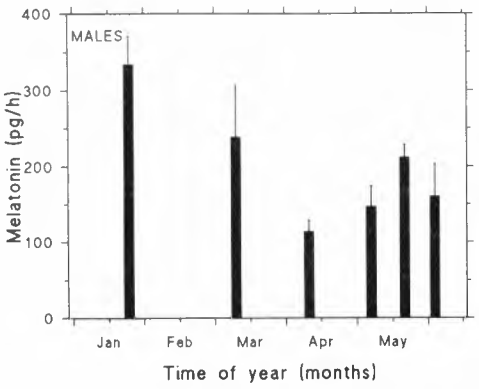

Fig. 2. Vernal melatonin excretion (mean ± SEM) in five adult male minks, colletion of nigt-time urin

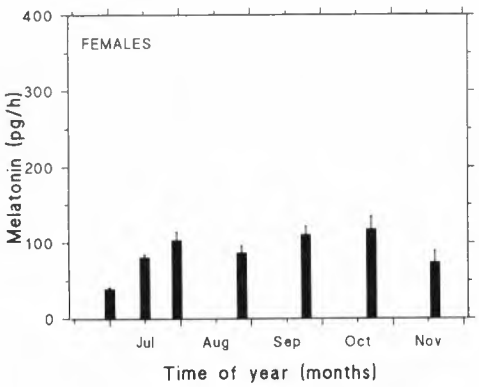

Fig. 3. Autumnal melatonin excretion (mean ± SEM) in five adult female minks, collection of night-time urine

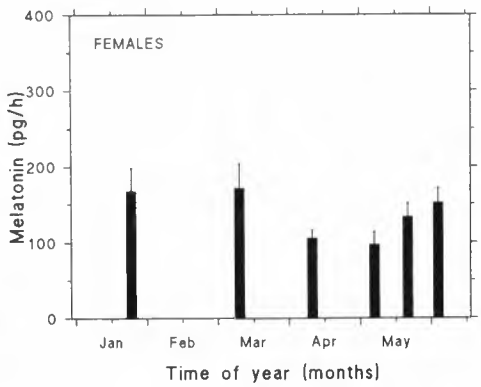

Fig. 4. Vernal melatonin excretion (mean ± SEM) in five adult female minks, collection of night-time urine

photoperiod and the daily melatonin secretion rate have been shown to be closely linked to the seasonal changes in reproduction and furring cycles in many mammals including mink. These physiological phenomena are easily altered by photoperiodic changes. Seasonal changes in melatonin secretion may include changes in the onset of secretion, its amplitude, duration and in the total amounts secreted.

Concerning the onset of winter fur growth, the increasing amounts of nocturnal melatonin secretion during the rest phase of the hair follicle seem to be crucial in triggering the onset of the growth, which can be advanced by constant-release implants of melatonin. The growth of hair follicles can be activated as soon as they have reached the telogen

phase. More hair follicles can be activated simultancously by exogenous melatonin but the last ones will not start to grow before the end of July. It has been demonstrated that in mink the melatonin secretion in autumn has to be sustained above a certain level until mid-October for the winter fur development to progress. After mid-October, when all winter hairs have started to grow, the level of melatonin secretion does not affect the maturation of winter fur (Valtonen et al. 1990). Concerning the onset of winter fur growth, it seems that increases in the duration and amplitude of melatonin secretion until a certain total amount or duration is reached for triggering the growth of all winter hair are crucial. After that the total amount of melatonin secretion may decrease without affecting hair growth. Concerning the onset of reproductive functions by seasonal changes in photoperiods and melatonin secretion, the critical component of the daily melatonin profile has not been clearly established. Changes in the duration, amplitude, time of onset and offset, and the shape of the daily secretion profile may be involved alone or combined. The quantitative night-time urine collection method used in the present study gives information only about the total amount of nocturnal melatonin secretion. This clearly decreased after September in males, which could be of biological significance to the onset of testicular recrudescence.

Melatonin secretion was high in males in January, coinciding with the time of peak testosterone secretion in normal males (Sundquist et al. 1984). An interrelationship of melatonin and testosterone secretion has been suggested by Newman et al. (1991) who found a daytime rise in melatonin coinciding in time and duration with the peak in circulating testosterone in the same fallow bucks under a natural photoperiod. Whether elevation of daytime melatonin contributes to the high amounts of melatonin secreted in male minks in January warrants further investigations.

Prolactin secretion follows the ambient photoperiod in mink (Martinet et al. 1982; Boissin-Agasse et al. 1988). This increases promptly during April, just after the significant decrease in melatonin secretion. The dependence of prolactin release on the circulating amonts of melatonin has been well demonstrated in mink, where normal increase in vernal prolactin concentrations can be inhibited by melatonin treatment which consequently delays gonadal involution, spring moult and progesterone increase and implantation (Martinet et al. 1981; Martinet et al. 1984).

Melatonin serves as an important transducer of photoperiodic information to the endocrine system and as such is intimately involved in the synchronization of seasonal reproduction and furring cycles. Various species may utilize the melatonin secretion pattern in different ways to induce gonadal involution or recrudescence and moulting periods. Therefore it is important to investigate the secretion pattern of melatonin in natural photoperiods throughout the year with frequent sampling regimes. The use of melatonin treatment for advancing winter fur growth has been successful, but in order to manipulate the breeding patter and adjust the breeding season and fecundity in mink with melatonin, specific information on the seasonal changes in timing, duration and amounts of melatonin secreted is needed.

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Pelage development in melatonin-treated mink

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The effects of melatonin on the autumn molt in mink skin were investigated by means of the changes in tyrosinase activity and histological parameters. Melatonin-treated mink molted twice in autumn and their hair activity, the amount of underfur and tyrosinase activity also exhibited twin peaks during the experimental period. Seasonal changes in tyrosinase activity were correlated with those in histological parameters in both control and melatonin-treated mink. These results indicate that exogenous melatonin accelerates not only hair production but also melanogenesis in mink skin.

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The seasonal molt is induced by the change of daylength and the pineal hormone melatonin acts as its organic messenger (Reiter 1988). Melatonin-treatment in mink induces an early autumn molt (Allain & Rougeot 1980; Rose et al 1984) and we previously reported the quantitative histological changes of the skin in melatonin-treated mink (Fukunaga et al. 1991).

The molt comprises not only hair production but also total skin events including the changes in skin components (Nishiumi et al. 1991). Melanin is one of such components of the hair or skin color and is produced in specialized cells, termed the melanocytes, differentiated from the neural crest. Melanin is synthesized in the melanocytes, by only one enzyme, tyrosinase.

In recent years several advanced studies have been carried out on hormonal and genetic control of tyrosinase (Burchill et al. 1988, 1990; Imokawa et al. 1988; Lamourex & Pendergast 1987). However, these works used mainly humans or mice as experimental models and their hair cycle is not common to the other mammals. Most mammals have a seasonal molting cycle and the studies that have been done on pigmentation of seasonal molting cycle are quite limited (Blumenkrantz & Blomstedt 1987; Weatherhead & Logan 1981). The purpose of the present study is to investigate the effects of exogenous melatonin on melanin synthesis and its relation to hair production in the autumn molt of skin.

MATERIALS AND METHODS

Male dark mink born in early May were used. The mink were classified in two groups. In early July, soon after the summer sostice, five melatonin pellets were implanted subcutaneously into the interscapular area of five mink. The others made up the control group. These silastic implants weighed 30-35 mg, contained 10% (w/w) melatonin (about 15 mg melatonin) and were prepared to release melatonin gradually for a period of about two months. After the implantation of melatonin, a skin biopsy was taken from the mid-dorsal region every two weeks. Skin samples were immediately stored in liquid nitrogen and kept till assay. The preparation of crude tyrosinase from mink skin and the tyrosinase activity assay were carried out by the method described by Hearing (1987), and by Lamourex & Pendergast (1987) with some modifications.

To follow the histological changes in the skin by means of an optical microscope, sections 10-20-μm-thick were cut vertically to the hair follicle using a cryostat and stained with hematoxylin and eosin. The following two parameters were used: (1) proportion of underfur in the active phase to the total amount of underfur (hair activity); (2) number of underfur hairs per follicle group. Whether the underfur was in the active phase or not was judged by the presence or absence of hair medulla.

RESULTS

1. Tyrosinase activity

(1) Incubation time dependency of tyrosinase activity:

In every sample, the tyrosinase activity of mink skin changed in a two-phasic manner during the incubation time for 24 h. Figure 1 illustrates the typical pattern of tyrosinase activity in which, for the first 12 h. at least, the absorbance showed a small increase or constant value but after that a steady rapid increase was observed.

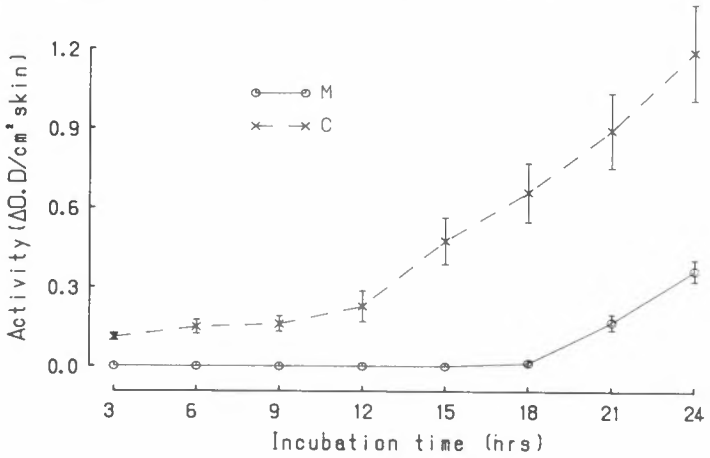

Fig. 1. Incubation time-related change in tyrosinase activity in mink. Crude extract of tyrosinase from skin was incubated at 37°C with Dopa. Activity was expressed as the change of absorbance at 475 nm and the mean values (n=5) are given. The vertical line represents SD

(2) Seasonal variation in tyrosinase activity:
In the low reaction phase (6-h. incubation), the values of enzyme activity were low but the seasonal changes in tyrosinase activity were clear in both the control and melatonin-treated groups (Fig. 2).

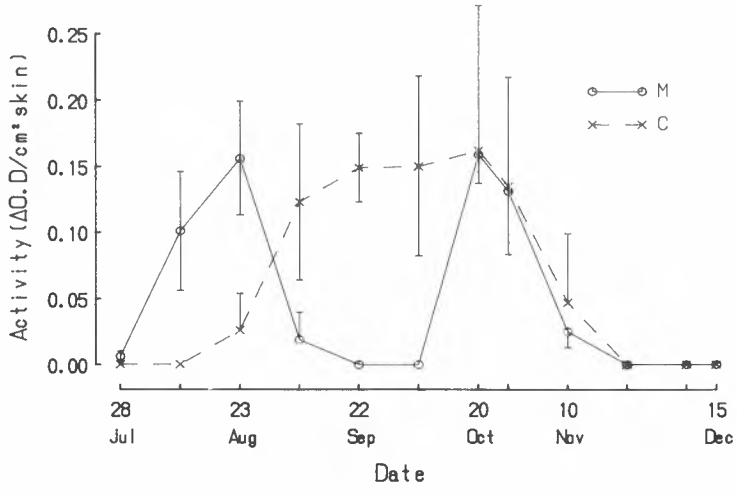

Fig. 2. Seasonal variations in tyrosinase activity in mink skin. The mean values after the 6-h. incubation period are given. The abbreviations are the same as those in Fig. 1.

