FEAR RESPONSE AND SOCIAL INTERACTIONS IN DAIRY GOATS HOUSED IN THREE DIFFERENT DENSITIES DURING PREGNANCY

FRYKTRESPONS OG SOSIALE INTERAKSJONER HOS MELKEGEIT OPPSTALLET I TRE ULIKE TETTHETER UNDER DREKTIGHET

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Forord

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Abstract

Farm animals are social and are willing to work for access to social companions. To separate and isolate animals from each other is a strong stressor, and will induce fear. The fear of novel and fear-eliciting situations in social animals can be reduced by being a part of a group. The response to handling will probably not only be a reaction to humans, but will also be affected by the environment, the novelty of the situation and the social context. The aim of this project was firstly to study the effect of three different densities (1 m^2, 2 m^2 and 3 m^2) on fear responses and social behaviour in a separation test and human approach test. Secondly we wanted to study the relationship between fear responses and different types of social interactions on the individual level. Fifty four pregnant goats were selected and used in this study. They were housed in three different densities with three groups in each density. Each of the groups consisted of six goats. The separation test lasted for two minutes were vocalizations and flight attempts were recorded. The human approach test was performed right after the separation test. The scores from this test were used to determine the individuals reactivity. A human entered the pen, took one big step to the left or right and waited for five second before approaching the goat. When the animal was within reach the goat was given the opportunity to smell the hand of the human. The human would then crouch together and hold this position for 10 seconds.

We predicted higher levels of vocalization and flight attempts in the second separation test in the high density. Our findings could not support this prediction. Number of vocalizations was significantly higher in the second separation test, but had no effect of density. Number of flight attempts was not affected by densities, but there was a significant difference between the two tests. We predicted that confidence score in the human approach test were affected by density. The results could not support this prediction. As predicted, the results showed a higher level of offensive initiated behaviours in the high level density than in lower densities. It will be difficult to make any conclusion about the level of fear in these tests since they go in different directions. The density had an effect on the number of offensive behaviour initiated and received. These results give us a clear indication that high densities give higher aggression among goats.

Sammendrag

Husdyr er sosiale dyr som ønsker å være i en flokk, og de viser sosial motivasjon for å få det til. Å isolerere dyr fra fra hverandre blir betraktet som en sterk stressor, som igjen vil medføre frykt. Frykten for det fremmede og fryktfremkallende situasjoner kan bli redusert ved at gruppe medlemmer er i nærheten. Responsen til håndtering vil mest sannsynlig ikke bare være en reaksjon på mennesker, men også en reaksjon på miljøet, det ukjente og den sosiale sammenhengen. Formålet med dette forsøket var først å studere effekten av tre ulike dyretettheter (1 m^2, 2 m^2 og 3 m^2) på fryktresponser og sosial atferd i en separasjonstest og human approach test. Det neste mållet var å undersøke forholdet mellom fryktresponser og ulike typer sosiale interaksjoner på individnivå. Femtifire drektige geiter ble valgt ut og brukt i forsøket. De ble oppstallet i tre ulike dyretettheter med tre grupper innen hver tetthet. I hver av gruppene var det seks geiter. Separasjonstesten hadde varte i to minutter, og vokaliseringer og flukt forsøk ble registrert. Human approach testen ble gjennomført dirkete etter separasjonstesten. Scoren geitene fikk i denne testen ble brukt til å bestemme et individs reaktivitet. En observatør gikk inn i bingen, tok et stort skritt til høyre eller venstre, ventet i fem sekunder før observartøren nærmet seg geita. Da geita var innen rekkevidde fikk den muligheten til å lukte hånda til observatøren. Deretter ville observatøren sette seg ned og huke seg sammen og holde denne posisjoen for 10 sekunder.

Det var forventet høyere nivå av vokalisering og flukt forsøk i den andre separasjonstesten i høy dyretetthet. Det ble ikke funnet støtte for denne prediksjonen. Frekvensen av vokaliseringer var signifikant høyere i den andre separasjonstesten, men det var ingen effekt av tetthet. Antallet flukt forsøk var ikke påvirket av tetthet, men det var derimot en signifikant forskjell mellom de to separasjonstestene. Det var også forventet at scoren i human approach testen hadde en effekt av dyretetthet, men resultatene kunne ikke finne støtte for denne prediksjonen. Som forventet viste resultatene et høyere nivå av offensive initierte atferder i høy dyretetthet enn i de lavere dyretetthetene. Det er vanskelig å trekke noen konklusjon om fryktnivået i disse frykttestene siden de peker i ulike retninger. Dyretettheten hadde en effekt på frekvensen av offensive initierte atferder og offensive mottatte atferder. Disse resultatene gir oss en klar indikasjon på at høyere dyretetthet gir høyere aggresjon blant geiter.

1.0 Introduction

1.1 Group life – cost and benefits

Farm animals are social and are willing to work for access to social companions. Groups with goats can vary widely in size, depending on the local environment conditions and the characteristic of the population. Among feral goats, the groups normally consists of two to 10 individuals, but in some populations, the group size might have a wide range from 50 to 100 individuals (Shackleton and Shank, 1984). The female and their juvenile offspring will form groups on a home range, while males will get separate from the female groups and form smaller group and share home range with the female groups (Dwyer, 2009). Living in groups implies that there will be some cost and benefits. This has been studied to a great extent in wild animals (see e.g. Krause and Ruxton, 2002). The greatest benefit of living in groups is the reduced predation risk through dilution (Roberts, 1996). An individual's risk of being attacked decreases with increasing group size, known as the dilution effect. With a larger group there will also be a greater chance of detecting predators. Because of the increased group size each individual will have more time to forage and rest than to look for predators (Krause and Ruxton, 2002). The confusion effect is another factor that is beneficial for groups. This means that it will be difficult for a predator to pick out a single prey in a group (Cresswell, 1994). Farm animals are rarely exposed to predators, but have still maintained a strong anti-predator behaviour trough the domestication (Penning et al., 1993; Newberry, 2001), and they show lower fear when grouped. Detecting food can be easier when living in groups than solitary. Shrader et al. (2007) observed that goats use social information from other goats to locate better foraging areas. The time spent on searching for food will decrease. Animals that rely on food that is unpredictable dispersed will have great benefits of living in groups (Mendl and Held, 2001). Ungulates have a more predictable dispersion of their food and the benefits will not be so great (O'Brien, 1988). Animals living in group will have the benefit of obtaining food that would not be obtainable otherwise, and food sources can be defended if necessary (Mendl and Held, 2001).

Living in groups will not just be beneficial, but also have some cost for the animals in terms of competition for important resources. There will be competition for the resources as food, mates and attractive resting places within a group (Alcock, 1997). Larger groups may also have the cost of spending more time searching for food to support the whole group (Estevez et al., 2007; Krause and Ruxton, 2002). Foraging in a group will almost always have a cost.

When food resources are discovered there will be less for each individual if the group is large (Krause and Ruxton, 2002). Majolo et al. (2008) found that large group of primates had to move further and they also spent more time on feeding than smaller groups of primates. When food sources are scarce there will be a risk of kleptoparasitism. Other group members can steal the food item before the animal can consume it. Some animals might get an advantage on this, if they can steal more than they get stolen from. As a result of this some animals might end up without resources (Krause and Ruxton, 2002). There will also be a competition when it comes to farm animals within groups. They will compete for food, water and the best access to these resources along with resting places, environmental enrichments and the opportunity to move freely. When competition for resources occurs there will often be aggression involved in the social interactions (Archer, 1988). When resources are limited there will be a higher competition and the level of aggression will increase as a result of this (Andersen, 1999; Bøe 2006).

1.2 Social strategies and fear

To cope is the way in which one reacts to a challenging situation, which will induce physiological stress reactions (Wechsler, 1995). Fraser and Broom (1990) have defined 'coping' as "Having control of mental and bodily stability. This control may be short-lived or prolonged. Failure to be in control of mental and bodily stability leads to reduced fitness". Fear and anxiety can be defined as "emotional states that are induced by perception of any actual danger (fear state) or potential danger (anxiety) that threatens the well-being of the individual" (Boissy, 1995: In Andersen et al., 2000). Farm animals kept in intensive housing systems will most likely at some point be exposed to challenging situations, and often they will not have possibility to avoid these situations by showing a specific behaviour. If the situation is ongoing, the animals will try out different coping strategies in an attempt to cope with the situation. The efficiency of the coping behaviour will be determined whether the challenging situation can be changed or not (Wechsler, 1995).

1.3 Measures of fear and social motivation

As mentioned, the majority of farm animals are gregarious and will form stable social groups. To separate and isolate animals from each other is a strong stressor (Boissy and Le Neindre, 1997; Dwyer, 2009). Isolation from the group might lead to agitation and flight attempts, running, high –pitched vocalization and rearing against the pen walls. Isolation from the group can be more stressful than being captured and restrained within the group (Dwyer, 2009). When exposed to fear-inducing situation, animals will show less signs of distress together with familiar group members (Boissy and Le Neindre, 1990). This coincides with the evidence found by Færevik et al. (2006); dairy calves preferred to stay close to familiar and unfamiliar calves rather than alone, but had the highest preference for familiar calves. In a separation test they observed that the presence of a familiar or unfamiliar calf had a significant effect on vocalization. With a familiar calf present there were no vocalizations. Social bonds are important for the animals, and they are willing to work for access to other conspecifics (Hovland, 2005). Lyons et al. (1988) found that human-reared goats had low levels of fear in comparison to dam-reared goats. The behaviour of the dam-reared goats' changed over time to resemble the human-reared goats' behaviour, but the individual differences stayed stable through the study. Siebert (2011) found that isolation had an effect on goats and suggests that goats should not be isolated if it is possible to be avoided. Goats that were partially separated vocalized more and had higher locomotion than completely isolated goats.

1.4 The relationship between social behaviour and fear

According to Fraser and Broom (1990) social behaviour is "the reciprocal interactions of two or more animals and resulting modification of individual activity". Dwyer (2009) claimed that goats are not aggressive except when the resources are limited. The dominant goats will maintain their dominance by subtle behaviours as eye contact and placing their head on the back of the other goat for displacement. Goats involved in fights might be interrupted by a third goat. It will serve as a mediator and split up the fight. This appears to happen in groups were the level of aggression is high (Andersen, 2011; Miranda-de la Lama et al., 2011). Schino (1998) found that affiliative behaviours will increase after a fight. This might mean that goats seek reconciliation to mend relationships in the group. In the same study they found that goats often would have renewed aggression after conflicts, and they suggest that

increased tension is the cause of the renewed aggression. Goats normally inhabit areas that often have a limitation in visibility; vocal communication is thereby an important tool to maintain in contact with the rest of the group. When isolated from the group, increased vocalization might be an increased effort to get back to the group or it might be distress or fear.

1.5 Social behaviour and fear when handling

The fear of novel and fear-eliciting situations in social animals can be reduced by being a part of a group. The response to handling will probably not only be a reaction to humans, but will also be affected by the environment, the novelty of the situation and the social context. (Grignard et al., 2000) Separation from the group will induce a higher level of stress (Siebert, 2011). Price and Thos (1980) conducted a study on sheep and goats that were short-term isolated. Goats that were put in an unfamiliar enclosure spent significantly more time rearing against the walls than the ones kept in the home pen. Number of vocalizations were not affected by an unfamiliar environment or being in the home pen. When the test were done in the presence of an observer there were significantly lower frequency of vocalizations, fewer bouts of rearing and less time spent on rearing. Vocalization is important in creating bonds between mother and infant and it will be helpful for the mothers to locate the infant's lying site (Poindron, 2003). Goats that are dam-reared will use longer time to approach humans and also spend less time in close proximity to the humans than human-reared goats (Lyons, 1989). Boivin and Braastad (1996) conducted a study on early weaning on goat kids' later response to humans. Kids that had experience with humans from an early age spent more time closer to humans later on. In the presence of a moving human the control kids were moving away from the human in contrast to the handled kids. This is an unwanted trait when it comes to animal production. The treated kids vocalized less than the control group at five months of age. The vocalization rate was highest when the kids were alone and the rate decreases when a human were present, both seated and moving (encounter test). For animals to develop social bonds is it important to recognise familiar individuals and to form a social memory (Lim and Young, 2006).

