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REGROUPING OF DAIRY GOATS IN LOOSE HOUSING

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Table of contents

1	Summary	1
2	Zusammenfassung	4
3	General introduction	8
4	The introduction of individual goats into small established groups has serious negative effects on the introduced goat but not on resident goats	16
	Based on: Patt et al. (2012) <i>Applied Animal Behaviour Science</i> 138, 47-59	
5	Behavioural and physiological reactions of goats confronted with an unfamiliar group either when alone or with two peers	50
	Based on: Patt et al. (2013) <i>Applied Animal Behaviour Science</i> 146, 56-65	
6	Factors influencing the welfare of goats in small established groups during the separation and reintegration of individuals	77
	Based on: Patt et al. (2013) <i>Applied Animal Behaviour Science</i> 144, 63-72	
7	General discussion	100
8	References	118
9	Acknowledgements	130
10	Curriculum vitae	131

1 Summary

In goat husbandry, several management procedures are associated with the regrouping of the animals. One of these is the introduction of unfamiliar goats into established groups, usually performed in order to restock the herd or increase its size. Another procedure consists of temporarily separating individual goats, e.g. during kidding or because of injury, and subsequently reintegrating them into their original groups. It has been noted that regrouping can be associated with negative welfare effects in goats, exhibited in intense agonistic interactions and reduced feeding times. Despite this, there is a lack of systematic research into the consequences of regrouping, especially under intensive housing conditions and distinguishing between the newly introduced/reintegrated goats and the pre-existing group members. Furthermore, because of differences in social behaviour between horned and hornless goats, horn status needs to be taken into account when assessing the effects of these management procedures. The aim of the present thesis was to assess effects associated with the regrouping of horned and hornless goats in loose housing under various conditions, and to make recommendations for the improvement of these management procedures in terms of the goats' welfare.

The first study (chapter 4) aimed to quantify the effect of introducing an unfamiliar animal into an established herd. To assess this situation, eight horned and eight hornless goats were introduced one at a time into established groups of six goats (experimental groups) over a five-day period. Two of the four experimental groups consisted of horned goats, the other two of hornless goats. Individual goats were always introduced into groups with the same (i.e. their own) horn status (four introductions per experimental group). Before and during the introduction period, data on social interactions, lying and feeding behaviour and concentrations of faecal cortisol metabolites were measured in both the introduced goat and the focal group members. Data concerning the location of the introduced goat within the pen were also recorded. During the entire introduction period, substantially

longer lying times, shorter feeding times and elevated concentrations of faecal cortisol metabolites were recorded for the introduced goats, which were also on the receiving end of a considerable number of agonistic interactions on the first day of the introduction period. These changes were more pronounced in horned than in hornless goats, and long lying times in niches were recorded for newly introduced horned goats in particular. Thus, the results showed that the welfare of goats introduced individually into small established groups was adversely affected for at least five days after introduction. By contrast, levels of welfare indicators for resident goats remained largely the same, indicating that they were not negatively affected by the introduction of an individual goat.

Because effects were more pronounced in horned than in hornless goats in the first study (chapter 4), the following studies (chapter 5, 6) specifically investigated regrouping in horned goats. The main objective of the second study (chapter 5) was to test whether the presence of familiar conspecifics reduced negative effects during social confrontations with unfamiliar conspecifics. Twelve goats (called confrontees) were confronted both alone and in the company of two familiar conspecifics with four unfamiliar established groups each consisting of six goats (24 confrontations in total). Confrontations were conducted in a neutral environment and each confrontation lasted one hour. Social interactions, level of activity and faecal cortisol metabolites concentrations were quantified in the confrontees and unfamiliar focal goats throughout the confrontations. The number of agonistic interactions experienced by confrontees was significantly lower when familiar conspecifics were present than when they were on their own. It may therefore be concluded that the presence of familiar conspecifics can mitigate the adverse effects associated with a social confrontation with unfamiliar goats.

The third study (chapter 6) investigated the effects associated with the separation and reintegration of individual goats with the objective to determine whether an increased level of contact with the original group could reduce the negative effects of both separation and reintegration for the goat that was separated as well as for the remaining group members. The effects of separation and reintegration were

tested by individually separating twelve goats from four experimental groups that consisted of seven goats. Each goat experienced two different treatments (24 separations in total). One treatment only allowed for acoustic contact with the group, whilst the other treatment also permitted visual and tactile contact. After the two-day separation period the goats were reintegrated into their groups and data were collected for a further three days (reintegration period). Social interactions, length of lying and feeding times and faecal cortisol metabolites concentrations were recorded before and throughout the separation and reintegration period in both the separated goats and focal group members. The results showed that both separation and reintegration adversely affected the welfare of the separated goat, which expressed shorter feeding times throughout the separation period as well as higher faecal cortisol metabolites concentrations during both periods, separation and reintegration. Increased contact mitigated these adverse effects, however: where both visual and tactile contact were possible, lying times were not lower during the separation period and faecal cortisol metabolite levels were generally lower than when only acoustic contact was allowed. Since effects were more pronounced and lasted longer during the separation than the reintegration period, it was concluded that the separation was more aversive than the subsequent reintegration. Behaviour and the level of faecal cortisol metabolites hardly changed in the remaining goats of the experimental groups, indicating that they were only slightly affected by the temporary separation and subsequent reintegration of an individual herd member. Taken together, the results of this thesis show that both the introduction of unfamiliar individuals into established groups and the temporary separation of individuals from the rest of the group should be avoided whenever possible in goat husbandry. If an introduction is unavoidable, it is advisable to introduce several familiar goats simultaneously. During separation, acoustic, visual and tactile contact with the remaining group members should be permitted in order to mitigate adverse effects on the welfare of the separated individual.

2 Zusammenfassung

In der Ziegenhaltung sind zahlreiche Managementmassnahmen mit der Umgruppierung von Ziegen verbunden. Zu diesen Massnahmen gehört unter anderem die Eingliederung unbekannter Ziegen in bereits bestehende Gruppen, um diese zu remontieren oder zu vergrössern. Zudem werden Ziegen beispielsweise zur Geburt oder aufgrund von Verletzungen, vorübergehend einzeln von der Gruppe separiert, um anschliessend wieder in ihre ursprüngliche Gruppe eingegliedert zu werden. Ein hohes Mass agonistischer Interaktionen und reduzierte Fresszeiten weisen darauf hin, dass sich Umgruppieren negativ auf das Wohlbefinden der Ziegen auswirkt.

Systematische Untersuchungen über die Auswirkungen des Umgruppierens unter intensiven Haltungsbedingungen fehlen jedoch nahezu völlig. Es mangelt insbesondere an Studien, die sowohl die Auswirkungen für die eingegliederten/wiedereingegliederten Ziegen als auch für die Gruppenmitglieder der Gruppe, in die die Ziege eingegliedert wurde, belegen. Da sich das Sozialverhalten von behornten und hornlosen Ziegen unterscheidet, muss der Hornstatus bei der Beurteilung der Auswirkungen dieser Managementmassnahmen berücksichtigt werden. Die vorliegende Arbeit verfolgte das Ziel, sowohl die Auswirkungen, die im Zusammenhang mit der Umgruppierung von behornten und hornlosen Ziegen in der Gruppenhaltung auftreten, zu beurteilen, als auch Möglichkeiten zu finden, wie diese Managementmassnahmen in Bezug auf das Wohlbefinden von Ziegen verbessert werden können.

In der ersten Studie (Kapitel 4) sollte quantifiziert werden, welche Auswirkungen die Eingliederung einer unbekanntes Ziege in eine bereits bestehende Gruppe hat. Um diese Situation beurteilen zu können, wurden acht behornste und acht hornlose Ziegen jeweils einzeln für fünf Tage in bestehende Gruppen (Versuchsgruppen), die aus je sechs Ziegen bestanden, eingegliedert. Zwei der vier Versuchsgruppen bestanden aus behornten Ziegen, die anderen beiden aus hornlosen Ziegen. Die Einzelziegen wurden jeweils in Gruppen mit dem gleichen (d.h. ihrem eigenen)

Hornstatus eingegliedert (vier Eingliederungen pro Versuchsgruppe). Vor und während der fünftägigen Eingliederungsphase wurden soziale Interaktionen, Liege- und Fressverhalten und Konzentrationen von Cortisolmetaboliten im Kot sowohl für die eingegliederte Ziege als auch für die Fokustiere in den Versuchsgruppen erhoben. Ausserdem wurde während der Eingliederungsphase die Lokalisation der eingegliederten Ziege innerhalb der Bucht der Versuchsgruppe erfasst. Während der gesamten Eingliederungsphase lagen die eingegliederten Ziegen deutlich länger. Ihre Fressdauer reduzierte sich stark und die Konzentrationen von Cortisolmetaboliten waren erhöht. Zudem waren die eingegliederten Ziegen am ersten Tag der Eingliederungsphase das Ziel zahlreicher agonistischer Interaktionen. Im Vergleich zu hornlosen Ziegen waren diese Auswirkungen bei behornen Ziegen deutlich ausgeprägter und auch eine lange Aufenthaltsdauer in Liegenischen wurde insbesondere für behornete Ziegen beobachtet. Die Ergebnisse zeigten, dass einzeln in bestehende Gruppen eingegliederte Ziegen für mindestens fünf Tage belastet sind. Im Gegensatz dazu scheinen die Ziegen der etablierten Gruppen durch die Eingliederung einer einzelnen Ziege nicht belastet zu sein, da sich die gemessenen Variablen in Folge der Eingliederung kaum veränderten. Da sich eine Eingliederung (Kapitel 4) stärker auf behornete als auf hornlose Ziegen auswirkte, lag der Fokus der beiden folgenden Studien (Kapitel 5, 6) auf Untersuchungen bei behornen Ziegen. In der zweiten Studie (Kapitel 5) wurde untersucht, ob die negativen Auswirkungen von sozialen Konfrontationen mit unbekanntem Ziegen durch die Anwesenheit bekannter Artgenossen reduziert werden können. Dazu wurden zwölf Ziegen (= konfrontierte Ziegen) sowohl alleine als auch zusammen mit zwei bekannten Artgenossen mit vier ihnen unbekanntem, aus je sechs Ziegen bestehenden Gruppen konfrontiert (24 Konfrontationen insgesamt). Die Konfrontationen fanden in einer neutralen Umgebung statt und dauerten jeweils eine Stunde. Während der Konfrontationen wurden soziale Interaktionen, das Ausmass an Aktivität sowie die Konzentrationen von Cortisolmetaboliten im Kot für die konfrontierten Ziegen sowie Fokustiere der unbekanntem Gruppen quantifiziert. Konfrontierte Ziegen erhielten deutlich

weniger agonistische Interaktionen, wenn sie zusammen mit bekannten Artgenossen konfrontiert wurden. Das Ergebnis zeigte, dass durch die Anwesenheit bekannter Artgenossen die Belastung einer Ziege während sozialer Konfrontationen mit unbekanntem Ziegen reduziert werden konnte.

In der dritten Studie (Kapitel 6) standen die Auswirkungen einer temporären Separation und der sich anschließenden Wiedereingliederung im Mittelpunkt. Es wurde untersucht, ob intensiverer Kontakt zur eigenen Gruppe die Belastung sowohl der separierten Ziege als auch der Gruppenmitglieder reduzieren kann. Dazu wurden von vier Versuchsgruppen, die je aus sieben Ziegen bestanden, insgesamt zwölf Ziegen einzeln separiert. Jede der zwölf Ziegen wurde zwei Separationsverfahren ausgesetzt (24 Separationen insgesamt). Während ein Separationsverfahren ausschliesslich akustischen Kontakt zur Gruppe zulies, war im anderen Separationsverfahren auch visueller und taktiler Kontakt zur Gruppe möglich. Nach der zweitägigen Separationsphase wurden die separierten Ziegen wieder in ihre ursprüngliche Gruppe eingegliedert und Daten für weitere drei Tage erhoben (Wiedereingliederungsphase). Zur Beurteilung der Belastung wurden vor und während der Separations- und Wiedereingliederungsphase sowohl für die separierten Ziegen als auch für die Fokustiere in der Gruppe soziale Interaktionen, Liege- und Fressdauer sowie die Konzentrationen von Cortisolmetaboliten im Kot erhoben. Die Ergebnisse zeigten, dass sowohl die Separation als auch die Wiedereingliederung für die separierte Ziege belastend waren. So war die Fressdauer während der gesamten Separationsphase reduziert und die Konzentrationen von Cortisolmetaboliten sowohl in der Separations- als auch in der Wiedereingliederungsphase erhöht. Das Ausmass der Belastung konnte jedoch durch intensiveren Kontakt zur Gruppe reduziert werden: Bestand visueller und taktiler Kontakt, so war die Liegedauer während der Separationsphase nicht reduziert und die Cortisolmetaboliten-Konzentrationen waren im Vergleich zu ausschliesslich akustischem Kontakt geringer. Da die Auswirkungen während der Separation stärker waren und auch länger anhielten, konnte gefolgert werden, dass eine Separation belastender war als die sich anschließende

Wiedereingliederung. Die verbleibenden Gruppenmitglieder schienen hingegen kaum durch die Separation eines einzelnen Gruppenmitglieds belastet zu werden, da sich ihr Verhalten und auch die Cortisolmetaboliten-Konzentrationen nur wenig veränderten.

Zusammenfassend weisen die Ergebnisse dieser Arbeit darauf hin, dass sowohl das Eingliedern unbekannter Ziegen in bestehende Gruppen als auch die vorübergehende Separation von der Gruppe vermieden werden sollte. Ist eine Eingliederung unvermeidbar, so ist es empfehlenswert, mehrere Ziegen gleichzeitig einzugliedern. Während der Dauer einer Separation sollte akustischer, visueller und taktiler Kontakt zur Gruppe ermöglicht werden, um das Ausmass der Belastung für die separierte Ziege zu reduzieren.

3 General introduction

Common management procedures in farming practice involve the regrouping of goats in order to restock the herd, increase its size, or because individual goats have to be temporarily separated, e.g. during kidding or because of injury. Hence, the extent of regrouping can range from introducing unfamiliar goats into an established group, to exchanging subgroups, to temporarily separating individual goats and subsequently reintegrating them into their original group. The shared element between different forms of regrouping is therefore a change in group composition.

3.1 Regrouping goats in farming practice

Introducing unfamiliar goats into established groups can increase agonistic behaviour (Addison and Baker 1982, Alley and Fordham 1994, Schwarz and Sambraus 1997), reduce the introduced goats' feeding times (Schwarz and Sambraus 1997), and increase blood cortisol concentrations (Ortiz and Alvarez 2007). Similarly, exchanging (sub)groups of goats was shown to increase the level of agonistic interactions (Fernández et al. 2007, Andersen et al. 2008). It is reasonable to assume that these procedures are perceived as stressful by the regrouped goats. Furthermore, increased levels of activity and vocalisation during (experimental) social separation (Price and Thos 1980, Carbonaro et al. 1992, Aschwanden et al. 2008a, Siebert et al. 2011) and increased levels of agonistic interactions when separated goats return to their group (Ramírez et al. 2007) indicate that such procedures negatively affect the goats' welfare. Like in goats, regrouping was found to reduce welfare in other farm animals, such as cows (e.g. Brakel and Leis 1976, Hasegawa et al. 1997, von Keyserlingk et al. 2008, Castro et al. 2012), sheep (e.g. Sevi et al. 2001, Guesdon et al. 2012), pigs (e.g. Ewbank and Meese 1971, Meese and Ewbank 1973, Tuchscherer et al. 1998, Coutellier et al. 2007), and horses (e.g. Søndergaard and Christensen 2009, Hartmann et al. 2012).

Results from previous studies on the effects of regrouping on goats' welfare emphasise the need for research into more acceptable ways of regrouping goats. However, although goats in farming practice are frequently subjected to such management procedures, the consequences of different regrouping events have not been assessed systematically. Moreover, effects of regrouping should be evaluated under intensive housing conditions, and it is necessary to distinguish between the effects on the regrouped goats on the one hand and on the pre-existing group members on the other hand. To date, the potential effects of regrouping on the latter have hardly been considered.

To explain why regrouping of goats in intensive housing is associated with impaired welfare and to explore new strategies to optimise regrouping, three topics are addressed in more detail in this General introduction. Firstly, considering the social organisation of goats living under natural conditions should help assess the capacity of goats to adapt to regrouping. Secondly, housing conditions that might intensify or reduce the negative effects of regrouping will be identified. Thirdly, regrouping strategies that have already been tested in other farm animal species will be reviewed to generate hypotheses for research in goats.

3.2 Social organisation of goats living under natural conditions

The available observations of goats under natural conditions agree on goats living in rather small, stable groups with a matrilineal organisation, i.e. the basic unit consists of females with their recent and often also previous offspring (= family groups) (Riney and Caughley 1959, Yocom 1967, Shank 1972, McDougall 1975, Shi et al. 2005). Even if family group members cannot always keep visual contact due to habitat characteristics or when being widely distributed while foraging, they are hypothesised to constantly maintain contact at least by acoustic signals (Yocom 1967, Siebert et al. 2011). Acoustic signals are important for mutual recognition between mother and offspring (Terrazas et al. 2003, Briefer and McElligott 2011, Briefer et al. 2012) and potentially for differentiating between familiar and unfamiliar individuals (Keil et al. 2012). Even during parturition, the only time

when female goats actively separate from the herd, females seem to choose birthing sites within the home range of the herd, especially in areas frequently used by the herd and close to the main night camp (O'Brien 1983). It thus seems that goats prefer staying in contact with the herd and keeping periods of separation as short as possible.

The social organisation between the family groups of a certain population is not clear. For example, whereas bucks move between different family groups (predominantly) in the mating season (Riney and Caughley 1959, McDougall 1975, Shi et al. 2005), such movement has not been described for female goats. Contact between family groups seems to be limited to forming temporary, loose associations when grazing the habitat (Riney and Caughley 1959, Yocom 1967, Shi et al. 2005). The size of these associations may vary when family groups fuse, fission, or dissolve while foraging (Shi et al. 2005). Whether associations only result from environmental factors, such as food dispersal, or whether goats within such associations are socially organised remains unknown. Although individuals within associations have been described to be dominant or subordinate based on the outcomes of agonistic interactions (Riney and Caughley 1959, Yocom 1967, Shank 1972, McDougall 1975, Shi and Dunbar 2006), it is unclear whether these outcomes are consistent outside the family group, i.e. whether dominance relationships are established between goats of different family groups. Riney and Caughley (1959), for example, were not able to observe a hierarchy outside family groups.

Altogether, this information suggests that neither the introduction of (individual) female goats into established groups nor the separation of females from the group complies with the social organisation of goats living under natural conditions. Thus, regrouping goats in farming practice could compromise the animals' welfare by overtaxing the goats' adaptive capacities.

3.3 The effects of housing conditions on the welfare of regrouped goats

Social and structural housing conditions, such as group composition and access to resources, can have substantial effects on the level of agonistic interactions between goats living in established groups. Consequently, housing conditions can modify the negative effects associated with regrouping. Under natural conditions, the overall level of agonistic interactions is low, and explicit agonistic interactions (e.g. fights, butts) within family groups as well as between goats within an association are observed rarely (Riney and Caughley 1959, Shank 1972, McDougall 1975, Shi and Dunbar 2006). In contrast, the level of agonistic interactions in intensive housing is high even in established groups and is a problem commonly reported by farmers. When goats are regrouped, this level increases even further. The increase of agonistic interactions between unfamiliar animals is hypothesised to reflect the establishment of dominance relationships (Barash 1977) as, for example, introduced goats need to find their position in the existing hierarchy (Addison and Baker 1982).

Group composition under intensive housing conditions, however, might make it difficult for the goats to establish dominance relationships. Rearing kids artificially disrupts the matrilineal organisation and prevents the goats from living in family groups. During rearing and as adults, goats are usually housed in groups consisting of animals that are more or less homogeneous in age, size, and weight (e.g. adult female goats in dairy farming). Because dominance relationships between animals are assessed based, in part, on physical characteristics (Scott 1948, Ross and Scott 1949, Donaldson et al. 1967, Hafez et al. 1969, Shi and Dunbar 2006), it can be argued that physically similar individuals have similar fighting abilities and are more likely to engage in explicit agonistic interactions (Maynard Smith and Parker 1976). The hierarchy in homogeneous groups can thus be expected to be more labile than in heterogeneous groups.

Another problem that goats face when being regrouped is associated with the limited access to important resources, such as food and lying spaces, under

intensive farming conditions. The restricted dispersal of resources within the housing system increases the competition between goats, e.g. in the feeding and lying area, and has been shown to increase the level of agonistic interactions even in established groups (Loretz et al. 2004, Andersen and Bøe 2007, Meisfjord Jørgensen et al. 2007, Aschwanden et al. 2009a).

Aschwanden et al. (2008a, b, 2009a, b) investigated measures to optimise loose housing for goats in order to reduce the level of agonistic interactions and to improve access to resources. Their results showed that providing structural elements, which function as a barrier and/or offer visual cover, helps to reduce agonistic interactions between group members. Further, grouping goats early in ontogeny helps to reduce the individual distance between group members, i.e. the minimum distance between a given pair of goats without either of them showing agonistic or avoidance behaviour (Hediger 1940). This is thought to be due to an increased level of social tolerance that results from the process of growing up together.

However, as both pen design and grouping goats early in ontogeny are means to reduce agonistic interactions in established groups, i.e. groups whose members have determined dominance relationships between each other, these measures might not be effective in mitigating negative effects associated with regrouping. Thus, it appears that high levels of agonistic interactions associated with the regrouping of goats are only an initial reaction. In order to effectively and permanently reduce the negative effects of regrouping, dominance relationships have to be established.

3.4 Research into regrouping in other farm animal species

Regrouping is associated with negative welfare effects not only in goats but also in several other farm animal species. To generate hypotheses for experimental studies in goats, it is thus worth considering which regrouping strategies have been studied in other species.