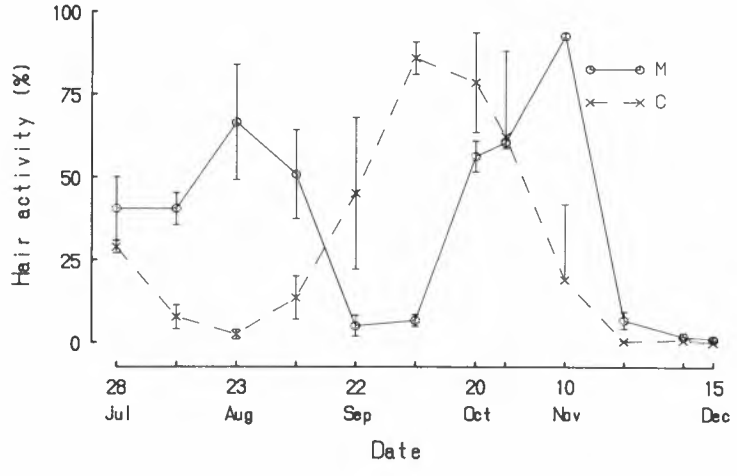

Fig. 3. Seasonal variations in the ratio of hair activity in mink. The vertical line represents SD

In the control group, there was a lull in tyrosinase activity from late July to early August, after which it was found that the activity increased markedly, reaching a plateau which was maintained for about two months. Then from the end of October the enzyme activity dropped and at the end of November it was undetectable. In contrast, the melatonin-treated group indicated sharp twin peaks, the first in late August and the second peak during the latter part of October. Tyrosinase activity was undetectable in the period between the two peaks. After the second peak, the enzyme activity fell and became undetectable at almost the same time as that in the control group.

2. Seasonal variations in the histological parameters

The values of hair activity at the follicular level are plotted in Fig. 3.

Hair activity of the melatonin-treated group exhibited two peaks, as was observed in the tyrosinase activity (Fig. 2). The first peak occurred at the end of August (66.5%), the second early in November and its value was greater than that of the first peak (92%). Between peaks, from mid-September to early October, hair activity decreased to about 6%. After the second peak, hair activity decreased markedly but a level of 0% was unachievable until the end of the experimental period.

In contrast to the hair activity, the notable seasonal changes in the amount of underfur were observed at the pore level rather than at the follicular level. Thus, in Fig. 4, only the pore level values were plotted.

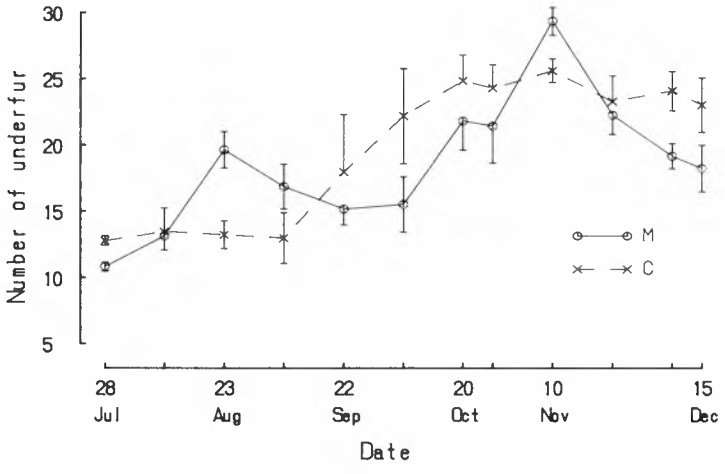

Fig. 4. Seasonal variations in the proportion of underfur per follicle in mink. The vertical line represents SD

In the melatonin-treated group, the amount of underfur also exhibited two peaks, the first was low (19.7) at the end of August and the second was high (29.4) early in November. Between peaks, from mid-September to early November, the amount of underfur was of relatively constant low value (at about 15).

DICUSSION

The melatonin-treated mink molted twice during the autumn and this phenomenon was confirmed by seasonal changes in the histological parameters and the tyrosinase activity that appeared on the graphs as twin peaks. The first early autumn molt occurred at almost the same as that recorded in our previous report on melatonin-treated mink (Fukunaga et al. 1991), but the second late autumn molt was an unexpected occurrence. Although we implanted a sufficient amount of melatonin (about 15 mg/head) similar to many other investigations, our implants had released all of the melatonin within a relatively short period (about 50 days) unlike the implants of other investigators (Allain & Rogeot 1980; Allain et al. 1981; Rose et al. 1984; Rose et al 1987). So it is assumed that after the induction of the first early autumn molt, the exogenous melatonin disappeared and the blood level of melatonin immediately dropped down to the normal level, but after that the endogenous melatonin increased with shortening days and induced the second late autumn molt. This speculation suggests that there is a critical dosage level and a critical time for inducing a single early autumn molt and arriving at an early pelting time of mink by means of exogenous melatonin.

The seasonal changes in mink skin tyrosinase activity are correlated with those of hair growth parametrized histologically. In both control and melatonin-treated groups, the tyrosinase activity was high during anagen and low or undetectable during telogen and in particular the 6-h incubation period showed the precise cyclicity associated with hair production. These results were consistent with the report in which mouse skin tyrosinase activity increased during anagen (Geschwind et al. 1972). But during any anagen, the increase in the tyrosinase activity begins slightly earlier than that of the histological parameters and the black pigments develop before the hair medulla is observable under the *microscope on mink skin sections*. So it seems that the melanogenesis occurs prior to hair procuction in mink skin.

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Quantitation of elastic fibres in the reticular dermis in mink using electronic image analysis

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Rasmussen, P.V. 1992. Quantitation of elastic fibres in the reticular dermis in mink using electronic image analysis. Norwegian Journal of Agricultural Sciences. Suppl. no. 9: 599-603. ISSN 0801-5341.

Examinations of mink pelts with reduced hair quantity on both hip areas (RHH) led to an illustration of factors of importance to the local stretching properties of the skin. At pelting, skin biopsies from hip and back were obtained from 14 control animals thought to have a low probability of developing RHH, and 42 animals with an expected high probability of developing RHH. Histological sections were made, and the elastic fibres in these sections were stained selectively according to a modified orcein method. The volume fraction of elastic fibres in areas of *stratum reticulare* between the hair groups was determined according to stereological principles. Microscopy and electronic image analysis were used. The individual volume fractions were between 0.4 and 0.7% and were normally distributed in both groups. On a group level, no genetic differences or correlation with the degree of RHH could be proved.

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For some time now elastic fibres have been known as an important part of connective tissue. This is especially valid for tissue with elastic properties such as artery walls, lung alveolae, elastic ligaments and skin (Ross & Bornstein 1971).

Mature, elastic fibres consist of two morphologically different components, an amorphous elastin component, constituting as much as 90% of the fibre, and a fibrillar component, primarily surrounding the amorphous part (Smith et al. 1982).

As far as skin is concerned, a suitable combination of the fibrous protein collagen, water and the basic substance polysaccharides provides high tensile strength. The elastic fibres, amounting to only a few percent of the dermal proteins, and their complicated network provide the skin with stretchability and the possibility of returning to the initial position after stretching.

From human investigations it is known (Smith et al. 1982) that individual variations exist as regards the structure and fibrous components of the connective tissue.

As hair and skin form an integral part of the mink pelt, knowledge of the structure and chemical composition of mink skin and thus its special properties is of course important. This applies for instance to mink that give pelts with reduced hair mass on both hip areas (RHH), resulting in a poorer quality of pelt.

Various investigations and assumptions (Lund 1988; Rasmussen & Lohi 1988; Olesen 1988) justified a comparative determination of the volume fraction of elastic fibres in the dermis, as this could be of importance to the local stretching properties of skin and thus also to the skin defect RHH.

MATERIALS AND METHODS

Biopsies, preparation and staining

The experiment included 56 Scanblack male kits: 14 control animals of parents which the year before did not produce any kits with RHH, and 42 experimental animals of parents, 50% of whose kits developed RHH skins in the preceding year.

At pelting, immediately after killing, skin biopsies were taken from each animal from hip as well as back. After fixation in a neutral formalin solution and normal procedures, the skin biopsies were embedded in paraffin wax and 5 μm thick sections were cut parallel to the median plane. The purpose of staining was to stain mature, elastic fibres selectively. The orcein method based on the recommendations of Romeis (1948) was used for staining, especially because this method is very selective and relatively simple to carry out (Lyon 1985). One gram of orcein, a cation dye, was dissolved in 100 ml 70% ethanol, to which 1 ml concentrated HCl was added. Differentiation was performed for two hours in pure methanol. Empirically this procedure gave the best selective dark brown staining of the elastic fibres.

Attention was focused on the reticular part of the dermis where the large (prime) elastic fibres are found (Frances & Robert 1984). The area between the individual hair groups was analysed. At the same time elastic fibres attached to the hair groups were deliberately excluded from the analysis.

Morphometry

By means of light microscopy combined with electronic image analysis (Uitto et al. 1983) have proved that with histological sections as the basic material, the volume fraction of elastic fibres can be determined stereologically from the area fraction of these fibres.

In this investigation five slides each with four serial sections, i.e. a total of 20 sections, were analysed for each biopsy. The samples from the hip were determined in duplicate. The sections were cut to represent a 300-μm thick slice of the biopsy. In each section the area fraction was only determined in one analytic field. The area of this field was 0.021 mm^2. In this way none of the fields analysed was identically positioned, and the individual fractions became mutually independent.

By means of the demo program in a Scan Beam sb1024 Image Processing Module the area fraction of selectively stained elastic fibres was determined quantitatively. The image in question was sliced in two: all pixel values greater than the threshold were coloured red, and all pixel values below the threshold (elastic fibres) were coloured green. The horizontal position of the cursor determined the threshold level, and the red and green percentages were written in the menu field. The results of the image analysis are illustrated in Figs. 1 and 2.

Geometrically, it can be proved that on the condition that the section thickness is

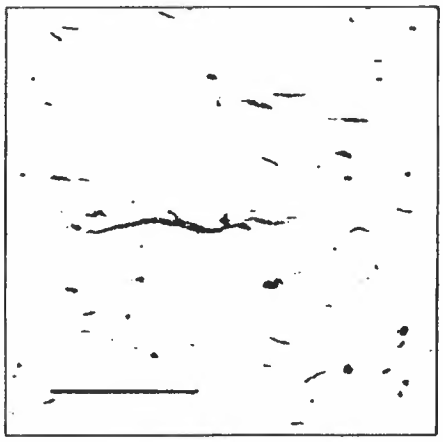

Fig. 1. Electronic image analysis. The area fraction of elastic fibres (black areas) is 2%. The volume fraction is then 0.44%. Scale = 50 μm

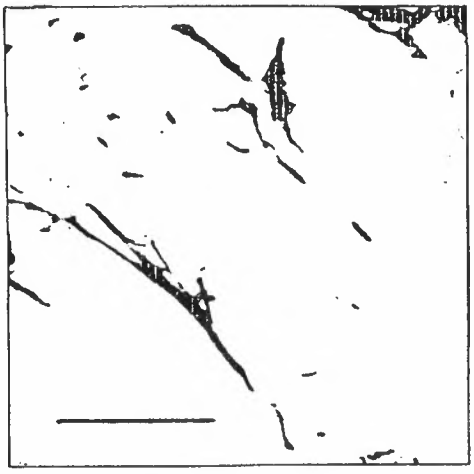

Fig. 2. Electronic image analysis. The area fraction of elastic fibres (black areas) is 5%. The volume fraction is then 1.10%. Scale = 50 μm

much smaller than the size of the objects measured, then the area fraction (Aa) = the volume fraction (Vv). When this is not the case the so-called "Holmes effect" (Weibel 1979) will apply, so that the volume fraction is stereologically overestimated.

