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The drawing on the cover is from Kjell Aukrust's *«Guttene på broen»*.

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# MAGNESIUM, GLUCOSE AND CHOLESTEROL IN SERUM OF PREGNANT EWES FED SILAGE AND BARN DRIED HAY

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Astrup, H.N. & Nedkvitne, J.J. 1987. Magnesium, Glucose and Cholesterol in serum of pregnant ewes fed silage and barn dried hay. Norwegian Journal of Agricultural Sciences 1: 75 – 80. ISSN 0801-5341.

Magnesium deficiency in ewes occured at the end of winter feeding. Serum magnesium increased with rise in serum glucose and decreased with the serum cholesterol when the ewe became deficient.

Key words:

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Magnesium deficiency can be expected when blood serum magnesium values fall below 2 mg %, and since availability and supply of minerals through the basic components of the feed ration are often insufficient, calves, cows and sheep may need magnesium supplements if deficiency and possible death are to be avoided.

Glucose level of serum is regulated by the liver. However, small variations occurring in the serum level seem to reflect that glucose and energy are available through feeding.

Cholesterol, too, is subjected to control in the body, the total level being affected by the level of fat in the feed.

It was observed that in pregnant ewes serum values of these parameters were affected. In one of the three experiments where average magnesium levels were lower than 2.0 mg %, the parameters correlated significantly.

*Preceding results*

General experience from comparisons of hay and silage had already been summarized when the present investigation was being carried out. Appetite feeding tended to give better uptake of hay than silage on a dry matter base. This, however, was influenced to a great extent by the feed quality of the two roughages, and by the stage of harvesting of the grass. Silage feeding generally reduced body surface temperature, and increased calculated moisture intake and the void urine volumes. Silage-fed ewes had more of a tendency to produce twins (Nedkvitne, 1969, Nygaard, Nedkvitne 1977). In the year preceding these experiments, magnesium serum values dropped during pregnancy both in hay and silage-fed ewes. Serum cholesterol became highest in silage fed-animals.

*Feeding of hay or silage in the present investigation*

Native bred Dala sheep were used in an experiment in 1966 – 67 on the Agricultural University farm (NLH) at Aas. In the two experiments 1966 – 67 and 1967 – 68, conducted at Edøy experimental farm in western Norway, the an-

imals were pure bred Cheviot sheep. The investigations were part of a programme comparing hay and silage as winter feed for ewes. The animals were kept indoors from 2 to 3 weeks before mating, towards the end of November, up to after lambing in the first half of April. Up until the end of February the ewes were group fed and offered feed to take up the same quantity of dry matter from hay and silage. From 6 weeks before lambing the animals then received roughage according to appetite, and additionally 0.2 kg concentrate mixture. At this time the animals were shorn. The ewes at Aas also had their wool shorn before mating. Silage and barn dried hay cut and prepared at comparable stages of grass growth gave the two roughages compared in the present study.

*Serum methods*

Blood samples taken from the vena jugularis at 0900 h were left for 2 h to coagulate in the cold room, and then centrifuged for 20 min at 1500 in 50 ml swing-out cups of the laboratory Christ centrifuge. To avoid contamination with red cells serum was carefully withdrawn using a pipette. Calcium and magnesium levels were determined by emission and atomic absorption spectro-photometry, respectively, using a Zeiss instrument. Inorganic phosphorus assayed in a colorimetric procedure after reduction of the phosphomolybdate ion was taken from Urbach by Zeiss-Opton (1951). The cholesterol was determined by Liebermann-Burchard's principle. Alkaline and acid phosphatase methods were as described in Sigma procedures (1963). Free fatty acids (FFA) were extracted by means of hydrochloric acid isopropanol and heptane, according to Dole, and then titrated with a micrometer syringe and N/100 alcoholic NaOH under nitrogen, with Nile blue as indicator. Glucose was assayed by the ortho toluidine colorimetric procedure on serum previously deproteinized by trichloro acetic acid. Polyenoic acids (PFA) were determined enzymatically by the procedure of McGee (1959).

*Weighing the animals*

In both Edøy experiments, growth and gain figures refer to differences in the weighings of Nov. 8 and April 8. At NLH, weight gain is cal-

Table 1. Results of experiments at Edøy.

| | 1966 – 67 | | 1967 – 68 | |
|---|---|---|---|---|
| | Silage Mean-SE | Hay Mean-SE | Silage Mean-SE | Hay Mean-SE |
| No. of ewes | 16 | 16 | 16 | 16 |
| Fat intake, g | 56 | 31 | 93 | 32 |
| Weights, kg | | | | |
| before mating | 68.1 – 1 | 69.1 – 1 | 68.0 – 2 | 67.4 – 2 |
| before lambing | 73.1 – 1 | 70.9 – 1 | 71.9 – 2 | 70.0 – 2 |
| Gain, kg | 4.3 – 0.6 | 1.8 – 0.6 | 3.5 – 0.7 | 2.6 – 0.6 |
| Wool yield, kg | 3.7 – 0.1 | 3.9 – 0.2 | 3.9 – 0.1 | 3.9 – 0.2 |
| Serum values | | | | |
| FFA, mEqv/kg | 2.0 – 0.2 | 1.9 – 0.2 | – | – |
| Glucose, mg % | 36 – 3 | 25 – 2 | 58 – 7 | 53 – 3 |
| Cholesterol, mg % | 115 – 6 | 93 – 4 | 80 – 3 | 62 – 1 |
| Mg, mg % | 2.04 – 0.05 | 1.97 – 0.05 | 211 – 0.0 | 2.01 – 0.05 |
| P, mg % | 5.1 – 0.2 | 5.4 – 0.4 | 5.7 – 0.2 | 4.6 – 0.3 |
| Ca, mg % | 9.5 – 0.2 | 9.2 – 0.1 | 10.0 – 0.1 | 9.6 – 0.2 |
| Alk. P.ase, units | 3.4 – 0.4 | 3.9 – 0.5 | 1.9 – 0.3 | 2.0 – 0.2 |

culated from regression lines derived from all weekly weight records through the same period of time.

## RESULTS AND DISCUSSION

The results of the Edøy and NLH trials are given in Tables 1 and 2, respectively. Fat intake of the Edøy animals was higher with silage than with hay. The accompanying rise in serum cholesterol in silage feeding was consistent throughout all experiments. Since the ewes at each places behaved differently, this could not have been caused by the intake of fat only. The serum magnesium level was little affected by the feeding at Edøy. There was rather a tendency for magnesium and other parameters to go in opposite directions in the two experiments. Result from both places were different in degree of magnesium deficiency.

The serum magnesium values in the NLH experiment were lowest, and silage feeding produced the more serious fall in magnesium (Fig. 1). One of the animals from this group died in tetanie at the end of the experiment, with its last serum value recorded at 0.8 mg %. In results from two average March records at NLH (Table 2), magnesium, calcium, phosphorus and alkaline phosphatase values were lower in the silage-fed animals than in the hay fed group. Judging by the results of the Edøy and NLH experiments, conditions must have been different. This is reflected by the observations on weight gain, the serum free fatty acids and the serum glucose values. At Edøy (Table 1) weight gain and serum glucose values indicated a better feed energy balance in favour of silage. At NLH hay produced the better gain. Forage intake in the appetite feeding period before lambing was followed closely in the NLH experiment and confirms the differences indicated by weight gain (Table 3). Better feed intake is followed by, and indicates, an improved mineral balance. This would be in accord with previous observations (Breirem et al. 1949, Hvidsten, Langebrekke 1972, Astrup et al. 1987). This again suggests a fundamental thesis in nutrition that mineral requirement is proportional not to mineral concentrations in the feed, but rather to the total

Table 2. Results of the experiment at NLH 1966 – 67.

| | Silage Mean – SE | Hay Mean – SE |
|---|---|---|
| No. of ewes | 16 | 16 |
| Weights, kg | | |
| before mating, Nov. 8 | 75.9 – 2 | 74.0 – 2 |
| before lambing, April 8 | 80.5 – 2 | 81.0 – 2 |
| Gain, kg | 4.6 – 1 | 7.0 – 1 |
| Twin birth | 15 | 10 |
| Wool yield, kg | 1.42 – 0.03 | 1.59 – 0.05 |
| Serum values | | |
| FFA mEqv./kg | 1.6 – 0.10 | 1.3 – 0.05 |
| Glucose, mg % | 57 – 1.4 | 65 – 1.5 |
| Cholesterol, mg % | 92 – 1.9 | 78 – 2.1 |
| Mg, mg % | 1.61 – 0.05 | 1.95 – 0.03 |
| P, mg % | 4.91 – 0.2 | 5.18 – 0.2 |
| Ca, mg % | 10.4 – 0.3 | 10.9 – 0.3 |
| Alk. P.ase, units | 3.4 – 0.2 | 3.9 – 0.3 |
| Acid. P.ase, units | 4.5 – 0.2 | 4.3 – 0.1 |

amount of minerals taken with the feed. An increase in feed level of nutrients does not seem to require more minerals, on the contrary, high feed levels will meet and satisfy the need for minerals.

This is also in accordance with a recent report on the feeding of silage to cows. Increasing the amount of silage taken improved mineral serum level in the deficient cow (Astrup, 1985).

Correlation of magnesium with cholesterol and glucose in serum was made throughout the whole pregnancy period (Table 4). A consistent high degree of correlation is seen, a negative coefficient with cholesterol and a positive correlation with glucose. The relations are significant and close when magnesium level is below 2 mg %. The all-versus-all correlation of serum values is compiled in Table 5 from records of March 15 at NLH.

The results confirm the role of cholesterol and magnesium. When the partial correlation coefficient of cholesterol is calculated it is increased from -0.39 to -0.56, with glucose kept constant. Indications show that fat intake, after all, interferes with serum magnesium level, behold it is low. Magnesium-fat interaction mechanisms in general have been discussed recently (Astrup, 1987).

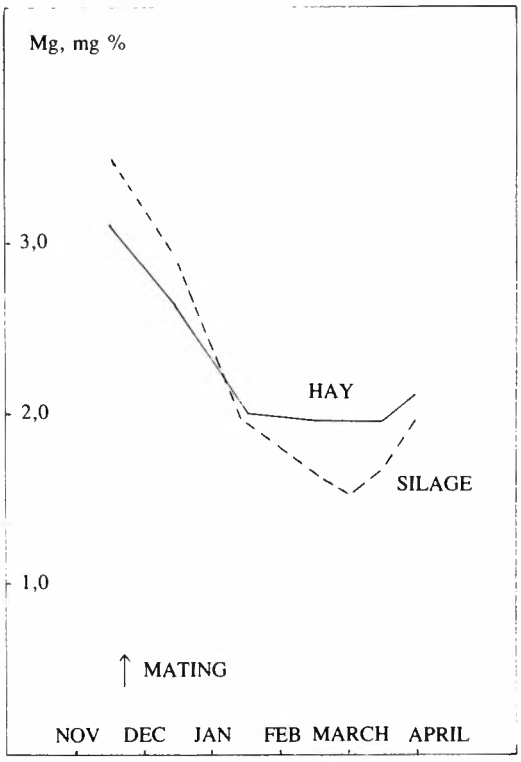


*Fig. 1. Serum magnesium levels throughout the pregnancy of sheep (winter 1966 – 67) at NLH.*

Table 3. Daily forage DM intake of ewes from 6 weeks before lambing in NLH experiment.

| Week before lambing | 5 | 4 | 3 | 2 | 1 |
|---|---|---|---|---|---|
| Silage, kg | 1.3 | 1.3 | 1.2 | 1.2 | 1.3 |
| Hay, kg | 2.1 | 2.0 | 2.0 | 2.0 | 2.0 |

Table 4. Coefficients of correlation of magnesium with glucose and cholesterol of ewes' serum during winter 1966 – 67 at NLH.

| Sample dato | Glucose | Cholesterol |
|---|---|---|
| Dec. 12 | 0.15 | 0.18 |
| Jan. 16 | 0.25 | -0.27 |
| Feb. 14 | 0.48** | -0.31 |
| March 1 | 0.37* | -0.33 |
| March 15 | 0.36* | -0.39* |
| March 29 | 0.30 | -0.34 |
| April 14 | 0.39* | 0.11 |

Indications given by glucose level seem to show that sufficient or deficient mineral intake is dependent almost entirely on the basic nutrients.

## SUMMARY

1. Three comparisons were made between silage and barn dried hay during the winters 1966 – 67 and 1967 – 68.

2. Each group of animals was fed roughage to give equal dry matter intake from mating to 6 weeks before departure. Appetite feeding of roughage was carried out the last 6 weeks of pregnancy.

3. Weight gains were better with hay feeding in one experiment. Serum glucose and free fatty acids reflected group differences. Serum magnesium was reduced during pregnancy and even further reduced with silage feeding in this experiment. Serum cholesterol values increased with silage feeding in all experiments.

4. Correlation study of serum glucose, magnesium and cholesterol in the final stage of pregnancy and at low magnesium level gave positive significant coefficients of correlation of magnesium with glucose and negative with cholesterol.

5. Since glucose reflects higher feed intakes, the feed level likely regulated the supply of magnesium and serum magnesium values. Since cholesterol reflects higher fat intakes, the fat intake in silage may have reduced the supply of magnesium and serum magnesium values.

## ACKNOWLEDGEMENT

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Table 5. Correlation coefficients of serum values of ewes (March 15), taken at the end of pregnancy winter 1966 – 67, at NLH.

| | Glucose | PFA | Alk.P | Ac.P | Cholesterol | FFA | P | Ca | Mg |
|---|---|---|---|---|---|---|---|---|---|
| Glucose | 1 | | | | | | | | |
| PFA | 0.20 | 1 | | | | | | | |
| Alk P.ase | 0.23 | -0.03 | 1 | | | | | | |
| Acid P.ase | -0.10 | -0.11 | -0.39* | 1 | | | | | |
| Cholesterol | -0.36* | 0.45** | -0.25 | 0.23 | 1 | | | | |
| FFA | -0.13 | 0.04 | -0.19 | 0.50** | 0.19 | 1 | | | |
| P | 0.24 | -0.19 | -0.14 | 0.02 | -0.21 | -0.28 | 1 | | |
| Ca | 0.32 | -0.36* | 0.13 | -0.12 | -0.27 | -0.02 | 0.12 | | |
| Mg | 0.36* | -0.21 | 0.39* | -0.28 | -0.39** | -0.25 | 0.24 | 0.47** | 1 |

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# FEED CONVERSION, PORK FAT SOFTENING AND LIVER MALONDIALDEHYDE REACTIVITY IN PIGS SUPPLEMENTED WITH COPPER

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Bacon pigs improved feed conversion at copper levels of 63, 125 and 250 ppm. At higher levels pork softening became evident. Malonedialdehyde reactivity was reduced in the liver.

Key words:

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Pigs fed on purefied diets readily demonstrate a copper deficiency but on practical feeds this is hardly ever encountered. The copper requirement at which deficiency signs are not seen is less than 4 ppm (ARC 1967). Toxification is unlikely, as the pig is almost indifferent to additions below 400 ppm in the dry feed. Supplements in the range 60 – 400 ppm, however, soften the pork fat, and improve feed conversion (Braude, Ryder 1973). The variations in these effects may be caused by copper availability changes or differing antagonisms in the pig.

Copper is a feared oxidation catalyst in oil refineries and steps in the production of milk, but does it also increase oxidation in the body?

Stimulation of growth and improvement in feed utilization were demonstrated in the present investigation. A series of 8 experiments with 283 pigs has been reported in Norway (Matre 1971) and the authors felt that a review would emphasize the quality aspects.

## EXPERIMENTALS

Eight experiments were conducted during the years 1962 – 69, 3 at the Agricultural University Farm, while 5 were carried out at Staur experimental farm, 100 km from Ås.

In the experiments, 125 and 250 ppm supplemented in separate groups, and treatment was repeated in 5 of the 8 experiments. Only 3 groups (13 pigs) were offered a 63 ppm supplement. Since subgroup treatment did not bias the results, the main group values are averaged (and reported here). Subgroup feeding involved exchanging the 90 % barley diet with one also containing 25 % durra and 5 % oats. Further factors were the addition of 12 ppm Zinkbacitracin, and the way of giving the copper supplement in one of the experiments (Matre 1971). Copper was given as $CuSO_4 . 5H_2O$ with the dry feed. A 2 % mineral mixture provided an additional 10 ppm Cu to all groups.

Health problems are integrated in the extension farm experiments. Animals are bought on

the market from various sources. The University Farm stock was more homogeneous and the facilities allowed individual feeding. To compensate, the number of animals in the groups at Staur experimental farm was increased, usually from 4 to 8 animals; 16 in the final experiment. Animal weights were recorded weekly.

## EXPERIMENTAL DESIGN

*Exp. 1, NLH, 1962*

| | | |
|---|---|---|
| Group | I | Control (protein premix + barley) |
| | II | 0.5 % copper sulphate in premix |
| | III | Control (Commercial mixes 1 and 2) |
| | IV | 0.1 % copper sulphate in both |

*Exp. 2, NLH, 1964*

| | | |
|---|---|---|
| Group | I | Control (25 % durra, 5 % oat) |
| | II | 0.025 % copper sulphate |
| | III | 0.05 % copper sulphate |
| | IV | 0.1 % copper sulphate |
| | V | Control (90 % barley) |
| | VI | 0.025 copper sulphate |
| | VII | 0.05 % copper sulphate |
| | VIII | 0.1 % copper sulphate |

*Exp. 3 – 6, Staur, 1964 – 5*

| | | |
|---|---|---|
| Group | I | Control (commercial mixes 1 and 2) |
| | II | 0.05 % copper sulphate |
| | III | 0.1 % copper sulphate |
| | IV | Control (as above, with 12 mg Zn bacitracin) |
| | V | 0.05 % copper sulphate |
| | VI | 0.1 % copper sulphate |

*Exp. 8, Staur, 1969*

| | | |
|---|---|---|
| Group | I | Control (commercial mixes 1 and 2) |
| | II | 0.1 % copper sulphate |

Commercial mixes 1 and 2 (all containing durra and oats) were purchased from A/S Felleskjøpet. The animals from Staur and Aas were slaughtered in Hamar and in Oslo, respectively.

## EXPERIMENTAL METHODS

The carcasses were judged for fat firmness, measured for fat thickness, and samples of back fat were taken the day after slaughter. Radial sectors were cut from the livers in order to obtain representative samples for copper analysis. The Cu content increased 30 % towards the edges of the organ.

Liver Cu was determined on ashed samples of homogenized liver using Zeiss atomic absorption equipment. Iodine value procedure was that of Wijs, reported by Hawk et al. 1949. Melting point was recorded when fat started sliding in the capillary tube in the water bath. The AOM (active oxygen method) was carried out, where air is bubbled through 20 ml samples of fat at 98 °C until rancidity is noticable, and confirmed by peroxide determination (peroxide value = 20) (AOCS). Peroxide value was analysed using the method of Lea (1938). The liver malondialdehyde assay with thiobarbituric acid reagent was carried out in accordance with a procedure undertaken by Bieri (1959).

## MALONDIALDEHYDE (MDA) IN LIVER

A 5 % homogenate of liver is prepared in ice-cold phosphate buffer (homogenized approx. 5 min using 0.5 g liver); 5 ml of homogenate is transferred to a 50 ml erlenmeyer flask and incubated by shaking for 1 h in an air temperature of 37 °C; 1 ml of the incubated medium is added to 1.5 ml of 10 % trichloroacetic acid in a centrifuge tube to precipitate the proteins. Mixing and centrifuging is carried out at 1500 revs for 10 mins and 2 ml of the clear supernatant is added to 2 ml 0.67 % thiobarbituric acid in a 5 ml volumetric flask and placed in a boiling water bath for 10 min. The mixture is cooled and the volume topped up to 5 ml with deionized

water. It is read at 535 nm and the results are recorded as optical density (OD).

*Notes:* Retain cold conditions during homogenization. Shaking traversely for approx. 120/min is satisfactory. Prepare TBA freshly during incubation stages.

*Reagents:* Thiobarbituric acid (TBA): 0.168 g TBA in 25 ml vol. flask. Add 20 ml deionized water and beat in water bath. Allow to cool and make up to the mark. Buffer pH 7.4. Prepare soln. A, 0.2 M $KH_2PO4$ (6.80 g/250 ml aq.) and soln. B, 0.2 M $KH_2PO4$ (8.71 g/250 ml aq). For 0.1 M buffer mix 19 ml A + 81 ml B and dil. to 200 ml with deionized water.

## RESULTS AND DISCUSSION

Average results of group treatments of increasing levels of Cu are given in Table 1 for the parameters followed in the experiments. The improvement in growth is evident. The corresponding corrected feed conversion values show an improvement of 5 % above the control by the copper supplements; improvement appears at all three levels of copper in the feed. The liver copper content was increased with level of the supplement. Fat deposition was not affected, as seen from fat thickness values. The points for fat firmness, iodine value and fat melting changed with the addition of copper and the parameters indicated that a softening of the pork fat did occur. The oxidative stability of the pork was low, but the copper feeding did not seem to reduce stability. The oxidative sta-

Table 1. Animal group average records in experiments with copper supplements.

| Copper in feed, ppm | 0<br>Mean-SE | 63<br>Mean-SE | 125<br>Mean-SE | 250<br>Mean-SE |
|---|---|---|---|---|
| No. of groups | 14 | 3 | 12 | 13 |
| No. of pigs | 99 | 13 | 75 | 96 |
| Days in trial | 106-2 | 107-4 | 104-1 | 104-2 |
| Initialw., kg | 23.0-0.4 | 23.3-1.6 | 23.2-0.6 | 23.0-0.5 |
| Final w., kg | 89.2-0.8 | 90.2-0.3 | 90.0-0.5 | 90.0-0.4 |
| Final w, kg, corr | 90.1-1.0 | 92.2-0.7 | 91.6-0.5 | 90.8-0.7 |
| Carcass w., kg | 65.8-0.7 | 67.3-0.5 | 66.8-0.4 | 66.4-0.5 |
| Carcass percent | 73.8-0.4 | 74.6-0.7 | 74.3-0.2 | 73.8-0.4 |
| Growth, g/day, 55 kg | 541-9 | 546-2 | 569-11 | 578-8 |
| Growth, g/day | 622-11 | 623-10 | 645-11 | 643-8 |
| Growth, g/day, corr. | 630-10 | 637-1 | 660-10 | 652-9 |
| Feed conv. kg/kg | 3.24-0.4 | 3.09-0.3 | 3.12–0.3 | 3.12-0.3 |
| Feed conv. kg/kg, corr | 3.20-0.4 | 3.01-0.2 | 3.04-0.3 | 3.06-0.2 |
| Liver Cu, ppm | 44-6 | 34-4 | 64-10 | 195-19 |
| Fat thickness, mm | 29.2-0.6 | 30.1-1.4 | 30.0-0.5 | 28.7-0.7 |
| Fat firmness, pts | 11.6-0.1 | 11.7-0.1 | 11.5-0.1 | 11.2-0.1 |
| Iodine value | 61.0-0.8b | 59.5-0.4 | 61.6-1.0a | 63.0-0.7a |
| Melting point, C | 39.2-1.1b | 39.4-0.6 | 36.7-1.4a | 35.4-0.6a |
| AOM, hrs | 2.7-0.4c | 3.4-1.1 | 2.9-0.6b | 2.5-0.3b |
| Liver MDA, 100xOD | 23.1-2.4b | 16.0.2-7 | 20.7-2.6b | 12.8-1.9b |

No. of groups, a = 7, b = 8, c = 11

Table 2. Intercorrelation of feed conversion fat softening and liver malondialdehyde (MDA) reactivity in pigs supplemented with copper.

| | Feed conv. | Liver Cu | Fat thickn. | Fat firmn. | Iodine value | Melting point | Liver MDA | Fat AOM |
|---|---|---|---|---|---|---|---|---|
| Feed conv. | 1 | | | | | | | |
| Liver Cu | -0.19 | 1 | | | | | | |
| Fat thickn. | 0.19 | -0.04 | 1 | | | | | |
| Fat firmn. | 0.22 | -0.49 ** | 0.31 * | 1 | | | | |
| Iodine value | -0.05 | 0.31 | -0.66 ** | -0.78 *** | 1 | | | |
| Melting point | 0.30 | -0.44 * | 0.66 ** | -0.82 *** | -0.86 *** | 1 | | |
| Liver MDA | -0.21 | -0.43 * | 0.02 | -0.05 | 0.28 | -0.29 | 1 | |
| Fat AOM | 0.29 | 0.01 | 0.60 ** | -0.50 * | -0.77 ** | 0.77 ** | -0.15 | 1 |

*, **, *** significance at 5, 1 and 0.1 levels respectively of percent probability.

Table 3. Subgroup feeding of whole barley compared with a mixed cereal diet containing durra and oats, in expt. 2.

| | 0 | 63 | 125 | 250 | mixed cereals | barley |
|---|---|---|---|---|---|---|
| No. pigs | 4 | 4 | 4 | 4 | 16 | 16 |
| Feed conv., kg/kg | 3.14 | 3.06 | 3.02 | 3.02 | 3.06 | 3.06 |
| Liver Cu, ppm | 37 | 37 | 67 | 246 | 96 | 99 |
| Fat thickn. mm | 3.14 | 3.13 | 3.07 | 3.08 | 30.0 | 31.6 |
| Fat firmn. pts | 11.9 | 11.5 | 11.5 | 11.3 | 11.5 | 11.7 |
| Iodine value | 58.7 | 59.5 | 59.6 | 61.0 | 60.4 | 59.3 |
| Melting point, C | 42.0 | 39.4 | 39.4 | 37.4 | 38.7 | 40.4 |
| AOM, hrs | 4.3 | 4.5 | 4.1 | 3.6 | 3.6 | 4.6 |
| Liver MDA, ODx100 | 22.3 | 18.7 | 14.1 | 6.0 | 16.5 | 14.0 |

bility of the liver was improved by the copper supplement, and most at the highest copper level. This evident from the assay of malondialdehyde (MDA) activity.

The intercorrelation results of copper feeding are given in Table 2. Improvement in feed conversion is apparently not closely related with copper deposition, nor with the softening characteristics of the fat. From Table 1 it can be seen that improved feed conversion is not dose dependent, and the change is noticeable even with the 63 ppm supplement. This indicates that the high levels are not necessary for growth stimulation. Such a mechanism can likely be explained by the presence of copper in the gut, rather than in the body. The close connection of fat thickness, iodine value, fat firmness melting point and oxidative stability is expected irrespective of the treatment. But liver Cu also correlates fat firmness, thickness with melting point and iodine value of back fat here, indicating a true relationship.

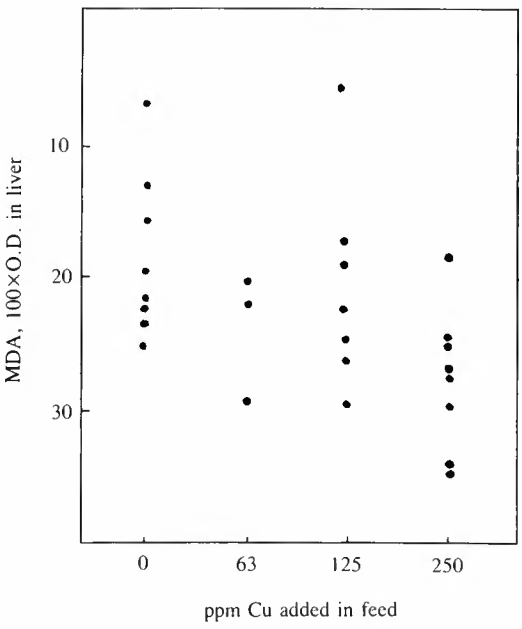


Fig. 1. Oxidative stability in pigs liver an increasing levels of copper supplements.

Pork fat stability was not related to Cu in liver or copper feeding. The softening of pork fat without interfering with oxidative stability may come about if the relative stable oleic acid level is raised. And oleic acid has been reported to increase (Taylor, Tomke 1964). Copper is found to raise dehydrogenase activity in rat tissues, and this provides an explanation for the direct effect of copper feeding on body lipids (Cunnam 1985). This, however, may not exclude that also polyenoic acids made from oleic acid in the animal may be raised. In fact, reduced stability of pig back fat by Cu feeding has been reported (Amer, Elliot 1967) of Cu feeding. The opposite effect on oxidative stability of liver is seen in the tables and in Fig. 1. The reduction in malonedialdehyde reactivity might seem unexpected, but is documented by these experiments.

Liver Cu compounds apparently possess antioxidative properties. The copper containing superoxide dismutase and ceruloplasmine and possible oxygen scavangers, and thus protect the tissue. A non-enzymatic radical capture may also be thought of. Ceruloplasmine is found to reduce malondialdehyde reactivity in the erythrocyte membrane (Gutteridge et al. 1980).

## SUBGROUP TREATMENTS

In experiment 2, barley as a sole carbohydrate source was compared with the trade mixtures containing durra and oats. Table 3 gives the results of feed conversion, pork characteristics and liver aldehyde. The barley fed pigs did not differ in feed conversion, fat thickness or MDA reactivity. However, a high barley diet making pork more solid is in accordance with previous findings (Astrup 1964).

In experiments 3 – 6 zinkbacitracin was used at a level of 12 ppm. The levels, in agreement with previous investigations, may have been too low (Homb 1959, Hvidsten 1961) to affect the results.

SUMMARY

1. Growth and feed conversion were improved when feeding 63, 125 and 250 ppm of copper in the dry feed to bacon pigs in 8 experiments with 283 animals.
2. The corrected feed conversion improved 5 %. This was also recorded at the low levels of supplementation.
3. Copper feeding caused pork softening without affecting the oxidative stability of the pork fat.
4. The copper feeding brought about better liver oxidative stability as seen on malondialdehyde reactive components.
5. Copper content of the liver increased with the raised copper levels in the feed.
6. Pork softening and liver stability changed in proportion to the copper supplementation.

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# EFFECTS OF VITAMIN D SUPPLEMENT ON COWS AND SHEEP

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Vitamin D tended to improve mineral level, production and weight gain in the cow. Pregnant ewes given vitamin D produced lambs with lowered alkaline phosphatase.

Key words: Cow, sheep, vitamin D.

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About 10 closely related compounds with vitamin D activity are produced when natural sterols are irradiated. Of significance in animal production, $D_2$ and $D_3$ are synthesized in plants and animals, respectively, when their sterols are subjected to light in UV wavelengths between 250 and 315 nm. In the cloudy weather of spring and autumn, the diffuse light is also of importance.

Vitamin D is also produced industrially; $D_2$ is made from ergosterol extracted from yeast, and irradiated. When cholesterol is dehydrogenated to 7,8 dehydrocholesterol and irradiated, vitamin $D_3$ is produced. Vitamin D is inexpensive, and its biopotential high. The IU is 0.025 μg of crystalline vitamin $D_3$.

## OCCURRENCE

Vitamin D content in hay varies in accordance with stage of harvest and its conditions of drying; sun-dried hay of late harvest has high activity, 1 000 IU or more per kg, while silages of early harvest may contain only 100 IU in dry matter. Irradiated yeast contains up to several million units. Herring meal has 4 000 IU per kg. In cod liver oil the content is up to 200 000 IU per kg, while milk at most contains 25 IU per kg. Storage capacity of the vitamin as a fat soluble vitamin is low. The hide is important, followed by blood and liver. Lenkeit & Günther (1967) report the ratio of the contents of these organs to be 3:1:1.

## THE MODE OF ACTION

Vitamin D, without being a coenzyme, acts as a biocatalyst on Ca metabolism. It is synthesized in the epiderm and, according to Günther (1968) has properties like a hormone.

The vitamin activates the bone substance such that Ca deposition and release are facilitated. In low doses, less than 10 IU per kg body weight, deposition dominates. Ca participates in a cycle which, in principle, is regulated by common biochemical mechanisms.

Parathormon from parathyroide increases Ca mobilization, while calcitonin in thyroidea en-

hances calcification, and will interfere in the cycle. The effect upon absorption in the gut is to stimulate the active uptake of Ca, and then of P. Very low doses are also effective here (Nicolaysen 1937).

Vitamin D acts on the kidney by increasing P resorption in tubuli, and may be a secondary effect of Ca and parathormon activity. The general vitamin effect is likely an increase in the membrane permeability of Ca.

The resulting changes within the cell bodies of Ca-concentration may cause reduction or increase in phosphatases in different tissues. Vitamin D also seems to affect Co-carboxylase, coenzyme A and level of ATP, lipid P, alkali reserve, and hexosamin synthesis in ground substance of bone and other collagenous tissues (Günther 1968). An early sign of rachitis in humans is the increased citric acid synthesis and excretion, and insufficient amino acid resorption in the kidneys.

Vitamin D is also affected by sex hormones – synergistic by estrogens and progesterol, antagonistic by the androgens. Vitamin D action is dependent of sufficient thyroxine production. There is interaction with the adrenal hormones. It is antagonized by vitamin A, while synergism is seen with B and K vitamins (Günther 1968).

By overdosing, however, vitamins A, B and E appear beneficial, as do cortison and estrogen. Fluor is an antagonist and inhibits Ca utilization from bones. More active than the vitamin D are its metabolites hydroxylated in 25, 24 and 25, 1 and 25 positions. Different target organs carry receptors for the metabolites (Haussler 1986).

## THE VITAMIN D DEMAND

Difficulties in establishing the exact requirement are caused by uncertainty in the effect of irradiation and level and availability of feeds. Antagonists appear not only to vitamin D, but also against Ca. The Ca-P relationship, phytic acid and phytic acid phosphatase activity in feed, fatty acids and oxalic acid affect Ca absorption.

Grown-up animals also have a demand to cover production and pregnancy. ARC-CAB (1980) recommend around 10 IU per kg live weight for milk cows. Günther (1968) suggests 5 000 – 8 000 IU daily. Hibbs & Conrad (1966) presume that 20 000 IU daily will affect health and production favourably if the animals are in negative P balance. Very large doses have been used in preventing milk fever. The large doses, however, sometimes appear toxic.

Vitamin D in feeding supplements of grown-up animals may be considered because of changes in the vitamin D levels of the feed in recent years. Less herring meal and less hay is given. The animals grow more rapidly, become larger and produce more. The demand is probably larger also when indoor feeding time is extended.

Recent reviews of Ca, P and vitamin D function and demand in growth and lactation have been carried out by Luthman (1974), Jørgensen (1974), Dobson & Ward (1974), Braithwaite (1976), Jonsson (1979), Kolb (1979), Lawson & Davie (1979), Olson (1979), Georgiewskii (1981), Miller (1981) and Deluca & Schoes (1983), Horst & Reinhardt (1983), Henry & Norman (1984), Haussler (1986). Particular mineral balance aspects have been treated by Ender et al. (1971), Astrup et al. (1973), Jørgensen (1974), Braithwaite (1976), Georgiewskii et al. (1981), Astrup (1985) and Block (1987).

## THE PRESENT INVESTIGATIONS

### A. MILK COWS AND VITAMIN D

The minimum dose necessary for avoiding racitic symptoms in dairy cows is very low. This is because the vitamin, even in very low concentrations, stimulates Ca deposition in bone substances.

It is possible to increase the content of Ca and P in blood with supplements of vitamin D, even the supply is adequate to prevent rachitis. The size of the increase might indicate whether the supply is liberal or not. If an extra supply favourably affects milk yield and body weight together with the changes in blood parameters, re-

commendations may be altered. The mobilizing property of vitamin D, appearing by increasing doses, may possibly benefit production.

An extra motivation for these experiments was that low P and Ca in the blood were seen in the cows of the experimental barn early in 1966. There were also indications of milk fever (Ekern 1966, Astrup et al. 1968 and Ekern 1972).

*Moderate feeding before calving*

The animals were being used in an experimental programme in which moderate and strong feeding levels before and after calving were being investigated (Ekern 1966). The present recommendations are to increase feed level before calving in order to alleviate the change to high levels after calving. When the animals were given 2 kg less concentrates than recommended before calving (moderate feeding), blood P and Ca levels dropped after the departure (Table 1). Silage was fed ad lib. It is noticeable that the combination of moderate feeding before calving with strong feeding afterwards brings about the lowest blood mineral values. Moderate feeding before calving appeared to depress blood minerals, while moderate or strong feeding afterwards had little effect.

A few animals showed signs of untrift, and were given Ca injections. This treatment alone restored blood picture for a short period, but a combination with injection of vitamin D tended to give a longer lasting effect.

*Milk fever*

Although signs of milk fever in the animals were not allowed to develop further, the ailment is known to have caused losses in other herds. The cause is put down by either maladjustment of the cow's hormones or an insufficient supply of minerals. Favourable results have been reported feeding AIV silage, cereals, phosphate and vitamin D at high levels before calving.

*NLH Experiment 1968*

Supplements of phosphorus, 100 g mono sodium phosphate ($NaH_2PO_4$) and the intramuscular injection of 1.9 million IU vitamin $D_3$ in oil emulsion were given.

At the end of the feed level experiment the 24 animals were subgrouped into three treatment groups of 8 animals each. After one month blood serum P-values had increased in all animals, and highest in the group given vitamin D injections (Table 2).

*NLH Experiment 1967*

Animals were selected from the University herd and distributed into blocks and groups on February 1. Blocks were balanced, as far as possible, according to age, stage of lactation, milk yield and blood serum values. On each treatment 16 animals were available:

A: Control.

B: 70 000 IU vitamin $D_3$ on the concentrate mixture once a week.

C: 1.2 million IU vitamin $D_3$ injected in the muscle.

Milk was weighed every second week. Half of the experimental animals were weighed weekly, and blood samples were taken and analysed as described for sheep (Astrup & Nedkvitne 1967) once a month – on March 8, April 6 and May 3.

Table 1. Serum minerals in animals fed moderate (M) and strong (S) levels of feeding before and after calving.

| | MM | MS | SM | SS |
|---|---|---|---|---|
| Animals | 6 | 6 | 6 | 6 |
| P, mg % | 2.32 | 2.08 | 3.42 | 3.12 |
| Ca, mg % | 11.1 | 10.2 | 11.0 | 11.5 |
| Mg, mg % | 2.07 | 2.02 | 2.01 | 2.13 |

Milk weights, milk fat percentage, and body weights were corrected for control periods before and after the experimental feeding. The corrected figures of serum assays had to be calculated from the pre experimental feeding period only (Table 3).

When calculated this way a tendency to raised serum values was found in the supplemented group and in the vitamin D injected animals. The significance is $0.1>P>0.05$ for Ca in the orally treated vitamin D group.

The only highly significant change ($P = 0.001$) is an increase in body weight in cows by supplementing vitamin D.