Repeatedly exposing animals to regroupings or social confrontations was investigated in cows (Raussi et al. 2005, 2006), sheep (Sevi et al. 2001), pigs (Giersing and Andersson 1998, Coutellier et al. 2007), and horses (Hartmann et al. 2009, Christensen et al. 2011). It has been studied whether increasing the number of regroupings will habituate the animals to the procedure and, in turn, will help establish dominance relationships more quickly (Raussi et al. 2005, Christensen et al. 2011). Further, it has been tested whether introducing several cows simultaneously instead of introducing them individually can reduce the associated negative effects by providing social support and/or via a dilution effect (O'Connell et al. 2008, Gygax et al. 2009, Neisen et al. 2009). In pigs, the aspect of social support and/or a dilution effect was investigated with respect to the familiarity of the introduced animals and the ratio between introduced animals and group members (Durrell et al. 2003, O'Connell et al. 2004). Another approach, studied in pigs and horses, examined whether exposure prior to the actual encounter or increasing weight heterogeneity between confronted individuals would make it easier to assess each other's fighting ability and thus reduce the amount of fighting during social confrontations (Rushen 1987, 1988, Jensen and Yngvesson 1998, Hartmann et al. 2009, 2011). In horses, it was also tested whether the amount of agonistic interactions when introducing an individual unfamiliar horse could be mitigated by confronting this horse with each group member separately (Hartmann et al. 2011). Moreover, introducing animals at different times of day has been investigated in cows and pigs, assuming that introductions during times of least activity could reduce the frequency and severity of agonistic interactions (Nakanishi et al. 1993, Barnett et al. 1994, Boyle et al. 2012).

Overall, the results of the above mentioned studies may allow concluding that even though some regrouping strategies were found to reduce associated negative effects, they could do so only to a certain extent. Further, similar strategies were not equally successful in all species and even provided inconsistent results within one species. Finding ways to effectively mitigate negative effects associated with regrouping thus remains an important topic in farm animal welfare research.

Hence, regrouping methods investigated in other species are valuable to identify approaches that would be useful to study in goats as well. It should be noted, however, that techniques that were found to be promising in one species cannot per se be applied to other species. This might be because the social organisation under natural conditions varies considerably between the different farm animal species. Consequently, to effectively address impairment of welfare by regrouping in goats, it is necessary to specifically investigate the situation in this species.

3.5 Aim of this thesis, study design, and research questions

The aim of this thesis was to provide basic knowledge on the effects of regrouping on the welfare of goats and to find methods that are less aversive. The focus was on small groups, as the problem of high levels of agonistic interactions seems to be more pronounced in small groups compared with larger groups (Andersen et al. 2011).

To understand how aversive the introductions of unfamiliar goats to a group are, the first study (chapter 4) assessed the effects of an introduction on the individually introduced goat as well as on the pre-existing group members. Further, it was investigated which variables were the most suitable ones to measure the effects associated with regrouping. Sixteen goats were introduced one at a time into small established groups over a five-day period (four introductions per group). As hornless and horned goats differ by showing agonistic interactions predominantly with and without physical contact (Aschwanden et al. 2008b), it was hypothesised that they would be differently affected by the introduction of an unfamiliar goat. Consequently, groups of both hornless and horned goats were included in this first study.

Based on the results of the first study, the following studies (chapters 5 and 6) focussed specifically on regrouping in horned goats. The question of the second study (chapter 5) was whether the presence of familiar conspecifics can reduce the negative effects for a goat during social confrontations with unfamiliar goats. Moreover, it was determined whether the reactions of a group of goats differ

when the group is confronted with one unfamiliar goat or several unfamiliar individuals at a time. Twelve goats were confronted both alone and accompanied by two familiar conspecifics with an unfamiliar small established group (24 confrontations in total) for one hour each. To exclude competition for resources, confrontations were conducted in a neutral environment.

The third study (chapter 6) investigated the separation and reintegration of horned individual goats. The question was whether an increased level of contact with the original group during separation could reduce the negative effects of separation and reintegration for both the goat that was separated and the remaining group members. The effects were tested by individually separating twelve goats from four experimental groups. The separation lasted two days, with each separated goat experiencing two treatments that differed by how much contact was maintained with the group (24 separations in total). Afterwards, goats were reintegrated into their original group.

Dominance relationships within each group were assessed prior to each experiment, and the goats' rank categories were considered in the analyses of all three studies. This was done because in goats, similar to most farm animal species (e.g. pigs and cows: Zanella et al. 1998, Olofsson 1999, Kongsted 2006, Sutherland et al. 2006, Lexer et al. 2009), negative welfare effects are not equally distributed among the goats of a group. Depending on the situation, goats of certain rank categories are more affected than those of different rank categories (Barroso et al. 2000, Loretz et al. 2004, Andersen and Bøe 2007, Fernández et al. 2007, Meisfjord Jørgensen et al. 2007, Aschwanden et al. 2009a). Additionally, due to the supposed interrelation between physical characteristics and social status, the introduced goat's former rank might influence how and/or to what extent it is affected by the different regrouping strategies.

4 The introduction of individual goats into small established groups has serious negative effects on the introduced goat but not on resident goats

Based on:

Patt A, Gygax L, Wechsler B, Hillmann E, Palme R, Keil NM (2013)

Applied Animal Behaviour Science 138, 47-59

Abstract

The introduction of an individual goat into an established group is likely to result in intense agonistic interactions, which may adversely affect the welfare of both the introduced goat and the resident goats. To assess this situation, we introduced eight horned and eight hornless goats one at a time over a five-day period into one of four experimental groups, two of which consisted of six horned goats and the other two of six hornless goats. Individual goats were always introduced into groups with the same horn status. Before and during the introduction period, we recorded agonistic and sniffing behaviour, the location of the introduced goat within the pen, time spent lying, duration of feeding and rumination, the occurrence of injuries, and the concentrations of faecal cortisol metabolites in both the introduced goats and three resident group members. In addition, we evaluated the dominance relationships of the introduced goats in their groups of origin as well as of the goats in the four experimental groups by direct observations made during the main feeding times before the start of the experiment. Data were analysed using generalised linear mixed-effects models with the explanatory variables day (i.e. day number of the observation period), presence of horns and rank category.

In general, group members were little affected by the introduction of a single goat. By contrast, newly introduced goats displayed considerably longer lying periods, considerably shorter feeding times, and elevated concentrations of faecal cortisol metabolites throughout the introduction period. Frequencies of agonistic

interactions and sniffing behaviour directed towards the introduced goats were high only on the day of introduction, and decreased to a low and constant level throughout the remaining introduction period. Changes were more pronounced in introduced goats with horns, with the highest cortisol metabolite concentrations measured in high-ranking introduced goats with horns. In conclusion, our results indicate that the welfare of goats individually introduced into small groups is seriously adversely affected for a minimum of 5 days.

Keywords

goat, grouping, loose housing, horns, stress, social behaviour, agonistic interactions

4.1 Introduction

Under natural conditions, goats live in fairly small, stable groups, which are reported to consist of between 4 and 6 (Shank 1972), 14 (Riney and Caughley 1959) and infrequently of more than 20 individuals (Yocom 1967). Movements of adult female goats between herds would appear to occur only occasionally (Riney and Caughley 1959). In goat husbandry, however, new animals are commonly periodically introduced into established groups (e.g. kids that have been reared to adulthood, additional goats that have been purchased). The introduction of new animals into established groups often leads to an increase in agonistic behaviour as has been described for most farm animals, e.g. cows (Brakel and Leis 1976, Hasegawa et al. 1997, von Keyserlingk et al. 2008, Neisen et al. 2009), pigs (Meese and Ewbank 1973), horses (Søndergaard and Christensen 2009), sheep (Sevi et al. 2001) and goats (Addison and Baker 1982, Alley and Fordham 1994, Schwarz and Sambraus 1997). In addition to a general increase in agonistic behaviour, newly introduced goats exhibit shorter feeding times (Schwarz and Sambraus 1997) as well as an increased concentration of cortisol in the blood (Ortiz and Alvarez 2007). All of the above indicates that a goat recently introduced into an established group will perceive this experience as stressful. Whereas it is known that frequent regrouping influences the welfare of individuals in a group (Fernández et al. 2007, Andersen et al. 2008, Sondresen et al. 2008), it is not known whether the introduction of a single goat might also be stressful for group members. Furthermore, social rank might influence the stress-related response of the introduced goat as well as that of the resident goats. In a study of cows, Arave and Albright (1976) concluded that the impact of an introduction is greater if the introduced animal is highly dominant as opposed to being of medium or low dominance. With goats, Alley and Fordham (1994) assume that the group members' response towards an introduced goat might be influenced by the social status of that goat prior to the introduction, and suggest that in particular, group members with a similar dominance ranking to the introduced goat are likely to react aggressively towards it.

Since the duration of a stressor is also important in terms of its consequences, the question of the effects of an introduction is closely related to the question of the time required for a newly introduced goat to integrate fully into a group. Based on the observation of aggressive behaviour, Alley and Fordham (1994) conclude that the time required for the introduced goat to integrate fully is 24 hours, while Addison and Baker (1982) put the figure at four weeks. The large difference may result from the fact that the studies varied in terms of the number of goats previously in the herd and number of newly introduced goats, which for Addison and Baker (1982) was seven and two, respectively, and for Alley and Fordham (1994) was 63 and one and 150 and one in two separate cases. In addition, since the goats of these two studies were introduced on pasture with very few spatial restrictions, the applicability of these two studies to intensive housing conditions may be limited.

The increase of agonistic behaviour following an introduction is assumed to be due to interactions occurring when each group member establishes a dominance relationship with the introduced animal (Barash 1977, Fernández et al. 2007). In goat housing, factors such as horn status (Aschwanden et al. 2008b), small herd sizes (Gaudernack Tønnesen et al. 2008), unstructured pens (Aschwanden et al. 2009a, b) and restricted resources (Loretz et al. 2004, Meisfjord Jørgensen et al. 2007) are discussed as important factors which increase the quantity and/or intensity of agonistic social interactions. The introduction of an unknown goat into an established group therefore raises issues of importance to animal welfare. The consequences of this introduction are unknown, both for the individually introduced goat and the remaining group members. The introduction of an unknown goat might be expected to be more problematic in the case of horned goats, and for small groups with intensive housing conditions.

The aim of the present study was therefore to assess possible adverse effects on welfare associated with the introduction of an individual goat into an established small group for both the introduced goat and the resident goats. We were interested in the changes in behavioural and physiological responses over time,

potential differences between horned and hornless goats, and the question of whether response is modulated by social rank. These questions were tested by consecutively introducing individual horned or hornless female goats into established groups of six female goats with the same horn status. Impact on welfare was monitored by recording agonistic and affiliative behaviour, lying behaviour and feeding behaviour, as well as by recording injuries sustained by both the introduced goats and three resident goats. Additionally, cortisol metabolites in faecal samples were measured to assess physiological stress responses. We expected these variables to change directly after the introduction of an unknown individual, and anticipated the possibility of further changes throughout the introduction period.

4.2 Methods

4.2.1 Animals and housing conditions

Eight groups of six non-lactating female goats kept in eight identically equipped pens (48 goats in total) were studied. The groups were formed in September 2009 from individuals of various Swiss milking breeds (Saanen, Toggenburger, Appenzeller, Chamois Coloured, St. Gallen Booted, Grisons Striped, Peacock, and Valais Blackneck) and their crossbreeds. The goats had been bought on several Swiss farms in 2005 and were born between 2000 and 2005. In four, three and one of the groups a maximum of two, three and four goats, respectively, originated from the same farm. The detailed genetic relationships of these individual were unknown but they were never siblings or mother-daughter pairs. Four of the eight groups consisted of horned and the remaining four groups of hornless individuals (unknown whether genetically hornless or dehorned). As the presence of horns is either desirable (e.g. St. Gallen Booted) or wholly undesirable (e.g. Appenzeller), it was not possible to include horned and hornless animals of each breed.

Nevertheless, the distribution of the breeds was balanced over the groups as much as possible.

Each pen had an overall area of 15.3 m² (approximately 3 m x 5 m), consisting of a deep-bedded straw area of 11.7 m² (approximately 3 m x 4 m) and a 0.5 m elevated feeding place (3.6 m²) divided by a wooden wall into two compartments of equal size (1.2 m x 1.5 m). Hay was fed ad libitum in the feeding area from a 3 m hayrack refilled twice daily at around 8.45 am and 5 pm. Assuming an animal/feeding-place ratio of 1:1, each goat had access to a 50 cm-wide feeding space during the control situation (6 goats per pen) and a 43 cm-wide feeding space during the experimental situation (7 goats per pen). A water trough, a licking stone providing minerals and vitamins, and a brush were supplied in each pen. The deep-bedded area was further structured by a wooden platform (2.5 m x 0.65 m, 0.55 m high) providing climbing opportunities and both elevated (above) and

protected (below) lying areas, as well as a freestanding partition in the centre of the pen (approx. 1 m in diameter and 0.8 m in height) also serving as a platform.

4.2.2 Experimental groups and goats to be introduced

The experiment was carried out within the home pens during the period of November 2009 to January 2010. Of the eight groups, two horned and two hornless were used as experimental groups to which unknown individuals were introduced. The other four groups (likewise two horned and two hornless) provided the animals which were introduced. All groups were housed in the same building, and therefore had visual as well as acoustic contact with one another. Because the experimental groups and the groups with the animals to be introduced were on opposite sides of a feeding alley, however, tactile contact was not possible. Horned and hornless goats were only introduced into horned and hornless groups, respectively. The experiment was licensed by the Cantonal Office (Frauenfeld, Thurgau, Switzerland, F4/09).

4.2.3 Dominance relationships

Dominance relationships were determined in each of the eight groups in order to test whether the effect on animal-welfare indicators associated with the introduction differed depending on an individual's rank in its group of origin as well as its rank in the assigned experimental group. The evaluations were carried out shortly before the start of the experiment according to the method described by Aschwanden et al. (2008b) and based on direct observations during morning and evening feeding times. Indicators for dominance and subordination were being the active party in agonistic behaviour and avoidance behaviour, respectively. A dominant goat forced another goat to leave its current position either through agonistic behaviour or implicit displacement (definitions as presented in Table 1). For each pair of goats, a clear unidirectional relationship was presumed if at least three agonistic interactions with the same goat being dominant were observed. If one of these three outcomes was contradictory (= bidirectional relationship), at least one additional agonistic interaction was observed for the pair concerned until

one goat was twice as often clearly dominant over the other. The following rank index (between 0 = omega and 1 = alpha) was then calculated for each goat: number of dominated group members/number of possible rank relationships (five for a group of six). Each goat was categorised as being either low-ranking (0.0 or 0.2), medium-ranking (0.4 or 0.6) or high-ranking (0.8 or 1.0).

4.2.4 Experimental procedure

In total, 16 different goats were introduced into the four experimental groups, i.e. four introductions took place in each of the groups. The individual goats were introduced into the pens by leading them through the stable doors at one end of the elevated feeding area. The four goats to be introduced into one experimental group were introduced in a randomised order with respect to rank. A series of introductions into all four experimental groups started around 8.30 am on experimental day 0, when the first goat was introduced to the respective experimental group. The second introduction was performed 15 minutes later, when another individual was introduced into the second experimental group. This procedure was repeated until the fourth individual was introduced in the fourth experimental group at around 9.15 am. One introduction period was five days in length (5 x 24 hours). After each introduction period, the introduced individuals were returned to their original groups. There followed a nine-day break before the next introductions took place.

In the days before each introduction period, data on the experimental groups and the individuals to be introduced were collected on two days for lying behaviour (days -7, -6), feeding behaviour (days -6, -5), rumination (days -6, -5), cortisol metabolites (days -4, -3) and once for injuries (day -1). Data on agonistic and affiliative behaviour were recorded on two days in the experimental groups (days -7, -5) and used as a reference for the following introduction period. On days 0-4, all individuals in a group - both the resident goats and newly introduced goats - were taken into account in the daily recordings of agonistic and affiliative behaviour. Lying, feeding and ruminating behaviour as well as faecal cortisol

metabolites and injuries were measured daily (days 0-4) for both the newly introduced goats and three resident goats representing the three rank categories of high, medium and low. These resident goats remained the same for all four repetitions to account for an effect of individuality. The location of the introduced goats within the pen was recorded daily (days 0-4).

4.2.4.1 Agonistic and affiliative behaviour

Agonistic and affiliative behaviour was directly observed between 8.30-11.30 am and again between 4-7 pm. During these two observation blocks - which included the peak feeding times - each of the four experimental groups was observed three times for 15 minutes at a time. The sequence of observation within the blocks was balanced such that groups were observed as equally as possible within the different 15 minute time slots. On day 0 (introduction day), the first observation slot for each experimental group included the 15 minutes following the introduction of the unknown individual, thereby ensuring that the first 15 minutes of contact between the group and the newly introduced goat in each of the four groups was not missed out. The order of introduction was balanced among the experimental groups, i.e. each experimental group occupied position one, two, three, and four once.

All goats in a group were observed simultaneously. The behaviours recorded are listed in Table 1, together with their definitions. For each agonistic interaction, we noted which goat initiated and which goat was on the receiving end of the behaviour, as well as whether the initiator was successful or not. An interaction was defined as successful if the recipient changed location as a result of it. For each affiliative interaction, we noted which goat was the initiator and which goat was the recipient.

Table 1: Definitions of observed social interactions.

Quality	Definition
Agonistic Behaviour	
Threat	A goat lowers her head, draws her chin into her chest and presents her horns/horn rudiments (Shank 1972), or a goat (vigorously) shakes her head at another goat. No physical contact is involved.
Implicit displacement	The mere approach of a goat causes another goat to move away from a given location without physical contact and without apparent threat prior to the displacement (Addison and Baker 1982).
Explicit displacement	Using physical contact (with biting, butting and fighting not considered), a goat pushes another goat out of the way (Addison and Baker 1982).
Butting	A goat butts another goat with the head or horns, but not standing opposite it as in a fight.
Biting	A goat bites another goat and pulls at parts of its body with her teeth (Tölü and Savas 2007).
Fighting	Two goats stand opposite one another, their heads raised. Simultaneously, the goats lower their chins against their throats by tilting their heads slightly to the right or left. From 1-2 m distance - possibly rearing up - the two goats clash heads (Shank 1972).
Affiliative Behaviour	
Sniffing	A goat places her muzzle close to the head/body of another goat with the wings of her nostrils moving.
Scratching	The goat touches the head or body of another goat with her head or horn, moving the latter rhythmically back and forth or up and down.
Licking	A goat touches another goat with her tongue.
Mock fighting	Two goats fight as described above, but the behaviour is playful because after the fight neither of the goats turns away to show her submission.

4.2.4.2 Location of the introduced goat

During the introduction period (days 0-4), the location of the introduced goat in the pen was noted at the beginning of each 15 minute slot. The possible locations were categorised as follows: elevated feeding place, deep-bedded area, on top of the freestanding partition, on top of the wooden platform, or below the wooden platform (lying niche).

4.2.4.3 Lying behaviour

Lying behaviour was recorded using a commercial 3D acceleration logger (MSR145WA, Modular Signal Recorder Electronics GmbH; 33 mm x 15 mm x 61 mm). A logger was attached to the left hind leg of each of the introduced goats and three of the resident goats in each experimental group. Acceleration in the direction of the y-axis, which was the axis parallel to the longitudinal axis of the goats' hind leg, was recorded once a second. The different positions of the goats' hind legs while lying down (almost horizontal) as opposed to standing and walking (almost vertical) meant that the amount of time (in hours) each goat spent lying could be calculated per 24-hour period.

4.2.4.4 Feeding and rumination behaviour

Feeding behaviour was recorded using a commercial logger (MSR145WS, Modular Signal Recorder Electronics GmbH, 20 mm x 15 mm x 61 mm) fitted with a pressure sensor combined with an oil-filled silicon tube. This tube was attached to the head collar, above the goat's nose. During mastication, pressure differences were transmitted through the oil-filled tube and detected by the pressure sensor. The signal was saved at a rate of 10 Hz. It was possible to differentiate between feeding and rumination owing to the differences in the characteristics of the pressure pattern generated by each (Scheidegger 2008, Nydegger et al. 2010). Because the logger had a maximum storage capacity of 29 hours, data had to be transferred daily. To enable this, daily feeding behaviour was only recorded for 21.75 hours a day. For purposes of analysis, data were extrapolated to the duration (no. of hours) of feeding and rumination per 24 hours. Loggers were

attached to the newly introduced goats as well as to three of the resident goats in each experimental group.

4.2.4.5 Cortisol metabolites

In order to monitor an acute stressor via the measurement of faecal cortisol metabolites, samples should be collected 12-15 hours after the event in question (Kleinsasser et al. 2010). Faecal sampling of both the newly introduced goats and three resident goats of each experimental group started at 8.30 pm. To ensure a sampling interval of 12 hours after introduction, samples were taken in the order of introduction on the morning of day 0. To account for a possible circadian rhythm of cortisol levels, faecal samples were taken at the same time on the evenings of days -4, -3, 0, 1, 2, 3 and 4. For the newly introduced goats, an additional sampling one week after their return to their original group (day 11) served as a second control.

The goats were successively attached at the hayrack and faeces were collected manually from the animal's anal channel/rectum. Each sample was immediately placed in a cooling box until sampling was completed. Afterwards, all samples were frozen and stored at -20°C until analysis. The concentration of cortisol metabolites in the faeces was determined by a group-specific 11-oxoetiocholanolone enzyme immunoassay (EIA) (Möstl et al. 2002) that measures metabolites with a 5 β -3 α -hydroxy-11-oxo structure. This EIA has been successfully validated for monitoring adrenocortical activity in goats (Kleinsasser et al. 2010).

4.2.4.6 Injuries

On each day of the introduction period, the introduced goats and the three resident goats were examined and the number and type of injuries recorded. It was differentiated between hairless patches, abrasions (the epidermis is scraped off), haematoma (blood collected under the skin), and wounds (all layers of the skin severed). Only injuries that had appeared since the previous recording were taken into account. In order to ensure that only injuries occurring during the

experiment were considered, the animals were examined on day -1 to determine the status quo.

4.2.5 Statistical analysis

In order to adequately reflect dependencies in the experimental design (nesting, repeated measurements), generalised linear mixed-effects models were used to evaluate the outcome variables. Statistical analysis was performed in R (version 2.12.2, R Development Core Team 2011) using the lme and glmer methods from the nlme (Pinheiro et al. 2009) and lme4 (Bates et al. 2011) packages, respectively. Fixed effects were the presence of horns (factor with two levels: yes, no), rank category (factor with three levels: high-, medium- or low-ranking) and day. The fixed-effect day was a factor with a varying number of levels: seven when there was a control measurement comparing resident goats before and after the introduction of a new goat, or investigating the impact of the introduction period on the introduced goat; five when only data the introduction period was relevant; and eight levels for cortisol metabolites, since there was an additional control measurement for the introduced goats after they were returned to their groups of origin.