The average cylinder diameter of mature, elastic fibres was determined at 1.4 μm, meaning that the area fraction of elastic fibres overestimated the volume fraction and therefore had to be corrected: Vv = Aa x K (Vv). If t is the section thickness and d is the cylinder diameter, the relative section thickness is g = t/d. Mathematically, it can be proved (Weibel 1979) that when the object can be regarded as long cylinders, which it can here, the correction factor K (Vv) = $1/(1+g) = 1/(1+(5/1.4)) = 0.22$.

RESULTS

The level of threshold adjustment and the quality of the sections can affect the area determined as elastic fibres. Therefore two different operators determined the area fraction (%) in the hip region of six different animals. For the two series of measures recorded, the coefficients of variation (CV) averaged 0.44 and 0.40. Mean values (%) were 2.9 ± 0.5 and 3.0 ± 0.4, respectively ($r = 0.9$; $p < 0.02$), and the general method was therefore considered reproducible.

Some skin biopsy preparations were unsatisfactory, with the result that the number of animals examined was reduced. For the hip area (sampling in duplicate) the mean volume fractions (%) of elastic fibres in the control group (N = 10) and in the experimental group (N = 36) were 0.50 ± 0.07 and 0.50 ± 0.06, respectively, and the minimum and maximum values (min; max) (0.40; 0.65) and (0.37; 0.62), respectively.

Statistically this meant that there was no difference between groups. Normal distributions appear from Figs. 3 and 4.

Stem	Leaf	#
6	5	1
6		
5	5	1
5	1224	4
4	6	1
4	124	3

Multiply Stem.Leaf by 10''-1

Fig. 3. The distribution of the volume fraction of elastic fibres in the hip area for the control group is shown by means of SAS (Proc Univeriate)

For the back area a smaller number of individuals was examined (single samples). The volume fractions (%) of elastic fibres in the control group (N = 6) and the experimental group (N = 14) had minimum and maximum values (min.; max) of 0.43; 0.59 and 0.43; 0.70, respectively. Data indicated that there was no difference between the two groups.

A t-test of 14 of the back samples of the experimental group against corresponding hip samples indicated that there was no difference between back and hip as regards the size of the volume fraction of elastic fibres: Mean values (%) were 0.56 ± 0.09 and 0.54 ± 0.06 ($p < 0.5$), respectively. The same facts apply to the control group.

A later subjective grouping of the pelts as regards the degree of RHH (grades 0, 1, and 2) gave correspondingly similar values for the volume fraction of elastic fibres for all three groups.

Stem	Leaf	#
62	24	2
60		
58	88	2
56	5667	4
54	024	3
52	26679	5
50	56677	5
48	0	1
46	055569	6
44	68	2
42	44	2
40	67	2
38		
36	58	2

Multiply Stem.Leaf by 10**-2

Fig. 4. The distribution of the volume fraction of elastic fibres in the hip area for the experimental group is shown by means of SAS (Proc Univariate)

DISCUSSION

This morphometric investigation showed that the volume fraction of elastic fibres in the reticular dermis can hardly be related to genetic properties concerning the possibility of developing the skin defect RHH. On a group level, no significant differences could be found.

Compared to similar morphometric examinations of human connective tissue the mean values calculated are low (Godeau et al. 1986; Uitto et al. 1983). This does not mean that the values are too low for the localities examined. Obviously the distributions shown seem to illustrate the normal variation of the volume fraction of elastic fibres here.

As the variation within the single individual is comparatively large, it is reasonable to perform a determination in duplicate.

Factors which might result in a systematic underestimation of the volume fraction of elastic fibres are the selectivity of the colouring method used, the size of the medium thickness of the fibres and thus of the correction factor K (Vv).

Stereological methods combined with electronic image analysis have been used to characterize local fractions of the elastic component of mink pelts, i.e. the elastic fibres, but other components can be characterized according to similar principles.

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Capillary electrophoresis as an efficient tool in studies of pelt glycosaminoglycans

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Michaelsen, S., Mai-Britt Schrøder & H. Sørensen 1992. Capillary electrophoresis as an efficient tool in studies of pelt glycosaminoglycans. Norwegian Journal of Agricultural Sciences. Suppl. no. 9: 604-612. ISSN 0801-5341.

Glycosaminoglycans (GAGs) and collagen glycoproteins are skin constituents assumed to be important for the properties of mink pelts. Exact structural information on GAGs in mink skin is needed, and seems to be obtainable by utilization of the structural features of GAGs. These provide analytical advantages by means of enzymatic cleavages by various lyases (EC.4.2.2.) which allow a gentle release of disaccharide units. High performance capillary electrophoresis (HPCE) based on cetyltrimethylammonium bromide (CTAB) has now been developed as an efficient method of analysis of these GAG-disaccharide units. The influence of varying separation conditions on separation parameters has been investigated. The results indicate the possibility of changing separation conditions according to the samples analysed. GAG-disaccharide units from various chondroitins and GAGs in mink skin have been analysed by the HPCE method after protease and chondroitinase treatments.

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Glycosaminoglycans (GAGs) and collagen glycoproteins are skin constituents assumed to be important for the properties of mink pelts (Lindahl & Höök 1978). GAGs,or chondroitins, seem to vary in their content of different sulphated carbohydrates as a function of the animal's age and various pathological stages (Poole 1986; Carney & Osborne 1991). Exact structural information on GAGs in mink skin is needed, but is not available owing to the instability of the compounds during the conditions required for acid hydrolysis and owing to lack of specificity of the methods normally used in such studies.

The structural features of GAGs provide analytical advantages by means of enzymatic cleavage by various lyases (EC.4.2.2.). This allows a gentle release of disaccharide units with 4.5-unsaturated uronic acid parts, which give a chromophore (λ_{max} at appr. 230 nm).

Determinations of individual chondroitin disaccharides have been based on techniques such as paper chromatography, TLC, paper or cellulose acetate membrane electrophoresis and HPLC (Seno *et al.1972*; Seno & Murakami 1982; Säämänen & Tammi 1984; Gherezghiher *et al.* 1987; Nomura *et al.* 1989). HPLC is superior to the other techniques. However, the disadvantages of HPLC include large sample volumes, large solvent volumes, long-term analysis, expensive columns, long equilibration and regeneration times of

columns, and an inability to resolve some of the isomers.

Recently, high performance capillary electrophoresis (HPCE) as free zone or micellar electrokinetic capillary chromatography (MECC) with SDS has been introduced to separate isomeric disaccharides (Carney & Osborne 1991; Al-Hakim & Linhardt 1991). Glycosaminoglycan disaccharides are well suited for separation by HPCE, as they are charged water soluble compounds with a characteristic absorbance at 232 nm. Generally, HPCE techniques are fast and inexpensive, with potentialities of high resolution of analytes only small sample volumes are needed, and HPCE techniques require only inexpensive fused-silica capillars (Carney & Osborne 1991; Michaelsen *et al.* 1992).

The present work describes an efficient HPCE method based on MECC with cetyltrimethylammonium bromide (CTAB), and developed for determination of individual GAG-disaccharide units. The studied parameters include evaluation of effects from temperature, voltage, pH, CTAB and electrolyte concentration. Results from enzymatic cleavages of GAGs from various chondroitins and mink skin are described, as is the separation of the obtained mixtures of disaccharides.

MATERIALS AND METHODS

The ABI Model 270 A Capillary Electrophoresis System (Applied Biosystems, USA) was used with a 750 mm x 50 μm i.d. fused-silica capillar. Detection was 522 mm from the injection end of the capillar and carried out as on-column measurements of UV absorption. Data processing was performed on a Shimadzu Chromatopac C-R3A (Kyoto, Japan).

Chondroitin disaccharides Δ^4-GlcUA→GalNAc (ΔDi-0S;**1**), Δ^4-GlcUA→4-O-sulpho-GalNAc (ΔDi-4;**2**), Δ^4-GlcUA→6-O-sulpho-GalNAc (ΔDi-6S;**3**), Δ^4-2-O-sulpho-GlcUA→4-sulfo-GalNAc (ΔDi-diS_B;**4**) (Fig. 1), chondroitin sulphate A from bovine trachea (appr. 70% and 30% C), chondroitin sulphate B from bovine mucosa (dermatan sulfate, appr. 85% and 15% A + C), and chondroitin sulphate C from shark cartilage (appr. 90% and 10% A), as well as chondroitinase ABC (chondroitin ABC lyase; EC 4.2.2.4) were obtained from the Sigma Chemical Company (St. Louis, USA). Pepsin and pancreatin were from Merck (Darmstadt, Germany). Skin samples were from the back of standard mink pelts and obtained from the National Institute of Animal Science, Research in Fur Animals, DK-8830 Tjele, Denmark, and the samples were stored at - 20°C until used.

Sodium tetraborate and sodium phosphate were from the Sigma Chemical Company and CTAB was from BDH (Poole, England). All chemicals were of analytical reagent grade.

Buffer preparations for the HPCE separations were performed according to Michaelsen *et al.* (1992). Samples were introduced from the cathodic end of the capillar by 1-sec vacuum injection. Separations were performed at 30-60°C and 10-30 kV. On-column UV detection was at 232 nm unless otherwise mentioned. Washing with buffer was done between each analysis for five minutes. After a number of analyses were carried out, the capillar was washed for five minutes with 1.0 M NaOH and for two minutes with water.

Calculations of relative migration times (RMT), normalized peak areas (NA) and relative normalized peak areas (RNA) were performed according to Michaelsen *et al.* (1992).

RESULTS AND DISCUSSION

GAGs are proteoglycans of high molecular weight which contain a high amount of long unbranched heteropolysaccharides, 90-95% by weight, attached as O-glycosides to serine side chains in the protein core through a trisaccharide unit. GAGs contain repeating disaccharide subunits in which one of the sugars is uronic acid (β-D-GlcUA, α-L-IdUA, or 2-O-sulpho-α-L-IdUA) or β-D-galactose attached through 1-3 or 1-4 glycoside bonds to 6-O or 4-O sulphate esters of glycosamines (β-D-GlcNAc, β-D-GalNAc; evt. SO_3^- instead of Ac; se Table 1). The structures of the disaccharide standards are shown in Fig. 1.

To establish the most favourable separation conditions for disaccharides present in various samples, a systematic investigation of the influence of changes in separation conditions on parameters such as migration times (MT), RMT and peak area (NA and RNA) was carried out.The initially applied separation conditions were a running buffer consisting of 18 mM borate, 30 mM phosphate, and 50 mM CTAB adjusted to pH 7.0, a temperature of 30°C, and a voltage set at 20 kV.

Table 1. Components of the GAG's invarious proteoglycans (Lindahl & Höök 1978; Silbert 1983

	Appr.MW of GAG ($x10^3$)	Compo-nents of repeat. units	Location of sul-phate	Sulphate pr disacch. unit	Linkage	Other sugar compo-nents	Examples of occurrence
Hyaluronic acid	4-8000	β-D-GlcUA β-D-GlcNAc	-	0	β-1,3 β-1,4	-	Skin, cartilage
Chondroition sulphate A	5-50	β-D-GalNAc	4	0.1-1.3	β-1,3 β-1,4	D-Gal D-Xyl	Skin, cartilage, bone
Chondroitin sulphate B (Dermatan sulphate)	15-40	(β-D-GlcUA) α-L-IdUA β-D-GalNAc	 2 4	1.0-3.0	β-1,3 α-1,3 β-1,4	D-Gal D-Xyl	Skin, heart valve, arterial wall
Chondroitin sulphate C	5-50	β-D-GlcUA β-D-GalNAc	 6	0.1-1.3	β-1,4 β-1,3	D-Gal D-Xyl	Skin, cartilage bone
Heparin	5-40	β-D-GlcUA α-L-IdUA α-D-GlcN	 2 6,N	1.6-3.0	β-1,4 α-1,4 α1,4	D-Gal D-Xyl	Skin, intestinal mucosa

Chondroitin disaccharide standards **1**, **2**, **3**, and **4** were dissolved in water and analysed by HPCE individually and as a mixture. The four disaccharides could be separated completely under the initially applied separation conditions (Fig. 2). The CTAB-MECC separation is based on hydrophobic and ion-pairing interaction of the negatively charged disaccharides and the positively charged CTAB micelles and the CTAB-covered capillary wall, as used

for glucosinolates (Michaelsen *et al.* 1991). Compared to the HPCE methods published, this CTAB system involves a reversal of the electro-osmotic flow in the capillar, which is very important for the separation to occur. Further details of the separation principle are given by Michaelsen *et al.* (1992).