Table 2. Serum phosphorus in animals from feed level experiment treated with phosphate or vitamin $D_3$ after calving (NLH 1966).

| | Control | P-suppl. | D-inj. |
|---|---|---|---|
| Animals | 8 | 8 | 8 |
| Serum, P, mg %: | | | |
| Control period | 2.66 | 3.24 | 2.33 |
| After 1 month | 4.99 | 5.05 | 7.75 |
| Increase | 2.32 ± 2.0 | 1.81 ± 1.7 | 5.42 ± 0.8 |

Table 3. Daily milk yield, milk fat, body weights and blood serum values in cows treated with vitamin D (NLH 1967).

| | Control | D-suppl. | D-inj. |
|---|---|---|---|
| Animals | 16 | 16 | 16 |
| Milk, kg | 20.4 | 21.1 | 20.9 |
| Milk fat, % | 3.9 | 3.9 | 3.9 |
| Body weights, kg | 562 | 577*** | 571 |
| Ca, mg % | 10.0 | 10.6 | 10.7 |
| P, mg % | 5.4 | 6.2 | 5.7 |
| Alk. P-ase, units | 1.47 | 1.60 | 1.85 |
| Mg, mg % | 2.2 | 2.1 | 2.1 |
| Cholesterol, mg % | 252 | 273 | 250 |
| FFA mM | 0.35 | 0.35 | 0.30 |
| Glucose, mg % | 74 | 72 | 75 |

Table 4. Daily milk yield and blood serum alkaline phosphatase, calcium and phosphorus in cows fed phosphor or vitamin D supplements (NLH 1968).

| | - P | + P | - D | + D |
|---|---|---|---|---|
| Animals | 20 | 20 | 20 | 20 |
| Milk, kg | 21.8 | 21.3 | 21.3 | 21.9 |
| Alk. P-ase, units | 0.95 | 0.74 | 0.80 | 0.69 |
| Ca, mg % | 10.4 | 10.7 | 10.5 | 10.5 |
| P, mg % | 5.9 | 6.0 | 6.1 | 5.8 |

*NLH Experiments 1968*

During the winter 1968, 40 animals were treated in 4 groups each of 10 animals in a factorial design, with the treatments:

A: Control.

B: 20 000 IU vitamin $D_3$ given daily on the concentrate.

C: 100 g calcium monophosphate given daily on the roughage.

D: Treatments B+C.

Milk was weighed 4 times in the 2 month period of supplementation. Blood samples were taken twice and analysed. Corrections to the results are made according to the pre-experimental determination in February (Table 4).

The results this time indicated an improvement in Ca feeding phosphorus, but not vitamin D. Differences were not statistically significant.

*Extension farm experiment 1968*

In a herd (Presterud farm) close to the University, the animals were fed indoors throughout the year. The cows were balanced in two groups, one with control animals, the other animals receiving a weekly supplement of 140 000 IU. Blood samples were taken twice in the supplement period. Again pre-period deviations were corrected for (Table 5). This time both Ca and P in serum showed a rise as a result of the treatment. The differences appeared statistically significant with respect to serum Ca increase.

## B. THE SHEEP AND VITAMIN D

The demand for supplements has been assessed as being just as much as for the cow (Günther 1968) because of the shielding wool coat of the sheep. Indications of a vitamin D deficiency were seen in the lambs in the spring of 1968, when early-born and late-born lambs were compared. The early-born lambs were kept indoors one month longer than those born in the later, but normal schedule. The lambs kept inside the shed developed a gait characteristic of rachitic animals. Out of the shed they soon recovered and showed high blood mineral levels.

Table 5. Daily milk yield, alkaline phosphatase, calcium and phosphorus in cows supplemented with vitamin D (Presterud 1968).

| | - D | + D |
|---|---|---|
| Animals | 15 | 15 |
| Milk, kg | 19.6 | 19.6 |
| Ca, mg % | 9.4 | 10.5 * |
| P, mg % | 7.3 | 8.3 |
| Alk. P-ase, units | 0.72 | 0.88 |

Table 6. Average serum values from two years' experiments with ewes (NLH and Edøy 1966, 1967).

| | - D | + D | Lambing | | Hay | Silage |
|---|---|---|---|---|---|---|
| | | | early | late | | |
| Animals | 60 | 60 | 60 | 60 | 60 | 60 |
| Ca, mg % | 9.8 | 9.7 | 9.8 | 9.6 | 9.8 | 9.7 |
| P, mg % | 5.0 | 5.3 | 5.2 | 5.0 | 5.2 | 5.1 |
| Alk. P-ase, units | 2.5 | 2.8 | 2.6 | 2.7 | 2.6 | 2.6 |

Table 7. Daily milk yield and milk minerals of two years' experiments with ewes.

| | - D | + D | Hay | Silage |
|---|---|---|---|---|
| Animals | 30 | 30 | 30 | 30 |
| Milk, kg | 2.9 | 2.8 | 2.8 | 2.8 |
| Ca, % | 0.174 | 0.182 | 0.178 | 0.176 |
| P, % | 0.152 | 0.151 | 0.152 | 0.151 |

Table 8. Serum values of twin lambs from ewes supplemented with vitamin D.

| | - D | + D | Hay | Silage |
|---|---|---|---|---|
| Animals | 20 | 22 | 20 | 22 |
| Alk. P-ase, units | 9.9 | 8.8*** | 11.2 | 8.9 |
| Ca, mg % | 11.2 | 11.5 | 11.2 | 11.5 |
| P, mg % | 12.2 | 13.1* | 12.3 | 13.0 |

Table 9. Serum mineral and alkaline phosphatase values in one-year ewes and their lambs, when ewes were treated with vitamin D.

| | Young ewes | | Lambs | |
|---|---|---|---|---|
| | - D | + D | - D | + D |
| Animals | 10 | 10 | 11 | 11 |
| P, mg % | 7.2 | 7.2 | 13.7 | 13.6 |
| Alk. P-ase, units | 3.2 | 3.0 | 12.1 | 9.5* |

Table 10. Effects of exponential increase of vitamin D supplement to ewes.

| Dose, IU | 0 | $10^2$ | $10^3$ | $10^4$ | $10^5$ | $10^6$ |
|---|---|---|---|---|---|---|
| Weight loss, % | 25 | 18 | 20 | 25 | 22 | 32 |
| Lambs wt., kg | 7.2 | 8.1 | 8.9 | 9.8 | 9.6 | 7.0 |
| Serum P, mg % | 5.8 | 5.9 | 4.8 | 5.3 | 4.7 | 9.5 |
| Serum Ca, mg % | 10.0 | 10.3 | 9.7 | 10.3 | 10.3 | 11.1 |
| Serum P-32, % | 0.021 | 0.021 | 0.023 | 0.023 | 0.025 | 0.042 |
| Faeces P-32, % | 1.5 | 2.1 | 1.9 | 2.1 | 2.0 | 1.5 |
| Urine P-32, % | 0.005 | 0.005 | 0.005 | 0.005 | 0.005 | 0.005 |
| Milk ashes, % | 0.73 | 0.76 | 0.77 | 0.81 | 0.86 | 0.76 |
| Radio acitivity, tails, % | 0.33 | 0.28 | 0.30 | 0.32 | 0.33 | 0.37 |
| Lamb tails, ashes, % | 59.3 | 59.6 | 59.9 | 58.5 | 58.5 | 59.5 |

*The pregnant ewe and vitamin D*

Supplements of 3 000 IU daily of vitamin D were given through the indoor feeding period in NLH and Edøy herds in 1966 and 1967.

Barn dried hay versus silage and early and late lambing in the experimental lay out were included (Table 6). A tendency towards raised serum phosphorus appeared not to be statistically significant by the treatment. Growth and lamb weights were not affected.

The ewes tended to improved milk content of calcium with the vitamin D supplement (Table 7).

*Lambs of vitamin D supplemented ewes*

In the last of the two-year treatments, only twin lambs were involved. The Ca, P and alkaline phosphatase values appeared to be high in all lambs. The lambs from vitamin D treated ewes had statistically significantly lower alkaline phosphatase levels and higher contents of Ca and P. Relatively, these changes, however, appeared small (Table 8).

The following year 10 out of 20 one-year-old ewes were treated with vitamin D, 5 000 IU a day for 6 weeks before lambing. As in the previous years, this had little effect on their blood serum values, But in lambs from ewes given vitamin D, also this year alkaline phosphatase was lowered. Ca and P in serum was slightly raised (Table 9).

Bone mineralization was investigated in the lambs. Their tails were cut off, dried and ashed. Mineral contents of the tails appeared not to be affected by the treatment of their mothers.

*The effect of increasing levels of vitamin D in the pregnant ewe*

In a two-week trial with 6 animals, increasing doses of vitamin $D_3$ were supplemented. The levels given daily were 0, 100, 1 000, 10 000, 100 000 and 1 000 000 IU to each of the respective animals. P-32 was used to assay digestibility of P according to Kleiber (1965) and has been reported elsewhere (Astrup et al. 1974).

Blood P and Ca improved only at the very high level of vitamin D. The blood content of P-32, however, showed a small but consistent dose response to the vitamin. The milk mineral contents of the ewes were not affected, nor mineralization of the tails of the lambs (Table 10).

## GENERAL DISCUSSION

The question of vitamin D supplements to the milk cow in the critical period after calving was encountered during experimental low-energy feeding before calving. The low-energy ration contained little phosphorus, and might have been the primary cause of the problem. Vitamin D injections raised the low blood P values and removed signs of milk fever.

The high latitude of our country may be one cause of the insufficient vitamin D supply. The present experiments indicated this to be true. In the experiments on the University farm animals, the Ca content of their blood was raised, and milk yield tended to be higher when treatments were given orally or injected.

The most significant results were obtained with the oral treatment. Highly statistically significant was the weight gain ($P<0.001$) by supplements of vitamin D. This enforces the tendency seen in milk production. The vitamin D supply may have stimulated the animals' appetite. A significant increase in blood Ca was seen in the last experiments in the herd of animals kept indoors all the year round.

The sheep experiments did not indicate especially high requirements of vitamin D, in spite of their shielding wool coat. No effect on production, weight gain or milk yield could be detected. However, pre-lambing supplements resulted in statistically significantly lower alkaline phosphatase in the lambs. This indicates a better rate of calcification and also might prevent stiff gait syndromes seen in lambs kept indoors in the spring.

While phosphorus and calcium levels in blood tended to be stimulated in the milk producing cow with vitamin D, alkaline phosphatase of blood was unchanged or only slightly raised, In the lambs with high bone growth the alkaline phosphatase values were high in blood, and vitamin D reduced the activity.

The milk cow alkaline phosphatase of blood is likely dominated by gut enzymes and reflects improved Ca and P uptake in the gut.

The sheep experiment giving exponential increasing vitamin D levels confirms that the high supplement is a third effect of the vitamin. It breaks down the homostatic regulation at the highest level given, and is pharmacological in nature.

## SUMMARY

1. Vitamin D treatment post-calving was seen to improve low serum P levels and signs of milk fever, when pre-calving energy and feed phosphorus level were low.

2. Vitamin D supplemented to milk cows tended to improve blood Ca levels, milk production and weight gain.

3. Vitamin D did not improve serum values and growth in pregnant ewes, but statistically significantly affected the alkaline phosphatase of their lambs.

4. Raising vitamin D levels in the pregnant ewe exponentially changed blood parameters only at the $10^6$ IU a day level.

5. Vitamin D supplements in the order of 10 IU to each kg live weight of the milk cow and the pregnant ewe are recommended as a result of these experiments.

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# EXPERIMENTS WITH *RUMEX* SPECIES

## Translocation of assimilates and MCPA related to carbohydrate content of the roots and herbicide effect

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Fykse, H. 1987. Experiments with *Rumex* Species. Translocation of assimiliates and MCPA related to carbohydrate content of the roots and herbicide effect. Norwegian Journal of Agricultural Sciences 1: 97 – 103. ISSN 0801 – 5341.

The distribution of $^{14}C$-labelled assimilates and MCPA, the amount of carbohydrates in the root system and the herbicide effect of MCPA were studied in *Rumex longifolius* DC., *Rumex obtusifolius* L. and *Rumex crispus* L. The species differed regarding carbohydrate amount and sinks of translocated assimilates. Quantitatively the movement of MCPA was poorly linked to that of assimilates. The susceptibility to MCPA varied with species and growth stage, but could be correlated neither with translocation of the herbicide or assimilates nor with the carbohydrate content ot the roots.

Key words: Carbohydrates, MCPA, translocation, herbicide effect

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Docks are troublesome weeds in many long-lasting meadows and pastures. Common species in Norway are *R. longifolius* DC., *R. obtusifolius* L. and *R. crispus* L., the first is the most important.

As the measures available against docks are often unsatisfactory, a research programme was initiated at the Norwegian Plant Protection Institute to study both the biological and the physiological aspects of the species mentioned above. The results of experiments on growth and regeneration have been published (Fykse 1986), and show that in several ways the three species behave differently.

This paper deals with experiments concerning distribution of assimilates and MCPA at different growth stages of docks in spring, as well as in the regrowth after cutting in summer. Distribution is evaluated for content of carbohydrates of the root system and for herbicide effect of MCPA.

## MATERIALS AND METHODS

The plants of this study were raised together with the plants used in the experiments on growth and regeneration (Fykse 1986). The treatments/samplings of plants for analysis of carbohydrates were done at the stages when the different tests of the study referred to started, i.e. (1) small rosettes, (2) large rosettes with stems beginning to expand, (3) stems 35 – 45 cm in height with inflorescences not or only

slightly visible, (4) the day of cutting (only analysis of carbohydrates), and in the regrowth when the plants had again reached stages 1, 2 and 3.

*Distribution of assimilates and MCPA*

The study was performed by applying $^{14}C$-labelled substances to a healthy leaf at the base of the plant.

Radioactive assimilates were synthesized in the plants when the leaf was treated with $^{14}CO_2$ (Fykse 1974), corresponding to 0.1 MBq per plant. Duration of the $^{14}CO_2$-gas exposure was two hours.

$^{14}C$-MCPA [(2-methyl-4-chlorophenoxy) acetic acid], carboxyl labelled, with specific activity 2.22 MBq/µM, was dissolved and diluted in 50 % ethanol to 3.7 KBq/µl. To study the translocation of MCPA under conditions as realistic as possible, the plants were pretreated with commercially formulated MCPA equivalent to 2 kg ai/ha in 250 l of water. Immediately after spraying 10 µl of the $^{14}C$-MCPA solution (37 kBq) was carefully mixed with spray droplets on the selected leaf.

Each treatment was repeated on four plants per species, the experimental period lasting for five days. At harvest, the treated leaf was cut off at the base of the petiole, and the rest of the plant divided into [1]) rosette including stem, if present, [2]) main root (connected directly to the stem with the treated leaf), and [3]) the remaining root system. The plant material was dried for 48 h at 90 °C. Further processing and counting of radioactivity were performed according to Fykse (1974).

*Carbohydrates in the roots*

At each developmental stage the root system of four plants per species was dug out, washed and stored at -20 °C until further processing.

Before analysis, the roots were dried at 40 °C for three days. The amount of carbohydrates was principally determined according to the method described by Allen (1974). However, to include starch in the total amount of sugars, extraction was done using 3 N $H_2SO_4$ instead of water.

*Herbicide effect of MCPA*

At the time when the plants in the translocation study were pretreated with formulated MCPA, 10 extra plants were sprayed with the same dosage – 2 kg ai/ha. During this operation untreated control plants were covered with plastic bags.

The numbers of living and herbicide killed plants were counted at different intervals, the last assessment in June the year after treatment.

*Statistical analyses*

The tests were done by means of Proc anova, Proc reg and Proc catmod of SAS (SAS Institute, 1985). Differences between tested subjects were considered significant when $P<=0.05$.

## RESULTS

*Distribution of assimilates and MCPA*

Figures 1 and 2 show the distribution of these compounds at stages 1, 2 and 3 in spring and after cutting in summer, respectively.

In all species an appreciable export of assimilates took place from the treated leaf to the rest of the plant. On average, *R. longifolius* and *R. obtusifolius* exported greater amounts of assimilates than *R. crispus*. However, these apparent differences between species were not statistically significant. Regarding the influence of season and stages of growth, the assimilate export was significantly greater in spring than in the regrowth after cutting, and was significantly higher at stages 2 (large rosettes) and 3 (stem 35 – 45 cm in height) than at stage 1 (small rosettes).

The rosette including a possible stem received significantly more assimilates than the main root and the rest of the root system. Between the two root fractions, however, no significant differences were observed.

Analysis of the interaction between species, plant parts, growth stages and time of treatment revealed that the rosettes of *R. longifolius* and *R. crispus* imported more assimilates in spring than after cutting. On the other hand, *R. obtusifolius* translocated during the whole ex-

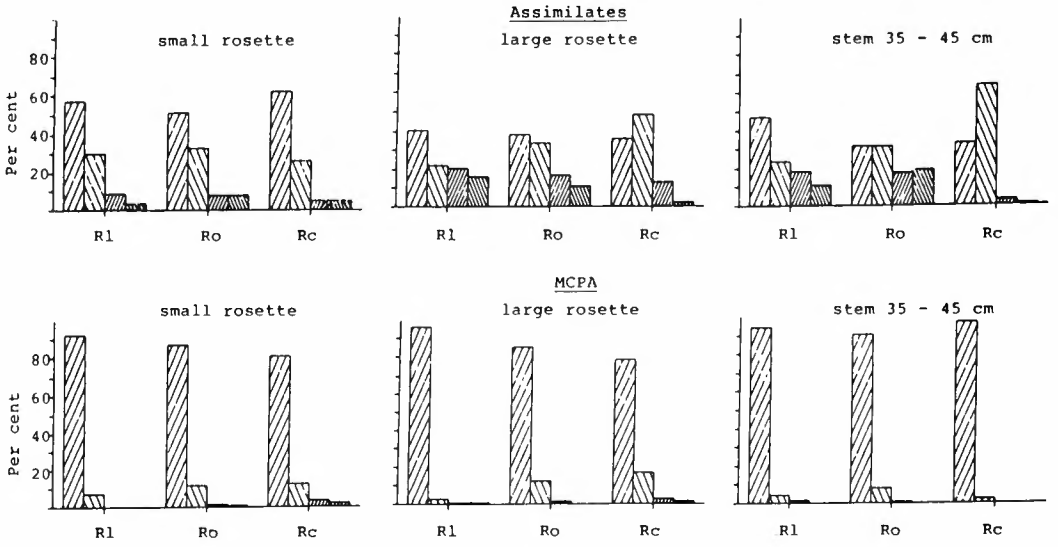


Fig. 1. Distribution of $^{14}C$-labelled assimilates and MCPA in some *Rumex* species in spring: R1 = *R. longifolius* DC, Ro = *R. obtusifolius* L. and Rc = *R. crispus* L.. ▨ = treated leaf, ▨ = rosette + poss. stem, ▨ = main root, ▨ = other roots.

perimental season significantly more assimilates into the roots than *R. crispus* – in spring even more than *R. longifolius*. This was particularly the case when the $^{14}CO_2$-exposure was done at the later two stages.

The export of $^{14}C$-MCPA from the treated leaf was rather limited. On the average, *R. longifolius* exported the highest amount of labelled material and *R. crispus* the lowest. These differences between species were not significant, however. On the other hand the export in spring was significantly greater than after cutting, and was greater when the plants were treated at stage 1 (small rosette) than at stage 3 (stem 35 – 45 cm in heigth).

Of plant parts other than the treated leaf, the rosette with or without stem imported significantly more $^{14}C$-MCPA than the main root, which in turn received significantly more radioactivity than the rest of the root system. However, very small amounts of $^{14}C$-MCPA were translocated into the roots.

The results show a significant correlation between the transport of assimilates and the transport of MCPA, i.e. assimilates and MCPA were translocated in the same *direction*. By studying the different plant parts separately, however, no significant correlation between import of assimilates and import of MCPA could be found. This lack of correlation between assimilates and MCPA also applied to the export from the treated leaf.

*Carbohydrates in the roots*

The content of soluble carbohydrates in relation to the growth stage of the plant is shown in Fig. 3. Marked differences between the species appeared. During the whole experimental period *R. obtusifolius* had significantly higher concentrations of carbohydrates in the root system than the other two species, and, in turn, *R. longifolius* had higher concentrations than *R, crispus*.

No significant differences regarding the content of carbohydrates in the roots appeared between the different stages of *R. longifolius* and *R. obtusifolius* in spring. In *R. crispus,* however, the carbohydrate content decreased significantly from stage 1 (small rosettes) to stage 2 (large rosettes). Between stage 2 and stage 3

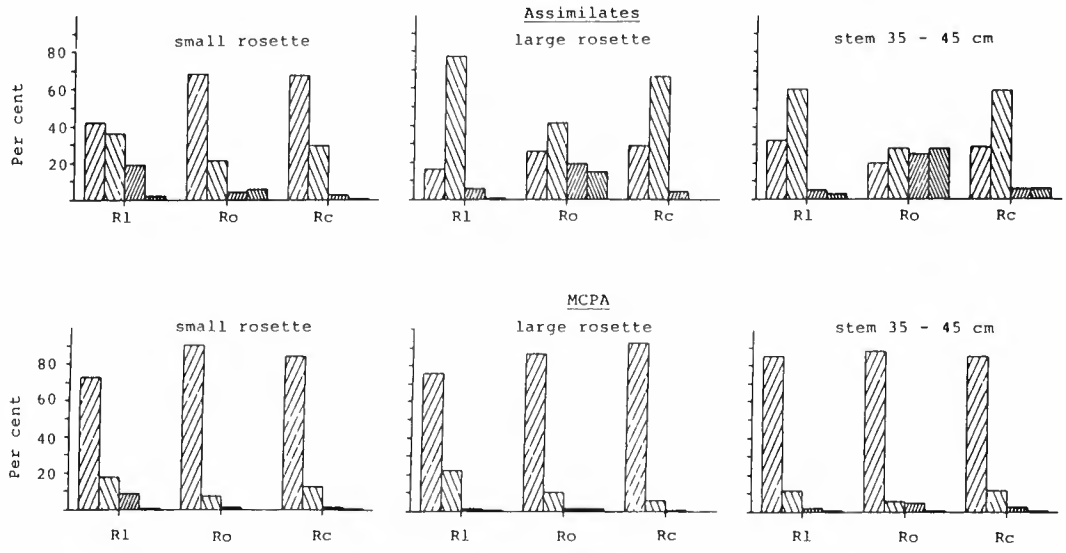


Fig. 2. Distribution of $^{14}C$-labelled assimilates and MCPA in some *Rumex* species after cutting. Legend as in fig. 1.

(stem 35 – 45 cm) a significant increase was observed.

After cutting, the content of soluble carbohydrates in the roots decreased in all species.

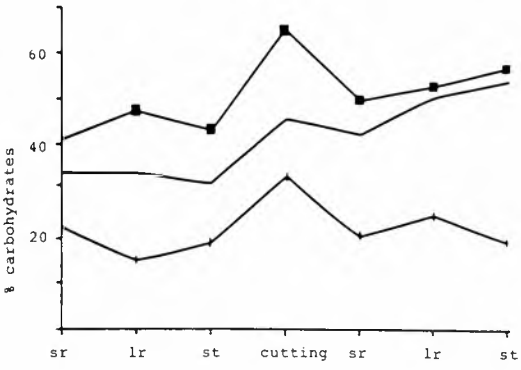


Fig. 3. Amount of soluble carbohydrates in the roots of some *Rumex* species at different developmental stages in spring and after cutting in summer: sr=small rosette, lr=large rosette, st=stem 35 – 45 cm. ——*R. longifolius* DC., -■-■-*R. obtusifolius* L. and -+-+-*R. crispus L.*

The reduction was statistically significant in *R. obtusifolius* and *R. crispus*. After this drop an appreciable increase took place in *R. longifolius* and *R. obtusifolius*, while in *R. crispus* a slight recovery was followed by a new decrease at the time of stem elongation.

*Herbicide effect of MCPA*

The results of this experiment are presented in Table 1. Great differences between the species regarding susceptibility to MCPA were observed. *R. longifolius* was significantly more resistant to the herbicide than the other two species, and *R. crispus* was the most susceptible.

*R. longifolius* demonstrated the highest sensitivity at stage 2 in spring. At the earlier as well as the later stage the herbicide caused only some twisting of the leaves and stems, but had no lasting effect. After cutting, the effect of MCPA at the earliest stages was equally good, but was inferior to the best effect in the previous period.

*R. obtusifolius* was most susceptible at the small rosette stage in spring. After cutting, however, the susceptibility appeared to be greatest at the latest two stages.

Table 1. Effect of MCPA, 2 kg ai per ha, on some *Rumex* species, expressed as percentage of the plants killed one year after treatment.

| Growth stages | *R. longifolius* | | *R.obtusifolius* | | *R. crispus* | |
|---|---|---|---|---|---|---|
| | In spring | After cutting | In spring | After cutting | In spring | After cutting |
| Small rosette | 0 | 40 | 60 | 30 | 100 | 100 |
| Large rosette | 60 | 40 | 40 | 90 | 100 | 100 |
| Stem 35 – 45 cm | 0 | 20 | 0 | 90 | 90 | 100 |

*R. crispus* turned out to be very sensitive to MCPA, and the differences in this respect between growth stages were negligible.

## DISCUSSION

### *Translocation*

The export of assimilates from the treated leaf during the experimental period did not differ principally between the species. Regarding the sinks for the exported material, however, the species did behave differently. *R. obtusifolius* translocated more of the assimilates into the root system than the other two species – a result which corresponded well with the greater root growth of this species (Fykse 1986). Conversely *R. longifolius* and *R. crispus* shipped relatively more of the assimilates into the rosettes. This was most clearly seen in spring, when these species also had the most vigorous development of leaves and stems (Fykse 1986).

In spring, the export of assimilates generally proceeded more rapidly than after cutting, and was higher in taller than in smaller plants. MCPA too was exported in greater amounts in spring, but the size of the plants had an opposite effect on the quantity translocated than in the case of assimilates. Further, more of the exported herbicide was translocated to the plant parts above ground. Only negligible amounts went down to the root system. Altogether, assimilates and MCPA were translocated in the same direction, the correlation, however, between the amounts of these substances, either exported from the treated leaf or deposited in other parts of the plant, was rather small.

These results correspond very well with those of similar experiments with *Sonchus arvensis* L., *Cirsium arvense* (L) Scop., *Tussilago farfara* L., *Achillea millefolium* L. and *Achillea ptarmica* L. (Fykse 1974, 1977 and 1980). They differ, however, from the general opinion expressed by among others, Müller (1969, 1976), of a very close connection between the translocation of assimilates and that of MCPA. Based on that opinion, Imhoff & Voigtländer (1979) and Kübauch et al. (1980) used studies on translocation of assimilates to determine the optimum time for controlling e.g. *R. obtusifolius* in practical situations, but with limited success.

The present experiments, in which the distributions of assimilates and MCPA were studied simultaneously, and in corresponding experiments with the other species mentioned above, provide evidence of the distribution of assimilates not being a good indicator of the quantitative distribution of MCPA. The herbicide is to a much greater extent than assimilates kept within the treated leaf – the more so the older the leaf.

### *Carbohydrates*

The amount of soluble carbohydrates in the root system differed very much between the species. *R. obtusifolius* had the highest content of carbohydrates and *R. crispus* the lowest – a result which is in full accordance with the import of labelled assimilates into the roots, and also with the growth and size of the root system of the

three species (Fykse 1986). During the initial period in spring, roots of *R. crispus* showed a substantial decrease in the carbohydrate content, demonstrating again the lower robustness of this species compared with the other two species, in which similar changes did not take place. The commencement of regrowth after cutting, however, reduced the amount of soluble carbohydrates in all species. Even the root weights decreased (Fykse 1986).

*Herbicide effect*

The effect of MCPA varied quite a lot between the species. *R. crispus* turned out to be the most susceptible. More unexpected, however, was the response of *R. obtusifolius,* which in several ways has proved to be a robust species, not least regarding the root system.

Comparing *R. longifolius* and *R. obtusifolius* showed a clear interaction between species and time of application. In spring, *R. longifolius* was more susceptible at stage 2 than at any other stage, while *R. obtusifolius* had reached this point even at stage 1. At stage 3, both species were resistant. After cutting, the opposite relation between species and time of application appeared.

In spring, *R. obtusifolius* developed very slowly. In fact it did not reach stage 1 until *R. longifolius* had reached stage 2. After cutting, however, this situation changed, *R. longifolius* now being the slower developing species (Fykse 1986). The question arises, therefore, whether species can be on an equal physiological level even when they differ morphologically, and further, whether the length of the growth period is equally or even more important to the herbicide response than the actual developmental stage. New field experiments have been set up to study these questions under more realistic conditions.

The accumulation of MCPA in the root system was, as mentioned, poorly correlated with the translocation of assimilates, and consequently with the concentration of soluble carbohydrates. Additionally, the amount of MCPA in the roots was very small, and the minor differences that occurred between treatments never had such a character that they could explain the variations in the herbicide effects. On the other hand, the ability of the upper part of the root to produce new shoots varied between the species and throughout the experimental period (Fykse 1986). No complete correspondence between reduced ability of sprouting and herbicide effect was detected. However, as with perennial weed species with vegetative reproductive organs (Fykse 1977), the effect of MCPA on *Rumex* spp. seems to be more closely linked to a reduction of the shoot producing ability of the root system than to its content of carbohydrates or the distribution of assimilates and the herbicide.

## SUMMARY

The distribution of $^{14}$C-labelled assimilates and MCPA, changes in the content of carbohydrates in the root system and the herbicidal effect of MCPA were studied in one-year-old plants of *Rumex longifolius* DC, *Rumex obtusifolius* L. and *Rumex crispus* L. The treatments/analyses were performed at different growth stages in spring and in the regrowth after cutting.

The quantities of assimilates and MCPA translocated were greater in spring than in the following period. Regarding the export of labelled material from the treated leaf, no differences between the species were found. However, *R. obtusifolius* loaded relatively more of the exported assimilates into the roots than *R. longifolius* and *R. crispus,* which preferred the above-ground parts of the plant. The translocation of assimilates increased with increasing plant size, while that of MCPA decreased. Generally the translocation of MCPA was small and quantitatively poorly correlated with the translocation of assimilates.

The highest content of carbohydrates was found in the root-system of *R. obtusifolius, R. crispus* showed the lowest, and in this species the content decreased during the early growth stages in spring. After cutting, an immediate reduction occurred in all species, but except for

*R. crispus* the content again increased during stem elongation.

*R. longifolius* showed the highest resistance to MCPA and *R. crispus* the lowest. The most susceptible stage differed between the species, but could not be correlated with translocation of assimilates and MCPA or with the content of carbohydrates in the root system.

## ACKNOWLEDGEMENTS

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# ANTHER CULTURE OF CABBAGE. INFLUENCE OF GROWTH TEMPERATURE OF DONOR PLANTS AND MEDIA COMPOSITION ON EMBRYO YIELD AND PLANT REGENERATION

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The number of anthers producing embryoids was enhanced three times by 1.0 $mgl^{-1}$ 2,4-Dichlorophenoxyacetic acid (2, 4-D) and 1-Napthaleneacetic acid (NAA) compared with lower concentrations av auxins. Inclusion of 0.05 $mgl^{-1}$ 6-Benzylaminopurine (BA) in the media had no beneficial effect on embryo yield. About 60 % of the embryoids developed green structures on a hormone-free medium. The addition of 10 % coconut water did not enhance greening of embryoids. Growth of donor plants at constant 18 °C was inferior to 18 °C/12 °C or constant 12 °C, which proved to be suitable.

Key words: *Brassica oleracea*, var. *capitata*, cabbage, anther culture, growth regulators, growth temperature.

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The production of homozygous lines via anther culture is considered to be a useful tool in breeding programmes. The aim of this work was to establish conditions for embryo yield and plant regeneration from anthers of the Norwegian spring cabbage cultivar «Ladi». The growth regulators of the anther culture medium first used by Keller and coworkers (1975), i.e. NAA 0.1 $mgl^{-1}$ and 2, 4-D 0.1 $mgl^{-1}$, have frequently been used when culturing anthers of *B. campestris* (Keller and Armstrong 1979, Keller *et al.* 1975), *B. napus* (Hansson 1978, Keller and Armstrong 1977, 1978) and *B. oleracea* (Keller and Armstrong 1981, Ockendon 1984, Orton and Browers 1985). This combination of growth, regulators was also used for the cultivation of *B. oleracea* var. *capitata* anthers (Chiang *et al.* 1985). Keller and Armstrong (1983), however, had indications that higher concentrations of auxins were beneficial when culturing *B. oleracea* var. *italica* anthers. For *B. napus* there are also indications that the addition of a cytokinin in low concentration may be beneficial (Lichter 1981). To our knowledge there are no reports on the influence of various concentrations of growth substances on the embryo yield of *B. oleracea* var. *capitata*. We therefore tested the effect of two different auxin

concentrations, and the addition of cytokinin, on the cultivation of *B. oleracea,* var. *capitata* anthers.

There are reports that growth temperature of the anther donor plants is of crucial importance for the embryo yield of *B. napus* and *B. oleracea* var. *gemmifera* (Dunwell *et al.* 1985, Ockendon 1984). Four different temperature regimes were tested in the work presented in this paper.

## MATERIALS AND METHODS

*Plant material*

Mature heads of the Norwegian spring cabbage cultivar «Ladi» taken from the field or seedlings grown in the greenhouse to the 9th leaf stage were vernalized at 5 °C in continuous light 26 $Wm^{-2}$ (Phillips TL 33 fluorescent lamps) for 8 weeks. After vernalization the plants were transferred to naturally lit phytotron compartment. The following temperatures were used; 18 °C 12 h day/12 °C 12 h night (standard treatment), 18 °C 8 h day/12 °C 16 h night, 24 °C 8 h day/12 °C 16 h night, and constant 12 °C, 18 °C, 21 °C or 24 °C. The plants were grown in a standardized potting compost and fertilized weekly with a complete nutrient solution. The plants started to flower after 2 – 4 weeks, depending on temperature. When testing growth regulators, buds were taken from one plant each day and an equal number of anthers were spread on the three different media to be tested. When testing growth temperature of donor plants, anthers from all plants were harvested daily during a 3-week period in July and August.

*Anther culture*

The methods were modified after Keller and Armstrong (1983). Buds were selected when the petals were ½ – ⅓ the length of the anthers, and surface sterilized in 10 % commercial bleach with a drop of Tween 20. They were then rinsed three times in sterile water. Eighteen anthers were placed in each 60x15 mm petridish with 2 ml of liquid culture medium. The anthers were then cultured for 24 h at 35 °C before being transferred to 25 °C. Incubation was in darkness. The number of embryoids was scored after 2, 3, 4 and 5 weeks. The culture medium was the same as that of Keller and Armstrong (1977), with 10 % sucrose, L-glutamine 800 $mgl^{-1}$and L-serine 100 $mgl^{-1}$. Four growth regulator combinations were tested. Medium 1: NAA 0.1 $mgl^{-1}$ and 2, 4-D 0.1 $mgl^{-1}$, medium 2: NAA 1 $mgl^{-1}$ and 2, 4-D 1.0 $mgl^{-1}$, medium 3: NAA 0.5 $mgl^{-1}$ and BA 0.05 $mgl^{-1}$, medium 4: NAA 1 $mgl^{-1}$1, 2, 4-D 1 $mgl^{-1}$ and BA 0.05 $mgl^{-1}$.

*Plant regeneration*

The embryoids were placed on a hormone-free medium (Keller and Armstrong 1977), with or without 10 % coconut water, and incubated at 12 h light 5 $Wm^{-2}$ (OSRAM L36 white fluorescent)/12 h darkness. Teh coconut water was prepared from about 10 coconuts. After heating to 70 °C for 10 min, the filtrate was frozen at 18 °C. Since only occasionally did the embryoids develop into normal looking plantlets, embryoid derived «seedlings» were routinely cut into 2x10 mm pieces and placed on a medium for regeneration. Irradiance was increased to 8 $Wm^{-2}$. The medium of Lillo and Shahin (1986) was used with 2 % sucrose and three different hormone combinations; trans-zeatin 1.0 $mgl^{-1}$ and Indole-3-aceticacid (IAA) 0.1 $mgl^{-1}$, or BA 1.0 $mgl^{-1}$ and gibberellicacid ($GA_3$) 0.1 $mgl^{-1}$ or 2-isopentyl-adenin (2iP) 4.0 $mgl^{-1}$. After 2 – 3 weeks, shoots formed without any callus formation. Shoots regenerated on all three media, but slightly more so on the BA/$GA_3$ medium. Shoots were transferred to a hormone-free medium for further growth and root formation. Almost all plants were successfully transferred to soil in growth chambers.

*Chromosome numbers*

Chromosome numbers of regenerated plants were determined by counting the chromosomes in the nucellar cells (Chiang *et al.* 1979).

## RESULTS AND DISCUSSION

During March and April anthers from plants grown at 18 °C 12 h day/12 °C 12 h night were placed on three different media. Medium 1 with low concentrations of auxin, medium 2 with higher concentrations of auxin and medium 3 with auxin and cytokinin. The results (Table 1) show that medium 2, with higher concentrations of auxins, gave about three times more embryo producing anthers than the other two. On medium 2, some anthers produced more than 30 embryoids. About 500 anthers were placed on media 1, 2 and 3 in introductory experiments. Although not as systematic as those presented in Table 1, also these experiments showed 3 to 4 times higher embryo yield on medium 2.

During May and June another medium was tested, the same auxin concentrations as medium 2 but with the addition of BA. No beneficial effect of BA was found (Table 1).

These experiments showed that NAA and 2, 4-D at 1 $mgl^{-1}$ enhanced embryo formation compared to 0.1 $mgl^{-1}$. The results are therefore in agreement with the work of Keller and Armstrong (1983) on *B. oleracea* var. *italica* that higher auxin concentrations stimulate embryo formation. Furthermore, BA at 0.05 $mgl^{-1}$ did not stimulate embryo formation in *B. oleracea* var. *capitata*, cv. Ladi, as was found for *B. napus* (Lichter 1981).

For *B. napus* there have been indications that 10 % coconut water stimulated the survival and growth of embryos in a $B_5$ medium with 1 % glucose (Keller and Armstrong 1978). For *B. oleracea* almost all embryos grew when placed on solid $B_5$ medium with 2 % sucrose, many developing roots but no leafy structures. The addition of 10 % coconut water did not improve

Table 1. The effect of hormones on embryo yield. Four different hormone combinations were used. Anthers were taken from plants kept at a temperature regime of 18 °C 12 h light/12 °C h dark. Anthers harvested during March and April were placed on media 1, 2 and 3; those harvested during May and June on media 2, 3 and 4.

| Time of harvesting March – April | Medium | Hormones mgl-1 NAA | 2,4-D | BA | Number of anthers cultured | Number of anthers producing embryoids | Number of embryoids |
|---|---|---|---|---|---|---|---|
| March – April | 1 | 0.1 | 0.1 | 0 | 1008 | 9 (0.9 %) | 11 |
| | 2 | 1.0 | 1.0 | 0 | 1247 | 50 (4.0 %) | 445 |
| | 3 | 0.5 | 0 | 0.05 | 918 | 10 (1.1 %) | 13 |
| May – June | 2 | 1.0 | 1.0 | 0 | 1398 | 40 (2.9 %) | |
| | 3 | 0.5 | 0 | 0.05 | 1398 | 21 (1.5 %) | |
| | 4 | 1.0 | 1.0 | 0.05 | 1398 | 39 (2.8 %) | |

Table 2. The effect of 10 % coconut water on the greening of embryoids. Embryoids obtained during June, July and August were placed on a medium with or without coconut water.

| Medium | Number of embryoids cultured | Number of green embryoids after 2 weeks | 3 weeks |
|---|---|---|---|
| With coconut water | 203 | 82 | 84 (40 %) |
| Without coconut water | 151 | 94 | 96 (62 %) |

greening of embryos; it rather inhibited the greening of embryos in cabbage (Table 2).

Chromosome counting of a random sample (25 plants) of regenerated plants showed that 72 % were diploids, 16 % tetraploids, 8 % haploids and 4 % triploids.