For all outcome variables dealing with resident goats only, random effects were repetition nested in animal nested in experimental group. If an outcome variable described interactions initiated by resident goats towards the introduced goats, interactions initiated by the introduced goat towards resident goats or the behaviour of the introduced goat, random effects were given as animal nested in experimental group. The effect of presence of horns was only considered when it appeared within an interaction with another fixed effect, because the number of groups that were either horned or hornless - two of each - was too small for inferences to be drawn, and error degrees of freedom thus did not allow for an accurate estimate.

For the analysis, a maximum model (all interactions), three intermediate models (each with one anticipated two-way interaction between presence of horns and

day, day and rank category, or presence of horns and rank category) and a minimum model (main effects only) were set up for both the resident goats and the introduced goats. The choice among the five models was based on the Bayesian information criterion (BIC) inferring the probability of the specific model given the data (Burnham and Anderson 2003). For most models, the main-effects model provided the lowest BIC values. If not stated otherwise, therefore, it is the results of the main-effects model that are presented. Model assumptions were checked using graphical analysis of residuals that focused on normality of errors and random effects as well as homoscedasticity of the errors in the case of normally distributed errors, and on normality of random effects and absence of bias in the mean errors for the generalised models.

To support interpretation and as a concession to more classical approaches, we still report the p-values in the models that include main effects. The effect of day is reported for all outcome variables to show changes over time, whilst presence of horns and rank category are only reported if their effect was significant. For more complex models with two- or three way-interactions, we do not report p-values: In models including statistical interactions, it is not possible to use the explanatory variable's p-value to interpret its effect when it appears in a interaction, and only the p-value of a variables' interaction with the highest number of terms is meaningful (Engqvist 2005). According to the BIC approach p-values were not the significant criteria for model selection and therefore single p-values of interactions were not helpful for identifying relevant effects.

4.2.5.1 Agonistic and sniffing behaviour

Three types of social interactions were distinguished: (a) both initiator and receiver were resident goats; (b) the initiator was a resident goat and the receiver the introduced goat; (c) the initiator was the introduced goat and the receiver a resident goat. Although several affiliative behaviours were included in the ethogram, they only manifested very sporadically. The exception to this was

sniffing, which was therefore included in the statistical analysis of affiliative behaviour.

4.2.5.1.1 Interactions directed against resident goats by other resident goats

Among resident goats, the number of agonistic and the occurrence of sniffing behaviours experienced at the receiving end per animal and day served as outcome variables. The number of agonistic behaviours was analysed using a linear mixed-effects model and log-transformation of data, whilst a generalised linear mixed model based on the binomial distribution was used in the case of sniffing behaviour.

4.2.5.1.2 Interactions directed against the introduced goat by resident goats

Outcome variables here were the number of agonistic and sniffing behaviours directed against the introduced goat by resident goats per day. For agonistic behaviour, we also analysed the proportion of agonistic interactions in which the introduced goats came out the losers in relation to all agonistic interactions directed against introduced goats by resident goats, as well as the proportion of agonistic interactions involving physical contact in relation to all agonistic interactions directed against the introduced goats by resident goats.

Linear mixed-effects models were used and data was log-transformed for number of agonistic interactions as well as number of sniffing behaviours. Data analysing the proportion of agonistic interactions lost and the proportion of those with physical contact was logit-transformed. Some goats were not on the receiving end of any interactions. To enable a log-transformation, these zeros, which might not be genuine zeros but represent values lower than the detection level, were replaced by a value lower than the detection level, i.e. smaller than the smallest observed value, before transformation.

4.2.5.1.3 Interactions directed against resident goats by the introduced goat

The occurrence of sniffing behaviour was used as an outcome variable in a generalised linear mixed model for the interactions directed against resident goats by the introduced goat. Data reflecting the agonistic behaviour of introduced goats are summarised numerically only because it occurred too rarely for quantitative analysis.

4.2.5.2 Location of the introduced goat

Since horned introduced goats were mainly recorded as occupying the niche below the wooden platform, the proportion of scans per day spent in this niche was calculated for the introduced goats and logit-transformed for analysis. To satisfy statistical assumptions, we omitted one outlier: one day (day 4) in which a specific introduced horned goat spent considerably less time in the niche than the other introduced horned goats and than on the other days.

4.2.5.3 Lying behaviour

Data for resident goats and introduced animals were analysed separately and two separate linear mixed-effects models were calculated for time spend lying (no. of hours) per goat per 24 hours. According to the BIC value, the best models were the ones with the three-way interaction. Compared to the other models tested (main-effects only and those with two-way interactions), the BIC value did not drop substantially until the three-way interaction was included in the model, when it was considered to be the relevant term in the analysis.

4.2.5.4 Feeding and rumination behaviour

Here again, data for resident goats and introduced goats were analysed separately, using linear mixed-effects models. Outcome variables were the duration of feeding and rumination per 24 hours (no. of hours). According to the BIC value, for resident goats the most suitable model for feeding and rumination was the one with the three-way interaction. Only after the three-way interaction was introduced did the BIC value drop substantially compared to the other models tested (main-effects only and those with two-way interactions). For rumination behaviour of introduced goats the difference of BIC value between the model with the two-way interaction between presence of horns and rank category and the main-effects model was < 2 . We therefore decided to choose the main-effects model.

Owing to technical problems during data collection, five days' worth of data for individual animals were missing and could therefore not be analysed. Another seven files, each relating to one animal and one day, were omitted from the analysis because mastication activity was below detection level.

4.2.5.5 Cortisol metabolites

The data for resident goats and introduced goats were analysed separately, using linear mixed-effects models. To satisfy model assumptions, concentrations of cortisol metabolites were log-transformed. Following the BIC criterion, the best model for the introduced goats was the one including the two-way interaction between presence of horns and rank category.

4.2.5.6 Injuries

The number of injuries in introduced goats was used as an outcome variable in a generalised linear mixed model. The number and distribution of injuries in focal resident goats are only summarised because they occurred too rarely for quantitative analysis.

4.3 Results

4.3.1 Qualitative observations

Immediately after introduction of the individual goat, resident goats would in many cases gather round and sniff at the introduced animal. Agonistic interactions directed towards the introduced goat followed soon thereafter, and in the beginning often involved several resident goats at different locations in the pen chasing the introduced goat around. Among the agonistic interactions, butts were observed most frequently and threats somewhat less often, whereas fights occurred only rarely. Horned introduced goats used the niche within minutes of introduction. Throughout the introductory period, introduced goats were mainly on the receiving end of agonistic interactions (mainly butts and threats), either when they went to eat or drink or left the niche to defaecate, or when resident goats competed with them for lying space in the niche. Introduced goats were sometimes observed eating some of the litter (straw and bits of hay that had been swept from the elevated feeding area to the deep-bedded straw area) while lying in the niche. In the evenings when faecal samples were taken, introduced goats began eating immediately for the short time they were tethered at the hayrack. Once the sampling was completed and the experimenter had left the pen, resident goats would often start directing agonistic interactions (butts) towards the introduced goat.

4.3.2 Agonistic and sniffing behaviour

4.3.2.1 Interactions directed against resident goats by other resident goats

The number of agonistic interactions per animal and day for which resident goats were on the receiving end from other resident goats was lower during the introduction period than on day -7 ($F_{6,570} = 6.22$, $p < 0.001$, day -5: OR (odds ratio) = 0.92, day 0: OR = 0.63, day 1: OR = 0.67, day 2: OR = 0.85, day 3: OR = 0.66, day 4: OR = 0.72, Figure 1a). Generally, low- and medium-ranking resident goats were on the receiving end of more agonistic interactions than high-ranking ones, who had an average of one agonistic interaction per animal and day ($F_{2,18} = 17.33$,

$p < 0.001$, medium-ranking: OR = 2.25, low-ranking: OR = 3.25) directed against them. The probability of the occurrence of sniffing behaviour in horned and hornless groups was lower on day -5 and day 1 than on the remaining days ($\chi^2_4 = 20.62$, $p < 0.001$, day -5: OR = 0.44, day 0: OR = 0.82, day 1: OR = 0.49, day 2: OR = 0.86, day 3: OR = 1.00, day 4: OR = 0.91, Figure 1b).

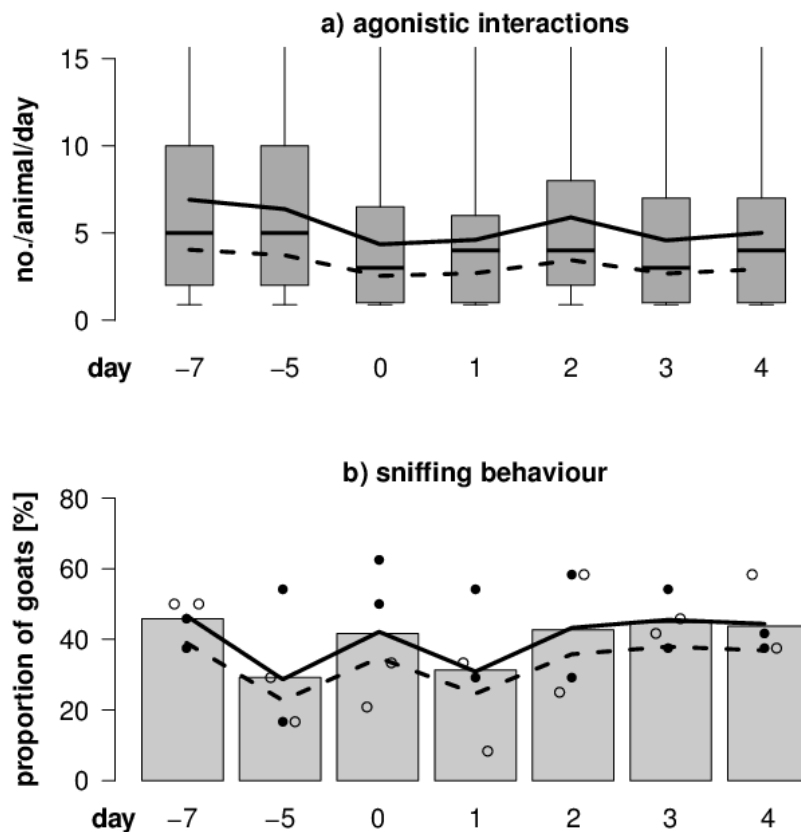


Figure 1: (a) Number of agonistic interactions directed against resident goats from other resident goats per animal and day.

Box-and-whiskers-plot, boxes = 1st and 3rd quartile, thick line = median, whiskers = range of data.

(b) Proportion of resident goats directing sniffing behaviour at other resident goats.

Circles: proportions in the four groups; open circles = horned goats, filled circles = hornless goats.

Solid lines = model estimates for hornless medium-ranking goats, dashed lines = model estimates for horned medium-ranking goats.

4.3.2.2 Interactions directed against the introduced goat by resident goats

The number of agonistic interactions directed against the introduced goat by resident goats was higher on day 0 than on days 1 to 4 ($F_{4,60} = 15.09$, $p < 0.001$, Figure 2a). All introduced goats were sniffed at on day 0. Compared to this day, there was a considerable decrease in the occurrence of sniffing behaviour, which

remained constant at a low level throughout the remaining days ($F_{4,60} = 170.84$, $p < 0.001$, Figure 2b).

The proportion of agonistic interactions involving physical contact decreased over the course of the introduction period ($F_{4,60} = 2.77$, $p = 0.035$, Figure 2c).

Low-ranking goats tended to direct agonistic behaviour with physical contact towards the introduced animals more often than high- and medium-ranking resident goats did ($F_{2,10} = 3.30$, $p = 0.08$, medium-ranking: OR = 1.47, low-ranking: OR = 1.93). Nevertheless, the proportion of agonistic interactions from which the introduced goat emerged as the loser remained more or less constant on a high absolute level of 74% (SD: 18%) throughout the introduction period ($F_{4,60} = 2.02$, $p = 0.10$).

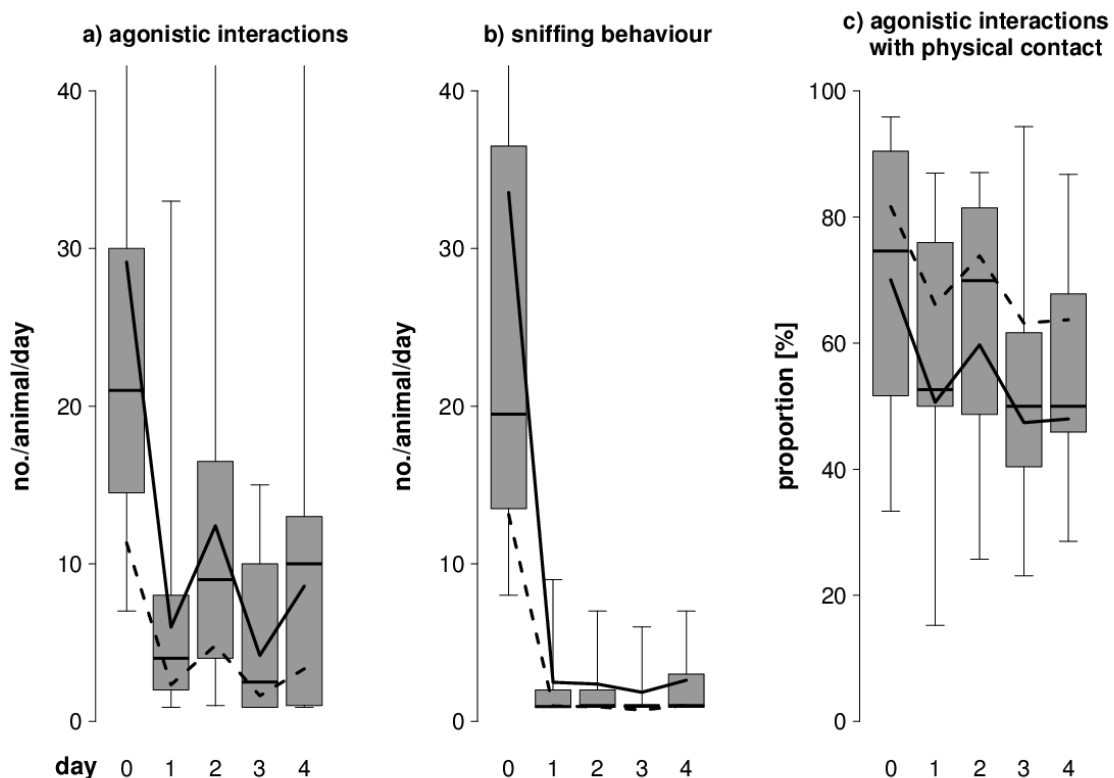


Figure 2: (a) Number of agonistic interactions directed against introduced goats by resident goats per animal and day.

(b) Number of sniffing behaviours directed against introduced goats by resident goats per animal and day.

Box-and whisker-plots, boxes = 1st and 3rd quartile, thick line = median, whiskers = range of data.

(c) Proportion [%] of agonistic interactions with physical contact out of all agonistic interactions directed against introduced goats by resident goats.

Solid lines = model estimates for hornless medium-ranking goats, dashed lines = model estimates for horned medium-ranking goats.

4.3.2.3. Interactions directed against resident goats by the introduced goat

Agonistic behaviour was very rarely demonstrated by introduced goats, and was never exhibited by introduced horned goats. By contrast, each of the eight introduced hornless goats performed between 0 and 35 (mean: 2.9) agonistic interactions on day 0.

The probability of introduced goats sniffing at resident goats was greatest on day 0 ($\chi^2_4 = 20.62$, $p < 0.001$). Horned introduced goats sniffed at resident goats only on day 0. Seven of the eight hornless and five of the eight horned introduced goats demonstrated sniffing behaviour towards resident goats on day 0. Most introduced hornless goats continued to sniff at resident goats on days 1 to 4 (day 1: six goats, day 2: five goats, day 3: three goats, day 4: six goats). On day 0, hornless goats sniffed on average 7.6 times at resident goats (SD: 3.8), whilst horned goats did so only once (SD: 1.07).

4.3.3 Location of introduced goat

Hornless introduced goats were less often recorded as being present in the niche below the wooden platform than their horned counterparts. Whereas four of the eight horned goats introduced lay in the niche below the wooden platform during all six scans on all five days of the introduction period, six of the eight hornless goats introduced were not once recorded as being in the niche. The behaviour of individual goats was very consistent, with the proportion of scans spent by introduced goats in the niche remaining the same throughout the introduction period ($F_{4,59} = 1.76$, $p = 0.15$).

4.3.4 Lying behaviour

All hornless resident goats spent less time lying on day 0 than on the control days and on days 1 to 4 of the introduction period. In the case of horned resident goats, only medium-ranking goats reacted in this way. By contrast, horned low-ranking resident goats spent more time lying on day 0 than on any other day. In horned high-ranking goats, the time spent lying appeared to increase continuously throughout the introduction period. Generally speaking, the lowest time spent lying per 24 hours was observed in horned and hornless medium-ranking resident goats (Figure 3a).

Once the introduction period (day 0) had started, the time which horned introduced individuals spent lying increased significantly with respect to the control situation, reaching values of between 21.7-23.3 hours per 24 hours. The variation between animals was small compared to the variation seen in the control measurements. For horned low-ranking introduced goats only, the time spent lying decreased somewhat towards the end of the introduction period (days 3 and 4). By contrast, the time hornless introduced goats spent lying increased to a markedly lower level and fairly steadily until days 3 and 4, after which there was a similar decrease to that observed in horned low-ranking introduced goats. Throughout the introduction period, the range of variation remained high in hornless introduced goats of all three rank categories (Figure 3b).

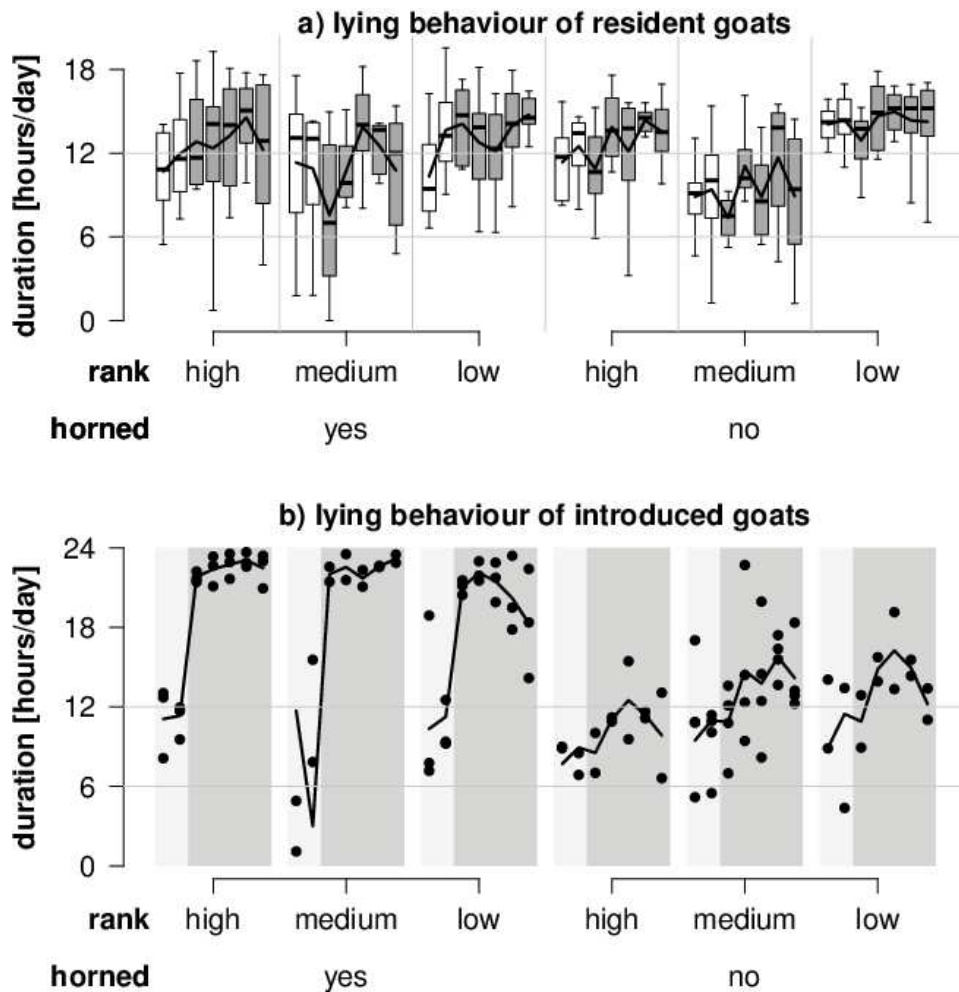


Figure 3: (a) Time (in hours) resident goats spent lying for 24 hours.

Box-and-whiskers-plot, boxes = 1st and 3rd quartile, thick line = median, whiskers = range of data.

(b) Time (in hours) introduced goats spent lying for 24 hours.

Filled circles = data of the individual goats of the indicated horn-status and rank category per day before/after introduction.

White boxes (resident goats (a)) or light-grey background (introduced goats (b)) = control situation before introduction (days -7 and -6); grey boxes (resident goats (a)) or grey background (introduced goats (b)) = introduction period (days 0-4). Solid lines indicate model estimates.

4.3.5 Feeding and rumination behaviour

It was observed that apart from hornless medium-ranking resident goats eating for a shorter length of time on day 0, whilst low-ranking individuals spent longer eating, resident goats were hardly influenced by the introduction of the individual goat. Irrespective of the introduction procedure, the following general patterns emerged: In horned resident goats, the time spent feeding decreased from high- to low-ranking individuals. The opposite effect was observed in hornless goats, i.e. the time spent feeding increased from high- to low-ranking individuals. Thus, whereas high-ranking resident goats fed for a similar amount of time, horned medium- and low-ranking goats spent less time feeding than hornless goats of the same ranking (Figure 4a).

The time resident goats spent ruminating was not influenced by the introduction of an unknown individual. In order to take into account the nesting of statistical data as well as the three-way interaction, mean values were merged by calculating the daily average rumination time for each resident goat per introduction period (days -6 and -5 as well as days 0-4). Afterwards, these values were averaged for the four experimental groups, whose values were in turn averaged. The mean length of rumination was 5.61 hours/24 hours (SD: 0.68) for resident goats. With the exception of horned low-ranking resident goats, which ruminated for a similar amount of time as horned medium-ranking resident goats, the pattern of rumination was very similar to the feeding pattern.

The time introduced goats spent feeding decreased significantly with the start of the introduction period (day 0). Although there is a slight increase in course of the introduction period, time spent feeding stayed at a low level until the end of the period ($F_{6,91} = 13.64$, $p < 0.001$, Figure 4b). Generally, medium-ranking introduced goats seem to feed for longer periods than high- and low-ranking ones ($F_{2,91} = 2.60$, $p = 0.08$).