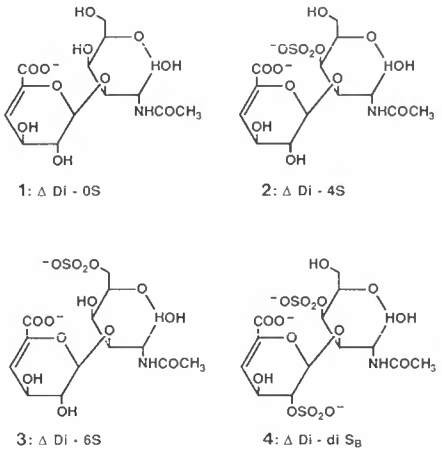

Fig. 1. Structure of the standard GAG-disaccharides used in HPCE analyses

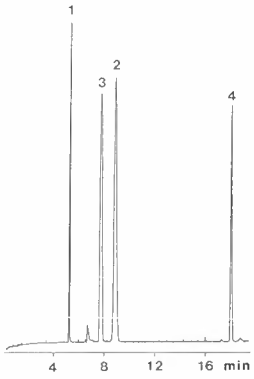

Fig. 2. Electropherogram of GAG-disaccharides dissolved in water. **1** = ΔDi-0S (0.45 mg/ml), **2** = ΔDi-4S (0.80 mg/ml), **3** = ΔDi-6S (1.6 mg/ml), **4** = ΔDi-diS_B (0.40 mg/ml). Buffer: 18 mM borate, 30 mM phosphate, and 50 mM CTAB (pH = 7.0); Temperature: 30 °C; Voltage : 20 kV

Applied voltages of 10, 15, 20, 25 and 30 kV were tested. MT values decreased rapidly with increasing voltages (Fig. 3), whereas RMT, NA and RNA values were constant. From the obtained results a voltage of 20 kV was chosen as a tradeoff between obtained separation and total time of analysis.

Increasing temperatures from 25, 30, 40, 50 and to 60°C reduced MT values, and had no effect on RMT values except for GAG-disaccharide 4, where RMT values increased slightly with temperature. NA values increased slightly, whereas RNA values were constant (Fig. 4). This is important with regard to quantitative determinations (Michaelsen *et al.* 1992). A temperature of 40°C was chosen.

Increasing the electrolyte concentration (borate plus phosphate, 3:5) from 16 to 48 mM produced interesting results for MT and RMT. MT values increased for GAG-disaccharides 1 and 4, and decreased for 2 and 3 (Fig. 5). RMT values increased for 1 and 4 and were constant for the other two GAG-disaccharides. NA and RNA values were constant. From these results an electrolyte concentration of 48 mM was chosen.

From these results it can be seen, that it is possible and easy to change MT and RMT according to what is determined by HPCE analyses of various samples. The effects causing the observed changes in the separation parameters, after applying various separation conditions, will not be dealt with in detail here. All together,no single effect can explain the changes observed, and a more thorough treatment of the effects involved can be found in Michaelsen *et al.* (1992) and Bjergegaard *et al.* (1992). However, the results indicate

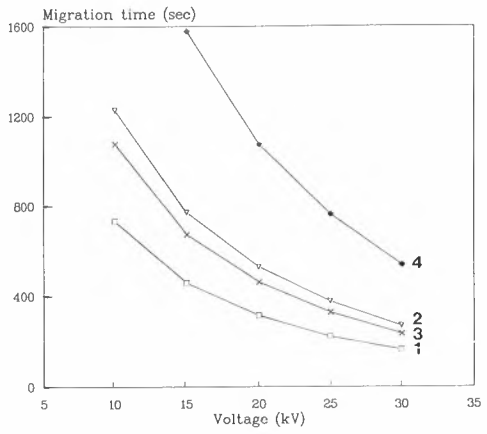

Fig. 3. The influence of applied voltage on migration times of GAG-disaccharides. Numbers refer to Fig. 1

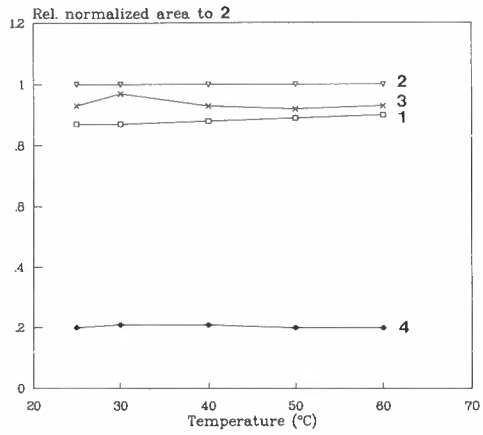

Fig. 4. The influence of temperature on relative normalized peak areas to the GAG-disaccharide **2**. Numbers refer to Fig. 1

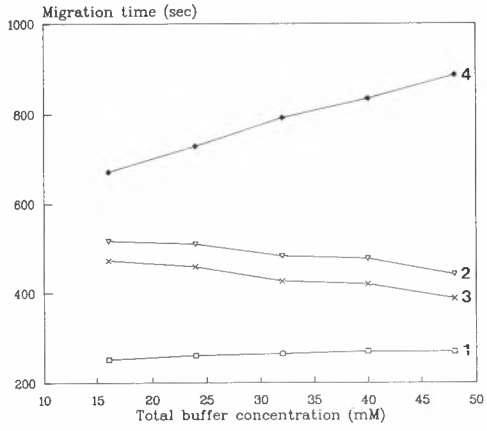

Fig. 5. The influence of electrolyte concentration (borate:phosphate = 3:5) on migration times of GAG-disaccharides. Numbers refer to Fig. 1

that complete separation can be obtained and quantitation can be performed from NA values. RNA values equal the use of internal standards in analyses, which is always recommended in order to obtain more accurate and reproducible results. In order to further evaluate and chose separation conditions for GAG-disaccharides, resolutions between peaks and the number of theoretical plates have to be determined, as revealed from ongoing work.

The ability to analyse for the composition of GAG-disaccharides in proteoglycans, e.g.in skin samples, necessitates an enzymatic cleavage of GAGs into disaccharides. Chondroitin sulphates A, B, and C (1 mg of each) were each treated with 0.30 mg of chondroitinase ABC in 100 μl water added 900 μl 50 mM Tris-HCL (pH = 8.0) buffer. The reaction mixtures were

incubated at 37°C for 18 h and the reactions were stopped by transfering the mixtures to 100 μl Dowex 50w x 8 columns and collecting the unretained solutes. Samples were then filtered through 0.2μm filters, which were washed with 2 x 0.5 ml water. The filtrates were analysed directly by HPCE or evaporated to dryness, redissolved in 100 μl water and analysed by HPCE.

Analyses carried out directly on filtrates gave well-separated peaks (Fig. 6), whereas the redissolved samples had too high concentrations of solutes to give proper HPCE separations. A further 5 or 10 dilutions of redissolved samples resulted in well-separated peaks. After evaporation, redissolving and dilution, however, at least two new peaks appeared between GAG-disaccharides 1 and 3 in all electropherograms. The two peaks were very narrow and not baseline separated. Instability of the GAG-disaccharides during evaporation of the solvent is most likely the reason for these additional peaks and further studies on this are currently being carried out.Identities of peaks shown in Fig. 6 are based on overspiking with reference standards (compounds 1-4) and on data from Nomura *et al.* (1989), Al-Hakim & Linhardt (1991) and Carney & Osborne (1991). The suggested structures are very likely based on this information and on the purity of GAGs according to the manufacturer.

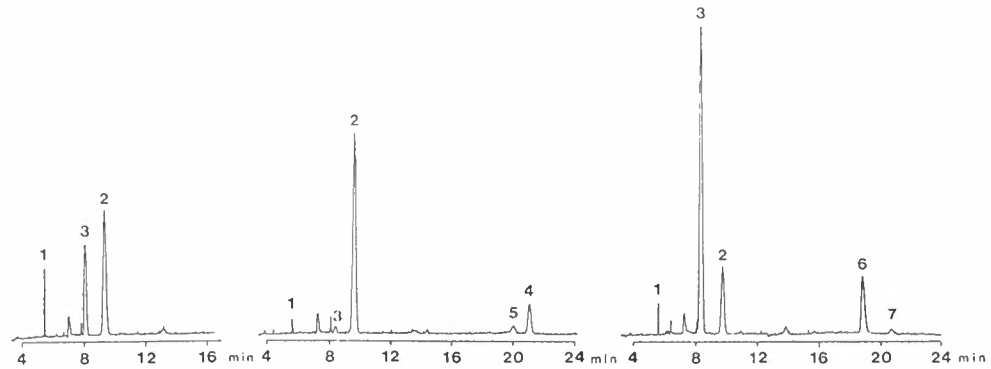

Fig. 6. Electropherograms of disaccharides obtained after chondroitinase ABC treatment of chondroitin sulphate A, B, and C. Numbers 1 - 4 refer to Fig. 1. 5 = ΔDi-diS; 6 = Δ^4-2-O-sulpho-GlcUA→6-O-sulpho-GalNAc (ΔDi-diS$_D$); 7 = Δ^4-GlcUA→4,6-bis-O-sulpho-GalNAc (ΔDi-diS$_E$)

In chondroitin sulphate B,α-L-iduronic acid dominates (Table 1). In disaccharides formed after chondroitinase cleavage of GAGs it is not possible to distinguish between β-D-glucuronic acid and α-L-iduronic acid originally present in GAGs, because of the identical structures of these compounds in disaccharides after enzymatic cleavage. Furthermore,after chondroitinase cleavage β-D-galacturonic acid would have an identical structure to the two above-mentioned glycuronic acids.

Four experiments were performed both with and without protease treatment of skin samples prior to chondroitinase treatment. The results indicated that the ratio between chondroitinase and skin amounts, as well as the protease treatments were of importance. A too low amount present or liberated from GAGs, to be detected properly by HPCE, was the result of the first chondroitinase (0.3 mg) treatment of a skin sample (12.9 mg; Fig. 7A). Increasing the chondroitinase and sample amounts to 3.2 mg and 53.6 mg, respectively, and treating the samples with the proteases pepsin and pancreatin prior to chondroitinase treatment improved the peak sizes (Fig. 7B). However, due to the instability of GAG-disaccharides during the applied evaporation and redissolving of samples (see above) several other peaks appeared. Peaks corresponding to intact disaccharides, when compared to the standards shown in Fig. 7C, are marked with numbers in Fig. 7B. Protease and chondroitinase teatments are now beeing further improvedand the problems with instability solved. Quantitative aspects of the various procedures are also being investigated.