1.6 Animal density and housing

The housing systems for animal production are based on large groups and high density of animals. That is to ensure a good economic return. It has been shown that high densities results in increased aggression, reduced performance and behavioural problems in several farm animal species (Al-Rawi and Craig, 1975; Hill et al., 2009; Fisher et al., 1997; Blanc and Thériez, 1998; Bøe et al., 2006; Kondo et al., 1989). Al-Rawi and Craig (1975) showed that agonistic behaviour in poultry was affected by area per animal. The level of non severe agonistic interaction and the total amount of agonistic behaviour were significantly affected. In the study done by Bøe et al. (2006) they found that reduced lying space for ewes affected number of displacements and a reduction in lying time. When the lying space was reduced from 0.75 m^2 to 0.5 m^2/ewe, lying time was significantly reduced. Displacements significantly increased as a result to the decreased lying space. The same results have been found for goat by Andersen and Bøe (2007). The goats decreased their lying time and increased their resting time in the activity area. Temporary isolation of individuals from the rest of the group is not uncommon in goat management (Price and Thos, 1980). If given the opportunity, farm animals have a strong tendency to perform synchronous resting behaviour and activity (Rook and Penning, 1991). Such needs should be taken into consideration when designing goat houses. Andersen and Bøe (2007) observed that goats spent less time in the resting area when it was small compared to medium and large size. As a result, the goats spent more time resting in the activity area, when the resting area was small. Resting against the pen wall occurred more frequent in pens with medium and large size resting area. The time spent resting against a pen wall increased from 68 % (small resting area) to 82 % (large resting area). They also observed lower frequency of displacements and overall aggression level when the lying area was organized into two levels rather than one. Furthermore they found that goats rarely rested with body contact. Ehrlenbruch et al. (2010) tested if additional walls in the pen would have an effect on goats resting behaviour and social interactions in goats. They found that goats prefer to rest against walls, but the additional walls had no effect on resting time and synchronization. It was also observed that goats rarely rested with body contact, which corresponds with the results of Andersen and Bøe (2007). Social distances will be influenced by the quality of the social bonds and the ages of the goats when grouped together. Goats will keep closer to other goats that show a positive behaviour, and stay further away from the ones that show more agonistic behaviour. Goats that are grouped together at a young age will have shorter intra-individual distance than goats grouped at adult age (Aschwanden et al., 2008). Fernández et al. 2007 found in French Alpine goats that the level of aggression increased after

all three regroupings, while the milk production decreased after the first regrouping. The level of aggression decreased to the same level as recorded before regrouping the day after regrouping.

1.7 Social structure and dominance

Fraser and Broom has defined social structure as "all of the relationships among individuals in a social group and their consequences for spatial distribution and behavioural interactions". Small ruminants use agonistic and affiliative social interactions as communication signals and mechanisms of recognition to maintain their social structure in the group (Miranda-de la Lama and Mattiello, 2010), and by maintaining the social structure they will get access to attractive recourses (Shackleton and Shank, 1984). Hurnik et al. (1995) has defined agonistic behaviour as "any behaviour indicative of social conflict such as threat, attack, and fight; or escape, avoidance appeasement, and subordination". Movement in free-ranging goats will most often be led by an older goat with experience, and they will mostly form a single line. The dominant goat will usually be in front, but not in the leading position since it can be a vulnerable position. By being up front she will obtain the feeding sources on arrival to the new location (Dwyer, 2009). Barroso et al. (2000) found a clear relationship between dominance and sub dominance in their study of the domestic goat. Throughout the trail period the relationships stayed stable, but there were some changes in position between some goats in the herd. In the same study they found evidence that, out in the field, the highest ranking goats will have more passive social interactions, but when placed in a goat house the aggressive interactions will increase between the goats.

1.9 Aim of study

The aim of this project was firstly to study the effect of three different densities on fear responses and social behaviour in a separation test and human approach test.

Secondly we wanted to study the relationship between fear responses and different types of social interactions on the individual level.

Predictions:

- A higher occurrence of offensive behaviour in high density groups.
- Higher level of vocalization and flight attempts in high density groups in the second separation test.
- Lower confidence score in the second human approach test in the highest densities.

2.0 Materials and methods

This project consists of four work packages and this thesis is part of work package 3 (WP 3). The aim of WP 3 is;

"to form an experimental model to test in a controlled manner how three major resource based factors that we see on a farm influence welfare of pregnant females and their offspring, namely animal density, group size and human handling."

http://www.animal-welfare-indicators.net, 21.02.2012

2.1 Experimental design

The effect of different densities on social behaviour of pregnant animals was investigated by using 54 goats. We tested at different densities and the group size was therefore kept stable throughout the study. There were three different densities with six animals in each group. There were tree replicates of each treatment:

1. 1.0 m^2 per animal (D1); (18 animals: 3 groups of 6: G1/1, G1/2, G1/3)
2. 2.0 m^2 per animal (D2); (18 animals: 3 groups of 6: G2/1, G2/2, G2/3)
3. 3.0 m^2 per animal (D3); (18 animals: 3 groups of 6: G3/1, G3/2, G3/3)

The goats' reactivity was tested before the treatment and used to form groups by their character. Animals with high, low and intermediate reactivity were evenly distributed amongst the different treatments. This ensured that none of the groups consisted of extreme animals only. Reactivity was measured with two tests; separation from group mates (separation test) and being approached by unfamiliar human (human approach test). Both tests were carried out immediately after each other and all six goats went trough the tests consecutively. The separation test and human approach test were replicated two weeks before the expected time of parturition. The goats' social strategy was observed when mixed into their treatment groups by observing their social interactions.

2.2 Animals and feeding

The animals in this study were of the breed Norwegian diary goat. All the goats were older than one year of age with mean age 2.79 years (± 0.7) (all given birth earlier) and dehorned. The mean weight at the start (November) of the project was 50.3 kg ± 7.71 and increased to 59.3 kg ± 7.98 at the end of project. Feeding was done twice a day; between 08.00 and 09.00 in the morning and in the afternoon between 14.00 and 15.00. During morning feeding, they were given silage and standard concentrate pellet diet. Each goat received 0.2 kg concentrate in November and this increased during the project to 0.6 kg at the end (January). Together with the concentrate they received 20 g minerals per day. In the afternoon they were fed with silage and hay. They had free access to water in water dispensers. Every pen had access to salt blocks (with copper). Their reactivity were tested before the treatment and used to form groups by their character.

2.3 Experimental pens

The study was conducted in an insulated building with mechanical ventilation at the Norwegian University of Life Science. Observations were done in the period from September 2011 until January 2012. The goat house usually holds between 6ºC and -10ºC. The pens were divided in two; one resting area with wooden floor and one activity/dunging area with expanded metal floor. The pens were cleaned once a day. Faces and urine was removed from resting area and a thin layer of sawdust was administered. Every pen had six eating places, so all goats could eat at the same time.

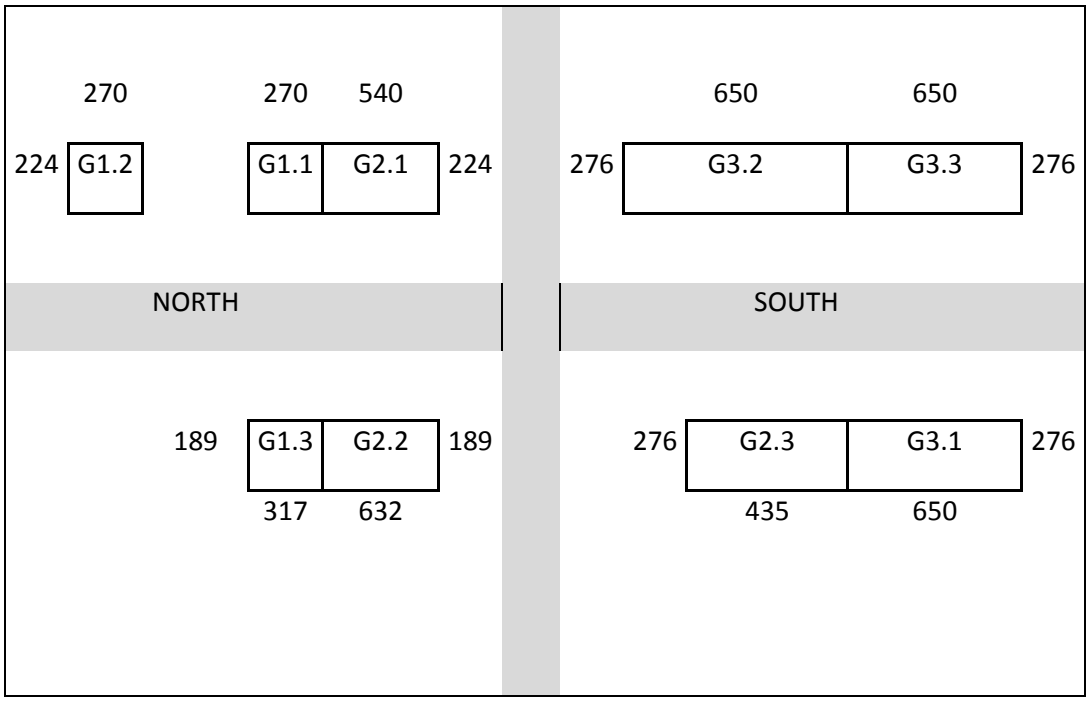

Fig. 1. Overview of the experimental pens in the goat house. The numbers inside the squares is the group number. The numbers on the outside of the squares are the measurements in centimetres of the experimental pens.

2.4 Behavioural observations

2.4.1 Separation test

The test was conducted in the home pen of the animals. All the goats were taken out and a solid wall with a door on it was brought in (Fig 2A). The wall was placed so it created a pen that was 6 m^2 and would serve as test area. The test animal was led back trough the door and into the test area. The behaviour was observed during the two minutes of separation. The behaviour was recorded by two video cameras, placed with different angles (Fig 2B).

Behavioural variables:

1. *Vocalisation* (frequency)
2. *Flight attempts* (frequency): number of escape attempts (rushing with the two forlegs on to one of the walls, either original or separation wall)

2.4.2 Human approach test

The test was performed right after the separation test. The scores from this test will be used to determine the individuals' reactivity. A human (an unfamiliar woman, wearing standard coveralls) entered the pen, took one big step to the left or right. Depending on the goat's position, the human took one step in the opposite direction of the goat. This would simplify the determination of the goat's behaviour, whether it was approaching the human or the door. The human stepped sideway without looking at the goat, standing still (looking down) for five seconds and then established eye contact with the goat, if possible. The human looked at the muzzle of the goat, raised the right arm to 45º forward with the palm down (Fig 2C). The goat was approached by the human by one step (30 cm) per second (recorded beat trough earplugs). When the animal was within reach the goat was given the opportunity to smell the hand of the human (even if the goat approached the human). If the goat did not escape, the human tried to pet it between the ears. If the goat escaped, the human followed the goat for one step. The human would then crouch together and hold this position for 10 seconds (Fig 2D).

Behavioural variables:

Reaction to stationary human

0 – the goat moves away from the person

1 – the goat does not approach nor avoids human

2 – the goat approaches human but does not get into physical contact with her

3 – the goat approaches the human and gets into physical contact with her

Reaction to approaching human

0 – no stroke, the goat flights when approached

1 – the goat smelled the hand of the human, but could not be stroked

2 – the goat could be stroked

3 – the goat approached the human before she arrived to it

Reaction to crouching human

0 – the goat moves away from the person

1 – the goat does not approach nor avoids human

2 – the goat approaches human but does not get into physical contact with her

3 – the goat approaches the human and gets into physical contact with her

A

B

C

D

Figure 2A, B, C, D. Overview over experimental pen during separation test and the procedure in the human approach test.