Similarly, the amount of time that introduced goats spent ruminating decreased significantly with the start of the introduction period ($F_{6,91} = 6.74$, $p < 0.001$). Low-ranking-introduced goats tended to ruminate for longer periods than

medium- and high-ranking goats ($F_{2,91} = 2.77$, $p = 0.07$). The mean rumination time for high-ranking introduced goats was 6.09 hours/24 hours (SD: 1.69) during the control measurements and 3.03 hours/24 hours (SD: 2.17) during the introduction period, implying a decrease of around 50% between the control measurements and the introduction period. The equivalent values for medium-ranking goats are 5.66 hours/24 hours (SD: 0.82) during the control measurement and 3.68 hours/24 hours (SD: 1.58) during the introduction period (decrease of around 35%), whereas low-ranking goats spent 5.09 hours/24 hours (SD: 1.01) ruminating during control measurement and 4.32 hours/24 hours (SD: 1.89) during the introduction period (decrease of around 15%).

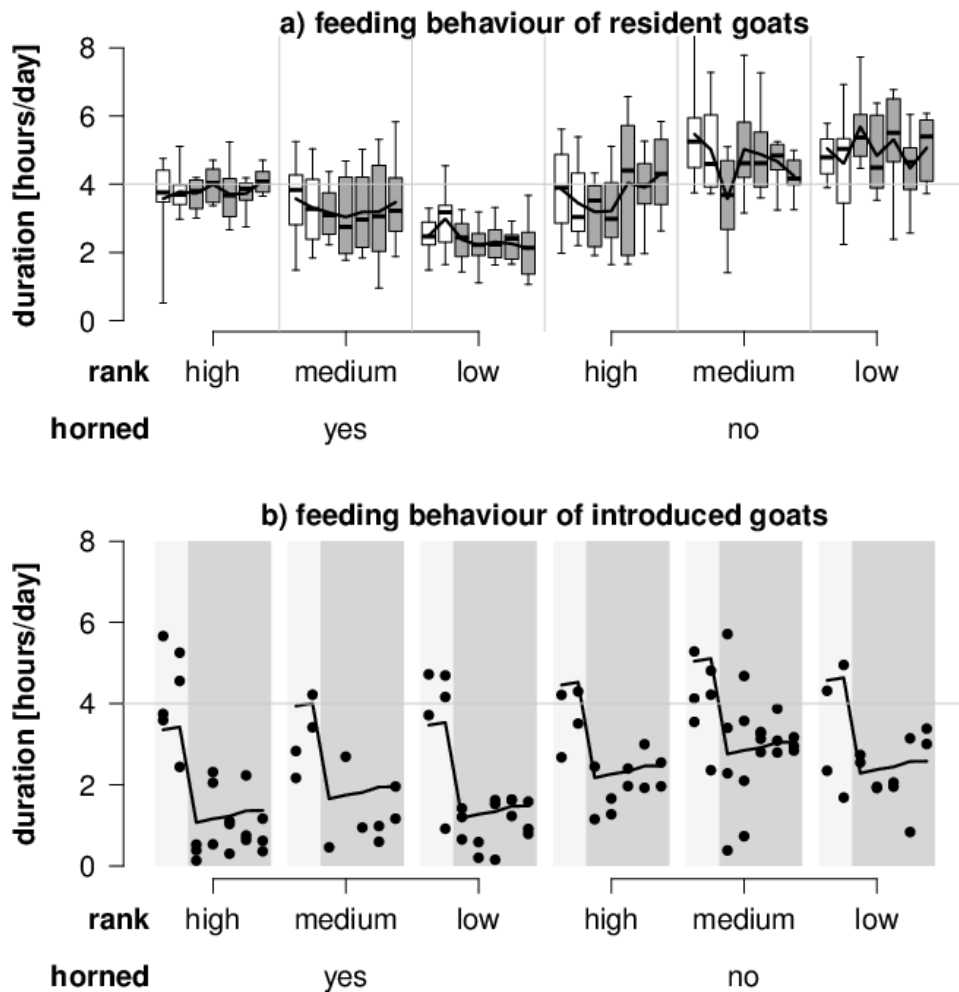


Figure 4: (a) Length of time (in hours) resident goats spent feeding for 24 hours.

Box-and-whiskers-plot, boxes = 1st and 3rd quartile, thick line = median, whiskers = range of data.

(b) Length of time (in hours) introduced goats spent feeding for 24 hours.

Filled circles = data of the individual goats of the indicated horn-status and rank category per day before/after introduction.

White boxes (resident goats (a)) or light-grey background (introduced goats (b)) = control situation before introduction (days -7 and -6); grey boxes (resident goats (a)) or grey background (introduced goats (b)) = introduction period (days 0-4). Solid lines indicate model estimates.

4.3.6 Cortisol metabolites

High-ranking resident goats exhibited higher cortisol metabolites concentrations than medium- and low-ranking animals (Figure 5a). Despite this, the introduction of unknown individuals had no influence on the concentrations of cortisol metabolites of resident goats. By contrast, the introduction led to an increase in cortisol metabolites in the introduced animals on days 1-4. After being returned to their original groups, the introduced goats' faecal cortisol metabolites concentrations returned to baseline concentrations by day 11 (maximum increase on day 2 compared to day -4: +63%, Figure 5b). High-ranking horned individuals had higher concentrations of cortisol metabolites than high-ranking hornless ones. The same was true for low-ranking goats. For medium-ranking goats, however, hornless animals had higher cortisol metabolites concentrations than horned individuals. In general, the level of increase of faecal cortisol metabolites in horned high-ranking introduced goats was higher than for both horned medium- and low-ranking goats and hornless goats (Figure 5b).

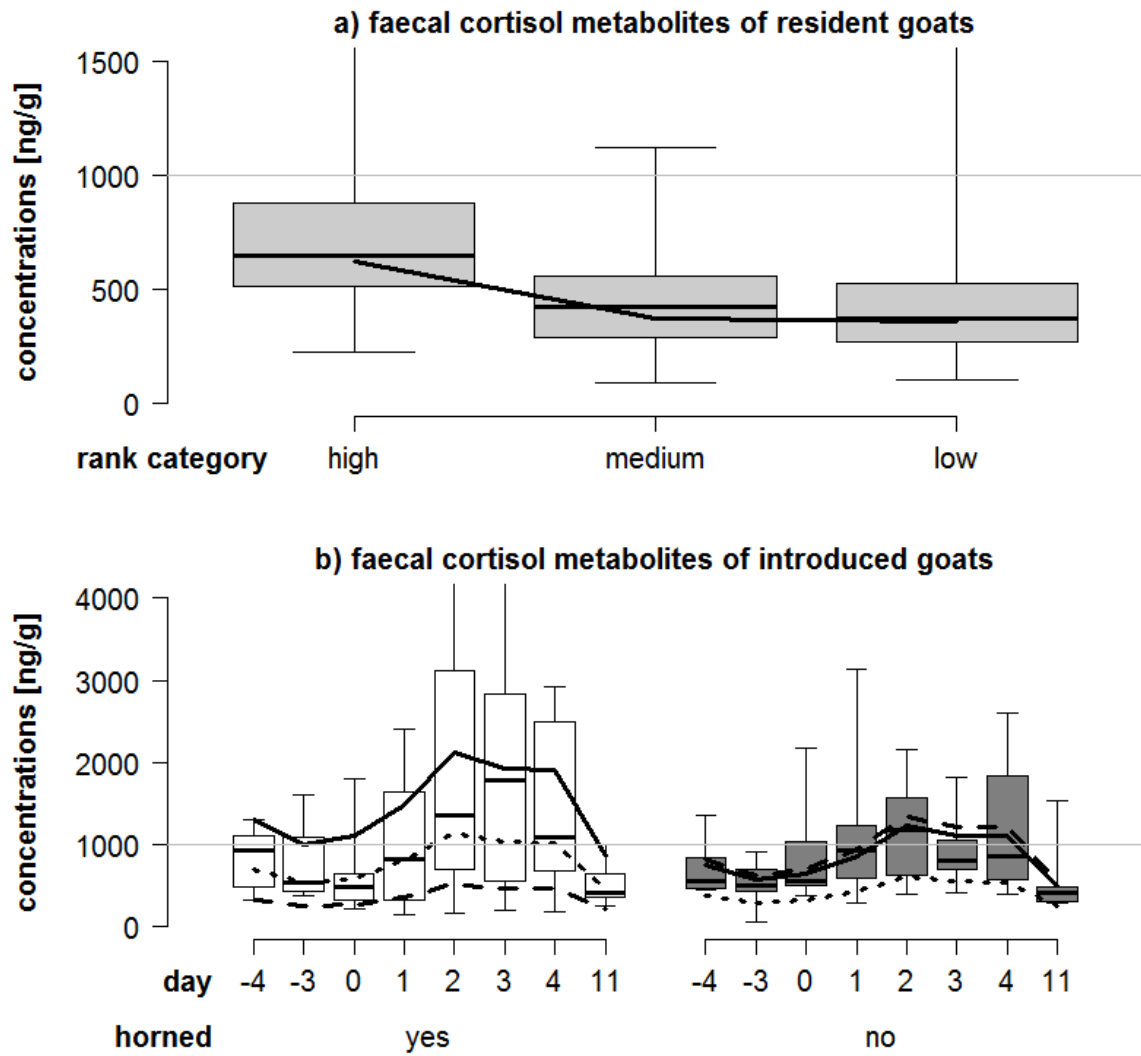


Figure 5: (a) Concentrations of faecal cortisol metabolites [ng/g] of resident goats with respect to rank category.

Solid line = model estimate averaged for horned and hornless goats.

(b) Concentration of faecal cortisol metabolites [ng/g] of introduced goats (white boxes) and hornless goats (black boxes) with respect to day and presence of horns.

Solid lines = model estimates for high-ranking goats; dashed lines = model estimates for medium-ranking goats; dotted lines = model estimates for low-ranking horned and hornless goats.

Box-and whisker-plots, boxes = 1st and 3rd quartile, thick line = median, whiskers = range of data.

4.3.7 Injuries

The risk of injuries in introduced goats was not influenced by day ($\chi^2_4 = 3.94$, $p = 0.41$).

In total, 29 injuries were recorded, 21 in introduced goats and eight in resident goats. Of the eight injuries to resident goats, three were of medium-ranking animals and five of low-ranking ones. For the introduced goats, four injuries occurred in high-ranking, eleven in medium-ranking and six in low-ranking goats. Twenty-one of the 29 injuries occurred in hornless and eight in horned goats. Injuries consisted of 11 haematomas, 17 abrasion injuries and one cracked horn. Of these injuries, 11 occurred on day 0, two on day 1, eight on day 2, and four on both days 3 and 4. Twenty-six injuries were in the head/cervical area, three close to the vulva. In hornless goats, all injuries were to the head/cervical region, especially at the base of the horns. The three injuries near the vulva were found in horned goats. A total of five injuries in four goats were recorded when the animals were examined to determine the status quo on day -1. All injuries (two encrusted abrasions, one haematoma and two abrasions) were to the head and cervical area.

4.5 Discussion

In this study, we assessed the effects of introducing an individual goat into an established group on the welfare of both resident goats and the introduced goat. We were interested in the development of variables over the course of the introduction period, potential differences between horned and hornless goats, and differences due to rank category. It was discovered that whilst the resident goats were only slightly affected by the introduction of a new goat, there were nevertheless serious negative effects for the introduced goats throughout the entire introduction period. Horned goats and especially those that had been high-ranking in their group of origin appeared to be more affected.

The lack of effect on resident goats of the introduction of a new goat could be seen in the group's virtually unchanged social, lying and feeding behaviour. Aggressive interactions between resident goats and the introduced goat occurred mainly on the first day of the introduction period and moreover appeared to be independent of the social rank of the introduced goat. Furthermore, injuries sustained by the resident goats were negligible, and the introduction of the individual goat had no effect on cortisol metabolites concentrations in resident goats. Viewed as a whole, resident goats did not appear to be adversely affected in welfare terms following the introduction of a new goat. This can be explained by the behavioural reactions of the introduced goats, who seemed intent on avoiding unnecessary contact with resident goats.

Introduced goats tended to isolate themselves from the group by hiding in the niche. Crowley and Grace (1988) reported that goats attempted to separate themselves in a similarly drastic fashion by jumping on top of hay racks or hiding under feeding boxes in a quarantine station where unfamiliar goats were assembled. Seemingly, the introduced goats had no other option but to take this self-isolating approach to minimise social interactions. Unlike free-range conditions, in which individuals can move away from their groups in response to a high rate of agonistic interactions, this option does not exist under housing conditions (Mendl and Held 2001). In some studies, integration is said to be

successfully accomplished once the amount of (physical) agonistic interactions drops to the pre-introduction level (Addison and Baker 1982, Fernández et al. 2007). Although the number of agonistic interactions and sniffing decreased significantly from day 1 after introduction onwards in our study, this would not appear to be synonymous with successful integration, particularly since introduced goats spent most of their time in the niche beneath the wooden platform, very rarely exhibited agonistic or affiliative behaviour, and differed greatly from both resident goats and their own control values in their feeding, ruminating and lying behaviour. Increased sniffing behaviour on day 0 shown by resident goats towards the introduced goats is likely to be an olfactory inspection rather than an affiliative form of social interaction, given that goats were mainly seen to sniff at the base of the other goats' horns where scent glands are located. Consequently, in the context of the introduction of a new individual, agonistic and sniffing behaviour must be considered along with other variables when assessing the degree and success of the introduced goat's integration.

The considerable increase in time spent lying, in particular in horned introduced goats, and the substantial reduction in time spent feeding and ruminating was closely connected with their isolation in the lying niche. According to Schwarz and Sambraus (1997), throughout a six-week observation period, the feeding durations of a group of introduced young goats never equalled those of the resident goats, but time spent feeding did increase consistently, reaching approximately 40% of the resident goats' times within one hour after feeding in the first week following introduction. Despite some slight improvement in feeding and rumination times for introduced goats in the course of the introduction period in the present study, the length of time spent feeding was far from a return to the control level. This restriction in feeding is highly relevant to the animals' welfare and a threat to their health, potentially causing ketosis (Stöber 2006). Since the goats in our study did not develop clinical signs of ketosis, however, it can be assumed that the onset of this metabolic disease was either delayed or remained subclinical, owing to the lower demand for energy from the (non-lactating) goats. Moreover, such a drop in

time spent feeding would interfere with milk production. Reduced performance has already been observed in studies on the re-grouping of goats (Fernández et al. 2007) and sheep (Sevi et al. 2001), as well as after the introduction of cows (Brakel and Leis 1976, von Keyserlingk et al. 2008).

In addition, the increased levels of cortisol metabolites throughout the introduction period demonstrated the considerable extent to which newly introduced goats were affected by the experimental procedure. This stress response could have been a response not only to altered feeding and lying behaviour but to social stress as well. Although the number of agonistic interactions experienced by the introduced goats decreased, these animals came out the losers in almost all of said interactions. Being continuously exposed to potential interactions and constantly defeated in these interactions was likely to be perceived as stressful by the goats, as was shown to be the case with rats in a study by Zelena et al. (1999). Nevertheless, in our study, after the introduced goats were returned to their original group, concentrations of faecal cortisol metabolites reliably returned to baseline concentrations within a week. It therefore seems that the five-day introduction period failed to result in a long-lasting increase in glucocorticoid concentration beyond the duration of the stressor.

The fact that the concentrations of faecal cortisol metabolites increased from day 1 onwards but not on day 0 itself is very likely due to the time interval we chose between the time of introduction and the first sampling. According to Kleinsasser et al. (2010), faecal samples should be taken around 12 to 15 hours after the stressor is experienced. As the time interval we chose was at the lower limit, it is likely that the time lag was too short, and should therefore be extended to at least 13 hours in further studies.

Hornless introduced goats seemed to suffer fewer adverse effects from the experimental procedure than their horned counterparts, initiating at least a few agonistic interactions with resident goats and spending less time in the niche. This difference between horned and hornless goats can be explained by their differing strategies with respect to agonistic behaviour. Whereas in horned goats agonistic

interactions without physical contact predominated, those with hornless goats more often involved physical contact (Aschwanden et al. 2008b). Consequently, hornless goats probably did not need to isolate themselves to the same extent as horned goats, leading to less dramatic changes especially in lying duration for the former.

Since introduced goats without horns sustained more injuries than those with horns, one might be tempted to conclude that hornless introduced goats were more adversely affected by the introduction. This, however, does not tally with the time spent in the niche, the lying and feeding behaviour or the cortisol metabolite concentrations. Presumably, this contradiction is once again caused by the above-mentioned differences in agonistic behaviour between horned and hornless goats. Hornless goats' interactions are accompanied by more physical contact, and in the case of fights and head butting, they are at considerable risk of injury, particularly to the head (unlike their horned counterparts, whose horns protect them from direct blows to the head). On the other hand, only horned goats sustained injuries on the vulva, which are potentially more serious in terms of economic value. Regarding injuries in general, although it is difficult to weigh up the relevant consequences for the goats' health, it is nevertheless important to analyse these consequences, as each injury could have a major impact on the welfare of the affected individual.

Whereas the extent of the increase in cortisol metabolites concentrations was similar in horned medium- and low-ranking individuals as well as in hornless high-, medium- and low-ranking ones, the increase was substantially greater in horned high-ranking goats. One reason for this might be that introduced goats with horns and a high dominance ranking were faced with the greatest discrepancy between their previous experiences in the original group and those after their introduction into a new group where there were heavy restrictions on their feeding, ruminating and resting behaviour and where they came out the losers in most of the agonistic interactions.

Most of the variables we tested seemed to be suitable for reflecting the adverse effects on welfare associated with the introduction of individual goats into established groups. However, agonistic and sniffing behaviour on their own appear to be less suitable indices, as the considerable decrease in this behaviour after day 0 might lead to the erroneous impression that adverse effects on welfare were limited to day 0. Due to the sharp decrease in feeding time, time spent ruminating was also reduced, so the latter could not give us any additional information. It is likely, however, that a gentler introduction to the group (e.g. on pasture where the resource food is widely distributed and food intake should not be limited for introduced goats) would not reduce rumination behaviour to the same extent, allowing it to act as a useful variable.

In conclusion, our results show that goats introduced individually into a small herd suffered impaired welfare, as clearly shown by most of the investigated variables. Introduced goats, for example, were on the receiving end of a relatively high number of agonistic behaviours from resident goats on day 0, emerged as losers in most of these confrontations, spent most of their time during the introduction period in the niche, and considerably increased the length of time they spent lying whilst substantially decreasing the amount of time spent feeding and ruminating. The increased concentrations of faecal cortisol metabolites found was indicative of an activation of the physiological stress axis. Lastly, introduced goats were at significant risk of being injured. By contrast, variables for resident goats scarcely changed after the introduction of the new unknown goat, indicating how little they were affected by the latter's introduction. It is essential, therefore, that acceptable methods be found for introducing individual goats into established small groups.

5 Behavioural and physiological reactions of goats confronted with an unfamiliar group either when alone or with two peers

Based on:

Patt A, Gygax L, Wechsler B, Hillmann E, Palme R, Keil NM (2012)

Applied Animal Behaviour Science 146, 56-65

Abstract

When introduced into a new herd, goats are confronted with unfamiliar animals. Their behavioural and physiological reactions during this confrontation are likely to differ depending on the presence or absence of familiar conspecifics (peers). To assess these reactions, we confronted 12 goats both alone and with two peers (confrontees) with established groups ($n = 4$ groups) consisting of goats unfamiliar to the confrontees (unfamiliar goats) ($12 \text{ goats} \times 2 \text{ confrontations} = 24$ confrontations in total). Each confrontation lasted for one hour. Agonistic interactions, sniffing behaviour and level of activity were recorded throughout the confrontations. In addition, concentrations of cortisol metabolites were measured in faecal samples taken in the evening before the confrontation and three successive samples after the confrontation. Before the start of the experiment, we evaluated the dominance relationships of the involved goats within their respective housing groups by direct observations made during the main feeding times. Data were analysed using generalised linear mixed-effects models with the fixed effects presence of peers (yes, no), rank category (high, medium, low) and repeated confrontation (numeric variable). For the analysis of activity level and concentrations of faecal cortisol metabolites, period (minutes 0-15, 16-30, 31-45, 46-60) and sample (control, 13, 14, 15 hours after the confrontation), respectively, were included as additional fixed effects. Unfamiliar goats directed fewer agonistic interactions towards confrontees when the latter were accompanied by peers compared to when they were alone (without peers: 57, with peers: 20 interactions per animal and confrontation). The same was true for the proportion of agonistic

interactions involving physical contact (without peers: 69, with peers: 53%) and the number of sniffing behaviours (without peers: 16, with peers: 9 interactions per animal and confrontation). On the other hand, confrontees with peers were more likely to direct agonistic and sniffing behaviour towards unfamiliar goats than those on their own. Confrontees with peers had lower concentrations of faecal cortisol metabolites after confrontations (without peers: 273, with peers: 198 ng/g). For confrontees (with and without peers), activity level was highest during the first 15 minutes of the confrontation and decreased over its course. For the unfamiliar goats, the activity pattern was similar but was modulated by rank, with higher values for low-ranking goats than for medium- and high-ranking ones. In conclusion, our results indicate that the presence of peers is advantageous for goats being introduced into groups of unfamiliar goats.

Keywords

goat, grouping, introduction, social support, agonistic interactions, social behaviour, stress

5.1 Introduction

The introduction of individual goats into small established groups has considerable negative consequences for the introduced goat's welfare, including a substantial increase in lying duration (more than 20 hours per day) and reduced feeding duration, as well as elevated levels of cortisol metabolites. Additionally, introduced goats received a considerable number of agonistic interactions on the first day of the introduction period (Patt et al. 2012 (chapter 4)). It is not known, however, whether these effects can be mitigated by introducing a goat together with familiar conspecifics (peers).

This question has been addressed for dairy cows in several studies (O'Connell et al. 2008, Gygax et al. 2009, Neisen et al. 2009) which aim to reduce the negative effects of introducing unfamiliar individuals, such as increased levels of aggression, impaired lying and feeding behaviour, and reduced performance (Brakel and Leis 1976, von Keyserlingk et al. 2008). In our opinion, the presence of conspecifics could benefit the animals being introduced, either by providing social support, i.e. enhancing their coping ability (reviewed by Rault 2012), and/or via a dilution effect, in which a more-or-less constant number of agonistic interactions is distributed equally among several animals, instead of being directed against just one (Neisen et al. 2009). Indeed, Neisen et al. (2009) reported fewer agonistic interactions after the introduction of a pair of heifers than after the introduction of just one. Further, heifers introduced as pairs were found to spend more time in the lying area (Gygax et al. 2009) and to spend more time lying when most animals of the herd were lying too (O'Connell et al. 2008). For dairy cows, therefore, the presence of conspecifics seems to be a promising approach to reducing the negative effects associated with introducing a new animal.