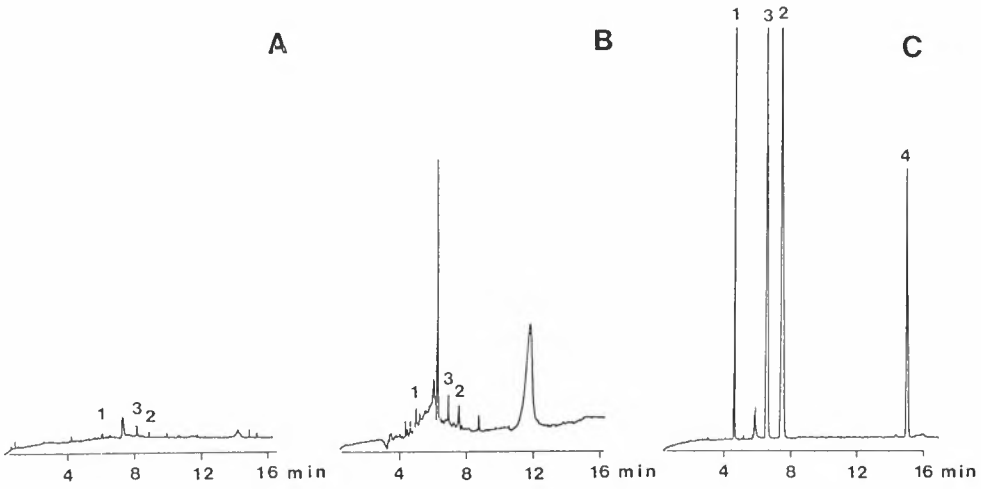

Fig. 7. Electropherograms of samples of mink skin obtained after enzyme treatments and of standard. A: Chondroitinase (0.3 mg) treatment of skin sample (12.9 mg) in 50 mM Tris-HCl (pH 8.0). HPCE conditions as initially applied. B: Pepsin and pancreatin treatment of a skin sample (53.6 mg) followed by chondroitinase treatment (3.2 mg) in 50 mM Tris-HCl (pH 6.8). Temperature at 40 °C, other HPCE conditions as initially applied. C: GAG-disaccharide standard. HPCE conditions as in B. Numbers refer to Fig. 1

All together protease and chondroitinase treatments of mink skin, combined with HPCE analyses, make it possible to determine the disaccharide composition in GAGs from mink skin.

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Skin length and skin quality

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Børsting, E. & N. Therkildsen 1992. Skin length and skin quality. Norwegian Journal of Agricultural Sciences. Suppl. no. 9: 613-619. ISSN 0801-5341.

The breeding objective in mink is to improve pelt price especially through increased pelt length and pelt quality. That is selection on traits scored on live kits. Experiments carried out at Research Farm "South" are in good agreement with other experiments and show that live body weight has a correlation to pelt length of 0.6 - 0.9. The live grading for pelt quality has a correlation of 0.3 -0.5 to grading of the raw pelt for quality. The length of the live kit has a lower correlation to pelt length than the body weight and it does not indicate the negative correlation to live grading for quality as clearly as the body weight does. Live body weight and quality grading of the live animal's pelt are concluded to be the best traits for indirect selection on pelt length and pelt quality.

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Skin length and skin quality are negatively correlated in mink. Large skins have a greater tendency towards a poorer quality than small skins. This is evident in skin data from *Danish Fur Sales* (Børsting & Therkildsen 1992). These authors found a Spearman rank correlation of -0.103*** between size and quality. (Note 1.)

The negative correlation can be biologically (genetic) or technically (pelting) determined or can be a combination of the two. Since both skin length and skin quality have an effect on the skin price, both skin characteristics will normally be included in the breeding programme.

When calculating breeding value for skin length and skin quality one uses indirect measurements. The measurements are undertaken on live animals and are used to express the skin length and quality.

If the negative correlation between skin length and quality is biologically determined, then it should be evident in the live animals, assuming that the indirect measurements are accurate enough.

If the live animal measurements do not show a negative correlation between "quality" and "length", it is either because the measurements are not accurate enough and/or because the negative correlation between skin length and skin quality is a result of pelting and blocking.

Live animal measurement and grading are used because

- they are easy and cheap to undertake
- loss of worthy breeding animals is avoided
- the generation interval becomes shorter

Direct measurement of skin length and quality can only be used in breeding work in connection with a progeny test. This will always result in a long generation interval, a complicated and expensive breeding programme, and loss of worthy breeding animals.

These disadvantages can be avoided if the skin length and quality can be predicted with reasonable confidence by measuring the live animals.

MEASUREMENT OF SKIN LENGTH AND QUALITY

Many investigations have been carried out on the relation between live animal and skin measurements.

Reference no.	7	8	9	1(x)
Measurement				
Weight at pelting - skin length	-	0.85	0.78***	0.83**
Length at pelting - skin length	-	0.66	0.55***	-
Live quality - skin quality	0.33***	0.42	-	0.47***
No. of animals	1315	311	218	461

(x) Weight in September

In 1990 and 1991 a corresponding investigation was undertaken at the Research Farm South with standard male kits.

Measurement	Correlations	
	1990	1991
Weight in Oct. - skin length	0.80***	0.89***
Body length in Oct. - skin length	0.70***	0.81***
Live quality - skin quality	0.49***	0.42***
No. of animals	275	302

The investigations mentioned above show that the correlation between body weight and skin length is 0.6-0.9. The correlation between live quality grading and skin quality is 0.21-0.49. Measurement of body length gives a poorer correlation to skin length than body weight in those studies where both were registered.

Body weight and subjective grading of quality are thus the best measurements one can use on live animals to predict skin length and quality. In most of the investigations, they are

strongly significantly correlated to skin length and quality. Body weight and subjective grading of quality on live animals can thus "see" the pelt length and quality without requiring the animal to be pelted first. Since skin length and quality are negatively correlated, they must also be able to "see" the negative correlation between these two characteristics.

CORRELATION BETWEEN LENGTH AND QUALITY

If measurements of live animals are to be used to judge potential breeding animals, they must not only be reliable expressions of the skin length and quality, but also be able to reveal the negative correlation. If the negative correlation between skin length and quality cannot be revealed, then the distribution of the selection pressure on the two properties will be incorrect, and the selection results cannot be predicted.

Correlations to the live measurements are referred to in several of the cited studies.

Lagerkvist & Lundheim (1990) found the following correlations:

Measurement	Correlations
Weight in Sept. - quality in Aug.	0.03
Weight in Sept. - quality in Nov.	0.06***
No. of animals	3028

O. Lohi (1989) found the following correlations:

Measurement	Correlations
(Skin) Skin length - skin quality	-0.17
(Live) Weight at pelting - live quality	-0.02
Length at pelting - live quality	0.20
No. of animals	313

At the Research Farm "South", the following correlations were found in 1988, 1990 and 1991:

Measurement	Correlations		
	1988	1990	1991
(Skin) Skin length - skin quality	-0.34***	-0.31***	-0.38***
(Live) Weight in Sept. - live quality in Nov.	-0.19***	-	-
(Live) Weight in Oct. - live quality in Nov.	-	-0.27***	-0.20***
(Live) Length in Oct. - live quality in Nov.	-	-0.10	-0.15***
No. of animals	461	275	302

It would seem that it is the animal weight and quality grading which best reflect the real skin length and quality. It is thus these two measurements on the live animals which "behave" in the same way as the skin length and skin quality.

The physical effects on the skins of skinning and blocking could possibly have an influence on the skin quality in a way that is dependent on the size of the animal. One could imagine that long skins are stretched differently from short skins, altering the quality so that the negative correlation between the skin quality and length occurred during pelting.

Of the 275 and 302 male skins studied at the Research Farm "South" in 1990 and 1991, both skinning stretch and blocking stretch were registered.

As mentioned before, correlations between skin length and skin quality of -0.31, -0.38 were found in 1990 and 1991, respectively.

If one corrects the two variables for the influence of the percentage stretch at skinning and blocking, the correlation between length and quality becomes -0.21*** and -0.22*** for the two years.

This can be interpreted to mean that the greatest part of the negative correlation between skin length and quality is biologically determined and not a result of physical effects at pelting.

MEASUREMENTS ON LIVE ANIMALS

If it is correctly assumed that the negative correlation between skin length and quality is biologically determined, then the measurements on the live animals should also demonstrate a negative correlation.

At the Research Farm South, weighing of the kits and subjective quality grading of the live animals have made up a regular part of the research plans since the farm started in 1986.

Aside from the previous results, the following correlations were found by Børsting & Therkildsen (1992) for standard kits (corrected for sex differences):

Year	Measurement	Line	No. of animals	Correlation
1986	Sept. weight-live qual.	-	1489	-0.17***
1987	Sept. weight-live qual.	14	613	-0.27***
		15	544	-0.29***
1988	Sept. weight-live qual.	14	683	-0.26***
		15	591	-0.36***
1989	Sept. weight-live qual.	14	636	-0.25***
		15	598	-0.16***
1990	Nov. weight-live qual.	14	614	-0.36***
		15	598	-0.39***
1991	Nov. weight-live qual.	14	626	-0.36***
		15	493	-0.27***

Each year, the grading of the animals is done by experienced, practical mink breeders, and the results confirm that the negative correlation between size and quality is found in the live animals.

COMBINATION OF SEVERAL MEASUREMENTS

The previously mentioned studies all revealed that the animal's length is a poorer measurement of the skin length than the animal's weight. However, one could imagine that a combination of weight and length could produce an even more precise prediction of the skin length.

This can be investigated by calculating regression equations on the material from the study of the 275 male skins at the Research Farm "South" in 1990 and the 302 skins from 1991.

R2 shows how much of the variation in the dependent variable (y) can be explained by the variation in the independent x (x's).

Equation (constant and b-values excl.)	R2		Sign
	1990	1991	
Skin length = weight in Oct.	0.63	0.79	+
Skin length = length in Oct.	0.48	0.66	+
Skin length = weight in Oct./length in Oct.	0.47	0.71	+
Skin length = weight in Oct. +length in Oct.	0.67	0.80	
Skin quality = live quality in Nov.	0.24	0.18	+
Skin quality = weight in Oct.	0.07	0.13	-
Skin quality = length in Oct.	0.02	0.08	-
Skin quality = weight in Oct./length in Oct.	0.08	0.12	-
Skin quality = weight in Oct.+ length in Oct.	0.08	0.13	

In 1990 and 1991 the weight could account for 63% and 79%, respectively, of the variation in the skin length. A combination of weight and length can increase the confidence in the determination of the skin length to 67% and 80%, respectively, if calculated with the following equation:

$y = a + b1 \times weight + b2 \times length$

and not by calculating the weight/length relation, which only gives an R2 of 47% and 71%, respectively. This modest increase can in no way pay for the costs connected with measuring the length.

The weight in October can account for 7-13% of the variation in the skin quality, whereas the length can only account for 2-8% of the variation in quality. This is a natural consequence of the fact that the animal's length is a poorer measure of the skin length, and therefore cannot demonstrate the negative correlation between skin length and quality as precisely as the animal's weight can.

DISCUSSION

Several studies have shown concurrently that weighing and subjective quality grading of live mink kits result in a significant correlation to skin length and quality. Comparisons of weight and length measurements on live kits also show clearly that the weight is the best measure of skin length. The negative correlation between skin length and quality is for the most part biologically determined. Therefore, the measurements applied to live animals, with the aim of selecting breeding animals, must be such that this correlation can be found. The measurement methods should therefore be aimed towards giving a true picture of the negative correlation and should not "overlook" the true correlation.

This condition is by and large met by weighing and subjectively grading the quality of potential breeding animals.

Note 1.

