# SOCIAL INTERACTIONS AND CORTISOL LEVEL IN BLOOD OF DAIRY GOATS (CAPRA HIRCUS) HOUSED IN THREE DIFFERENT DENSITIES DURING PREGNANCY

# SOSIALE INTERAKSJONER OG KORTISOLNIVÅ I BLOD HOS MELKEGEITER (CAPRA HIRCUS) OPPSTALLET I TRE ULIKE TETTHETER UNDER DREKTIGHETEN

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## Forord

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***~~ A journey of a thousand miles begins with a single step ~~***

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Ås, mai 2012.

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Marte Flor Kjøren

Marte Flor Kjøren og Charlotte Lyngwa, 2012

# Abstract

Domesticated goats (*Capra hircus*) are social species that live in groups. Throughout the world goats are kept in different systems, and on different densities when housed indoors. There is no Norwegian requirement stating the amount of space accessible for each goat, except in the ecological driven farms, where 1.5 $m^2$/goat is required by law. Usually, 0.6 $m^2$/goat is considered sufficient in Norwegian dairy goat husbandry. High levels of displacements from resources, chasing, threats and physical encounters can be an indication of social stress in a group and can be induced by among other aspects high density.

The aim of this study was to investigate whether there is any effect of different stocking densities on social interactions and cortisol level in blood of pregnant goats modeling common densities applied in different countries. We used fifty-four pregnant goats divided in nine groups to investigate these questions. The group size was kept constant of six goats, but the area available for each individual varied from 1 $m^2$, 2 $m^2$ or 3 $m^2$, meaning that they were housed in 3 different group densities: 6 $m^2$, 12 $m^2$ or 18 $m^2$. The experiment was carried out in three replicates of observations of social interactions (November, December and January), and three replicates of blood sampling to determine the cortisol level (October, December and January). Both basal levels of cortisol, (collected before the experiment started) and two samples during the actual treatment were conducted.

The results showed that only the behaviour “threat” was affected by density, which increased in the highest density of 1 $m^2$ per goat. Time period had an effect on most of the behaviours tested, meaning a change in the rate from one observational period to another. Density and time period together affected one behaviour, namely butting. Different groups that were housed within different densities affected the number of “clashing” and the number of “displacements from rest”. The level of cortisol was not affected by density, the opposite of what we predicted. Cortisol level correlated negatively with positive behaviours. Total amount of agonistic behaviours declined over time, while positive behaviours increased over time. The cortisol level was highest at the beginning of the treatment period. We concluded that cortisol in blood plasma might not be the best indicator of long-term stress.

Housing goats in relatively high densities can lead to increased amounts of aggressive interactions, but within the range of densities 1- 3 $m^2$ per goat, as tested in the present project, the effects were only moderate.

## Sammendrag

Domestisert geit (*Capra hircus*) er sosiale dyr som lever i grupper. Geiter holdes på ulike måter i forskjellige deler av verden, og i forskjellige dyretettheter når de blir oppstallet i fjøs. Det norske lovverket har ingen minstekrav om tilgengelig plass per dyr, bortsett fra i økologisk produksjon, hvor minstekravet er 1.5 $m^2$. Vanligvis er det ansett som tilstrekkelig med 0.6 $m^2$ per geit i norsk geiteproduksjon. Høyt antall fortregninger fra ressurser, jaging, trusler og fysiske trefninger mellom dyra kan være en indikasjon på sosialt stress i en gruppe, og kan blant annet induseres av høy dyretetthet.

Målet med denne studien var å undersøke om ulike dyretettheter kan gi effekt på sosiale interaksjoner og kortisolnivå hos drektige geiter ved anvendte tettheter brukt i forskjellige land. I forsøket ble det brukt femtifire drektige geiter oppstallet i ni forskjellige grupper for å få svar på disse spørsmålene. Gruppestørrelsen ble holdt konstant på seks dyr, men arealet som var tilgjengelig per dyr varierte mellom 1 $m^2$, 2 $m^2$ og 3 $m^2$, som innebar at de ble oppstallet i 3 ulike tettheter; 6 $m^2$, 12 $m^2$ eller 18 $m^2$. Forsøket ble utført med tre observasjonsperioder av de sosiale interaksjonene (november, desember og januar), og tre gjentak med blodprøvetaking for å bestemme kortisolnivåene (oktober, desember og januar). Det ble samlet både basalverdier av kortisol (før selve forsøket ble igangsatt), samt to prøver som ble tatt etter at observasjonsperioden var i gang.

Resultatene viste at atferden "trussel" var affektert av dyretetthet, og økte i den høyeste tettheten på 1 $m^2$. Observasjonsperiode påvirket de aller fleste av de observerte adtferdene, som betyr at de økte eller sank fra en periode til en annen. Tetthet og observasjonsperiode påvirket sammen den ene atferden, nemlig "stange". Forskjellige grupper oppstallet innen de forskjellige tetthetene viste å påvirke antallet av "stanging" og "fortregning fra hvile". Kortisolnivået var ikke signifikant påvirket av de ulike dyretetthetene, som var det omvendte av våre prediksjoner. Kortisolnivået korrelerte med positive interaksjoner.

Totalt sett gikk antallet agonistiske atferder ned over tid, mens de positive atferdene økte over tid. Kortisolnivået var høyest i den første observasjonsperioden. Vi konkluderte med at kortisol i blodplasma ikke nødvendigvis er den beste indikatoren på langtids-stress.

Å oppstalle geiter i relativt høye tettheter kan føre til økt antall aggressive interaksjoner, men innen 1-3 $m^2$, som i dette forsøket, er effektene heller moderate.

Contents

# 1.0 Introduction

## 1.1 Costs and benefits of group living.

Many of our farm animals are social species who prefer to live in groups; this also includes domesticated goats (Capra hircus). Group living involves both positive and negative aspects; the benefits of group living can be lower predation risk, increase time for resting and foraging, provide companionship, social thermoregulation, social grooming, social learning and facilitation, interactions in play (especially in younger animals, which stimulates motor skills) and social feeding. The most apparent benefits of living in a group, at least for animals living in the wild, might be increased vigilance and dilution, and thereby lowering of the predation risk. The more animals available to scan for predators, the more time an individual will have for feeding. Active group defense is also a benefit of the group (Krebs and Davies, 1993). For animals that are group living species the significance of being around conspecifics are great, proven by motivational studies of calves (Holm et al., 2002) and foxes (Hovland et al., 2008).

The negative side of group living can be competition for resources, mainly food, but also other valuable resources like shelter, resting places, water, and favored areas in the pen (Estevez et al., 2007). Living in a group may also increase the attack rate on the group for wild living species as living in a larger group increases visibility (Krause and Ruxton, 2002). Other negative aspects of group living can be social stress inducing aggression (Archer, 1979), increased pressure of pathogens and parasites, misdirected parental care, kleptoparasitism (stealing food from conspecifics) and simply getting in the way of each other (Krause and Ruxton, 2002). The optimization between costs and benefits can lead to the individual`s decision about whether to join or leave a social group, this can be dependent on both non-social aspects (e.g. food availability) and social factors (e.g. sex ratio, group size). Additionally, in farm animals, some of the costs of living in a group can be lower feed intake, reduced resting time, lower reproductive success, reduced growth and lowered immune suppression having negative effects on production (milk, meat etc.), (De Groot et al., 2001; Andersen et al., 2008; Correa et al., 2010), especially if area is limited.

The costs and benefits of living in groups has been studied extensively in wild animal populations (Pulliam and Caraco, 1984), where the survival of the animals often depends on the possibilities of the group to discover prey and good foraging spots (benefits), and where the costs of the group is usually competition for food. Studies of domestic (farm) animals also show a similar perspective, where the costs of living in a group are mainly associated with the competition for available resources. The greatest difference between these two aspects is that the farm animals are regulated in terms of spatial surroundings while free ranging animals can easily self-regulate their group size therefore avoiding some of the negative effects of group living (Estevez et al., 2007).

Resource monopolization will be favored when resources are limited or clumped, which often can be the case in farm environments where animal density and group size is a result of economic assessment rather than an assessment from the animal's point of view (Estevez et al., 2007). Grouping and regrouping in modern dairy herds is done according to age, nutrient requirements, lactation period, milk yield or other aspects, as a common management practice to enhance productivity. This practice may disturb the social structure of the herd temporarily, and in that way have a negative effect. High levels of displacements from resources, chasing, threats and physical encounters is an indication of social stress in a group (Correa et al., 2010). However, one of the reasons humans are able to hold animals in rather large flocks or groups in restricted areas is that the animals are provided with access to food continuously and/or that the food available is evenly distributed between individuals.

Aggression as a negative aspect of group living can be seen in correlation with the area provided per animal. This will be further explained in the context of group size and animal density. Shackleton and Shank (1984) stated that *agonistic* behaviour serves at least two functions: "for immediate gain or protection of resources and for determining and maintaining dominance relations that will determine future access to resources." Aggression between individuals in a group can be seen as a result of controversy either over food or other resources (especially when resources are scarce) or in the context of assessing dominance relationships within the group (Krause and Ruxton, 2002). Both causes are likely to have a negative effect when area is limited (Estevez et al., 2007; Miranda-de la Lama and Mattiello, 2010). Another negative effect of limited surroundings for the animals is that it might trigger social stress within the group (Held et al., 1995; Hughes et al., 1997; Hedenskog et al., 2002). Social stress refers to stress induced by conspecifics (Archer, 1979).

In farming environments introduction of new animals into established groups is known to cause aggression (Andersen et al., 2008; Correa et al., 2010). High levels of chasing, fighting and displacement are good indicators of social stress within a group (Andersen et al., 2008). Continuously regrouping following exposure to a novel stimulus in combination with little or no possibilities to escape can lead to intensive fighting between individuals. Alternatively, they will have little time to rest, possibly leading to lower feed intake and weight gain (Bøe et al., 2006; Andersen et al., 2008). Continuously regrouping farm animals can also lead to social instability (Estevez et al., 2007; Andersen et al., 2008). Regrouping of unacquainted animals can also lead to lesions as a result of the above mentioned reasons (Andersen et al., 2000).

Although domesticated animals are kept in captivity, often with little or restricted accommodations, they have a strong anti-predator behavior (Hansen et al., 2001; in Estevez et al., 2007).  Hopewell et al., (2005) stated that animals living in rather predator-free areas might have lost some of the cues concerning anti-predator behaviour, but their anti-predator behaviour is not absent even though they have been living without predators in the areas for several generations. When animals are held in captivity they can harm themselves and their conspecifics because both physical and psychological injuries can occur as a result when stressed or frightened animals try to escape from their restricted area (Boissy and Bouissou, 1995).

The costs and benefits of living in a group or a flock are many and some reasons are more relevant for one species than others, but it also depends on group, population and season. Pay-offs will nevertheless differ between dominant and subordinate individuals in the same group (Krebs and Davies, 1993).

## 1.2 Group size and animal density, effect on social interactions.

Social behaviour of goats is widely studied and described (Shackleton and Shank, 1984; Barroso et al., 2000; Andersen et al., 2008; Correa et al., 2010) but there are few studies conducted on group size and animal density of goats. Finding the optimal group size and densities of group living farm animals has proven to be difficult, but it is desired to secure high welfare levels for the animals (Estevez et al., 2007). The optimal space allowance per animal concerning health and welfare can often be higher than the economic profit-making point. This gives farmers a difficult starting-point, since they face a trade-off between economic interests and animal welfare-related concerns (Vanhonacker et al., 2009).

Much research on optimal group sizes has been done in husbandry farming (Estevez et al., 2002; Andersen et al., 2004) and understanding the relevance of different environmental factors such as access to resources and space is important for understanding how the most optimal environment for larger groups of farm animals can be created. Both increasing group sizes and increasing animal density can influence behaviour and production in most farm animals (Estevez et al., 2007; Miranda-de la Lama and Mattiello, 2010) however sometimes these concepts are confused and therefore results of different research work may be of less significance (Estevez et al., 2007). In the following, group size is defined as *"the number of individuals that form a group"* while animal density is defined as *"the number of individuals per unit of space"* (Estevez et al., 2007).

Animal production systems of today are often recognizable by both large numbers of animals per unit space and big groups, as this is often the most economically viable way of farming (Estevez et al., 2007; Vanhonacker et al., 2009). Group housing requirements are, in general, lower than single-pen housing. Acknowledging this, but at the same time understanding that limited space may trigger aggression and fighting over resources, we see why both group size and animal density can affect not only the economic aspect, but also the welfare and production of the animals. It is widely reported that high densities have a negative impact in farm animals regarding behavioural problems, performance and output, alongside the negative effects of increased aggression in itself (Lewis and Hurnik, 1990; Beattie et al., 1996; Fisher et al., 1997; Pettit-Riley and Estevez, 2001; Fregonesi and Leaver, 2002; Loretz et al., 2004; Andersen and Bøe, 2007; Li et al., 2007; Hill et al., 2009).

Barroso et al., (2000) found that the frequency of aggressive interactions was twice as high in 90 goats when kept in a barn as when they were kept on pasture, which imply that the space available can have caused some of the differences (animal density was lowered when the goats were at pasture, but groups size might also have been smaller, because the goats were free to choose who they interacted with). Andersen et al. (2011) found that both affiliative interactions such as resting and exploring, and agonistic interactions was negatively correlated with group size.