In studies on the consequences of regrouping sheep and cows (Sevi et al. 2001, Schirmann et al. 2011) it was also found that adverse effects were stronger for animals having to deal with relocation to a new environment in addition to the grouping, whilst remaining in the home pen appeared to give residents an advantage over the relocated individuals. These two aspects potentially apply to

both the residents' and the introduced animals' perspective and are must be considered as confounding factors. Consequently, in order to specifically test the effects of the presence of familiar conspecifics during social confrontations, it is essential to use a place that is equally familiar to all of the involved animals. In the present study, we assessed whether the presence of familiar conspecifics reduces negative effects during social confrontations with unfamiliar goats. The confrontations took place in a neutral arena, so as to exclude effects of residency and relocation to a new environment as potentially confounding factors. We were interested in the behavioural and physiological responses caused by confrontation both with and without peers. Moreover, we investigated whether these responses were modulated by the social rank of both confrontees and unfamiliar goats. We addressed these questions by consecutively confronting goats (= confrontees) which were either alone or with two peers, with an established group of six goats (= unfamiliar group). We expected confrontees with peers to be less affected by the confrontation than those introduced on their own. This might potentially be reflected in their being on the receiving end of fewer agonistic interactions, as well as in lower levels of activity and of faecal cortisol metabolites. Furthermore, we anticipated that unfamiliar goats would react differently when faced with an individual confrontee as opposed to a confrontee accompanied by two peers.

5.2 Methods

5.2.1 Animals and housing conditions

Four established groups of six (= unfamiliar groups) and four groups of three (= confrontees) horned, non-lactating female goats were used in the experiment. Confrontees were either confronted with an unfamiliar group individually or in the presence of the two peers with which they were housed. The goats had been grouped prior to the study in early March 2011 from individuals of various Swiss milking breeds (Saanen, Toggenburger, St Gallen Booted, Grisons Striped, Peacock, Nera Verzasca and Valais Blackneck) and their crossbreeds. Two goats were part Anglo Nubian. The animals were born between 2003 and 2009, and the experiment was conducted from May to July 2011. As far as possible, group composition was balanced in terms of breed, age and weight.

All groups were housed in the same building in identical pens, and had acoustic and visual contact as described in Patt et al. (2012) (chapter 4). Two groups of unfamiliar goats were each housed adjacent to a group of confrontees, but separated by a wooden wall to prevent tactile contact. The total area of each pen was 15.3 m² (approximately 3 m × 5 m), consisting of a deep-bedded straw area of 11.7 m² and an elevated feeding place (3.6 m²) divided by a wooden wall into two equal-sized compartments. The deep-bedded area of each group of unfamiliar goats and each group of confrontees was further structured by a wooden platform and a freestanding partition providing climbing opportunities, and both elevated and protected lying areas. Hay was provided ad libitum in the feeding area from a 3 m hayrack refilled twice daily at around 8.45 am and 5 pm. One water trough, one licking stone and a brush were provided in each pen.

5.2.2 Dominance relationships

Shortly before the start of the experiment, the dominance relationships of the goats in each group ('unfamiliar' goats and 'confrontees') were evaluated by direct observation during morning and evening feeding times according to the method used by Aschwanden et al. (2008b). With the help of the rank index (between

0 = omega and 1 = alpha), each goat was categorised as either low- (0.0-0.2), medium- (0.4-0.6) or high-ranking (0.8-1.0).

5.2.3 Confrontations

Based on the results of a previous study (Patt et al. 2012 (chapter 4)) confrontations of unfamiliar goats lasting longer than a few hours had serious effects in terms of animal welfare. Additionally, confrontations of unfamiliar goats may bear a risk of being injured. However, in the study of Patt et al. (2012) (chapter 4) the registered injuries were only mild. Thus, we considered confrontations lasting one hour to be acceptable for the present study and were prepared to terminate confrontations if a goat was attacked with risk of being injured. As the experimenter was present during all confrontations, interference could have been instantly but in effect was never required. The experiment was approved by the Cantonal Veterinary Office (Frauenfeld, Thurgau, Switzerland, Approval F4/09).

Twenty-four confrontations were initiated, each of which lasted for one hour. One-half of these confrontations (= 12) was initiated between a single confrontee and an unfamiliar group, whilst the other half (= 12) was between a confrontee accompanied by two peers, and an unfamiliar group. Each of the four unfamiliar groups was faced with three of the four groups of confrontees, as well as with each confrontee of the fourth group (six confrontations per unfamiliar group). This fourth group of confrontees was a different one for each unfamiliar group, so that each confrontee was confronted on her own once. Each confrontee therefore experienced a total of four confrontations (3 × with peers, 1 × without). The order of confrontations with or without peers was balanced across the 24 initiated confrontations from the viewpoint of both the confrontees and the unfamiliar group. Furthermore, each group/goat had a break of at least one day in between two confrontations.

5.2.4 Experimental room and experimental procedure

The confrontations took place between 9-10 am in a separate indoor arena about 150 m from the home pens. To habituate the goats to the experimental room, each group was taken to it four times over the two weeks preceding the start of the experiment, and was allowed to explore this indoor arena for an hour each time. By the end of the habituation, goats followed the experimenter readily into the arena and moved calmly within the arena. Further, vocalisation was reduced considerably over the habituation period. Hay was offered during the first two habituation sessions. Afterwards and during confrontations, and so as to eliminate resource-based aggression, goats were not offered any food.

During confrontations, space per goat was kept at a constant 3 m². Consequently, the arena measured either 21 m² (5 m × 4.2 m) when the confrontee was confronted on her own, or 27 m² (5 m × 5.4 m) when she was confronted in the company of her two peers. The arena was further structured by two freestanding partitions identical to those in the goats' home pens (approximately 1 m in diameter and 0.8 m in height). These partitions allowed the goats to avoid each other more effectively during confrontations, similarly to such structural elements in loose housing that have been shown to effectively reduce agonistic interactions between goats (Aschwanden et al. 2009a). On the confrontation days, the unfamiliar group was always led into the arena first. A few minutes later, the corresponding group of confrontees was led into the arena, and either all three goats were confronted (= with peers), or two goats were led back and the remaining confrontee was confronted alone (= without peers) with the unfamiliar group.

5.2.5 Data recording

To assess the effects associated with confronting a goat with or without peers, we measured the frequency of social interactions, activity values, and concentrations of faecal cortisol metabolites. For faecal cortisol metabolites, control values were collected on the day before each confrontation (day -1). Social interactions were

recorded individually for all goats involved in the confrontations, whilst activity values and concentrations of cortisol metabolites were recorded in focal goats. In the 'unfamiliar' groups, the same three focal goats representing the three rank categories (high, medium and low) were chosen for recordings in all confrontations. In the 'confrontee' groups, each of the three goats served as a focal animal once during the three confrontations with peers.

5.2.5.1 Agonistic and affiliative interactions

During confrontations, the social behaviour of all goats was monitored continuously by direct observation. For agonistic interactions, we distinguished between whether physical contact was involved (head butt, fight and explicit displacement) or not (threat and implicit displacement). Sniffing, scratching, licking and mock fighting were recorded as affiliative behaviours. The definitions of the recorded behaviours are given in Patt et al. (2012) (chapter 4). For each interaction, both initiator and recipient were noted. In the case of agonistic interactions, we also noted whether the initiator was successful, i.e. whether the recipient moved location. Thus, a goat lost an interaction if she was forced to leave her current location by another goat either explicitly (e.g. fight, butt, threat) or implicitly, e.g. after being approached by another goat which did not show obvious agonistic behaviour.

5.2.5.2 Activity values

To measure activity, we recorded acceleration values using a commercial 3D acceleration logger (MSR145WA, Modular Signal Recorder Electronics GmbH; 33 mm × 15 mm × 61 mm). The logger was attached to the goat's left-hind leg to minimise risk of injury when goats stepped onto freestanding partitions. Acceleration in the direction of the y-axis (= the axis parallel to the longitudinal axis of the goat's hind leg) was continuously recorded at a measurement range of twice the earth's gravity acceleration (= m/s^2) and a rate of 10 Hz. Due to the way the logger was attached to the goats' hind leg, acceleration values observed while standing quietly equalled -1 g. When the leg was moved, acceleration values

reached both higher and lower values than the -1 g. We were only interested in deviations from the value at rest and we thus added +1 to the original values and then took their absolute values. These latter values were summed across the time period of interest (basically calculating an area under the curve).

Before the start of the experiment, we validated activity values with respect to different levels of activity. The increasing level of activity from 'standing' to 'walking regularly interrupted by halts' to 'continuous walking' was reliably reflected in the activity values. The lower (0.25) and upper (0.75) quartile of activity values reached 6.68-13.34 summed gravity acceleration/minute, 20.73-34.21 summed gravity acceleration/minute and 83.73-104.00 summed gravity acceleration/minute for standing, interrupted walking, and continuous walking, respectively.

During confrontations, acceleration values were recorded with the acceleration logger, using the same settings (2 g, 10 Hz) and position on the goat's left-hind leg. To take into account changes of activity over time, the confrontation was divided into four successive 15-minute periods (period 15, period 30, period 45, period 60) for analysis. The acceleration values were calculated for each 15-minute period, providing an activity value per 15 minutes (summed gravity acceleration/15 minutes [m/s^2]).

5.2.5.3 Cortisol metabolites

To monitor an acute stressor by measuring faecal cortisol metabolites, samples should be collected within 12-15 hours after the event in question (Kleinsasser et al., 2010). As delay times vary between 12 and 15 hours, samples should be taken several times in succession to allow measurement of the effect of a short-term stressor (i.e. one hour in our case). Samples were therefore collected 13, 14 and 15 hours after the start of a confrontation beginning at 10 pm. To account for a possible circadian rhythm of cortisol levels, faecal samples were also taken at 10 pm on the control day (day -1). The samples were collected manually from the animal's rectum, with the goats being successively attached to the hayrack using a

halter. Each sample was immediately put into a cooling box until the sampling was completed. Afterwards, all samples were frozen and stored at -20°C until analysis. The concentrations of faecal cortisol metabolites were determined by a group-specific 11-oxoaetiocholanolone enzyme immunoassay (EIA, Möstl et al. 2002). This EIA has been successfully validated for monitoring adrenocortical activity in goats (Kleinsasser et al. 2010).

5.2.6 Statistical analysis

5.2.6.1 Model selection

In order to adequately reflect dependencies in the experimental design (nesting, repeated measurements), generalised linear mixed-effects models were used to evaluate the outcome variables. Statistical analysis was performed in R (version 2.14.1, R Development Core Team 2011) using the lmer and glmer methods from the lme4 package (Bates et al. 2011), as well as the function dredge of the MuMIn package (Barton 2012) to perform all subset analyses. The statistical approach taken here is based on the use of Akaike's information criterion (AIC) which provides probabilities for each of several concurrent models given the data (Burnham et al. 2011, Garamszegi 2011, Symonds and Moussalli 2011). In our case, AIC was further corrected for small sample sizes (AIC_c). For each outcome variable, an all-subsets analysis was conducted, ranging from the minimal model including a constant (intercept) only (Burnham et al. 2011, Dochtermann and Jenkins 2011) to the model including all fixed effects and their interactions. The former model corresponds to the null hypothesis that no explanatory variable has an influence, and that the responses vary randomly around a general mean. The choice among the different models was based on the Akaike weight (w_i), which can be interpreted as the probability of a given model to fit the data best within the set (all weights together add to one). For all outcome variables, the optimal model based on the Akaike weight (w_i) is shown in Table 1 and was at least 1.3 times more likely than the next best model. If models with a similar mode probability were nested and had similar AIC_c values, we followed the advice of choosing the

simpler model (Richards et al. 2011). I.e. for Agonistic U-U and Cortisol U, we chose the second best model with model probabilities of 0.91 and 0.86 in relation to the best model.

Model selection is thus based on the models' relative fit within the set given the data. To visualise the relative strength of the best fitting model within the set, we also report the evidence ratio of the chosen model in comparison to the null model (E_0 in Table 1). Thus, ER_0 provides a measure of how much more likely the best fitting model is than the null model (Symonds and Moussalli 2011). This statistical approach takes into account that any model is only an approximation of the hypothesis investigated. Thus single fixed effects are no longer 'significant', but the chosen model as a whole represents the approximation that is most likely to explain the obtained data and has to be presented. By considering effect sizes of fixed effects it can be decided whether or not the observed changes are biologically relevant (Symonds and Moussalli 2011).

5.2.6.2 Outcome variables, random and fixed effects

Social behaviour was analysed from the recipient's point of view. Three types of social interactions were distinguished: (a) both recipient and initiator were unfamiliar goats; (b) the recipient was a confrontee or a peer, and the initiator was an unfamiliar goat; and (c) the recipient was an unfamiliar goat, whilst the initiator was a confrontee or a peer. Since most types of agonistic interactions (head butts, fights, explicit displacements, threats and implicit displacement) occurred too rarely to be analysed separately, they were analysed under the comprehensive term 'agonistic interactions'. Most of the various sorts of affiliative interactions included in the ethogram were observed only sporadically. The exception was sniffing, which was therefore analysed.

The following were analysed as outcome variables (for information regarding transformation of the different variables, see Table 2):

- Number of agonistic interactions received by unfamiliar goats from other unfamiliar goats (Agonistic U-U, abbreviations as used in the tables) over the course of one confrontation (no./animal/confrontation),
- Number of agonistic interactions (Agonistic U-C) and number of sniffing interactions (Sniffing U-C) received by the confrontee or one of her peers from unfamiliar goats over the course of one confrontation (no./animal/confrontation),
- Proportion (%) of agonistic interactions lost by a confrontee or peer in relation to all classifiable agonistic interactions received by a confrontee or one of her peers from an unfamiliar goat (Agonistic lost U-C),
- Proportion (%) of agonistic interactions involving physical contact in relation to all agonistic interactions received by a confrontee or one of her peers from an unfamiliar goat (Agonistic physical U-C),
- Whether agonistic interactions (Agonistic C-U) and sniffing (Sniffing C-U) were received by an unfamiliar goat from a confrontee or one of her peers,
- The activity values (summed gravity acceleration/15 min) of focal unfamiliar goats (Activity U) and the confrontee (Activity C) for each period,
- The concentrations of faecal cortisol metabolites (ng/g faeces) of focal unfamiliar goats (Cortisol U) and the confrontee (Cortisol C) using each sample taken at 13, 14 and 15 hours after confrontations individually.

In all models, random effects consisted of the recipients being either confrontees or unfamiliar goats nested in their housing group and crossed with the group identity of the unfamiliar group or confrontees' group, respectively. For activity values and concentrations of cortisol metabolites, the sequence number of the confrontation was also nested within recipient.

Model assumptions were verified using graphical analysis of residuals focusing on normality of errors and random effects as well as homoscedasticity of the errors in

the case of normally distributed errors, and on normality of random effects and absence of bias in the mean errors for the generalised models.

The presence of peers (factor with two levels: yes, no), rank category (factor with three levels: high, medium and low) and the fact that goats were confronted repeatedly (repetition as a numeric variable) were explanatory variables in all models. A fourth explanatory variable was added in some models: period (factor with four levels: period 15, 30, 45 and 60) in the case of activity values, and sample (factor with four levels: control, 13, 14 and 15 hours after confrontation) in the case of faecal cortisol metabolites.

Table 1: Criteria used to select the best-fitting model of each outcome variable analysed.

Outcome variable¹	Selected models²	AIC_c³	w_i⁴	ER₀⁵
Agonistic U-U (no./animal/confrontation)	Presence of peers × rank + repetition	237.30	0.31	> 62.00
Agonistic U-C (no./animal/confrontation)	Presence of peers + repetition	110.00	0.64	> 128.00
Agonistic lost U-C (proportion, %)	Presence of peers	177.30	0.34	26.15
Agonistic physical U-C (proportion, %)	Presence of peers	153.60	0.51	> 102.00
Sniffing U-C (no./animal/confrontation)	Presence of peers	109.80	0.63	3.94
Agonistic C-U (yes/no)	Presence of peers + repetition	163.50	0.31	> 62.00
Sniffing C-U (yes/no)	Presence of peers	195.70	0.40	2.50
Activity U (m/s ²)	Presence of peers + rank × period + repetition	289.20	0.12	> 24.00
Activity C (m/s ²)	Period + repetition	173.70	0.48	> 96.00
Cortisol U (ng/g)	Presence of peers × repetition	276.80	0.12	6.00
Cortisol C (ng/g)	Presence of peers + rank	110.10	0.29	> 58.00

¹ U-U: initiator = unfamiliar goat, recipient = unfamiliar goat; U-C: initiator = unfamiliar goat, recipient = confrontee/peers; C-U: initiator = confrontee/peers, recipient = unfamiliar goat; U = unfamiliar goat, C = confrontee.

² Fixed effects included in the best-fitting model.

³ Akaike's Information Criterion value corrected for small sample sizes.

⁴ w_i: Akaike weight which can be interpreted as the probability of a given model to fit the data best within the presented set.

⁵ ER₀: Evidence ratio between the chosen model and the null model (including the intercept only).

5.3 Results

5.3.1 Qualitative observations

Confrontations were characterised by many short intervals of high levels of activity and agonistic interactions. During these intervals, which had no recognisable trigger, unfamiliar goats directed many agonistic interactions towards confrontees and/or their peers, and chased them around. Unfamiliar goats often ran towards confrontees and/or their peers at full speed and butted them. Although many agonistic interactions that unfamiliar goats initiated against the confrontee or one of her peers involved physical contact, the mean number of fights was low with only 3.5 initiated fights per confrontation. In between these intervals, confrontees and peers tended to remain mostly along the outer wall of the arena, in corners, or on the wooden partitions. Where peers were present, they and the confrontees tended to stay close together, often maintaining bodily contact. During confrontations, goats were almost never observed to lie down. The 24 confrontations resulted in a total of eight injuries (seven abrasions and one haematoma) on five different goats (four confrontees and one unfamiliar goat).

5.3.2 Effects of the presence of peers (yes vs. no)

Whether or not a peer was present appeared to be important for all outcome variables, except for the activity of confrontees (Table 1: Activity C). Confrontees were on the receiving end of the greatest number of agonistic interactions per confrontation initiated by unfamiliar goats - around 54 - in the absence of peers. Accordingly, the number of instances of both agonistic interactions and sniffing behaviour was substantially lower for confrontees with peers as opposed to those on their own (Table 2: Agonistic U-C, Figure 1a and Sniffing U-C, Figure 1b). When looking at proportions rather than the absolute number of agonistic interactions, the proportion of agonistic interactions involving physical contact in relation to all agonistic interactions that were directed by unfamiliar goats towards confrontees and/or their peers was slightly lower where confrontees were with peers rather than alone (Table 2: Agonistic physical U-C, Figure 2a). Although the proportion of

agonistic interactions from which the confrontees and/or their peers emerged as losers in relation to all agonistic interactions was generally high, it was even higher when confrontees were confronted together with peers as opposed to without (Table 2: Agonistic lost U-C, Figure 2b). Moreover, although the activity values of the confrontees did not vary depending on whether or not they were accompanied by peers (Activity C), concentrations of faecal cortisol metabolites were lower when peers were present (Table 2: Cortisol C).

Looking at the agonistic interactions initiated by confrontees and/or peers, it is apparent that very few unfamiliar goats were on the receiving end of agonistic interactions initiated by an unaccompanied confrontee (Figure 3a). The proportion of unfamiliar goats receiving agonistic interactions were much higher when confrontees were accompanied by peers as opposed to being confronted on their own (Table 2: Agonistic C-U). Confrontees and/or peers directed sniffing behaviour towards a higher proportion of unfamiliar goats than agonistic interactions.

Moreover, there was a greater probability of confrontees sniffing at unfamiliar goats when the former were accompanied by peers rather than alone (Table 2: Sniffing C-U, Figure 3b). Fewer agonistic interactions were directed against low-ranking unfamiliar goats by other unfamiliar goats when confrontees were accompanied by peers than when they were alone (Table 3: Agonistic U-U). Furthermore, the activity values of unfamiliar goats were somewhat higher when the confrontee was accompanied by peers (Table 2: Activity U). Whereas concentrations of faecal cortisol metabolites in the unfamiliar goats (Cortisol U) decreased between confrontations when the confrontee was alone, they remained constant when the confrontee was with peers. Thus, when the confrontee was alone concentrations were reduced from 379 ng/g [274; 509] during the 1st confrontation to 202 ng/g [148; 271] during the 6th confrontation. When the confrontee was with peers, concentrations during the 1st confrontation were 274 ng/g [201; 368] compared to 279 ng/g [206; 377] during the 6th confrontation.

Confrontation of goats either alone or with two peers

Table 2: Estimates and 95% confidence intervals¹ for the fixed-effects presence of peers of all outcome variables analysed with main-effects models.

Outcome variable	Transformation	Presence of peers	
		Without peers	With peers
Agonistic U-U ² (no./animal/confrontation)	log	-	-
Agonistic U-C (no./animal/confrontation)	log	58 [54; 62]	20 [18; 23]
Agonistic lost U-C (proportion, %)	logit	77 [43; 95]	92 [75; 98]
Agonistic physical U-C (proportion, %)	logit	69 [40; 90]	53 [27; 79]
Sniffing U-C (no./animal/confrontation)	log	16 [9; 26]	9 [6; 13]
Agonistic C-U ³ (yes/no)	logit link function	17 [8; 34]	54 [33; 73]
Sniffing C-U ³ (yes/no)	logit link function	43 [31; 56]	65 [49; 78]
Activity U ⁴ (m/s ²)	log	40 [27; 61]	46 [31; 70]
Activity C (m/s ²)	log	-	-
Cortisol U (ng/g)	log	-	-
Cortisol C (ng/g)	log	273 [186; 387]	198 [138; 282]

¹ all values rounded to zero decimal places.

² See Table 3 for estimates and 95% confidence intervals of the interaction between the fixed-effects peers and rank.

³ Using generalised models with binomial error distribution.

⁴ See Table 4 for estimates and 95% confidence intervals of the interaction between the fixed-effects period and rank.

Table 3: Estimates and 95% confidence intervals for agonistic interactions between unfamiliar goats (Agonistic U-U; no./animal/confrontation).