* means $0.05 > = p > = 0.01$
** means $0.01 > p > = 0.001$
*** means $0.001 > p$

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Effects of dietary zinc, silicium and selenium on mineral content of fur in silver foxes during fur maturity

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The aim of our work was to study the influence of salts of zinc ($ZnSO_4 .7H_2O$), silicium ($C_7H_{14}O_3NSiCL$) and selenium ($C_{19}H_{22}Se$) on the mineral content of fur of silver foxes during the period of fur maturity. The mineral ingredients were added to the feed ration in the form of a saline solution and were administered from birth to the period of fur maturity. Mineral elements from samples of fur were determined by means of dispersion and röntgen fluorescent spectrometry as follows: K, Ca, Mn, Fe, Cu, Zn, Br, Rb, Sr and Pb. Our results revealed that zinc significantly increases the content of K, Ca, Mn, Fe, Cu, Sr; silicium increases K, Ca, Mn, Fe, Cu, Br, Rb, Sr and selenium increases K, Mn, Fe, Cu, Br, Rb, Sr in comparison with the control group. It was also found that the content of Pb was significantly decreased in all the trial groups.

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The value of farm-reared fur-bearing animals depends on their hair quality. Not only organic substances but also mineral elements help to form the fur. Since fur samples can be taken painlessly and need no special storage, fur is a suitable subject for research. Most mineral elements are more highly concentrated in the fur where they are firmly connected with proteinous structures than in the organs, tissues and blood where their content is not stable. This property of fur can be used to advantage in the optimalization of mineral nutrition and in research on the relationship between mineral element concentrations and efficiency traits. The addition of microelements to feed rations significantly improves the quality of the fur (Samkov 1972; Michajlov 1973; Sewell 1974; Madsen 1975; Icypov 1983; Berestov et al. 1984; Mertin et al. 1991a), and has a positive influence on female reproduction and the growth of kits (Oksansen 1981; Icypov 1983; Mertin 1989a,b; Balakirev 1990). Saba et al. (1982), Berestov et al. (1984). Hornshaw et al. (1985), Mertin et al. (1990, 1991b,c,d) carried out investigations on the content of mineral elements in the

fur of fur-bearing animals. Bialkowski & Saba (1985) and Työppönen et al. (1988), investigated the mineral content in fur and blood serum while Blus & Henny (1990) investigated the mineral elements in organs.

MATERIALS AND METHODS

Our aim was to determine the influence of microadditives of zinc, silicium and selenium salts on the mineral composition of the fur of silver foxes during the period of fur maturity.

The experiment took place in the Department of Fur Animal Breeding of the Research Institute of Animal Production in Nitra. Trial foxes were kept under the usual conditions of standard cage technology, i.e. in cages made of zinc-coated netting, located in two rows in sheds. The animals were clinically healthy during the experiment.

At the age of 6-7 weeks the young animals were divided into four groups with four animals in each group (one control group and three trial groups). The influence of zinc was observed in the first trial group. Zinc sulphate ($ZnSO_4.7H_2O$) was added in the amount of 13.0 mg per kg dry matter of feed daily as a saline solution, in order to make the mineral content homogenous in the feed. In the second trial group 6.0 mg silicium salt in the form of 1-chlormethyl-silitranium ($C_7H_{14}NSiCl$) was administered in a similar way. In the third trial group, the animals were given selenium salt 9-phenyl symmetrical-octanhydrose-lenozanthen as a chemical ($C_{19}H_{22}Se$) dissolved in salad oil. This was administered twice weekly with a seven-day interval. The nutritional value of the feed rations is presented in Table 1. Samples of feeding mixture were taken monthly and the concentrations of observed mineral elements were determined. Average values are given in Table 2.

Table 1. Content of digestible nutritive substances in feeding doses (g.418 KJ-1 ME)

Index	Month							
	IV	V	VI	VII	VIII	IX	X	XI
Digestible protein (g)	10.7	10.3	10.3	8.6	8.6	9.1	9.2	9.3
Digestible fat (g)	3.5	3.8	3.8	4.2	4.2	3.7	3.6	3.9
Digestible saccharides (g)	5.8	5.5	5.5	5.4	5.4	6.2	6.5	6.1
ME . place^{-1}, day^{-1} (KJ)	2092	2510	2510	2929	3096	2594	2176	1966

In the period of fur maturity we cut an approximately 2 g sample of fur from the dorsal part of the body. The mineral elements Ca, K, Mn, Fe, Cu, Zn, Br, Rb, Sn and Pb were determined by means of dispersion and röntgenofluorescent spectrometry (Tumanov & Stepanok 1986) in samples of fur and feeding mixture.

The concentration values of the observed elements thus obtained were processed to the basic variance-statistical characteristic ($M \pm SD$), and by means of a subsequent t-test the arithmetical means of the groups were compared and the significance of differences was assessed.

Table 2. Content of investigated mineral elements in feeding doses (per kg dry matter)

Element (mg · kg^{-1})	M ± SD
K (g · kg^{-1})	4.00 ± 0.35
Ca (g · kg^{-1})	2.26 ± 0.06
Fe	0.007 ± 0.001
Mn	49.30 ± 8.00
Cu	4.93 ± 0.50
Zn	43.93 ± 3.08
Br	2.95 ± 0.30
Rb	0.350 ± 0.118
Sr	8.48 ± 0.65
Pb	0.508 ± 0.115

RESULTS

The arithmetical means of the concentrations of observed mineral elements in the fur of silver foxes in the period of fur maturity after the application of zinc, silicium and selenium into feed ration are presented in Table 3.

From the achieved results it was found that the addition of silicium salt had the greatest influence on the concentration of observed mineral elements. Significant differences in content on the level of significance ($p \leq 0.01$) were observed in Fe, Mn, Br, Cu, Sr, K, Ca and on the level of significance ($p \leq 0.01$) for Rb.

The highest concentrations were found in iron, 555.5 ± 69.7 and 93.2 ± 8.1 mg. kg^{-1}, and the lowest content in calcium, 0.107 ± 0.030 and 0.048 ± 0.005%, absolutely expressed, compared with the control group.

The addition of selenium salt to the feed ration had a significant influence on the increase in concentrations of Fe, Mn, Cu, Sr, K ($p \leq 0.01$) and Br, Rb ($p \leq 0.05$). The group given selenium salt had the greatest increase in concentration of iron (634.3 ± 248.2 mg.kg^{-1}) compared with that of the control group (93.2 ± 8.1 mg.kg^{-1}), but the lowest concentrations of calcium (0.067 ± 0.017 and 0.048 ± 0.005%) were also observed.

Table 3. Content of investigated mineral elements in hair of silver foxes (M ± SD)

Element (mg.kg^{-1})	1 Control) group (n=4)	2 Zn (n=4)	3 Si (n=4)	4 Se (n=4)	t-test
K (%)	0.173 ± 0.005	0.340 ±0.069	0.395 ±0.068	0.333 ±0.107	1:2^{++}, 1:3^{++}, 1:4^{++}
Ca (%)	0.048 ± 0.005	0.112 ±0.042	0.107 ±0.030	0.067 ±0.017	1:2^{++}, 1:3^{++}
Mn	43.70 ± 2.58	78.58 ±12.60	87.38 ± 3.59	79.08 ±30.25	1:2^{++}, 1:3^{++}, 1:4^{++}
Fe	93.2 ± 8.1	429.0 ± 79.8	555.5 ± 69.7	634.3 ±248.2	1:2^{++}, 1:3^{++}, 1:4^{++}
Cu	8.20 ± 0.94	21.15 ± 2.80	24.08 ± 7.03	18.88 ± 1.51	1:2^{++}, 1:3^{++}, 1:4^{++}, 2:3^{+}
Zn	172.3 ± 4.5	183.8 ± 3.8	170.0 ± 7.0	182.0 ± 6.0	2:3^{+}
Br	21.28 ± 0.44	26.63 ± 4.62	25.20 ± 1.27	24.65 ± 4.10	1:3^{++}, 1:4^{+}
Rb	2.58 ± 0.18	3.40 ± 0.75	3.30 ± 0.45	3.50 ± 0.58	1:3^{+}, 1:4^{+}
Sr	2.16 ± 0.09	3.58 ± 0.64	4.14 ± 0.57	2.95 ± 0.57	1:2^{++}, 1:3^{++}, 1:4^{++}, 3:4^{+}
Pb	2.51 ± 0.09	0.315 ±0.045	0.326 ±0.021	0.275 ±0.074	1:2^{++}, 1:3^{++}, 1:4^{++}

+ $P \leq 0.05$, ++ $P \leq 0.01$

Similarly, the addition of zinc salt increased the concentration of some mineral elements in the fur of silver foxes compared with that of the control group. On the level of

significance $p \leq 0.01$ the content of Fe, Mn, Cu, Br, K and Ca increased. The highest concentration was found in iron (429.0 ± 79.8 and 93.2 ± 8.1 $mg.kg^{-1}$) and the lowest in calcium (0.112 ± 0.042 and 0.048 ± 0.005%) by analogy with previous trial groups.

The content of lead decreased significantly in all trial groups. This fact was most marked with the application of selenium (0.275 ± 0.074 and 2.51 ± 0.09 $mg.kg^{-1}$).

After an analysis of the results, we found that there were significant differences in the concentration of mineral elements also among the trial groups, namely between the second (Zn) and third (Si) groups in the content of Cu (21.15 ± 2.58 and 24.08 ± 7.03 $mg.kg^{-1}$) and Zn (183.8 ± 3.8 and 170.0 ± 7.0 $mg.kg^{-1}$) and between the third (Si) and fourth (Se) groups in the concentration of Sr (4.14 ± 0.57 and 2.95 ± 0.57 $mg.kg^{-1}$) $p \leq 0.05$.

DISCUSSION

The organic substances and mineral elements which together form the fur of fur-bearing animals are necessary factors in the metabolic reactions for the building and regulation processes which closely influence the manifestation of functions of organisms. Studies dealing with the problems concerning the influence of mineral nutrition in fur animals together with the content of mineral elements in fur are sporadic in the literature. However, these problems are elaborated on in studies on the larger farm animals and on poultry.

Works in which it is mentioned that the changes in the concentration of elements in fur are dependent on their concentration in feed prove that the chemical composition of fur reflects the level of mineral elements in the organism. Application of mineral additives causes an increase in the content of some elements and the absence of additives causes a decrease in the content. This dependence was discovered for manganese, zinc (Taucin & Svilane 1965), iron (Grün et al. 1978), selenium (White & Sommers 1985), potassium and sodium (Brochart 1971). We reached similar results in our work, too. After the application of zinc salt in the feed rations of silver foxes during ontogenesis, the concentrations of Fe, Mn, Cu, Br, K and Ca increased in their fur in the period of fur maturity and the silicium salt increased the concentrations of Fe, Mn, Cu, Sr, K, Br and Rb compared with those of the control group. A significant increase in the concentrations of Fe, Mn, Cu, Sr, K, Br and Rb was also observed with the application of selenium salt. It was found that all the applied microadditives very markedly increased the content of iron, a fact that was confirmed by Hornshaw et al. (1985). They added copper or zinc salt to the feed rations of dark and pastel mink.

These authors state that the content of iron and manganese in the fur of mink increased with the application of these salts. The concentrations of zinc and copper did not rise, however. The results of our analyses indicate that the addition of salts in the feed significantly increases the observed mineral elements or tends to increase their concentration, with the exception of lead. The content of lead in the fur of foxes was significantly decreased in all the trial groups.

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Application of a aluminosilicate-V for degreasing fur coats

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Application of vermiculite improves the appeal of the furs and is ecologically advisable. Furthermore, it should be noted that vermiculite is chemically inert, harmless, sterile, ecologically pure and cheap.

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The aim of our investigations was to develop an effective method of degreasing the fur coats of mink in order to improve their marketability. Taking into consideration the species peculiarities of subcutaneous grease and the adsorption properties of vermiculite, a degreasing mixture for the coats of fur-bearing animals was developed.