Increased group size is shown to effect the time-budgets of animals allowing them to spend more time in activities such as resting and grooming and less time in activities such as vigilance. This has been shown in poultry (Newberry et al., 2001) and in mammals (Hopewell et al., 2005). Hopewell et al., (2005) states that feeding in large groups and keeping vigilant is more important for animals in an environment with a high predation risk. An increase in time spent on grooming, resting, and foraging may be the positive effect of increasing group sizes on farms, but there is a good possibility that the negative aspects will overwhelm the positive ones. Big groups in combination with high density can lead to social conflicts, increased aggression, decreased access to resources (especially for low status individuals), lowered immune responses, therefore negatively affecting the reproductive success (Turner et al., 2000; Estevez et al., 2007).

In goats, as well as in other social species, so called “interventions” have been reported by animals not participating in a fight with those who are fighting. The individuals performing these behaviours are described as “problem solvers” because of their ability to act as social mediators in a conflict. This type of behaviour is observed both in negative and positive interactions (Andersen et al., 2011) and is documented in goats by Keil and Sambraus, (1998) who stated that it was the highest ranking goats that mainly performed this behaviour. Affiliative and other positive interactions are important for the stability of the group. The most high-ranked individuals are the ones who seem to be capable of stopping aggressive interactions between others. As a result they will monopolize resources and control the social interactions in the group, thus to cohere the group (Miranda-de la Lama and Mattiello, 2011). To our knowledge, a correlation as not yet been supported between intervention behaviour and animal density, but intervention behaviour has been correlated with group size. According to Andersen et al. (2011), intervention behaviour occurred more commonly in smaller group sizes (n=six) opposed to bigger groups (n= 12 or 24) when goats were exposed to different group sizes.

## 1.3 Social behaviour and reproductive success in goats

Both wild and domestic goats are highly social animals and live in small to moderate group sizes. They are matrifocal, meaning that it is the females and their offspring who remain together in the home range, while smaller groups of males will segregate from the flock but still share some of the overlapping homeland (Dwyer, 2009).

Social behaviour is defined as all of the interactions between two or more individuals in a group that modify the activity of the group (Fraser and Broom, 1990, modified by Miranda-de la Lama and Mattiello, 2010). The social behaviour of small ruminants has been studied but sheep and goat behavior are quite different. Goats tend to be much more reactive and they have an anti-predator-strategy which is rather opposite from sheep: goats will turn against the predator and defend themselves and their offspring, while sheep will flee. These strategies also become evident in relation to their offspring, where lambs use a “follower-strategy” and goat kids are “hiders” in the early period following parturition. Goat kids develop more of a follower-strategy after 2-4 days and will thereafter be closely attached to their mothers (Shackleton and Shank, 1984; Dwyer 2009) if they are not separated for economical or farming reasons.

Individuals separated from the flock show increased cortisol levels as a sign of emotional stress (Kannan et al., 2002). Keeping goats in social isolation is highly stressful, this is shown by the goats displaying behaviours associated with agitation; escape attempts and high-pitched vocalization, and is more stressful to the animal than being restrained within the social group. Moreover, human contact and management can cause severe distress for animals in large groups who have experienced minimal handling (Dwyer, 2009), which is perhaps more relevant in countries with large free-ranging flocks, unlike in Norway. Social recognition in goats is an important aspect of their complex social structure. Recognition is mainly based on visual, vocal and olfactory cues. Goats have two scent-glands on their feet and one on the tail, which ensures scent marking, but it is not fully understood how important scent marking is for their social recognition (Dwyer, 2009).

Affiliative behaviours in goats help establish bonds between individuals, which further improves the cohesion of the group (Schino, 1998; Andersen et al., 2011). Some known affiliate behaviours include resting together, allo-grooming, sniffing, muzzle-muzzle-contact, muzzle-body-contact and exploring and licking the base of the udder (Schino 1998; Miranda-de la Lama and Mattiello, 2010). Goats develop more cohesion when group dynamics are kept stabile over time and they continue to develop affinity towards each other as long as the composition of the group is not altered. This stability also implies that agonistic behaviors are kept low (Miranda-de la Lama and Mattiello, 2010). Maintenance of the social structure of the group relies in continuous social signals such as vocalizations and reconciliations between the members. Short intra-individual distances are therefore a part of maintaining social communication between individuals (Clutton-Brock et al., 1982; In Miranda-de la Lama and Mattiello, 2010).

Another aspect of group cohesion is social facilitation, meaning that members of one group participate in the same behaviours. This simultaneity in behaviours indicates not only that the group is highly synchronized but it has also been seen as a signal on positive welfare in the animals performing it (Andersen and Bøe, 2007; Ehrlenbruch et al., 2010). One of the behaviours most commonly seen and defined within social facilitation is feeding behaviour (Collins and Sumpter, 2007; in Spinka 2012). Following behaviour also constitutes a great part of what defines goat behaviour as goats tend to form lines or files when moving on pasture. Usually it is the oldest, more experienced goat who leads, but the one with the highest rank (who also can be old and experienced) usually follows one step behind, since the most vulnerable place to be during a move is in the front. Engaging in these kinds of synchronized behaviours might have derived from the anti-predator strategy using the dilution-effect to confuse potential predators (Dwyer, 2009).

Agonistic behaviours are necessary for establishing and maintaining dominance relationships within the flock or group in social species and its biological function is to help an individual gain access to resources (Blanchard et al., 1993; in Miranda-de la Lama and Mattiello, 2010; Lindberg, 2001). One function of dominance relationships can be to reduce aggression within the group, since aggressive displays are energetically costly and can cause physical injuries to the animals involved (Syme and Syme, 1979 in; Barroso et al., 2000; Lindberg 2001).

It is normal to expect an individual to either perform or receive agonistic behaviour such as threats or actual aggressive interactions towards others when grouped together in limited areas such as a pen. The level of aggression is higher when area is limited, and in intensive goat production systems, where area per goat restricted the levels of aggression is found to be higher than in less intensive systems (Orgeur et al., 1990; in Miranda-de la Lama and Mattiello, 2010). Dwyer (2009) also states that goats are not often involved in agonistic behaviour unless they are competing for limited resources. This coincides with Barroso et al. (2000), who stated that goats compete more when resources are scarce. The establishment of a social hierarchy within the group prevents continuous agonistic interactions, but it does not prevent aggression entirely (Alados and Escos, 1992; in Barroso et al., 2000). Alternatively, Aschwanden et al. (2009) states that goats have strict rank relationships therefore causing frequent social conflicts. Miranda-de la Lama et al. (2011) stated in their experiment that goats have a clearly hierarchical system. The tendency to form a strict hierarchical group between goats with a high level of agonistic interactions can be seen as a direct result of human intervention inducing higher levels of agonistic interactions than seen in feral or wild living goats (Shackleton and Shank, 1984).

Dominance behaviour within a goat flock is mostly subtle, where eye contact and pressing ones chin on the back of another goat often is enough to displace each other. But if the disagreement escalates, goats can rear up on their hind legs and clash together. Other agonistic behaviors they conduct are threats (positioning their head, stare), kicking with the forelegs, butts with the horns, displacements, clashing, biting and making rumble vocalizations (Shackleton and Shank, 1984; Addison and Baker, 1982). It is more relevant with agonistic behaviours between male animals, and this is also true for the buck. At least in free ranging and wild living groups of goats, male individuals fight and engage in displays, fighting over access to females. The goat scull is designed to withstand the physical impacts (protection of the brain) the clashes causes (Dwyer, 2009). A buck housed in husbandry systems does not always have the opportunity to be together in a flock, or to be housed together with other bucks. One of the primary reasons that goats/bucks developed horns is for the use as rank symbols, which in turn ensures prevention of high-intensity fights between animals of equal status. The animals only need to assess the size of the horns to decide whether or not it is profitable to engage in a fight (Dwyer, 2009). Barroso et al., (2000) also found that horns in goats greatly affected rank in a herd of 35 animals. The goats occupying the highest positions in the social hierarchy did not only have horns, they were also the most aggressive ones.

The production from the goats in a milk and meat-production environment can also greatly be affected by the social rank/status of the animal, producing best when they are positioned in the middle of the hierarchy (Barroso et al., 2000; Miranda-de la Lama and Mattiello, 2010).The way social behaviour affects production traits has been little investigated. Barroso et al., (2000) found that the hierarchy of dominance in a stable flock of 90 grazing goats actually affected their production, both milk-yield and meat (by measuring weight of the kids). The production of milk per day was highest for the middle-ranking goats as was the number of born kid per goat. This was the same for the weight of the kids as newborn, and after the first month of life. Barroso et al., (2000) explained the good results of the intermediate goats with *(this goat)"…may suffer from less social pressure than the animals of inferior status and, at the same time not have to exert energy in continual aggression to maintain its position as with the most dominant animals".* Csermely and Wood-Gush (1987) noted the same behaviour in group-housed sows' pre parturition, where high ranking sows spent more time defending the food than actually feeding. In female mountain goats, Côtè and Festa-Bianchet (2001) found similar results as Barroso et al., (2000), indicating that social rank within the group appeared to have effects on the goat`s reproductive success, especially for younger females. Superior animals had greater chance to successfully reproduce, but social rank did not affect kid survival over time.

## 1.4. Housing, Norwegian conditions, challenges and regulations

Housing conditions for Norwegian milking goats differ from semi-intensive and extensive production systems in other countries because of the given natural conditions in this country, meaning that the goats need to be housed indoors during the winter. Usually, this means that the goats are kept indoors from approximately September to May, depending on where in the country the farm is located due to the different climatic conditions.

Housing in insulated buildings with no access to outdoor areas during the winter season, and extensive use of expanded metal grating and little use of bedding is predominant in Norwegian herds (Simensen et al., 2010). It is about 38 000 Norwegian milking goats in Norway in 2011, most of them are of the Norwegian dairy breed (Norsk melkegeit) (nsg.no). It is around 380 farms in the country (ssb.no) where goats are held for milk production mainly, but also for meat production and for maintenance of the landscape. This fact implies that the livestock per farm is rather small, around 100 goats on average. Most of the goat farms are located either in the county of Troms (in the north) or in the county of Møre og Romsdal or in Sogn og Fjordane (the west) (snl.no).

The Norwegian production system on goats suggests that this is a rather intensive production, where one wants the most efficient production possible due to high building costs and other inputs. Usually, housing conditions for goats in Norway are isolated barns with pens, where the manure is dropped in a cellar beneath. The ground in the pen is usually partly hardwood floors or completely expanded metal, but for the kidding season it is most common to seal the floor with straw bedding. Not using straw for the elder goats is of practical causation; because most of the Norwegian dairy goat production is located in areas where access to straw is limited. The advantage with the use of pens with expanded metal is that the animals are kept relatively clean, and therefore requires a low work input (Bøe et al., 2007). Goats stalled in countries with a warmer climate are more often housed on deep straw bedding (Touissant, 1997). For Norwegian conditions, space requirements for adult goats are considered to be higher in pens with deep straw bedding than in pens with expanded metal. This is according to Gjestang et al. (1999), who argue that 0.90 $m^2$/head is sufficient for adult milking goats stalled on expanded metal flooring, while 1.20 $m^2$/head for goats stalled in deep straw bedding is considered suitable for such conditions.

Norwegian regulations have few requirements for the housing and design, others than that the lying space shall be comfortable, non-draught and dry, and kids and lambs shall have solid floor with satisfactory thermal conductivity. All animals should be able to be lying down simultaneously (FOR 2005-02-18 nr 160: Forskrift om velferd for småfe). Goats are very sensible to draughts (Touissant, 1997). The requirements for small ruminants also states that the pens shall be designed in order to maintain normal behaviour in the animals, including normal mobility, and that they shall be kept in groups/flocks (FOR 2005-02-18 nr 160: Forskrift om velferd for småfe). The requirement of group-holding is for the sake of their mental well-being, since small ruminants are highly social creatures that use the social presence of conspecifics to provide social support and reduce distress behaviours (Rault, 2012).

The regulations set no demands for accurate available area per animal, others than the total area per animal must be adapted to the animal's individual needs (FOR 2005-02-18 nr 160: Forskrift om velferd for småfe). Since the requirements does not state any accurate amount of area that should be provided, the farmer himself/herself is primarily free to decide what`s the proper amount of space for their herd. It has been common to allocate approximately 0.6 $m^2$ per animal under Norwegian indoor housing for goats when they are stalled in loose housed pens, according to Pettersen (2005) in a final report from a project for the health services in Norwegian dairy goats. The requirements for goats kept in ecological driven farms in Norway are a minimum of 1.5 $m^2$, and half of this area shall be solid floor (www.mattilsynet.no), but this requirement only affects about 1250 goats (800 of whom are milking goats), that is included in the ecological production in 2011 (www.debio.no). Other countries like Switzerland have a minimum of 1.5 $m^2$ per adult goat in their requirements (Bundesamt für Veterinärvesen, Switzerland).