2.4.3 Social interactions

The goats were individually marked with collars of different colours (purple, grey, green, red, blue and yellow). Social interactions were observed for one hour after mixing. The observations of all social interactions were made continuously. The frequency of these observations was noted for each individual. The social behaviours were defined in this ethogram (based on Andersen and Bøe, 2007):

1. Frontal clashing: 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. Push: 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 on / exploring: nose in contact with another goat
7. Grooming: grooming by using an other goat for this activity (the other can be either a passive recipient or take part actively in the mutual grooming)
8. Displacement from food: physically forcing another goat to leave its feeding place, or passively displacing the other goat simply by approaching that individual
9. Displacement from rest: physically forcing another goat to leave its resting place, or passively displacing the other goat simply by approaching that individual.

Social behaviour was observed again one week after mixing when social relationships were stable and the rank order established. There were tree observations periods during the treatment period. The observations were done over four consecutive days. Observations were conducted twice a day and each observation period lasted for one and a half hours and was done in the morning (between 08.00 and 09.00) and afternoon (between 14.00 and 15.00) just after the goats were fed. Each pen was observed twice during the four day period. During

these observations the initiator and recipient of the social interaction were noted. After observations the behaviours were divided into different categories:

- Offensive initiated
    - Frontal clash initiated
    - Butt initiated
    - Push initiated
    - Threat initiated
    - Displacement from food initiated
    - Displacement from rest initiated
- Offensive received
    - Frontal clash received
    - Butt received
    - Push received
    - Threat received
- Defensive initiated
    - Withdraw initiated
    - Displacement from food received
    - Displacement from rest received
- Defensive received
    - Withdraw received
    - Displacement from food initiated
    - Displacement from rest initiated
- Positive initiated
    - Nosing initiated
    - Grooming initiated
- Positive received
    - Nosing received
    - Grooming received

The observations were conducted by tree different researchers. Test trial of social observations had been conducted previous to the actual observations, to ensure a high inter-observer reliability.

One goat aborted one week before expected parturition. She was therefore excluded from the last observational data set.

The success – and activity indices were calculated as follows:

$$\text{Succes index} = \frac{Frequncy\ \ initiated}{(Frequency\ \ received + initiated)}$$

$$\text{Activity index} = \frac{Frequency\ initiated}{\sum Frequency\ initiated\ \ (group)}$$

Dominance rank (DOM) was calculated with Matmat. It is based on the displacement behaviours (from food and from resting place) between pairs of goats.

2.5 Statistics

To analyse the effect of treatment, age and pigmented vs. white on vocalisation and flight attempts during separation test, a mixed model analysis of variance were used in SAS. The class variable in the analysis were density (small: 1 m^2/goat (D1), medium: 2 m^2/goat (D2) and large: 3 m^2/goat (D3)), pens (three levels) and pigmented/white. Pen within treatment was specified as a random effect in the model. Density, age and pigmented/white were specified as fixed effects. The difference between the first and second test were calculated and used in the statistical procedure. The box plots are made in MiniTab. The model that was used in separation test was also used for the human approach test. The mean score for the human approach test were calculated and used in the statistical procedure. The mean score from the first and second human approach test was also calculated and used in the statistical procedure.

To analyse the response of the different variables to density, age and pen, a generalized linear mixed model were used in SAS. The class variables in the analysis were as mentioned in the former analysis, except for pigmented/white (not included). Pen within density were set as a random effect, and the fixed effect were set as density and age. There were used a LS means test to find the differences between the means.

Pearson's correlation test in SAS was used to test for the correlation between different variables. To compare differences between variables the wilcoxon signed rank test were used.

3.0 Results

Density and age had no significant effect on either the vocalizations, flight attempts or the scores obtained in the human approach test (Tab. 1).

Table 1. Effects of the density, age and pigmentation on the behaviour shown in the separation and human approach test.

	Density		Age		Pigmented/White	
Variable	$F_{2,6}$	P	$F_{1,43}$	P	$F_{1,43}$	P
Vocalization 1	0.21	ns	0.38	ns	0.56	ns
Vocalization 2	0.68	ns	0.11	ns	0.28	ns
Vocalization 2-1	0.27	ns	0.15	ns	0.07	ns
Flight attempt 1	1.18	ns	0.02	ns	0.64	ns
Flight attempt 2	1.42	ns	0.01	ns	0.21	ns
Flight attempt 2-1	0.32	ns	0.06	ns	2.43	ns
Human appr. 1	0.61	ns	1.91	ns	4.99	<0.05
Human appr. 2	0.74	ns	2.88	ns	10.31	<0.005
Human appr. 2-1	2.76	ns	0.00	ns	0.23	ns

3.1. Separation test

Pigmentation had no effect on the vocalizations and flight attempts (Tab. 1).

3.1.1. Vocalization

Number of vocalizations was lower in the first test, 410 times against 510 in the second separation test, and there was a significant difference between the two tests ($P < 0.05$). In the

second test vocalization was highest in 2 m^2 with an average of 11.1 ± 3.0 (Fig 2). The difference between the first and second test is presented in figure 5. It shows the significant difference in frequency of vocalization between the two separation tests. The frequency distribution shows that 66.7 % (36 individuals) of the goats vocalized between 0 to 5 times during the first separation test (Fig. 3). The rest of the frequency distribution was evenly distributed. The frequency distribution of the second test shows a similar distribution as in the first separation test. Thirty one (57.4 %) goats vocalized between 0 to 5 times during the second separation test (Fig. 4). The rest of the frequency distribution was evenly distributed.

The calculated difference in vocalization between first and second test was not significantly affected by density, age or pigmentation (Tab. 1).

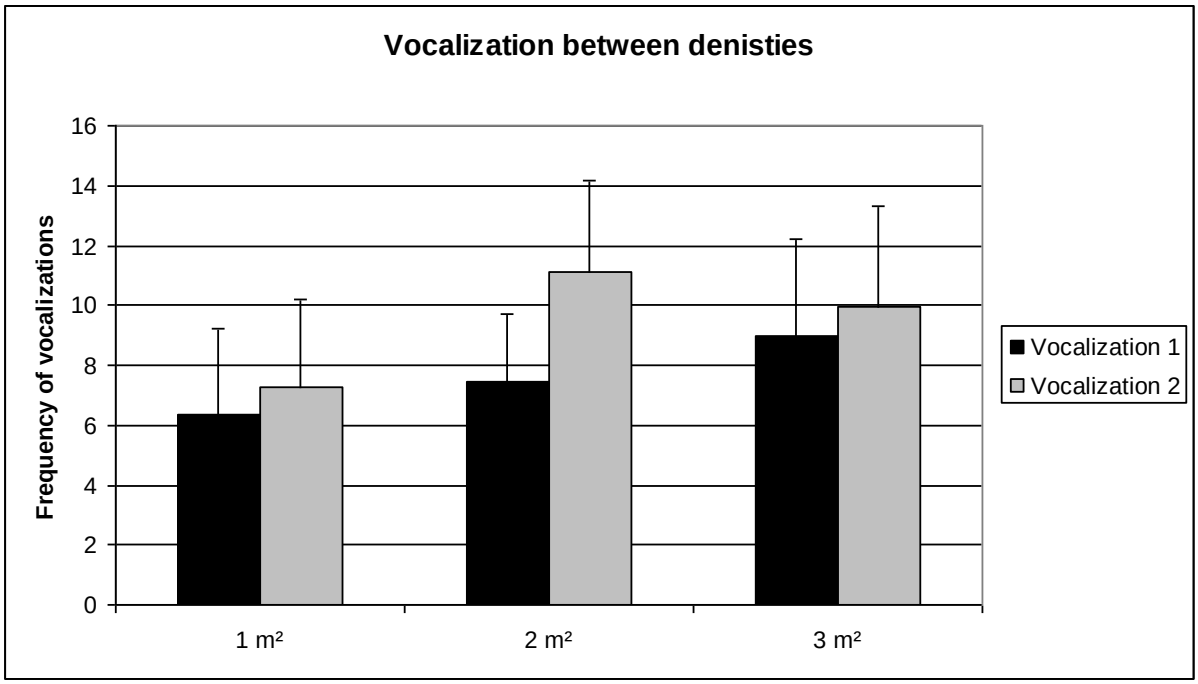

Figure 2. Frequency of vocalizations in different densities.

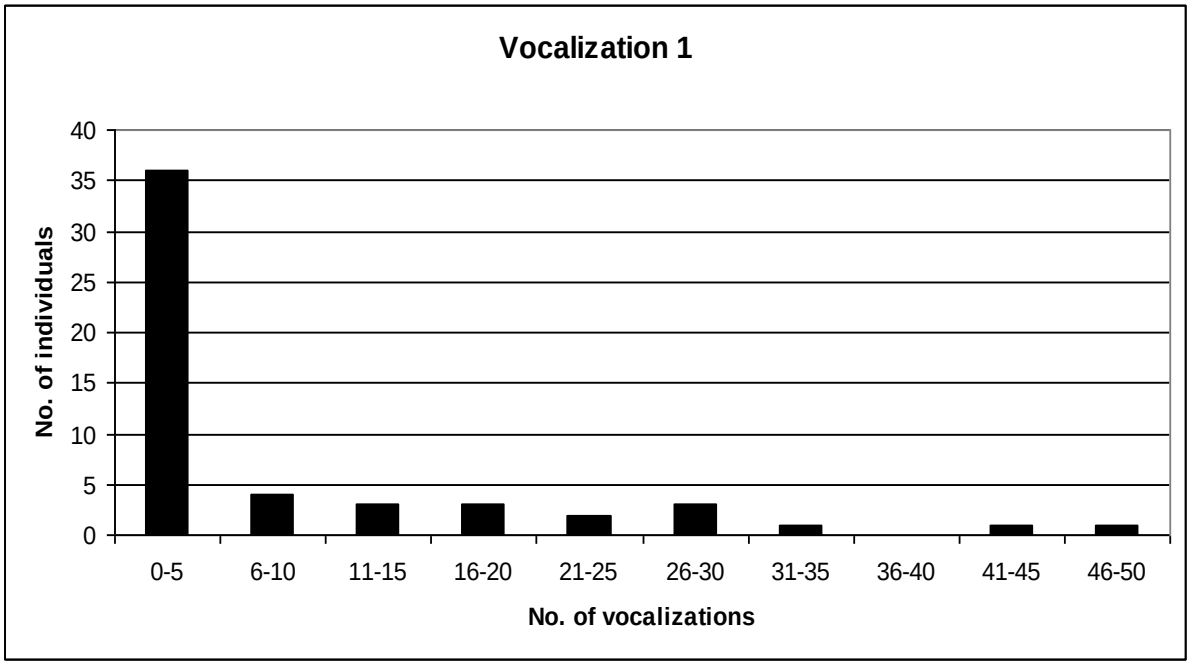

Figure 3. Frequency distribution of the vocalizations during the first separation test.