MATERIAL AND RESULTS

After heat treatment at 1000°C, aluminosilicate-vermiculite acquires high adsorption properties regarding fat-like substances.

Grease is removed from the flesh side of the fur- and the hair coat in order to soften and loosen the fur and to obtain a fluffiness that enhances its appeal.

To process the fur coats of the mink sawdust and vermiculite were mixed in the proportion 1:10. The adsorption dynamics over a four-hour period is presented in Table 1. It was found that sawdust absorbed grease in 120 min. A clearer picture emerged in the investigations of adsorption on the hair coat (Table 1). Volumetric mass of the control specimens grew only for an hour (104 g). During the subsequent two hours it remained stable.

In experimental specimens the obtained result increased for three hours until the maximum point (123 g) was reached.

It should be noted that although there was 9.0-23.8% less residual degreasing mixture on the experimental fur coats, the time required for combing practically remaining unchanged.

Having studied the adsorption dynamics of the degreasing mixture over a three-hour period during the processing of the hair coats of polar fox furs, we are convinced of the advantages of the degreasing mixture including vermiculite (Table 2). A favourable effect of 15-19% was found with the experimental hair coats. The advantage of the suggested method (25-33%) was borne out by the detection of traces of degreasing mixture in the polar fox furs.

Table 1. Adsorption of grease from the flesh side of the fur- and hair coat at degreasing

Number of measurements	Volume of mixture (cm^3)	Duration of processing of furs (min)	Weight of the applied mixture (g) control	trial
		Flesh side of the fur coat		
1	500	00	99.3	94.3
2	500	30	100.0	99.8
3	500	60	100.6	100.4
4	500	90	101.3	102.8
5	500	120	102.4	104.5
6	500	150	102.4	107.6
7	500	180	102.5	109.1
8	500	210	102.5	109.6
9	500	240	102.6	110.4
		Hair coat		
1	500	00	90.0	90.1
2	500	15	95.1	94.6
3	500	30	96.8	100.3
4	500	45	99.9	105.4
5	500	60	103.6	107.2
6	500	75	103.6	109.4
7	500	90	103.7	110.8
8	500	105	103.7	114.6
9	500	120	103.9	116.4
10	500	135	103.9	118.6
11	500	150	103.9	119.5
12	500	165	104.0	120.0
13	500	180	104.0	122.9

Table 2. Adsorption of grease from the hair coat during degreasing of the fur coat of the polar fox

Number of measurements	Volume of degreasing mixture (cm^3)	Duration of fur coat processing (min)	Weight of degreasing mixture (g) control	trial
1	500	00	90.0	90.1
2	500	15	95.2	96.3
3	500	30	103.5	101.3
4	500	45	103.8	105.3
5	500	60	103.8	108.0
6	500	75	103.0	109.0
7	500	90	103.6	112.6
8	500	105	103.8	114.6
9	500	120	103.8	118.8
10	500	135	103.7	120.0
11	500	150	103.9	121.8
12	500	165	103.8	123.9
13	500	180	103.8	123.9

With manual degreasing, adsorption properties of vermiculite were manifested much more clearly (Table 3). The air-dried substance of the flesh side of the polar fox fur contained 20.5% less grease and that of the hair coat contained 2.3% less grease.

Table 3. Adsorption of grease during manual degreasing of polar fox furs

Number of measurements	Volume of degreasing mixture (cm^3)	Weight of the spent degreasing mixture (g)	
		sawdust	vermiculite
1	500	24.68	40.32
2	500	26.01	38.06
3	500	31.16	42.10
4	500	28.64	36.36
5	500	30.42	41.02
6	500	31.27	42.84
7	500	26.38	37.69
8	500	29.34	40.73
9	500	29.05	42.95
Mean	500	28.55	40.23
trial/control	(%)	100.0	140.9

Thus, the effectivity of the new method of degreasing the coats of fur-bearing animals is dependent on the replacement of the vital stuff-sawdust. Saving the sawdust is proportional to the application of vermiculite (from four to ten times).

The absorption volume of the suggested degreasing mixture for the flesh side of the fur coat was 7-20.5% and for the haircoat 15-19% higher; with the manual method of degreasing it was 2-4% higher.

Surface morphology and innervation of defective guard hairs of American mink - *Lutreola vison* (Schreber, 1774)

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Vaněk, Milan & Jitka Hanzlová 1992. Surface morphology and innervation of defective guard hairs of American mink - *Lutreola vison* (Schreber, 1774). Norwegian Journal of Agricultural Sciences. Suppl. no. 9: 629-636. ISSN 0801-5341.

Hair samples with typical defective bent quard hairs were resected from the dorsal somatic regions of six males and five females of *L. vison*. In electronographs from transmission electron microscopy the shape and structure of cuticular cells in the proximal segment (stem) of the guard hairs were the same as those of clinically healthy animals. Lancet-like segment, uneveness and conspicuous sharpness of apical parts of the cuticular scales were clearly seen. Another distinctive character was unhomogeneity up to destructivity of the cuticular cell structure in comparison with guard hairs that were unchanged pathologically. The material was also treated under the usual transmission electron microscopy. The following types of nerve endings were observed: (1) Bundles of non-myelinized nerve fibres in mesenchyme hair sheaths, the so-called free penicillate nerve endings. Non-myelinized axons are are surrounded by processes of Schwann's cells, (2) Pilo-Ruffini's complexes, i.e. a greater number of axonal endings containing numerous mitochondria, surrounded by a narrow fringe of Schwann's cells and associated peripherally with numerous collagen fibrils.

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Several authors have dealt with the morphological structure of defective hairs (guard hairs) in *Lutreola vison*. The "metallic" defect is the most well-known and frequent abnormality. This defect of mink fur was described in detail by Openshaw & Ellis (1988). From the morphological aspect, the above authors characterize metallic guard hairs as: (a) tip-splitting and brittle; (b) displaying aneurysm or stenosis of their lancet-like part or angle deformation. Furthermore, they describe triplicate breaking of the guard tip in detail. They write that, in the lancet-like segment, metallic defective guard hairs were bent, not straight like the guard hairs from the adjacent undefective somatic regions. Reiten (1973) and Blomstedt & Lohi (1980) describe these guard hairs as being bent in the form of hooklets, contrary to normal ones. A study of the morphological and histological characteristics of metallic defective fur (guard hairs) was carried out by Blomstedt & Lohi (1980) with the following results. In transverse histological section, in the neck part of the lancet-like (subapical) segment, the transverse contour is more circular but, in undefective hairs, it is

elliptical, with a thicker cuticular layer on the convex side. The scale surface of the epidermiculae in the subapical segment is very uneven (rough), and the transverse histological section runs across three scales (cells). while in undefective guard hairs, across four. Cuticular cells in this segment are higher and are characterized by sharp apical apexes.

Wu et al. (1977) examined guard hairs under SEM from normal and defective somatic regions, so-called mink fur "singe". They stated that the cuticle of normal guard hairs consists of overlapping scales of different shape and size. In fur "singe", a marked tip-splitting of the guard hairs was observed. Wu et al. (1977), studying this defect of guard hairs in electronographs from SEM, state that the evaluation of their structure in transversal section is more important when compared with normal guard hairs. Ebbersten (1973), studying metallic and normal mink hairs by means of light microscopy, confirmed these results. In Scandinavia, problems connected with "singe" defect were studied by Udris (1971, 1972), Ebbersten (1973) and Reiten (1973). They found that this anomaly of guard hairs occurs mainly in dark mink types. According to Reiten (1973) , there are no correlations between "singe" and the length of the guard hairs, or the size and weight of the pelt, or the number of young in the litter, or the type of feeding. A more significant effect of this defect in Finblack males than in females is claimed by Lohi (1973) and Udris (1976). Similarly, Udris (1971, 1973a, 1973b), Reiten (1973) and Backus (1982) write that "singe" occurs more often in mink males than in females.

Another abnormality of mink fur is "red hip". According to Wu et al. (1977), the cause of this defect is failure in hair exchange (moult) in the hip region. They found that the gradual disappearance of hairs causes the reddish shade of hairs covering the hips. Stout et al. (1971) argued against the idea that dietary stress brings about "red hip", but claimed that a significantly important role is played here by the mode of hair moult. From the morphological aspect, the "red hip" defect is evaluated in electronographs from SEM by Wu et al. (1977), who write that these pathological guard hairs are often split close to their tip when cuticular cells are absent. The latter is the result of the presence of older hairs, which tend to be more abrasive. It is a fact that this splitting of the distal segment in guard hairs is also typical of the "singe" defect, where its appearance of course, is more striking.

MATERIALS AND METHODS

In our study, samples from 11 adult *L. vison* were used (six males and five females) and the material was collected in the months of November, December and January. All eleven animals were clinical cases with typical defective guard hairs bent in form of hooklets.

To examine the surface structure of the guard hairs under the electron microscope scanner (SEM), we used samples resected from the dorsal somatic regions, of approximately 1 cm^2. To study nerve endings under the transmission electron microscopy (TEM), we resected samples from regions with defective hairs, from the dorsal somatic regions.

Before examination under the electron microscope scanner, we first stripped the selected defective guard hairs of any surface corpuscles. Washing in petroleum ether for 30 min. proved to be ineffective, because fat and dust particles still remained in hollows of the cuticular scales. For this reason, the hairs were cleaned with acetone for 20 min.;

this medium was stirred continuously and surface particles were wiped off, from the base to the tip of the guard hairs using cotton-wool tampons. Nevertheless, despite this treatment, no perfect purification of the cuticular cell surface was achieved.

In order to prevent hair recontamination, all further manipulation was carried out with the aid of pincers. The guard hairs were then mounted with conductive cement on to aluminium handles. In this way, the prepared hair samples were metallized by 24 carat Au, 40-50 mm in diameter, of the apparatus of SCD 030 type, working on the principle of ionic steaming of the Sputering system, which is a product of the firm Balzers Union Switzerland. Samples were photographed under TEM Tesla BS 300.

The material was also prepared following the usual procedure for examination by transmission electron microscopy. Small skin particles were fixed in 3% glutaraldehyde solution in 0.1 M phosphate buffer (ph 7.4) for 2 h. Fixation was completed by 2% OsO_4 in phosphate buffer. After that the material was dehydrated with acetone and alcohol, and then embedded in Durcupan.

Ultrathin sections for clarifying electron microscopy were cut on the ultramicrotome Ultracut E - Reichert Jung. The sections were contrasted with a water solution of uranylacetate and lead citrate, examined and photographed under TEM Tesla BS 500.