Andersen and Bøe (2007) showed that goats prefer to rest against a wall, and without body contact with their conspecifics in general, and that time spent resting in the resting area decreased when it became smaller (from 1.5$m^2$ to 0.5 $m^2$). Touissant (1997) recommends a space requirement for adult milking goats to be 1.5 $m^2$, and with a minimum of 0.5 $m^2$. Space allowance per animal does not only affect the behaviour when they are housed in a group, but also affects the air temperature and humidity in the building. Touissant (1997) further recommends a total of air volume per goat to be 9 $m^3$. When goats are housed in a confined space it is also likely that the air pollution increases, so that it is desirable to find the ideal area where animals are ensured with enough space, but where they are also ensured with enough air volume. The Norwegian regulations for sheep and goats states that the climate inside the barn should be of low concentrations of dust and harmful gases, and this should be secured by using a mechanical ventilation system (FOR 2005-02-18 nr 160: Forskrift om velferd for småfe ). All living particles in the air inside the barn like microorganisms, molds, bacteria and viruses can be pathogenic, and overcrowding negatively affects the indoor climate (Touissant, 1997). Many of the goat barns in Norway and their ventilation system are of elder date, which can affect the indoor climate negatively. It is also rather usual to override the ventilation system in severe winters, to keep the warm air inside, and thereby not replace sufficient amounts of air, which again leads to poorer air quality.

The goats have a great ability to adapt to different temperatures (Touissant, 1997). The lower critical temperature for goats is not well documented, but a general perception is that goats are more resistant to lower temperatures rather than high temperatures. Touissant (1997) argue that optimal air temperature for goats in a building is between 10-18 ° C and that the temperature should not be under 6°C or exceed 27°C. According to Bøe et al., (2007) adult milking goats spend more time being active and eating when the ambient air temperature is dropped from 10-12 ° C to minus 8-12°C. One could expect that the most probable cause for many Norwegian goat farmers to choose insulated barns over uninsulated barns would be for the sake of their own wellbeing, and not for the goats comfort, at least in terms of the rigid winters in Norway.

## 1.5. Stress and levels of cortisol in blood

Stress is a natural part of life, and is not only good or bad. All organisms have developed different mechanisms to cope with stress (Moberg, 2000), but information about stress in goats is still scarce (Nwe et al., 1996). In everyday term, stress is often used in a wide range to describe situations where the individual cannot cope with the demands and difficulties it is faced with, further leading to physical or neurological disorders (Archer, 1979). The term distress (stress) can also be used by some researchers and scientists as a way of describing “negative stress”. Since “stress” is a term that is known to the general public, it will also be used in this thesis.

Stressors produce an interference with the homeostasis of the individual, and to restore balance, an adaptive response is triggered (Zimerman et al., 2011). Stress- producing agents are called stressors (Archer, 1979). The term stress is widely used in biology and everyday life, but in this thesis one statement from Toates (1995) is appropriate to use giving a definition onto stress; *"a protracted failure of the animal to maintain alignment between its reference values and the actual state of the world"*.

With this statement one understands that distress erupts when the animal is no longer capable of coping with stressors in the environment, and as a result biological functions of the individual will be affected. Moberg (2000) states that;

*"When the biological cost of coping with the stressor diverts resources away from other biological functions, such as maintaining immune competence, reproduction or growth, the animal experiences distress"(stress).*

The biological function of the stress response is to mobilize various biological resources such as glucocorticoids and fatty acids and convert them into useful energy-substrates such as glucose (Moberg, 2000). The glucose is needed for different biological functions, e.g. increasing heart and respiratory rates during e.g. a fight- or flight-response in the animal, but also for continuous energy supply to neural tissue in a more long-term stress response (Martini and Nath, 2009). The costs of coping with stressful situations, either short term or long term, will usually be in trade-off with the cost of other biological activities (Bakken et al., 1998).

According to Olsson and Hydbring- Sandberg (2011) plasma cortisol-levels are one of the measurements that are often used in evaluating physiological reactions of stress. Cortisol is the primary glucocorticoid (hormone) in the body of mammals (Sjaastad et al., 2003), and glucocorticoid hormones are of the most essential hormones in the long-term phase (lasting longer than days or weeks) of stress (the resistance phase) in the individual (Martini and Nath, 2009). It's the hypothalamic-pituitary-adrenocortical (HPA) system that is regarded as the body`s primary stress-responsive neuroendocrine system, and this system can either adapt or become dysregulated when exposed to a prolonged stressor (Hennessy et al., 2009). Heightened levels of circulating glucocorticoid hormones (cortisol among others) are adaptive for coping with stressors (Sapolsky et al., 2000 in; Hennessy et al., 2009).

Both glucocorticoids (like cortisol) and catecholamines (e.g. adrenaline) are two of the "front-line" hormones to overcome stressful situations, and therefore they can also be used as parameters of adrenal activity, caused by stressful events or disturbance. The concentration of glucocorticoids can be measured in various body fluids or excreta (Möstl and Palme, 2002). Hormones like cortisol are transported via the blood, and can therefore easily be measured. Nwe et al. (1996) also states that plasma levels of both cortisol and catecholamine compounds can be considered as ways of measuring the effects of stress in an animal. Alam et al., (1986; in Nwe et al., 1996) states that plasma cortisol has been used as a reliable measurement of determining stress response. Moberg (2000), states that; "…under carefully controlled experimental conditions cortisol can be a reliable indicator of stress."

A stress response can be appropriate if the animal meets with acute stressors which it can escape from. This is usually not the case when farm animals are kept under restrained conditions in barns or feedlots; where they are unable to remove themselves from a potential or potential stressor. In such case the stress response will continue over time, and essentially lead to a higher risk of developing pathologies, and thereby reduced production/reproduction, reduced growth or abnormal behaviour (Bakken et al., 1998). Or, said in another way; extreme stress which is beyond the animal's stress-coping ability might also produce mal-adaptive or pathological effects (Andersen, et al., 2008).

Dealing effectively with the stressor or the potential dangerous situation is the real function of any stress reaction, regardless in which way it is done. Behavioural responses like fighting the stressor, or physiological responses like secreting adrenalin or cortisol throughout the adrenocortical response are both adaptive responses, which are working together trying to minimize the effect of the stressor on the body (Archer, 1979).

Most farm animals are social animals living in groups, and this also applies for goats. The social environment they live in is usually enriching for them, meaning that the social companionship from a peer can be regarded as a positive welfare "initiator" (Galindo et al., 2011). Further, the ability of a social partner to reduce the stress response can be referred to as "social buffering", meaning that the presence of social companionship has positive effects on the individual and its reactions during stress response (Hennessy et al., 2009). But the state of stress can also be induced by conspecifics; this is further defined as social stress (Archer, 1979). It commonly occurs when animals are reared together with little available area. Social stress also includes the stress one animal experiences by being repeatedly attacked by conspecifics, and the continued presence of the attackers (Archer, 1979). Social stress is therefore a term that can be linked to crowding.

According to Lindberg (2001) the lack of adequate space for an animal in a group can also affect the level of aggression and thereby the state of stress. This is due to the fact that submissive animals might be prohibited from retreating from an aggressive encounter, or to retreat in defeat. This can further lead to prolonged fights, because the distance the submissive animal has to retreat before its submissive behaviour is recognized might not be effective in the area available. To be housed together with individuals that are on top in the hierarchy without the opportunity to retreat if there are aggressive encounters, is likely to be a highly stressful state over time. Sufficient space is extremely important for animals to establish a hierarchy (Lindberg, 2001). The chronic stress some animals experience can therefore be based mainly on their spatial surroundings.

When animals in a group are constrained to a certain area with high animal density and no possibility to escape, it has been demonstrated that they alter their behaviours to e.g. avoid feeding/drinking at the same time as higher ranking animals, or they will decrease their feeding/drinking intake (Loretz et al., 2004; Ehrlenbruch et al., 2010; Jørgensen et al., 2007). Jørgensen et al., (2007) also found that the total time of queuing increased, and both aggressive interactions and displacements increased. Behavioural changes can also be one of the effects of the hormonal changes due to the stress response (Archer, 1979).

It is suggested that stressors can lead to increased mortality and negative effects on reproduction like smaller reproductive organs, further leading to decreased reproductive output (Archer, 1979). Confinement and high densities in combination can give rise to stress, which can be measured by e.g. elevated cortisol concentration in faeces or cortisol level in blood (physical response). Stress response can also be measured at other levels, like heavier adrenal glands (anatomical response), or changes in time-budget (behavioural response) as it was shown by the work of Li et al., 2007.

Sapolsky (1994) argues that excessive excretion of glucocorticoids due to the stress response can affect the bone mass of the body; giving a greater chance of getting osteoporosis. This is due to Sapolsky proved in female monkeys, where social stress is proved to lead to loss of bone mass. Sapolsky further argues that social stress also leads to plaque formation in coronary arteries, which in turn can contribute to heart attacks. This development of atherosclerosis arises from the overactive sympathetic nervous system component of the stress response, and was also proved in monkeys by the work of J. Kaplan.

Kaplan`s research demonstrated that living as a subordinate individual in a stable hierarchy of monkeys exposed them to continuous stress. Sapolsky concluded that individual coping styles were critical for how the individuals responded to social stress with or without getting physical impacts. Toates (1995) also states that stress is a condition which makes hormonal levels rise over time, increases levels of stereotypies and can give body indices of pathology. These pathological changes can happen as a result of the adaptive response in the animal; simply because the different hormone systems respond to stressors, and their responses can therefore affect both physical and mental health over time (Moberg and Mench, 2000).

## 1.6 Aim of the study and predictions

The aim of this study was to examine the effects of three different densities (1 $m^2$, 2$m^2$ or 3 $m^2$) on social interactions and cortisol-levels in the blood of pregnant goats. The study was conducted from the first third of the pregnancy of the goats, and we looked at both agonistic and affiliate, social interactions between the goats. We predicted that it would be more agonistic interactions in the groups with the highest densities. We also predicted that the cortisol level would be highest in the groups with highest density, as a result of a possible higher level of social stress. It is also likely to find higher amounts of social behaviours (especially agonistic behaviours) in total in the first observational period, compared to the middle and last observational period, as the goats then are assessing each other's strength, and establishing a new hierarchy (Lindberg, 2001).

Our prediction or hypothesis is important to answer, mostly because it can have considerably practical implications. It is also interesting because little research on the effect of different densities in goats has been conducted earlier.

## 2.0 Material and Method

### 2.1 Project description

This thesis is a part of a larger project called AWIN (Animal Welfare Indicators) which is a collaboration of researchers and institutes that work with behaviour and welfare. The different researchers and Institutes focusing on this research come from Spain, Portugal, Italy, USA, Brazil, Scotland, Norway, Great Britain, Czech Republic and Germany. The project is divided in four different work packages, and this thesis is a part of the work package no. 3, which focus on examining how prenatal and early postnatal social environments effects development and welfare. More specifically, the focus will be on the effects of group size and animal density during pregnancy on behaviour and welfare of ewes, goats and their offspring. The research is financed by the EU 7 Framework Program (FP7-KBBE-2010-4) (http://www.animal-welfare-indicators.net/site/)

### 2.2 Summarized experimental plan

Fifty-four pregnant goats divided in nine groups were tested for the impact of different densities on their social behaviours during their pregnancy of approximately 145 days. The group size was kept constant of six goats, but the area available for each individual varied from 1 $m^2$, 2 $m^2$ or 3 $m^2$, meaning that they were housed in 3 different group densities;

1. 1.0 $m^2$ per animal (18 animals: 3 groups of 6: G1.1, G1.2, G1.3)
2. 2.0 $m^2$ per animal (18 animals: 3 groups of 6: G2.1, G2.2, G2.3)
3. 3.0 $m^2$ per animal (18 animals: 3 groups of 6: G3.1, G3.2, G3.3)

### 2.3 Animals, housing, management

A total of 60 Norwegian dairy goats were mated or inseminated, and 54 of them were chosen for the experiment after the use of ultrasound investigation to confirm their pregnancy. They were grouped so that each group contained goats of different weight, age, and with a similar date of parturition.

The 54 pregnant goats used in the experiment were of the Norwegian dairy goat (NKG). They all originated from the same experimental herd, resident in the goat barn at the Norwegian University of Life Sciences in Ås, in southeastern Norway. The goat barn is insulated, mechanically ventilated, and all the pens used in the experiment contained expanded metal flooring, and a resting area with solid hardwood floors. The barn usually holds an ambient temperature of +6/-10°C during wintertime.

All of the goats were dehorned, and they were between 2 and 5 years old, with a mean age of 2. 8 years (±0.7). The mean weight of the goats was 50. 3 (±7.71) kg at the start of the observations in November, and increased to a mean of 59.3 (±7.98) kg in January. The goats were also previously familiar with each other, as they have been on pasture together from May to September before the experiment started. Each goat was individually marked, and all of the goats within the nine experimental groups received a colored collar for an easier individual identification. The colors used were purple, grey, red, yellow, green and blue.