During July and August anthers were harvested from plants grown at four different temperature regimes (Table 3). Plants were also grown at continuous 24 °C and 24 °C 8 h day/12 °C 16 h night. However, flowering was too poor at these temperatures for inclusion in the experiment. Plants grown continuously at 18 °C did not respond as well as those grown at 12 °C or combinations of 18 and 12 °C. For the three temperature regimes including 12 °C, a better reponse was obtained during August than during July. There was no consistent relationship between individual plants (genotypes) responding well in July and those responding well in August (Table 3), pointing to the complex influence of both physiological and genetic factors on the response of the anthers to tissue culture.

These experiments show that growth conditions of the plants are important for the number of anthers responding in culture, lower temperatures being preferable. This is in agreement with the results of Ockendon (1984) and Dunwell *et al.* (1985).

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Table 3. The effect of growth temperature of the donor plants on embryo yield. Plants were grown at four different temperature regimes. Anthers were harvested during July and August and placed on medium 2.

| Temperature regime | Plant number | Number of anthers cultured | | Number of anthers producing embryoids | | Number of embryoids | |
|---|---|---|---|---|---|---|---|
| | | July | August | July | August | July | August |
| 12 °C – 24 h | 1 | 288 | 180 | 0 | 8( 4.4 %) | 0 | 26 |
| | 2 | 288 | 180 | 1(0.3 %) | 9( 5.0 %) | 1 | 11 |
| | 3 | 288 | 180 | 7(2.4 %) | 12( 6.7 %) | 10 | 15 |
| | Total | 864 | 540 | 8(0.9 %) | 29( 5.4 %) | 11 | 52 |
| 18 °C – 8 h/12 °C – 16 h | 4 | 288 | 180 | 1(0.3 %) | 1( 0.6 %) | 1 | 1 |
| | 5 | 288 | 180 | 0 | 18(10 %) | 0 | 63 |
| | 6 | 288 | 180 | 15(5.2 %) | 6( 3.3 %) | 29 | 14 |
| | Total | 864 | 540 | 16(1.9 %) | 25( 4.6 %) | 30 | 78 |
| 18 °C – 12 h/12 °C – 12 h | 7 | 288 | 180 | 1(0.3 %) | 4( 2.2 %) | 1 | 5 |
| | 8 | 288 | 180 | 7(2.4 %) | 10( 5.6 %) | 13 | 18 |
| | 9 | 288 | 180 | 0 | 19(10.6 %) | 0 | 32 |
| | 10 | 288 | 180 | 12(4.2 %) | 3( 1.7 %) | 16 | 7 |
| | Total | 1152 | 720 | 20(1.7 %) | 36( 5.0 %) | 30 | 62 |
| 18 °C – 24 h | 11 | 288 | 180 | 4(1.4 %) | 2( 1.1 %) | 6 | 4 |
| | 12 | 288 | 180 | 3(1.0 %) | 1( 0.6 %) | 3 | 3 |
| | Total | 576 | 360 | 7(1.2 %) | 3( 0.8 %) | 9 | 7 |

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# ENZYMES AND OFF-FLAVOURS: PALMITOYL-CoA HYDROLASE, LIPOXYGENASE, α-OXIDATION, PEROXIDASE, CATALASE ACTIVITIES AND ASCORBIC ACID CONTENT IN DIFFERENT VEGETABLES

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The enzyme activities of palmitoyl-CoA hydrolase, lipoxygenase, α-oxidation, peroxidase, catalase and the ascorbic acid contents measured in twenty-two different vegetables, are discussed in relation to the necessity of blanching the vegetables before freezing to prevent flavour deterioration. Palmitoyl-CoA hydrolase is suggested as a blanching indicator.

Key words: palmitoyl-CoA hydrolase, lipoxygenease, α-oxidation, peroxydase, catalase, ascorbic acid, vegetables.

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Flavour changes in unblanched, frozen vegetables are claimed to be related to the activity of the enzymes peroxidase, catalase, esterase and lipoxygenase (Svensson, 1977).

Peroxidase (EC 1.11.1.7) and catalase (EC 1.11.1.6) are widely distributed in higher plants. An empirical relationship is established between these two enzymes and the development of off-flavours and off-odours in raw and unblanched vegetables (Burnette, 1977).

Esterases, which release fatty acids, belong either to the group of thiolester hydrolases (EC 3.1.2) or to the group of carboxylic ester hydrolases (EC 3.1.1). The number of hydrolases so far recognized in higher plants is limited. There is also considerable confusion in the literature on the nomenclature of such enzymes (Galliard, 1978).

Fatty acids released by hydrolytic enzymes may be attacked by oxidative enzymes (lipoxygenase and α-oxidation), and volatile products with characteristic flavour properties may result. Although desirable flavours may be produced in edible plants by such processes, frequently the result of these degradative reactions are undesirable off-flavours (Galliard, 1975).

Lipoxygenase (ED 1.13.11.12) occurs in seeds, roots and leaves (Stumpf, 1976). The enzyme attacks the *cis, cis*-1,4-pentadiene moiety of linoleic and linolenic acids, and volatile alchohols and aldehydes are formed.

Alpha-oxidation involves decarboxylation of

fatty acid to a long chain aldehyde which in turn is rapidly oxidized by a $NAD^+$ long-chain aldehyde dehydrogenase to the corresponding free fatty acids. This acid can then be recycled into a sequence for further α-oxidation (Stumpf, 1976).

Peroxidase and catalase are normally used as blanching indicators due to their heat resistance compared to other enzyme systems. However, in our studies of blanched carrot cubes stored up to 13 months at -20 °C (Baardseth & Slinde, 1983a), good quality retention was obtained when the thiolester hydrolase (palmitoyl-CoA hydrolase) was no longer present, while activity of catalase (9 %) and peroxidase (6 %) remained. Unblanched carrot cubes developed an off-taste which was characterized as stearin, paraffin or soap.

Most vegetables have to be blanched before freezing due to the development of off-flavours. Swede, however, has been found to have good storage stability both when stored fresh (Berg & Lentz, 1977) and when stored frozen unblanched (Baardseth, 1978). It is claimed that the high ascorbic acid content protects the fresh and frozen swede and acts as an antioxidant (Slinde et al., 1983).

The aim of this study was to compare the activities of palmitoyl-CoA hydrolase, lipoxygenase, α-oxidation, peroxidase and catalase in different vegetables. The need for blanching of vegetables is discussed based on these enzyme activities and the ascorbic acid content of the vegetables.

## MATERIALS AND METHODS

The vegetables listed in Table 1 were grown at the Agricultural University of Norway and harvested at maturity.

All chemicals were of analytical grade. [1-$^{14}$C] palmitoyl-CoA (specific activity 57 μCi $mg^{-1}$) and [1-$^{14}$C] palmitic acid (specific activity 213 μCi $mg^{-1}$) were purchased from Amersham International, Amersham, England.

The vegetables were cut with a knife and 40 g homogenized with an Ultra-Turrax TP 18/10 with 40 ml buffer (15 mmol HEPES (N-2-hydroxyethylpiperazine-N'-2-ethansulphonic acid) per litre, 0.1 nmol DTT (dithiothreitol) per litre, pH 6.3) at 4 °C. After filtration through cheese-cloth, the resulting filrate was centrifuged at $6.2 \cdot 10^5 \cdot g_{av} \cdot min$ (15 000 rpm; 30 min, $R_{av}$ = 8.3 cm) and the supernatant analysed for palmitoyl-CoA hydrolase, peroxidase, catalase and lipoxygenase activities.

Palmitoyl-CoA hydrolase activity (EC 3.1.2.2) was assayed by the release of [1-$^{14}$C] labelled fatty acid from the CoA ester (Baardseth & Slinde, 1983b; Berge & Farstad, 1979). Peroxidase activity (EC 1.11.1.7) was determined at 420 nm using guaiacol and hydrogen peroxide as substrate, as described by Lu & Whitaker (1974). Catalase (EC 1.11.1.6) activity was measured at 230 nm using hydrogen peroxide, having an absorbance at 230 nm = 1.0, as substrate (Bergmeyer et al., 1974). The enzyme assays were carried out at 25 °C using a Shimadzu UV-300 spectrophotometer with a 1 cm cell. Lipoxygenase activity (EC 1.13.1.12) was measured as oxygen consumption with a Clark oxygen electrode at 25 °C (Yellow Spring Instr. Biological Oxygen Minitor), using linoleic acid as substrate (Galliard & Phillips, 1971). Brij 58 was used to emulsify the linoleic acid. The substrate concentration used in the assay was 1 mmol per litre and 15 mmol HEPES per litre (pH 6.3).

For α-oxidation activities, a buffer containing 5 mmol HEPES per litre, 1 mmol EDTA (ethylenediamine tetra-acidic acid) per litre, pH 7.2 (one part vegetables, one part buffer) was used. After filtration through cheese-cloth, the resulting filtrate was centrifuged for $1.6 \cdot 10^4$ $g_{av} \cdot min$ (3 000 rpm, 20 min, $R_{av}$ = 8.3 cm) and the α-oxidation activity measured in the supernatant. To measure $^{14}CO_2$ production (α-oxidation) from $^{14}$C-labelled fatty acid, the incubation technique described by Galliard & Matthew (1976) was used with 58 μmol [1-$^{14}$C] palmitic acid per litre; the reaction time varied from 15 min up to 20 hr at 25 °C.

For the ascorbic acid analyses one part vegetable was homogenized with one part of 1 % oxalic acid. After filtration through cheese-

cloth, the resulting filtrate was centrifuged at $6.2 \cdot 10^5$ $g_{av} \cdot min$ (15 000 rpm; 30 min, $R_{av}$ = 8.3 cm) and the supernatant analysed for ascorbic acid. Ascorbic acid was determined by HPLC with a reversed phase $C_{18}$ Waters Radial column. The mobile phase was 0.05 mol $KH_2PO_4$ per litre (pH 4.5) and the detector was set at 254 nm (Williams et al., 1973).

Protein was determined by the Kjeldahl method, and dry matter by drying under vacuum at 70 °C overnight.

## RESULTS

All vegetables testet contained palmitoyl-CoA hydrolase activity (0.05 to 3.63 μmol $min^{-1}$ 100 g wet $weight^{-1}$) and lipoxygenase activities (1 to 45 μatoms oxygen $min^{-1}$ 100 g wet $weight^{-1}$) (Table 2). For palmitoyl-CoA hydrolase especially potato, carrots, peas and snap peas showed high activities, while the highest lipoxygenase activities were found in peas and potato.

Alpha-oxidation activities were found ranging from 0 to 63.6 nmol $min^{-1}$ 100 g wet $weight^{-1}$. Alpha-oxidation activity was especially pronounced in kale and cucumber. In about one third of the vegetables no α-oxidation activity was detected.

The activities of peroxidase observed in this study ranged from 0 to more than 2 600 ΔA $min^{-1}$ 100 g wet $weight^{-1}$ and catalase from 0 to about 400 ΔA $min^{-1}$ 100 g wet $weight^{-1}$ (Table 2). With the exception of red pepper, all vegetables were found to contain peroxidase, while catalase activities were not found in red pepper, snap beans or potato. Especially for peroxidase there was a wide range in the activities, with brussels sprouts and swede as the vegetables

Table 1. Vegetable varieties used in the experiment.

| Vegetable | Botanical name | Commercial variety |
|---|---|---|
| Onion | *Allium cepa* | Jumbo |
| Leek | *Allium porrum* | King Richard |
| Celeriac | *Apium graveoleus* var. *rapaceum* | Iram |
| Beetroot | *Beta vulgaris* | Boltardy |
| Swede | *Brassica napobrassica* | Bangholm |
| Kale | *Brassica oleracea* var. *acephala* | Smaragd |
| Cauliflower | *Brassica oleracea* var. *botrytis* | 54925 K |
| Cabbage | *Brassica oleracea* var. *capitata* | Toten Amager |
| Brussels sprouts | *Brassica oleracea* var. *gemmifera* | Lunet |
| Broccoli | *Brassica oleracea* var. *botrytis* f.*asparagoides* | Corvet |
| Green pepper/ Red pepper | *Capsicum annuum* | Sempra |
| Cucumber | *Cucumis sativa* | Farbis $F_1$ |
| Carrots | *Daucus carota* | Nantes Duke |
| Crispheaded lettuce | *Lactua sativa* var. *capitata* | Italia |
| Parsley | *Petroselinum crispum* | Clivi |
| Snap beans | *Phaseolus vulgaris* | Fran Toccata |
| Peas | *Pisum sativum* | Tristar |
| Snap peas | *Pisum sativum* var. *sacchara'tum* | Sweet snap |
| Tomato | *Solanum lycopersicum* | Alfresco $F_1$ |
| Potato | *Solanum tuberosum* | Beate |
| Corn | *Zea mays* var. *saccharata* | Aztec |

Table 2. Enzyme activities, ascorbic acid, protein and dry matter in some vegetables per 100 g wet weight.

| Vegetable | Palmitoyl-CoA hydrolase µmol $min^{-1}$ | Lipoxygenase µatoms oxygen $min^{-1}$ | α-oxidation nmol $min^{-1}$ | Peroxydase $\Delta A$ $min^{-1}$ | Catalase $\Delta A$ $min^{-1}$ | Ascorbic acid mg | Protein g | Dry matter g |
|---|---|---|---|---|---|---|---|---|
| Onion | 0.05 | 1.3 | 0 | 2 | 14 | 12 | 1.8 | 12.5 |
| Leek | 0.79 | 8.2 | 0 | 12 | 58 | 17 | 2.1 | 9.2 |
| Celeriac | 0.10 | 4.1 | 0 | 11 | 61 | 3 | 1.8 | 11.1 |
| Beetroot | 0.05 | 3.6 | 0 | 39 | 5 | 3 | 1.8 | 12.4 |
| Swede | 0.56 | 19.9 | 0.5 | 1 281 | 23 | 43 | 2.1 | 10.7 |
| Kale | 0.16 | 12.7 | 63.6 | 105 | 147 | 9 | 4.2 | 13.0 |
| Cauliflower | 0.10 | 5.8 | 1.9 | 150 | 12 | 29 | 2.0 | 6.6 |
| Cabbbage | 0.46 | 9.3 | 7.1 | 804 | 12 | 16 | 1.5 | 7.1 |
| Brussels sprouts | 0.16 | 7.9 | 0.9 | 2 656 | 59 | 31 | 5.4 | 16.0 |
| Broccoli | 0.28 | 8.0 | 1.4 | 406 | 40 | 44 | 3.6 | 9.9 |
| Red pepper | 0.71 | 24.3 | 0 | 0 | 0 | 170 | 1.3 | 8.0 |
| Green pepper | 0.08 | 17.8 | 0 | 10 | 14 | 100 | 1.0 | 6.2 |
| Cucumber | 0.76 | 15.9 | 41.3 | 12 | 2 | – | 0.8 | 2.7 |
| Carrots | 2.56 | 1.8 | 1.5 | 8 | 40 | 1 | 0.8 | 11.1 |
| Crispheaded lettuce | 1.65 | 13.0 | 0.3 | 3 | 31 | 4 | 1.1 | 4.5 |
| Parsley | 0.17 | 1.0 | 0 | 39 | 28 | 53 | 3.5 | 15.7 |
| Snap beans | 0.30 | 10.9 | 1.6 | 132 | 50 | 9 | 2.0 | 9.4 |
| Peas | 2.26 | 44.9 | 16.5 | 289 | 403 | 19 | 6.3 | 32.1 |
| Snap peas | 1.55 | 7.9 | 1.6 | 379 | 0 | 39 | 3.3 | 13.5 |
| Tomato | 0.09 | 18.1 | 0 | 24 | 0 | 44 | 1.6 | 5.0 |
| Potato | 3.63 | 31.8 | 0 | 20 | 17 | 12 | 2.2 | 23.9 |
| Corn | 0.34 | 5.9 | 3.3 | 201 | 239 | 8 | 3.8 | 26.9 |

having highest activities. Peas, corn and kale were high in catalase activity.

Among the vegetable tested, red and green pepper had especially high ascorbic acid concentration, while carrots. celeriac, beetroot and crisphead lettuce contained small amounts of ascorbic acid.

The relationships between the various enzyme activities and between the enzyme activities and the ascorbic acid concentration of the vegetables, were examined by linear regression analysis. Calculations were made on the basis of wet weight, dry matter or protein concentration. The analysis revealed no significant correlation ($P>0.05$) between any of the enzyme activities or between any of the enzyme activities and the ascorbic acid concentration.

## DISCUSSION

Long-chain thiolester hydrolase activities have previously been found in leek (Abdul-Karim et al., 1982), spinach leaves (Joyard & Stumpf, 1980), potato tuber (Huang & Stumpf, 1971), and carrots (Baardseth & Slinde, 1983b) with specific activities similar to the palmitoyl-CoA hydrolase activity found in the present study.

Palmitic-, linoleic- and linolenic acid are the fatty acids mainly released by esterases (Bengtsson & Bosund, 1966; Galliard et al., 1976). The further breakdown of fatty acids can occur through α-oxidation. Laties et al. (1972) found α-oxidation in potato and Galliard & Matthew (1976) in cucumber with palmitic acid as substrate. Inhibition of the α-oxidation in cucumber was observed when peroxidase and ascorbic acid were added to the reaction medium (Galliard & Matthew, 1976). This may explain why α-oxidation activity was not detected in some of the present vegetables.

Free linoleic- and linolenic acid can also be further broken down by lipoxygenase. Rhee & Watt (1966) and Pinsky et al., (1971) found high lipoxygenase activities in peas and potato while the activity in onion and carrots was low. The present results confirm this since the highest activity was found in peas, potato and red pepper, and the lowest activity in parsley, onion and carrots. However, lipoxygenase is also inhibited by ascorbic acid (Bonnet & Crouzet, 1977), which can again explain the wide range in lipoxygenase activities found.

Peroxidase and catalase activities in plants have been extensively studied (Whitaker, 1972; Burnette, 1977; Yamaguchi et al., 1980) and low or no detectable activities of peroxidase in lettuce, onion and carrots, and of catalase in tomato and beans have been reported. Except for the beans this agrees well with the results obtained in this study.

The results from the present study indicate that no general relationship can be found between any of the enzyme activities and the ascorbic acid concentration.

The vegetables which are reported to develop off-flavour after storage at -20 °C are peas (2 weeks), beans (4 weeks), corn (2 months) (Lee et al., 1955), peas in pods (4 weeks) (Lee et al., 1956), brussels sprouts (within 3 months) (Adams, 1977), cauliflower (within 3 months) and carrots (within 6 months) (Baardseth, 1978). Swede, leek and onion can be stored for up to 15 months at -20 °C without developing off-flavours (Baardseth, 1978). Kozlowski (1979) and Adams (1983) reported that unblanched red and green pepper, parsley, cabbage, celeriac, cucumber and tomato also store well at -20 °C. The vegetables which are claimed to store well unblanched all have a strong characteristic taste which might cover a development of lipid oxidation.

If we try to correlate reported storage ability and the level of enzyme activities and ascorbic acid, again no general relationship can be found between the level of one single enzyme activity, as measured *in vitro,* and the tendency of various vegetables to develop off-flavours and off-odours. It seems that the enzyme(s) responsible will have to be determined in each species. The level of the different enzyme activities, ascorbic acid, protein and dry matter will also vary betweeen varieties (Galliard & Rayward-Smith, 1977) and within a variety (Baardseth & Slinde, 1981). However, the use of lipid degradation enzymes such as palmitoyl-CoA hydrolase as a

blanching indicator may be favourable in cases where lipid degradation products are responsible for off-taste and off-odour. An advantage of using blanching indicators less heat resistant than perioxidase and catalase will be reduced energy cost due to lower blanching temperatures. This also implies smaller losses of nutrients (Bengtsson, 1984). However, practical experiments should always be carried out to see if a new blanching indicator could be used.

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# CAUSES OF VARIATION IN THE LENGTHS OF GROWTH PERIODS AND THE HEAT SUM REQUIREMENTS OF CEREAL CULTIVARS

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The effects of sowing date, temperature, rainfall, hours of sunshine, and global radiation on the lengths of the growth periods and the required heat sums were investigated for wheat, barley and oat cultivars grown in field for 17 to 34 years. The effects of the variables were calculated using the partial and multiple correlation and regression analyses technique. Early sowing lengthened the period from sowing to heading and increased the heat sums required by the cultivars. For the heading to ripeness period no such effects were observed, except for the oat, when late sowing increased both the growth period and the heat sum.

Higher temperatures shortened the growth periods for all cultivars. Higher temperatures increased heat sum requirements for the sowing to heading period and reduced the heat sums for the heading to ripeness period. Higher rainfall extended growth periods and increased heat sums for all cultivars. The oat reacted most strongly to variations in rainfall.

Higher global radiation shortended the growth periods and reduced heat sums for the wheat cultivar. The results were not significant for the other cultivars.

Increased sunshine hours shortened the period from heading to ripeness for the barley and wheat cultivars. For the barley the heat sums were also reduced. For the sowing to heading period, hours of sunshine had no significant effects. There were no cases where the oat responded significantly to variations in global radiation or sunshine hours.

Key words: Crop failure, global radiation, rainfall, sowing date, sunshine hours, temperature.

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On the Northern border of cereal growing the lengths of the growth periods and the heat sum of the season are limiting factors in commercial cereal production. Under adequate moisture conditions the yield of the cultivars is very closely proportional to the lengths of the growth periods. In order to obtain maximum yield, therefore, it is important to select cultivars which can utilize the largest possible part of the growth season but without risking crop failure due to incomplete ripening.

Annual fluctuations in climatic conditions

and the response of the cultivars to fluctuations such as the lengths of growth periods and the heat sum required for ripening are important basic factors in cultivar recommendations.

It is generally accepted that at higher temperatures cereal cultivars grow faster and complete their life cycle in a shorter time. However, considerable variation which cannot be ascribed to temperature is also recorded. The heat sum required by the cultivars, which is supposed to be a fairly constant value characterizing the cultivar, also shows great variation. In order to estimate the risk of crop failure because of incomplete ripeness, the length of the climatic growth period is related to the date of ripeness of cultivars which have different requirements regarding the lengths of growth periods and heat sums.

In the investigations an attempt is made to clarify the effects of date of sowing, temperature, rainfall, sunshine hours and global radiation on the lengths of growth periods and the heat sums required by the cultivars.

## MATERIAL AND METHODS

The six-rowed spring barley cv. Lise, the two-rowed spring barley cv. Møyjar, the spring wheat cv. Runar, and the spring oarts cv. Mustang were selected for this study. The cultivars were grown for 26, 19, 25, and 34 years respectively. Over these years the effects of date of sowing, temperature, and rainfall on the lengths of the growth periods and on the heat sums required by the cultivars were determined. For a period of 17 years for all cultivars the parameters sunshine hours and global radiation were also included in the study as possible causes of variation in the lengths of growth periods, and the heat sum requirements.

The growth periods of the cultivars were categorized as follows:

1. Sown to headed.
2. Headed to ripe.
3. Sown to ripe.

For these three periods the following variables were recorded separately:

Dependent variables:

1. Lengths of growth periods in days.
2. Heat sums required by the cultivars using 0 °C as a base temperature.

Independent variables:

1. Date of sowing.
2. Temperature in centigrade, means for the periods.
3. Rainfall in mm per day, means for the periods.
4. Sunshine hours per day, means for the periods recorded by use of the Campell – Stokes heliograph.
5. Global radiation in Cal/cm$^2$/day measured as the sum of direct and diffuse shortwave radiation on a horizontal surface, means for the periods.

The developmental stages of the cereal cultivars were defined as follows.

*Headed* when 50 percent of the spikes were completely out of the boots.

*Ripe* when the moisture content of the kernels was 38 percent of fresh weight.

As a description of the cultivars the lengths of the growth periods and the required heat sums are given in Table 1. The means and the standard deviations are calculated over 17 years of observation.

In Table 2 meteorologic parameters for the growth periods of cv. Lise are given as means of 17 years. For the other cultivars the values may be slightly different.

*The effects of date of sowing, temperature and rainfall on the lengths and growth periods and on the heat sums required by cereal cultivars*

The statistical methods employed in studying the effects of the different independent variables on the lengths of the growth periods and on the heat sum requirements were partial and multiple correlation and regression coefficients. By definition, the partial correlation coefficient expresses the effect of one independent variable

at constant values of the others. The interpretation of the results, however, is complicated because of strong correlations between some of the independent variables. Simple correlation coefficients between sunshine hours and global radiation range from r = .75** to r = .91** for the different growth periods, those between rainfall and sunshine hours from r = -.46 to r = -.61 and those between rainfall and global radiation from r = -.63 to -.66. For the headed to ripe period there was significant negative correlation between temperature and rainfall, positive correlation between temperature and sunshine hours and again, positive between temperature and global radiation. For the other growth periods the correlation coefficients were lower and non-significant.

During the growth season the temperature increased from date of sowing to July 16th and then it decreased. The rainfall increased slightly throughout the season. Because of these seasonal changes the temperature is strongly cor-

Table 1. Lengths of growth periods and heat sum required by the cultivars.

| | Growth periods | | | | | |
|---|---|---|---|---|---|---|
| | Sown to headed | | Headed to ripe | | Sown to ripe | |
| | Mean | SD | Mean | SD | Mean | SD |
| *Lise* | | | | | | |
| Lengths of growth periods, days | 59.2 | 5.04 | 33.6 | 4.02 | 92.8 | 5.95 |
| Heat sums | 764.6 | 34.5 | 542.3 | 41.0 | 1 306.9 | 58.5 |
| *Møyjar* | | | | | | |
| Lengths of growth periods, days | 60.5 | 5.43 | 38.0 | 4.41 | 98.5 | 5.97 |
| Heat sums | 785.0 | 26.9 | 611.8 | 43.4 | 1 396.8 | 54.5 |
| *Runar* | | | | | | |
| Lengths of growth periods, days | 57.2 | 6.40 | 47.8 | 7.55 | 105.0 | 7.57 |
| Heat sums | 735.2 | 37.4 | 757.5 | 75.2 | 1 492.7 | 84.5 |
| *Mustang* | | | | | | |
| Lengths of growth periods, days | 62.1 | 6.14 | 37.7 | 7.92 | 99.8 | 7.74 |
| Heat sums | 814.6 | 44.6 | 604.5 | 93.7 | 1 419.1 | 97.4 |

Table 2. Meteorologic parameters of the growth periods. Means of 17 years for the cv. Lise.

| | Growth periods | | | | | |
|---|---|---|---|---|---|---|
| | Sown to headed | | Headed to ripe | | Sown to ripe | |
| | Mean | SD | Mean | SD | Mean | SD |
| Temperature | 13.0 | 1.16 | 16.2 | 1.13 | 14.1 | .78 |
| Rainfall | 2.08 | .64 | 2.51 | .94 | 2.25 | .56 |
| Sunshine hours | 8.68 | 1.07 | 8.09 | 1.37 | 8.43 | .94 |
| Global rad. | 469 | 40.5 | 460 | 49.6 | 465 | 34.6 |
| Date of sowing (May 1st = 1.0) | 2.1 | 8.7 | | | | |

related with date of sowing (r = .77**) in the sown to headed period. The rainfall is also positively correlated with date of sowing. For the same reasons both sunshine hours and global radiations are negatively correlated with date of sowing. However, these correlations, based on 17 years of observation, are weaker.

The effects of date of sowing, temperature, and rainfall on the lengths of growth periods of barley, wheat, and oats cultivars calculated over periods from 19 to 34 years are given in Table 3. The effects of the same factors on the heat sums required by the cultivars are given in Table 4.

*Effects of date of sowing*

As can be seen from Table 3, early sowing lengthened the period from sowing to heading in all cultivars. The effects on the heat sums are similar (see Table 4). The reason for this reaction may be the lower soil temperatures in relation to the measured air temperature at early sowing dates.

For the headed to ripe period the effects of date of sowing are small and varied, except for the oat cultivar, where late sowing lengthened the period and significantly increased the heat sum. This reaction, specific to the oat cv. is hard to explain. The reason, however, may be due to the fact that late sowing results in lower temperatures and higher rainfall during ripening and the oat shows disproportionately slow ripening under such conditions.

*Effects of temperature*

As expected the temperature had a strong and highly significant effect on the lengths of all growth periods; each 1.0 °C increase in temperature shortened growth periode by 6 to 8 days.

The effects of temperature on the heat sums required by the cultivars are the opposite in the two growth periods. For the sown to headed period the correlation coefficients between temperatures and heat sums are positive for all cultivars. The reason may be that the soil temperature curves lag behind the air temperature in high temperature seasons.

For the headed to ripe period the same correlation coefficients are negative for all cultivars. This shows that the ripening process is strongly influenced by the temperature.

The opposite effects of temperature on the heat sum requirements in the two growth periods resulted in a negligible effect for the whole growth period.

*Effects of rainfall*

For all cultivars and in all growth periods increasing rainfall lengthened the growth periods. For the whole growth period the effects were strong and highly significant. One millimetre of rain per day, or approximately 100 mm for the period, increased the growth period by 3 to 4 days for barley and wheat and by 7 days for the oat cultivar.

The rainfall also had a strong effect on the heat sum requirements which increased by 50 – 60 day degrees for barley and wheat cultivars and by 110 day degrees for oat.

It is not clear why rainfall should delay plant development and ripening. No doubt it is partly due to the fact that rainfall reduces the temperature more in the soil and in the lower plant zone than at the 2.0 m level where the air temperature is measured, i.e. an effect of bias in temperature recording.

The multiple correlation coefficients in Table 3 vary from .84 to .94 indicating that 71 to 88 percent of the variation in the length of the growth periods can be ascribed to the effects of date of sowing, temperature and rainfall. In Table 4 the r-values are .40 to .84. Hence, the independent variables accounted for 15 to 70 percent of the variation in heat sum requirements. In both Tables the values of *a* are given making it possible to construct the equations and calculate the length of the growth periods and the heat sum required by the cultivars for any given value of the independent variables.

*The effects of sunshine hours and global radiation on the lengths of growth periods and the heat sums required by cereal cultivars*

Sunshine hours and global radiation were recorded in addition to date of sowing, temperature, and rainfall over a period of 17 years. Be-

cause of the annual variation in climatic and other factors, the length of the experimental periods had an influence on the results. In order to estimate the effects of sunshine hours and global radiation more precisely the effects of all 5 independent variables were calculated on the basis of the same 17 years of observation. In Table 5 the effects on the length of the growth periods are presented, and in Table 6 the effect on the heat sum requirements are given.

The multiple correlation coefficients in Table 5 vary from .79 to .97 indicating that 63 to 94 percent of the variation is accounted for. In Table 6 the corresponding coefficients are .47

Table 3. Lengths of growth periods of cereal cultivars as influenced by date of sowing, temperature and rainfall. Partial and multiple correlation and regression coefficients. Notation of variables, see below.

| | | Cultivars | | | |
|---|---|---|---|---|---|
| | | Lise 26 years | Møyjar 19 years | Runar 25 years | Mustang 34 years |
| *Sown to headed* | | | | | |
| Date of sowing | r | -.51** | -.35 | -.50* | -.22 |
| | b | -.19 | -.19 | -.27 | -.12 |
| Temperature | r | -.75** | -.65** | -.69** | -.67** |
| | b | -2.77 | -3.37 | -3.22 | -4.00 |
| Rainfall | r | .11 | .30 | .29 | .40* |
| | b | .38 | 1.31 | 1.27 | 1.85 |
| | R | .89** | .92** | .91** | .84** |
| | $R^2$ | .79 | .85 | .82 | .71 |
| | a | 94.6 | 102.3 | 97.5 | 112.3 |
| *Headed to ripe* | | | | | |
| Date of sowing | r | .38 | -.11 | .33 | .49** |
| | b | .11 | -.00 | .13 | .23 |
| Temperature | r | -.78** | -.76** | -.85** | -.78** |
| | b | -2.83 | -2.55 | -5.39 | -3.42 |
| Rainfall | r | .22 | .64** | .12 | .50** |
| | b | .56 | 2.11 | .42 | 2.02 |
| | R | .91** | .93** | .94** | .92** |
| | $R^2$ | .83 | .86 | .88 | .84 |
| | a | 78.2 | 74.2 | 132.7 | 88.1 |
| *Sown to ripe* | | | | | |
| Date of sowing | r | .05 | -.10 | .17 | .38* |
| | b | .02 | -.06 | .12 | .25 |
| Temperature | r | -.86** | -.70** | -.76** | -.78** |
| | b | -6.17 | -6.57 | -8.87 | -8.27 |
| Rainfall | r | .61** | .58** | .48* | .75** |
| | b | 3.30 | 4.11 | 3.99 | 7.16 |
| | R | .89** | .87** | .87** | .89** |
| | $R^2$ | .79 | .75 | .75 | .80 |
| | a | 172.2 | 182.7 | 222.4 | 202.2 |

Notation of variables.
Dependent variable: Lengths of growth periods, days
Independent variables: Date of sowing
: Temperature, °C
: Rainfall, mm per day

to .89 which shows that 22 to 81 percent of the variation in heat sum requirements can be ascribed to the effect of the 5 independent variables. The multiple correlation coefficients are somewhat higher compared to the three independent factors in Tables 3 and 4. However, the lower number of degrees of freedom, because of shorter experimental periods, and the higher number of independent variables, render the results less significant.

Table 4. Heat sum required by cereal cultivars as influenced by date of sowing, temperature and rainfall. Partial and multiple correlation and regression coefficients. Notation of variables, see below.

| | | Cultivars | | | |
|---|---|---|---|---|---|
| | | Lise 26 years | Møyjar 19 years | Runar 25 years | Mustang 34 years |
| *Sown to headed* | | | | | |
| Date of sowing | r | -.48* | -.35 | -.20 | -.22 |
| | b | -2.18 | -2.47 | -4.79 | -1.54 |
| Temperature | a | .58** | .34 | .50* | .15 |
| | b | 22.23 | 17.75 | 23.45 | 8.56 |
| Rainfall | r | .11 | .36 | .39 | .43* |
| | b | 5.07 | 20.31 | 21.59 | 26.19 |
| | R | .59** | .40 | .63** | .46* |
| | $R^2$ | .35 | .16 | .40 | .21 |
| | a | 465.7 | 516.6 | 403.2 | 663.0 |
| *Headed to ripe* | | | | | |
| Date of sowing | r | .31 | -.02 | .33 | .45* |
| | b | 1.34 | -.08 | 2.07 | 3.05 |
| Temperature | r | -.26 | -.14 | -.54** | -.32 |
| | b | -9.27 | -5.13 | -33.58 | -13.53 |
| Rainfall | r | .21 | .60* | .16 | .59** |
| | b | 8.16 | 32.64 | 8.69 | 36.93 |
| | R | .62** | .75** | .80** | .82** |
| | $R^2$ | .39 | .56 | .64 | .67 |
| | a | 672.4 | 615.3 | 1 267.0 | 730.1 |
| *Sown to ripe* | | | | | |
| Date of sowing | r | .01 | -.10 | .09 | .34 |
| | b | .04 | -.79 | .95 | 2.90 |
| Temperature | r | .14 | .05 | -.09 | -.18 |
| | b | 7.40 | 5.23 | -10.40 | -15.45 |
| Rainfall | r | .60* | .57* | .48* | .80** |
| | b | 47.21 | 60.44 | 58.15 | 110.24 |
| | R | .65** | .60* | .61** | .84** |
| | $R^2$ | .42 | .35 | .38 | .70 |
| | a | 1 090.3 | 1 187.5 | 1 511.7 | 1 398.9 |

Notation of variables.
Dependent variable: Heat sum, day degrees, °C.
Independent variables: Date of sowing
: Temperature, °C
: Rainfall mm per day

Table 5. Lengths of growth periods of cereal cultivars as influenced by date of sowing, temperature, rainfall, sunshine hours and global radiation. Partial and multiple correlation and regression coefficients. Notation of variables, see below.

| | | Cultivars | | | |
|---|---|---|---|---|---|
| | | Lise | Møyjar | Runar | Mustang |
| *Sown to headed* | | | | | |
| Date of sowing | r | -.68* | -.34 | -.34 | .07 |
| | b | -.38 | -.20 | -.23 | .08 |
| Temperature | r | -.40 | -.59* | -.57* | -.59* |
| | b | -1.36 | -3.01 | -2.90 | -4.52 |
| Rainfall | r | .28 | .25 | -.24 | -.26 |
| | b | 1.13 | .93 | -1.03 | -1.68 |
| Sunshine h. | r | -.25 | -.14 | .19 | .39 |
| | b | -.72 | -.52 | 1.47 | 6.49 |
| Global rad. | r | -.02 | .05 | -.40 | -.48 |
| | b | -.00 | .00 | -.09 | -.25 |
| | R | .93** | .94** | .95** | .93** |
| | $R^2$ | .87 | .89 | .91 | .87 |
| | a | 82.30 | 100.84 | 126.97 | 186.03 |
| *Headed to ripe* | | | | | |
| Date of sowing | r | -.01 | -.34 | .01 | .17 |
| | b | -.00 | -.06 | .00 | .14 |
| Temperature | r | -.73** | -.81** | -.91** | -.53 |
| | b | -2.30 | -2.26 | -5.16 | -3.38 |
| Rainfall | r | -.11 | .42 | -.08 | .32 |
| | b | -.33 | 1.15 | -.29 | 3.20 |
| Sunshine h. | r | .40 | -.69 | -.28 | -.01 |
| | b | 1.39 | -1.76 | -.80 | -.06 |
| Global rad. | r | .01 | .48 | -.41 | -.05 |
| | b | .00 | .02 | -.03 | -.01 |
| | R | .90** | .95** | .97** | .87** |
| | $R^2$ | .81 | .90 | .94 | .75 |
| | a | 82.44 | 75.42 | 149.91 | 91.58 |
| *Sown to ripe* | | | | | |
| Date of sowing | r | -.48 | -.21 | .01 | -.39 |
| | b | -.28 | -.14 | .01 | -.39 |
| Temperature | r | -.53 | -.63* | -.46 | -.20 |
| | b | -3.39 | -5.85 | -6.69 | -2.11 |
| Rainfall | r | .53 | .47 | .01 | .72** |
| | b | 3.74 | 3.60 | 1.07 | 11.46 |
| Sunshine h. | r | -.07 | -.14 | .14 | -.26 |
| | b | -.43 | -.85 | 2.00 | -4.76 |
| Global rad. | r | -.24 | -.10 | -.37 | .19 |
| | b | -.05 | -.01 | -.14 | .10 |
| | R | .92** | .90** | .79** | .90** |
| | $R^2$ | .84 | .81 | .63 | .81 |
| | a | 157.54 | 188.08 | 244.89 | 97.83 |

Notation of variables.
Dependent variable: Lengths of growth periods, days
Independent variables: Date of sowing
: Temperature, °C
: Rainfall, mm per day
: Sunshine, hours per day
: Global radiation cal/cm$^2$/day

Table 6. Heat sum required by cereal cultivars as influenced by date of sowing, temperature, rainfall, sunshine hours, and global radiation. Partial and multiple correlation and regression coefficients. Notation of variables, see below.

| | | Cultivars | | | |
|---|---|---|---|---|---|
| | | Lise | Møyjar | Runar | Mustang |
| *Sown to headed* | | | | | |
| Date of sowing | r | -.68* | -.38 | -.28 | .12 |
| | b | -4.89 | -2.95 | -2.42 | 1.79 |
| Temperature | r | .73** | .42 | .32 | .01 |
| | b | 43.19 | 24.71 | 18.38 | .38 |
| Rainfall | r | .29 | .33 | -.26 | -.28 |
| | b | 15.27 | 15.99 | -14.43 | -24.35 |
| Sunshine h. | r | -.20 | -.20 | .31 | .44 |
| | b | -7.11 | -9.98 | 32.62 | 97.93 |
| Global rad. | r | -.09 | .13 | -.50 | -.52 |
| | b | -.10 | .14 | -1.55 | -3.66 |
| | R | .74** | .47 | .74** | .75** |
| | $R^2$ | .54 | .22 | .55 | .56 |
| | a | 288.36 | 454.63 | 982.35 | 1 744.15 |
| *Headed to ripe* | | | | | |
| Date of sowing | r | -.02 | -.32 | .05 | .12 |
| | b | -.09 | -1.06 | .20 | 1.53 |
| Temperature | r | -.16 | -.01 | -.64* | -.24 |
| | b | -5.66 | -.15 | -35.37 | -21.11 |
| Rainfall | r | -.12 | .35 | -.02 | .26 |
| | b | -5.86 | 16.16 | -1.11 | 40.86 |
| Sunshine h. | r | -.33 | -.67** | -.23 | .02 |
| | b | -18.44 | -28.90 | -.12 | 3.18 |
| Global rad. | r | -.05 | .47 | -.32 | -.10 |
| | b | -.08 | .41 | -.39 | -.45 |
| | R | .70** | .84** | .89** | .73** |
| | $R^2$ | .49 | .70 | .81 | .54 |
| | a | 835.23 | 628.1 | 1 596.12 | 1 027.76 |
| *Sown to ripe* | | | | | |
| Date of sowing | r | -.42 | -.20 | -.02 | -.39 |
| | b | -3.65 | -1.98 | -.39 | -5.69 |
| Temperature | r | .46 | .16 | .06 | .39 |
| | b | 41.96 | 17.01 | 10.58 | 64.96 |
| Rainfall | r | .53 | .47 | .12 | .73** |
| | b | 55.66 | 52.52 | 19.75 | 169.82 |
| Sunshine h. | r | -.02 | -.15 | .10 | -.29 |
| | b | -1.43 | -13.38 | 21.22 | -76.54 |
| Global rad. | r | -.26 | -.10 | -.32 | .23 |
| | b | -.75 | -.22 | -1.77 | 1.76 |
| | R | .79** | .71** | .59* | .87** |
| | $R^2$ | .62 | .51 | .35 | .75 |
| | a | 955.60 | 1 258.80 | 1 936.10 | -31.75 |

Notation of variables.
Dependent variable: Heat sum, day degrees, °C
Independent variables: Date of sowing
: Temperature, °C
: Rainfall, mm per day
: Sunshine, hours per day
: Global radiation cal/cm$^2$/day

Table 7. Effect of sunshine hours and of global radiation on the lengths of growth periods and on the heat sums. Increase in information in terms of 100 $R^2$.

| | Global radiation | | | Sunshine hours | | |
|---|---|---|---|---|---|---|
| | S – H | H – R | S – R | S – H | H – R | S – R |
| *Lengths of growth periods* | | | | | | |
| Lise | .66 | 7.42 | 5.23 | 1.57 | 11.05* | 4.32 |
| Møyjar | .06 | 0 | 1.61 | .25 | 5.72 | 1.83 |
| Runar | 4.40* | 4.76* | 9.69 | 2.99 | 4.08* | 4.65 |
| Mustang | 5.40 | .29 | .89 | 3.81 | .24 | 1.55 |
| *Heat sums* | | | | | | |
| Lise | 3.36 | 18.76 | 10.96 | 4.86 | 24.76* | 8.19 |
| Møyjar | .04 | .01 | 4.54 | 1.99 | 15.47* | 5.23 |
| Runar | 25.56* | 9.85* | 14.63 | 14.98 | 8.56 | 7.73 |
| Mustang | 20.01 | 1.16 | 1.02 | 13.73 | .72 | 1.98 |

Table 8. The risk of incomplete ripeness of the cultivars. Mean and variation in the date of ripeness and the date of termination of the climatic growth period.

| | Lise | | Runar | | Mustang | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| 1. Date of sowing[1]) | 3.3 | 9.0 | 3.3 | 9.0 | 4.8 | 9.8 |
| 2. Growth periods, days | 94.0 | 5.8 | 107.6 | 8.6 | 103.1 | 9.6 |
| 3. Heat sum, day-degrees | 1 311 | 51.4 | 1 511 | 78.7 | 1 447 | 101.5 |
| 4. Date of ripeness[2]) | 6.6 | 10.9 | 20.1 | 14.8 | 17.6 | 17.0 |
| 5. End of climatic growth period[2]) | 50.0 | 5.2 | 50.0 | 5.2 | 50.0 | 5.2 |
| Diff. between 4 and 5 | 43.4 | 13.2 | 29.7 | 16.5 | 32.3 | 18.5 |
| Days to spare for 5 % risk of incomplete ripeness | 20.8 | | 1.7 | | .7 | |
| Coef. of variation, days | | 6.2 | | 8.0 | | 9.3 |
| Coef. of variation, heat sum | | 3.9 | | 5.1 | | 7.3 |

[1]) No. of days after May 1st.
[2]) No. of days after August 1st.