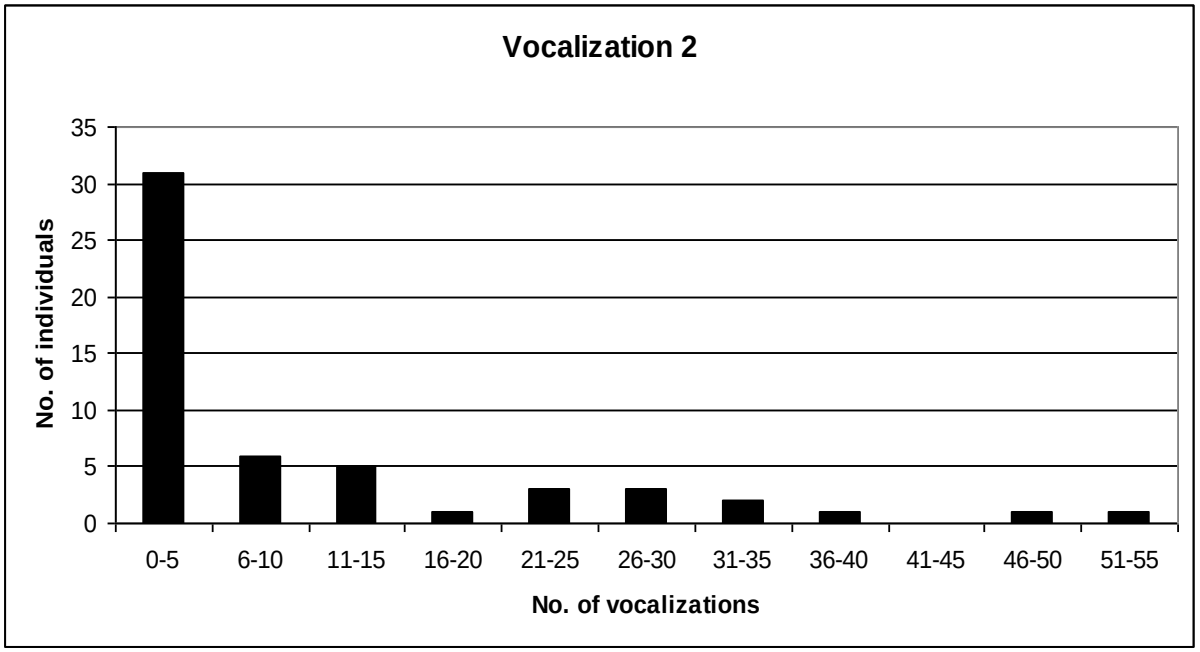

Figure 4. Frequency distribution of the vocalizations during the second separation test.

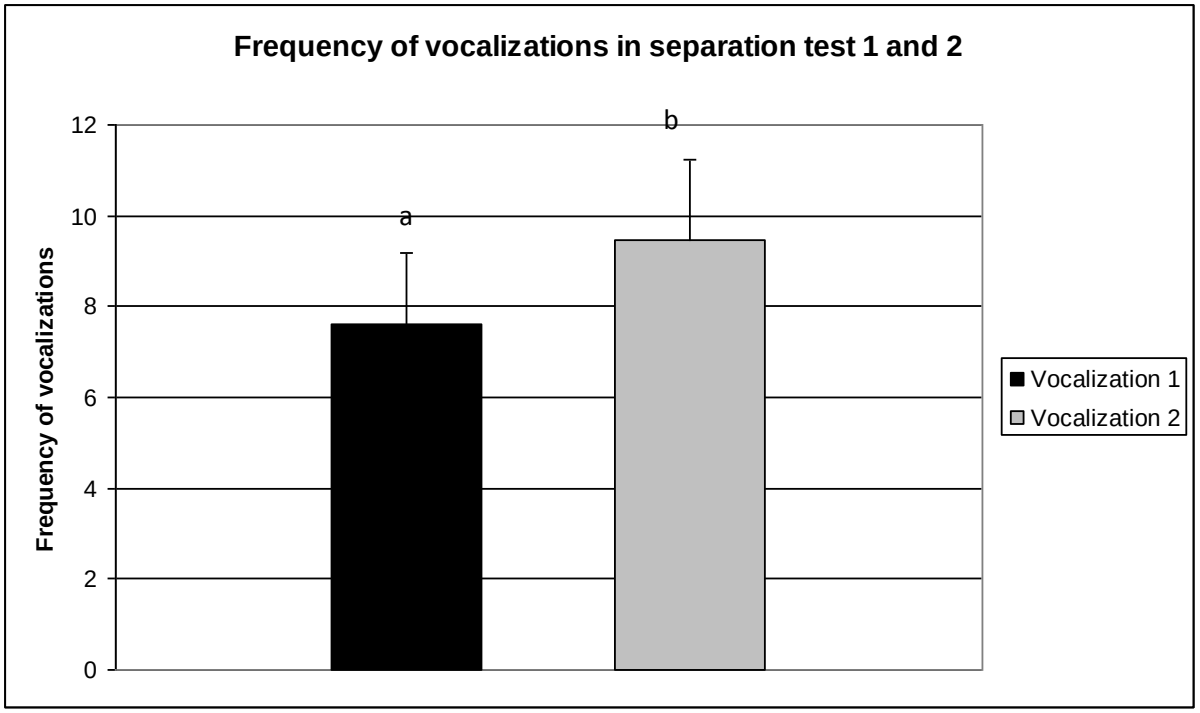

Figure 5. The difference in vocalisations between first and second separation test. The different letters show the significant difference.

3.1.2. Flight attempts

Number of flight attempts was higher in the first test, 90 times against 44 times in the second test. There was a significant difference between the two tests ($P < 0.001$). The significant difference in frequency of flight attempts in the first and second test is presented in figure 9. Between the densities, number of flight attempts was highest in 2 m^2 in the first test with an average number of 2.2 ± 0.4. The average number in 1 m^2 was 1.4 ± 0.3, and the same average number was found for 3 m^2 1.4 ± 0.2 (Fig 6). Also in the second test, 2 m^2 had the highest average number of flight attempts 1.6 ± 0.6 and 1 m^2 had the lowest average number with 0.2 ± 0.1. The frequency distribution shows that 43 goats (79.6 %) perform 0 to 2 flight attempts, and the peak is with one flight attempt with 18 (33.3 %) individuals (Fig. 7). The last 11 goats (20.4 %) perform between three to six flight attempts each. The frequency distribution of the second test has a different distribution from the first test. It has one peak with 40 goats (74.1 %) that performed zero flight attempts (Fig. 8). The remaining 14 goats (26.0 %) performed between 1 to 7 flight attempts.

The calculated difference in flight attempts between the first and second test were not significantly affected by density, age or pigmentation (Tab. 1).

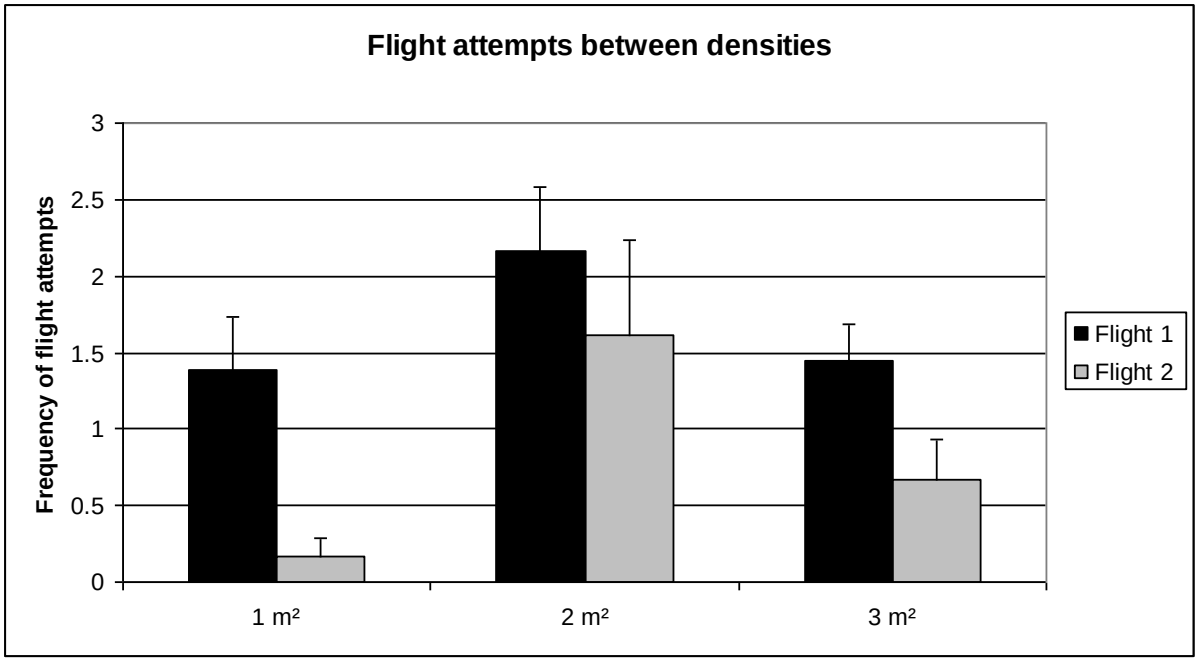

Figure 6. Frequencies of flight attempts between the densities.

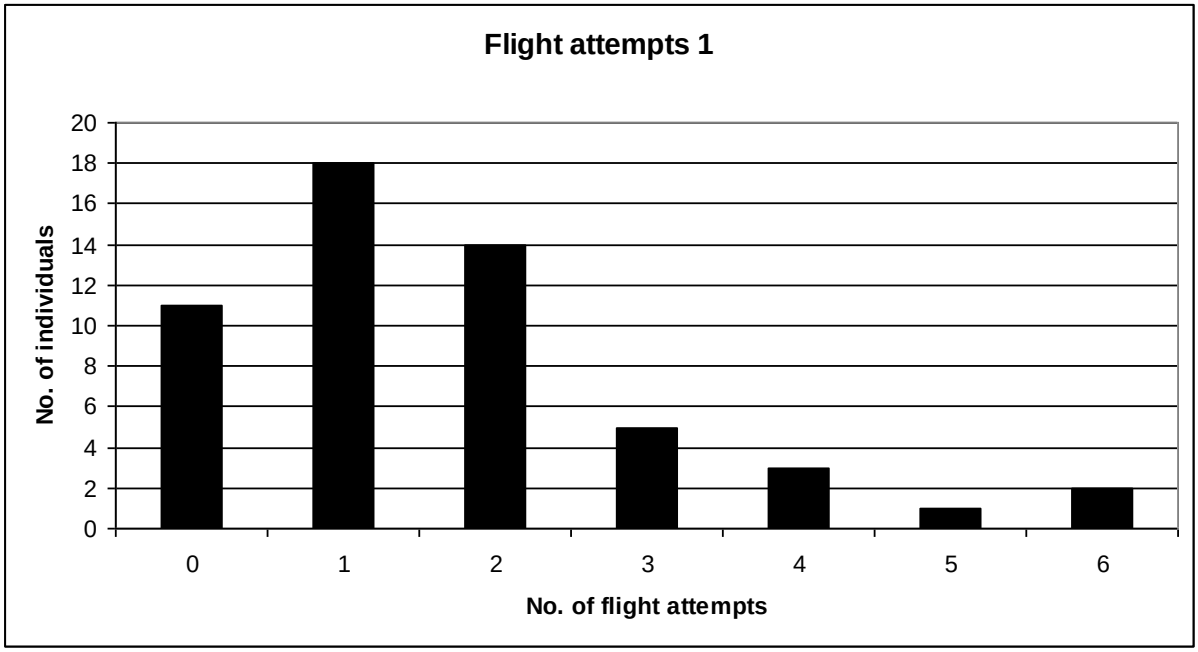

Figure 7. Frequency distribution of the flight attempts during the first human approach test.