The goats were all accustomed to human contact and handling, and were fed twice a day, usually somewhere between 08.00 and 09.00 in the morning, and between 14.00 and 16.00 hours in the afternoon. At the start of the project, they were only fed silage, but due to some problems with soft faeces they were in the end of the pregnancy fed with silage in the morning. In the afternoon they were fed both silage and hay. All of the goats received 0.2 kg of concentrate each per day during the morning feeding from the start of November, and this amount was increased to 0.6 kg/goat/day immediately before parturition. The goats received 20 g of minerals each day together with the concentrate, but they also had free access to minerals through salt blocks with copper. Their pens were cleaned out once a day, usually in the morning, and a layer of sawdust was added in the solid resting area to ensure a dry surface.

Lactation period ended right before they were put to the experimental pens. The kidding started in the end of January, after the last observational period of behaviours.


***Fig.1. A, B, C, D. Illustration of: A) Registration during blood-sampling B) Overview of the pen group 2.2 was housed in, two feeding places are seen down to the right C) Illustration of the pen-design and flooring D) The process of extracting blood-samples***

## 2.4 Experimental pens

The nine groups were housed in two different main rooms in the barn, one containing five of the experimental pens, and the other room containing four of the pens (Fig.2). The experimental pens in the same treatments (density) had similar total amount of space (6 $m^2$, 12 $m^2$ or 18 $m^2$/pen), but had a slightly different shape due to the design of the barn. The goats in the different pens were allowed to have vocal and visual, but not tactile contact. The design of the pens in the two sides of the barn made it possible for the goats in the 4 pens and in the 5 other pens to have visual contact over the feeding area between the different pens. The original number of feeding places was blinded with wooden wallboards, leaving only six places available in every experimental pen, giving a total number of one goat per available feeding place. All the goats had free access to water, since the experimental pens contained at least one water dispenser or several nipple drinkers.

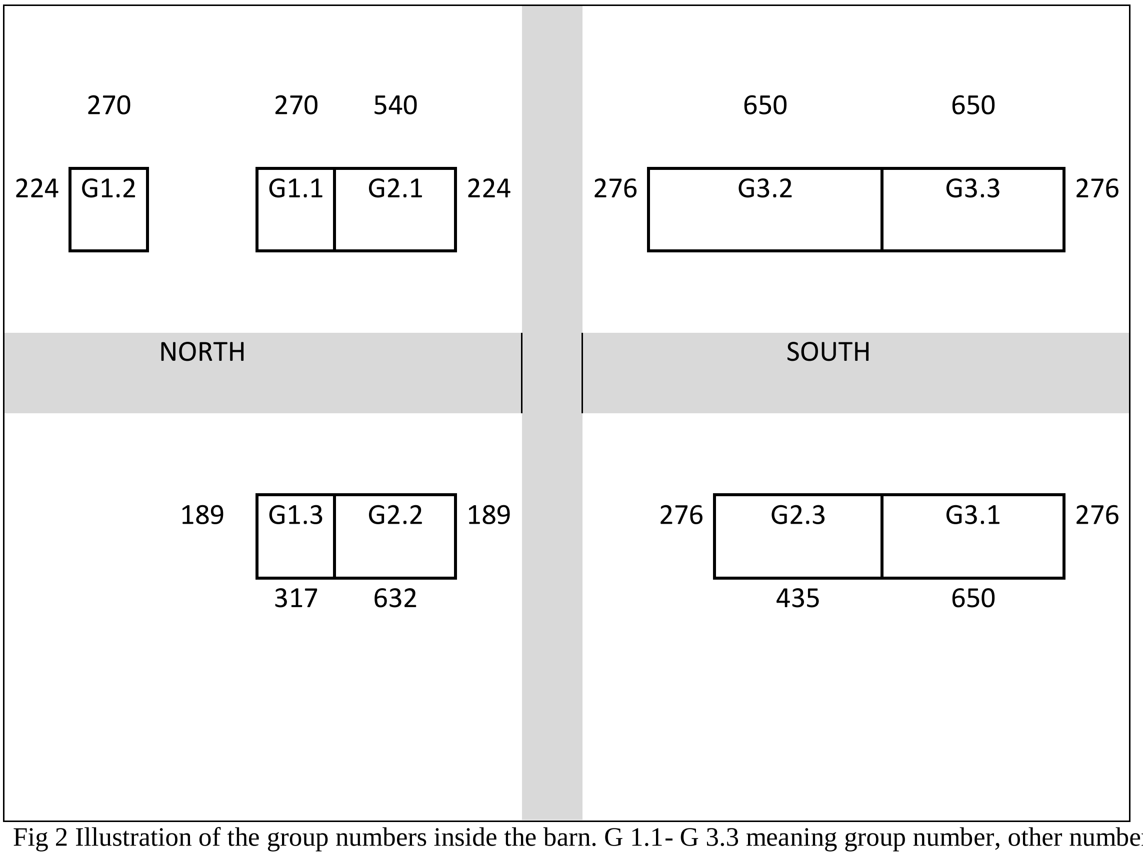


Fig 2 Illustration of the group numbers inside the barn. G 1.1- G 3.3 meaning group number, other numbers are measures in cm showing the size of the pens.

## 2.5 Behavioural observations, interval and methods

Social behaviour was observed for three replicates, conducted a total of three times during the pregnancy of the goats. The behavioural observations were conducted in week 45 (November) and 50 (December) in 2011, and in week 5 (January) in 2012. The observations were conducted in three periods (for four consecutive days) during the experimental period. The behavioural observations were carried out for one and a half hours twice a day after the morning and afternoon feeding of the goats. Total time of observational recordings of the behaviours was therefore 108 hours throughout the experimental period. The first behavioural study was conducted one week after the goats were mixed into their respective groups to ensure that the rank order was fully established. The observational test was conducted by three different persons. Definitions of the different social behaviours were set before the observational tests started and shared observations were carried out to ensure high interobserver reliability.

The behaviours were scored by using an ethogram containing nine different behaviours which were scored using continuous sampling. During the observations of the behaviour both the initiator and recipient of the social interactions were noticed. The ethogram was based on previous studies on social interactions in goats (e.g. Andersen and Bøe, 2007). The behaviours were defined as follows:

1. Frontal clash: a position where the actor is rearing onto the hind legs with the head and torso twisted followed by descending forcefully onto the front legs delivering a powerful strike forwards and downwards reaching the head of the receiver
2. Butting: contact (sudden and forceful movement) with the head towards another goat
3. Pushing: pressing the head to any part of another goat, slowly
4. Threatening: pawing or rushing towards, or directing the forehead towards the opponent without physical contact, biting or attempt to bite another goat
5. Withdrawing: moving the head and/or body away from another goat (after a social interaction)
6. Nosing/exploring: nose in contact with another goat

7. Grooming: grooming by scratching or rubbing itself towards another goat (the other can be either passive recipient or take part actively in the mutual grooming)

8. Displacing from food: physically forcing another goat to leave its feeding place, or passively displacing the other goat simply by approaching that individual

9. Displacing from resting place: physically forcing another goat to leave its resting place, or passively displacing the other goat simply by approaching that individual

Appendix no. 1 shows how the form used to register the behaviours was designed.From the observed behaviours frequency of three additional behavior categories were calculated:

Offensive behaviours: the sum of the frequency of frontal clash, butting, pushing, threatening, displacement from food and displacement from resting place.

Defensive behaviours: the sum of frequency of withdrawal, received displacement from food, received displacement from resting place.

Positive behaviours: the sum of the frequency of nosing/ exploring and grooming.

Overall, offensive, defensive and positive behaviours were regarded as different behavioural categories.

## 2.6 Blood sampling, interval and methods

Basal cortisol levels in the blood were measured by blood samples of all of the 54 goats, once every third of the pregnancy, each time for two consecutive days, making a total of 321 blood samples. Three samples were not analysed because they were mixed up in the lab. In week 43 there were gathered blood samples to confirm the basal values of the cortisol before the experiment started. The other blood samples were taken in week 50 (December), and in week 5 (January). All the blood samples were labelled individually with the number of the goat. The blood samples were collected in the morning, usually during one hour before the morning feeding, approximately between 7 and 8 a.m. Blood samples were drawn from all the goats via jugular venepuncture, and taken immediately after each other, starting with different animals/pens each time. The samples were collected as gently as possible, trying our best not to arouse the goats. Sampling of one goat took approximately 30 sec. (Andersen et al., 2008).

All of the blood samples were kept in a cold room at 4° C for 24 h after they were collected and thereafter spinned in a laboratory so that the plasma part of the samples could be extracted. The samples were spinned for approximately 15 minutes at room temperature at 3000 rotations/minute. Two samples from each blood sample were thereby put into Eppendorf tubes. All of the tubes were individually marked with the number of the goat and date of sampling, and then put in a freeze at -18°C. When all 324 original samples were collected, processed and labelled they were sent off to "Hormonlaboratoriet", medisinsk klinikk at Aker Hospital for further investigation of the cortisol levels. The average of the cortisol level values from the two consecutive days of sampling from both basal values (October), and the first period (December) and the second period (January) was calculated and used later in the analyses.

## 2.7 Statistical analyses

One goat delivered a stillborn kid one week before expected parturition, and was thereby excluded from the rest of the observational data, which implies the last behavioural observation in January. Three blood samples were mixed at one point during the work at the lab, meaning that information about three goats from the first blood sampling also was excluded.

The data from the morning and afternoon observations was merged together, and all the data from observations/each day was put in to and processed in Microsoft Office Excel 2010. The figures and tables were also made in Excel. Further, all of the data was processed in the statistical analysis program JMP.8.

Parametric statistics in JMP: To include both fixed and random effects we applied the mixed model REML (Restricted or residual maximum likelihood). We investigated if there was any connection between the thirteen variables (frontal clash, butting, pushing, threatening, withdrawing, nosing/ exploring, grooming, displacement from food, displacement from resting place, offensive behaviours, defensive behaviours, positive behaviours, cortisol level), with the fixed effects (density, time period, density* time period). Group within density (nine different groups at three different levels of density) was specified as random effect. The level of significance was 0.05 ($P< 0.05$).

To investigate whether the cortisol level in December and January correlated with any of the behaviours offensive, defensive or positive within every observational month, we used a Pearson correlation analysis in Microsoft Office Excel 2010.

# 3.0 Results

## 3.1 Overview

Offensive behaviours were not significant in context of time period, but showed a tendency to be affected of density.

In total, one observed behaviour was affected by density; threat. Both push and offensive behaviours showed a tendency to be affected by density (Table 1). None of the other defensive or positive behaviours were affected by density. With respect to butting there were significant interactions between density and time period (Table 1).

For “group within density”, the only behaviours that differed significantly were clashing and displacement from resting place.

Although not significant, the frequency of clashing was highest in the density of 1m², while displacement from resting was highest in the 3 m² density (Table 1).

Time period had significant effect on butting, threats, withdrawal, nosing/ exploring, grooming, displacement from food, displacement from resting place, offensive, positive and defensive behaviours, and cortisol level (Table 1).

The level of cortisol correlated with positive behaviours. The low level of cortisol correlated with high amount of positive behaviours, meaning that the cortisol was lower when the amount of positive interactions increased. The level of cortisol was also affected by period (Fig. 9), meaning that cortisol was lower in January than in December in average for all the goats.

Table1. Overview; F-value and P-value for all behaviours and cortisol for all fixed effects and the random effect

| | Density | | Time period | | Density*Period | | (Group) density |
|---|---|---|---|---|---|---|---|
| Variables | $\chi^2$ | P-value | $\chi^2$ | P-value | $\chi^2$ | P-value | P-value |
| clash | 0.69 | ns | 2.7 | 0.07 | 2.003 | 0.096 | <0.05 |
| butt | 1.824 | ns | 13.72 | <0.0001 | 6.551 | <0.0001 | ns |
| push | 3.899 | 0.082 | 2.472 | 0.087 | 0.417 | ns | ns |
| threat | 5.477 | 0.044 | 7.487 | 0.001 | 0.795 | ns | ns |
| withdraw | 1.607 | ns | 3.676 | 0.027 | 0.449 | ns | ns |
| nose expl | 0.062 | ns | 13.191 | <0.0001 | 0.766 | ns | ns |
| groom | 0.682 | ns | 12.383 | <0.0001 | 0.509 | ns | ns |
| disp food | 0.247 | ns | 15.637 | <0.0001 | 0.647 | ns | ns |
| disp rest | 0.885 | ns | 13.792 | <0.0001 | 0.173 | ns | <0.05 |
| offensive | 4.822 | 0.056 | 14.105 | <0.0001 | 1.302 | ns | ns |
| positive | 0.052 | ns | 16.83 | <0.0001 | 0.753 | ns | ns |
| defensive | 0.177 | ns | 3.308 | 0.039 | 0.342 | ns | ns |
| cortisol | 1.15 | ns | 6.808 | 0.002 | 0.332 | ns | ns |

Threat was the only behaviour that showed a significant difference between the densities of 1 $m^2$, 2 $m^2$ or 3 $m^2$, and was highest in groups within the density of 1$m^2$ (Table1, Fig.4, Fig.5). All of the offensive behaviours (frontal clash, butt, threat, displacement from food and displacement from rest) showed a tendency to be affected by density, although not significant (Table 1).