Presence of peers × Rank		
High	Medium	Low
Without peers		
1.1 [0.8; 1.5]	1.3 [0.9; 1.8]	7.7 [5.7; 10.5]
With peers		
0.9 [0.7; 1.3]	1.2 [0.9; 1.6]	3.4 [2.5; 4.6]

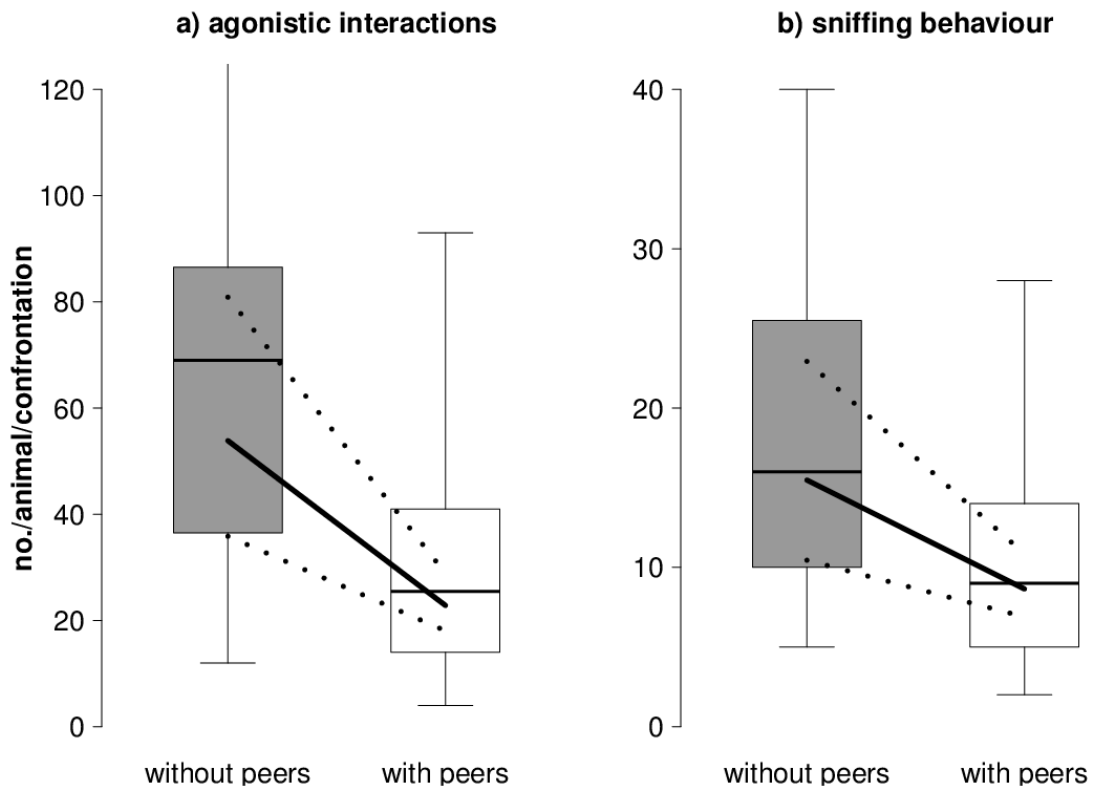


Figure 1: (a) Total number of agonistic interactions and (b) number of sniffing behaviours directed by unfamiliar goats towards confrontees confronted either individually (without peers) or accompanied by peers (with peers).

Box-and-whiskers plot: boxes = 1st and 3rd quartile, thick line = median, whiskers = range from minimum to maximum value. Solid lines = model estimates, dotted lines = 95% confidence intervals. Model estimate of agonistic interactions takes repeated testing into account.

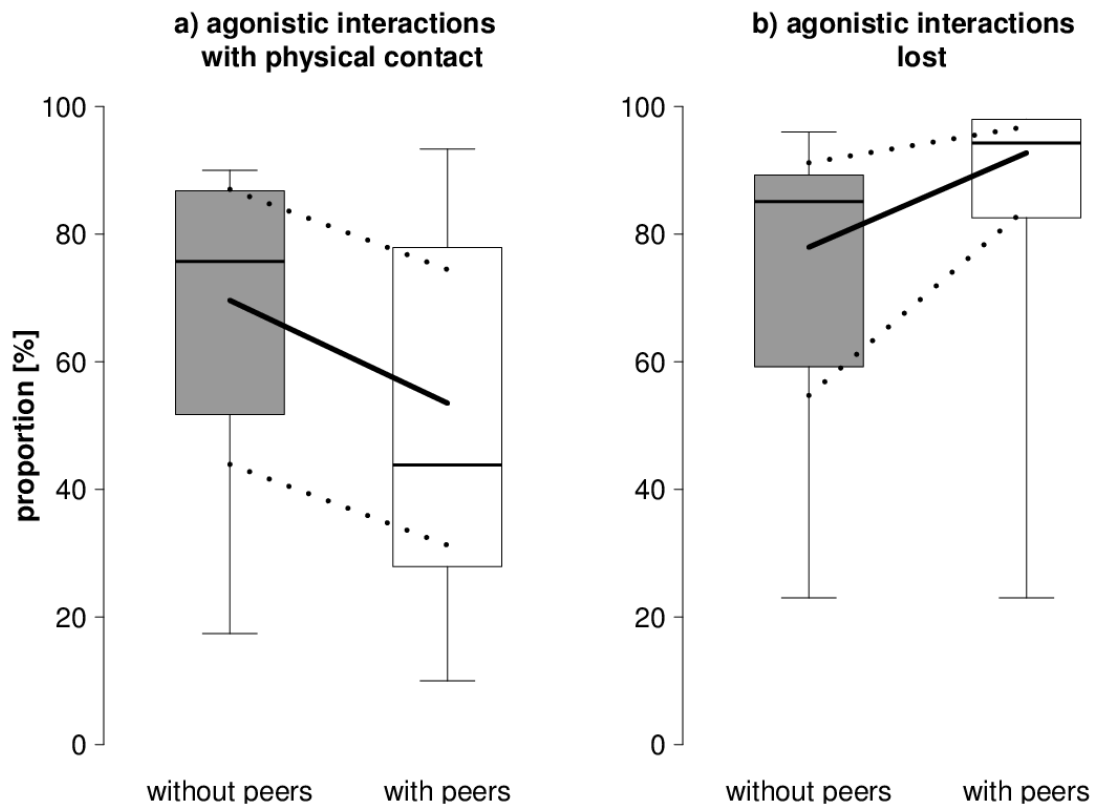


Figure 2: (a) Proportion [%] of agonistic interactions with physical contact and (b) proportion [%] of agonistic interactions lost by the confrontee or one of her peers out of all agonistic interactions directed by unfamiliar goats towards either individually confronted goats (without peers) or the confrontee or one of her peers (with peers).

Box-and-whiskers plot: boxes = 1st and 3rd quartile, thick line = median, whiskers = range from minimum to maximum value. Solid lines = model estimates, dotted lines = 95% confidence intervals.

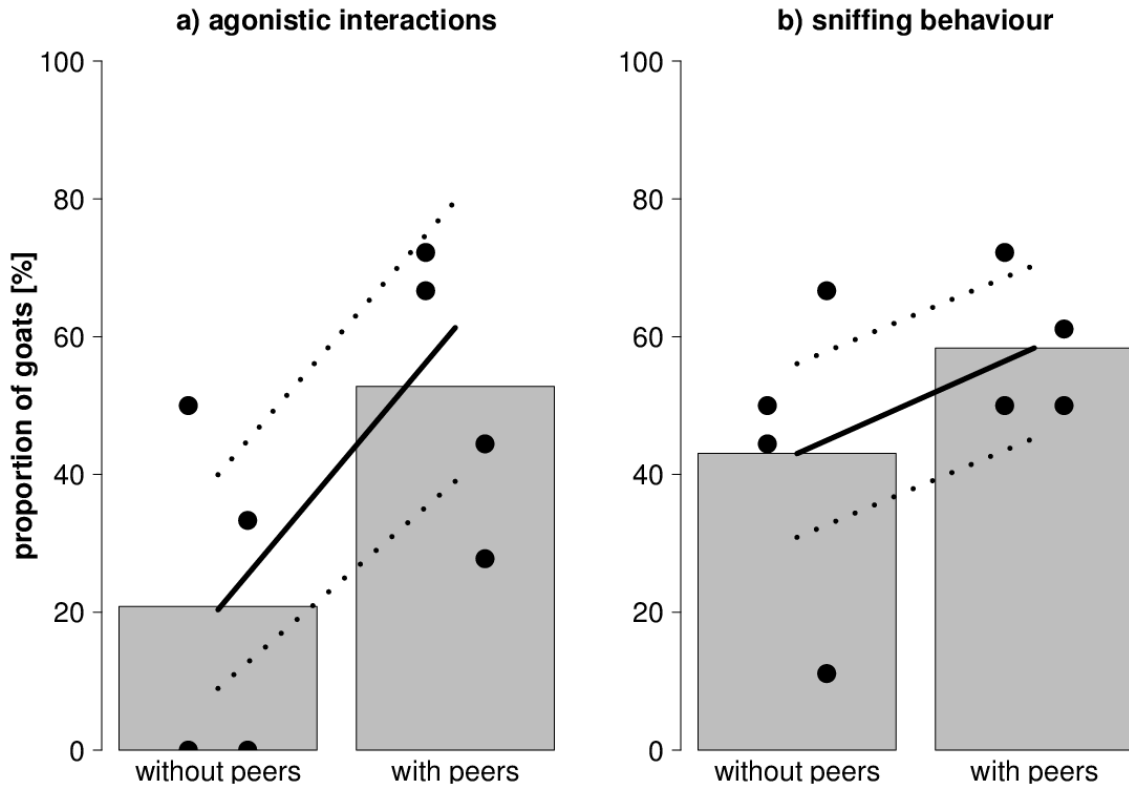


Figure 3: Proportion of unfamiliar goats towards whom (a) agonistic interactions and (b) sniffing behaviour were directed either by individually confronted goats (without peers) or by confrontees accompanied by peers (with peers). *Filled circles = proportion in the four experimental groups. Solid lines = model estimates taking repeated testing into account, dotted lines = 95% confidence intervals.*

5.3.3 Effects of repetition

Apart from the unfamiliar goats' cortisol metabolites concentrations (Cortisol U), where the effect of repetition interacted with presence of peers, repetition was included as a main effect in the best-fitting models of some other variables. Being confronted repeatedly reduced the number of agonistic interactions initiated by unfamiliar goats and experienced by confrontees and/or peers (Agonistic U-C, a reduction from 44 interactions [23; 84] during the 1st confrontation to 18 interactions [9; 34] during the 4th confrontation), as well as reducing the proportion of unfamiliar goats being on the receiving end of agonistic interactions initiated by confrontees and/or peers (Agonistic C-U, i.e. a reduction from a proportion of 50% [30; 70] unfamiliar goats during the 1st confrontation to 22% [11; 41] during the 6th confrontation). Similarly, activity values of both

unfamiliar goats and confrontees decreased with repeated confrontations (Activity U, i.e. from 50 m/s² [33; 70] during the 1st confrontation to 36 m/s² [24; 52] during the 6th confrontation; Activity C, i.e. from 70 m/s² [42; 109] during the 1st confrontation to 20 m/s² [9; 50] during the 4th confrontation). Despite this, the number of agonistic interactions between unfamiliar goats increased slightly (Agonistic U-U, i.e. from 1.6 interactions [1.2; 2.2] during the 1st confrontation to 2.1 interactions [1.5; 2.9] during the 6th confrontation).

5.3.4 Effects of period (period 15, 30, 45, 60)

The confrontees' activity values were higher during the first period of a confrontation than during the following three periods (Activity C, period 15: 112 m/s² [72; 181], period 30: 57 m/s² [36; 90], period 45: 50 m/s² [31; 80], period 60: 33 m/s² [20; 53], Figure 4). In focal unfamiliar goats, the decrease in activity values from period 15 to period 60 interacted with social status, since the activity levels of low-ranking goats were higher than those of medium- and high-ranking goats, especially in period 15 (Table 4: Activity U).

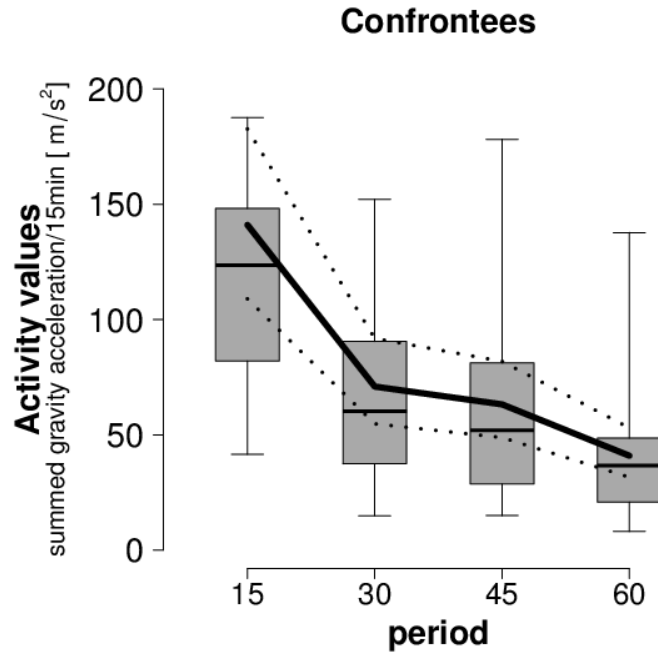


Figure 4: Activity values for confrontees with respect to the four 15-minute periods during a confrontation.
Box-and-whiskers plot: boxes = 1st and 3rd quartile, thick line = median, whiskers = range from minimum to maximum value. Solid lines = model estimates taking repetition/repeated testing into account, dotted lines = 95% confidence intervals.

Table 4: Estimated effects and 95% confidence intervals¹ for activity values of unfamiliar goats (Activity U; m/s²).

Rank × Period			
Period 15	Period 30	Period 45	Period 60
High			
50 [33; 79]	33 [22; 52]	27 [18; 42]	24 [16; 38]
Medium			
75 [48; 120]	41 [26; 64]	38 [25; 61]	39 [25; 60]
Low			
99 [62; 162]	58 [37; 93]	45 [29; 75]	36 [23; 58]

¹ all values rounded to zero decimal places

5.3.5 Effects of rank (high, medium, low)

Rank as a main effect was included in the best-fitting model of the confrontees' cortisol metabolites concentrations (Cortisol C). Further, rank was included in two other models: agonistic interactions between unfamiliar goats (Table 3: Agnostic U-U), where rank interacted with the presence of peers, and in the case of unfamiliar goats' activity values (Table 4: Activity U), where it interacted with period. Concentrations of faecal cortisol metabolites decreased along with the goat's rank, from high- to medium- and low-ranking goats (high-ranking: 316 ng/g [223; 451], medium-ranking: 275 ng/g [195; 381], low-ranking: 143 ng/g [102; 202]).

5.4 Discussion

In the present study, we tested whether the presence of peers reduced the negative effects of social confrontation experienced by confrontees when confronted without peers. In addition, we aimed to test whether these two confrontation paradigms had different effects on the reactions of the unfamiliar goats with which the confrontees were faced.

Our results show that the presence of peers reduced the total number of agonistic interactions, as well as the proportion of agonistic interactions involving physical contact directed against the confrontees and/or peers by unfamiliar goats.

Moreover, the confrontees' cortisol metabolites concentrations were lower in the presence of peers than in their absence. Consequently, the presence of familiar conspecifics was shown to be advantageous for goats during confrontations.

This advantage might be the result of both social support (Rault 2012) and a dilution effect (Neisen et al. 2009), with the data providing indications for both. On the one hand, the number of agonistic interactions directed by unfamiliar goats towards confrontees accompanied by two peers is roughly one-third of the number directed at non-accompanied confrontees, and suggests a dilution effect.

On the other hand, the observation that confrontees lost more agonistic interactions when accompanied by peers than when unaccompanied, but still had lower levels of cortisol metabolites, might suggest an element of social support. In a study of pigs, it was shown that losing most agonistic interactions is associated with higher cortisol concentrations than winning most interactions (Mendl et al. 1992). In addition, the increased probability of agonistic interactions being directed against unfamiliar goats by confrontees accompanied by peers could be interpreted as a result of social support. As the positive effect of peers was linked with familiarity in our study, it would be interesting to know if the effect of the presence of peers could also be confirmed by using unfamiliar goats. However, when introducing sows that were familiar to one another into a dynamic group the amount of aggression between residents and introduced sows was lower than was

the case when introducing sows that were unfamiliar to one another (Durrell et al. 2003).

Both in the present study and an earlier study in which goats were introduced individually into established groups (Patt et al. 2012 (chapter 4)), goats were housed in the same building and had acoustic and visual contact. Thus, they were not completely unfamiliar to each other. Between mothers and their offspring and within established groups, acoustic and visual contact seems to be important to recognise each other and to keep in contact (Siebert et al. 2011, Briefer et al. 2012, Keil et al. 2012, Patt et al. 2013 (chapter 6)). However, in both studies familiarisation between goats was only based on acoustic and visual contact and seemed to be minimal as it did not allow goats to establish dominance relationships quickly after introductions or confrontations. The fact that acoustic and visual contact did not ease confrontations in the earlier and the present study additionally highlights the difficulty of introducing goats into established herds. During the confrontations, we observed a high proportion of agonistic interactions involving physical contact. Far more of these were initiated by unfamiliar goats than by confrontees and/or peers, irrespective of the rank of the unfamiliar goats. This finding is unusual for horned goats in stable groups, where agonistic interactions without physical contact normally predominate (Aschwanden et al. 2008b). Rather than being associated with the establishment of dominance relationships, the agonistic interactions during confrontations might have been motivated by unfamiliarity, intended to drive away unfamiliar animals, as has been suggested by Puppe (1998) in the context of mixing unfamiliar pigs. This hypothesis is also supported by the qualitative observation that confrontees and peers stayed mainly along the outer wall of the arena, in corners, or on the wooden partitions. We had also found a similar picture in an earlier study, where goats introduced individually into groups minimised social interactions by hiding in lying niches (Patt et al. 2012 (chapter 4)).

The higher concentrations of cortisol metabolites in unaccompanied confrontees than in goats accompanied by two peers might be explained by the higher number

and larger proportion of agonistic interactions involving physical contact experienced by confrontees without peers. In pigs, physical agonistic interactions during social confrontations were shown to increase cortisol concentrations (Otten et al. 1999), and agonistic interactions with physical contact are characterised by a higher heart rate than those without physical contact (Marchant et al. 1995). However, the mere fact of being confronted on their own could also have caused the higher concentration of faecal cortisol metabolites in confrontees, as separation from the group has itself been shown to activate the hypothalamic-pituitary-adrenal axis (Guesdon et al. 2012, Patt et al. 2013 (chapter 6)). Nevertheless, since the factor sample was not included in the best-fitting model for cortisol metabolites, it must be assumed that the differences between the reference values and the samples 13, 14, and 15 hours after the stressor were not statistically relevant.

A number of the unfamiliar goats' reactions varied depending on whether or not confrontees were accompanied by peers. Increasing the ratio of confronted to unfamiliar goats from 1:6 to 1:2 led to a reduced level of agonistic interactions directed against low-ranking unfamiliar goats, a higher proportion of agonistic interactions lost by confrontees and/or peers, and a higher level of activity values in unfamiliar goats. Furthermore, whereas unfamiliar goats' concentrations of faecal cortisol metabolites decreased when repeatedly confronted with just one goat, they remained more or less constant when confronted with three goats. All in all, this indicates that unfamiliar goats paid more attention to three goats than to one, as well as putting more effort into agonistic interactions and being more restless when faced with three goats. Consequently, it would be interesting to investigate whether increasing the number of peers would further increase the impact on unfamiliar goats while decreasing the impact on confrontees, and whether such effects depend on group size. Further, it would be of practical relevance to investigate independently the potential positive and negative effects of remaining in the home environment and being relocated to a novel environment.

In conclusion, the results of our study show that the presence of familiar conspecifics mitigates the adverse effects associated with social confrontations taking place in a neutral environment. Given our experimental design, however, it is necessary to verify whether the results are also valid for goats introduced into larger groups. Nevertheless, we recommend putting the husbandry procedure tested in our experiment into practice when unfamiliar goats are introduced into established herds. This would involve introducing groups of goats familiar with one another instead of individuals, as well as mixing animals in a neutral environment (e.g. on pasture) rather than in their home pen, provided that all animals are equally accustomed to the location.

6 Factors influencing the welfare of goats in small established groups during the separation and reintegration of individuals

Based on:

Patt A, Gygax L, Wechsler B, Hillmann E, Palme R, Keil NM (2013)

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Abstract

As a goat's separation from or reintegration into its group is likely to have an adverse effect on the welfare of both the separated goat and the remaining goats in the group, management procedures need to be carried out in a way that minimises their negative impact. In the present study, we tested the effects of two treatments of separation and reintegration by individually separating 12 goats from four experimental groups, each composed of seven horned, non-lactating female goats. In the no-contact treatment the separation allowed for acoustic contact with the group only, whereas in the contact treatment tactile and visual contact was also possible. The separation lasted two days, with each separated goat experiencing both treatments (i.e. there were 24 separations in total). Per group, one separated goat was of high, medium and low rank, respectively. The effects of separation and reintegration were assessed by evaluating social interactions, lying and feeding behaviour, and the concentration of cortisol metabolites in faecal samples of separated and resident goats. Data were collected during three phases: a reference period (days -7 to -1), a separation period (days 0 and 1) and a reintegration period (days 2, 3 and 4).

Separated goats fed less and had higher concentrations of faecal cortisol metabolites during the separation than during the reference period. In addition, goats undergoing the no-contact treatment spent less time lying during separation. On the first day of the reintegration period, newly reintroduced goats were more likely to display agonistic and sniffing behaviour towards resident goats and had higher concentrations of faecal cortisol metabolites than during the reference

period. The faecal cortisol metabolite levels of the recently separated goats tended to be higher and the probability of recently separated goats displaying sniffing behaviour towards resident goats was higher in the no-contact than in the contact treatment. By contrast, resident goats were scarcely affected by the separation and reintegration of a group member. The rank of both separated and resident goats influenced behaviour per se, but did not interact with treatment or day. In conclusion, our results indicate that separation had a greater impact on the welfare of the individual goats than did reintegration. To mitigate negative effects on the goats' welfare, it may therefore be advantageous to allow tactile, visual and acoustic contact during separation.