Based on the results from the 17-year period, the gain in information by adding sunshine hours or global radiation to the three previous independent variables is calculated and the results presented in Table 7.

As can be seen from Tables 5 and 6, and from Table 7, more intense global radiation shortened the growth periods for spring wheat and reduced the heat sums significantly. This is the case for the sowing to heading and the heading to ripeness periods, but not for the whole growth period. For the other cultivars this effect is not significant.

Increased sunshine hours significantly short-

ened the period heading to ripeness, for the cultivars Lise, Møyjar, and Runar. For the two barley cultivars the heat sums are also reduced significantly. For the sowing to heading period there are no significant effects. The oat as did not react significantly to the variation in global radiation or in sunshine hours.

*The risk of crop failure because on incomplete ripeness*

In estimating the chances of the crop attaining complete ripeness, two factors are important. One is the mean date of ripeness of the crop and the annual variation of this date. The variable includes the effects of date of sowing and the effects of all climatic variables which influence the rate of crop development.

The other factor is the mean date of the termination of the climatic growth period and its annual variation. The termination of the climatic growth period for cereal cultivars is assumed to be the date when the decreasing curve of the daily mean temperature reaches 10.0 °C. As a mean of a period of years this date can easily be determined. For each year it may be more difficult because of periodic fluctation in temperature around 10.0 °C at the end of the growth period. In such cases only the days with mean temperature above 10.0 °C are considered.

In Table 8 the risk of incomplete ripeness for the cv. Lise, Runar and Mustang is calculated on the basis of observations from the period 1960 – 85, 26 years in all. The Table shows that the standard deviation (SD) of date of sowing is approximately 9 days and that the SD of the growth periods for the latest cultivars is of the same magnitude. It may also be seen that the relative variation (coefficient of variation) is less for the heat sums than for the growth periods in days required by the cultivars. The variation in heat sums, however, is surprisingly high especially for wheat and oat which react most strongly to variation in rainfall and temperature.

The SD of the date of ripeness, which is the result of the combined variation in dates of sowing and the length of the growth periods, is in the range of 11 – 17 days. The SD of the date of the termination of the climatic growth period, namely 5.2 days, is low compared to the variation in date of ripeness of the crop which varied from 10.9 to 17.0 for the different cultivars.

The difference between the date of ripeness and the date of the end of the climatic growth period and the combined variation of these two factors determine the likelihood of the cultivars reaching ripeness. Accepting a 5 percent chance (one tail test) of incomplete ripeness the cv. Lise had a margin of 20.8 days, while the wheat and the oat cultivars had only 1.7 and 0.7 days, respectively, to spare.

The validity of the calculated risk of incomplete ripeness is based on the assumption that the 26-year period is a random sample of the long time climatic conditions. The daily mean temperature of May – September for the 26-year period was .16 °C above the 100-year average (1874 – 1973). The amount of rainfall, too was slightly above the long time average. The sum effect of these deviations on the available growth period is negligible.

## DISCUSSION

For a comparison of the results presented in sections III and IV with results of other investigations obtained under Nordic growing conditions two papers are of particular interest. One is by Lallukka & Mukula (1977) and the other by Vik (1913). In both papers very detailed observations are presented allowing analysis of some variables by the same technique as described in section II.

*Date of sowing*

The investigations detailed on in this paper show that early sowing lengthens growth periods and increases heat sums for all cultivars for the sowing to heading period. For the heading to ripeness period early sowing has no effect on growth period and heat sum except for oat where late sowing increases both these characters. Lallukka & Mukula (1977), too found that early sowing had a tendency to extend the

sowing to heading periods and increase the heat sums for wheat and barley but not for oat. The heading to ripeness periods were shortended and the heat sums reduced. The effects, however, were not significant.

*Temperature*

Higher temperatures shortened the growth periods by 6 – 8 days for the different cultivars, increased the heat sums of the sowing to heading period, and reduced the heat sums for the heading to ripeness periods. For the whole growth period the effects were negligible. By comparison, Lallukka & Mukula (1977) found that an increase of 1.0 °C in temperature reduced the growth periods by 5 – 6 days for all cultivars. Higher temperature also reduced the heat sums of the heading to ripeness period for all cultivars. For the whole growth period, however, the effects were small. In a 12-year study of spring wheat Vik (1913) found that an increase of 1.0 °C temperature shortened the sowing to ripeness period by 5.2 days. The temperature had no significant effects on the heat sum. Data from 60 outlying yield trials, partly located in low temperature areas, showed that a 1.0 °C increase in temperature reduced the growth periods by 10.0 days for wheat, 10.4 days for oat. 8.4 days for two-rowed barley, and by 7.3 days for six-rowed barley. An insignificant reduction in heat sums was also observed.

*Rainfall*

Higher rainfall delayed growth and increased heat sums in all growth periods for all cultivars. For the barley and wheat cultivars the effect of 1.0 mm per day or approximately 100 mm for the whole growth period, was 3 – 4 days and 50 – 60 day degrees, and for the oats 7 days and 110 day degrees. Lallukka & Mukula (1977) found that a 100 mm rainfall delayed growth and increased heat sums by 1.2 days and 13 day degrees for barley, by 4.3. days and 66 day degrees for wheat and by 7.6 days and 109 day degrees for oat. In his 12 years of observations on wheat, Vik (1913) found that higher rainfall delayed ripening by 7.2 days and increased the heat sum by 102 day degrees for each 100 mm of rainfall during the growth period. In another part of his material the effects were recorded as 1.4 day and 19 day degrees for six-rowed barley, 2.4 days and 33 day degrees for two-rowed barley, 5.6 days and 78 day degrees for wheat, and 5.0 days and 68 day degrees for oat.

*Sunshine hours*

Duration of sunshine had no noticeable effect on growth rate and heat sums in the pre-heading growth period. The ripening periods were shortened by longer duration of sunshine for barley and wheat. For barley the heat sum was also reduced. Oat did not respond significantly to variation in sunshine hours. In the material reported by Lallukka & Mukula (1977) longer duration of sunshine had a tendency to reduce growth periods and heat sums for all cultivars. The effects were strongest for wheat and barley, less for oat.

*Global radiation*

More intensive global radiation shortened growth periods and reduced heat sums significantly for wheat. For the other cultivars no responses were significant.

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# SELECTION FOR LITTER SIZE IN MINK

## I. Background, analyses of the base population and design of the experiment

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Einarsson, E.J. 1987. Selection for litter size in mink. I. *Background, analyses of the base population and design of the experiment.* Norwegian Journal of Agricultural Sciences 1: 131 – 153. ISSN 0801-5341.

In 1978, a selection experiment on litter size in dark mink was started at the experimental farm and lasted for six generations. In addition to the trait of selection, litter size at birth, the study included other reproductive traits, kit postweaning traits, body size and pelt characteristics. Divergent selection, based on an index, was practised for high (H) and low (L) litter size at birth, while a randomly selected line was kept as a control (C). The general selection procedure is described together with analyses of traits from the base population. Predicted selection response per generation in litter size at birth was calculated at 0.12, -0.08 and 0.00 kits in the H-, L- and C-lines, respectively. The expected inbreeding per generation was calculated at about 1.1 percent in the selection lines and 0.7 percent in the C-line. The aim of the experiment was to observe how intensive divergent selection for litter size at birth affect the selected trait itself and to determine correlated responses in other economically important traits.

Key words: Mink, population, reproduction, selection.

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Throughout the history of fur farming the relatively strong selection pressure for fur characteristics and body size in mink breeding have led to a low or sometimes even negative selection differential for litter size. This is thought to be one of the main explanations for the low gain in litter size observed over a long period of time. The economic importance of reproduction in mink necessitates that litter size should be a matter of major concern in the selection of breeding animals. It is therefore necessary to find out how litter size responds to intensive selection and whether such selection affects other economically important traits. On this basis, a selection experiment for litter size at birth in dark mink was started from a large and representative base population. The experiment included divergent selection and a randomly selected control line. The results from the selection experiment, both direct and correlated responses, and the development of an applied selection index of litter size in mink are presented and discussed in the four following papers in this series (II-V). The present paper (Part I) deals with the background of selecting for litter size and the basis of the present selection experiment for litter size at birth in mink.

## SELECTION FOR REPRODUCTIVE TRAITS AND ASPECTS ON REPRODUCTION IN MINK

Reproduction is essential to both wild and domestic populations. It provides the propagation and survival of the species. The fecundity varies from one species to another and is often inversely proportional to the probability that individual offspring will contribute to the next generation. Natural selection for reproductive rate has often resulted in adaption of species and therefore deserves more attention from the animal breeders as well as from the evolutionary biologists (Parkin, 1979).

In domestic animals, many traits, including the reproductive traits, come about by artificial selection. However, it was early argued that litter size was difficult to change by artificial selection, mainly due to the apparent low heritabilities involved. This was also a common point of view in mink breeding.

In 1955, Falconer reported on a successful selection experiment for litter size in mice. In the following years, Falconer and other scientists carried out selection experiments for litter size in mice with varying success. In general, low realized heritabilities were obtained (reviewed by Joakimsen & Baker, 1977). These low heritability estimates for litter size were later shown to be caused partly by maternal effects, or more precisely, by the negative covariance between direct and maternal effects for litter size. After reducing this negative effect by standardizing litter size, Falconer (1963) found a heritability of 0.21 for litter size in mice. Among others, Joakimsen & Baker (1977) obtained a high response when selecting for litter size in mice with standardized litter size. Successful selection experiments for fertility, mainly for litter size and ovulation rate, have also been reported for species such as cattle, sheep, pigs, rabbits and poultry. The relevant literature has been reviewed by Steine (1974), Kolstad (1980), Land (1980), Rouvier (1980) and Mgheni (1982).

Reproduction in mink is as essential as it is in other farm animals. Increased pelt production per breeding animal will increase the farmer's profit through both increased pelt sales and a more efficient utilization of feed, equipment and labour, assuming that increased litter size has no drastic negative effects on other economically important traits.

Experimental work on reproduction in mink was initiated by Hansson (1947) and Enders (1952). Breeding programs, including reproductive traits, have more recently been discussed by Einarsson (1978, 1979a, b), Rønningen et al. (1980), Einarsson & Elofson (1981), Elofson & Einarsson (1984) and Christensen et al. (1984). Many reports have been given on heritability estimates of litter size in mink as reported and reviewed by Einarsson (1981a), but none of them consider genetic parameters on the relationship between litter size and other economically important traits. Lack of knowledge about how litter size responds to intensive selection and how this affects other economically important traits are therefore limiting factors. Although a few genetic analyses on reproduction have been conducted, no selection experiments for reproduction in mink have been reported.

In order to clarify the problems of including litter size in the complete selection program for mink, a selection experiment for litter size at birth was started. The principal aim was to determine the direct response in litter size at birth, and the correlated responses in other traits when practising divergent selection. It was also aimed at estimating the genetic parameters involved.

The possibility of being able to change litter size in mink appears to be promising because of the large and reasonable heritability of the trait and the relatively short generation interval (Einarson, 1981a). Furthermore, there seem to be no negative correlations between direct and maternal effects for litter size (Einarsson, 1981a). This should increase the possibility of changing the litter size in mink and, compared to pigs, allow a simpler selection programme.

As pointed out by King (1966), breeders may have multiple trait objectives in mind, but their effort tends to be concentrated on one single trait. This was stated for poultry, but is even

more relevant in mink breeding. The selection of breeding animals normally takes place in November when the winter coat is judged. The main focus at this time is on fur quality and body size; little attention is paid to fertility which was observed «a long time ago». These facts necessitate the development of a complete and practical selection program for fur bearing animals.

The physiology of reproduction in mink has been extensively studied, emphasizing induced ovulation and delayed implantation (Hansson, 1947; Enders, 1952; Venge, 1973; Møller, 1973; Murphy & Moger, 1977; Pilbeam et al., 1979; Papke et al., 1980; Martinet et al., 1981; Murphy et al., 1981; Einarsson, 1985; Elofson et al., 1988). A complete review on reproductive physiology of female mustelids is given by Murphy (1987).

In the northern hemisphere the mink comes into heat at the beginning of March and the sexual season terminates at the end of the month. As in other mammals, light seems to play an important role in the reproduction of mink. It seems to be the total amount of light that controls the reactions; however, some effect may be caused by variations in light intensity and wave length (reviewed by Mejborn, 1980; Einarsson, 1981b). In the latter review it was concluded that the mink was a short-day breeder, but with the need for successive increase in day length in order to obtain the normal maturity of eggs and sperms. The gametogenesis probably initiates at the embryonic stage, which means that the number of oogonia and oocytes is determined about the time of birth (Murphy, 1987).

Earlier it was believed that cyclic heat periods occured during the breeding season in mink and that matings should take place at the top of these heat cycles. It has been shown, however, that after the female mink comes into heat, she remains in that condition until mating occurs or the breeding season terminates (Elofson et al., 1988). Repeated heat cycles will only occur if one or several matings take place within the breeding season and with an interval of at least six to seven days.

It is not known whether the initial number of follicles persists until mating occurs or if follicular development and atresia go on continuously so that there are enough follicles to be ovulated when copulation takes place (Murphy, 1987). According to Murphy, histological observations support the latter explanation. However, this explanation has to be in accordance with the interval of six to seven days which the new pool of follicles needs for development.

Along with certain other mammals mink have induced ovulation. The inducing factor is normally the act of mating and ovulation occurs on average 48 h post coitum with variations from 33 to 72 h (Hansson, 1947; Enders, 1952; Venge, 1959).

According to Venge (1956) and Adams & Rietveld (1981) a copulation of less than 6 min duration may give reduced reproduction results. The authors speculations were that the increased number of empty females was caused partly by reduced transportation of sperms and partly by too few spermatozoa being ejaculated from the males. Åhman (1966), however, reported the best reproductive result from females with a copulation time of less than 10 min, which he thought was the result of females being mated at the top of one of the cyclic heat periods.

Another peculiarity in the reproduction of the mink is delayed implantation. Fertilization takes place at an early stage during the six days the eggs requires to pass through the oviduct, arriving in the uterus about eights days post coitum. At that time the blastocyst, consisting of about 300 cells, normally delays the development while it becomes almost metabolically inactive until implantation is initiated (Hansson, 1947; Enders, 1952; Duby, 1969; Lanman, 1970). The delay of implantation is caused by delayed function of the corpora lutea (Hansson, 1947; Møller, 1973). The initiating of the corpora lutea is determined by luteotrophic effects of hypophyseal prolactin triggered by photoperiod (Hansson, 1947; Murphy et al., 1980; Papke et al., 1980; Martinet et al., 1981; Murphy et al., 1981). Activation of the corpora lutea results in progesterone production and the plasma

progesterone increases above 10 ng/ml about 32 days prior to parturition (Møller, 1973; Einarsson, 1985). Because of the delayed implantation the length of the gestation period in mink may vary from a minimum of 38 days, as the blastocyst reaches the uterus 8 days post coitum and the embryo requires 30 days from implantation to birth.

Several experiments and analyses have shown that the later the last mating takes place, the shorter the gestation period will be, as a result of shortening the delay before implantation (Pearson & Enders, 1944; Hansson, 1947; Venge, 1973; Elofson et al., 1988). As Einarsson (1985) has pointed out, however, it has to be taken into consideration that a late mating in the estrous period will also delay the date of parturition. Einarsson (1985) found that the date of increase in plasma progesterone was affected by the date of mating. Concannon et al. (1980) claimed that the initial increase in plasma progesterone occurred around the vernal equinox regardless of the time of mating, which would then result in an almost constant date of birth. This is difficult to accept when it is known that the parturition takes place within an interval of about 14 days and that the increase in progesterone must vary over a period of similar length (Einarsson, 1985), since the implantation period is relatively constant (Enders, 1952).

The effect of the length of the gestation period on litter size at birth is often found to be negative, but not always distinct. Some analyses show a scala-dependent effect of gestation length on litter size at birth (Dukelow, 1966; Baud & Hoogerbrugge, 1976; and partly Elofson et al., 1988). Einarsson (1981b) found a significant reduction in litter size with increased gestation length in single mated females, but not in the remated ones. Bowness (1968) and Jonsson (1971) found a relationship between the day of mating and litter size at birth in females mated once, while Hansson (1947) found a certain though irregular tendency towards reduced litter size by increased gestation length.

The implantation zones in the uterus of the mink are clearly visible until about the time of weaning. However, a few zones, especially in the uterus from females losing all embryos, may be difficult to discover even after the uterus has been opened and coloured with Haematoxylin. In an unpublished experiment, Einarsson found that in females sacrificed at different times from parturition to the time of pelting, the only reliable results were found up until weaning and that the zones were impossible to discover in December. The implantation zones indicate the number of implanted embryos from about 23 days prepartum and are valuable in estimating embryonic loss (Hansson, 1947; Nyberg, 1979; Einarsson, unpublished).

There have only been a few investigations into prenatal loss in mink, and the total loss from ovulation to birth varies from 50 to 66 percent of the ovulated eggs (Hansson, 1947; Enders, 1952; Venge, 1966). However, the pre- and postimplantation losses were not examined in the same females. As discussed by Einarsson (1982) and Murphy (1983), there are several factors which have an influence on the survival of the embryo in mink.

In order to get the necessary nutrition each embryo requires a certain space on the uterus wall, called the «embryonic disc». This may limit the number of embryos that will develope fully. The regression of litter size on the number of ovulated eggs, or the number of implanted foetuses, may therefore be curvilinear, with negative regression coefficients for the highest number as found in pigs (Blichfeldt & Almlid, 1982). A previous analysis on mink showed no such negative regression coefficients, indicating that the number of embryos implanted could increase (Einarsson, 1982).

It is known that pig blastocysts have a luteotrophic effect, through the production of estradiol 17-β, from about day 12 up to days 25 to 30. With less than four blastocysts implanted the corpora lutea degenerates and a new ovulation can take place in the female. No such effects are found in mink (Møller, 1973); any effects may possible be more complicated along with delayed implantation. However, a postulation of a trigger or a luteotropic effect from the blastocyst in mink should not be entirely discounted and this merits further investigation.

The accumulated postnatal kit mortality is often found to be about 10 percent when analysing field data (Einarsson, 1986). However, the real figures as observed under controlled experiments usually appear to be about twice as high (Einarsson, 1980b). Late first examination of the litter after birth and inprecise control of the nest box causes underestimated figures.

It has been found that litter size has an influence on body size in mink. The increase in litter size of one kit was found to reduce the body weight at pelting by 20 grams in the males ($p < 0.05$) (Einarsson, 1980a). The same increase in litter size also reduced the body length which resulted in significantly reduced skin length by 0.31 cm in the males and 0.18 cm in the females.

Maternal effects could have an influence on reproductive results, as is known in pigs (Vangen, 1980). However, in mink it was concluded that under normal conditions there is no evidence of any negative correlation between direct and maternal effects on litter size, either genetic or environmental (Einarsson, 1981a).

## ANALYSIS OF THE BASE POPULATION

The experiment was carried out at the experimental fur farm, Institute of Animal Science, Agricultural University of Norway. Some dark mink of the jetblack genotype were introduced into the standard mink population at the experimental farm from 1967 to 1969. The population of dark mink at the experimental farm has been relatively closed since 1970, with the introduction of only a few males into the population during the recent years. However, the population is assumed to be relatively outbred, with no indications of inbreeding depression. This population of dark mink was the basis for the selection experiment for litter size and is referred to as the base population. In addition to that of 1978 two analyses conducted by Reiten for the period 1972 – 75 and by Einarsson for the period 1975 – 77 were included. The specific population of dark mink in 1878, where the first selection took place, is referred to as the foundation stock. The number of parturient females per year was on average 350 in the period up to 1978.

The number of females in the foundation stock is shown in Table 1. Females of the genotype finblack and females mated with two different males were excluded. The breeding animals forming the foundation stock were of different ages as shown below;

| Age, years | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| No. of males | 36 | 19 | 14 | 3 | 4 |
| No. of females | 111 | 84 | 35 | | |

Compared to the one-year-old females the litter size as birth was 0.5 kits higher in the two-year-

Table 1. Number of females available in the foundation stock (1978).

| | | |
|---|---|---|
| Dark mink females of the standard and jetblack genotypes | | 299 |
| Died before mating | 7 | |
| Unmated | 2 | |
| Died between mating and parturition | 6 | |
| Mated females until parturition | | 284 |
| Barren females | 41 | |
| Litters in which all kits were lost preweaning | 12 | |
| No. of females with litters at weaning | | 231 |

old females and 0.4 kits lower in the three-year-old females. Each male mated from one to ten females, with the average at three.

The breeding animals in 1978 were used in feeding experiments, but the selection procedure was conducted so that it would not be influenced by different feeding regimes. An outline of the results from some of these feeding experiments is given by Skrede (1981a, b).

*Description of traits*

*a) Reproductive traits*

The reproductive traits are presented in Table 2. The traits were observed from 284 females during the mating season and from 231 parturient females in the foundation stock (see Table 1). The distribution of litter size at birth in the foundation stock is presented in Figure 1. Only 0.7 percent of the females refused to mate and 15.1 percent of the females were empty.

The mating began on March 6 and terminated on March 25. The females were carried to the male's cage for mating. When the first mating took place before March 18, the females were given the opportunity to remate 8 – 10 days later. If the first successful mating took place on or after March 18, the females were allowed to remate the day after.

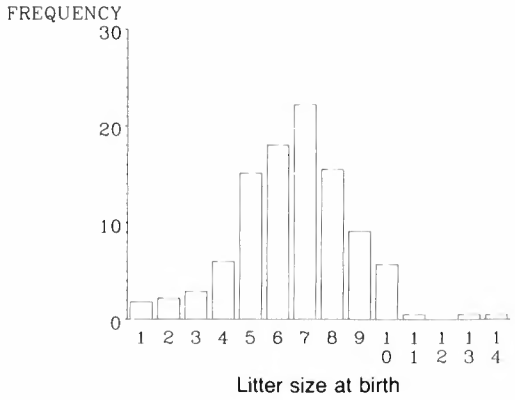


Fig. 1. Distribution of litter size at birth per whelped female in the foundation stock.

Table 2. Reproductive traits in the foundation stock, means and standard deviations (SD).

| | means | SD |
|---|---|---|
| Remated females, % | 72.5 | |
| First mating; | | |
| Date[a] | March 11 | 4.8 |
| Length of copulation, min | 35 | 22 |
| Length of gestation from first mating, days | 52 | 5.1 |
| Second mating; | | |
| Date[a] | March 19 | 2.3 |
| Length of copulation, min | 65 | 30 |
| Length of gestation from second mating, days | 44 | 2.7 |
| No. of kits per parturient female | | |
| Total at parturition | 6.58 | 2.06 |
| Alive at parturition | 6.05 | 2.03 |
| Alive at 7 days postpartum | 5.45 | 2.07 |
| Alive at 21 days postpartum | 5.40 | 2.22 |
| Alive at 42 days postpartum (weaning) | 5.35 | 2.20 |
| Mortality from birth to weaning, %[b] | 18.8 | |
| No. of kits at weaning, per mated female | 4.33 | |

[a] Standard deviation in days.

[b] Including dead kits at first examination.

The litters were observed and the kits counted as soon as possible after birth, and generally before 10 hours postpartum. The nest boxes were thoroughly searched to check on the total number of kits born. The kits were counted again 2, 7, 21 and 42 days postpartum. Each mother nursed her own kits, all kept together until the time of weaning at 42 days postpartum. At 21 days postpartum the kits were sexed. They were resexed at 42 days and individually marked at the same time.

Kit mortality in the foundation stock is presented in Table 2 and the results are in accordance with the figures from other populations, as reported by Einarsson (1980b).

The reproductive results at the experimental farm throughout the years are shown in Figure 2. In spite of the fluctuations, the observations were representative of a mink population, though with slightly better results compared with those from Norwegian mink farms (Streitlien , 1978; Einarsson, 1986).

*(b) Body weight and body length*

Body weights of both kits and breeding animals in the foundation stock are presented in Table 3 together with body length. The body weight at birth and at 21 days postpartum was based on litter weight, i.e. the total litter weight was divided by the total number of kits weighed. Standard deviation was therefore not calculated and sex differences were not observed. Body weight at weaning and at later points of time were individually measured. The body weight of the selected animals in Table 3 was taken from parturient females and breeding males.

The development of body weight of the kits in the foundation stock is presented in Figure 3, which also shows the average body weight for the period 1970 – 76. The increase in body weight at pelting from 1970 was on average 42 grams and 27 grams per year for males and females, respectively.

An analysis of the base population, based on the years 1975 – 78, showed that on average

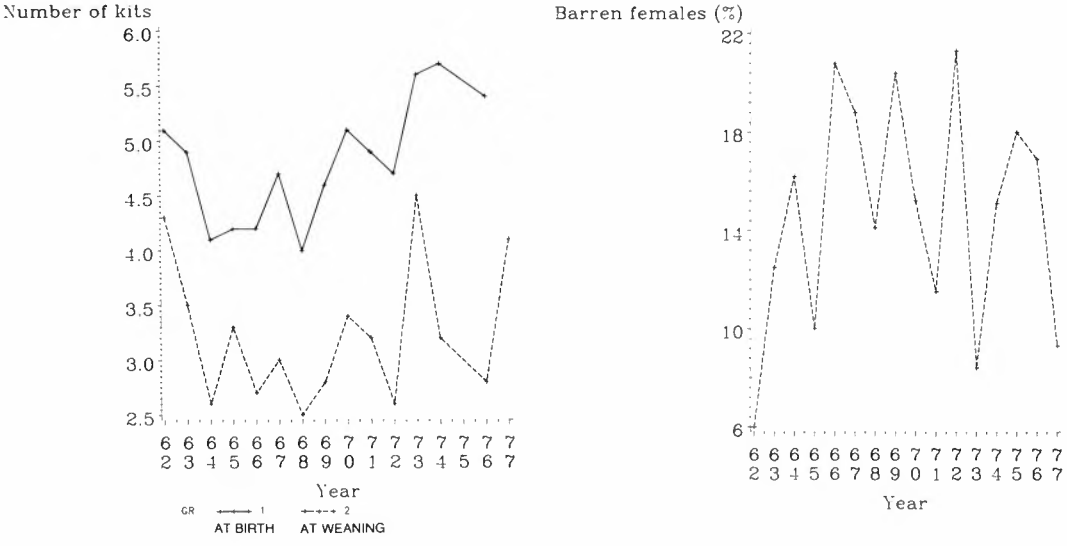


Fig. 2. Reproduction results at the experimental farm during the years 1957 – 77. Up to 1969 the figures included all colour types, from 1970 dark mink only. (Figures from 1957 to 1969 by Rimeslåtten, 1971). The Figure to left shows litter size at birth per parturient female and litter size at weaning per mated female, while the Figure to right shows the percent of barren females.

the male body weights were reduced by 11.1 grams at weaning and by 20 grams ($p<0.05$) at pelting, when litter size at birth increased by one kit (Einarsson, 1980a). The corresponding reductions in female body weight were not significant at pelting, but significantly reduced by 9.4 grams at weaning ($p<0.01$). The reductions were not so marked in litter sizes of 3 to 7 kits. Variation in litter size explained 0.5 – 1.5 percent of the variation in body weight at pelting. These results concurred with earlier investigations (Venge, 1960; Hoogerbrugge & Baud, 1975; Reiten, 1975).

The body length of both males and females increased by about 1.5 cm during the last 20 years, according to earlier analyses at the experimental farm (Rimeslåtten reported by Reiten, 1978; Einarsson, 1980a). In a previous analysis on the base population, body length was found to be reduced by 0.15 cm for the males and 0.08 cm for the females, respectively, when litter size was increased by one kit (Einarsson, 1980a). These reductions were halved when including litter size of 3 to 7 kits only. The results were in agreement with earlier investigations at the experimental farm (Rimeslåtten, 1961; Reiten, 1978).

Table 3. Body weight and body length in the foundation stock, means and standard deviations (SD).

| | Males | | | Females | |
|---|---|---|---|---|---|
| | means | SD | | means | SD |
| Body weight of kits, g | | | | | |
| Birth (within the first day)[a] | | | 10.4 | | |
| 21 days postpartum[a] | | | 119 | | |
| 42 days postpartum (weaning) | 377 | 93 | | 329 | 66 |
| July 5 | 841 | 119 | | 632 | 78 |
| August 17 | 1 452 | 226 | | 922 | 135 |
| September 28 | 1 906 | 302 | | 1 116 | 157 |
| October 19 | 2 054 | 307 | | 1 190 | 163 |
| At pelting, November 22 | 2 085 | 334 | | 1 169 | 170 |
| Body length at pelting, cm | 43.9 | 1.6 | | 38.1 | 1.3 |
| Body weight of parturient females, g | | | | | |
| December 2 | | | | 1 090 | 164 |
| January 12 | | | | 1 026 | 122 |
| March 2 | | | | 945 | 113 |
| Parturition | | | | 1 074 | 113 |
| 21 days postpartum | | | | 1 010 | 143 |
| 42 days postpartum (weaning) | | | | 941 | 130 |
| Body weight of breeding males, g | | | | | |
| December 8 | 2 086 | 213 | | | |
| January 24 | 2 150 | 271 | | | |
| March 2 | 2 155 | 190 | | | |

[a] Based on total litter weight and average of both sexes.

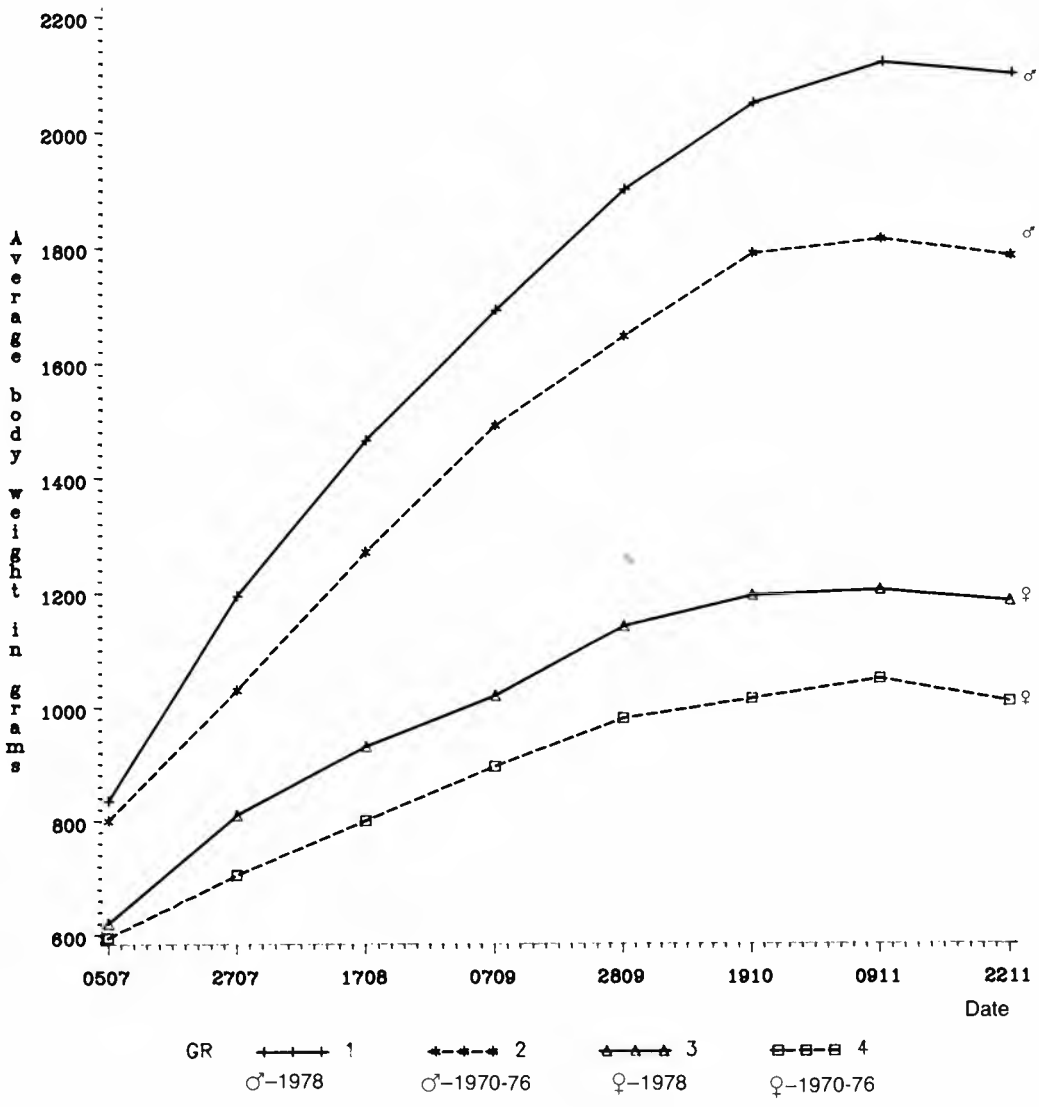


Fig. 3. Growth curves of mink kits, males and females. (Figures from 1970 til 1976 by Reiten, 1978).

*(c) Pelt characteristics*

Pelt characteristics of the foundation stock are presented in Table 4 and include 317 males and 346 females which were pelted at the farm in 1978. Studies of the quality characteristics of the winter fur were mainly based on dried undressed skins. The judging and measurements were practised according to the methods outlined by Reiten (1976, 1977a) and revised by Skrede (1978). The general fur quality was judged by professional fur graders at the Oslo Fur Auctions. All other traits were judged and measured at the experimental farm. A more detailed description of the traits is given below.

Skin length: Length of the skin measured from tip of the nose to base of the tail.

Skin weight: The weight of fleshed and dried skin.

Fur length: Average length of hairs, separate for guard hairs and underfur. Measured on the back, about 8 cm from base of tail.

Fur density: Number of hairs per unit area, separate for guard hairs and underfur. Subjectively judged from 0 (poorest) to 10 points (best).

Fur colour: Subjectively graded from 1 (black) to 7 points (pale).

Hair quality: Expresses the smoothness and fineness of the hairs (silkiness). From 1 (poorest) to 10 points (best).

Metallic: A fur anomaly characterized by irregular light reflection caused by abnormal guard hairs. Subjectively judged from 0 (free of metallic) to 5 points (poorest).

Wet belly: Caused by urinary tract disorder. Occurence and severity was judged from 0 (no wet belly) to 5 points (severe wet belly).

General fur quality: Judged according to the standard quality grading at the Oslo Fur Auctions. Subjectively graded from 0 (poorest) to 4 points (best). 0 = III grade; 1 = II grade; 2 = I grade; 3 = Saga; 4 = Saga selected.