## 3.2 Variables

Frontal clashing

There was no effect of density on the behaviour clashing. There was a tendency for time period on clashing, but no interaction between density and time period. Group within density significantly affected clashing, as it was highest in the highest density, and group 1.1 and 1.3 differed from group 1.2 (Table 1). Clashing was a behaviour that was not much used, in average for the whole experimental period; each goat performed clashing 0.34 times. Nearly half of the goats interacted in this type of behaviour; 25 goats, and the behaviour was performed 54 times in total of the whole observational period.

Butting

There was no effect of density on the behaviour butting, but there was a strong significance for time period and butting. Density and time period also affected this behaviour significantly (Fig 3), but there was no effect of group within the density (Table 1). Butting was performed in average 7.03 times per goat during the whole experimental period. The butting was performed most in the first observational period of November with 12.13 behaviours in average/ goat, for the second period in December it was conducted 4.59 times in average/ goat, and in the last time period in January it was conducted 4.39 times/ goat.

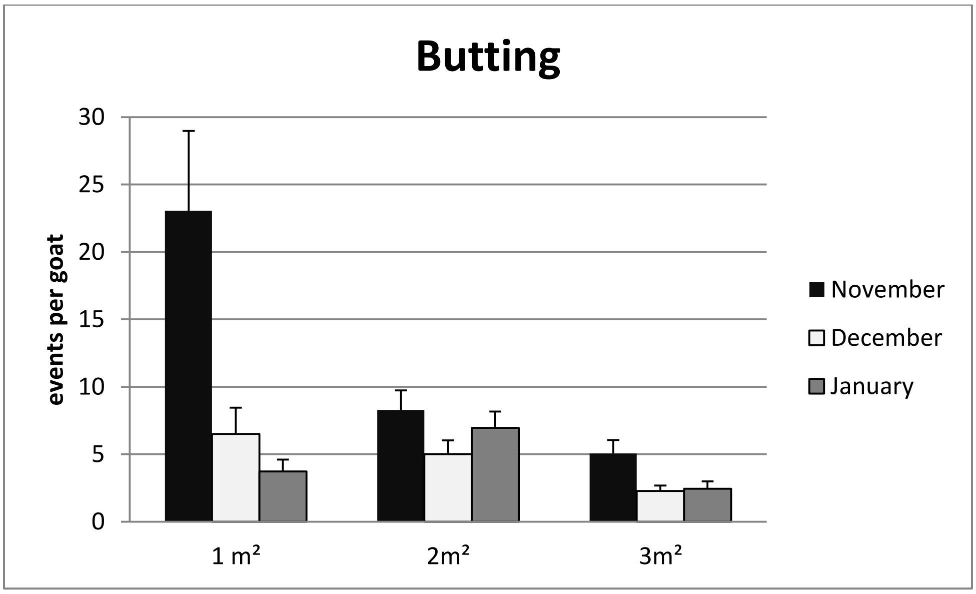


Fig 3 Average number of buttings within three densities and time periods

Butting is one of the offensive behaviours that were not used much; except from the high occurrences in November (time period 1). We registered between 0 and 83 butts on the individual level in general within any of the three different observation periods (Nov, Dec., or Jan.). The number of butts recorded in group 1.1 could have had some impact on the overall results, because one goat in this group performed butting 108 times throughout the length of the whole experiment (total in the whole experimental period). Butting was significant in the context of period, where the highest level was observed in November (Fig. 3).

The average number of butts/ goat was 12.1 in November, 4.6 butts/ goat in December, and 4.4 butts/ goat in January. For the density, the goats performed in average 11.09 butts per head in the highest density, 6.74 butts per head in the middle density, and 3.26 butts per head in the lowest density within the whole experimental period. In group 1.1 butting were observed 10.3 times in average/ goat for the whole experimental period, while the other groups were ranked between 1.2 and 4.4 behaviours of butting in average/ goat, with a total of 3.5 buttings for all groups in average for the whole period.

Pushing

Pushing was not significant in relation to density, but showed a tendency to be affected. This was the same for time period, where pushing showed a tendency. Density and time period in interaction did not have any effect on pushing, and neither did group within density (Table 1).

For the whole experimental period, push was expressed 14.6 times in average per goat in the highest density of $1m^2$, 10.5 times per goat/ average in the medium density of $2m^2$, and 10.2 times per goat/ average in the low density of $3m^2$ (Fig.4). For the time period, pushing was expressed 13.79 times per goat/ average in November, 11.42 times per goat/average in December, and 10.22 times per goat/ average in January.

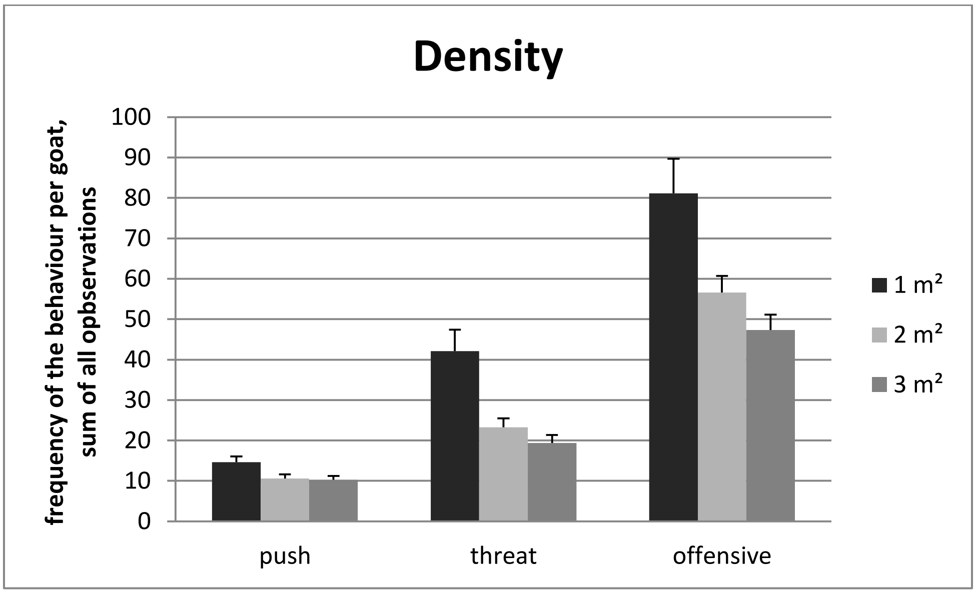


Fig 4 Effect of density on three behaviours that differed between treatments

Threats

Threats were significant in relation to density. In relation to time period threats showed a strong significance. For the density and time period threats was not significant, the same was true for the effect of group within density for threats. Overall, there were significantly more threats at the highest density of $1m^2$ (Table 1, Fig.5). Threats were displayed 42.1 times per goat in the high density ($1m^2$) during the whole experimental period, while displayed 23.2 times for the medium density ($2m^2$), and 19.4 times per goat in the low density of $3m^2$ (Fig.4). For each individual, the number of threats in total was between 0 and 170 within either of the periods registered.

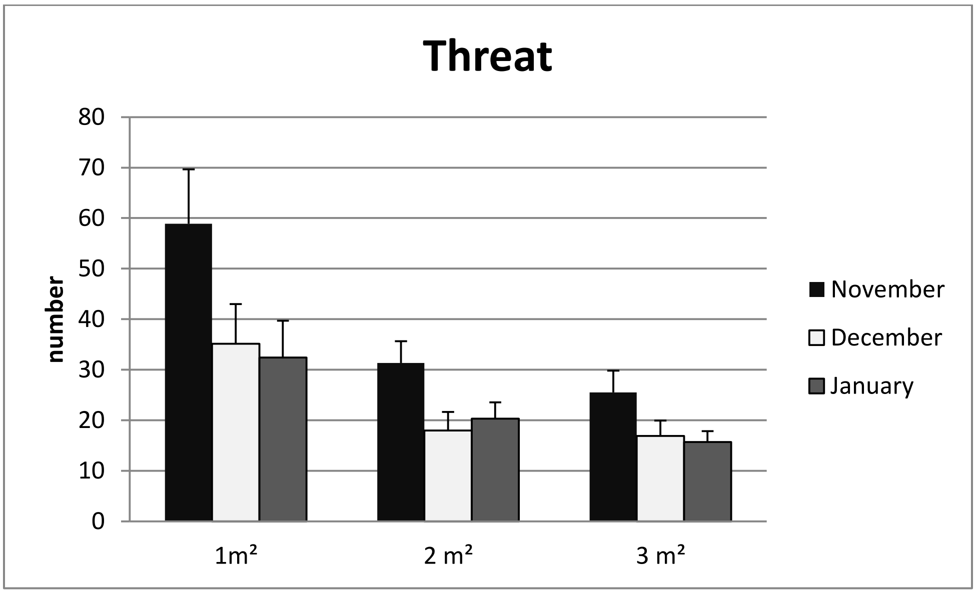


Fig 5 Number of threats in average in all three densities and period

Withdrawal

Withdrawal was not significantly affected by the density, but did show significant affect by the time period. Density and period did neither affect withdrawal, and this was the same for group within density (Table1). Withdrawal was carried out most in the third time period, in January. The highest amount of withdrawal was observed in January with an average of 7.87 per head. For November the average of withdrawals was 4.96, and for December the average was 4.72 withdrawals, meaning that the number of withdrawals increased from November to January.

Density affected the number of withdrawal with being highest in the highest density of 1 $m^2$, with 8.88 per/ head, and 4.03 per / head in the middle density of 2 $m^2$, and 4.63 per/ head in the lowest density of 3 $m^2$.

Nosing/ exploring

Nosing and/or exploring was not influenced by the density, but did show a strong significance regarding time period. Density and time period did not have any effect on nosing / exploring, neither did group within density (Table 1). Nosing/ exploring was displayed 5.31 times in average/ goat in the high density, 4.87 times in average/ goat in the middle density, and 5.53 times in average/ goat in the low density. For the different time periods, nosing/ exploring was displayed 4.79 times in average/ goat in November, 3.31 times in average/ goat in December, and 7.61 times in average/ goat in January (Which was significant with the density).

### Grooming

Grooming was not significant in relation to the density, but it was strongly significant in relation to the period. Grooming was not affected by both density and time period, nor was it affected by group within density (Table 1). The average number of grooming per goat was 2.52 in the highest density, 3.85 in the middle density, and 2.31 in the low density for the whole experimental period. For the time period, the average numbers of grooming was 1.35 in November, 1.72 in December, and rose to 5.61 in average for January, which was also strongly significant.

### Displacement from food

Density did not affect the amount of displacements from food that was carried out. Time period did on the other hand give a strong significance on displacements from food, where it was displaced most in the first time period. Density and period did not affect displacements from food, neither did group within density (Table 1). In November it was conducted in average 19.53 displacements from the food per goat, in December it was conducted 13.16 times in average/ goat, and in January displacements from food was halved down to 9.33 times in average/ goat.

### Displacement from resting

It was no effect of density on the displacements from rest, but time period gave a strong significance. Density and time period did neither give an effect. Group within density gave a significant affect (Table 1), and the most displacements from rest happened in the group that was housed in pen 3.2. The numbers of displacements from rest in the high density was 0.20 per goat in average, for the middle density it was 0.22 displacements per goat, and for the low density it was 0.31 per goat. Average numbers of displacements from rest for the first period was 0.03, for the second period it was 0.11, and for the third period it was 0.59 times in average per goat.

Offensive behaviours

For offensive behaviours there was not any significance in relation to density, but it showed a tendency to be influenced by density. Period did significantly affect the offensive behaviours. Offensive behaviours were not affected by density and time period, neither by group within density (Table 1). The effect of period was shown with the highest levels of offensive behaviours rising in the first period in November.

Offensive behaviours was expressed 81.2 times per goat in the high density (1m$^2$) for the whole experimental period, and 56.6 times per goat in the medium density (2m$^2$), and 47.3 times per goat in the low density (3m$^2$) (Fig.4).

Offensive behaviours were registered with between 47 and 81 events per head in average within all of the three test periods. On individual level, the highest number of offensive behaviours was recorded with 203 single offensive advances within all of the periods. Five goats were registered without any offensive interactions at all within one of the periods, and all goats except one were registered with at least one offensive interaction in total for the whole experiment.

Positive behaviours

There was no effect of density on the positive behaviours, but time period significantly affected them. Density and time period did not affect the positive behaviours, and neither did group within density (Table 1). More positive behaviours were observed in the last observation period in January, than in the other two periods (Fig. 9). The highest amount of positive behaviours performed was measured at 60 behaviours in total for the whole experimental period, and was conducted by one goat in group 3.3. The average amount of positive behaviours was 8.13 per goat for the whole experimental period. 21 goats did not perform any positive behaviour at all during the whole time of observation through three time periods.

Defensive behaviours

The defensive behaviours was not affected by the density, but was significantly affected with time period. For both density and time period no affect was found, neither did group within density affect the defensive behaviours (Table 1). Defensive behaviours performed in average per goat throughout the whole experiment were 19.98, while defensive behaviours during the first time period in November were 24.16. It was performed an average of 18 defensive behaviours in December, and 17.79 in average per goat in January, meaning that there was a decrease in defensive behaviours from the start of the experiment to the end of the experiment.