Keywords

goat, grouping, isolation, loose housing, stress, social behaviour, agonistic interactions

6.1 Introduction

Practical farming conditions require the temporary separation of individual goats from the herd, e.g. when kidding, or owing to injury or impaired claw health.

Despite this fact, it is widely acknowledged that social isolation is stressful for gregarious animals. In previous studies, the increase in vocalisations and general activity found in goats during separation experiments was interpreted as contact-seeking behaviour, or as a sign of fear and stress (Price and Thos 1980, Carbonaro et al. 1992, Aschwanden et al. 2008a, Siebert et al. 2011).

It is known that the introduction of a single unfamiliar goat into a small established group has an adverse effect on the welfare of the introduced goat (Patt et al. 2012 (chapter 4)). Because agonistic interactions occur after the temporary separation of group members (Ramírez et al. 2007), as well as when several subgroups are reunited (Fernández et al. 2007), even the reintegration of a familiar goat temporarily separated from its group is likely to have a negative influence on welfare. According to Ramírez et al. (2007), agonistic interactions are elicited after a minimum separation interval of two days. With pigs, it has been shown that high-ranking individuals could be separated from their group for longer periods of time than low-ranking ones without being involved in fights on their return (Ewbank and Meese 1971). It is therefore possible that the occurrence of such interactions is also modulated by the rank of the separated goats. These management procedures should be carried out in such a way as to minimise the negative effects of separation and reintegration. In natural (O'Brien 1983) as well as confined conditions (Lickliter 1985, Ramírez et al. 1995), female goats actively separate from the herd during parturition only. Observing behaviour prior to and shortly after parturition therefore serves to identify aspects that may help to optimise this procedure. The doe separates from the herd several hours prior to kidding until about 12-24 hours after giving birth. She then leaves the kids at their lying-out site and rejoins the herd. Until the kids join the herd about one week later, the doe leaves the herd for brief periods only, in order to suckle her offspring (Lickliter 1984, 1985). With feral goats, O'Brien (1983) observed that does chose

birthing sites within the home range of the herd, specifically in areas frequently used by the herd and close to the main night camp. It would therefore seem that goats prefer to stay in contact with the herd and to keep the period of separation as short as possible.

In the present study, it was hypothesised that (i) both separation and reintegration are less stressful if the separated individual has visual and tactile contact with the group through bars as opposed to having only acoustic contact, and (ii) that these responses might be modulated by the social rank of the separated goats, of the resident goats, or of both. We therefore compared this contact treatment to a situation in which the separated goat had acoustic contact only with its herd (no-contact treatment) in order to find a more acceptable way of temporarily separating individual goats.

To assess the welfare effects of the two treatments, we recorded behavioural and physiological responses during separation and reintegration in the separated goats, as well as in three focal resident goats. Each of the three focal residents and the three separated goats of each group were of different social rank.

6.2 Methods

6.2.1 Animals and housing conditions

The experiment was carried out between March and July 2010. Four groups of seven horned, non-lactating female goats kept in four identically equipped pens were used (28 goats in total). The goats were grouped in January 2010 from individuals of various Swiss dairy breeds (Saanen, Toggenburger, St Gallen Booted, Grisons Striped, Peacock, and Valais Blackneck) and their crossbreeds. Two goats were part Anglo-Nubian. The distribution of the breeds was balanced across the groups as far as possible. The total area per pen was 13.7 m² (approximately 3 m × 5 m), consisting of a deep-bedded straw area of 10.1 m² and an elevated feeding place (3.6 m²) divided by a wooden partition into two compartments of equal size. Ad libitum access to hay was offered in a 3 m hayrack that was refilled twice daily at around 8.45 am and 5 pm. Assuming an animal/feeding-place ratio of 1:1, feeding space was 43 cm per goat. A water trough, a mineralised salt block and a brush were provided. The deep-bedded area was further structured by an L-shaped wooden platform in the corner between the rear wall and one side wall (measuring respectively 1.75 m and 1 m × 0.65 m × 0.55 m high) which provided climbing opportunities as well as elevated and protected lying areas above and below, respectively. In addition, a freestanding partition in the centre of the pen also served as a platform (approximately 1 m in diameter and 0.8 m in height).

6.2.2 Dominance relationships

Shortly before the start of the experiment, the dominance relationships of the goats in each group were assessed by direct observations made during morning and evening feeding times according to the method used by Aschwanden et al. (2008b). With the help of the rank index (between 0 = omega and 1 = alpha), each goat in each group was categorised as being either low-ranking (0.00-0.33), medium-ranking (0.34-0.66) or high-ranking (0.67-1.00).

6.2.3 Separation treatments

In the experiment, individual goats were temporarily separated from their groups. Two distinct separation treatments were applied. In the contact treatment, the separated goat was housed separately in a pen inside the group's home pen whilst retaining visual, acoustic, and limited tactile contact with its pen mates through metal bars, while in the no-contact treatment the separated individual had only acoustic contact with its group.

During the separation period, the separation pen was accommodated in a section of the deep-bedded straw area in the home pen (Figure 1a and 1b). The space for the remaining six goats was reduced to 11.8 m² (8.2 m² deep-bedded area and 3.6 m² elevated feeding place), with the space available per animal kept constant. By contrast, feeding space per goat increased to 50 cm (6 goats at the 3 m hayrack). The pen for separated goats measured 3.5 m². Part of the partition consisted of bars to allow contact with group members, while part was of wood to allow the separated goats to withdraw if wished. Hay was available in a 1 m-long hayrack and water was supplied in a bucket.

The separation pen for the no-contact treatment consisted of a lying hutch on the outside wall of the barn with a deep-bedded straw area of 2.4 m² and a 1.1 m² outdoor exercise area (3.5 m² in total). Feed and water supply was similar to that of the contact treatment. Since two of the four separated goats per experimental period were in the no-contact treatment, two of these pens were used at the same time. The two pens were adjacent to each other and allowed visual, acoustic and tactile contact with an unfamiliar goat through bars. Because isolation (no contact with conspecifics) is known to be an extremely potent stressor for gregarious animals, the Swiss Animal Welfare Ordinance requires that individually housed goats at minimum be provided with visual contact with conspecifics. The average daily temperature during the experiment was 12.4°C, with no extreme weather conditions occurring. The experiment was approved by the Cantonal Veterinary Office (Frauenfeld, Thurgau, Switzerland, F4/09).

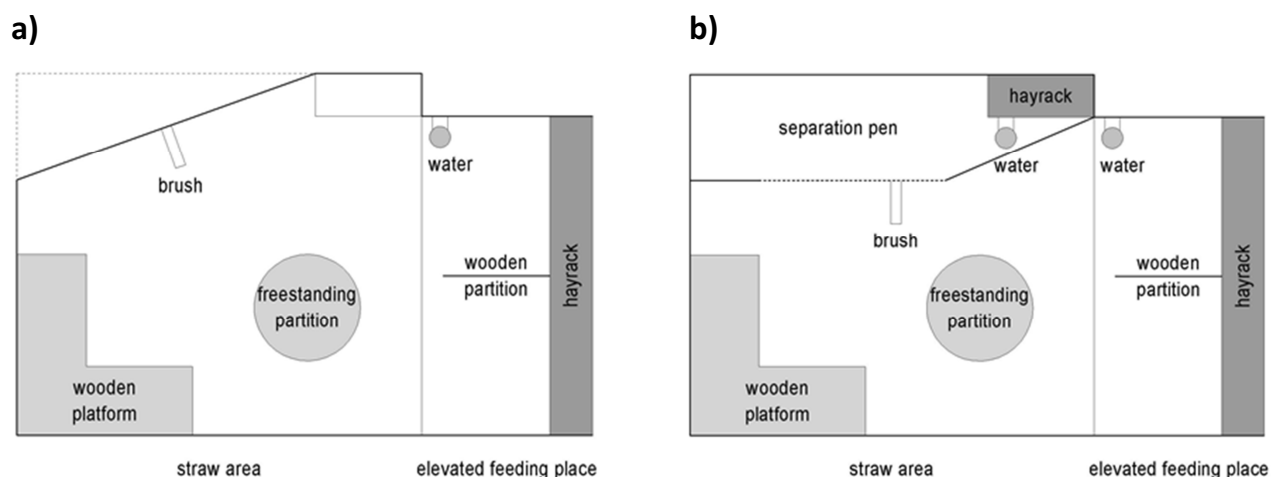


Figure 1: Layout of pen (true to scale) during (a) reference and reintegration periods and (b) separation periods.

In (a) and (b), grey highlighting indicates that hayracks contain hay. In (b), the dotted section of the separation pen's partition indicates the bars through which visual and tactile contact between separated and resident goats was possible.

6.2.4 Experimental procedure

Three goats per group were consecutively separated from their group twice for two days at a time. After each of these two-day periods, they rejoined their original group. Each separated goat underwent both treatments (contact and no-contact), with a gap of at least 14 days between treatments. Accordingly, 24 separation-reintegration procedures were accomplished in total, i.e. six separations per group. The three goats that were separated reflected the three rank categories (high, medium and low), and were chosen for the separation in a balanced order across groups and treatments.

Each experimental period was divided into three phases: a reference period (days -7 to -1), a separation period (days 0, 1) and a reintegration period (days 2, 3 and 4). After each experimental period there was a break of two days before the start of the next experimental period. To ensure that the same amount of space was available to the remaining residents during the separation phase, the separation pen was installed in each of the four experimental groups on day 0, regardless of the current treatment. The separation started when the first goat was put in either separation pen at around 8.30 am. Fifteen minutes later the second goat was separated, and this procedure was repeated until the fourth

individual was separated from its group at around 9.15 am. The order of separation was balanced between groups, i.e. each group was in position one, two, three and four once. The order of regrouping corresponded to the order of separation, and regrouping was also performed at 15-minute intervals.

6.2.5 Data recording

To monitor effects on welfare associated with the separation and reintegration of an individual goat, we measured social behaviour (frequency of agonistic and affiliative interactions), lying duration, feeding duration, and the concentrations of faecal cortisol metabolites. Reference values were collected twice for social behaviour (days -7, -5), lying duration (days -7, -6), feeding duration (days -7, -6) as well as concentrations of faecal cortisol metabolites (days -5, -4). Data were collected daily for all variables during both the separation period (days 0, 1) and the reintegration period (days 2, 3 and 4). An additional faecal sample serving as a second reference point was taken from the previously separated goats one week after reinstatement in their group (day 11).

During the separation period, social-behaviour data were measured among the six remaining residents in each group, whilst during the reference and reintegration periods data were measured from all seven goats per group assigned to their respective roles in the experimental period (those individuals about to be separated or previously separated versus residents). All other variables were measured in the goat to be separated and three of the remaining resident goats (focal residents). In order to control for effects of individuality, these focal residents remained the same for all six repetitions. They were never separated and reintegrated, and represented the three rank categories of high, medium and low.

6.2.5.1 Social behaviour

Social behaviour was directly observed between 8.30 and 10.30 am and between 5 and 7 pm, which included the peak feeding times. Each of the four experimental groups was observed twice for 15 minutes during both of these observation blocks. The sequence of the groups within the blocks was balanced such that

groups were observed equally during the various 15-minute time slots. On day 0 (start of separation) and 2 (start of reintegration), the first observation slot for each group included the first 15 minutes after the individual goat was separated and reintegrated, respectively.

During direct observation, all goats in a single group were observed simultaneously. Fighting, butting, explicit displacement, implicit displacement and threatening were recorded as agonistic interactions and sniffing, scratching, licking and mock fighting as affiliative behaviours. The definitions of the recorded behaviours are given in Patt et al. (2012) (chapter 4). For each interaction, the initiator and receiver were noted. With agonistic interactions, it was also noted whether the initiator was successful (i.e. if the receiver changed location).

6.2.5.2 Lying and feeding behaviour

Lying and feeding behaviour (duration in hours per 24 hours) was recorded according to the method described in Patt et al. (2012) (chapter 4). Owing to technical problems during data collection, seven days' worth of feeding data distributed among three different individuals was missing.

6.2.5.4 Cortisol metabolites

In order to monitor an acute stressor via measurement of faecal cortisol metabolites, samples should be collected within 12-15 hours after the event in question (Kleinsasser et al. 2010). Thus, faecal sampling of the separated goats and the focal residents of each experimental group started at 8.30 pm, and was performed according to the order of introduction on the morning of day 0. To account for a possible circadian rhythm of cortisol levels, faecal samples were taken at the same time on all days.

Goats were attached successively at the hayrack and samples were collected manually from the animals' anal channel/rectum. Each sample was immediately put into a cooling box until completion of sampling. All samples were then frozen and stored at -20°C until analysis. On one sampling day it was not possible to obtain faecal samples from two animals (two focal residents of the same group,

one high-, one low-ranking). The concentrations of cortisol metabolites were determined by a group-specific 11-oxoetiocholanolone enzyme immunoassay (EIA) (Möstl et al. 2002). This EIA has been successfully validated for monitoring adrenocortical activity in goats (Kleinsasser et al. 2010). The assay's sensitivity was 4.5 ng/g faeces and its intra- and interassay coefficients of variation were between 8.1% and 10.3%.

6.2.6 Statistical analysis

6.2.6.1 Explanatory variables, random effects and model selection

Generalised linear mixed-effects models were used to evaluate the outcome variables in order to adequately reflect dependencies in the experimental design (nesting, repeated measurements). Statistical analysis was performed with R (version 2.12.2, R Development Core Team 2011) using the lme and glmer methods from the nlme (Pinheiro et al. 2009) and lme4 (Bates et al. 2011) packages, respectively. Model assumptions were checked using graphical analysis of residuals focusing on normality of errors and random effects, as well as homoscedasticity of the errors in the case of normally distributed errors, and on normality of random effects and absence of bias in the mean errors for the generalised models.

Fixed effects were treatment (factor with two levels: no-contact, contact), rank of both separated goats and residents (factor with three levels: high, medium or low) and day. The fixed effect day was a factor with a varying number of levels according to the number of measurements taken for a given outcome variable. For all outcome variables dealing with (focal) residents, random effects were treatment nested in repetition nested in animal nested in experimental group. If an outcome variable described social interactions initiated by residents with the previously separated goats, or the situation of the separated goats, or social interactions with residents initiated by the previously separated animals, random effects were treatment nested in separated animal nested in experimental group.

For each analysis, we set up a maximum model (all interactions), three intermediate models (each with one anticipated two-way interaction either between treatment and day, day and rank, or treatment and rank), and a minimum model (main effects only). The choice among the five models was based on the Bayesian information criterion (BIC), reflecting the probability of the whole model given the data (Burnham and Anderson 2003). To aid in the interpretation of the main-effect models, the subsequent presentation of results is based on p-values being significant or representing a trend.

6.2.6.2 Outcome variables

Social behaviour was analysed from the recipient's point of view. As most forms of agonistic interactions occurred too rarely to analyse them separately, the different forms were summed up for analysis. Although several affiliative behaviours were included in the ethogram, they only manifested themselves very sporadically. The exception to this was sniffing, which was therefore included in the statistical analysis.

As outcome variables, we analysed (for information regarding transformation of the different variables, see Table 1 and Table 2):

- whether agonistic interactions (Agonistic R-R) and sniffing (Sniffing R-R) were received by a resident from other residents,
- the number of agonistic interactions (Agonistic R-S) per day received by the previously separated goat from residents and whether the previously separated goat received sniffing (Sniffing R-S) from residents,
- whether agonistic interactions (Agonistic S-R) and sniffing (Sniffing S-R) were received by residents from the previously separated goat,
- the lying duration (hours/day) of focal residents (Lying R) and of separated goats (Lying S),
- the feeding duration (hours/day) of focal residents (Feeding R) and of separated goats (Feeding S),
- the concentrations of faecal cortisol metabolites (ng/g) of focal residents (Cortisol R) and of separated goats (Cortisol S).

Table 1: Estimated effects as well as test statistics (F-test and F-values for models with normally distributed errors and likelihood-ratio tests, χ^2 -values for models based on binomial distribution) and p-values of the fixed-effect treatment for all outcome variables (main-effects models and model including the interaction with day for Lying S).

Outcome variable	Trans-formation	Error distribution	Type of effect ¹	Absolute values no-contact	Treatment		F-/ χ^2 -value	p-value
					Estimated effects			
					no-contact	contact		
Agonistic R-R ² (no./animal/day)	-	binomial	OR	0.18	1.00	1.22	$\chi^2_1 = 0.65$	0.419
Agonistic R-S (no./animal/day)	log	normal	m	3.94	1.00	0.91	$F_{1,27} = 0.63$	0.436
Agonistic S-R (yes/no)	-	binomial	OR	0.24	1.00	0.98	$\chi^2_1 = 0.009$	0.925
Sniffing R-R (yes/no)	-	binomial	OR	0.06	1.00	0.59	$\chi^2_1 = 3.29$	0.069
Sniffing R-S (yes/no)	-	binomial	OR	0.61	1.00	0.60	$\chi^2_1 = 2.76$	0.097
Sniffing S-R (yes/no)	-	binomial	OR	0.10	1.00	0.47	$\chi^2_1 = 6.25$	0.012
Feeding R (hours/day)	-	normal	a	3.42	0.00	-0.12	$F_{1,35} = 0.59$	0.448
Feeding S (hours/day)	-	normal	a	3.27	0.00	0.28	$F_{1,11} = 1.21$	0.294
Cortisol R (ng/g)	log	normal	m	319.13	1.00	0.89	$F_{1,35} = 2.50$	0.123
Cortisol S (ng/g)	log	normal	m	350.02	1.00	0.81	$F_{1,11} = 3.87$	0.075
Lying R (hours/day)	-	normal	a	12.92	0.00	0.22	$F_{1,35} = 0.52$	0.474
Day -7 x no-contact								
Lying S (hours/day)	-	normal	a	12.10	0.00	1.16	$F_{6,131} = 4.44$	< 0.001
					Day -7	0.00	0.13	
					Day -6	0.80	0.55	
					Day 0	-4.43	0.72	
					Day 1	-0.40	1.16	
					Day 2	-0.38	-0.70	
					Day 3	0.72	0.68	
					Day 4	0.63	1.13	

¹ a = additive, m = multiplicative, OR = odds ratio; additive = add the quoted estimated effect size to the value of the no-contact treatment; multiplicative = multiply the estimated effect by the value of the no-contact treatment; odds ratio < 1 = decreased probability, > 1 = increased probability in relation to the no-contact treatment.

² R-R: initiator = resident, receiver = resident; R-S: initiator = resident, receiver = separated goat; S-R: initiator = separated goat, receiver = resident; R = resident; S = separated goat.

Table 2: Estimated effects as well as test-statistics (F-test and F-values for models with normally distributed errors and likelihood-ratio tests, χ^2 -values for models based on binomial distribution) and p-values of the fixed-effect day for all outcome variables analysed with main-effects models.

Outcome variable	Reference ¹											F-/ χ^2 -value	p-value
	Separation					Reintegration							
	Absolute values Day 'a' ²	Day 'a'	Day 'b'	Day 0	Day 1	Day 2	Day 3	Day 4	Day 11	Day 11			
Agonistic R-R ³ (no./animal/day)	0.22	1.00	0.73	0.11	0.04	2.73	1.48	0.94	-	$\chi^2_6 = 120.87$	< 0.001		
Agonistic R-S (no./animal/day)	3.62	1.00	0.96	-	-	1.06	1.13	1.05	-	$F_{4,220} = 0.57$	0.682		
Agonistic S-R (yes/no)	0.22	1.00	0.82	-	-	1.94	1.42	1.00	-	$\chi^2_4 = 10.92$	0.028		
Sniffing R-R (yes/no)	0.04	1.00	1.54	0.32	0.32	5.16	0.66	1.36	-	$\chi^2_6 = 44.78$	< 0.001		
Sniffing R-S (yes/no)	0.46	1.00	1.76	-	-	2.87	1.76	1.46	-	$\chi^2_4 = 5.97$	0.202		
Sniffing S-R (yes/no)	0.04	1.00	1.54	-	-	5.20	0.66	1.36	-	$\chi^2_4 = 27.37$	< 0.001		
Feeding R (hours/day)	3.31	0.00	0.14	0.22	-0.06	-0.04	-0.13	0.20	-	$F_{6,426} = 1.82$	0.095		
Feeding S (hours/day)	3.61	0.00	0.11	-0.75	-0.68	-0.02	-0.15	0.14	-	$F_{6,131} = 3.42$	0.004		
Cortisol R (ng/g)	285.62	1.00	0.91	1.13	1.15	1.06	1.15	0.99	-	$F_{6,425} = 3.20$	0.004		
Cortisol S (ng/g)	278.93	1.00	0.95	1.10	1.83	1.54	1.03	0.91	0.97	$F_{7,161} = 5.90$	< 0.001		
Lying R (hours/day)	13.22	0.00	0.76	-0.47	-0.27	-0.67	-0.39	-0.23	-	$F_{6,423} = 2.60$	0.018		
Lying S (hours/day) ⁴	-	-	-	-	-	-	-	-	-	-	-		

¹ Day 'a' and 'b' represent day -7 and -5 for social behaviour, -7 and -6 for lying duration and feeding duration and -5 and -4 for faecal cortisol metabolites.

² Transformation, error distribution and type of effect as stated in Table 1.

³ R-R: initiator = resident, receiver = separated goat; R-S: initiator = resident, receiver = separated goat; S-R: initiator = separated goat, receiver = resident;

R = resident; S = separated goat.

⁴ See Table 1.

6.3 Results

The main-effects model was rated as the best-fitting model for all but one model. For lying duration of separated goats (Lying S) the best-fitting model also included the interaction between treatment and day.

6.3.1 Effects of separation treatment (no-contact vs. contact)

With separated goats, the separation caused a marked reduction in lying duration (Lying S) in the no-contact treatment - specifically on day 0 - compared to reference measurements and to the contact treatment. This was borne out by our qualitative observations during data recording, with separated goats in the no-contact treatment being more restless (i.e. moving about in the separation pen and rearing and jumping against the wall of the pen) than when in the contact treatment. Also on day 2, lying duration (Lying S) was lower in both treatments, but more so for goats previously separated in the contact treatment (Figure 2, two-way interaction between day and treatment, i.e. to obtain the value for goats in the contact treatment on day 0, 0.72 hours must be added to the absolute value for goats in the no-contact treatment on day 'a' (day -7), Table 1).