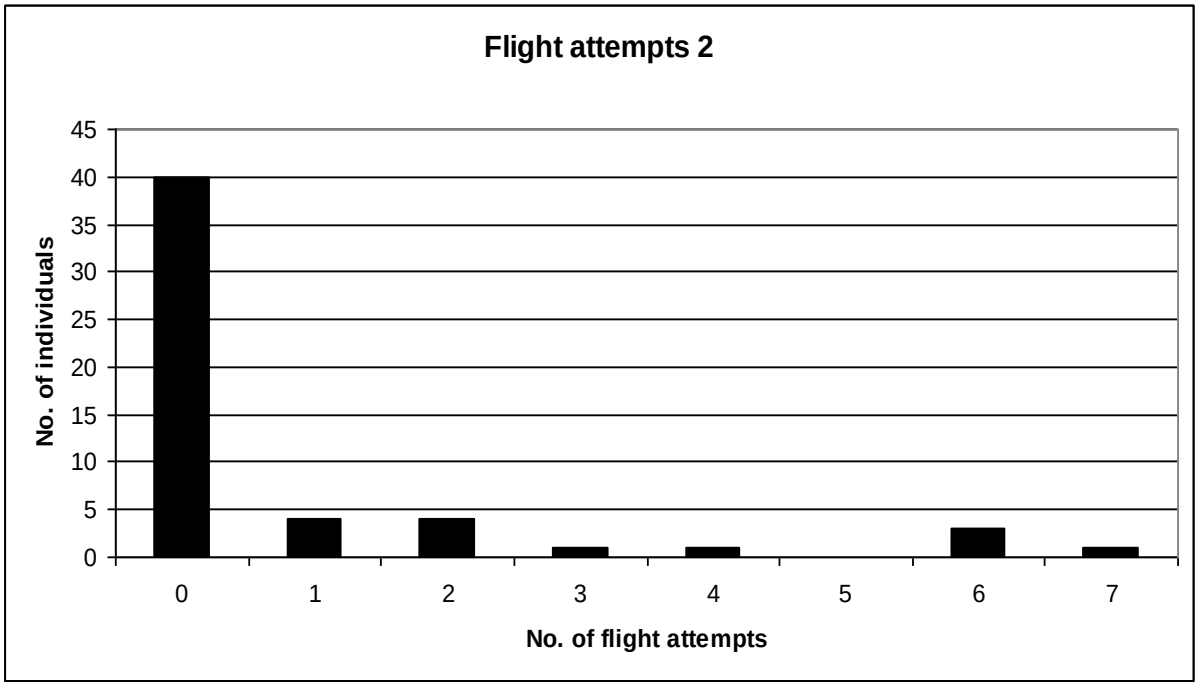

Figure 8. Frequency distribution of the flight attempts during the second human approach test.

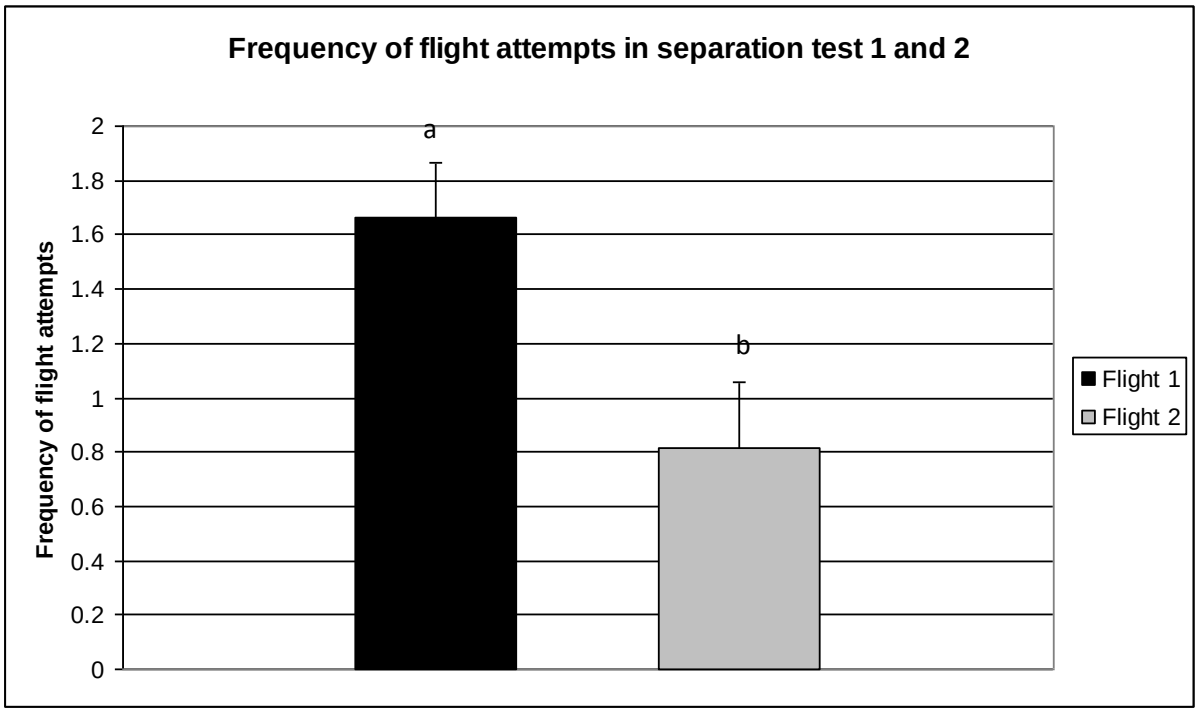

Figure 9. The difference in flight attempts between first and second separation test.

3.2 Human approach test

3.2.1. Human approach test 1

The confidence score obtained in total in the first human approach test was 72.5 and the average score was 1.34 (Fig 10). The score in the human approach test is and average of three scores; stationary human, approaching human and crouching human. The frequency distribution shows that 35 (64.8 %) of the goats did not approach nor avoided the stationary observer, and six (11.1 %) goats approached the stationary observer and made physical contact (Fig. 11). When approached, 26 (48.1 %) goats fled before the observer could pet them, and 16 (29.6 %) could be petted when approached. When the observed crouched down 13 (24.1 %) goats fled away, while 19 (35.2 %) approached the observer and made physical contact with the observer. The goats reactivity shown in the first human approach test was significantly affected by their pigmentation (Tab. 1; Fig 12). The pigmented goats had a higher score in the first human approach tests and were seeking contact more often with humans during the test. Number of pigmented goats was 19 and 35 white goats.

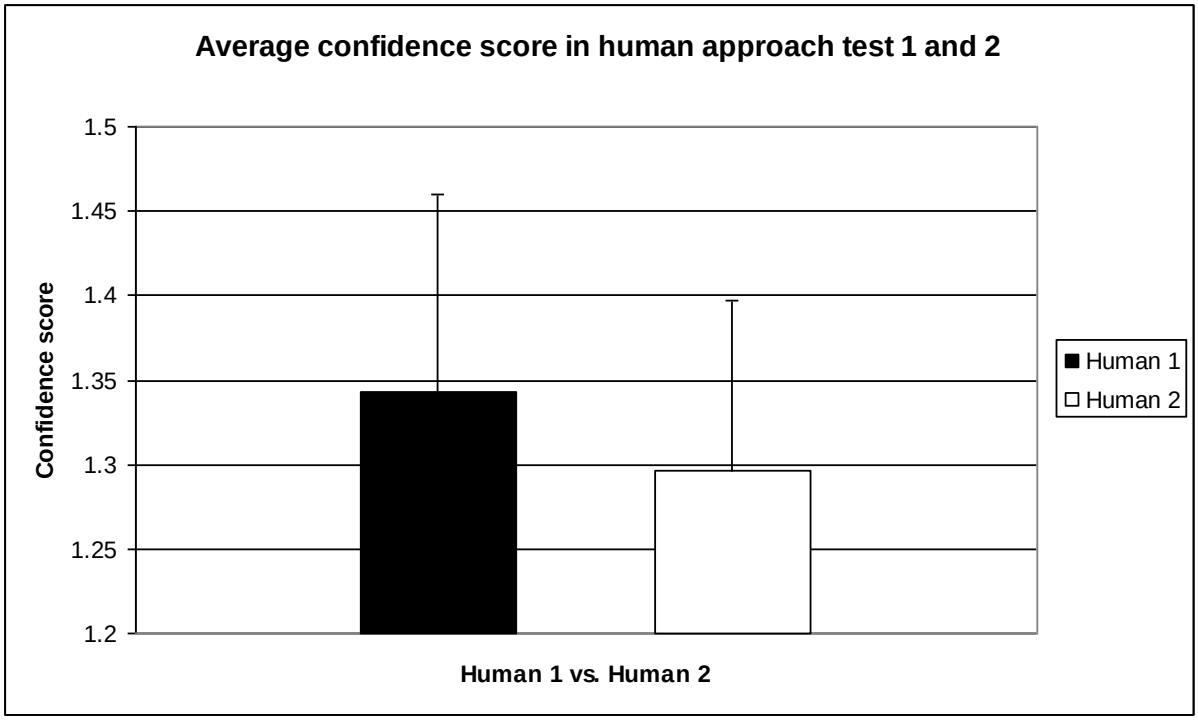

Figure 10. Average confidence score from human approach test 1 and human approach test 2.

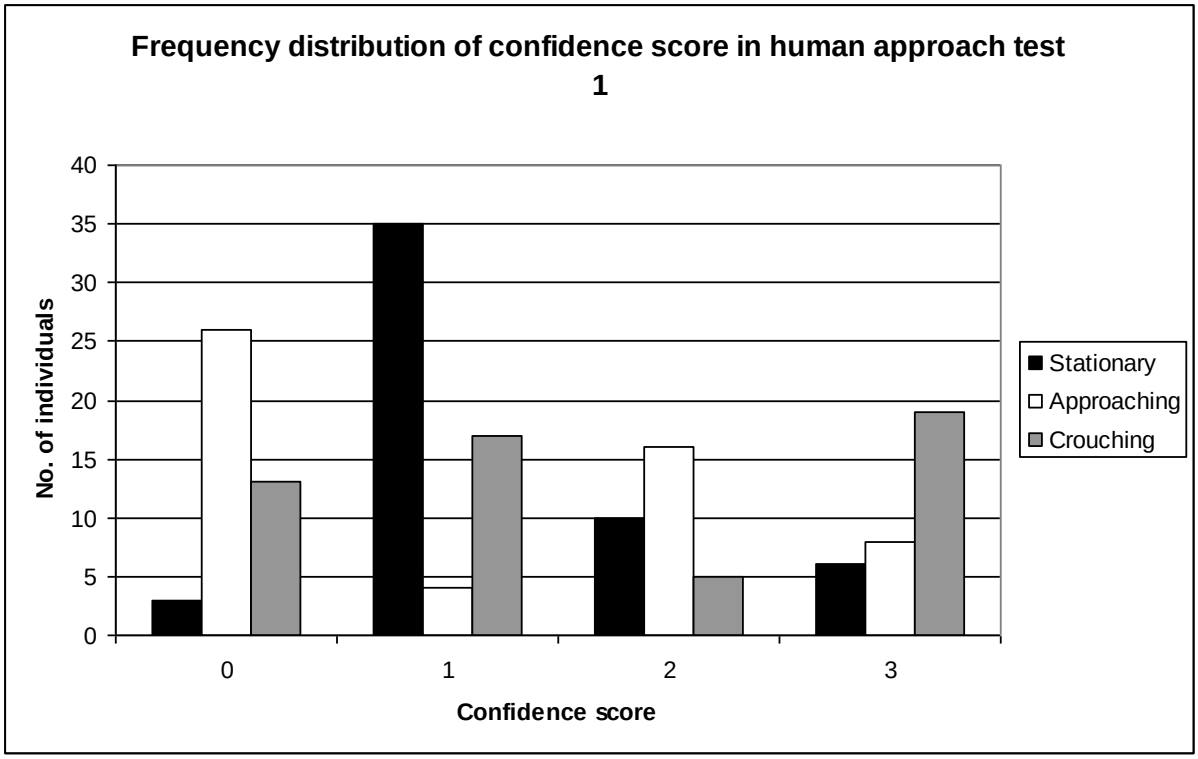

Figure 11. Frequency distribution of the different confident scores obtained in human approach test 1.