Cortisol

The level of cortisol did not get affected by the density, but was significantly affected by the time period. Density and period did not affect the values of cortisol. Group within density did not affect the level of cortisol (Table 1).

### 3.3 Effect of group within density

The level of threat was highest in group 1.1, where the average was 58.9 threats per goat in the first time period in November. For December the average number of threats was 35.11 in group 1.1, while the average was 32.38 in January for group 1.1. Three of the most offensive goats within this group performed more threats in average separately than the remaining three did in total. Within the rest of the groups the average number of threats was between 15.7 and 35.1 for all the goats within all the time periods. The level of threat was also quite high in group 1.3 (Fig. 6).

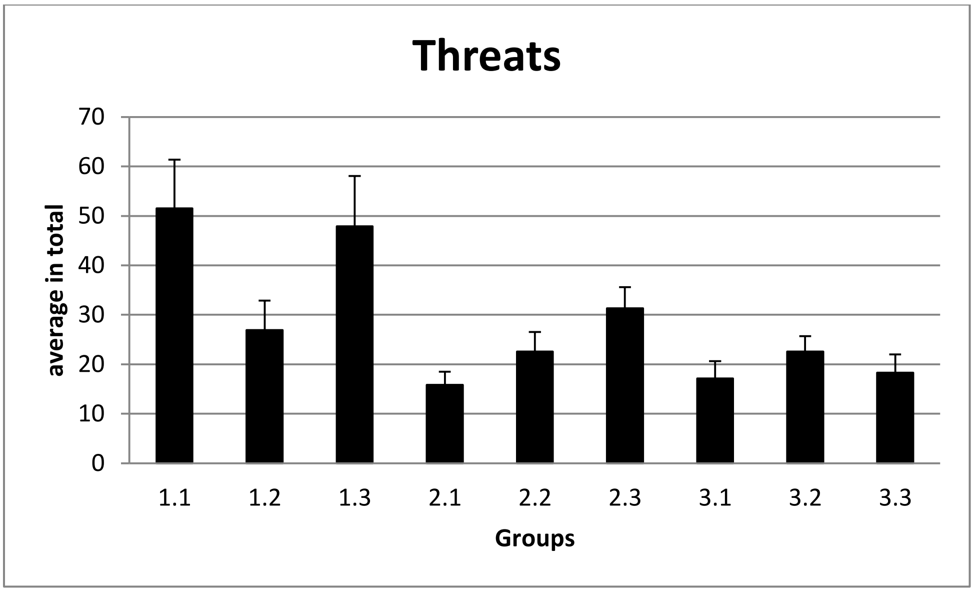


Fig 6 Average numbers of threats in every group through the whole observational period

Two goats did not display any threats within the hours of observation, but the ten goats that displayed the most was registered with more than 102 threats within all of the observational periods. The one goat that displayed the most threats in total during the experiment displayed 317 threats, and belonged to group 1.1. Out of the ten goats that displayed the most, six of them belonged to group 1.1.

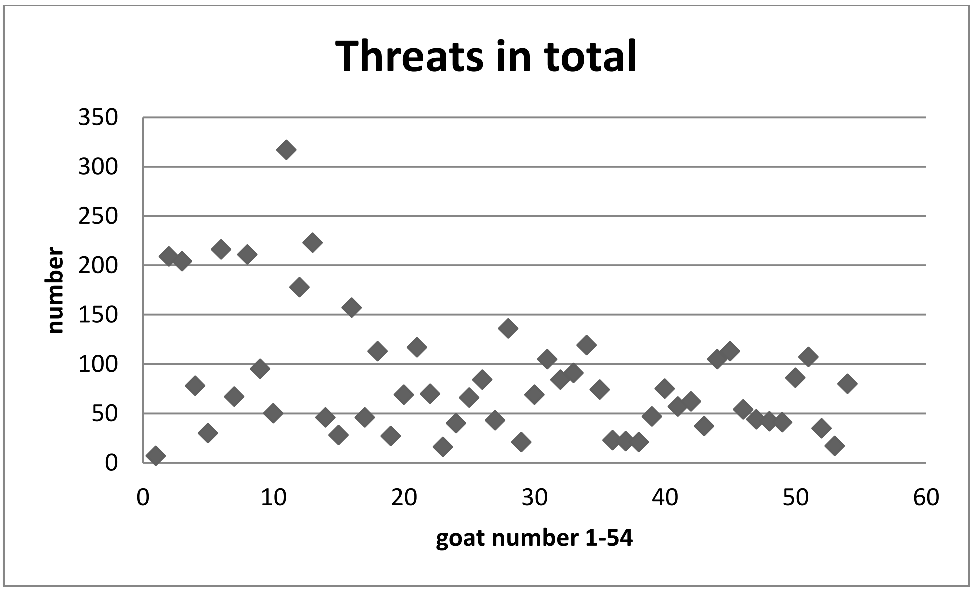


Fig 7 Number of threats per goat in total for the whole experiment

## 3.4 All of the behaviours

Within all of the behaviours, the offensive behaviours were the only ones that showed a tendency to be dependent by density (Table 1, Fig.8). Offensive behaviours were affected by the density, showed by a higher observation rate of offensive behaviours in the groups with high density (1 $m^2$).

Offensive behaviours were observed on average 117.6 times in the 1 $m^2$- groups in the start of the experimental period (November), while it was observed 74.2 times in average for the 2 $m^2$- groups, and 61.8 for the 3 $m^2$- groups. By the end of the experimental period (January), the average numbers of offensive behaviours had declined to 58.7 for the 1 $m^2$- groups, 47.4 for the 2 $m^2$- groups, and 36.3 for the 3 $m^2$- groups. This means that offensive behaviours nearly halved from the start to the end of the experimental period.

Overall, the average registered behaviours in the groups of 1 $m^2$- were 592.8, against the 2 $m^2$- groups with 416 behaviours, and the 3 $m^2$- groups with 378.3 (Fig.10).

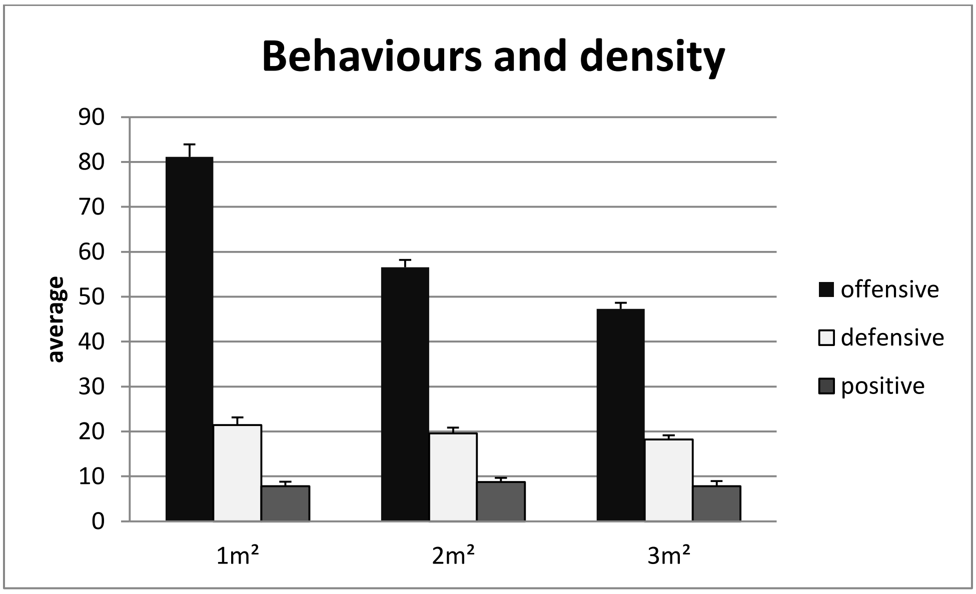


Fig 8 All behaviours, average for each density throughout the experimental period

Neither defensive behaviours nor positive behaviours were significantly affected by density (Fig.8). For period, all of the different behaviours were affected, and the effect of period on offensive behaviours and positive behaviours were strongly significant (Table1, Fig.9).

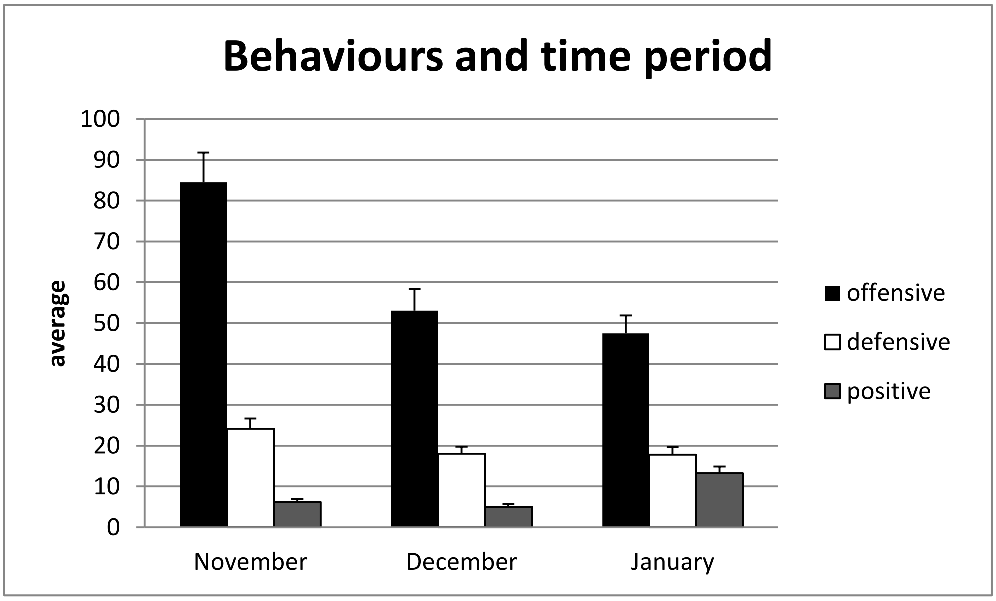


Fig 9 All behaviours in relation to time period

There were no interaction effects between density and time period for any of the behaviours, except from butting (significant effect) and clashing (showed a tendency).

There was a reduction in all of the offensive behaviours from the start of the time period to the end, except from the positive behaviours, which showed an increase from December to January (Fig.9) The level of behaviours in total was also higher in the groups with high density (1 $m^2$) (Fig.10).

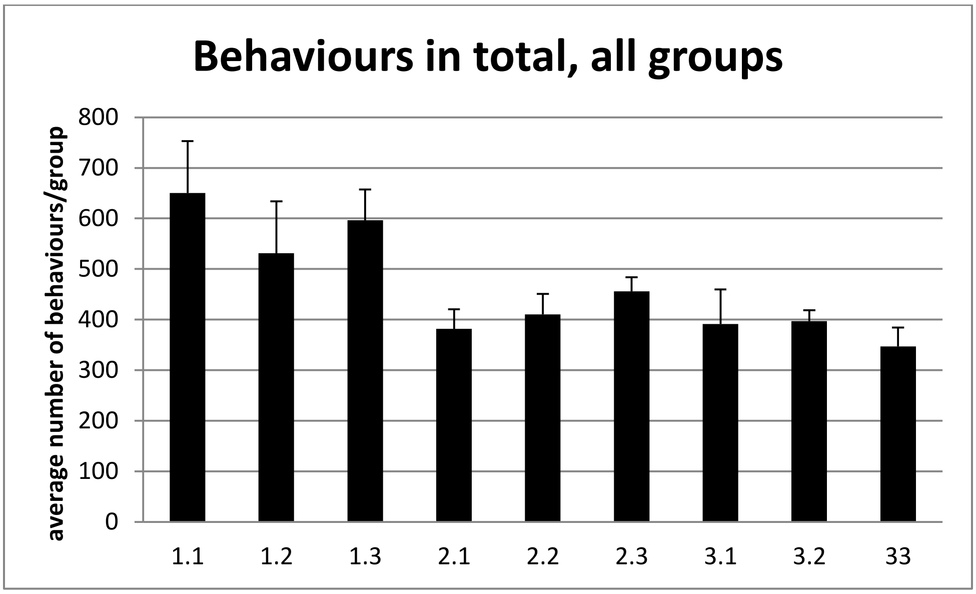


Fig 10 All behaviours in average for all groups within the length of the whole experiment

For the whole experiment we registered an average of 462.4 of all of the different behaviours in total/ group. Twenty-one goats did not perform positive behaviour like nosing/ exploring or grooming at all while they were observed, fourteen did not perform defensive behaviours at all, while 1 goat (in group 2.1) did not perform offensive behaviours at all while she was observed.

### 3.5 Cortisol measures

The level of cortisol in the blood plasma was significantly affected by period (Table 1, Fig.12), but not by density (Table 1, Fig. 11).

The cortisol levels were higher in the first period, December, with an average of 28.69 nmol/l. The cortisol-levels were not significant in relation to the effect of both period and density or by group within density (Table1, Fig.13).

The cortisol level showed a moderate correlation with the positive social behaviours; nosing on/ exploring ($R = -0.283$, $P < 0.05$) and grooming ($R = -0.328$, $P < 0.05$). There was no correlation between any of the other behaviours and the level of cortisol.