Table 4. Pelt characteristics in the foundation stock, excluding the selected breeding animals, means and standard deviations (SD).

| | Males | | Females | |
|---|---|---|---|---|
| | means | SD | means | SD |
| Skin length, cm | 71.6 | 4.20 | 59.9 | 3.81 |
| Skin weight, g | 145 | 22.9 | 83 | 12.8 |
| Length of guard fur, mm | 22.9 | 1.51 | 21.5 | 1.25 |
| Length of underfur, mm | 14.3 | 0.88 | 13.6 | 0.86 |
| Density of guard fur, points | 4.8 | 0.79 | 5.2 | 0.80 |
| Density of underfur, points | 4.7 | 0.80 | 5.1 | 0.90 |
| Fur colour, points | 4.7 | 0.95 | 4.7 | 0.93 |
| Hair quality, points | 4.5 | 0.84 | 5.2 | 0.88 |
| Metallic, points | 1.4 | 1.22 | 0.6 | 0.86 |
| Wet belly, points | 1.2 | 1.22 | 0.1 | 0.48 |
| General fur quality, points | 2.4 | 0.84 | 2.4 | 0.90 |

See text for definition of points.

The judging of fur colour was carried out at the experimental farm and also at the Oslo Fur Auctions. The correlation between these respective assessments was 0.64 over the two sexes, but 0.68 and 0.61 for males and females, respectively.

Einarsson (1980a) found that the skin length was significantly reduced by 0.31 cm for males and 0.18 cm for females when litter size at birth increased by one kit. Except for the skin weight of the males no effect of litter size on other pelt characteristics was observed.

*Genetic and phenotypic parameters of the traits*

In order to give a more detailed presentation of the base population, analyses of genetic and phenotypic parameters from previous years at the experimental farm were included. Body weight, body length and fur characteristics were analysed by Reiten (1977b, c) and litter size was studied by Einarsson (1981a).

The estimates of heritability for litter size at birth were based on 960 females at the experimental farm in 1975 – 77 and are presented in Table 5. The average heritability estimate was 0.2, while the average litter size at birth was 5.7, 5.4 and 6.0 for the three years, respectively. As discussed by Einarsson (1981a), using regression of daughter on dam for estimating heritability for litter size in mink was preferable with limited family size, since the correlation between direct and maternal effects for litter size could be disregarded. The estimation method using daughter nested within dam and sire could also be preferred, but on average it requires more than two daughters per dam and more than three dams per sire.

Genetic and phenotypic parameters on body weight, body length and pelt characteristics of dark mink at the experimental farm during the years 1972 – 75 were reported by Reiten (1977b, c). The number of animals included in

Table 5. k-values and heritability estimates (± S.E.) for litter size at birth in dark mink at the experimental farm (Einarsson, 1981a).

| Year | Age (y.) | Method 1[a] | | | | | | Method 2[a] | Method 3[a] |
|---|---|---|---|---|---|---|---|---|---|
| | | $k_1^b$ | $k_2^b$ | $k_3^b$ | $h_S^2$ | $h_D^2$ | $h_{S+D}^2$ | $h^2$ | $h^2$ |
| 1975 | 1 – 4 | 1.31 | 1.51 | 2.60 | 0.21±0.28 | -0.40±0.59 | -0.08±0.25 | 0.13±0.10 | |
| | 1 | | | | | | | 0.09±0.21 | |
| | 2 – 4 | | | | | | | 0.27±0.27 | |
| 1976 | 1 – 4 | 1.37 | 1.44 | 3.09 | 0.21±0.26 | 0.13±0.53 | 0.17±0.24 | 0.25±0.14 | |
| | 1 | | | | | | | 0.30±0.28 | |
| | 2 – 4 | | | | | | | 0.11±0.33 | |
| 1977 | 1 – 4 | 1.36 | 1.52 | 3.22 | -0.07±0.24 | -0.07±0.59 | -0.07±0.26 | 0.27±0.13 | |
| | 1 | | | | | | | 0.27±0.35 | |
| | 2 – 4 | | | | | | | 0.48±0.30 | |
| 1975 – 1976 | 1 – 4 | | | | | | | | 0.29 |

[a] Method 1: Daughter nested within dam and within sire.
Method 2: Regression of daughter on dam.
Method 3: Regression of daughter on granddam.

[b] $k_1$ = number of dams within sire
$k_2$ = number of daughters within dam
$k_3$ = number of daughters within sire.

Table 6. Heritability estimates of body weight, body length and pelt characteristics (Reiten 1977c).

| | $h_s^2$ | |
|---|---|---|
| | Males | Females |
| Body weight[a] | 0.42* | 0.48* |
| Body length[a] | 0.52* | 0.43* |
| Skin length | 0.48* | 0.43* |
| Skin weight | 0.56* | 0.50* |
| Length of guard fur | 0.80* | 0.71* |
| Length of underfur | 0.62* | 0.63* |
| Density of guard fur | 0.25* | 0.19* |
| Density of underfur | 0.37* | 0.29* |
| Fur colour | 0.45* | 0.47* |
| Hair quality | 0.64* | 0.16 |
| Metallic | 0.64* | 0.35* |
| Wet belly | 0.25* | 0.00 |
| General fur quality | 0.41* | 0.37* |

[a] Measured on live animals in November. All other traits were measured on the skins.
* Heritability estimates larger than twice the mean error.

Table 7. Phenotypic correlations between different pelt characteristics. Males below and females above the diagonal (Reiten 1977b).

| | Skin length | Skin weight | Length of guard fur | Length of underfur | Density of guard fur | Density of underfur | Fur colour | Hair quality | Metallic | Wet belly | General fur quality |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Skin length | | .67 | -.10 | -.08 | -.04 | -.01 | .02 | .08 | .14 | .10 | -.40 |
| Skin weight | .82 | | .02 | .10 | .08 | .21 | -.02 | .03 | .08 | .12 | .07 |
| Length of guard fur | -.07 | .07 | | .52 | .03 | .10 | .12 | -.15 | -.13 | -.06 | .02 |
| Length of underfur | .01 | .15 | .61 | | .16 | .22 | -.01 | .10 | -.09 | .03 | .18 |
| Density of guard fur | -.16 | -.08 | -.12 | .06 | | .37 | -.18 | .33 | -.02 | -.04 | .35 |
| Density of underfur | .03 | .23 | .02 | .16 | .27 | | .00 | .20 | -.03 | .00 | .33 |
| Fur colour | .06 | .04 | .13 | .05 | -.13 | .01 | | -.15 | -.20 | .15 | .03 |
| Hair quality | -.11 | -.16 | -.08 | .17 | .12 | .10 | .12 | | -.21 | -.16 | .33 |
| Metallic | .24 | .25 | -.16 | -.14 | -.16 | -.01 | -.24 | -.13 | | .07 | -.20 |
| Wet belly | .07 | .04 | -.05 | -.04 | -.02 | -.15 | .10 | -.17 | .00 | | -.14 |
| General fur quality | -.11 | -.04 | .21 | .03 | .38 | .25 | .02 | .39 | -.12 | -.15 | |

these analyses were 2 452 males and 2 396 females. The numbers of skins were 2 153 from males and 1 585 from females. Table 6 shows the heritability estimates of pelt characteristics based on half sib correlations.

Heritability estimates based on the dam component were generally higher than those obtained using the sire component. This indicates maternal influence on the traits.

Phenotypic correlation coefficients between body weight, body length and pelt characteristics were estimated by Reiten (1977b) and are presented in Table 7. There are positive correlations between the different quality traits and general fur quality. However, negative correlations were found between skin length and general fur quality for both sexes and between skin length and length of guard fur, density of guard fur and hair quality for the males.

## DESIGN OF THE SELECTION EXPERIMENT

From the 231 litters in the foundation stock, 60 females and 25 males were selected for each line based on the size of the litter in which they were born. After the selection lines were established, selection took place within closed lines only, as shown in Figure 4. The total number of animals to be used in the experiment was limited to about 150 whelping females per year.

The selection trait in the experiment was litter size at birth, defined as the total number of kits per litter observed at the first examination. One-year-old females and males were used in the experiment to keep the generation interval at just one year. However, in the last generation half of the breeding females were two-year-olds. Two selection lines were established, one for increased litter size (H) and one for decreased litter size (L), and in addition an unselected line was kept as a control line (C).

Just as in similar experiments on many other species, the selection in the present experiment was based on female fertility. The breeding animals were selected according to a reproduction trait measurable in the female only. Litter size

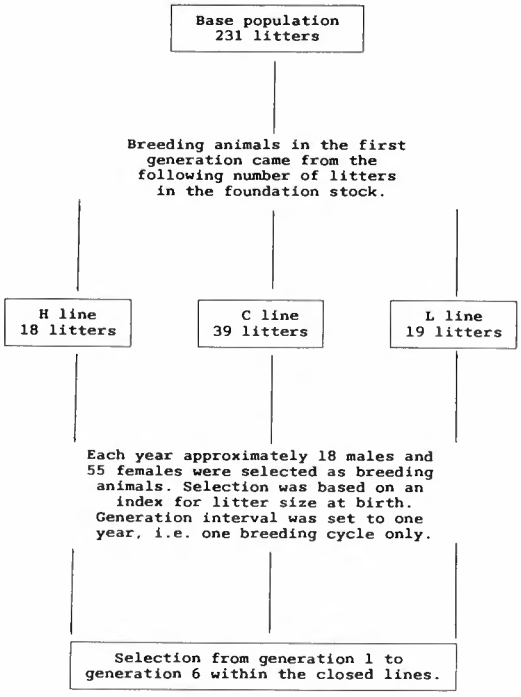


Fig. 4. Outline of the selection experiment.

at birth means the final result of a series of different factors, starting with the ovulation rate (Einarsson, 1980b). Litter size at birth was chosen as the selection trait since it was the first phenotypic reproduction trait to be observed. Although density, mobility and the fertilizing capacity of the semen are important factors affecting litter size, male fertility was not directly included in the selection and thereby regarded as a random effect. However, any correlation between female and male fertility could through the selection have an influence on the male reproductive characters also.

To reduce the rate of inbreeding, a maximum of one male and three females were selected from the same litter in the selection lines, except in the last generation and partly the year before. In order to check environmental trends which could affect the traits, an unselected control line was established. In the present experiment, the method of Gowe et al. (1959) and King et al. (1959) was used to set up the control

line. Each male was to be represented in the next generation by one son and a number of daughters equal to the total number of breeding females divided by the number of breeding males, expressed as the proportion of dams to sires (D/S). Each female was to be represented by one daughter and the probability of being represented by a son was the proportion of the number of sires to the number of dams (S/D). The animals selected according to this method were randomly selected within sire and dam. Mating between close relatives was avoided in all the lines.

The selection of breeding animals was based on information from relatives, combined in a selection index. The selection index used was constructed according to the recommendations given by Hazel (1943), Osborne (1957a, b) and Henderson (1963).

The indices were based on litter size information from the full- and half sisters of the breeding male and female (sire and dam) in addition to the dam's own record. This is the optimal use of information with a fixed generation interval of one year. The pedigree index of the kits was based on the index of the dam (D) and the sire (S), assuming no relationship between them.

$$I_D = b_1(P_1 - \bar{P}) + b_2(\bar{P}_2 - \bar{P}) + b_3(\bar{P}_3 - \bar{P})$$
$$I_S = b_4(\bar{P}_4 - \bar{P}) + b_5(\bar{P}_5 - \bar{P})$$

The indices were standardized to a mean of 100, with a standard deviation of 10. The pedigree index for the litter was then calculated as:

$$I_P = \tfrac{1}{2}(I_D + I_S)$$

where,

$\bar{P}_1$ = litter size of the dam (in which the kits were born)

$\bar{P}_2$ = mean litter size of dam's full sisters

$\bar{P}_3$ = mean litter size of dam's half sisters

$\bar{P}_4$ = mean litter size of sire's full sisters

$\bar{P}_5$ = mean litter size of sire's half sisters

$\bar{P}$ = mean litter size of the selection line for each respective generation

$b_i$ = partial regression coefficient (i = 1, 2, 3, 4, 5).

The number of full sisters in the present study varied from 0 to 2, excluding the dam herself, and the number of half sisters varied from 0 to 6, excluding the dam and her full sisters. The parameters used in the index calculation were: $h^2 = 0.15$, $\sigma_P = 2.10$, $\sigma_G = 0.81$ and $\sigma_{om} = 0$ (Einarsson, 1981a), where $h^2$ is the heritability, $\sigma_P$ and $\sigma_G$ the standard deviation of phenotype and genotype, respectively, and $\sigma_{om}$ the covariance component between the direct and the maternal effect for litter size. The computer program SELIND, developed by Cunningham (1968), was used to calculate the b-values in the index.

The use of control lines in selection experiments was discussed by Hill (1971, 1972a, b, c, 1980), who concluded that control lines in general seemed to serve a useful function. Compared to other farm animals and especially laboratory animals, the environment of fur animals is less controlled with regard to housing, management and food. It is obvious therefore that environmental trends could affect the traits studied in the experiment. The use of an unselected control line was therefore considered desirable in order to make allowance for environmental effects, especially when comparing results over generations.

It is favourable to practice divergent selection when symmetric response is assumed, and especially concerning a trait of low heritability, with a small population and with an experiment carried out over a relatively limited time (Hill, 1980). The use of two divergent selection lines and a control line has been the traditional design of such selection experiments in selecting for litter size, used among others by Joakimsen & Baker (1977) in their experiment with mice.

Hill (1980) discussed the use of replicates in selection experiments. There are advantages in using replicates, an example being that the variances among the lines can be estimated directly and independently of the parameter estimates. The problem of using only two replicates of

each line, is that the variance for the lines has to be estimated, with the number of replicates minus one degree of freedom (Hill, 1980). However, to obtain sufficient data for estimating genetic parameters (Robertson, 1959a, b), the population used in the current experiment allows for a maximum of three lines. The number of animals in each line had also to be above a minimum in order to avoid a rapid increase in inbreeding.

When selecting for reproductive traits, with relatively low heritability, it is important to increase the accuracy of the selection (Maijala, 1971). This can be done by using information about litter size from different relatives (Osborne, 1957a, b). The correlation between the calculated breeding value and the true breeding value will then be increased. Furthermore, another advantage of using an index with information from relatives, is that there is the possibility of calculating indices for both sexes for traits measurable in one sex only.

As discussed by Liljedahl et al. (1979), the performance of a female breeding candidate can be included in, or excluded from her full sister's mean, and the full sister's mean can be included in, or excluded from her half sister's mean. These models were called the including and excluding models. Quite different P-matrices will be obtained by each of these two models, though the correlation between the index and the true breeding value will be the same. By using the including model, the sibling group will consist of both full and half sisters and the average relationship within the sire group will differ by 0.25. Einarsson's (1981a) calculation of the relationship was from 0.28 to 0.32 for half sib groups in mink. The relatively high figures and the variation in relationship, due to family structure, favour the use of the excluding model. In the present experiment the excluding model was used, having the advantage of the simplest P-matrix and a relationship of 0.5 and 0.25 within the full and half sister groups, respectively.

The animals were kept under conventional ranch facilities and management conditions. From weaning, at 6 weeks of age, until pelting, two animals of each sex were kept together in all-net cages. After pelting time the breeding animals were housed individually. Before whelping double bottoms were inserted into the female cages, and the wooden nest boxes were filled with hay and wood shavings. Observations on reproductive traits, body size and fur characteristics were generally as described for the foundation stock.

*Feeding*

Adult animals were fed once a day on the top wire. The kits were fed on a plate at the cage bottom from 25 days postpartum. The ingredients and calculated contents of the diet are shown in Table 8. The diets were composed specially for the selection experiment and were used throughout the entire experiment. The quality of the ingredients may vary from one batch to another, especially in the slaughter offal. The calculated contents could therefore differ slightly, but the varity was apparently limited.

From December until mating the animals were on restricted feeding in order to be in the right condition for the mating season. From mating until parturition the animals were given amounts of feed slightly below the level of voluntary consumption. *Ad libitum* feeding was practised from parturition. Water was supplied *ad libitum*.

*Health conditions*

Only animals that were in normal healthy condition were used in the experiment. Obviously sick animals, including those with «extreme wet belly», were excluded. The animals were tested for plasmacytosis (Aleutian disease) using both the non-specific Mallen-test and the specific agar-test during the selection experiment. In November 1978 the results from all kits in the farm showed 27 percent Mallen-positive reactors, while the specific agar-test showed 88 percent serapositives.

Animals that died during the experiment were necropsied at the National Veterinary Institute, Oslo.

Table 8. Feed composition (A. Skrede).

| Diet | Breeder diet (approx. Dec. 10 – June 25) | Grower diet (approx. June 25 – Dec. 10) |
|---|---|---|
| *Ingredients (%)* | | |
| Fish filleting scrap | 29.6 | 22.0 |
| Fish meal | 5.0 | 6.0 |
| Slaughter offal | 20.6 | 10.0 |
| Blood (cattle and pigs) | 4.6 | 5.0 |
| Liver (cattle and pigs) | 5.7 | – |
| Precooked cereal | 6.2 | 12.0 |
| Lard | 2.8 | 5.0 |
| Vitamin mixture[a] | 2.3 | 2.0 |
| Water | 23.2 | 38.0 |
| *Calculated contents (% of ME)*[b] | | |
| Protein | 44 | 34 |
| Fat | 44 | 46 |
| Carbohydrate | 12 | 20 |

[a] Containing grass meal, brewers yeast, Torula yeast and wheat bran fortified with the following vitamins per 100 g: Vit. A, 30 000 I.U.; vit. $D_3$, 3 000 I.U.; DL-α-tocopherol acetate, 60 mg; thiamine $NO_3$, 20 mg; riboflavin, 12 mg; niacinamide, 12 mg; Ca-pantothenate, 12 mg; pyridoxine HCl, 12 mg; folic acid, 1 mg.
[b] ME = metabolisable energy.

## PREDICTION OF CHANGES DURING SELECTION

The expected selection response in litter size at birth per generation was calculated using the formula:

$$\Delta G = \frac{r_{TI} \cdot i_I \cdot \sigma_G}{L}$$

where

$\Delta G$ = expected genetic response in the litter size at birth

$r_{TI}$ = correlation between the calculated index and the true breeding value (the square root of the «heritability» of the index)

$i_I$ = difference between the mean of selected animals and population mean divided by the standard deviation, all in index units

$\sigma_G$ = genetic standard deviation of litter size at birth

L = generation interval

The generation interval was fixed at one year and the genetic standard deviation of litter size at birth set at 0.81. Selection intensity was expressed by selection differences in standard deviations of the index ($i_I$) and calculated separately for each sex and line. The selection intensity was adapted to allow replacement of the lines only. The average index value for each line was calculated to include all litters present at weaning. Litters in which all kits died before weaning were consequently excluded, as they could not contribute to the following generation. The selection of breeding animals was based on between litter selection according to the index. According to this procedure, the average index value for the breeding animals of each line was calculated over litters. If the cal-

culated mean index value had been based on selected individuals, then the figures would have been the same for the sires, because only one male was selected per litter. For the females, however, the figures would have been about similar in the C- and H-lines, but somewhat higher in the L-line. In the L-line, the number of kits selected from each litter would increase with increased litter size, resulting in an increased average value of the index.

The expected figures of the family structure and the calculated $r_{TI}$-values are given in Table 9 for both sexes within each of the three lines. The average family structure differs somewhat within the lines throughout the experiment, a fact which is often caused by variation in the distribution of litter size.

The predicted selection response in litter size at birth is given in Table 10. The difference in the absolute value of the predicted response between the selection lines was mainly thought to be an effect of the difference in selection intensity. This is partly explained by the limited number of kits from small litters (low indices) compared to the number of kits from large litters. The level and the distribution of litter size in the base population could thus influence the responses in the selection experiment. The selection intensity was assumed to be about equal in both sexes because of between-litter selection and the selection of only one male per litter. The difference in the expected changes of litter size between the sexes was due to the difference in the accuracy of the dam and sire index, as the selection trait is measurable in females only.

As pointed out by Hill (1971, 1980), one of the most important criteria in selection experiments for estimating genetic parameters is that there should be no change in the genetic variances and covariances within the population during the selection. Another important factor is that inbreeding is kept at a low level. The expected inbreeding in the L- and H-lines was calculated by the formula;

$$\Delta F = \frac{1}{8 \cdot N_S} + \frac{1}{8 \cdot N_D}$$

Table 9. The expected average number of full sisters and half sisters (excluding model) and the estimated correlation between the calculated index and the true breeding value ($r_{TI}$).

| Line | Sex | Ind.obs. | No. of full sisters | No. of half sisters | $r_{TI}$ |
|---|---|---|---|---|---|
| Low | Female | 1 | 1.0 | 2.4 | 0.22 |
| Low | Male | 0 | 1.6 | 1.8 | 0.13 |
| Control | Female | 1 | 0.5 | 2.1 | 0.21 |
| Control | Male | 0 | 1.0 | 2.7 | 0.12 |
| High | Female | 1 | 1.4 | 2.8 | 0.23 |
| High | Male | 0 | 1.7 | 2.7 | 0.14 |

Table 10. Predicted selection response for litter size at birth per generation in the three lines.

| Line | Selection response | | |
|---|---|---|---|
| | Dams | Sires | Average |
| Low | -0.09 | -0.06 | -0.08 |
| Control | 0.0 | 0.01 | 0.00 |
| High | 0.13 | 0.11 | 0.12 |

where $\Delta F$ is the increase in inbreeding per generation, $N_S$ is the number of sires and $N_D$ the number of dams per generation.

These calculations based on the number of breeding animals in the experiment show that the average inbreeding per generation would be approximately 1.1 percent in both the selection lines.

It is important to know how inbreeding develops during a selection experiment, especially when selecting for reproductive traits. It is known that ovulation rate declines in mice by increased inbreeding of the dam and that preimplantation mortality increases due to increased inbreeding in both the embryo and the dam (McCarthy, 1967). Inbreeding may also affect maternal influence, expressed as maternal effects (reviewed by Einarsson, 1977). After three generations of full sib mating in mink, there was an average reduction of 0.5 kits at weaning per mated female per 10 percent increase in the inbreeding (Johansson, 1969).

Since the control line is needed to account for environmental trends, the stability of this line is essential in estimating genetic change in the selection lines. When using the previously mentioned design of a control population, the rate of inbreeding can be calculated by the formula (Gowe et al., 1959).

$$\Delta F = \frac{3}{32 \cdot N_S} + \frac{1}{32 \cdot N_D}$$

The inbreeding in the C-line was estimated to be 0.7 percent per generation. It should be pointed out that the assumption that each male should be represented by the number of daughters equal to the number of dams divided by the number of sires in the line (D/S), cannot always be fulfilled in mink breeding. It may also happen that a sire has only daughters or a dam has no daughters at all. The calculated inbreeding can therefore be underestimated. However, the inbreeding in the C-line will be lower than in the selection lines. The estimated inbreeding may also be affected by how successfully the random mating is conducted.

Hill (1971) stated that the accuracy of a realized heritability estimate is largely a function of the total number of individuals recorded and selected in the entire experiment. This plays a more important role than the duration of the experiment or the number of respective individuals recorded per generation. There is little advantage in larger numbers in the last generation. In the current experiment the aim was to keep the same number of breeding animals in all the lines and over the entire experiment.

The proportion between the drift variance and the sampling error of measurement may be estimated as the number of individuals tested in the line (M) divided by the effective population size (Ne) (Nordskog et al., 1974). In the present experiment about 45 females per generation were expected to give birth in the control line and Ne was then calculated at 72. The ratio of M/Ne at 0.63, is probably underestimated because of overestimation of Ne in the control line. Nevertheless, this figure indicates that the error variance of individual measurements is higher than the drift variance. However, one should bear in mind that genetic drift accumulates over the generations. According to Hill (1972a), it is impossible to separate genetic change from environmental change when using only a single control line. A stable environment will give indications but no proof of genetic change.

Directional change may occur in the control line as a result of natural selection. Hill (1972b) comments on a paper by Dickerson (1965), who points out that there was little evidence of such change except perhaps during the one or two first generations of relaxation after selection. In the current experiment the selection trait was not under intense selection in the base population, and both the control and the selection lines came from the same base population. This implies that only small or negligible directional changes are likely to be involved in the present control line.

The variance of the selected trait can change during selection. According to Hill (1972c), the variance with unlinked loci will be reduced by a total of about $h^2 \cdot \sigma^2/2$ for a wide range of selec-

tion intensities. On the other hand, Robertson (1977) stated that, assuming litter size is controlled by polygenes, the variance and response will not be reduced by selection since the change in initial frequency at each locus is small. This problem is also discussed by Fimland (1979), based on Bulmer's theory (1971). Fimland pointed out that the additive genetic parameters stabilize after a few generations of selection. This may produce some problems when realized heritabilities have to be estimated in short selection experiments. Accordingly, an estimate of realized heritability based on the entire period of the first few generations will be biased. Fimland (1979) recommends that selection experiments should be divided into several subsamples for the purpose of estimating realized heritabilities in different stages of the selection process. In the current experiment, however, factors like management, housing and population size reduce the importance of such an analysis.

Different reports have been given on limits in selection response (Robertson, 1960; King, 1980). However, in a selection experiment for litter size in mink it would require several generations to outline any approach to a possible limit and this is not the aim of this study.

Based on experiments, analyses and experience from mink breeding there is no expectation of correlated responses in fur characteristics. However, as described earlier in this paper, correlated responses can be observed in traits connected with body size. Whether these traits or other reproductive traits are affected by the selection for litter size at birth remains to be seen, but the current selection experiment with mink may cast some light on these interesting questions.

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# SELECTION FOR LITTER SIZE IN MINK

# II. Direct response in litter size at birth

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This paper presents the direct response from a six-generation selection experiment for litter size at birth in dark mink. A significant difference in litter size at birth was observed between the high and low lines during the last year of the experiment. The selection response in these two divergent selection lines, expressed as deviation from the control line, was asymmetric and thought to be caused by instability in the control line. The cumulative effective selection differential in the last year was about – 4 kits, 2 kits and 12 kits in the L-, C- and H-lines, respectively. The estimated realized heritability for litter size at birth was 0.11, which was underestimated because of the effects of inbreeding and natural selection. It is postulated that some developmental defects in the embryo and early kit life could affect the subsequent oogenesis, which could result in reduced reproductive capacity of the female.

Key words: Inbreeding, mink, oogenesis, realized heritability, reproduction, selection.

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In fur production different groups of traits should be taken into consideration when selecting breeding animals. However, fertility has not been given its place in relation to its economic importance. To design a national mink breeding program, it is necessary to increase the work put into genetic investigations on reproductive aspects in mink.

A few papers have been published where estimates of genetic parameters on reproductive traits in mink have been included (Moore, 1954; Venge, 1961; Johansson, 1965; Rosberg, 1978; Narucka & Gedymin, 1978; Pastirnac, 1980; Einarsson, 1981; Clausen, 1985). However, there is a lack of information on both the possibility of changing litter size by selection and on the correlated effects on other reproductive traits, growth and fur characteristics.

Several scientists have drawn attention to the physiology of reproduction in mink and especially to the peculiarities induced ovulation and delayed implantation. First of all the work of Hansson (1947) must be mentioned along with that of Enders (1952), Onstad (1967) and Venge (1973). Several papers have later been published in the field of reproductive endocrinology in mink, among others those by Møller (1973), Allais & Martinet (1978) and Murphy et al. (1981). Many of the effects on and aspects of reproduction will be discussed in this paper,

with the major emphasis on a genetic point of view.

The scientific investigation of genetic aspects of fertility is based on a selection experiment, selecting divergently for litter size at birth. Three lines were kept, a high line selected for increased litter size (H), a low line selected for decreased litter size (L) and a randomly selected control line (C). The background and the design of the experiment, including analyses of the base population, are given in Part I of this series (Einarsson, 1987). Correlated effects from the single-trait selection are presented in two subsequent papers in this series, while the development of an applied selection index is discussed in the final paper.

The present paper focuses on the selection response in litter size at birth, the estimates of realized heritability obtained in the present experiment and analyses of the effects of inbreeding and natural selection.

## MATERIAL AND METHODS

### *Experimental conditions*

In 1978 the selection experiment began with the establishing of the three closed lines of dark mink from a foundation stock of 231 litters, and terminating after six generations of selection at the end of 1984.

The selection criterion for the kits was a pedigree index based solely on litter size at birth, including litter information from relatives in the parental generation. Breeding animals were kept for one year only, in order to obtain as low a generation interval as possible, except for the last year when half of the females were two years old. The animals were kept under conventional ranch facilities and management conditions. The breeder diet, fed from mid-December to the end of June, contained approximately 44 percent protein, 44 percent fat and 12 percent carbohydrate, given as a percent of metabolizable energy. Further details about the base population, experimental design, animal management and feed composition are given in Part I of this series (Einarsson, 1987).

The numbers of breeding females giving birth throughout the experiment are given in Table 1.

One female in the L-line in 1980 and two females in the H-line in 1982 had defective uteri. In 1981 another female in the L-line and one female in the C-line were mistakenly placed and mated in a wrong line. A very sick female in the H-line was euthanasied on May 7 in 1984 and at the autopsy 8 fully developed foetuses were found. All 6 females were omitted in the tables giving the number of breeding animals, in the data presented and in the analyses.

The number of females given for 1984 includes both one- and two-year-old females. The two-year-old females therefore appear in the figures for both 1983 and 1984. The numbers of one-year-old females whelping in 1984 were 26, 26 and 23 in the L-, C- and H-lines, respectively.

From the beginning of the experiment, a maximum of three females and one male were allowed to be selected from the same litter in the selection lines. This was practised until the last two years, when these restrictions were removed. In the control line, however, a maximum of one male and two females were selected from the same litter during the whole experiment. The design of the selection procedure in the C-line is described in details in Part I.

### *Registration and definition of traits*

In order to clarify some of the terms used in the current and subsequent papers in this series, the following list of definitions is given.

| | |
|---|---|
| Successful mating | – observed mating with a duration of at least 15 min |
| Empty female | – female which mates successfully, but fails to give birth; in some papers referred to as a barren female |
| Infertile male | – a male mating at least three females, all of whom were empty and without implantation zones in the uterus wall |

| | |
|---|---|
| Litter size at birth | – total number of kits at first examination including both live and dead kits |
| Dead kits at first examination | – dead kits observed max. 10 h postpartum. (Often incorrectly defined as stillborns) |
| Stillborns | – dead kits at first examination in which the lungs sunk in the lung-floating test |
| Females without implantation zones | – autopsied mated females lacking implantation zones in the uterus wall |
| Females giving birth/whelped | – females with kits observed at the first examination, including dead kits |

The nest boxes were inspected at least three times a day during the whelping period. Both the nest box and the cage were thoroughly inspected in order to find all the kits born. In the last three years of the experiment, dead kits were collected and weighed at the first examination.

The testes were palpated early in March and only males with two normal testes were allowed to mate. Sperm testing was conducted by collecting semen from the females when mating was interrupted after about 20 min. In 1983 testes from all the breeding males were subjected to histological study, and microscopic examination of the seminiferous epithelum was conducted.

Mating took place from March 6 to March 25. Females mated before March 18 were given the opportunity to remate 8 to 11 days later. If the first mating took place on or after March 18, the females were allowed to remate the day after. The females were always remated with the same male, and attempts were made for one remating only. Further information about mating and gestation is given in subsequent paper in this series.

All the animals were tested for plasmacytosis (Aleutian disease) (see Porter et al., 1980) at the end of October or at the beginning of November. Both the non-specific Mallen-test and the specific agar-test (Hansen, 1980) were used. The latter test was carried out at the laboratory of the Norwegian Fur Breeders Association. This laboratory also conducted the quantitative agar-test of the breeding animals in 1982, 1983 and 1984, and of the breeding males in 1981. In this test the blood was collected by heart puncture from males after mating and from females after weaning; it was centrifuged and frozen at -70 °C. The samples were analysed to obtain titers, up to dilution of 1:2 048 of the serum. Blood samples from all breeding females over the last year were also analysed at the Veterinary University of Norway, both concerning a quantitative agar-test and percentage of γ-globulin. The breeding females from the last year were thoroughly examined for possible effects of plasmacytosis. After weaning the females were autopsied and the liver, kidneys and spleen were removed and weighed. A sample from the first two organs was fixed in formalin and examined at the Veterinary University. Animals that died during the experiment were autopsied at the National Veterinary Institute, Oslo.

*Statistical methods*

In order to study the possible trends of given traits during the experiment the following regression equation was applied.

$$Y_j = a + b \cdot X_j + e_j$$

where

| | |
|---|---|
| $Y_j$ = | a given dependent variable for the *j*th individual |
| a = | a constant |
| b = | the sample regression coefficient of $Y_j$ on $X_j$ |
| $X_j$ = | the independent variable of the *j*th individual, a fixed effect (often the generation number) |
| $e_j$ = | the random error associated with the *j*th individual |

The following model was used for analysing the possible effects of generation, line and their interaction on a given trait.

$$Y_{ijk} = a + g_i + l_j + c_{ij} + e_{ijk}$$

where

$Y_{ijk}$ = an observation on a given trait
$a$ = a constant
$g_i$ = a fixed effect of *ith* year (generation, however, two generations last year)
$l_j$ = a fixed effect of *jth* line
$c_{ij}$ = interaction between *ith* generation and *jth* line
$e_{ijk}$ = the random error associated with the ijk*th* female giving birth

If no significant interaction was found then the analysis was based on a model excluding the element of interaction. The results of the analysis were based on the type III sum of squares (SAS, 1985), where the other elements in the model are included before the given element is analysed.

With the two class variables, line and generation, a hierarchical model will include the element of interaction between year and line in the effect of the element line within the year. The sum of squares for both models would be the same.

Differences between the lines in the last generation or between the first and the last year within the line for given traits were analysed by a model similar to the previous one. This model would then include only the one component to be analysed ($Y_{ij} = a + l_i$, or $g_i$, $+ e_{ij}$).

Also in this analysis the type III of sum of squares was used.

The inbreeding of both the breeding animals and the kits was calculated using a computerized inbreeding program. For each generation a new degree of inbreeding was calculated for each individual by indetifying the last common ancestor of the parents. The earliest information about potential ancestors was given by including parents in the foundation stock. The elements included in the different models used to analyse the effect of inbreeding on litter size are presented together with the results. However, the models were organized in the same way as those presented previously.

The reproduction results in 1983 were abnormal because of difficult matings, increased number of empty females and unusual distribution of litter size. Reproduction data from 1983 were therefore omitted in the analyses and in several of the results presented. This problem is thoroughly discussed later in this paper.

## RESULTS

The mean and standard deviations of litter size at the first examination, expressed both as the total number of kits and the number of living kits, are presented in Table 1. In the last generation the litter size at birth was on average 6.00, 6.58 and 7.35 kits for one-year-old females in the L-, C- and H-lines, respectively, and 6.41, 6.90 and 7.20 kits for the two-year-old females in the same lines. The overall mean of litter size at birth in the experiment was 6.72 kits with a standard deviation of 2.46.

Results from the analysis of the effect of generation and line on litter size at birth are given in Table 2, where it can be seen that there was a significant effect of generation and line, but not of the interaction between them. By using a hierarchical model of year and line within year, the mean of the squares was found to be 13.5 and 5.6 for the respective elements. Only the effect of year was then significant. Further analysis on difference of the selection trait between the individual lines and years was conducted by using LS-means. Differences between lines were significant ($p = 0.0033$) in 1984 only, and then between the two selection lines. An analysis of differences between years showed a significantly ($p<0.03$) lower litter size in 1983 compared to the other years, except for 1981. No significant differences were observed between the other years.

The distribution of litter size at birth for all lines and years is shown in Figure 1. The frequency of litters with one kit only was high in 1983, especially in the H-line.

The divergent selection response in litter size at birth, expressed as deviation from the control line, is presented in Figure 2. The response is

Table 1. Number of females giving birth and number of kits at first examination, given both as total number and number of kits alive, measured per whelped mink female. Figures given in italic denote the standard deviation.

| Year | Line | No of females giving birth | No. of kits at first examination | | | |
|---|---|---|---|---|---|---|
| | | | total | | alive | |
| | | | mean | SD | mean | SD |
| 1979 | L | 45 | 6.82 | *2.83* | 5.82 | *2.28* |
| | C | 44 | 6.70 | *2.91* | 5.77 | *2.65* |
| | H | 50 | 7.40 | *2.08* | 6.10 | *2.22* |
| 1980 | L | 42 | 6.74 | *2.56* | 5.88 | *2.73* |
| | C | 46 | 7.09 | *1.84* | 6.24 | *1.84* |
| | H | 47 | 7.17 | *2.35* | 6.17 | *2.29* |
| 1981 | L | 38 | 6.34 | *2.56* | 5.55 | *2.55* |
| | C | 44 | 6.50 | *2.48* | 5.52 | *2.39* |
| | H | 49 | 7.10 | *2.20* | 6.34 | *2.39* |
| 1982 | L | 48 | 6.33 | *2.31* | 5.10 | *2.63* |
| | C | 41 | 6.85 | *2.55* | 5.66 | *2.70* |
| | H | 42 | 7.05 | *2.24* | 6.21 | *2.41* |
| 1983 | L | 37 | 6.00 | *2.24* | 5.08 | *2.53* |
| | C | 44 | 6.55 | *3.00* | 5.30 | *2.96* |
| | H | 32 | 5.66 | *2.86* | 4.56 | *2.64* |
| 1984 | L | 53 | 6.21 | *2.41* | 5.11 | *2.45* |
| | C | 47 | 6.72 | *2.39* | 6.38 | *2.46* |
| | H | 43 | 7.28 | *2.14* | 6.44 | *2.28* |
| 1979 – 1984 | L | 263 | | | | |
| | C | 266 | | | | |
| | H | 263 | | | | |

Table 2. Analysis of effect of generation, line and interaction component on litter size at birth.

| | d.f. | MS | Significance level, p |
|---|---|---|---|
| Generation | 5 | 13.52 | 0.047 |
| Line | 2 | 18.82 | 0.044 |
| Line×generation | 10 | 4.20 | N.S. |
| Error | 774 | 6.00 | N.S. |

d.f. = degrees of freedom.
MS = mean of squares.
N.S. = when $p>0.05$.