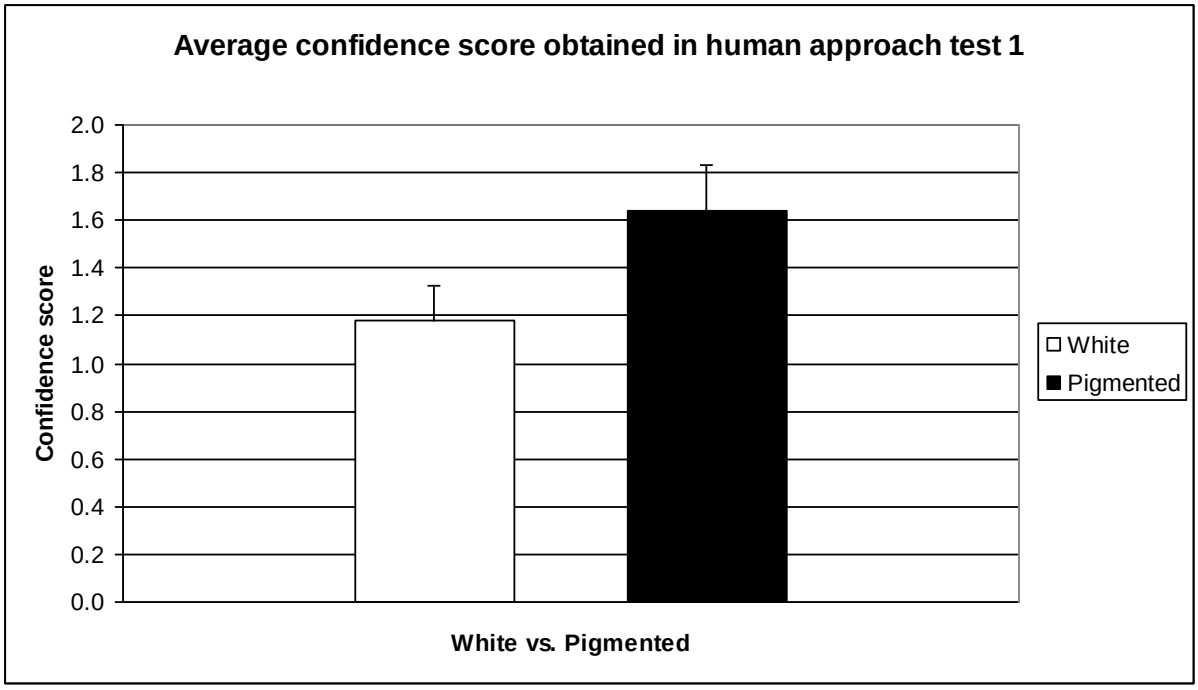

Figure 12. Behaviour shown by white and pigmented animals in the first human approach test.

3.2.2. Human approach test 2

The sum of the confidence scores obtained in human approach test 2 was 70.0, and the average score was 1.30 (Fig. 10). There was no significant difference between the two human approach tests. The frequency distribution shows that 38 (70.4 %) goats did not approach nor avoided the stationary observer, and only three (5.6 %) goats approached the stationary observer and made physical contact (Fig. 13). When the goats were approached, 25 (46.3 %) of the goats fled, while five (9.3 %) approached the observer and were able to be petted before the observer arrived the goat. Thirty (55.6 %) goats did not approach nor avoided the crouching observer, while 14 (26.0 %) approached the observer and made physical contact. The goats' reactivity in the second human approach test was significantly affected by their pigmentation (Tab 1; Fig. 14). As in the first test the pigmented goats had a higher confidence score than the white ones. The pigmented goats were more often seeking contact with the observer.

The difference between the first and second human approach test were not affected by density, age or colour (Tab. 1).

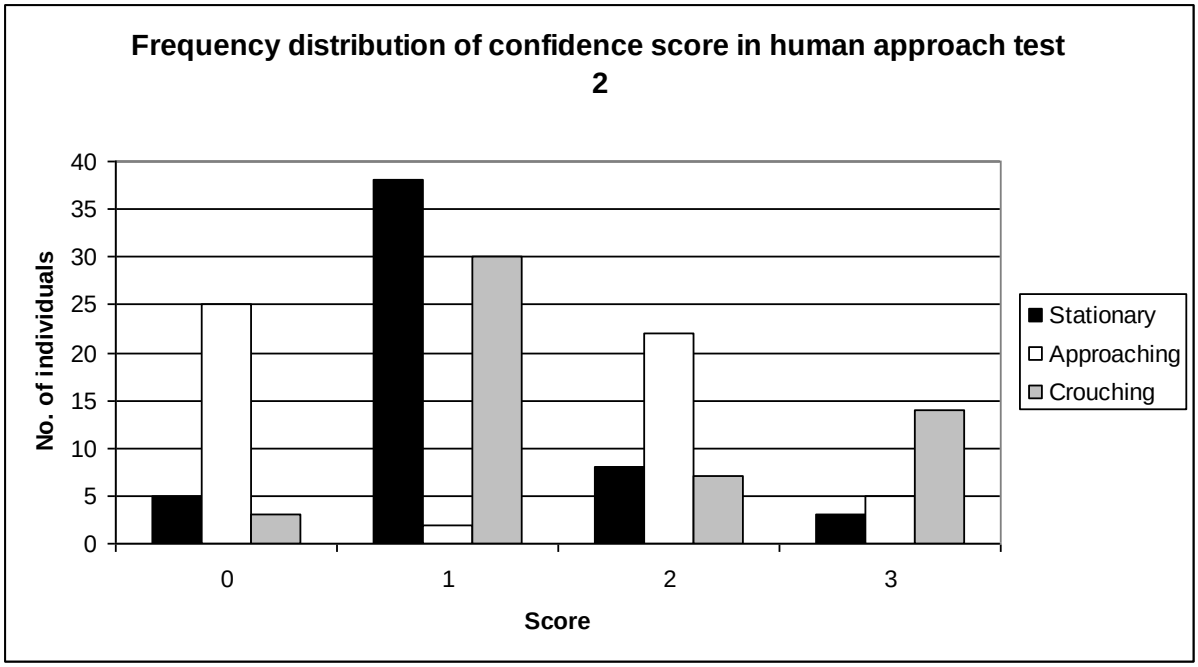

Figure 13. Frequency distribution of the different confident scores obtained in human approach test 2.

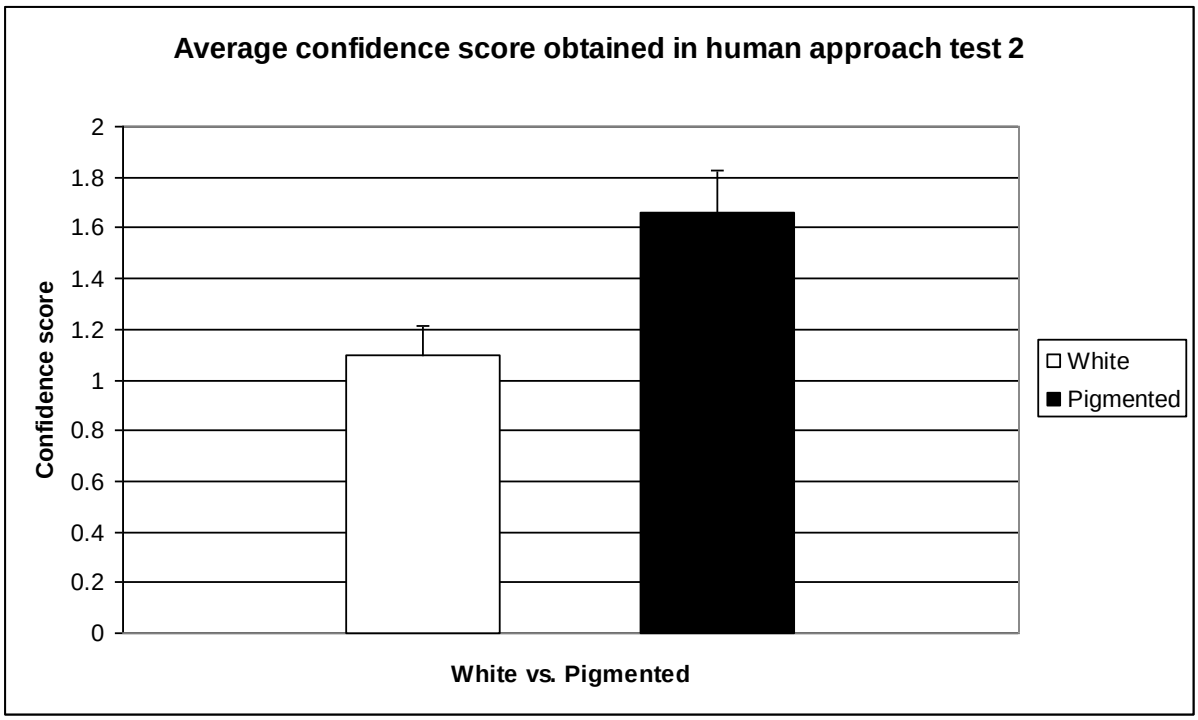

Figure 14. Behaviour shown by white and pigmented animals in the second human approach test.

3.3 Social interactions

3.3.1 Social behaviour shown in the stable social group?

The density and age had a significant effect on the number of offensive behaviour initiated (Tab. 2). Goats in the highest density (1 m^2) initiated significantly more offensive initiated behaviour than the goats in lower density (3 m^2). On average it was initiated 243.5 (43.86 %) interactions in the high density groups, while in the other two densities there were 169.7 (2 m^2; 30.6 %) and 141.9 (3 m^2; 25.6 %). It was a significant difference between 1 m^2 and 3 m^2 ($P < 0.05$; Fig 15), and the frequency of offensive behaviour was highest in 1 m^2 (Table 2 shows an overview over the results given from the generalized mixed model). The results showed that group as random effect had a significant effect on the offensive initiated behaviours ($P < 0.05$).

Number of offensive received behaviour was significantly affected by density and age (Tab. 2). There was a significant difference between 1 m^2 and 2 m^2 ($P < 0.05$; Fig 16), and between 1 m^2 and 3 m^2 ($P < 0.05$; Fig. 16). As expected from the results in offensive initiated, there is a higher frequency of offensive received in the highest density. In the highest density (1 m^2) there were initiated 204.9 (48.2 %) offensive received interactions, in comparison to density 2 m^2 where the average number was 122.5 (28.7 %) and 99.3 (23.3 %) in density 3 m^2. As in offensive initiated, there was a significant effect of group as random effect ($P < 0.05$).

None of the other variables were affected by the density, but there was an effect of age. Seventeen of the goats were two years old; 32 were three years old; four goats were four years old and one goat was five years old. (The age distribution within offensive initiated, defensive initiated and positive initiated are shown in fig. 17; 18; 19). The age frequency of offensive initiated and defensive initiated will at some extent reflect each other. In offensive initiated behaviours one goat in three years had a high frequency of interactions (545 interactions). In defensive initiated behaviours one goat initiated 209 interactions with other group members. Within positive initiated behaviours one goat initiated 89 interactions with other group members. Some variables had a significant effect of group as a random effect: defensive initiated behaviours (group 1.1; ($P < 0.05$); group 2.1; ($P < 0.05$)), defensive received behaviours (group 1.1; ($P < 0.05$)), positive initiated behaviours (group 1.3; ($P < 0.05$); group 3.3; ($P < 0.05$)), positive received behaviours (group 1.3; ($P < 0.05$)) and flight attempts 2 (group 2.3 ($P < 0.05$)). SI, ACT and DOM were not significantly affected by density or age.

Table 2. Effect of density and age on different test variables.