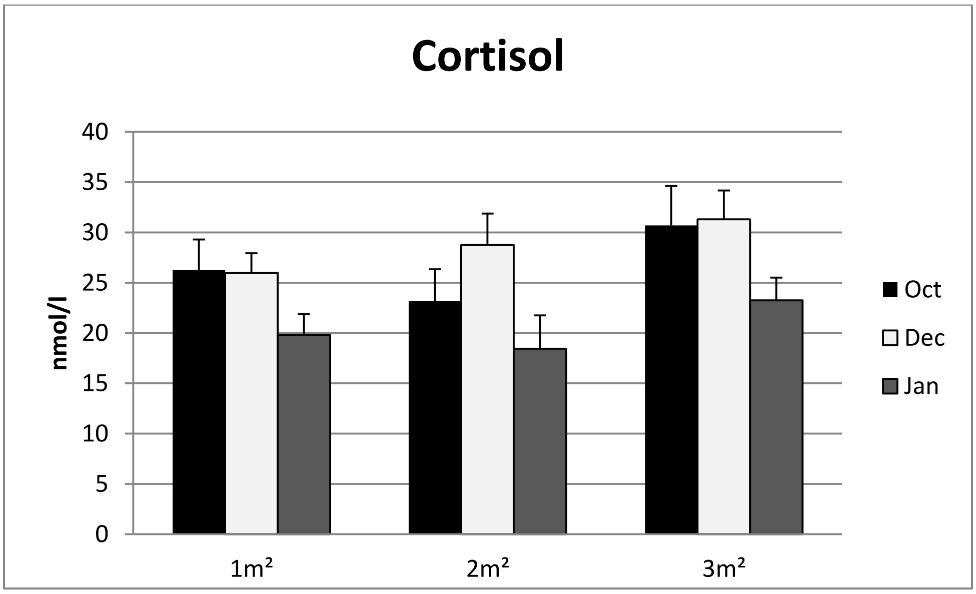


Fig 11 The effect of density in relation to period on cortisol level in both periods (December and January)+ basal value (October)

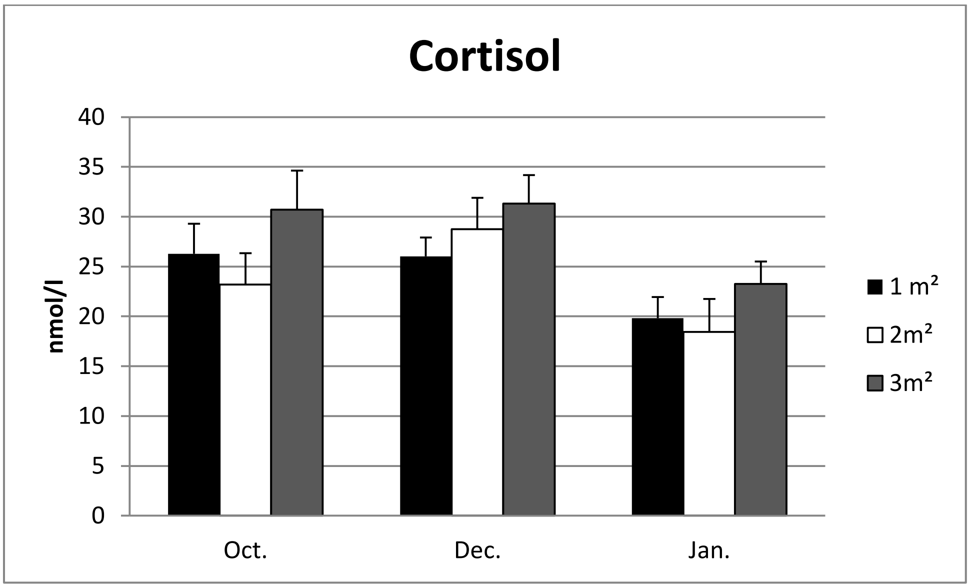


Fig 12 The effect of time period in relation to density on cortisol level in both periods (December and January) + basal value (October)

The lowest level of cortisol was measured in group 2.1 with 20.88 nmol/ l in average for all of the goats in that pen and for the whole experimental period. The highest level was found in group 3.2 with an average of 34.22 nmol/ l for the whole period (Fig.13).

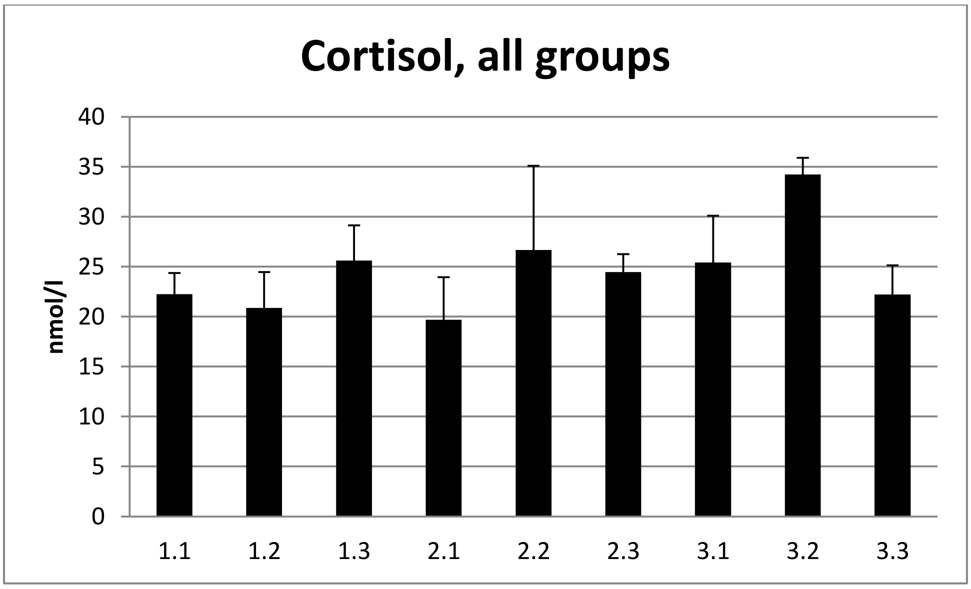


Fig 13 Average level of cortisol in each group, for the whole experimental period

The lowest level of cortisol measured for each day was found at day 6 in group 2 (Fig.14). There was no difference between any of the values of cortisol within the three groups belonging to the same density ($1m^2$, $2m^2$ or $3m^2$).

The first two days of sampling is the basal values taken before the goats were mixed in new groups. The basal cortisol values ranges between 23.6 and 27.0 nmol/ l for the three densities on day one, and between 23.4 and 27.8 nmol/ l on day two (Fig.14).

For the experimental values of cortisol from December (day 3 and 4), the values were more widespread; between 19.91 and 39.22 nmol/ l for each of the groups. The cortisol values from January (day 5 and 6) ranged between 16.29 and 28.62 for each of the groups (Fig. 14).

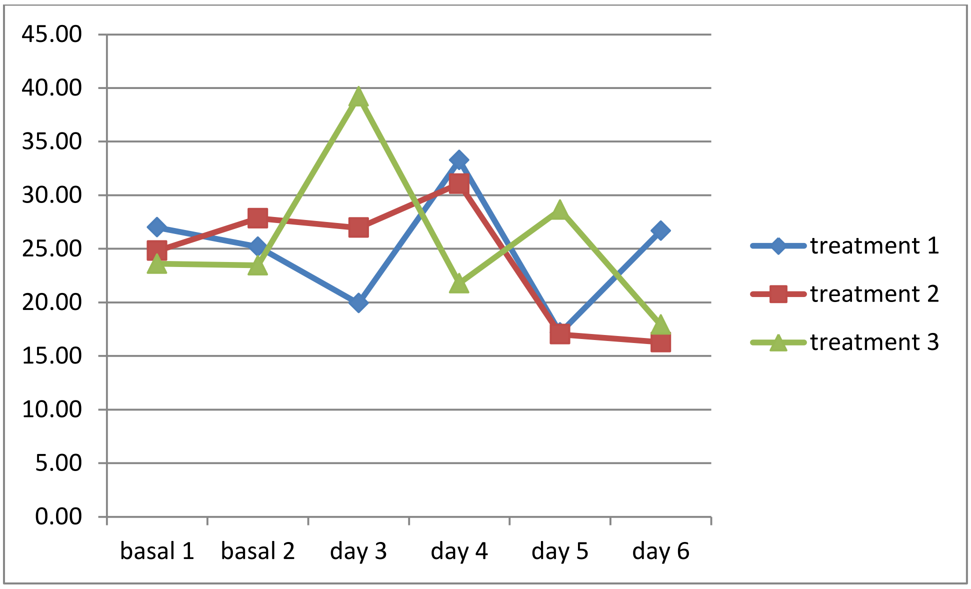


Fig 14 Average level of cortisol (nmol/l) from two days of blood sampling (basal values) and from four days of experiment in density 1 $m^2$, 2 $m^2$ and 3 $m^2$

# 4.0 Discussion

## 4.1 Behavioural results

Three of the behaviours measured in this thesis proved to be affected by density; both threats, pushing and offensive behaviours all together. As threats and push are defined within the offensive behaviours this was not surprising, but it also implied that our predictions was in compliance with the results. The highest amount of agonistic behaviour throughout the length of the whole experiment was found in all the three groups housed within the highest density of 1 $m^2$. Our results also showed accordance between the conducted social behaviours in total with the highest density, although the results were not as clear as expected. We could might have seen greater differences if the experiment was carried out with a bigger difference between the densities. The results between the different densities were not surprising, as competition between the individuals will increase when space is scarce. This was shown by e.g. Li et al. (2007), who found that decreasing the enclosure size was a part of what affected behaviour negatively, and also what affected the adrenocortical reaction in captive animals. Archer (1979) stated that social stress could induce more agonistic behaviours in animals, and this coincides with our results in terms of the agonistic behaviour. These results are also equal with Correa et al. (2010), who claims that high levels of social behaviours in a group is an indication of social stress.

The offensive behaviours were the ones that were registered with the highest amount of performances when compared to defensive and neutral behaviours (Offensive behaviours was in general recorded with an average of approx. 62 vs. defensive behaviours with approx. 20, and neutral behaviours with 8). This can be due to the fact that the three defensive behaviours we registered were withdrawal in general, being displaced from food and being displaced from rest after an agonistic encounter. If the goat that was compromised did engage in the fight or responded to it with the same strength, this behaviour would also be registered as an agonistic one. Nor was it surprising that the amount of threats performed was higher than the rate of pushing, since goats will spend as little energy as possible in performing costly agonistic behaviours. Subtle threats as eye contact and positioning of the head can be sufficient for the goat to displace a rival (Shackleton and Shank, 1984; Addison and Baker, 1982), and this type of communication will also make the goats able to save energy and limit the risk of injury. One goat that was housed in the highest density had however embraced this theory a bit too much; she distinguished herself with being the one goat with definite most aggressive interactions, and most threats in general of the whole experiment (317 threats).

According to Barroso et al. (2000), this would be a goat placed high in the hierarchical system. On several occasions we observed her resting in front of most of the eating area when she had finished eating, continually preventing the other goats from approaching the food. This type of displacement behaviour was also observed and described by Jørgensen et al. (2007), where goats were laying in the feeding area and thereby displacing other goats without physical contact. Nordmann et al. (2011) observed this displacement behaviour in goats that simply were standing in front of the feeding area. Jørgensen et al. (2007) concluded that low-status-goats experience a higher cost with increased competition than what high-status goats do when area is limited. The same is also supported by Loretz et al. (2004), who underpinned how low-ranking goats experience limited access to food caused by the high-ranking goats dominating several eating places at once. This is especially prominent when feeding space is insufficient, and highlights the importance of providing sufficient space for goats in a high-production environment. It is shown that high density in combination with decreasing access to food can influence the production level of goats (Barroso et al., 2000).

Social stress induces more agonistic behaviour in animals, but still; aggression is also a basic feature when unfamiliar individuals are placed in a new group (Lindberg, 2001). It is highly possible that this is the main reason for our results to show the highest amount of agonistic interactions in the beginning of the period. Moreover, animals living in groups will also compete for mobility, especially when area is limited (Archer, 1987). According to Krause and Ruxton (2002), the main reason for goats to compete against each other with aggressive interactions will either be because they are competing for valuable resources, or for assessing dominance relationships, which later on will help them in assessing more resources. It is mostly the male animals that interact and engage in aggressive encounters and agonistic behaviours (Dwyer, 2009), but it would also be expected of females to interact in disputes or fights when they are recently mixed, especially if resources as food and area are limited. For farming reasons we mainly keep female goats and bucks separated, but this does not mean that the goats not compete with each other. It is a great fitness advantage to secure its own accessibility to the resources, and sometimes the only way to achieve this is to involve in aggressive and potentially damaging fights. The goats do not have to be entirely aggressive in all their actions, but to maintain their social position it is clearly an advantage to be more aggressive in the first periods of interactions, and thereby reduce the level of aggressive encounters to more subtle signs as threats (Lindberg, 2001). This can to some extend also help explain why we registered the most threats in the first observational period. As stated by

Lindberg (2001), and Alados and Escos (1992; in Barroso et al., 2000), the hierarchy is established by aggressive interactions, and thereafter the “role” of the hierarchy is to prevent even further aggressive interactions, even though it cannot prevent aggressive encounters entirely. Barroso et al. (2000), stated that a social hierarchy within a herd is what permits successful coexistence, but there will always be some degree of conflicts. Low status individuals will either way be most vulnerable to suffer from reduced access to resources as well as be inhibited of activity. According to Shackleton and Shank (1984), the high levels of agonistic interactions between goats can be seen as a result of human interaction, and it’s conceivable that our housing of the goats is the strongest reason for them to interfere in such high levels of agonistic interactions as they did. Still, it is to point out that we tried our best to place the goats equally in their respective group due to differences in weight, age and reactivity type.