RESULTS AND DISCUSSION

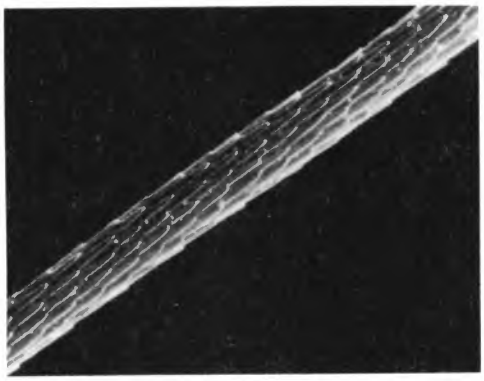

Fig. 1. Arrangement of cuticular scales in the proximal segment of defective guard hair from the dorsal somatic region of *L. vison* ♀. Magnification 100 x

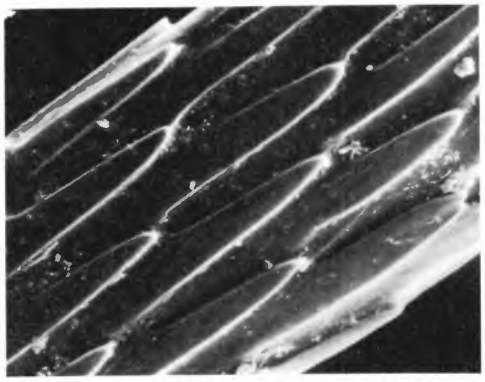

Fig. 2. The same photograph as in Fig. 1, magnification 600 x, showing the characteristic pointed apical part of the cuticular scales

On the basis of evaluation of pathologically changed *L. vison* guard hairs in electronographs from SEM, we can give the following reults. The shape and structure of cuticular cells in the proximal segment (stem) of the guard hairs (Figs. 1 and 2) are the same as those in clinically healthy animals with undefective hairs. This fact can be compared with the results obtained by Debrot et al. (1982) and Sorbanova (1988) in adult American mink, and by Zurková (1989) in *L. vison* kits. In the photographs of the lancet-like segment of defective guard hairs, we found a marked uneveness and distinct sharpness of the apical parts of the

cuticular scales. Another very conspicuous characteristic in this segment was unhomogeneity up to destructivity in the cuticular cell structure as compared with guard hairs that were an changed pathologically (Figs. 3 and 4).

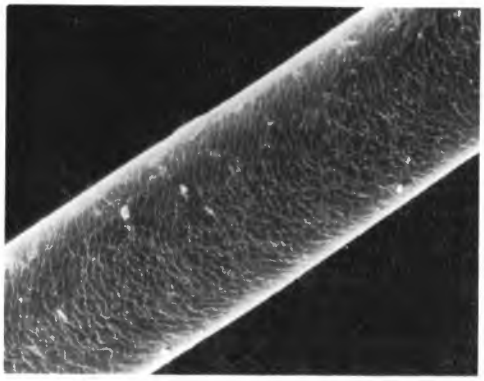

Fig. 3. Arrangement of cuticular scales in lancet-like (distal) segment of defective guard hair from the dorsal somatic region of *L. vison* ♀. Magnification 100 x

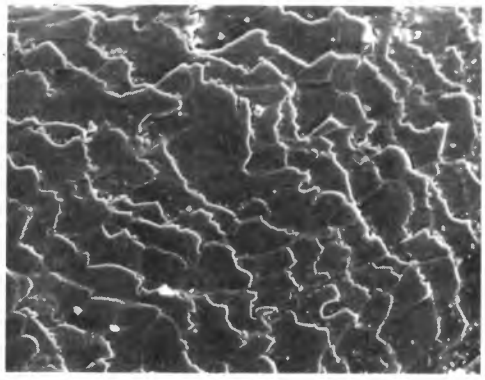

Fig. 4. The same photograph as in Fig. 3, magnification 600 x, showing the shape and structure of the cuticular scales

Examining under TEM skin biopsies which contained defective guard hairs, we found the following types of nerve endings: (a) Bundles of non-myelinized nerve fibres in mesenchyme hair sheaths, the so-called free penicillate nerve endings. Non-myelinized axons are surrounded by processes of Schwann's cells. These endings contain a few axoplasmatic organelles and are surrounded by the zone of collagen fibres situated parallel or vertical to the nerve endings; (b) Pilo-Ruffini's complexes, i.e. a greater number of axonal endings containing numerous mitochondria surrounded by a narrow fringe of cytoplasmatic Schwann's cells and associated peripherally with numerous collagen fibrils. These endings lie in the mesenchyme hair sheaths close to the glass-like membrane; (c) in the mesenchyme hair sheaths, there were, moreover, a relatively large number of non-myelinized fibres (several of them were situated close to the glass-like membrane) as well as nerve stems which contained non-myelinized fibres with myelin. The observed types of nerve endings are shown in Figs. 5-11.

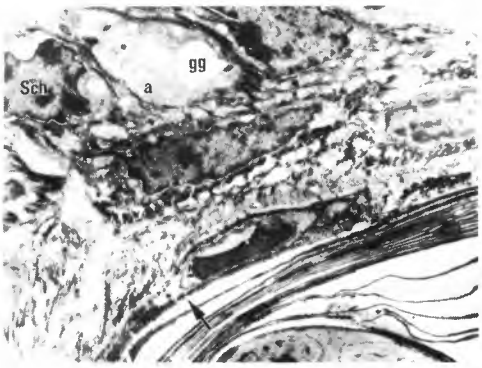

Fig. 5. Bundle of non-myelinized nerve fibres, so-called free penicillate nerve formations (endings) in hair Magnification 5000 x. a - axon, Sch - Schwann's cell,gg - glycogene granule, ---> glass-like membrane

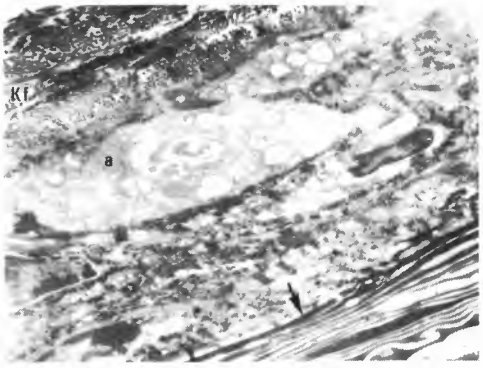

Fig. 6. Sensitive nerve formations from mesenchyme hair sheaths, close to glass-like membrane. Endings contain a few axoplasmatic organelles and are surrounded by the zone of collagen fibrils. Magnification 5000 x. a - axon, kf - collagen fibrils, ---> glass-like membrane

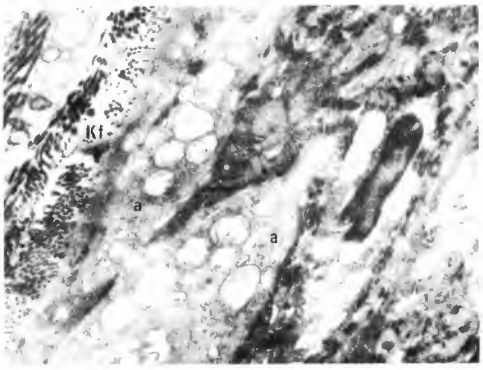

Fig. 7. Detailed photograph (identical with Fig. 6), axons and collagen fibrils are clear in transversal and longitudinal sections. Magnification 9000 x. a - axon , kf - collagen fibrils

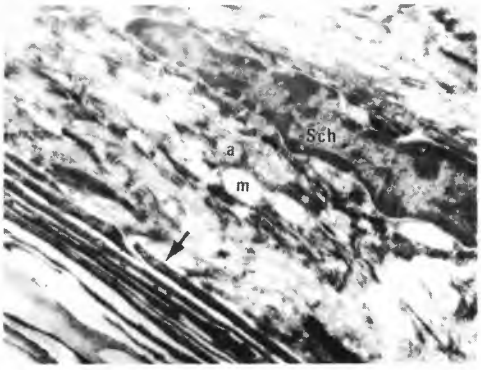

Fig. 8. Non-myelinized nerve fibre in mesenchyme hair sheath, close to the nucleus of Schwann's cell, with mitochondria in advanced stage of vacuolization. Magnification 9000 x. a - axon, Sch - Schwann's cell, m-mitochondria, ---> glass-like membrane

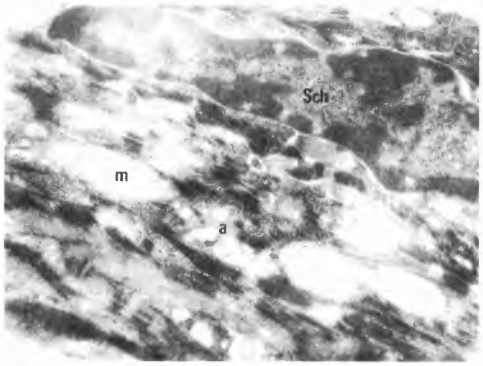

Fig. 9. Detailed photograph (identical with Fig. 8), vacuolization of mitochondria and basal nucleus of Schwann's cell are clear. Magnification 12000 x. a - axon, m - mitochondria, Sch - Schwann's cell

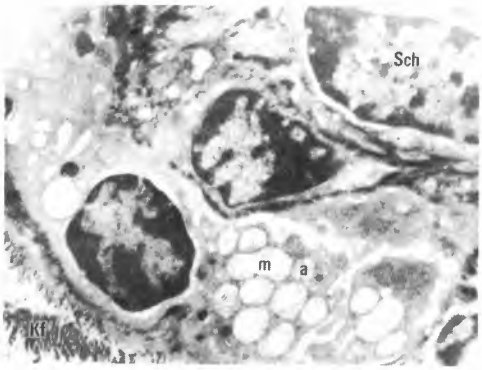

Fig. 10. Sensitive nerve formations with greater accumulation of mitochondria and distinct Schwann's cells, surrounded by collagen fibrils. Magnification 10000 x.a - axon, m - mitochondria, Sch - Schwann's cell, kf - collagen fibrils

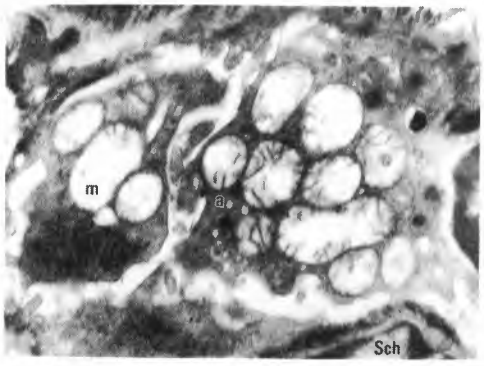

Fig. 11. Magnification 16000 x (identical photograph as in Fig. 10), partial vacuolization of mitochondria can be seen. a - axon, m - mitochondria, Sch - Schwann's cell

No sexual or age-conditioned differences were found in our results obtained by means of SEM and TEM examinations of the study material.

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Articles will usually be organized as follows: (1) introduction, (2) materials and methods, (3) results, (4) discussion and (5) summary. Up to three grades of headings can be used to divide up the text. Articles must not exceed 20 manuscript pages, and two copies should be submitted to the managing editor.

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All illustrations, line drawings as well as photographs, shall be considered as figures. Figures shall be numbered consecutively with Arabic numerals. Letters, numerals and symbols must stand out clearly and be in relation to each other. Make sure they are large enough to take reduction. Before preparing line drawings the author should decide wether they are to be of 1 column, 1½ columns, or 2 columns with so that lettering, etc., after reduction, will be the same size on all drawings. Photographs should be submitted as near to their printed size as possible. If enlargement or reduction is significant in an photograph, the scale should be given on the back and not in the legend. The legend should make the general meaning comprehensible without reference to the text. Figure legends, shall be typewritten together on one or more sheets of paper.

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- Only the first named author's initials and surname are inverted.
- The ampersand symbol is used between author's names.
- The date after the author's name is the year of publication.
- The issue number is given in parentheses after the volume number and only in cases where each issue of a particular volume begins on p. 1.
- A colon is used before page numbers in journal articles.
- The year of publication suffices in cases where the volume number is missing.
- In references to a book, the name of the publisher and the place of publication are given after the title of the book. If more than one edition of a book has been published, the edition used must be indicated. The number of pages should be given.
- It is recommended not to abbreviate the titles of periodicals, but in cases where this has nevertheless been done, abbreviations should be in accordance with the World List of Scientific Periodicals and/or BUCOP British Union Catalogue of Periodicals.

ABBREVIATIONS

Use standard abbreviations where available, otherwise abbreviations given in the text should be explained at their first mention. Quantities and units of measurement shall be in accordance with «Systéme International d'Unites» (SI).

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