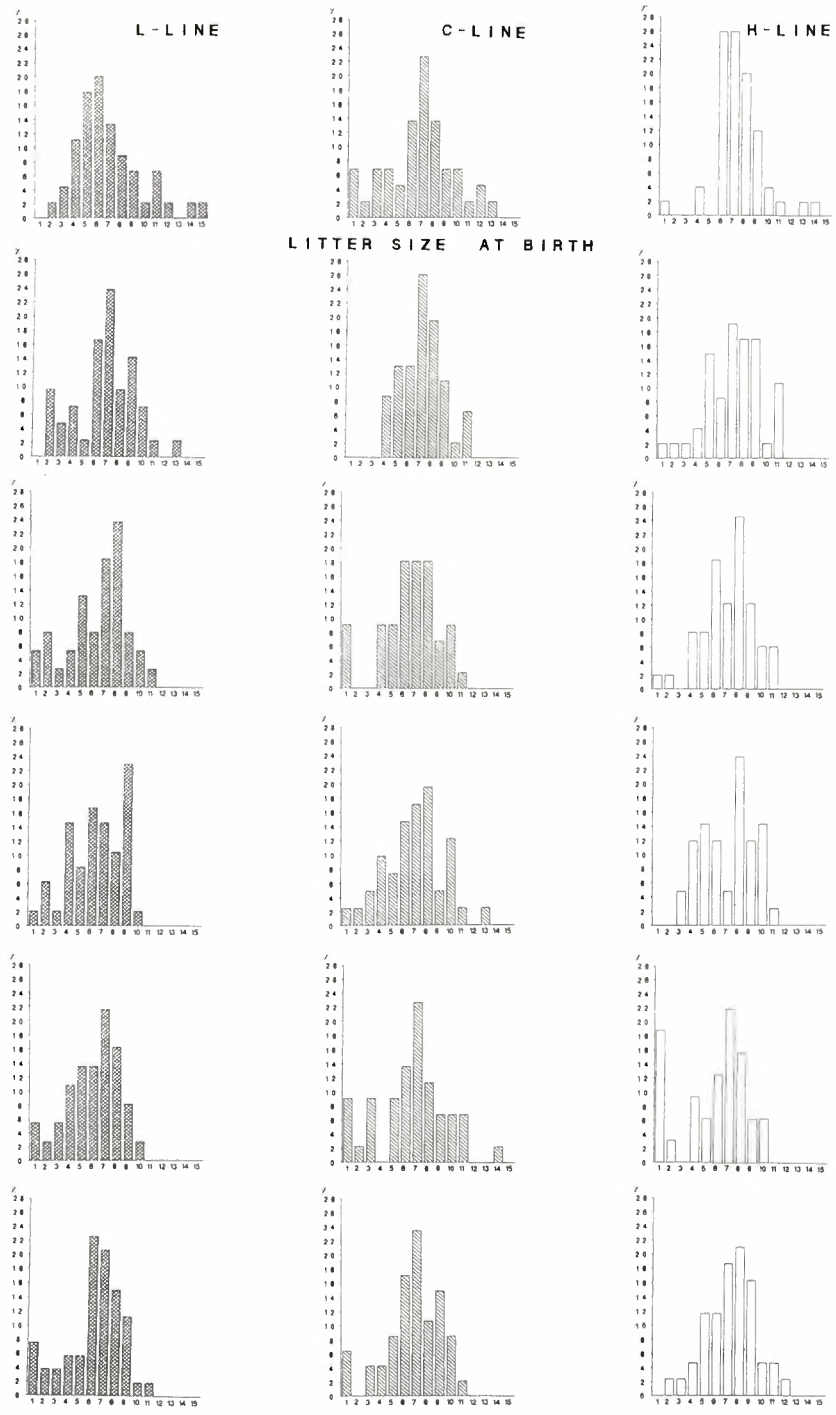



Figure 1. Distribution of litter size at birth in the three lines of mink during the six years. Results from the L-line are given in the first column, C-line in the second and H-line in the third. The first generation is in the upper row while the last generation is given the lowest row.

plotted against the years. In this figure, data from 1983 were excluded. The two selection lines fluctuated divergently compared to the C-line, although the differences were not significant (see earlier). By comparing the two selection lines, there appear to be common environmental fluctuations, or an unstable C-line.

In Figure 3 the effective cumulative selection differential is presented separately for each line and each sex. The effective selection differential expresses the within-line difference between the population mean and the mean of parents, weighed according to their contribution to the next generation to give birth. In 1984, when parents from two generations were used, the parent mean was calculated as the average for the two age classes. As seen from Figure 3 the selection differential was high in the H-line (between 1 and 3 kits each year). In the L-line, however, it was difficult to maintain a strong selection differential. In four of the six years a selection differential of between just 0.0 to 0.3 kits per year was obtained. The selection differential in the C-line was expected to be approximately zero, but increased to 2.39 in the last generation.

The difference between the population mean and the simple mean value of the selected parents, gives the expected selection differential, or the unweighed selection differential. By comparing the effective and the expected selection differentials, the effect of natural selection could be estimated. The average values of the ratio effective/expected selection differential for the L-, C- and H-lines were found to be 0.85, 1.09 and 1.00, respectively. This indicates that natural selection has worked against the reduction of litter size in the L-line and thereby reduced the response from artifical selection. The natural selection was slightly positive in the C-line, while there was no effect in the H-line. There were only small variations in the ratio factor between generations in the C- and H-lines, while the ratio in the L-line varied from 0.4 to 1.0.

The selection response in litter size at birth, expressed as deviations from the C-line and plotted against the effective cumulative selec-

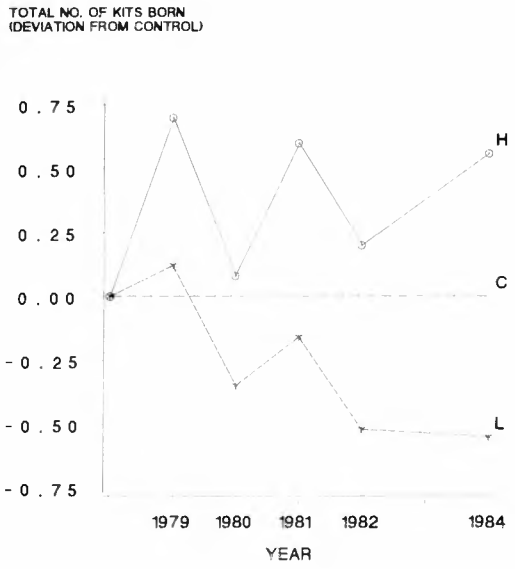



Figure 2. The selection response in the high- (H) and low lines (L), expressed as deviation from the control line (C), and plotted against the years. The data in 1983 were excluded.

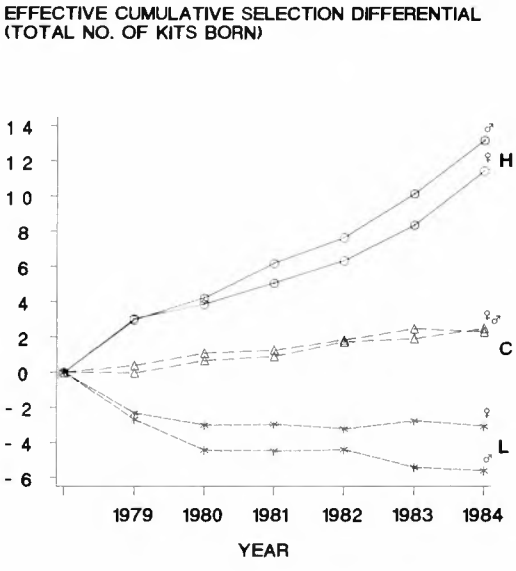



Figure 3. The effective cumulative selection differential for each line and sex, separately, plotted against the years.

tion differential, is shown in Figure 4. Both selection responses and selection differentials are expressed as deviation from the C-line. They are consequently corrected for the positive, accumulated selection differential in the C-line. Figure 4 clearly shows the response, for both increased and decreased litter size at birth, although some fluctuations were observed.

In Figure 5, the selection response is presented as the difference between the two selection lines and plotted against the effective cumulative selection differential. Because of possible instabilities in the C-line, this figure may give a better picture of the total selection response obtained.

The mean litter size at birth within the lines during the experiment is shown in Figure 6, where the results from the last year are also split and presented separately for the two generations.

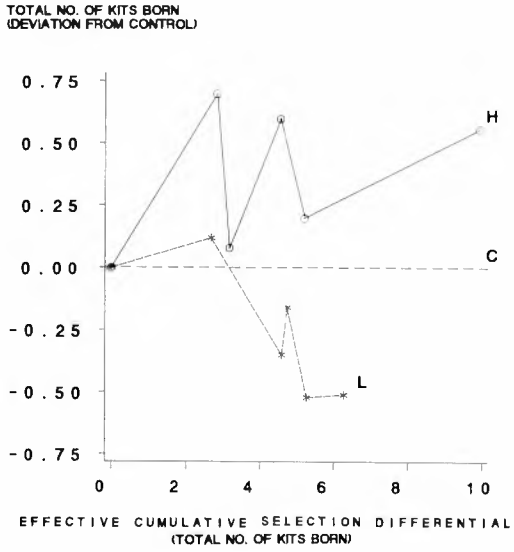



Figure 4. Selection response in litter size at birth plotted against the effective cumulative selection differential, both expressed as deviations from the C-line. The data for 1983 were excluded from the plotting, but are included in cumulative selection differential.

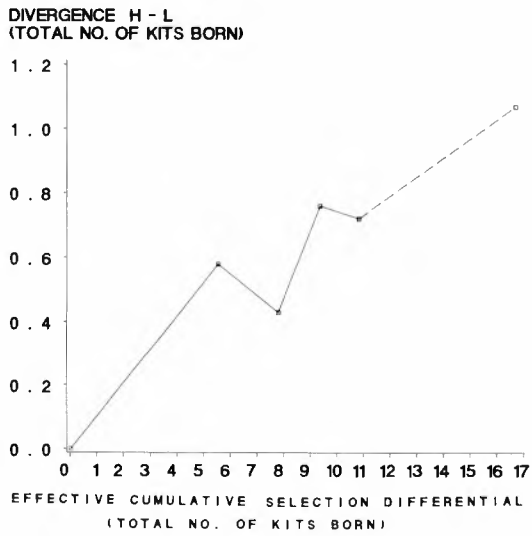



Figure 5. Selection response in litter size at birth, expressed as divergence between the H- and L-lines and plotted against the effective cumulative selection differential. Data from 1983 were excluded in selection response and therefore the broken line for the last two generations.

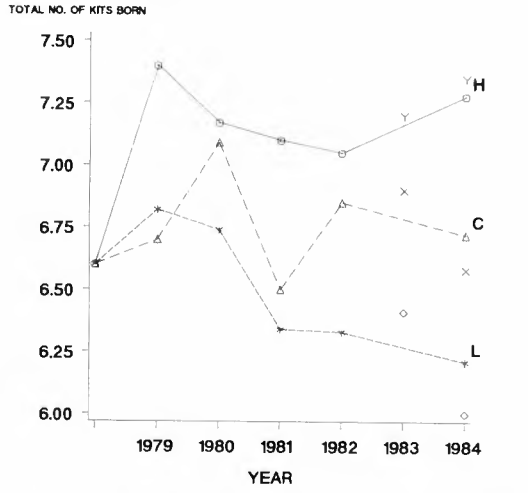



Figure 6. Mean litter size at birth for each line and year. The extra plots in 1983 represent the twoyear-old females in 1984 and the extra plots in 1984 the one-year-old females the same year. The symbols used in the last two years are Y for the H-line, X for the C-line and ◇ for the L-line.

The numbers of litters in which all the kits were dead at the first examination were 12, 13 and 8 in the L-, C- and H-lines respectively, during the entire experiment. For all lines the average litter size for these lost litters was 3.0. The litters with only one kit represented 45 percent of all these litters, increasing to 58 percent when litter size of two kits was added.

The analysis of the differences, given as LS-means, comparing the first and last generations within lines and between lines in the last generation is presented in Table 3.

No significant differences in litter size at birth were observed between the first and last generations when comparing LS-means within lines. However, it should be noted that litter size at birth was highest in the first year during the whole experiment, in both selection lines. Significant differences in litter size at birth were observed the last year between the H- and L-lines.

*Realized heritability*

The realized heritability of litter size at birth or, more precisely, of the selection index for litter size at birth, was calculated as twice the regression coefficient fitted to the lines in Figures 4 and 5. Since selection of individuals was based on the performance of parents and their sisters the regression coefficient was doubled when estimating heritability. The results are given in Table 4. The standard error of the estimated realized heritability is twice that of the regression coefficient. The best estimate of the heritability of the index is probably the one based on the divergence between the two selection lines; estimated to $0.14 \pm 0.03$, excluding data from 1983.

In order to express the heritability of the trait litter size at birth, an average family size was fixed in both the L- and the H-line. Family size corresponds to the number of relatives included in the index calculations. In the H-line, the litter size of the female herself, 3 full sisters and 5 half sisters was involved (1, 2, 2 in the excluding model). The corresponding figures in the L-line were the female herself, 2 full sisters and 4 half sisters (1, 1, 2 in the excluding model). A similar family structure was assumed on the sire side an the $r_{TI}$-value for the litter was calculated according to the following formula:

$$r_{TI_P} = \sqrt{0.25\, r^2_{TI_D} + 0.25\, r^2_{TI_S}}$$

Table 3. Differences in LS-means for litter size at birth between the first and the last generation within lines, and between lines in the last generation. The last generation includes both one- and two-year-old females.

| | Litter size at birth, no. of kits |
|---|---|
| Within lines (first-last generation) | |
| L | 0.61 |
| C | -0.02 |
| H | 0.12 |
| Between lines (last generation) | |
| H – C | 0.56 |
| C – L | 0.51 |
| H – L | 1.07* |

* = $p<0.05$.

where

$r_{TI_i}$ = the accuracy of the calculated index for litter size (correlation of true breeding value and index)

P = progeny, D = dam, S = sire

The $r_{TI}$-value based on mass selection is the square root of the heritability of the trait. Compared to index selection with the family structure as discussed, the ratios for $r_{TI}$ from these two selection methods were 0.79 and 0.73 in the L- and H-lines, respectively. The realized heritability for litter size at birth in the two selection lines when correcting for the ratio of the $r_{TI}$-values was then 0.11 in both lines, excluding data from 1983.

When calculating the regression coefficients of selection response in litter size at birth on years, or generations, the following results were obtained for the complete data H – C = 0.045 ± 0.038, L – C = -0.098 ± 0.038. When excluding data from 1983 the corresponding figures were 0.106 ± 0.041 and -0.094 ± 0.044.

The material and the family structure were too limited to obtain reliable estimates of genetic parameters of traits measured on the breeding animals. This view was borne out by the analysis, based on different models which included different elements. Within each trait, between one third and two thirds of the heritability estimates were negative, while some were above one. The standard errors were extremely high and several genetic and phenotypic correlations were above one or below minus one. However, the genetic and phenotypic correlations calculated between litter size could be studied at different points of time up until three weeks postpartum. Negative variance components were found between litter size at birth and the following three examinations, while the genetic correlations between litter size at 2, 7 and 21 days postpartum approached 1.0. The phenotypic correlations were 0.66, 0.63 and 0.61 for litter size at birth and for the three later points of time, and 0.97, 0.96 and 0.98 for the combinations 2/7, 2/21 and 7/21 days postpartum, respectively. All estimates were based on the sire component.

*Inbreeding within lines*

The degree of inbreeding was calculated during the experiment for all breeding animals in the three lines. There was no increase in average inbreeding during the first two years, but thereafter there was an increase (as shown in Table 5). The degree of inbreeding is given separately for each sex and for the year in which the animals became parents.

For 1984 the figures included one-year-old females only. The average inbreeding of females giving birth in 1984 was about the mean of the value for the two last years.

In 1983 the highest degrees of inbreeding in a single breeding animal that became a parent,

Table 4. Regression coefficients and standard errors for selection response in litter size at birth on effective cumulative selection differential, and realized heritabilities for the selection index. The results are given both for the entire material and when excluding data from 1983.

| Material on litter size at birth | High H – C | Low L – C | Divergence H – L |
|---|---|---|---|
| All years, b ± S.E. | 0.037±0.025 | -0.07±0.030* | 0.052±0.10*** |
| realized $h^2$ [a] | 0.07 | 0.15 | 0.10 |
| 1983 excluded, b ± S.E. | 0.072±0.026* | -0.070±0.034* | 0.069±0.014*** |
| realized $h^2$ [a] | 0.15 | 0.14 | 0.14 |

[a] The realized heritabilities estimated refer to the selection index for litter size at birth.

* = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$.

were 7.0, 6.3 and 9.4 in the L-, C- and H-lines, respectively. The corresponding figures in 1984 for one-year-old breeding animals were 11.1, 7.4 and 29.3, respectively. As seen from Table 5 the average inbreeding was fairly similar in the two selection lines, but only about half that in the C-line.

The inbreeding of the kits born was calculated for the two last years. The average results within the lines are presented in Table 5. In 1983 the inbreeding of litters varied from 1.2 to 20.7 in the L-line, from 0.6 to 9.0 in the C-line and from 1.4 to 29.3 in the H-line. The corresponding figures in 1984 varied up to 17.7, 14.7 and 30.2, respectively. The two-year-old females in 1984 had on average a lower degree of inbreeding than the one-year-old females and this explains the similar inbreeding between progeny and dams in the last year in two of the lines.

The effect of the inbreeding of the dam on litter size at birth was analysed both by including and excluding the effect of generation. As presented earlier, the only significant difference between generations for litter size at birth was between 1983 and the other years, except 1981. When excluding the effect of generation and when analysing across all lines, the regression coefficient for inbreeding on litter size at birth was -0.05 ($p<0.09$). In the model including the effect of generation, the regression coefficient for inbreeding on litter size at birth was only -0.01 and effect of generation was -0.14. Although the level of significance was 2.4 percent

Table 5. The average percent of inbreeding in breeding animals calculated per generation and given for each line and sex, separately. The animals included are only those that became parents. The average percent of inbreeding for the kits is given for the two last years only.

| Year | Line | Degree of inbreeding of the breeding animals | | Degree of inbreeding of the kits born[b] |
|---|---|---|---|---|
| | | Females[a] | Males | |
| 1979 – 1980 | | 0.0 | 0.0 | |
| 1981 | L | 1.0 | 0.0 | |
| | C | 0.0 | 0.0 | |
| | H | 0.0 | 0.0 | |
| 1982 | L | 2.2 | 2.1 | |
| | C | 0.6 | 0.7 | |
| | H | 1.6 | 1.8 | |
| 1983 | L | 2.6 | 3.1 | 5.2 |
| | C | 1.8 | 2.4 | 3.1 |
| | H | 4.4 | 4.1 | 5.7 |
| 1984 | L | 6.9 | 6.4 | 6.3 |
| | C | 2.6 | 3.7 | 3.6 |
| | H | 6.5 | 6.4 | 8.9 |

[a] The percent of inbreeding for females in 1984 is given for one-year-old females only.

[b] The average percent of inbreeding for the kits born was calculated over mating combinations resulting in kits born.

for the model used, it was only about 80 percent for the component of inbreeding. Analysis within lines showed the strongest effect in the C-line and a similar effect within the two selection lines. The regression coefficients of the dam's inbreeding on litter size at seven days postpartum were reduced in both models by 0.02, indicating a more limited effect of the dam's inbreeding on litter size throughout the nursing period.

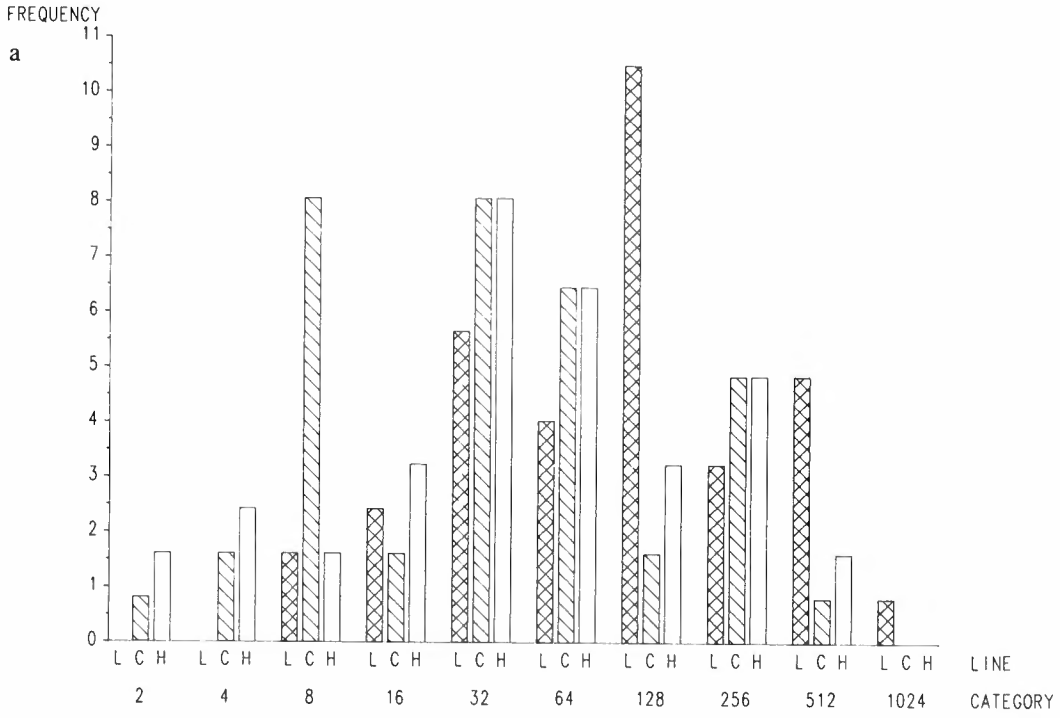
The effect of the inbreeding of both the dam and the foetus on litter size at birth was analysed. The model also included the effect of interaction between these two elements in addition to the effect of generation. The regression coefficients of the elements on litter size at birth were found to be -0.12 ($p = 0.12$) for inbreeding of the dam, -0.04 ($p = 0.45$) for inbreeding of the foetus and 0.01 ($p = 0.24$) for the interaction between them. However, the level of significance for the model was only 27 percent. The corresponding regression coefficients on litter size at 7 days postpartum were -0.14 ($p = 0.06$), -0.08 ($p = 0.10$) and 0.01 ($p = 0.06$), with a significance level for the model of 0.3 percent.

*Plasmacytosis*

Some results from the plasmacytosis test are shown in Figure 7. These presentations are commented on in the discussion.

## DISCUSSION

A significant difference in the selection trait was observed between the two selection lines during the last year, when the litter size at birth was 1.07 kits higher in the H-line than in the L-line. Although significant differences between lines were not observed within the other years, the litter size at birth was largest in the H-line during the entire experiment and, except for the first year, smallest in the L-line. The da-



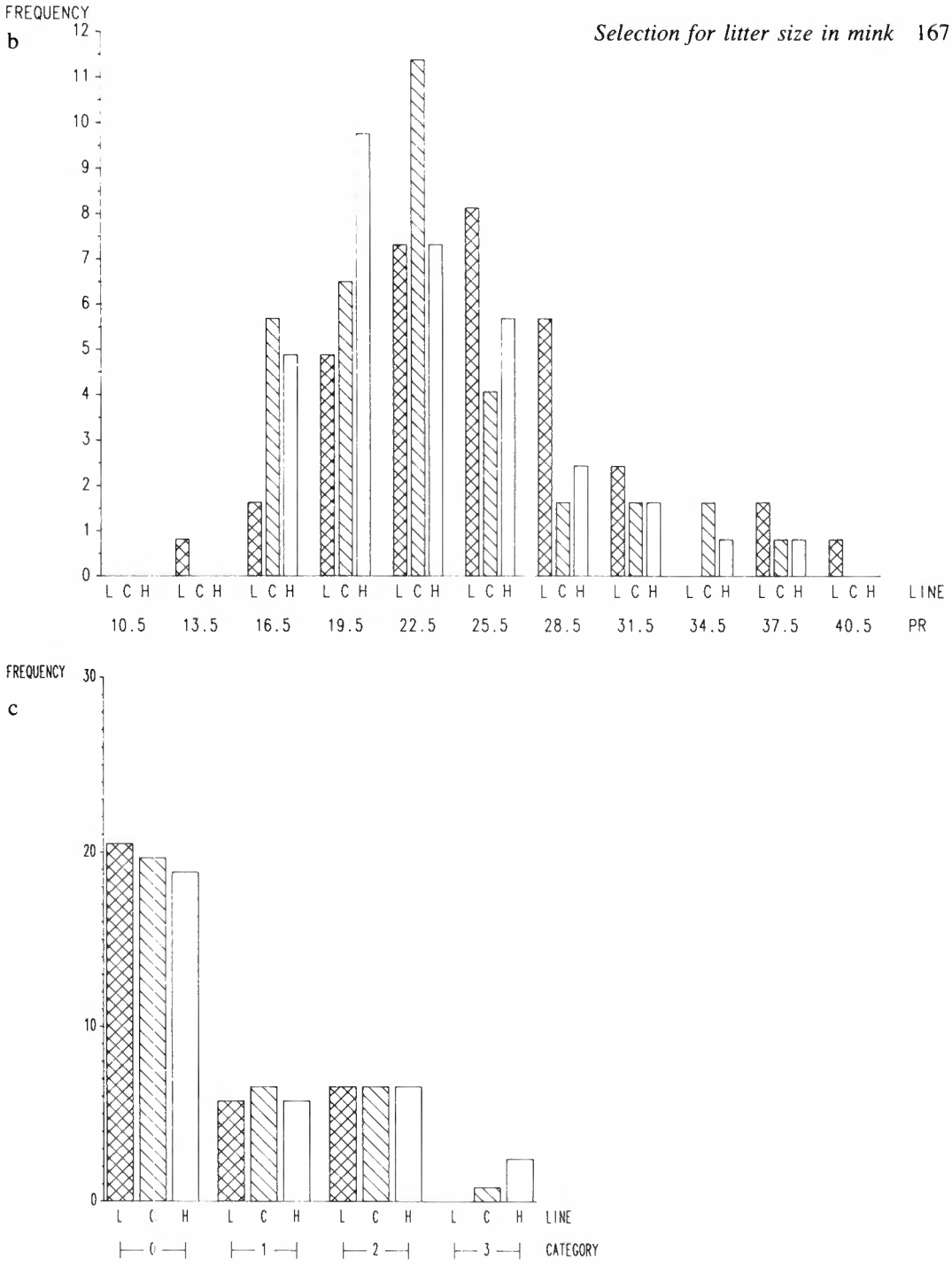



Figure 7. Results from the plasmacytose testing of breeding females giving birth in the last year.
a. The distribution of titers with dilutions of serum from 1:2 to 1:1024, using the specific agar-test.
b. The distribution of categories of percent γ-globulin.
c. The distribution of categories for severity of lesions in the kidneys (0 = free, 1 = +, 2 = ++, 3 = +++).

ta from 1983 were excluded, and will continue to be so in the following discussion. The effect of excluding the data from 1983 appears clearly in Table 4, where the realized heritability is halved comparing the H- and C-line, but less reduced comparing the two selection lines. However, as will be discussed, the results in 1983 and especially in the H-line, were so abnormal that to include them only would complicate the interpretation of the effect of selection.

The large response observed in the H-line in the first generation can be explained by the high selection differential. However, the high negative selection differential in the L-line did not result in a similarly large negative response. On the contrary, during the first generation the largest average litter size at birth was observed within the L-line, and it was the only year in which litter size at birth increased compared to the previous year. This is thought to be partly due to the foundation stock, which was not completely homogeneous at the time of selection. It often happens in selection experiments for litter size that the lines fluctuate during the first generations. Maternal effects are not believed to have caused the response in the L-line in the first year of the present experiment.

Because of the reproductive failures in 1983, the two-year-old females in 1984 could be chosen to represent the genotype of the previous generation. These females were not selected according to previous reproductive performance and could therefore represent a random sample of that generation. Two-year-old females are usually found to have higher reproduction results than one-year-old females and this could be assumed to off-set the effect of being mated to males selected from a later generation. In Figure 6 the litter size at birth for the two-year-old females in 1984 is plotted for 1983, while only the results from the one-year-old females in 1984 are plotted that year. This presentation shows an even stronger selection response than presented in previous Figures. The response is even more marked if differences are expressed as deviations from the C-line and as differences between the two selection lines. This implies that the realized heritability which was calculated from the observed response will be underestimated.

Figure 2, showing the selection response as a deviation from the control line, gives the impression that both the selection lines fluctuate more or less on a paralell throughout the years. It could therefore be questioned as to whether it is the C-line that fluctuates and therefore cause asymmetry. The presentation of the divergent selection response supports such an argument. A stable control line would be expected to have the two divergent selection lines symmetrically around it, assuming similar divergent selection response.

However, corresponding figures were observed when comparing the average values of different reproductive traits, and body weight of kits and breeding animals between the foundation stock and the last year in the C-line. The only differences observed were in kit mortality, percentage of remated females and body weight, all in favour of the foundation stock. This indicates a relatively stable control line.

The positive accumulated selection differential which was observed in the C-line could not be responsible for any of the fluctuations, only for a small but stable and positive response. No other effects, such as feeding, equipment, housing or general management, have been found as causes for the fluctuations in the C-line. The selection procedure for the C-line should also help to maintain a stable line. It has to be emphasized that compared to other selection experiments one must consider the less stable environment in a mink farm and also the variations occurring in small selection experiments involving reproductive traits. However, the effect of selection is quite clear from the total divergent response presented.

When calculating the effective selection differentials, all the breeding animals that gave birth were included. It could be argued for the inclusion of only those animals with progeny at the time of weaning, or putting it more extremely at the time of sexual maturity, as only they could contribute to the subsequent generations. However, assuming that the kits in all litters have the same possibility of reaching the age of

sexual maturity, the inclusion of those giving birth should be accurate enough. An exception to this assumption could be for a litter size of one or possibly two kits.

The effective cumulative selection differential was high in the H-line, particulary in the first year and in the last two years. In the first year this was due to the large number of litters in the foundation stock and the last two years because the number of individuals selected from the same litter was no longer limited. This also emphasizes the contradiction that occurs between obtaining a high selection differential and maintaining a low degree of inbreeding. In a short-term selection experiment, the selection differential should be given high priority. In a long-term selection experiment more care has to be taken to avoid a rapid increase in inbreeding, especially of reproductive traits.

In the L-line, the selection differential was strong for the first year only, when selecting from the large foundation stock. It was difficult to increase the cumulative negative selection differential in the following years, especially for the females. The change in limitation of number of kits selected from the same litter in the last two years had a minor effect in the L-line since the limited number was seldom reached in the small litters. However, some effect was seen for the males. The difference in cumulative selection differential between the L- and the C-lines increased during the years. Apart from the first generation, this was not due just to the selection in the L-line, but also because of an increased selection differential in the C-line. The increased selection differential in the control line was anticipated as small litters have less likelihood of being represented by both sexes at the time when selection takes place, and using the described procedure to reproduce the C-line results in overrepresentation of large litters.

It is interesting to observe how natural selection, expressed as the ratio between the effective and the expected selection differential (Falconer, 1981), behaves in the different lines. In the L-line it «resists» having the litter size reduced, which should be a logical reaction in a population with no environmental limits for increased reproduction (mainly food resources). Natural selection works to increase the reproduction in the C-line, even with artificial attempts to keep reproduction at a stable level. While under artificial selection for increased litter size, natural selection seems to have «no objections». The effect of natural selection in all three lines therefore seems to favourable to the increasing of litter size. It also implies that in a population with no artificial selection pressure for litter size, it will tend to be slightly increased. As pointed out by Falconer (1981), one should bear in mind that natural selection as defined here includes the effect of differences in fertility among parents only and not the differences of viability among the offspring. Such viability may be affected under some environmental conditions.

To the best of the author's knowledge no other selection experiments for reproductive traits have been conducted, either for mink or for any other species of specialized fur animals. However, several selection experiments for reproductive traits have been conducted with other multiparous species, mainly with mice, but also with pigs (reviewed by Vangen, 1981) and rabbits (Mgheni, 1982). When comparing results from different species one must be aware of the differences in reproductive function.

As pointed out by Joakimsen & Baker (1977), selection responses are dependent on several factors, the heritability for the selection trait, the phenotypic standard deviation and the selection intensity, but also the method of selection with the between family selection being superior to within family selection, outbred foundation population and population size. It could be claimed that the limiting factor in the present experiment was the population size. It could also have been the selection intensity, especially in the L-line.

Joakimsen & Baker (1977) found that the increase in litter size in the high line of mice was due almost entirely to an increase in ovulation rate, while the decrease in the low line was almost equally due to a decrease in ovulation rate and an increase in embryo loss. In a

long-term selection experiment for increased litter size at birth in mice, Bakker et al. (1978) found that the increase was mainly caused by higher ovulation rate. However, both reduced pre- and postimplantational losses also contributed to the increase.

Durrant et al. (1980) found increased ovulation rate and decreased early embryonic death when selecting for increased litter size in mice. However, no difference was observed when preimplantation loss was compared to the control line. It was not possible to observe ovulation rate in the present experiment since it is impossible to count the number of corpora lutea at the time of weaning. The embryonic loss together with kit loss are presented and discussed in the next paper in this series (Part III). It should be mentioned that Falconer (1963) suggests that ovulation rate and embryo survival are controlled by different genes. A close relationship between these traits would then occur if the genes were linked, i.e. that they are present in the same chromosome, or affected by common gene-products such as hormones.

It is known from other selection experiments, mainly for growth (Calvert et al., 1986), that major-genes can emerge and it is therefore important to be aware of this possibility. One of the problems, however, of utilizing a major-gene in selection for improved reproduction, is that fixation occurs rapidly and the variance and response obtained by selection is reduced. This does not occur when polygenes are involved (Robertson, 1977). As discussed previously, a large effective population size is also favourable for avoiding loss of desirable genes and depletion of additive genetic variance (Eisen, 1980).

*Reproductive results in 1983*

The reproduction data from 1983 were excluded from analyses and graphic illustrations of the results from the present experiment. This exclusion was based on the fact that the 1983-reproduction was widely variant from other years. There were difficulties in mating the females as many of them did not appear to be in heat, followed by a large number of empty females and a high frequency of small litter sizes at birth. These abnormalities were especially observed in the H-line. The following discussion concerning the reproduction results is included not only to spotlight the situation in 1983, but also as a general discussion concerning possible causes of variations in reproduction in the mink.

During the mating period in 1983 more than half of the females in the H-line refused to mate on the first attempt, while in the other two lines this was observed for about one-fifth of the females. Several females in the H-line were abnormally forced by the male to mate. This was also partly observed in the C-line but only at the beginning and at the end of the mating period. Though the average length of copulation did not differ from that of the other years, several matings in the H-line were doubtful or of short duration. Eight females had copulations of less than 15 minutes and six of them were empty.

Both Venge (1956) and Adams & Rietveld (1981) claim that a copulation of short duration, often less than six minutes, could result in poorer reproduction. They claim that the number of empty females increases as a result of reduced transportation of sperms, and also because of low concentration of the spermatozoa ejaculated. The short matings observed in the present experiment could also indicate that the females were not in heat and therefore would either not accept mating or only a short, enforced one. This argument was supported by the high increase in the number of empty females observed in 1983.

Uteri of the empty females euthanasied on May 16 were found to be pale, with a diameter varying between 1.5 and 3.5 mm. A large number of follicles were found in the ovaries of all the empty females, primary, secondary and tertiary follicles, the latter about 0.3 to 0.4 mm in diameter. Hansson (1947) found that the follicles were about 0.9 to 1.0 mm in diameter at the time of ovulation. It would appear that in several of the females the heat cycle was delayed in 1983. However, in the present experiment degeneration of the follicles was in progress and the size might therefore have been larger in

March. The ovaries were sectioned in order to search for remnants of tissue from corpora lutea as an indication that ovulation had taken place. Such tissue was found in 3 out of 11 females in the L-line, in 2 of 7 females in the C-line and in 12 of 19 females in the H-line. If remnants of luteal tissue indicate that ovulation has taken place, then 46 percent of the empty females did not ovulate. This conclusion was also supported by observations on implantation zones in the empty females which showed zones in only two females although no males were defined as sterile. The failure in ovulation could also result in females ovulating only a few eggs, finally resulting in both empty females and a higher frequency of small litters, which was also observed that year. It is argued therefore, that follicular development was delayed in many of the females in 1983.

Examination of the sectioned testes from the males euthanasied on March 25, showed that all the males had a normal development of the seminiferous epithelium and that sperms were found in normal to large number. The diameter of the testes varied, but within those variations observed earlier (Onstad, 1967).

Further analyses of the breeding animals showed no effect from the abnormal reproductive results of any of the ancestors, of size of the litter in which they were born, or of the day of mating. The weight of the breeding animals during the winter did not show any great differences from the other years. The reduction in body weight of the breeding females from December to March was not significantly different within the lines between empty females, females giving birth and females giving birth to one kit only. Although it is known that change in premating body weight, differences in feeding intensity and flushing may affect the reproductive results in one-year-old females (Tauson, 1985), this is not thought to have affected the reproductive results in 1983.

As described in the first paper in this series (Part I), it is clear that light plays an important role in the reproduction of the mink. Meteorological data were therefore studied for 1982 and 1983 for both the number of hours of sunshine and the global radiation. An increase in the temperature during the first weeks of March has been shown to decrease the gestation length (Bura et al., 1981). There was, however, no information from the meteorological data that could explain any of the negative effects on reproduction in 1983, either light or temperature. It seems that Stevenson's statement (1946, referred to by Hansson, 1947), that there is no effect of sunlight on sexual phenomena in mink, still has validity.

There was no indication that the feed composition or any of the feedstuffs used affected the reproductive results in 1983. It is known that both levels of dietary content and vitamins or minerals and quality of feed ingredients under special conditions may affect reproduction results in the mink (Rimeslåtten, 1964; Skrede, 1978). In the present experiment the same composition of feed was used during all the years (Einarsson, 1987). In 1983, 24 females outside the present experiment, 19 of them one-year-old, were used as a control group in a feeding experiment at the experimental farm (Skrede & Nes, 1983). These dark females were given the same feed as all females in the selection experiment, in the same farm and under the same environmental conditions. According to Skrede & Nes (1983) this group had normal reproduction with only one empty female, 6.8 kits at birth per whelped female and 14 percent kit mortality up to weaning. It could therefore be concluded that the feed did not alter the reproduction results in the selection experiment in 1983 compared to the other years.

Environmental contaminants could affect different subtraits of reproduction, as reviewed by Ringer (1981). In the present experiment there were no indications of such effects.

No clear difference was observed in the frequency of plasmacytosis, either in the other years or between the lines within 1983 (see later discussion).

It should be obvious from the examinations conducted on the empty females and from experience of the matings that some developmental disturbances occurred regarding both heat and ovulation. Since no blood samples

were collected to analyse the hormones involved in the reproduction, no final conclusions could be drawn about their function. However, there may have been some disturbance in the hormone levels in some of the females, which resulted in a delay or total failure in the final development of the follicles, thus causing few or no eggs to be ovulated. This would again explain the fact that some females had normal reproduction, some had small litters, others had a weak heat with or without ovulation and ultimately some did not come into heat at all.

As previously discussed, since the disturbances in the development of the follicles cannot be explained, it may be possible that they took place at an earlier stage. Both prenatal and especially the postnatal mortality were high during 1982, when the 1983-breeding animals were born. Together with the low average kit weight it indicated that conditions for the progenies were unfavourable. The preweaning growth of the kits was low, indicated by the low average body weights both at 21 days postpartum and at weaning. In the H-line the average kit weight of the females that became empty in 1983 was significantly lower compared both to the line-average and the females giving birth.

Against the background of these observations and indications of a situation with a suboptimal environment, resulting in disturbances in the development of embryos and kits, the following hypothesis is proposed. The oogenesis had been disturbed and these disturbances were manifested the subsequent year in the breeding female, as follicular failures resulting in disturbed heat development and ovulation.

The gametogenesis is not the same in both sexes. It begins during embryonic development, but while the production of sperms continues throughout the fertile life through stages of spermatogonia, spermacytes, and spermatids, the number of oogonia and oocytes is determined in the embryonic stage (Wartenberg, 1974). As reviewed by Rojas et al. (1984) it is known that male germ cells do not enter the meiotic prophase before early puberty, while the onset of meiosis in the female occurs in the foetal stage.