Barroso et al. (2000), coinciding results showed that goats compete more for resources when they are scarce. Food that is distributed twice per day can be regarded as limited by the goats, and will therefore be fought over. Even resources that we can look at as fairly evenly distributed, does not have to be that from the goats` perspective. Goats can also constantly compete over resources that never seem to be evenly distributed, like the best resting place in a pen, and this can give rise to a constant higher level of agonistic behaviour. Yet, the most obvious resource given to the goats in our experiment would be the food. It is also a fact that we conducted our behavioural observations right after the goats had been fed, so that we would be present at the times we expected it to be the most intense competition for resources. As mentioned in the introduction, farmers emphasize the importance of distributing the food evenly and continuously. But this is not always the case, and the goats would maybe eat what they considered to be the best silage first, then they would finish the rest whenever they felt hungry again. Barroso et al. (2000) has stated that goats behave more as specialists when the food quality is improved, and this underpins our perception that the goats will compete more over more valuable food. When distributing the food only twice a day it is expected to get a certain competition over the best silage. Providing adequate feeding space is therefore important (Miranda-de la Lama and Mattiello, 2010). In our experiment we ensured that all the groups of six animals had access to only one eating place per animal. This was done in order to secure equal conditions between the different pens they were housed within. Jørgensen et al. (2007) showed that less than one feeding place per animal increased time queuing, lowered the rate of feeding, and increased number of agonistic interactions over the roughage. On the contrary, Loretz et al. (2004) found that increasing the numbers of goats per eating place from 1 to 2 would not increase the levels of agonistic interactions in the group, but it did show a significant decrease in the time spent eating per goat. This means that having more than one goat per feeding place is not recommendable. The practical approach to this would be the design of the pen and feeding place: in our research pens of 1 $m^2$ the length of the feeding rack would hypothetically be long enough to secure the feeding place of 9 goats if we dealt with the Norwegian feeding space allowance of 0.4 m/goat as a sufficient feeding rack area. But the pen would in this case probably be too small to house nine fully grown goats without them standing like herrings in a barrel. To secure sufficient eating time for those low ranking animals, providing partitions would be a positive initiative, as demonstrated by Aschwanden et al. (2009), for positively affect simultaneous feeding time and lowering aggression whilst feeding.

As emphasized by Andersen et al. (2008), and Correa et al (2010), introduction of new animals into a group is known to cause aggression and high levels of agonistic interactions. This was avoided in our research, since we started our observation over a week after the goats were put together in their new groups, and since all of the goats had previously been together at pasture. It is possible that our work still was influenced by the mixing, and that our research would have benefitted from waiting one more week, to ensure that the grouping of the goats itself would not affect the results. According to Alley and Fòrdham (1994), a new goat added to the heard/group can be accepted as soon as 24h after the introduction, but this calls for sufficient space so that the new member can isolate itself from the group to avoid repeated aggression and "provocation" if necessary. This was also emphasized by Lindberg (2001) who drew inferences between the importance of sufficient space for the animals and the resulting high levels of aggression. Our experiment, however, differed from this research, because all of the goats in one groups was introduced to each other at the same time, instead of placing one goat into an already existing group like Alley and Fòrdham (1994) did. In our experiment the results on agonistic behaviours still suggest that 1 $m^2$ per goat might be insufficient for the (re-)grouping to go smoothly and without high amounts of agonistic interactions. In the research of Alley and Fòrdham they used several acres to ensure that the goats had the possibility to withdraw from agonistic peers if necessary. Sufficient space to establish a hierarchical system and thereby lower the agonistic encounters seems to be extremely important in goats, as proved by Lindberg (2001), and Aschwanden et al. (2009). Our flock of research goats was familiar with each other before the experiment started, but still we observed a rather high prevalence of agonistic behaviours in the start (The number of offensive behaviours in average per goat was nearly halved from November to January). We can only speculate if the results would have been different in terms of the agonistic behaviours (higher) if we rather used goats that were totally unfamiliar with each other. As we also predicted, the amount of social interactions would decrease from the first observational period to the last, and this was consistent with the actual results, but it was most obvious for the offensive behaviours. The same was true for defensive behaviours, but this was logic, since defensive behaviours (expressed by a goat defending herself) mainly will follow the offensive ones, expressed by a goat that actively impose agonistic behaviours onto others. Additionally, at the last observational period, the goats might have rested more frequently due to their emerging pregnancy, and it could therefore be less agonistic interactions.

However, it was interesting to note that the positive behaviours which we consider being affiliative behaviours, increased to the end of the experiment in January. The number of nosing/ exploring and grooming doubled from November to January, calculated with the average number per goat (From 6 to 13). These are interesting observations, as affiliative behaviour can help to improve the cohesion of the group (Andersen et al., 2008; Miranda-de la Lama and Mattiello, 2010). Goats grouped as juveniles freely chose a smaller inter-individual distance than goats grouped as adults (Aschwanden et al., 2008), and this proves that goats benefit in a social/affiliate context from being familiar to each other. This could have been an influence the goats in our experiment were subjected to, meaning that they might express less agonistic behaviour than what totally unfamiliar individuals could have done in the same management situation. Neither way, observing affiliative behaviours like nosing/ exploring and grooming is positive, and indicates that the goats in our research groups started to establish bonds between each other. In that way aggressive interactions are reduced, as we also found by the reduction in offensive behaviours from 84.5 an average in November, to 47.5 offensive behaviours in January. We also noted several occasions of intervention behaviour between the goats as we observed them, but this is not evident from the data material.

One other indication of positive welfare in the goats would be the amount and length of simultaneous resting. This was not a part of what we recorded during our observations, but we did note the numbers of resting that was disturbed by a peer (displacement from resting place). We could on the other hand emphasis the fact that we observed more disturbance of resting in the last observation period than the first and second one, and these observations went from an average per goat of 0.03 in November to 0.11 in December and increased to 0.59 in January. The goats might increase their resting time at the end of the experimental period due to the late phase of pregnancy.

Unfortunately, we suspect that there was done a bias to the research that might could have influenced the outcome; that the observations was done somewhat later after the feeding in January than what was done in November and December due to misunderstandings between us and the people in charge of feeding the goats.

## 4.2 Cortisol

The overall results from the measures of plasma cortisol revealed that the amount of cortisol was significantly affected by period, but not by density; the opposite of our prediction. The level was highest in the first experimental measuring in December. The first cortisol measure was taken one month after the regrouping of the goats, indicating that there was a higher level of stress in all groups even one month after regrouping than what was measured in time period number two, January. All of the goats in all densities showed similar or slightly higher amount of cortisol level after the regrouping compared to the basal values. But also, the cortisol was lower than the basal values in all densities in the second sampling, when closing up to the parturition. The level of cortisol was moderately correlated to the positive behaviours, which was an interesting finding.

We took blood-samples of the goats before the start of the treatment to confirm basal values. Doing that, we saw that the values were quite evenly distributed between the goats that later was grouped in the different densities, meaning that the spread between the individuals was low. The cortisol values in our experiment varied from 4.0- 64.8 nmol/l of the basal values, and between 0.6-75.8 nmol/l for the experimental period. Olsson and Hydbring- Sandberg (2011) measured between 17 (±1) and 49 (±6) nmol/l in the plasma cortisol of goats exposed to fear-eliciting stimuli (as a stressor), similar to our measurements. To measure plasma cortisol with the designation of nmol/l implies that one must know the molecular weight of cortisol for satisfactory to recalculate the number into other terms, as e.g. the term “ng/ml”. Andersen et al. (2008) measured between 2-10 ng/ml of cortisol concentrations in adult goats exposed to social instability, while Nwe et al. (1996) measured between 42-166 ng/ml cortisol concentrations in goats exposed to transportation stress.

According to Nwe et al. (1996); Moberg (2000); Olsson and Hydbring- Sandberg (2011), blood cortisol levels has been widely used to predict the level of stress in farm animals, but for our research this was obviously not sufficient. The use of cortisol as a measure of chronic stress might not be the best way to confirm influences of long term stress. Also, in our experiment we used measures of plasma concentrations of cortisol as the only physical measurement of social stress in goats; it might have been even more reliable to use other measures of physical stress in addition. E.g. both saliva, urine (Ekiz et al., 2012), faeces (Li et al., 2007) and hair contains cortisol, and can thereby be used as non-invasive measures for measuring physical effects of stress in an animal. Zimerman et al., (2011), concluded that the use of a single indicator to characterize a stressor might not be sufficient. There may also be differences between the way of measuring chronic and acute stress, since taking blood-samples involves both physical restraint and possible stress from the venipuncture itself, measurements can also be influenced by that, and thereby not show the appropriate measures.

Using cortisol as a measurement of stress is a common method, but several authors tend to regard the level of cortisol in blood as a short term measurement (with a half-life of less than 24 h), of physical reactions in the body of individuals subjected to stress (Broom et al., 1996; Grigor et al., 2004). We can therefore argue that cortisol level in blood is insufficient as a long term-indicator to measure if the goats in our experiment suffered from long term social stress. On the contrary, the work of Creel (2001; in Li et al., 2007), stated that agonistic interactions can provoke a large increase in cortisol, and that this level of cortisol-secretion will be persistent. It is possible that the pregnancy induced some of the effects we saw in level of cortisol (decrease when closing up to parturition), as Vierin and Bouissou (2002) suggested in their paper; how the stress-reaction was lower in pregnant than non-pregnant sheep when exposed to different fear-eliciting stimuli. Andersen et al. (2008), did not find significant differences in cortisol levels between goats housed in unstable versus stable groups, and explained this mainly with that the treatment only produced a moderate level of stress. But it was also emphasized that goats and sheep have been reported to have a sudden decline in cortisol concentration when exposed to the same stressor over time (E.g. Roussel et al., 2004: in Andersen et al., 2008). Nwe et al., (1996) proved the decline in cortisol measures in goat to decline to basal values 3 hours after the stress response was ended (transportation stress). Broom et al., (1996) found a greater concentration of plasma cortisol in the first 3 hours of journey in sheep exposed to transportation stress, and Grigor et al., (2004) found that plasma cortisol levels in calves returned to normal levels after 1.5 hours of lairage. This underpins the possibility of cortisol measures in blood to be a less reliable indicator of long-term stress.

Andersen et al. (2008), put forward the theory of that our farm animals could be highly indirectly selected to have a strong coping ability regarding social stress, as they have been subjected to high density condition and unstable social environment for several generations. Nevertheless, animals are able to display physical signs of social stress, at least if the stress is extremely above the coping-ability of the animal. Therefore it is up to us what methods we develop to find out the best way possible to measure these physical signs. Maybe can other methods than cortisol measure in blood plasma be a better way of determining the effects of long term stress.

The cortisol-levels in this experiment were not significant in relation to the effect of both period and density. However, cortisol did show a moderate correlation with positive behaviours, meaning that the higher amount of positive behaviours that was performed by the goats, the lower was their cortisol level. Cortisol did not show any correlation towards the other behaviours. Altogether, it seemed that the social stress imposed by agonistic behaviours would not be sufficient to elevate the plasma cortisol in the goats up to detrimental levels. Maybe would the positive interactions help counteract the negative effects of the agonistic behaviours.

## 5.0 Conclusion

The results in this thesis showed that increased density affected offensive behaviours negatively; meaning increased the number of offensive interactions. Threats were the only behaviours that were significantly influenced by density.

Total amount of agonistic behaviours declined over time, while positive behaviours increased over time. The cortisol level was highest in the first observational period. We concluded that cortisol in plasma might not be the best indicator of long-term stress.

Housing goats in relatively high densities can lead to increased amounts of aggressive interactions, but within the range of densities 1- 3 $m^2$ per goat, as tested in the present project, the effects were only moderate.

## 6.0 References


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# Appendix 1

Pen: Observer:

Date: Time:

| | | INITIATOR | | | | | |
|---|---|---|---|---|---|---|---|
| | | GOAT 1 | GOAT 2 | GOAT 3 | GOAT 4 | GOAT 5 | GOAT 6 |
| RECIPIENT | GOAT 1 | | | | | | |
| | GOAT 2 | | | | | | |
| | GOAT 3 | | | | | | |
| | GOAT 4 | | | | | | |
| | GOAT 5 | | | | | | |
| | GOAT 6 | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | **Codes of behaviours** | | | |
| | 1 frontal clashing | 4 threatening | 7 grooming | 2 butting | 5 withdrawing | 8 displ from food |
| | | | 3 push | 6 nosing/explore | 9 displ from rest | |