	Density		Age	
Variable	$F_{2,6}$	P	$F_{1,44}$	P
Offensive Initiated	5.41	<0.05	109.64	<0.0001
Offensive Received	6.49	<0.05	126.70	<0.0001
Defensive Initiated	0.18	ns	109.53	<0.0001
Defensive Received	0.09	ns	73.53	<0.0001
Positive Initiated	0.14	ns	30.07	<0.0001
Positive Received	0.13	ns	4.21	<0.05
Vocalization 1 (Voc1)	0.07	ns	227.24	<0.0001
Vocalization 2 (Voc2)	0.57	ns	178.55	<0.0001
Flight 1	1.80	ns	7.97	<0.01
Flight 2	1.55	ns	4.55	<0.05
Human appr. 1 (H1)	0.26	ns	0.37	ns
Human appr. 2 (H2)	0.25	ns	0.34	ns
Success index (SI)	0.01	ns	0.07	ns
Activity index (ACT)	0.00	ns	0.01	ns
Dominance rank (DOM)	0.81	ns	0.14	ns

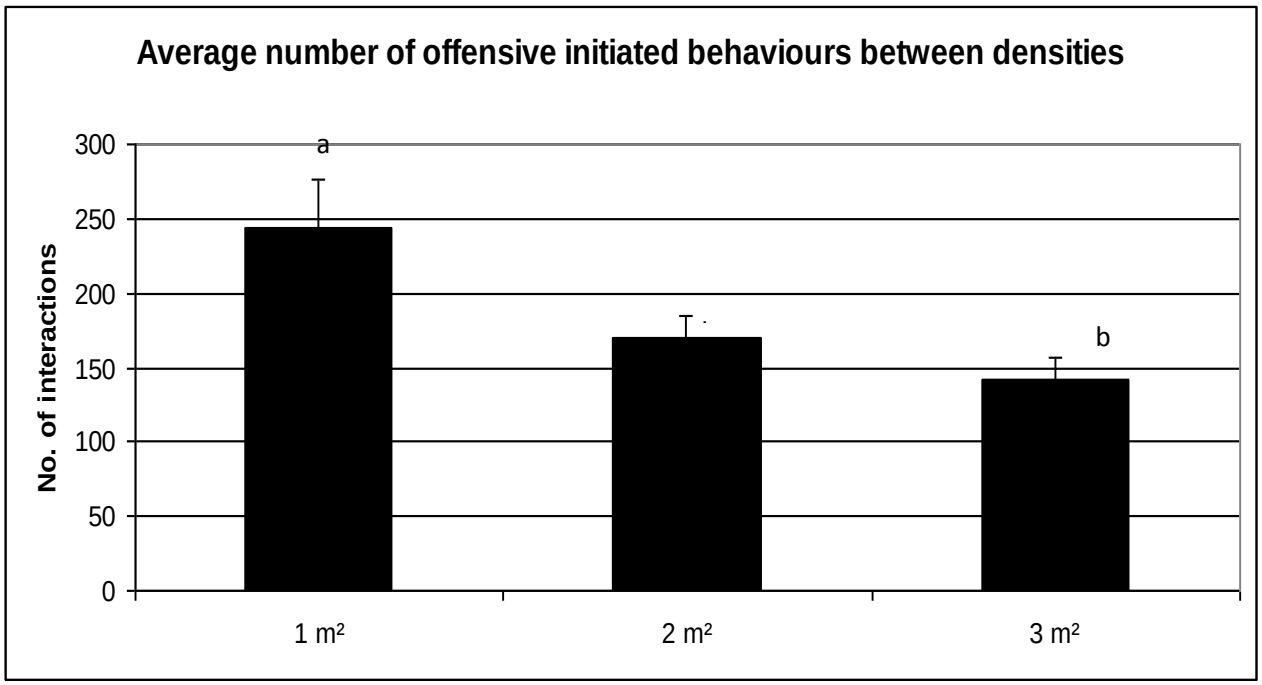

Figure 15. The difference between the densities regarding offensive initiated behaviours. The letters shows the significant difference.

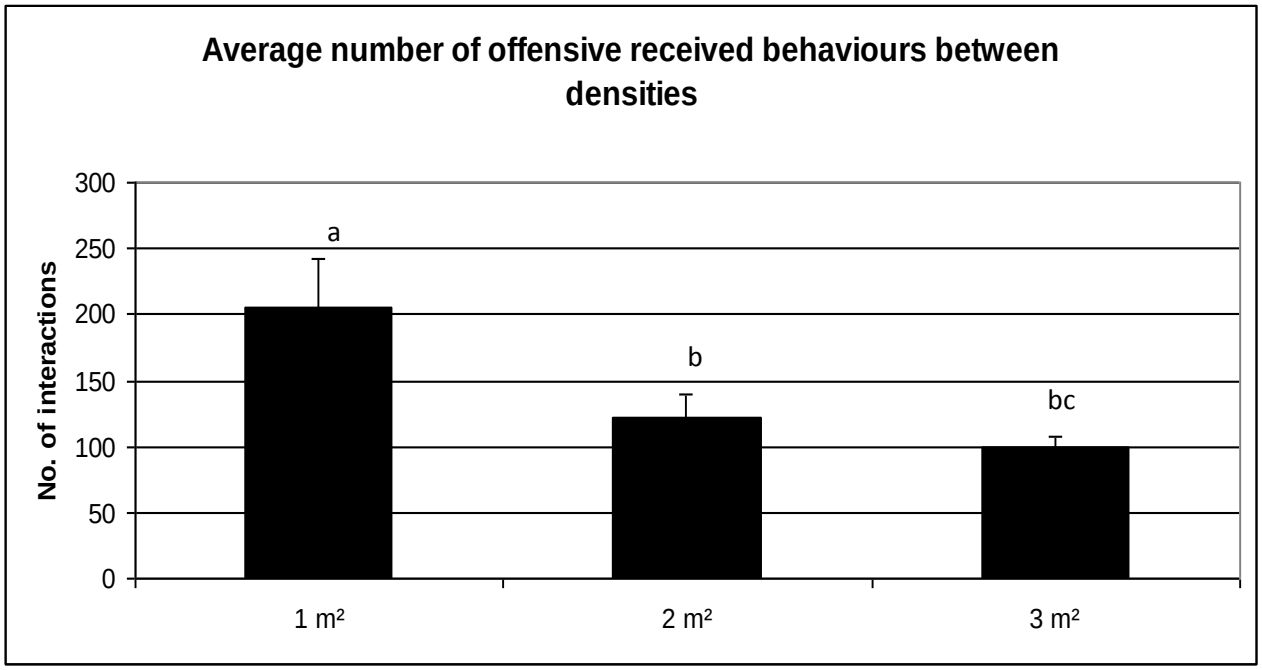

Figure 16. The difference between the densities regarding offensive received behaviours. The letters shows the significant difference.

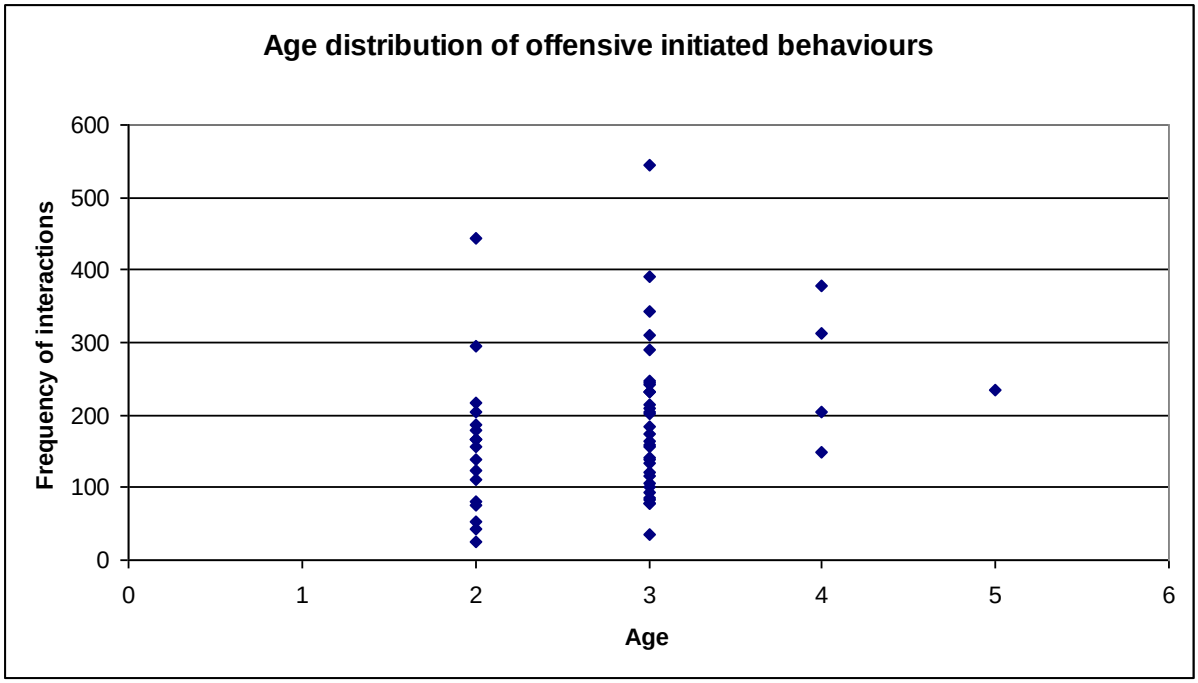

Figure 17. Age distribution of offensive initiated behaviours.

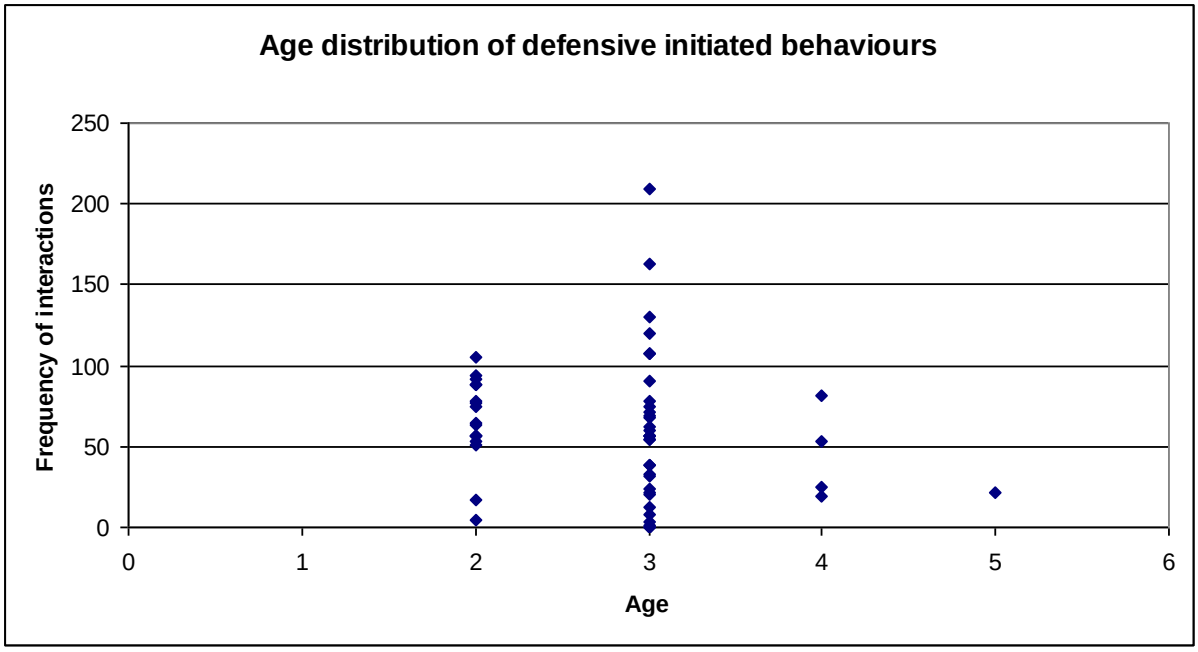

Figure 18. Age distribution of defensive initiated behaviours.