Studies of testicular development in mink (Onstad, 1967; Venge, 1973), showed a small increase in testes weight to about 0.5 gram per testis from birth until 200 days postpartum and thereafter a rapid increase to about 2.8 grams by the end of February. The changes in testes weight during the subsequent years in a male are cyclical, with lowest weight about 0.6 grams per testis. The first spermatogonia were present in the males at the age of 5½ months and spermatocytes or even spermatids were found 1½ months later. In January spermatozoa were present in the epididymis and the testes were fully developed at the end of February (Onstad, 1967).

It is known that the oogenesis in mammals such as rat, mouse, guinea pig, hamster, rabbit, ferret and cat begins at the end of the embryonic period or during the first week postpartum, and has a duration from one to three weeks (reviewed by Byskov, 1978). It is also found that during the oogenesis the retarded cells could degenerate. A high frequency of degeneration could therefore reduce the number of eggs that could develop up to ovulation. According to a figure given by Austin & Short (1972) the first meiotic division in the mink female begins at birth or just after birth, approximately about the same time as in the rabbit, ferret, vole and hamster. For these species Byskov (1978) reports the onset of oogenesis from the day of birth in the golden hamster to 6 days postpartum in the ferret. The oogenesis in mink should be investigated, especially since it is postulated that it could be of great importance to the reproductive results.

In the work of Hansson (1947), development from the primary follicle to the ovulated oocyte is described in nine different stages. The primordial follicle starts to grow and during the first stages granulosa cells are formed, while theca layer-cells are formed at the third stage. Zona pellucida also begins to form around the egg. In the theca layer differentiation takes place and theca externa and theca interna are formed. Up to the sixth stage the growth of the oocyte is almost linear and reaches a diameter of about 0.08 mm out of a total of 0.1 mm. The total follicle grows only slightly until this point,

being about 0.2 mm of the maximum 1.0 mm and up to 1.75 mm at ovulation (Hansson, 1947). During the last three stages the follicle grows rapidly and during the sixth stage the Graafian follicle, also called the tertiary follicle, is formed. Follicles failing to reach this stage will never ovulate.

It is during the formation of the Graafian follicle, under the stimuli of the follicle stimulating hormone (FSH) and the luteinized hormone (LH), that estrogen is produced and estrus initiated. Increased estriol secretion is triggered by the LH-secretion which results in the ovulation. It is from the fifth stage of the follicle development that the disturbances seem to take place, and this is postulated to be caused by a delay or failure of the earlier stages, again based on disturbances in late embryonic and early kit development. This is supported by the observations in part of the present experiment, showing high kit mortality and low kit body weight in 1982 and reproductive female disturbances in 1983 where several of the females had a pale uterus, no sign of tissue from corpora lutea and small follicles.

As discussed earlier male fertility would not be affected in the same way as indicated for female fertility and no disturbances in male fertility were observed in the current experiment.

More attention should be paid to the oogenesis in mink when investigating whether disturbances in the embryonic or kit development might affect the females reproductive capacity. Assuming the relevance of the hypothesis, it should be underlined that there could be maternal influence on the daughter's reproductive capacity under poor environmental conditions, but not necessarily depending on the size of the litter in which they were born. Kit body weight and general development should then be included in the selection program.

This hypothesis seems reasonable from an evolutionary point of view, as a factor in reducing a population under a given limitation, such as feeding resources.

Rutledge (1980) postulated a similar hypothesis for the possibility of increasing the ovulation rate in swine by reducing the size of litter in which they were raised. His hypothesis was that by offering a better environment in a small litter size it would provide conditions to avert degeneration of follicles during the different stages and thereby give a larger pool of primordial follicles. This was then assumed to increase the ovulation rate and the litter size for several parities. van der Steen (1983) found that results from experiments with swine were in line with the previous hypothesis.

*Diseases*

The non-specific Mallen-test for the virus disease plasmacytosis showed that during the experiment the positive reactors increased from an average of about 16 percent in all lines at the first generation to 40 percent in the L-line, 35 percent in the C-line and 50 percent in the H-line at the end of the experiment. From the specific agar-test it was seen that in all lines the frequency of serapositives increased from less than 90 percent to a 100 percent level within three years, remaining so during the rest of the experiment. Both these tests were based on all the kits before time of pelting. All kits selected from the foundation stock were Mallen negative.

The quantitative agar-test on the breeding animals in the last years showed varying degrees of antibody production. These tests gave no indication either that differences were present between the lines or that any general trend was seen within lines.

The main number of animals reacted negatively within the three classes of antibody titers, which were diluted to 1:64, 1:128 and 1:256. The percentages of breeding males within these three classes during the last four years were 75, 80, 60 and 67 percent in the L-line, 67, 80, 73 and 65 percent in the C-line and 82, 76, 77 and 58 percent in the H-line. The classes above and below these three classes were almost equally distributed. The distribution of the antibody titers for the females giving birth in the last year is shown in Figure 7a, for each line separately. For the empty females an increase to about 30 percent was found in the classes above 1:256.

No clear trend was observed when plotting

the quantitative test results against litter size at birth. There was, however, a slightly increased kit mortality with higher antibody titers. These observations concur with results obtained from agar-positive and agar-negative females in other experiments at the experimental farm. The sera negative females had a lower average number of kits born, but these differences were almost eliminated at weaning.

The γ-globulin level was also measured in the females giving birth in the last year, and these distributions are shown in Figure 7b. Neither was there any clear trend seen between these measurements and litter size at birth.

A final examination was conducted on organs from all females that whelped in 1984. The liver, kidneys and spleen were weighed and the two first examined microscopically. The average weights of these organs were similar in the three lines, on average liver 43 g (S.D. = 8), kidneys 6.5 g (S.D. = 1.1) and spleen 3.5 g (S.D. = 1.6). In plasmacytotic animals the plasma cells infiltrate several organs, but appear first in bone-marrow, the lymph nodes and spleen. However, it is not easy to estimate the number of such cells in these organs and in routine diagnostic work liver and kidneys are preferred. Lesions in these organs were graded by severity from + to +++ (1, 2, 3) (Nordstoga, 1972). The distribution of the categories, including free of lesions (0) is shown in Figure 7c for kidneys from females giving birth in the last year. A higher frequency of the category free of lesions was found for liver in the H-line, and it was lowest in both categories 1 and 2. When comparing the categories of examination to litter size at birth no trend in any differences was found, either for kidneys or for liver.

All the analyses and examinations on plasmacytosis during the selection experiment were conducted to investigate whether the disease could have confounded the observed selection response. It could be concluded that selection for litter size in the present experiment did not affect frequency of serapositives, either within or between the lines. No effects of the plasmacytosis on the selected trait or closely related traits, could be seen in the present experiment.

Factors such as optimum feeding, environmental conditions and the use of one-year-old animals may have contributed to the results observed. The results also indicate that selection for increased litter size will not eliminate or even reduce the disease. The virus observed in the present population is assumed to be of intermediate virulence (Hyllseth, personal communications). It has to be emphasized, however, that the farms should increase their efforts in culling out animals with the disease plasmacytosis and follow the recommended eradication program (Hansen, 1980).

*Realized heritability for litter size and effect of inbreeding*

The most reliable estimate of realized heritability for the index of litter size at birth was thought to be the one based on the divergence H – L and with data from 1983 excluded. Both the abnormal reproduction situation and the fluctuations in the C-line were then eliminated. The standard error of the heritability estimate was only 0.03 calculated as twice the standard error of the regression coefficient. The standard error of the regression of response on cumulative selection differential is thought to be biased downwards because of the positive correlation among generations caused by genetic drift. The standard error of the estimate could also be calculated by the formula of Osborne (1957):

$$\text{S.E. } (h^2) = \sqrt{32\ h^2/T}$$

where T = number of females having litter.

Applying this formula the standard error was twice the previous one.

The realized heritability expressing litter size at birth was found to be 0.11 in both the selection lines. The family structure which was assumed to be realistic should cover the average picture within the two selection lines. The calculated $r_{TI}$-values for the kits of 0.25 and 0.27 in the L- and the H-lines, respectively, are well in accordance with those expected when the experiment was designed (Einarsson, 1987). However, some variations in family structure

might have been present, but were not supposed to have biased the estimate. In general it is a problem using index selection to increase the accuracy of the selection and so separate the realized heritability of the index and the trait under selection.

The estimated realized heritability of 0.11 for litter size at birth in mink, obtained in the present experiment, is in relatively good concurrence with realized heritabilities from selection experiments for litter size in mice (0.08 to 0.22) in pigs (0.00 to 0.25), reviewed by Vangen (1981) and in rabbits (0.13) Mgheni (1982). In several analyses for litter size at birth in pigs, the heritability estimates ranged from 0.04 to 0.18 (Johansson, 1981), in accordance with values found for mink (Einarsson, 1981).

The average degree of inbreeding in the breeding animals during the last generation was almost identical to the 6.6 percent predicted in the two selection lines (Einarsson, 1987). In the C-line the calculated average inbreeding was lower than the predicted 4.2 percent. However, as presented earlier, the variation in the degree of inbreeding between the individuals was high, the greatest being 30 percent of inbreeding.

In an experiment with five generations of fullsibling mating, McCarthy (1967) studied the inbreeding depression on litter size at birth in mice. He found that increased inbreeding of the dam both reduced the number of ovulated eggs and increased the preimplantation mortality, while inbreeding of the litter increased the preimplantation mortality, but not the postimplantation mortality. An interaction between levels of inbreeding of the dam and of the litter increased preimplantation mortality. The physiological explanation for the decline in ovulation rate was suggested to be due to reduced levels of gonadotrophic hormones in inbred dams.

When using the model which includes both the effect of inbreeding from dam and foetuses in the present experiment, a reduction of about 0.1 and 0.05 kits, respectively, was found per percentage increase in the coefficient of inbreeding. Assuming these are representative figures, though the model was insignificant, and assuming a linear effect, the total effect of inbreeding on the selection trait for the last year could be calculated for each line as:

$$(F_{D1983} + F_{D1984})/2 \cdot b_1 + F_{F1984} \cdot b_2$$

where

$F_D$ = dams inbreeding at generations 5 and 6, respectively
$F_F$ = foetuses inbreeding at generation 6
$b_i$ = the regression coefficient of litter size on degree of inbreeding

A similar number of one- and two-year-old breeding females was assumed for the last year. The total effect of inbreeding reduced litter size at birth by 0.8 kits in the L-line, by 0.4 kits in the C-line and by 1.0 kits in the H-line. When using the model including the inbreeding of the dam only and a regression coefficient of -0.05, the reductions in litter size was 0.24, 0.10 and 0.27 kits in the respective lines. The component of inbreeding of the sire was not directly included in the model, but indirectly by contributing to the inbreeding of the foetuses. The inbreeding of the sires could be assumed to affect only the fertilizing capacity directly and to have small effect at the given level.

In mink it was found earlier that 10 percent inbreeding reduced the litter size at weaning by about 0.5 kits (Johansson, 1969). The same figure was given for mice in a review by Vangen (1984).

When using regression coefficients which are more in agreement with earlier reports -0.05 and -0.03 for the components of inbreeding of dam and foetus, the total inbreeding depressions on litter size at birth according to the previous formula were calculated at -0.43, -0.22 and -0.54 in the L-, C- and H- line, respectively.

The reduction in litter size at birth caused by the inbreeding underestimates the effect of artificial selection in the H-line, but overestimates the response in the L-line. However, the difference in response between the two selection lines and the estimate of the realized heritability based on the divergence between the H- and L-lines will only be biased by the difference in the

effect on inbreeding on the selection trait in the two selection lines. On the other hand the deviation of the H-line from the C-line will be underestimated, while overestimated when comparing the L- and C-lines, because of lower inbreeding depression in the C-line. The latter differences will strongly depend on the level of the effect of inbreeding.

As pointed out earlier, there was an effect of natural selection for litter size in the C- and L-lines. This leads to a small overestimation of the response in the C-line and to an underestimation of the response in the L-line. The total effect will thereby overestimate the difference between the H- and C-lines and underestimate the difference between the C- and L-lines. On the basis of the previous discussion the effect of inbreeding and natural selection on the response to litter size at birth could be summerized as follows.

| Line | Effect on the observed selection response, caused by | |
|---|---|---|
| | inbreeding | natural selection |
| Low | + | - |
| Control | - | + |
| High | - | 0 |

where + is overestimated and - is underestimated.

It should be emphasized that the underestimation in the H-line means that the response would have been higher if the reported effects were to be excluded. In the L-line on the other hand, overestimation means that the response would have been higher in the negative direction (lower).

The total effect of both the inbreeding and the natural selection implies that the observed response was underestimated in the H-line, while assuming approximately the same effect of the two components, would have no affect on the observed response in the other two lines. The estimates given for the realized heritability for litter size in mink based on the divergence between the two selection lines, will be underestimated by about 30 percent. The figure for heritability of litter size in mink is therefore probably about 0.16.

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# SELECTION FOR LITTER SIZE IN MINK

## III. Prepartum and preweaning observations

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The paper presents the correlated responses in prepartum and preweaning traits from a six-generation selection experiment for litter size at birth in dark mink. In the last generation a significant difference of 1.07 kits at birth was observed between the two divergent selection lines. This difference favouring the line selected for increased litter size at birth, increased at later periods and was 1.37 kits at weaning per whelped female. Embryonic mortality and kit mortality were highest in the line selected for increased litter size at the beginning of the experiment but decreased to lowest in the last generation: the opposite effect was observed in the line selected for decreased litter size. Preweaning kit body weight was not significantly affected by the selection for litter size, but higher kit mortality was observed with low kit body weight, especially with low birth weight.

Key words: Litter size, mink, reproduction, selection experiment.

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When changing litter size at birth by artificial selection, it is necessary to know whether the litter size at later points of time will be affected, either directly or indirectly, as a correlated response. In fur production the economic importance of litter size is in the final number of animals recorded per breeding unit at the time of pelting. Several traits are involved in kit survival, expressed both by dam and progeny and resulting from environmental and genetic factors. It is also important to know whether selection for litter size affects other traits during the reproduction period or on preweaning traits. Effects of litter size on preweaning performance in mink have been discussed in earlier reports (Venge, 1960; Hoogerbrugge & Baud, 1975a; Leoschke, 1982; Reiten, 1978; Einarsson, 1980b), and genetic parameters for reproductive traits have been estimated (Moore, 1954; Venge, 1961; Johansson, 1965; Rosberg, 1978; Narucka & Gedymin, 1978; Pastirnac, 1980; Einarsson, 1981a; Clausen, 1985). These works are based on analyses of populations from experimental or commercial mink farms. It was therefore important to analyse both the direct and the correlated effects of litter size based on an experiment conducted for genetic selection only.

The genetic aspects of fertility were studied through a selection experiment, selecting divergently for litter size at birth. This included a line selected for increased litter size (H), a line selected for decreased litter size (L) and a randomly selected control line (C). The back-

ground, analyses of the base population and design of the experiment are given in Part I of this series (Einarsson, 1987a). The direct response is presented and discussed in Part II (Einarsson, 1987b), where significant difference in litter size at birth was found between the selection lines in the last generation. Correlated effects in postweaning traits and development of an applied selection index are presented in Part IV and V of this series.

In the present paper correlated responses are presented for traits observed after the selection of breeding animals took place in December up until weaning. This includes information on mating, gestation, empty females, embryonic loss, kit mortality, litter size at different times during preweaning, and general development before weaning.

## MATERIAL AND METHODS

### *Experimental conditions*

Selection experiment began in 1978 when three closed lines were formed from a foundation stock of 231 litters of dark mink. The selection experiment lasted for six generations.

The selection of the kits was based on a pedigree index including the trait litter size at birth, with information from relatives in the parents' generation. The breeding animals were kept for one year only, in order to keep the generation interval at the lowest possible level, except for the last year when half of the females were two years old. The animals were kept under conventional ranch facilities and management conditions. The breeder diet, fed from mid December until the end of June, contained approximately 44 percent protein, 44 percent fat and 12 percent carbohydrate, given as percent of metabolisable energy. Other details about the experimental design are given in the papers I and II of this series (Einarsson, 1987a, b).

The numbers of breeding animals throughout the experiment are given in Table 1. In the first column the number of females includes only those females with successful matings, while the second column includes only females giving birth. The differences in number of females between these two categories cover the number of empty females. Throughout the experiment a total of nine mated females died before giving birth. Neither these females, nor the seven that died between the time of selection in December and mating, are included in Table 1. In total, seven females were unmated during the experiment, two in the L-line, two in the C-line and three in the H-line.

Since the two-year-old females were kept for the last generation, they appear in the figures for both 1983 and 1984. The numbers of one-year-old females whelping in 1984 were 26, 26 and 23 in the L-, C- and H-lines, respectively. The same situation also appears in other tables where the number of females for 1984 are given.

The first column of number of males in Table 1 includes all males which had at least one successful mating, while the next column refers to the number of males siring offspring. Between 2 and 3 males per line were each year taken out as spares. When necessary these were used to replace males that died from selection to mating or males refusing to mate. The number of males refusing to mate was low, only 1 to 2 each year.

It was planned for each male to mate on average three females. However, as seen from Table 2, the number of matings per male varied from one to eight. Males that mated one or two females only were generally unwilling to mate several females.

During the whelping period the nest boxes were thoroughly inspected at least three times a day, normally within 10 h after birth. Dead kits found at the first examination were defined as stillborn if the lungs failed to float in water. Females that lost their litters included females that lost all kits in the litter prior to weaning, also called «zero-females». Females with only dead kits at the first examination were classified as females giving birth. Other reproductive terms are defined in the Part II of this series.

Litter size was determined at birth, but also at 2, 7, 21 and 42 days postpartum. Accumulative

kit mortality to weaning was calculated from litter size at birth.

Dead kits at the first examination were collected and weighed during the last three years of the experiment. The lungs were placed in water and those lungs that floated indicated kits alive at birth, while the lungs that sunk indicated stillborn kits. This is referred to as the lung-float test.

The litters were weighed on the day of parturition and again at 21 days postpartum. The average body weight of kits at these two periods was calculated as litter weight divided by number of kits weighed. The first sexing of the kits

Table 1. Numbers of breeding females and breeding males during the experiment.

| Year | Line | Number of females | | | | Number of males | |
|---|---|---|---|---|---|---|---|
| | | Mated | Giving birth | With kits at weaning | Autopsied[a] | In mating | As sires |
| 1979 | L | 51 | 45 | 43 | 23 | 16 | 15 |
| | C | 49 | 44 | 43 | 22 | 18 | 17 |
| | H | 53 | 50 | 43 | 23 | 16 | 13 |
| 1980 | L | 52 | 42 | 39 | 17 | 15 | 14 |
| | C | 55 | 46 | 44 | 18 | 15 | 14 |
| | H | 54 | 47 | 45 | 17 | 15 | 14 |
| 1981 | L | 47 | 38 | 35 | 20 | 15 | 13 |
| | C | 48 | 44 | 41 | 21 | 13 | 12 |
| | H | 52 | 49 | 46 | 23 | 15 | 15 |
| 1982 | L | 52 | 48 | 40 | 43 | 12 | 12 |
| | C | 49 | 41 | 36 | 39 | 14 | 12 |
| | H | 52 | 42 | 33 | 41 | 13 | 11 |
| 1983 | L | 48 | 37 | 33 | 0 | 14 | 13 |
| | C | 51 | 44 | 36 | 0 | 15 | 15 |
| | H | 52 | 32 | 35 | 0 | 13 | 11 |
| 1984[b] | L | 58 | 53 | 47 | 42 | 17 | 15 |
| | C | 57 | 47 | 43 | 42 | 14 | 14 |
| | H | 52 | 43 | 42 | 41 | 18 | 16 |
| Sum | L | 308 | 263 | 237 | 145 | 89 | 82 |
| | C | 309 | 266 | 242 | 142 | 89 | 84 |
| | H | 315 | 263 | 236 | 145 | 90 | 80 |
| Total | | 932 | 792 | 715 | 432 | 268 | 246 |

[a] The number of autopsied females includes only females giving birth. In addition, all empty females were autopsied.

[b] Number of females in 1984 includes about half of the females from the previous year. Sum and total refer to number of observations on the females.

took place 21 days postpartum. At weaning, 42 days postpartum, the kits were resexed, individually marked and weighed. They were marked by tattooing the pads of the forelegs according to the commonly used method at the experimental farm (referred by Einarsson, 1979).

Autopsies of females that whelped were carried out immediately after weaning and included determination of total number of teats, number of active teats and number of implantation zones in the uterine horns. The implantation zones were remnants of the placenta (placenta zonaria) in the uterine mucosa. The visibility of the zones was most readily recognized when the uterus was transilluminated.

Autopsies of empty females were completed between May 20 and May 27, at the same date within the years. The uteri were examined for implantation zones.

Only males with two normal testes, proven by palpation before mating, were selected as breeding males. In 1980 two males in the C-line and in 1983 one male in the L-line were culled out because they had only one testis. To test the males for sperm, semen was collected from the female after an interrupted mating of about 20 minutes. No males were defined as infertile based on the results from the test. Only a few males had a low score in quantity and mobility, judged through the microscope. These males were not allowed to mate more than three females. The males were defined as infertile when

Table 2. Distribution of males classified for number of mated females per male. Only females with successful matings are recorded.

| Year | Line | Number of mated females per male | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 1979 | L | 2 | 5 | 2 | 3 | 2 | 2 | - | - |
| | C | 4 | 6 | 2 | 3 | 3 | 1 | - | - |
| | H | 3 | 2 | 4 | 4 | 1 | - | 2 | - |
| 1980 | L | - | 1 | 3 | 6 | 2 | - | - | 1 |
| | C | 2 | 2 | 2 | 2 | 3 | 2 | 1 | - |
| | H | 2 | 2 | 1 | 4 | 6 | - | - | - |
| 1981 | L | - | 4 | 6 | 3 | 1 | - | - | - |
| | C | 1 | 1 | 4 | 1 | 5 | 1 | - | - |
| | H | 2 | 2 | 5 | 2 | 2 | 1 | 1 | - |
| 1982 | L | 1 | 1 | 1 | 3 | 5 | - | 1 | - |
| | C | 2 | 2 | 3 | 2 | 4 | - | 1 | - |
| | H | 2 | 1 | - | 5 | 4 | - | - | 1 |
| 1983 | L | 1 | 1 | 3 | 9 | - | - | - | - |
| | C | 1 | 2 | 6 | 2 | 4 | - | - | - |
| | H | - | 1 | 3 | 6 | 2 | 1 | - | - |
| 1984 | L | - | 2 | 6 | 9 | - | - | - | - |
| | C | 1 | 1 | 3 | 3 | 3 | 2 | 1 | - |
| | H | 1 | 5 | 7 | 4 | 1 | - | - | - |

they had mated with at least three females, that all had uteri without implantation zones. At the termination of the mating season the males were autopsied. Both body weight and testes weights were recorded.

Matings took place from March 6 to March 25. Females mated before March 18 were given the opportunity to remate 8 to 11 days later. If the first mating took place after March 17, the females were allowed to remate on the following day. The females were always remated to the same male, and attempts were made for one remating only. The duration of copulation was recorded. The length of gestation was calculated from the date of the last mating.

The body weight of the breeding animals was recorded in January, February and March of each year. The number of breeding animals with body weights recorded was approximately the same as the number mated (see Table 1). Small variations in numbers were caused by the death of some animals during the winter. Females giving birth were weighed at parturition and again at 21 and 42 days postpartum. The latter two weighings also included females that lost their litters before weaning.

Animals that died during the experiment were autopsied at the National Veterinary Institute, Oslo. This included breeding females that died up until the end of June. The autopsy results are not presented as they gave no valuable information relating to the selection experiments.

*Statistical methods*

In order to study possible trends of given traits during the experiment, or the effect between traits, the regression coefficients from the following model were analysed

$$Y_j = a + b \cdot X_j + e_j$$

$Y_j$ = a given dependent variable for the *jth* individual
$a$ = a constant
$b$ = the sample regression coefficient of $Y_j$ on $X_j$
$X_j$ = the independent variable of the *jth* individual, a fixed effect (often the generation number)
$e_j$ = the random error associated with the *jth* individual

The described linear regression model was normally used, however, curvilinear regression was also conducted on parts of the material by introducing the element $b_2 \cdot X^2$.

For analysing possible effects of year, line and interaction between them on defined traits, the following model was used

$$Y_{ijk} = a + g_i + l_j + c_{ij} + e_{ijk}$$

where
$Y_{ijk}$ = an observation on the trait
$a$ = a constant
$g_i$ = a fixed effect of *ith* year (generation, however, two generations last year)
$l_j$ = a fixed effect of *jth* line
$c_{ij}$ = interaction between *ith* year and *jth line*
$e_{ijk}$ = *the random error associated with the ijkth* mink

If the interaction was nonsignificant in the model, the analysis was based on a model excluding the element of interaction.

With the two class-variables line and year, a hierarchical model will include the element of interaction between year and line in the effect of the element line within the year. The hierarchical model will give the same sum of squares as in the model used. The results from the analyses were based on type III sum of squares (SAS, 1985), where the other elements in the model were included before the given element was analysed.

Differences between the lines in the last generation or between the first and the last year within the line for given productive traits were analysed by excluding the other trait and the interaction component from the previous model ($Y_{ij} = a + l_i$, or $g_i$, $+ e_{ij}$).

Also in these analyses type III of sum of squares was used.

When estimating genetic parameters a nested analysis was used and composed as follows

$$Y_{ijk} = a + s_i + d_{ij} + e_{ijk}$$

where

$Y_{ijk}$ = an observation on the k*th* daughter of the j*th* dam mated with the i*th* sire
$a$ = a constant
$s_i$ = effect of the i*th* sire
$d_{ij}$ = effect of the j*th* dam mated with the i*th* sire
$e_{ijk}$ = the random error associated with the k*th* daughter within the j*th* dam and i*th* sire

Some analyses were also conducted by nesting the trait of daughter within sire and within year.

If the significant level is not given, then the terms significant or not significant refer to a 5 percent level.

## RESULTS

The average number of kits per whelped mink female and standard deviation at different times postpartum are given in Table 3.

The divergent selection response in litter size at birth, expressed as deviation from the control line and plotted against the generation number, is presented in Figure 1. Data from 1983 were excluded as discussed in Part II of this series. In the last generation the difference in litter size at birth between the two selection lines was significant ($p<0.05$).

*Kit mortality and reproductive traits*

The kit mortality at different intervals from birth to weaning is presented in Figure 2. The accumulated kit mortality to weaning, grouped for one-year-old and two-year-old females in 1984, was 35.8, 29.3, 30.7 and 34.2, 23.3, 21.6 in the L-, C- and H-lines, respectively. For the complete data set the average accumulated percent kit mortality was 14.4 at the first examination, 25.8 the first two days postpartum, 28.9 the first week, 30.7 the first three weeks and 32.4 to weaning. On average, 0.96 kits per litter were found dead on the day of parturition.

The numbers of litters with dead kits and destroyed litters at the first examination and lost litters during preweaning are presented in Table 4. At least one dead kit was found at the first examination in about half of the litters. About 10 percent of the litters were entirely lost from birth to weaning, including litters in which all the kits were dead at the first examination. The number of those litters with only dead kits was over the whole experiment 12, 13 and 8 in the L-, C- and H-lines, respectively. The average litter size for these lost litters was 3.0 kits and the cumulative percentages of litters consisting of one kit was 45 percent and of two kits 58 percent.

The kits found dead at the first examination in the last three years were subjected to the lung-float test. Results from this investigation are given in Table 5. On average from a total of 310 kits, 85 percent of the kits found dead at the first examination were defined as stillborn.

The data collected from the autopsied females, which all gave birth, are presented in Table 6. The postimplantation loss was expressed as the difference between the number of implantation zones and the total number of kits born. The percentage values are given in Figure 3 and were on average 13.6 percent for the whole experiment. The postimplantation loss was highest in the H-line the first year, but changed to become the lowest compared to the other two lines in both 1982 and 1984. An average of 8.05 implantation zones were found, with the highest number in the H-line, the lowest in the L-line. More than one embryo on average was lost during the postimplantation period. During all years and within all three lines there was a higher proportion of implantation zones (1.2:1) in the right uterine horn compared with the left.

The total number of teats varied from 3 to 12 and the number of active teats from 0 to 10. The average total number of teats of the breeding females was 7.2, with a similar number on both rows. The number of active teats was on average 1.5 less than the total number. In 1982 the number of teats was counted on all kits at three

Table 3. Average number of kits per whelped mink female, registered at different times postpartum (pp.). The figures given in italics denote the standard deviation.

| Year | Line | At first examin. | | 2 d. pp. | 7 d. pp. | 21 d. pp. | 42 d. pp. (weaning) |
|---|---|---|---|---|---|---|---|
| | | Total | Alive | | | | |
| 1979 | L | 6.82 | 5.82 | 5.00 | 4.98 | 4.93 | 4.87 |
| | | *2.83* | *2.28* | *2.43* | *2.39* | *2.37* | *2.58* |
| | C | 6.70 | 5.77 | 5.26 | 5.09 | 5.02 | 4.95 |
| | | *2.91* | *2.65* | *2.36* | *2.44* | *2.41* | *2.62* |
| | H | 7.40 | 6.10 | 5.15 | 5.10 | 4.94 | 4.86 |
| | | *2.08* | *2.22* | *2.54* | *2.40* | *2.74* | *2.46* |
| 1980 | L | 6.74 | 5.88 | 5.00 | 4.86 | 4.88 | 4.71 |
| | | *2.56* | *2.73* | *2.76* | *2.65* | *2.62* | *2.50* |
| | C | 7.09 | 6.24 | 5.54 | 5.39 | 5.30 | 5.17 |
| | | *1.84* | *1.84* | *2.06* | *2.13* | *2.10* | *2.03* |
| | H | 7.17 | 6.17 | 5.47 | 5.34 | 5.28 | 5.15 |
| | | *2.35* | *2.29* | *2.37* | *2.26* | *2.25* | *2.17* |
| 1981 | L | 6.34 | 5.55 | 4.97 | 4.82 | 4.68 | 4.58 |
| | | *2.56* | *2.55* | *2.33* | *2.43* | *2.37* | *2.33* |
| | C | 6.50 | 5.52 | 5.02 | 4.82 | 4.75 | 4.70 |
| | | *2.48* | *2.39* | *2.46* | *2.48* | *2.46* | *2.45* |
| | H | 7.10 | 6.34 | 5.94 | 5.76 | 5.61 | 5.49 |
| | | *2.20* | *2.39* | *2.39* | *2.44* | *2.36* | *2.35* |
| 1982 | L | 6.33 | 5.10 | 3.94 | 3.52 | 3.33 | 3.27 |
| | | *2.31* | *2.63* | *2.37* | *2.43* | *2.35* | *2.38* |
| | C | 6.85 | 5.66 | 4.61 | 4.34 | 4.30 | 4.10 |
| | | *2.55* | *2.70* | *2.69* | *2.73* | *2.56* | *2.61* |
| | H | 7.05 | 6.21 | 5.07 | 4.70 | 4.55 | 4.33 |
| | | *2.24* | *2.41* | *3.15* | *2.98* | *2.97* | *3.11* |
| 1983 | L | 6.00 | 5.08 | 4.30 | 4.22 | 4.00 | 3.84 |
| | | *2.24* | *2.53* | *2.59* | *2.65* | *2.58* | *2.59* |
| | C | 6.55 | 5.30 | 4.32 | 4.11 | 4.05 | 3.98 |
| | | *3.00* | *2.96* | *2.70* | *2.73* | *2.71* | *2.65* |
| | H | 5.66 | 4.56 | 3.84 | 3.44 | 3.41 | 3.34 |
| | | *2.86* | *2.64* | *2.68* | *2.73* | *2.72* | *2.66* |
| 1984 | L | 6.21 | 5.11 | 4.60 | 4.30 | 4.15 | 4.04 |
| | | *2.41* | *2.45* | *2.54* | *2.53* | *2.48* | *2.42* |
| | C | 6.72 | 6.38 | 5.68 | 5.47 | 5.19 | 4.96 |
| | | *2.39* | *2.46* | *2.54* | *2.59* | *2.52* | *2.47* |
| | H | 7.28 | 6.44 | 5.84 | 5.67 | 5.58 | 5.35 |
| | | *2.14* | *2.28* | *2.18* | *2.23* | *2.31* | *2.25* |

weeks of age and the average number was 7.40 in the L-line, 7.38 in the C-line and 7.47 in the H-line. Variations from 5 to 11 teats were observed. In 48 percent of the kits the teats were not paired.

*Empty females*

All empty females where autopsied in order to investigate the uterus (Table 7). During the whole experiment a total of 139 females or 14.9 percent of the mated females were empty. In 1983, 37 females were empty, while the total number of empty females per year varied between 13 and 26 for the other years.

Implantation zones were found in 38 of the 139 empty females, equal to 27.3 percent. Excluding the females mated with the 20 infertile males, as defined earlier, the average percent of empty females with implantation zones was 31.9. The two percentage figures were 35.3 and 43.9 respectively, when the results from 1983 were excluded. The average number of implantation zones in empty females was 5.1, almost three zones less than in females giving birth.

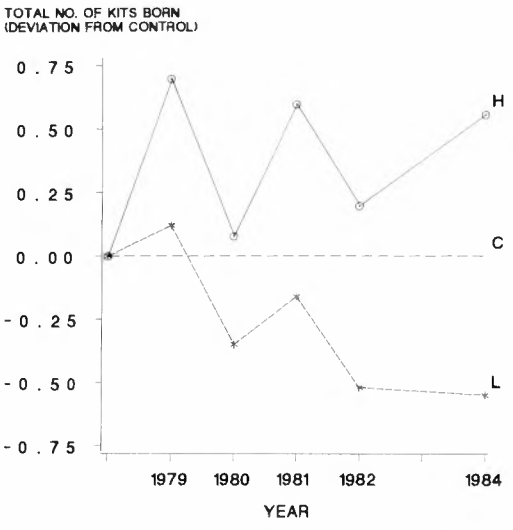


Figure 1. The selection response in high- (H) and low line (L) expressed as deviation from the control line (C), and plotted against the years. The data in 1983 were excluded.

*Mating and gestation*

Data about matifig, length of gestation and date of birth are given in Table 8. Sixty percent of the females allowed remating after at least seven days from the first mating, calculated for the entire data set. The average length of gestation was 46 days, varying from 38 to 58 days in individual females. The distribution of gestation length for each line and year is given in Figure 4.

The standard deviation within line and year for date of mating was about 2.5 days, for date of birth 3 days, for gestation length 3.5 days and for copulation time 20 minutes.

In 1984, the females that mated for the first time after mid-March and remated the following day gave 0.35 fewer kits at birth in the L-line and 0.22 kits more in the H-line, compared to the females that mated early and were given the opportunity to remate 8 days later. The females that mated early only once had lower litter size at birth, 0.14 and 0.20 below the line average for the L- and H-lines, respectively, and on average had the longest gestation length by about 50 days.

The effect of gestation length on litter size at birth was analysed. When the component of generation was included in the model, it was found to be significant for the entire experiment, but not within lines. However, the regression coefficient of length of gestation on litter size at birth was estimated at -0.16 $\pm$ 0.02, whether the generation component was included or excluded. Within lines the following regression component were found, -0.16, -0.20 and -0.14 in the L-, C- and H-lines, respectively. All these regression coefficients and the models were highly significant. In addition to these linear regressions a curvilinear regression was conducted but gave no significant effects.

For pregnancies of fewer and more than 50 days the regression coefficients were -0.12 and -0.42, respectively, regardless of the component of generation in the model. These regression coefficients and the models analysed over lines were highly significant. When analysing within lines, similar regression coefficients were found.

*Testes weights*

Data on testes weight and the proportion between the weight of the left and right testes are given in Table 9, together with body weight on the day of autopsy. The variation in average testis weight between years, especially for the high figures the first year, is probably due to the sampling method of the testis. This will not affect the comparison between the lines within the year, however, as the procedure of cutting out the testis was equal within each year. The standard deviation of the weight of one testis within line and year varied from 0.21 to 0.82 grams. Differences between average testis weight between the lines have to be about 0.2 grams to be significant at the 5 percent level. In the last generation the testis weight was on average significantly lower in the H-line compared with the L-line; however, at the same time the body weight was significantly lower in the H-line. The weights of the right and the left testes were about the same.

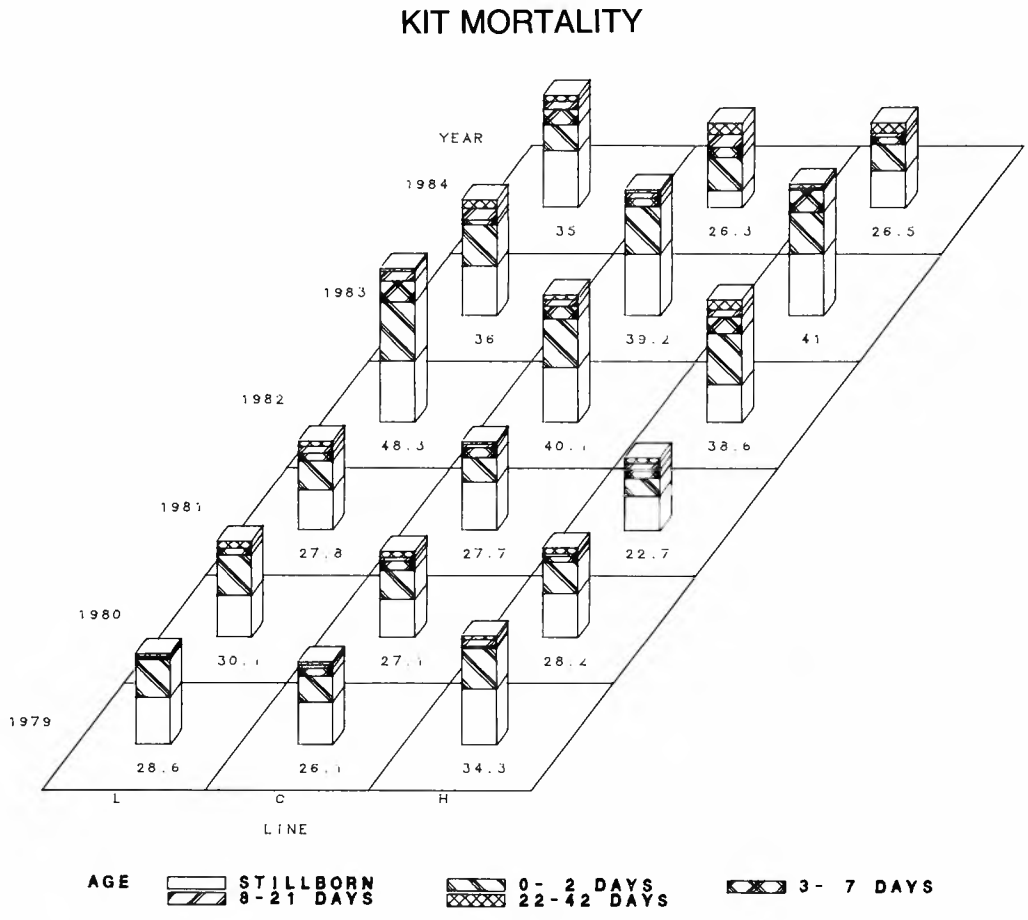


Figure 2. Kit mortality at different intervals from birth to weaning for each line and year. The lowest part of the pillar, denoted as stillborn, presents percent of dead kits observed at the first examination.

Table 4. Data concerning kit mortality, registered as litter-traits.

| Year | Line | Litters with dead kits at 1. examin. | | Destroyed litters at 1. examination | | Lost litters to weaning | |
|---|---|---|---|---|---|---|---|
| | | no. | % | no. | % | no. | % |
| 1979 | L | 19 | 42.2 | 0 | 0.0 | 2 | 4.4 |
| | C | 21 | 47.7 | 1 | 2.3 | 1 | 2.3 |
| | H | 26 | 52.0 | 2 | 4.0 | 5 | 10.0 |
| 1980 | L | 16 | 38.1 | 1 | 2.4 | 3 | 7.1 |
| | C | 23 | 50.0 | 0 | 0.0 | 2 | 4.3 |
| | H | 23 | 48.9 | 1 | 2.1 | 2 | 4.3 |
| 1981 | L | 17 | 44.7 | 3 | 7.9 | 3 | 7.9 |
| | C | 20 | 45.5 | 1 | 2.3 | 3 | 6.8 |
| | H | 20 | 40.8 | 1 | 2.0 | 3 | 6.1 |
| 1982 | L | 29 | 60.4 | 4 | 8.3 | 8 | 16.7 |
| | C | 22 | 53.7 | 3 | 7.3 | 6 | 14.6 |
| | H | 20 | 47.6 | 0 | 0.0 | 9 | 21.4 |
| 1983 | L | 16 | 43.2 | 2 | 5.4 | 4 | 11.7 |
| | C | 23 | 52.3 | 5 | 11.4 | 8 | 18.2 |
| | H | 20 | 62.5 | 3 | 9.4 | 7 | 21.9 |
| 1984 | L | 26 | 49.1 | 2 | 3.8 | 6 | 11.3 |
| | C | 13 | 27.7 | 3 | 6.4 | 4 | 8.5 |
| | H | 16 | 37.2 | 1 | 2.3 | 1 | 2.3 |
| Sum | L | 123 | 46.8 | 12 | 4.6 | 26 | 9.9 |
| | C | 122 | 45.9 | 13 | 4.9 | 24 | 9.0 |
| | H | 125 | 47.5 | 8 | 3.0 | 27 | 10.3 |
| Total | | 370 | 46.7 | 33 | 4.2 | 77 | 9.7 |

Table 5. Data and number of kits observed as dead at the first examination. Kits with lungs that sunk were defined as stillborn.

| Year | Line | No. of kits | Lungs floating, | | Average body weight, g |
|---|---|---|---|---|---|
| | | | n | % | |
| 1982 | L | 40 | 10 | 25.0 | 7.4 |
| | C | 38 | 5 | 13.2 | 7.9 |
| | H | 30 | 8 | 26.7 | 8.2 |
| 1983 | L | 31 | 1 | 3.2 | 9.4 |
| | C | 49 | 6 | 12.2 | 8.1 |
| | H | 26 | 5 | 19.2 | 8.3 |
| 1984 | L | 45 | 6 | 13.3 | 8.9 |
| | C | 19 | 1 | 5.3 | 7.5 |
| | H | 32 | 5 | 15.6 | 6.4 |
| Sum | L | 116 | 17 | 14.7 | |
| | C | 106 | 12 | 11.3 | |
| | H | 88 | 18 | 20.5 | |
| Total | | 310 | 47 | 15.2 | |

Table 6. Litter size at birth, implantation zones and number of teats observed on autopsied female mink. All autopsied females gave birth. Proportion of teats is for total number.

| Year | Line | No. of females | Litter size at birth[a] | Implantation zones | | | Teats | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | mean | SD | prop. r/l[b] | tot. | active | prop. r/l[b] |
| 1979 | L | 23 | 7.22 | 8.48 | *3.34* | 1.01 | 7.35 | 5.70 | 0.99 |
| | C | 22 | 6.64 | 7.95 | *2.77* | 1.41 | 7.27 | 6.18 | 0.95 |
| | H | 23 | 7.39 | 9.13 | *2.03* | 1.08 | 7.39 | 6.35 | 1.10 |
| 1980 | L | 17 | 5.65 | 6.06 | *2.56* | 1.15 | 7.24 | 4.82 | 0.89 |
| | C | 18 | 6.89 | 7.72 | *1.87* | 1.29 | 6.78 | 5.77 | 0.91 |
| | H | 17 | 7.35 | 8.18 | *2.00* | 1.25 | 7.18 | 6.35 | 0.97 |
| 1981 | L | 20 | 7.00 | 7.85 | *2.32* | 1.21 | 7.30 | 6.40 | 1.00 |
| | C | 21 | 7.14 | 8.24 | *1.84* | 1.34 | 7.19 | 5.90 | 0.96 |
| | H | 23 | 6.87 | 7.70 | *2.03* | 1.21 | 7.13 | 6.22 | 1.02 |
| 1982 | L | 43 | 6.49 | 7.88 | *1.90* | 1.05 | 6.88 | 4.88 | 0.94 |
| | C | 39 | 7.00 | 8.54 | *1.96* | 1.23 | 7.38 | 5.44 | 0.99 |
| | H | 41 | 7.12 | 8.05 | *2.52* | 1.23 | 7.54 | 5.71 | 0.99 |
| 1983 | Non autopsied | | | | | | | | |
| 1984 | L | 42 | 6.50 | 8.12 | *2.46* | 1.13 | 6.81 | 5.09 | - |
| | C | 42 | 7.07 | 8.38 | *2.74* | 1.26 | 7.24 | 6.00 | - |
| | H | 41 | 7.34 | 8.41 | *1.97* | 1.23 | 7.17 | 5.56 | - |
| Sum | L | 145 | 6.57 | 7.68 | | 1.11 | 7.12 | 5.38 | 0.96 |
| | C | 142 | 6.95 | 8.17 | | 1.31 | 7.17 | 5.86 | 0.95 |
| | H | 145 | 7.21 | 8.29 | | 1.20 | 7.28 | 6.04 | 1.02 |
| Total | | 432 | 6.91 | 8.05 | | 1.21 | 7.19 | 5.76 | 0.98 |

[a] Litter size at birth only for females autopsied.

[b] Proportion between right and left uteri horn and between right and left row of total number of teats, respectively.

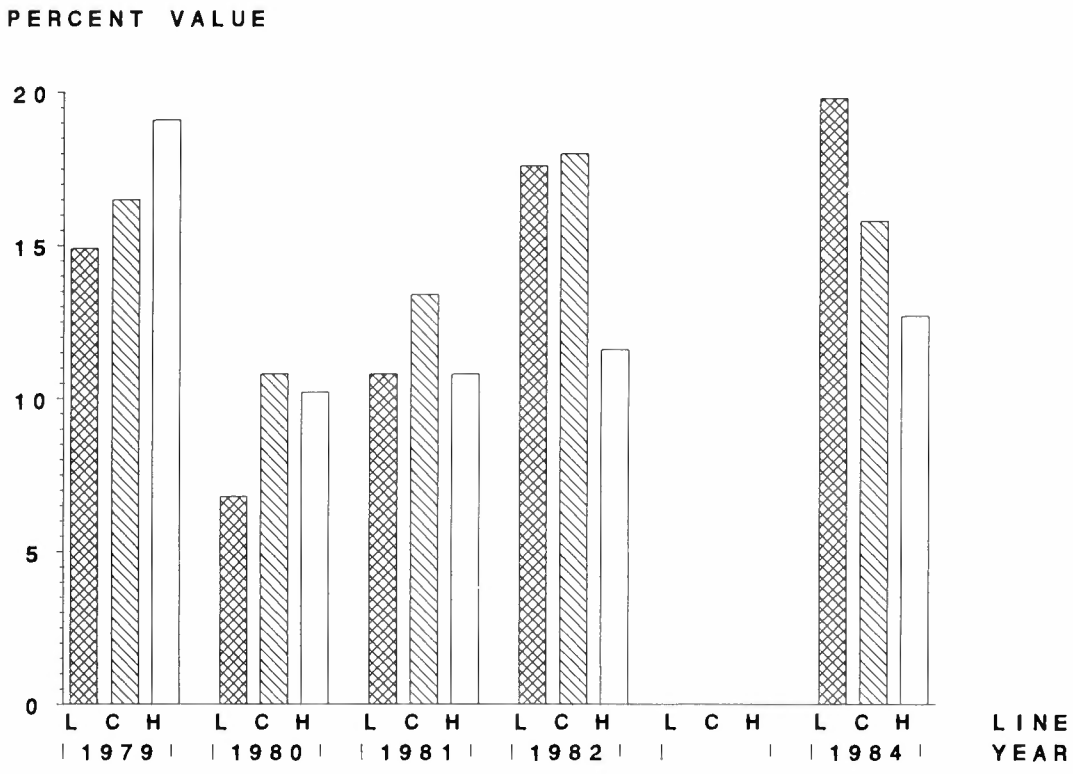


Figure 3. Prenatal mortality, calculated as the difference between the number of implantation zones and the total number of kits born to the autopsied females. This expresses the average prenatal mortality during the last 23 days of the pregnancy, approximately.

Table 7. The number of empty females, implantation zones and number of males mating with females becoming empty.

| Year | Line | Empty females | | | Implantation zones | | Males with empty females[b] |
|---|---|---|---|---|---|---|---|
| | | no. | % | (%)[a] | in no. of females | no. of zones | |
| 1979 | L | 6 | 11.8 | | 2 | 2, 9 | 5×1, 1×2 |
| | C | 4 | 8.3 | | 1 | 5 | 5×1, 1×2 |
| | H | 3 | 5.7 | | 0 | | 2×1, 1×2 |
| 1980 | L | 10 | 18.9 | (12.2) | 2 | 8, 8 | 2×1, 2×2, 1×4 |
| | C | 9 | 16.4 | ( 8.0) | 2 | 8, 10 | 4×1, 1×5 |
| | H | 7 | 13.0 | ( 7.8) | 0 | | 4×1, 1×4 |
| 1981 | L | 9 | 19.1 | (13.6) | 2 | 4, 6 | 2×1, 2×2, 1×3 |
| | C | 5 | 10.2 | | 4 | 1, 1, 6, 11 | 6×1 |
| | H | 3 | 5.8 | | 1 | 10 | 3×1 |
| 1982 | L | 4 | 7.7 | | 4 | 4, 6, 8, 11 | 4×1 |
| | C | 8 | 16.3 | | 4 | 1, 7, 7, 9 | 9×1 |
| | H | 10 | 19.2 | (10.6) | 1 | 3 | 5×1, 1×5 |
| 1983 | L | 11 | 22.9 | | 1 | 4 | 2×1, 3×3 |
| | C | 7 | 13.7 | | 1 | 5 | 5×1, 1×2 |
| | H | 19 | 37.3 | | 0 | | 3×1, 2×2, 3×4 |
| 1984 | L | 5 | 8.6 | | 1 | 1 | 1×2, 1×3 |
| | C | 10 | 17.5 | | 6 | 1, 2, 3, 4, 6, 7 | 4×1, 2×2, 1×3 |
| | H | 9 | 19.2 | | 6 | 1, 1, 1, 1, 4, 7 | 6×1, 1×4 |

[a] Figures calculated when excluding females mated with infertile males. Males were defined as infertile when mating with at least 3 females, all with uteri horns without implantation zones.

[b] Number of males with empty females. The first figure denotes number of males while the second figure denotes number of empty females per male, i.e. 5×1 means five males each with one empty female after mating.

Table 8. Data on mating, gestation length and date of birth.

| Year | Line | Date of mating, March | | Length of copulat. min. | | Remating % | Gestation length, days[c] | Date of birth, May |
|---|---|---|---|---|---|---|---|---|
| | | 1[a] | 2[b] | 1[a] | 2[b] | | | |
| 1979 | L | 11. | 18. | 41 | 67 | 84.6 | 44.4 | 2. |
| | C | 11. | 20. | 37 | 56 | 59.3 | 46.4 | 2. |
| | H | 11. | 20. | 38 | 53 | 63.0 | 46.1 | 2. |
| 1980 | L | 12. | 20. | 54 | 77 | 59.6 | 46.3 | 2. |
| | C | 12. | 19. | 41 | 68 | 65.5 | 45.9 | 1. |
| | H | 12. | 19. | 42 | 64 | 60.0 | 47.1 | 2. |
| 1981 | L | 12. | 19. | 46 | 49 | 76.6 | 46.8 | 4. |
| | C | 13. | 19. | 47 | 34 | 54.0 | 46.8 | 2. |
| | H | 13. | 20. | 40 | 42 | 67.3 | 46.1 | 3. |
| 1982 | L | 11. | 20. | 45 | 75 | 73.1 | 46.2 | 4. |
| | C | 11. | 20. | 38 | 68 | 78.0 | 43.6 | 1. |
| | H | 12. | 19. | 33 | 59 | 75.0 | 45.9 | 2. |
| 1983 | L | 10. | 19. | 46 | 78 | 72.9 | 45.2 | 1. |
| | C | 11. | 19. | 32 | 79 | 66.7 | 44.9 | 1. |
| | H | 12. | 20. | 34 | 61 | 60.8 | 45.8 | 2. |
| 1984 | L | 15. | 20. | 48 | 52 | 70.7 | 47.5 | 5. |
| | C | 16. | 20. | 42 | 50 | 61.4 | 45.8 | 4. |
| | H | 15. | 19. | 46 | 48 | 60.4 | 47.3 | 4. |

[a] First mating.
[b] Second mating.
[c] Gestation length was calculated from the last mating.

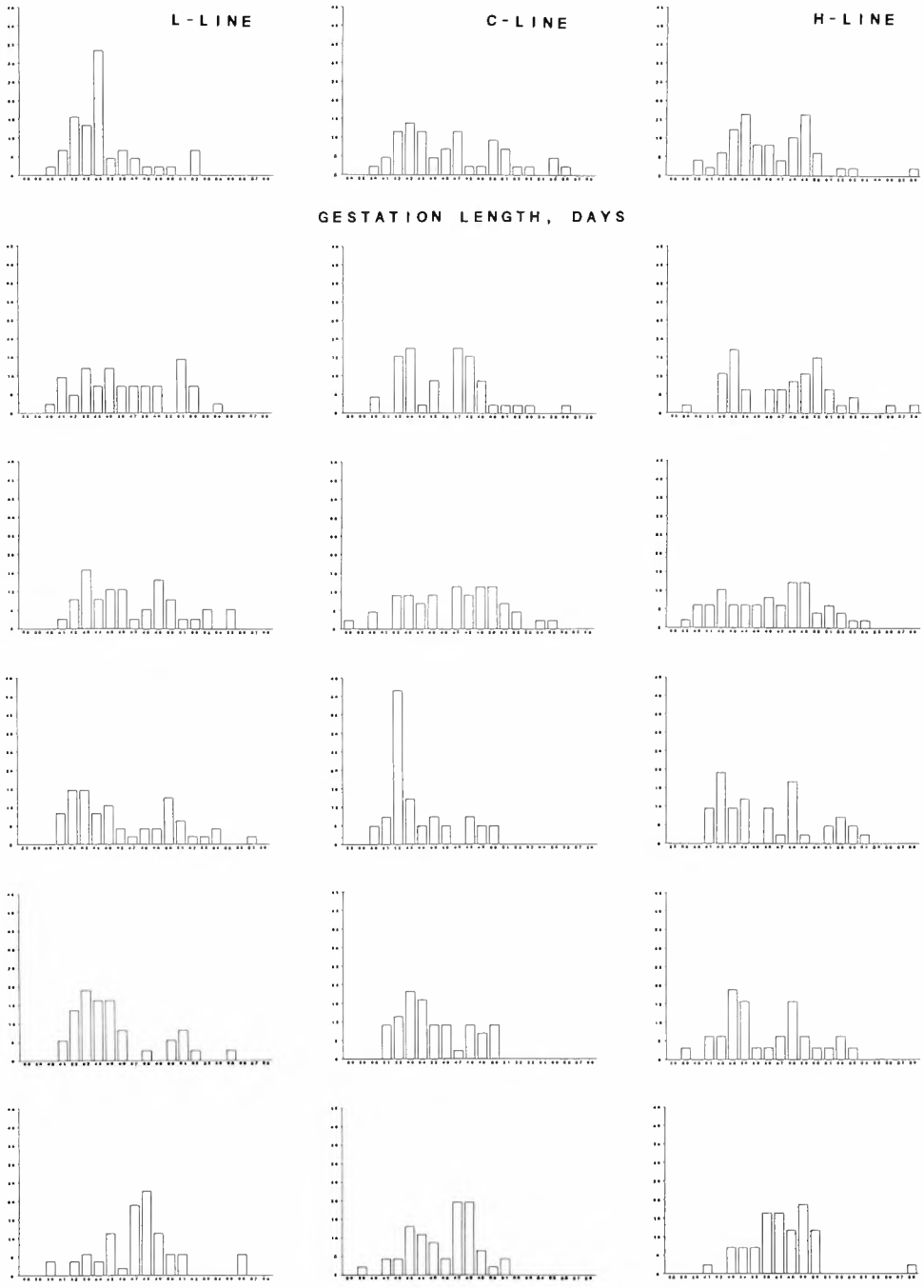


Figure 4. Distribution of the length of the gestation in mink, given for each line within the year. The gestation period was defined as the interval from the last mating to parturition.

*Preweaning kit body weight*

The average kit body weights at the day of birth, 21 days postpartum and at weaning (42 days postpartum) are given in Table 10, together with sex ratio at 21 days of age.

The average body weight at birth of the kits in a litter varied from 5.5 to 15.7 grams. No significant difference was observed between lines in the last generation, either in average body weight at the day of birth or at 21 days postpartum. At 42 days postpartum, significantly lower average body weight was observed for females in the H-line compared to the C- and the L-lines, while for males it was lower only when compared to the L-line. There was relatively high variation in average body weight at weaning for both females and males between years.

The overall average kit body weight at birth was 9.8 grams, at 21 days postpartum it was 119 grams and at weaning, 311 and 358 grams for females and males, respectively.

The standard deviation for kit body weight was on average about 1.6 grams at birth, 18 grams at 21 days of age, and 60 and 80 grams at weaning for females and males, respectively.

The sex ratio varied between 0.8 and 1.2, including variations within lines over years.

Table 9. Average body weight and testis weight of the breeding males registered postcoitum.

| Year[a] | Line | Body weight, g | Testis weight, g | | Proportion r/l testis |
|---|---|---|---|---|---|
| | | | right | left | |
| 1979 | L | 1 900 | 3.31 | 3.41 | 0.97 |
| | C | 1 807 | 3.27 | 3.42 | 0.96 |
| | H | 1 857 | 3.23 | 3.09 | 1.05 |
| 1980 | L | 1 848 | 2.96 | 3.05 | 0.97 |
| | C | 1 784 | 2.85 | 2.86 | 1.00 |
| | H | 1 690 | 2.70 | 2.71 | 1.00 |
| 1981 | L | 1 790 | 2.68 | 2.76 | 0.97 |
| | C | 1 761 | 2.67 | 2.51 | 1.06 |
| | H | 1 728 | 2.44 | 2.61 | 0.93 |
| 1982 | L | 1 791 | 2.91 | 3.18 | 0.92 |
| | C | 1 834 | 2.63 | 2.83 | 0.93 |
| | H | 1 813 | 2.85 | 2.87 | 0.99 |
| 1983 | L | 1 713 | 2.53 | 2.75 | 0.92 |
| | C | 1 837 | 2.70 | 2.74 | 0.99 |
| | H | 1 801 | 2.53 | 2.55 | 0.99 |
| 1984 | L | 1 756 | 2.39 | 2.61 | 0.92 |
| | C | 1 712 | 2.29 | 2.35 | 0.97 |
| | H | 1 671 | 2.07 | 2.21 | 0.94 |

[a] Date of autopsy was between March 26 and March 28, except in 1984 when it took place on April 6, but always on the same date within the year.

*Body weight of breeding animals*

The average body weight of breeding females and breeding males at certain times between selection and December to mating is given in Figure 5. The body weight of females was on average 1.18 kg at birth and 1.13 kg at 21 days postpartum. The only significant differences between lines were observed in female body weight at birth in 1982, with the highest body weight in the C-line and the lowest in the L-line. The average standard deviation was about 125 grams.

*Analyses of within-line differences between the first and last years and between-line differences in the last year*

Differences given as LS-means were analysed and are presented in Tables 11 and 12.

As can be seen from Table 11 no significant differences, either in litter size at birth or at 21 days postpartum, were observed between the first and last years when comparing LS-means within line. However, it should be noted that litter size at birth was highest in the first year of the experiment, in both selection lines. Litter size

Table 10. Average preweaning body weight of kits (g) at different times postpartum (p.p.) and sex ratio at 21 days p.p. Body weight at birth and at 21 days p.p. is given as the average over the sexes and based on the total litter weight. Body weight at weaning is based on individual weighings.

| Year | Line | Average kit body weight | | | | Sex ratio |
|---|---|---|---|---|---|---|
| | | at birth | 21 d.p.p. | 42 d.p.p. Females | 42 d.p.p. Males | 21 d.p.p. |
| 1979 | L | 10.4 | 127 | 340 | 386 | 0.90 |
| | C | 10.1 | 124 | 317 | 363 | 0.84 |
| | H | 10.0 | 115 | 309 | 346 | 1.12 |
| 1980 | L | 10.0 | 123 | 310 | 371 | 1.08 |
| | C | 10.2 | 128 | 332 | 371 | 1.12 |
| | H | 9.9 | 118 | 311 | 362 | 1.23 |
| 1981 | L | 10.2 | 124 | 347 | 397 | 1.07 |
| | C | 10.6 | 126 | 338 | 384 | 1.20 |
| | H | 10.0 | 118 | 304 | 338 | 0.94 |
| 1982 | L | 8.9 | 111 | 279 | 317 | 1.05 |
| | C | 8.9 | 121 | 278 | 318 | 1.15 |
| | H | 9.5 | 116 | 253 | 282 | 1.12 |
| 1983 | L | 9.8 | 105 | 323 | 380 | 0.78 |
| | C | 9.1 | 124 | 346 | 412 | 0.93 |
| | H | 9.3 | 111 | 316 | 370 | 1.14 |
| 1984 | L | 9.5 | 113 | 313 | 362 | 1.24 |
| | C | 9.5 | 117 | 304 | 345 | 1.22 |
| | H | 9.9 | 113 | 276 | 331 | 0.95 |

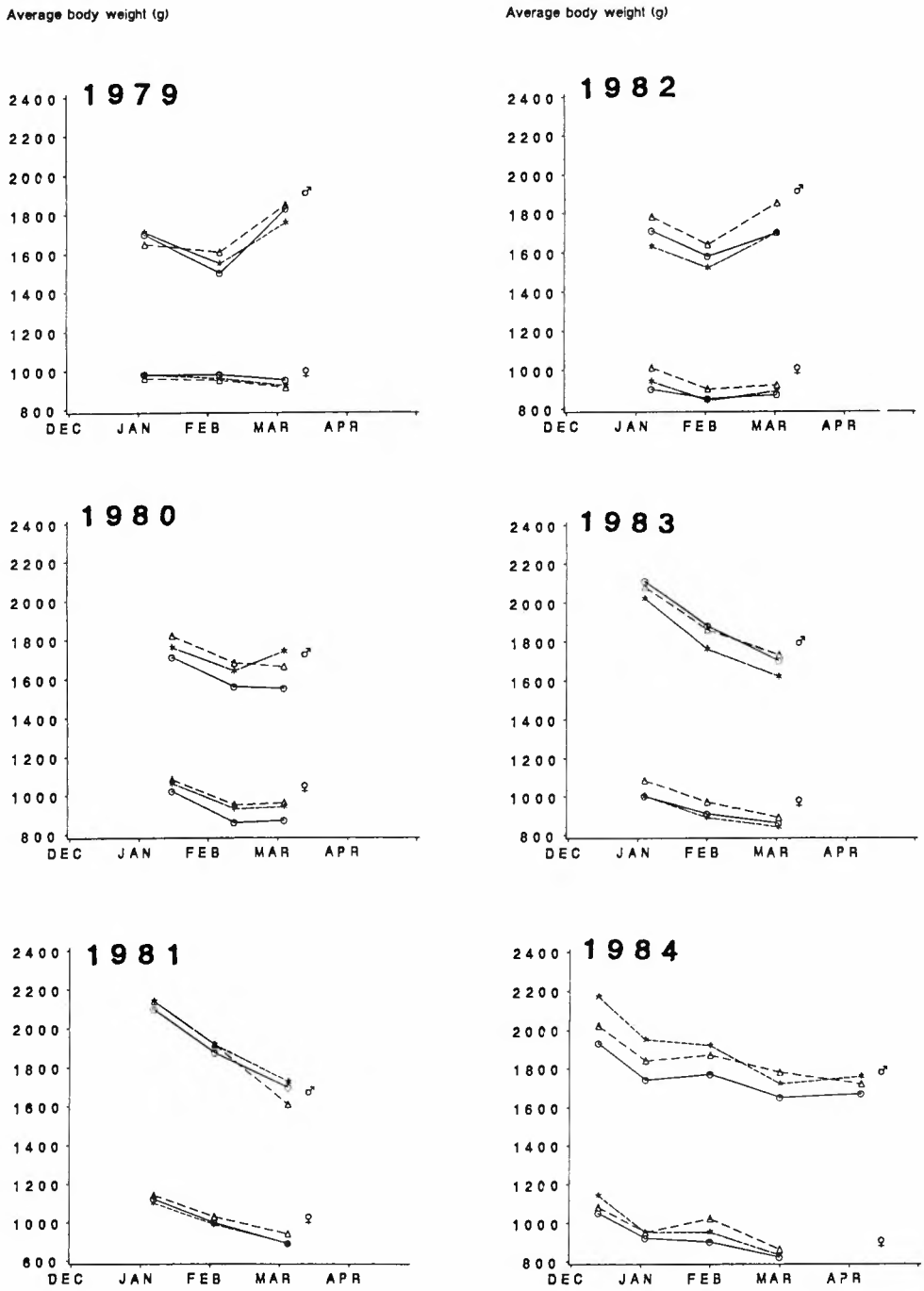


Figure 5. Average body weight of breeding females and males before mating in the different lines during the experiment. The first year (1979) is top left and the last year (1984) is bottom to right. In 1984 only one-year-old females were weighed.

at weaning was higher in the last year in all lines, but differed significantly only in the L- and C-lines. When expressing the litter size at weaning per whelped female, as given in Table 3, the differences between 1979 and 1984 were 0.83, -0.01 and -0.49 within the L-, C- and H-lines, respectively.

The length of gestation was significantly different between the years within the L-line only, but indicated no general trend in increased length of gestation.

The average body weight of the kits at birth and at 21 days postpartum tended to decrease from the start of the experiment as indicated by the figures given in Table 11.

Significant differences were observed in the last year between the selection lines in litter size at birth, at 21 days postpartum and at weaning. Litter size at 21 and 42 days postpartum were significantly lower in the L-line than in the C-line. The figures presented in Table 12 are based only on litters present at these two times. The

Table 11. Differences in LS-means for some reproductive traits and preweaning kits' body weights, between the first and the last year within lines.

| Trait[a] | Within line differences comparing data from 1979 and 1984 | | |
|---|---|---|---|
| | L | C | H |
| Litter size at birth, no. | 0.61 | -0.02 | 0.12 |
| Litter size at 21 days p.p., no. | 0.78 | -0.17 | -0.94 |
| Litter size at weaning (42 d.p.p.), no. | - 1.00* | -1.93*** | -0.49 |
| Implantation zones, no. | 0.36 | -0.43 | 0.71 |
| Gestation length, days | - 2.88*** | 0.52 | -1.04 |
| Kits body weight at birth, grams | 0.88** | 0.67* | 0.27 |
| Kits body weight at 21 days p.p., grams | 13.59*** | 6.62 | 2.83 |

[a] Litter size at different times postpartum (p.p.) is per litter at the given time and not per whelped female.
* = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$.

Table 12. Significance of difference in LS-means for some reproductive traits and preweaning kits' body weight, between the lines in the last generation.

| Trait[a] | Line differences | | |
|---|---|---|---|
| | H – C | C – L | H – L |
| Litter size at birth, no. | 0.56 | 0.51 | 1.07* |
| Litter size at 21 days p.p., no. | 0.04 | 0.99* | 1.03* |
| Litter size at weaning (42 d.p.p.), no. | 0.06 | 0.87* | 0.93* |
| Implantation zones, no. | 0.03 | 0.26 | 0.29 |
| Gestation length, days | 1.31* | -1.47* | -0.16 |
| Kits body weight at birth, grams | 0.42 | -0.05 | 0.37 |
| Kits body weight at 21 days p.p., grams | -3.58 | 3.88 | 0.30 |

[a] Litter size at different times postpartum (p.p.) is per litter at the given time and not per whelped female.
* = $p<0.05$.

differences in preweaning litter size per whelped female increased between the selection lines and was 1.24, 1.37, 1.42 and 1.31 for 2, 7, 21 and 42 days postpartum. Gestation length was longer by about 1.4 days in the H- and L-lines compared to the C-line.

## DISCUSSION

### *Prenatal and postnatal loss*

The litter sizes at different times until weaning are dependent on the number of kits born and kit mortality. The significant differences in litter size at 21 days postpartum and at weaning observed between the selection lines in the present experiment are therefore the result of a compounding of these traits.

The litter sizes given in Table 12 do not include females that lost their entire litter prior to the date of observation. Kit mortality is included only for litters present at observation. The differences in litter size decreased slightly from birth to weaning due to litter loss. As can be seen from Table 12 the differences between the H- and L-lines in litter size at 21 days and 42 days postpartum were similar to those between the C- and L-lines. This was largely explained by the increased postnatal mortality in the L-line.

The most interesting observation from the figures on kit mortality is the change between the two selection lines. During the first year the highest accumulated kit mortality was observed in the H-line. However, in the other years (except for 1983), kit mortality in the H-line was lower than in the L-line and about the same level as in the C-line. It is commonly found that with increased litter size the kit mortality, in absolute figures, increases (see review Einarsson, 1982). However, no negative regression coefficient of litter size at birth on litter size at weaning, not even at a high level of litter size, could be found in mink (Einarsson, 1982).

The highest postnatal kit mortality was observed during the first two days. The figures on kit mortality found in the present experiment are generally in accordance with the results from other experiments (Udris, 1968; Udris & Olden, 1977; Pedersen, 1978; Skrede, 1978; Howell, 1979; Einarsson, 1980a, Skrede, 1981; Tauson, 1985). It must be taken into consideration that, except for the last year, only one-year-old females were used. It was observed that the kit mortality was about 10 percent higher in yearlings than in older females (Einarsson, 1980a). The figures for the total accumulated kit mortality in mink, observed on commercial farms, are not comparable due to inconcutive and infrequent inspections of the nest boxes. The early dead kits could therefore go unobserved and kit mortality during the first two days would be underestimated.

In about half of the litters, at least one kit was found dead at the time of the first examination. However, during the experiment (except for 1983) there was a positive change in the H-line compared to the other selection lines, particularly in the last generation. Only a few litters were entirely lost when the first examination was conducted and more than half of these litters included only one or two kits. This concurs with earlier analyses, which showed a higher percentage kit mortality in the smallest litter sizes (see Einarsson, 1980a).

During the last three years an average of 85 percent of the kits found dead at the first examination were defined as stillborn. The lowest percentage of stillborn kits was found in the H-line. Using the lung-float test Pedersen (1978) found that 55 percent of the kits found dead at the first examination were stillborn. In that experiment, however, the litters were checked only once a day, which gave a longer interval from birth to the first examination. This would decrease the stillborn figure, since it included an increased number of kits that died postpartum. It could also increase the number of lost dead kits.

The implantation zones are invaluable for estimation of the embryonic loss from about 23 days prepartum (Einarsson, 1987a). In the present experiment it was found that on average 1.1 embryos were lost in the females giving birth during this period. In general a higher loss was observed in the L-line than in the H-line

and the differences in estimated prenatal mortality changed during the selection to lowest in the H-line (see Figure 3). This concurs with the changes mentioned earlier in postnatal loss. In a divergent selection experiment on litter size in mice, Batemann (1966) summarized that after 12 generations of selection; females from the less fertile strain were particularly prone to preimplantational loss of eggs. It was not known whether this was due to fertilizational or implantational failure. The preimplantation loss was found to be 7 to 10 percent, in relation to the number of corpora lutea (ovulated eggs). In other selection experiments for litter size in mice, prenatal loss was affected by the selection and most particularly in the low lines (Joakimsen & Baker, 1977; Bakker et al., 1978; Durrant et al., 1980).

As discussed in Part I of this series, the regression of litter size on the number of implanted embryos was found to be linear (Einarsson, 1982). Similar analyses from the whole selection experiment confirm these results.

It has been shown that prolactin is the inducing or luteotropic factor for activating the corpora lutea in mink from the delayed condition (Murphy et al., 1981). The activating factor of the prolactin is not exactly known, although light plays an important role. It is thought that there is no luteotropic effect from the blastocysts in mink, however, there could be some interaction between blastocysts and dam which should be investigated in more detail.

The percentage of empty females varied between 6 and 19 in the different lines within the year. These figures were to be expected when considering almost only one-year-old females. The data are too limited to draw any conclusions regarding the effect of selection on failure to whelp. From the present experiment and earlier results (Nyberg, 1979; Skrede, 1980), it can be concluded that about half of the empty mink females have had implanted embryos.

*Teats*

There were only small variations in the total number of teats between both years and lines, though a tendency to an increasing difference between the two selection lines could be seen. The differences observed in active teats are thought to be caused by the difference in litter size. In the present experiment there was no indication of increased kit mortality when the number of kits exceeded the number of active teats, as argued by Pedersen (1978). Although it is not necessary to have one teat per kit, assuming a minimum number of teats, it would be important to cull out breeding animals with quantitative or qualitative failure of the teats, especially when increasing litter size further. Both the total number and the number of active teats on the breeding females found in the present experiment were about 0.5 less than for females representing all age classes (Hoogerbrugge & Baud, 1975b) and about 0.8 higher compared to the one-year-old females in Pedersen's experiment (1978). Elofson & Swensson (1982) found an average of 7.5 teats in 3-week-old mink kits with variations from 3 to 11 teats. The number of normally developed teats in the breeding females in the same experiment was found to average at 6.4 with a range from 2 to 10. The total number of teats is difficult to examine on live animals after weaning. If included in the selection programme it should be conducted on the kits of both sexes before the age of three weeks.

*Mating and gestation*

The percentage of remated females may be an indication of the females willingness to mate, assuming the same mating possibilities for all females. It was observed that females in the H-line and partly in the C-line were more difficult to mate during the last years. No plausible explanation has been found for this observation. The later date of the first mating during the last year was observed because half of the females were given their first attempt to mate after March 14. This is also the explanation for the delayed date of birth that year.

The overall mean gestation length was 46.0 days. A difference of about 1.2 days in the gestation length was significant at the 5 percent level. Some significant differences were observed between the lines within the year, but

they did not show any general trend. The shortest gestation period was 38 days, which implies that development from ovulation to birth was continuous, i.e. without any delay in implantation (Einarsson, 1985). This female was mated on day 74, remated on day 83 and whelped on day 121, giving birth to 10 kits.

The delay before implantation is shortened with late mating in the estrous period, and therefore results in a shortning of the length of gestation (see Pearson & Enders, 1944; Hansson, 1947; Venge, 1973; Elofson et al., 1988). However, a late mating in the estrous period will affect the time of increase in plasma progesterone and will delay the date of parturition (Einarsson, 1985). This also explains the later time of birth in the present experiment during the last year caused by the late-mated females.

The results which showed different effects on litter size after a delay of about ten days in implantation are in accordance with observations and calculations presented by Dukelow (1966), Baud & Hoogerbrugge (1976) and partly by Elofson et al. (1988). Einarsson (1981b) found a significant reduction of litter size with an increased length of gestation in single mated females, but not in the remated ones. Bowness (1968) and Jonsson (1971) did not find any relationship between the day of mating and litter size at birth in females mated once, while Hansson (1947) did find a definite but irregular relationship. However, it must also be taken into consideration that in general large litters have a shorter gestation period than small ones. It could be concluded that a gestation period within about 48 days would only slightly affect the litter size at birth, while a prolongation above that level could lead to a gradual decrease in the litter size at birth. This implies that with 8 days post coitum to reach the uterus (Hansson, 1947) and 30 days from implantation to birth (Enders, 1952), a delayed period of up to 10 days would not influence the viability and implantation of the blastocysts. This concurs with the result observed in experiments with overyectomized rats (Weitlauf & Greenwald, referred to by Lanman, 1970), where delayed implantation could occur during the lactation period.

*Testes weight*

In the present experiment no correlated response in testes weight was observed as a result of selection for litter size at birth in the females, as was the case in mice (Joakimsen & Baker, 1977). Bateman (1966), however, found that male fertility was not related to the observed response from the increase in the litter size in mice.

The observations on testes weight are in accordance with those of Venge (1959) and Onstad (1967).

*Preweaning kit body weight*

The average kit body weight at birth was at about the same level in the H-line during the experiment, but slightly reduced in the L- and C-lines in the last three years. The average kit body weight at 21 days postpartum in the L-line showed a significant decrease, while the kits in the H-line remained at the same low level. It should be emphasized that the latter mean body weights were based on the litter weight, and that relatively large variations in individual body weight existed. These two traits could therefore be mainly defined as maternal traits. It is even more difficult to find any specific trend in the average body weights at the time of weaning. It was observed during the experiment that kits with low preweaning body weights, especially at birth, had a reduced chance of surviving the first days. This observation was also supported by the low average body weight at birth and high kit mortality in 1982. The average body weights of the kits observed as dead at the first examination were lower than those alive (see Table 5 and 10). This is an indication of the relationship between a low birth weight and the kits' chance of survival. However, it must be considered that the dead kits could have lost some weight before the weighings. No effect of the selection of litter size was present in preweaning kit body weight. It is known that increased litter size reduces average kit body weight (Einarsson, 1980b). However, as argued earlier, results obtained from analyses within given populations cannot be directly compared with correlated responses in selection experi-

ments. This should be taken into consideration particularly when selecting for a trait of high fitness-value, connected or even depending on several other sub-traits, such as the body weight of the individuals.

*Genetic parameters*

It was not possible to obtain reliable genetic parameters from the traits measured on the breeding animals. In the hierarchical analysis, the number of breeding females within sire varied greatly, averaging within line and year between 2.6 and 5.3. The smallest family sizes were naturally observed in the C-line, but they were also small in the L-line. The mean heritabilities of litter size at different times prepartum over line and generation varied between 0.2 and 0.3. However, standard errors were about three times the heritability estimates and several variance components of sire were negative.

The same problems as previously described were also present when estimating heritabilities for average kit body weight at birth and at 21 days postpartum, estimated at 0.41 and 0.36, respectively.

*Analyses within and between lines*

The analysis of within line differences between the first and the last years of the experiment depends on the level of the traits within these two years, as pointed out earlier. The variations and indications of trends as a result of the single trait selection for litter size have been discussed for each separate trait.

Assuming that observations made during the last year were representative both for the lines and for changes during the years of selection, the results given in Table 12 should be invaluable in the prediction of correlated effects from the selection. The difference between the H- and L-lines is important because the only significant difference in the selection trait observed occurred during the last generation. Significant differences were also observed in preweaning litter size, resulting from the selection for litter size at birth. Expressed as litter size per whelped female the difference increased from 1.07 kits at birth to 1.31 kits at weaning, involving prenatal and postnatal mortality.

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