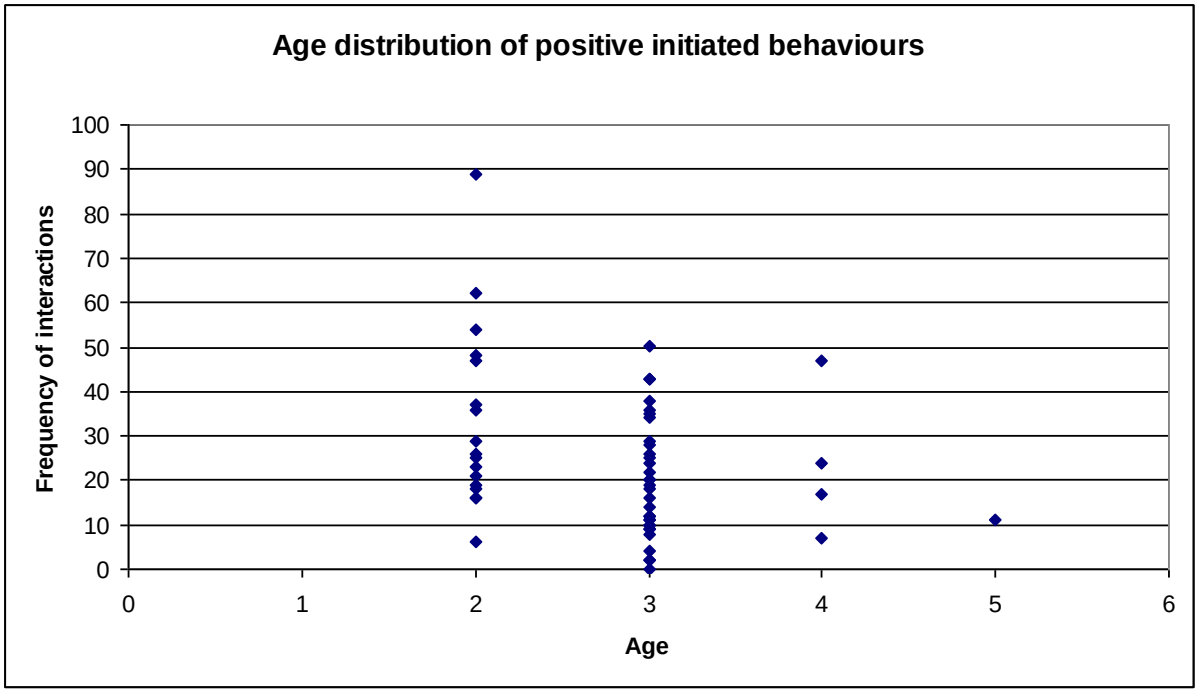

Figure 19. Age distribution of positive initiated behaviours.

3.3.2. Correlation between test variables.

Overall, the correlations between the variables from the different tests were low (Tab. 3). The offensive initiated behaviours had a positive correlation to the success index. Goats that performed offensive behaviours were more successful. The activity index was also positively correlated with offensive initiated behaviours. The ones that were offensive were also more active.

Defensive initiated behaviours had a moderate negatively correlation to offensive initiated behaviours. Goats that are exposed to offensive behaviours will show a defensive initiated behaviour. Defensive initiated behaviours are also highly negatively correlated to the success index. Goats that showed a defensive initiated behaviour had a low success.

There was a positive correlation between vocalization 1 and vocalization 2. Goats vocalizing in the first test were also vocalizing in the second test. Vocalization 1 is moderate correlated to the human approach test 1. The ones that vocalized had a higher average confidence score. This also applies for vocalization during the second separation test. Vocalization 2 is moderate correlated to human approach 1 and 2.

Flight attempts 1 and 2 had a moderate correlation in between them. Goats that fled in the second test had also tried to flee in the first separation test. Flight attempts in the second

separation test had a moderate correlation to the vocalization in first and second separation test. Goats that tried to flee were also the ones that vocalized more. There is an moderate correlation between flight attempts 2 and human approach 2. Goats that tried to escape in the second separation test were the ones with higher score in human approach 2.

The average confidence score in the two human approach test has a moderate correlation between them. Goats with higher score obtained higher score in the second test as well.

Success index and the activity index are moderately correlated. Dominance rank was not correlated to any of the different variables in the different tests.

Table 3. Overview over correlation between initiated social behaviour, fear response, confidence score and indices.

	Offensive ini.	Defensive ini.	Positive ini.	Vocal 1	Vocal 2	Flight 1	Flight 2	Human 1	Human 2	SI	ACT	DOM
Offensive ini.	1.00											
Defensive ini.	-0.40 0.00	1.00										
Positive ini.	-0.06 0.66	-0.01 0.93	1.00									
Vocal. 1	0.04 0.75	0.20 0.15	0.06 0.65	1.00								
Vocal. 2	0.01 0.93	0.17 0.21	0.16 0.24	0.69 <.0001	1.00							
Flight 1	-0.11 0.41	0.01 0.93	-0.01 0.93	0.24 0.08	0.12 0.37	1.00						
Flight 2	-0.04 0.80	-0.10 0.49	0.20 0.15	0.41 0.00	0.35 0.01	0.39 0.00	1.00					
Human 1	0.15 0.29	-0.04 0.75	0.12 0.40	0.34 0.01	0.36 0.01	-0.25 0.07	0.2 0.16	1.00				
Human 2	0.25 0.07	-0.10 0.45	0.26 0.06	0.27 0.05	0.39 0.00	-0.08 0.56	0.3 0.0	0.65 <.0001	1.00			
SI	0.58 <.0001	-0.59 <.0001	-0.17 0.23	-0.11 0.41	-0.08 0.55	-0.20 0.14	0.0 1.0	0.08 0.56	0.09 0.49	1.00		
ACT	0.67 <.0001	-0.13 0.34	0.12 0.37	0.07 0.60	0.13 0.35	-0.08 0.57	0.00 1.00	0.19 0.18	0.19 0.16	0.48 0.00	1.00	
DOM	-0.26 0.06	0.0950 0.49	-0.21 0.13	-0.08 0.59	-0.04 0.75	0.09 0.53	0.01 0.9	0.00 0.98	-0.05 0.71	-0.11 0.41	-0.12 0.37	1.00

4.0 Discussion

The separation test and human approach test are widely used in assessing fear in animals (Siebert et al., 2011; Price and Thos, 1980; Lyons, 1989; Boivin and Braastad, 1996).

We predicted higher levels of vocalization and flight attempts in the second separation test in the high density where vocalization and flight attempts was used as a measurement of fear. There were expected higher levels of fear in the high densities based on earlier studies that shows higher levels of offensive behaviours in high density groups (Al-Rawi and Craig, 1975; Kondo et al., 1989). Our findings could not support this prediction. The number of vocalizations was significantly higher in the second separation test, but had no effect of density. The increased number of vocalizations in the second test might be explained with establishment of groups and stable social rank order. Vocalization is important for the social establishment in a group as shown by Siebert et al. (2011) where partially isolated goats vocalized and reared against the walls more than completely isolated goats. This might indicate that separation from the rest of the group will cause fear and work as a stressor (Boissy and Le Neindre, 1997; Dwyer, 2009). Since goats are characteristically vocal animals they will have a low threshold for using vocalizations when visual contact is disrupted and by that try to keep in contact with group members. Social bonds are important for the animals, and they are willing to work for access to other conspecifics (Hovland, 2005).

Results from the flight attempts show a significant difference in the first and second separation test, where the level of flight attempts was higher in the first separation test. Price and Thos (1980) also found that goats reared less in the second test than in the first one. In the study by Siebert et al. (2011) they could not find any significant difference between the isolation sessions. An explanation for the decreased level of flight attempts in our study might due to their physical condition. When the second test was conducted it was close to parturition and their body weight had increased since the first separation test. Flight attempts in the second test would thereby cost more energy than in the first separation test.

To my knowledge there have been done little, if none studies on the effect of density has on fear in goats.

The confidence score in the human approach test was not significantly affected by density in contrast to the prediction. We expected lower confidence score in high densities in the second human approach test. Also, there was no significant difference in the confidence score between the two human approach tests, and there was a correlation between the first and second human approach test. This indicates that observer had no effect on the human approach test since the observers never approach the same goat twice. The correlation test also showed a correlation between the two human approach tests, which also indicates that the behaviours recorded in the two tests, did not differ widely. Boivin and Braastad (1996) found that goat kids who had an early experience with humans from an early age spent more time closer to humans later on. Lyons (1989) found similar results; human-reared kids stayed in closer proximity to observer during encounter test and had a lower latency time than dam-reared goat kids. The goats in our experiment are used to handling and contact with humans on a daily basis, and this might play a role in our results. The results might have been different if the goats were not used to handling and human contact. There was a moderate correlation between the separation test and the human approach test, and can indicate a stability in the goats temperament. Lyons et al. (1988) found that human-reared goats had low levels of fear in comparison to dam-reared goats, while the opposite was found for dam-reared goats. The dam-reared goats' behaviour changed over time to resemble the human-reared goats' behaviour, but the individual differences stayed stable through the study.

We discovered that pigmentation in the goats played a significant role towards the confidence score. The pigmented goats were less fearful towards the observer and had a higher confidence score than white goats. Ducrest et al. (2008) found that darker wild vertebrates were more aggressive and more resistant to stress. This is not corresponding with our findings, but our results should be taken with some consideration. Most of the goats in the study were white (35 goats) and the rest (19 goats) were pigmented. The number of pigmented goats is low, which might influence the reliability of the results.

As predicted, the results in this study showed a higher level of offensive initiated behaviours in the high level density than in lower densities. This is consistent with results found in earlier studies (Bøe et al. 2006; Al-Rawi and Craig, 1975; Jørgensen et al., 2007). There will be less space for eating, resting and to move freely around when space per animal is reduced, which in turn will lead to competition to gain access to the most attractive feeding places and resting places. When resources are limited, goats will compete more intensely to obtain access (Barroso et al., 2000). Animals that are faced with a competitor will flee or fight, but with a

reduced space per animal there will be no place to flee. They will have to face their competitor and the number of social interactions will increase as a result of this. Competition is often associated with aggression and will be more pronounced in higher densities (Archer, 1988). In a competitive environment, as in a farm production environment, one goat can monopolize feeding places and resting places to their own benefit (Jørgensen et al., 2007). During observations in the goat house we observed goats lying in front of the feeding place, and thereby blocking other goats from the feeding place. Dominance is reinforced by the use of more aggression towards the other group members. During the observations we observed that goats mediate between two goats that were aggressive towards each other. The intruding goat would normally stand between the two aggressive goats, or lay its head on one of the aggressive goats back. It could also touch one of the aggressive goats with its forehead, almost like a push, but not so forceful. This social behaviour was not recorded in this study, but Andersen et al. (2011) and Miranda-de la Lama et al. (2011) found results that support our personal observations.

All social interaction in our study was affected by age, and most of them strongly affected. This will most likely have to do with the social structure in the group, as proven by Barroso et al. (2000) and stated by Dwyer (2009); age and body weight had a strong influence on the social rank in the group. The oldest and heaviest goats were the most dominant ones in the social structure of the group.

There is no correlation between the separation test, human approach test and the social behaviour in the groups. This can indicate that the human approach test is interspecific and the animals exposed to the test might habituate to the situation. One animal's individual character might influence the results in a test like human approach test and separation test (Forkman, 2007). Flight attempts in the separation test might be attempts to investigate and an attempt to achieve visual contact with its group members. The social behaviour was measured within the group and without any object of novelty.

There would be no effect of the success index, activity index or the dominance rank on the density because it was calculated within a group. The success index and activity index were positively correlated and showed that higher activity would have a positive impact on the success within the group. The success index and activity index were both positively correlated with the offensive initiated interactions and implies that goats with a high activity are more offensive towards other goats in the group and by that has a higher success.

The goats in our study are accustomed to handling and being housed large parts of the year because of climate reasons. The results might have been different by using feral goats that is not accustomed to handling or to be housed with quite limited space. Our goats are also familiar with each other from earlier housing and time spent out on the pasture. There might be differences between species in goats when it comes to social structure and behavioural traits. While looking at studies in goats, one have to have in mind the variety of managements, form free ranging to intensive managements, in goat production.

5.0 Conclusion

The results showed that there was no effect of density on fear responses as vocalization, flight attempts, or the confidence score obtained in the human approach test. There was a difference between the first and second separation test for both vocalization and flight attempts. Vocalization increased in the second test, while the flight attempts decreased. It will be difficult to make any conclusion about the level of fear in these tests since they go in different directions.

The density had an effect on the number of offensive behaviour initiated and received. These results give us a clear indication that high densities lead to higher aggression among goats.

6.0 References

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