Apart from the lying duration of separated goats, treatment did not interact with day or rank, but influenced the following outcome variables: The concentration of faecal cortisol metabolites of the separated individuals (Cortisol S) tended to be lower in the contact treatment than in the no-contact treatment (i.e. to obtain the value for the contact treatment, the absolute value for goats during the no-contact treatment must be multiplied by 0.81, Table 1). For residents, the probability of being sniffed by the previously separated goats was lower during the contact treatment than during the no-contact treatment (i.e. the odds ratios for Sniffing S-R were 0.47 times lower during the contact treatment than the no-contact treatment, Table 1). Similarly, the probability of residents displaying sniffing behaviour among themselves or towards previously separated goats tended to be lower during the contact treatment (Sniffing R-R, Sniffing R-S). During

data recording, tactile contact between the separated goats and residents during separation in the contact treatment was only very rarely observed.

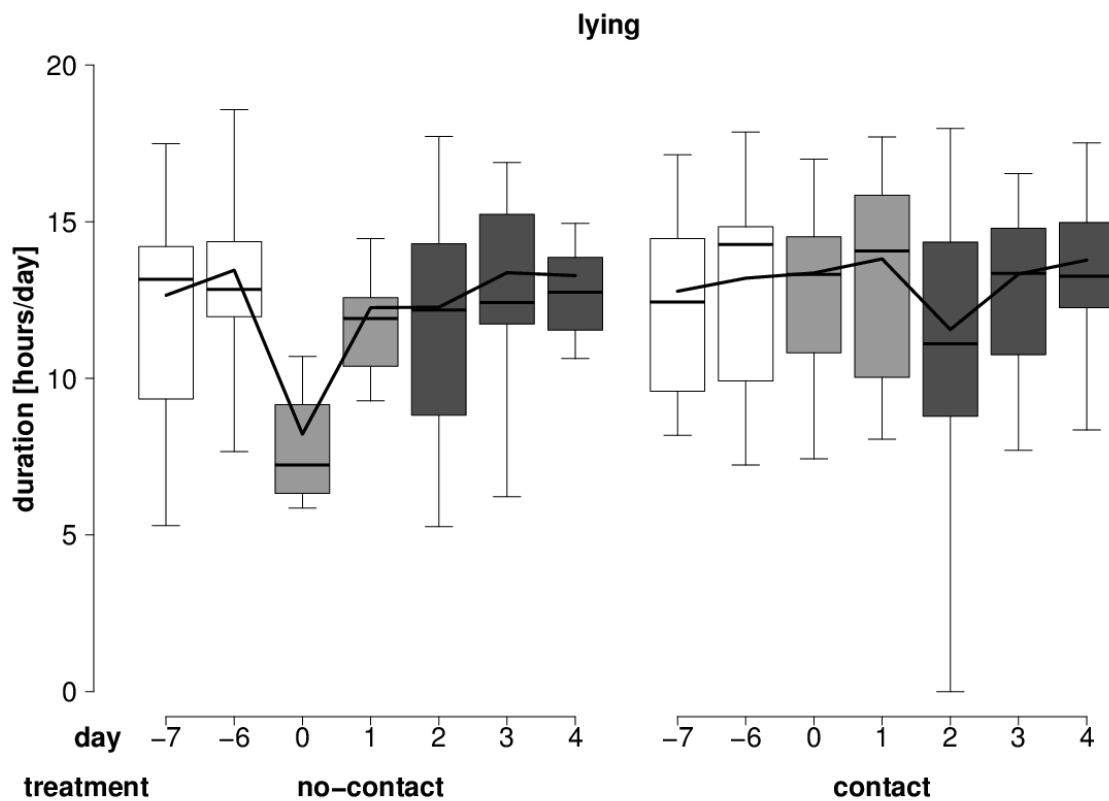


Figure 2: Lying duration [hours/day] for separated goats with respect to the no-contact and contact treatments.

Box-and-whiskers plot: boxes = 1st and 3rd quartile, thick line = median, whiskers = range from minimum to maximum value. White boxes = reference situation before separation (days -7 and -6), light grey boxes = separation period (days 0 and 1), dark grey boxes = reintegration period (days 2, 3 and 4). Solid lines = model estimates for medium-ranking goats.

6.3.2 Effects of day (separation and reintegration periods)

Separation from their group caused a reduction in feeding duration (Feeding S) on both separation days in both treatments, whilst feeding duration was only slightly lower on days 2 and 3 of the reintegration period (Figure 3). During separation, the concentrations of faecal cortisol metabolites (Cortisol S) was slightly elevated on day 0, and significantly higher on day 1. During reintegration, concentrations of cortisol metabolites were elevated on day 2, but no longer on days 3 and 4 (Figure 4). On the first day of reintegration, the probability of previously separated

goats displaying agonistic interactions (Agonistic S-R) or sniffing behaviour (Sniffing S-R) towards residents was greater than in the reference period. The probability of resident animals interacting agonistically amongst themselves (Agonistic R-R) was lower during the two separation days than during the reference period. By contrast, on days 2 and 3 (reintegration period) the probability was higher. Similarly, the probability of residents sniffing one another (Sniffing R-R) was lower during the separation period and higher on the first day of reintegration (day 2) than on reference days. Lying duration (Lying R) was slightly lower throughout the separation period than on reference days. Moreover, the concentration of faecal cortisol metabolites increased to a certain extent in focal residents (Cortisol R) on days 0 and 1 (separation) as well as on day 3 (reintegration).

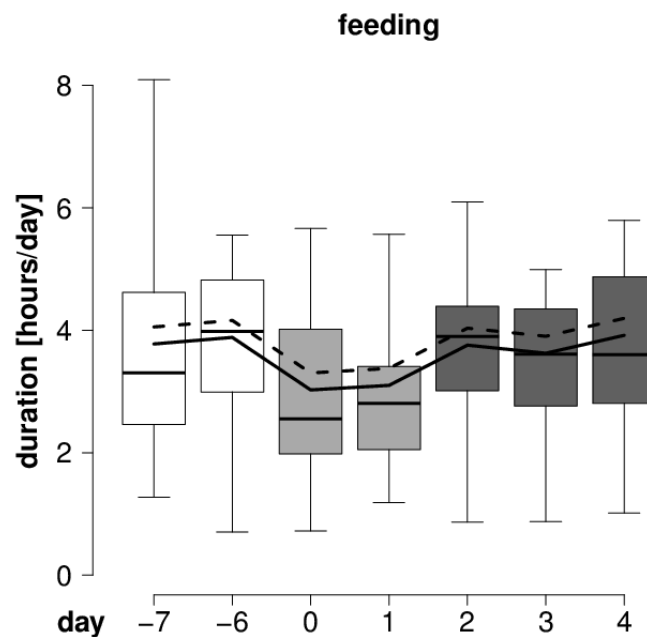


Figure 3: Duration [hours/day] separated goats spent feeding.

Box-and-whiskers plot: boxes = 1st and 3rd quartile, thick line = median, whiskers = range from minimum to maximum value. White boxes = reference situation before separation (days -7 and -6), light grey boxes = separation period (days 0 and 1), dark grey boxes = reintegration period (days 2, 3 and 4). Solid line = model estimate for medium-ranking goats during the no-contact treatment, dashed line = model estimate for medium-ranking goats during the contact treatment.

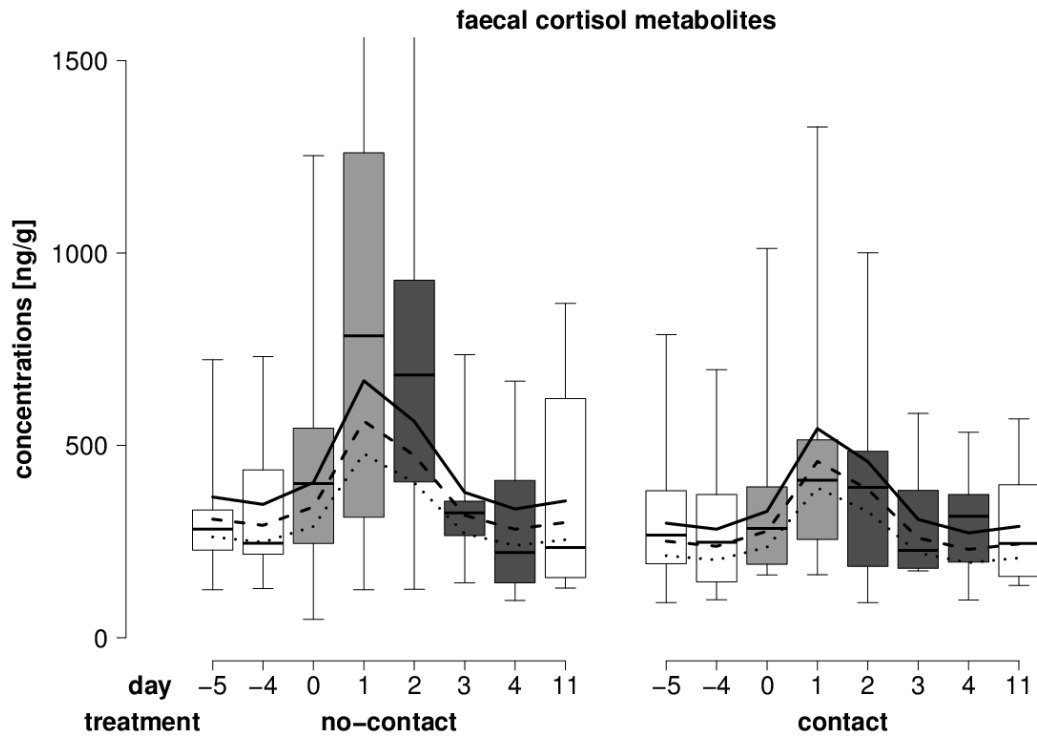


Figure 4: Concentrations of faecal cortisol metabolites [ng/g] of separated goats with respect to the no-contact and contact treatments.

Box-and-whiskers plot: boxes = 1st and 3rd quartile, thick line = median, whiskers = range from minimum to maximum value. White boxes = reference situation before separation and one week after reintegration (days -5 and -4 and 11), light grey boxes = separation period (days 0 and 1), dark grey boxes = reintegration period (days 2, 3 and 4). Solid lines = model estimates for high-ranking goats, dashed lines = model estimates for medium-ranking goats, dotted lines = model estimates for low-ranking goats.

6.3.3 Effects of rank

Rank influenced social behaviour as a main effect, but did not interact with treatment or day, the effects of separation and reintegration therefore being similar for all rank categories. Both the number of agonistic interactions by residents directed towards separated goats (Agonistic R-S, $F_{2,22} = 22.05$, $p < 0.001$, medium-ranking: multiplicative estimated effect = 2.66, low-ranking = 4.70) and the probability of residents being on the receiving end of the agonistic interactions initiated by other residents (Agonistic R-R, $\chi^2_2 = 10.86$, $p = 0.004$, medium-ranking: OR = 4.04, low-ranking = 7.35) or by separated goats (Agonistic S-R, $\chi^2_2 = 15.77$, $p < 0.001$, medium-ranking: OR = 5.40, low-ranking: OR = 8.19) generally increased as the rank of the individual in question decreased. The concentrations of faecal cortisol metabolites tended to decrease from high-, to medium-, to low-ranking resident individuals (Cortisol R, $F_{2,6} = 5.06$, $p = 0.052$, medium-ranking: multiplicative estimated effect = 0.88, low-ranking = 0.61). Similarly, the feeding duration of separated goats (Feeding S, $F_{2,6} = 4.69$, $p = 0.059$, medium-ranking: additive estimated effect = -0.20, low-ranking = -1.32) tended to decrease from high-, to medium-, to low-ranking goats.

6.4 Discussion

In the present study, we tested the effects of two different methods of separation and a subsequent reintegration on the separated goat itself, as well as on the residents. We were interested in changes in behavioural and physiological responses over time, potential differences between two separation treatments, and the question of whether response is modulated by social rank. It was shown that the welfare of the separated goats was adversely affected by both the separation and - to a lesser extent - the reintegration, whilst these management procedures had only minor effects on the residents. In addition, we found that the effects were less pronounced in the contact treatment than in the no-contact treatment.

6.4.1 Effects of treatment: no-contact vs. contact

Comparing the two methods of separation in terms of their welfare effects on separated goats, our results suggest that the no-contact treatment had a greater impact on separated individuals. Only when they were assigned to the no-contact treatment did the separated goats spend a significantly lower amount of time lying. After reintegration, the probability of previously separated goats sniffing resident group members was considerably greater than in the contact treatment. The more limited contact was during the foregoing separation, the more likely goats were to initiate contact with their group mates upon their return. Moreover, the separated goats' cortisol metabolites levels tended to be higher in the no-contact treatment over the entire experimental period.

Because the effects of separation and reintegration were similar for all rank categories independently of the applied treatment, rank does not seem to be an important factor when separating individual goats in farming practice. So bearing in mind all findings, we can conclude that the separation was stressful for goats of all rank categories but allowing them tactile, visual and acoustic contact during this period can reduce the negative effects of both the separation and the subsequent reintegration. This is highly relevant in farming practice, where sick or injured

goats at times need to be separated from the group, as stress itself can increase susceptibility to disease and/or negatively influence the course of the latter (Dhabhar 2009, Verbrugghe et al. 2011). Furthermore, spending less time lying, as separated goats did during the no-contact treatment, could slow down or even impede recovery from medical conditions of the claw or leg. This tallies with the results of Siebert et al. (2011), which showed that goats having acoustic and olfactory contact with group mates had a more active response pattern than those that were separated without any sensory contact with their group.

In order to give definitive management recommendations on separating goats, however, we would need to know whether adverse welfare effects can be reduced by visual contact alone, or whether the opportunity for additional tactile contact is essential. If visual contact alone proved to be sufficient, this would be especially advantageous for the separation of goats with infectious diseases. In sheep, it has been shown that the adverse effects of a 15-minute separation were lower when the separated individuals had visual contact with their group than when there was no contact (Baldock and Sibly 1990). Furthermore, simply showing a picture of a conspecific's face could reduce the adverse effects of short-term isolation in sheep (da Costa et al. 2004), and even exposure to a mirror image might have at least some effect on reactions to social separation in sheep, heifers and horses (Parrott et al. 1988, Piller et al. 1999, Kay and Hall 2009).

6.4.2 Effects of separation

Separation effects were quite obvious in the separated goats: they fed less and had significantly increased concentrations of cortisol metabolites, indicating the adverse effect of the separation on the separated individual. This is highlighted by the fact that feeding duration decreased in low-ranking goats to a similar extent as in the other rank categories (main effect). In intensive conditions, low-ranking goats normally feed less than high-ranking animals, as the former are the first to be negatively affected when access to food is limited (Loretz et al. 2004, Meisjord Jørgensen et al. 2007), and this also appeared to be the case in our study. Contrary

to our observations, low-ranking goats would be expected to feed more during separation, because hay was then freely available. Thus, all of the separated goats may have fed less owing to a stress response associated with the separation. Specifically, it is known that the secretion of corticotropin-releasing hormone (CRH) reduces appetite in vertebrates (Heinrichs and Richard 1999, Carr 2002). Although we do not know how goats would behave during a longer period of separation, feeding for almost 1 hour less, as was the case in the present study, is likely to lead in the long term to health problems, reduced performance, or both.

6.4.3 Effects of reintegration

Day 2 saw a decrease in lying, and to a minor extent, feeding duration for the previously separated goats, as well as an increase in their cortisol metabolites levels, indicating that reintegration also had a negative impact on them. As both the decrease in lying duration and the increase in cortisol metabolites concentrations were significantly greater during separation than during reintegration, however, we may conclude that the reintegration period had less of an effect on the separated goats. Since concentrations of cortisol metabolites reliably returned to baseline concentrations for all animals by the second day of the reintegration period, adverse welfare effects associated with reintegration seem to be limited in duration. The increased agonistic interactions between resident goats, as well as of previously separated goats towards residents, might be indicative of the goats' attempts to assert their position in the group hierarchy. The goats' sniffing behaviour on the first day of reintegration could be interpreted as a form of olfactory inspection. Olfactory cues have previously been identified as being important for recognition between doe and kid (e.g. Romeyer et al. 1994), for distinguishing between individuals (Baldwin 1977), or for determining group membership (Keil et al. 2012).

6.5 Conclusions

Our results show that a two-day separation period has negative effects on the separated goats' welfare, leading to a substantial reduction in time spent feeding and a clear activation of the hypothalamic-pituitary-adrenal axis, irrespective of the remaining level of contact allowed with the group. The subsequent reintegration also adversely affected the previously separated goats, but to a lesser extent, as effects were mainly limited to the first day of the reintegration period and were less pronounced than during the separation period. The 'acoustic contact only' treatment had disadvantages compared to the treatment which also allowed for visual and tactile contact, since goats in the no-contact treatment spent considerably less time lying during separation, and tended to have generally higher concentrations of cortisol metabolites. Consequently, if an individual goat must be separated from its group, it appears that allowing visual, acoustic and tactile contact with members of the latter will mitigate the adverse welfare effects of the separation.

7 General discussion

The aim of this thesis was to provide basic knowledge of the consequences of regrouping for the welfare of goats and to identify solutions that are less aversive than currently used methods. The first part of the discussion develops recommendations that can be derived from the main results and then specifies remaining open questions (chapter 7.1). Afterwards, it is discussed whether the chosen outcome variables were suitable to reflect the effects of regrouping (chapter 7.2). The next chapter (7.3) focuses specifically on the results of agonistic interactions and discusses the assumed underlying motivations for these interactions. Assuming that the motivation to accept introduced animals as group members is a precondition for group formation, the fourth part of the discussion assesses whether regrouping approaches investigated so far facilitate this process (chapter 7.4). The last part of the discussion offers ideas for future research into facilitating group formation (chapter 7.5).

7.1 Recommendations and open questions based on the outcomes of the project

To assess the effects of the different methods of regrouping on the goats' welfare and to discuss the resulting recommendations for less aversive approaches, the separation (and subsequent reintegration) of a goat from its group and the introduction of a goat into an unfamiliar herd had to be distinguished. In the third experiment (chapter 6), by investigating the effects associated with the separation of a goat from her group, it was found that a separation, despite allowing acoustic, visual, and tactile contact, negatively affected the separated goat. Furthermore, not only the separation but also - to a lesser extent - the reintegration had a negative impact on the welfare of the separated goat. This was expressed by shorter feeding times throughout the separation period as well as higher faecal cortisol metabolites concentrations during both the separation and the reintegration periods. Decreasing the level of contact to the original group

increased the negative effects of both separation and reintegration for the separated goat. When the separated goat was allowed only acoustic contact, lying times were lower during the separation period, and faecal cortisol metabolites concentrations were generally higher than when visual and tactile contact also were possible. To give management recommendations on the contact intensity necessary to reduce the associated negative welfare effects, it would be relevant to investigate if visual contact alone was sufficient or if the opportunity for additional tactile contact was essential. This knowledge would be especially valuable when a separation is necessary due to an infectious disease.

Nevertheless, from the results of this study, it can be derived that to reduce the adverse effects of temporary separation (and subsequent reintegration) of individual goats in farming practice, it is advisable to allow the separated goat acoustic, visual, and tactile contact to her group.

The results of the first and second study, described in chapters 4 and 5, respectively, addressed aspects associated with the introduction of unfamiliar goats. Results from the first study showed that introducing horned and hornless goats individually into established groups severely impaired the introduced goat's welfare (chapter 4). During the entire five-day introduction period, each introduced goat had considerably increased lying durations, shorter feeding durations, and elevated concentrations of faecal cortisol metabolites. On the first day of the introduction period, each goat additionally received a considerable number of agonistic interactions. In the second study, horned goats were confronted with unfamiliar groups of goats in a neutral environment either alone or in the presence of familiar conspecifics, aiming at mitigating negative effects associated with social confrontations (chapter 5). Results demonstrated that negative welfare effects of social confrontations could be reduced when goats were accompanied by familiar conspecifics. The number of agonistic interactions confrontees received when familiar conspecifics were present was reduced considerably, and the levels of cortisol metabolites were slightly lower. Taken together, the results of these two studies suggest that neither horned nor hornless

goats should be introduced individually into established groups. If an introduction is unavoidable, it can be recommended to introduce several familiar goats simultaneously, as the presence of familiar conspecifics can mitigate the adverse effects of the initial social confrontation.

In the context of this recommendation, several aspects need further investigation. The study described in chapter 5 was conducted by confronting the animals in a neutral arena away from the goats' home pen and with rather ample space. Therefore, it is necessary to examine the relevance of these two factors. In the meantime, however, employing this husbandry procedure would imply introducing animals on pasture or in an outdoor run, provided that all animals are equally accustomed to the location. As limited access to important resources is very likely to add to the negative effects of regrouping (chapter 3.3), it would also be interesting to test whether introducing goats on pasture can help to increase feeding duration of introduced goats. With regard to the advantageous effects of familiar conspecifics, it remains to be investigated whether unfamiliar conspecifics mitigate adverse effects on the goats' welfare to a similar extent. Additionally, it would be of practical importance to know whether the ratio of confronted goats to group members determines the extent to which negative effects are mitigated, and further, whether the beneficial effects are also valid for larger groups. Finally, although negative effects during the initial social confrontation were mitigated when a goat was accompanied by familiar conspecifics, the results do not allow predicting the duration introduced goats are exposed to negative welfare effects after an introduction. In other words, it is not yet known how long it takes until the introduced goats are integrated into the pre-existing group.

7.2 Suitability of chosen welfare indicators

To be able to assess the consequences of the different forms of regrouping for goats, the chosen welfare indicators have to reliably reflect the associated effects. In the studies presented here, this seemed to be the case for most outcome variables. Further, the results obtained for the different outcome variables were

not contradictory and, therefore, allowed drawing consistent conclusions. In the following, the suitability of each variable as a welfare indicator is discussed in more detail.

Lying duration indicated the adversity of regrouping both when it was substantially increased compared to control values during the introduction of an unfamiliar goat (chapter 4) and when it was decreased during separation of a goat from her herd (chapter 6). An increase or decrease in lying duration compared to reference values might therefore reflect the different qualities of impairment caused by the two regrouping procedures, i.e. introduction versus separation. The considerable increase of lying duration observed after the introduction was caused by the introduced goat being mostly located in the lying niche that she hardly left. A decreased lying duration, measured during the separation period when the separated goat was allowed only acoustic contact to her group, appeared associated with higher arousal and restlessness, potentially triggered by the goat's motivation to get back to her group.

The substantial reduction of **feeding duration** in the introduced goat, as described in the first study (chapter 4), reached a magnitude potentially resulting in health problems and demonstrated to what extent introduced goats were affected by this regrouping procedure. However, by measuring feeding duration in the separation experiment (chapter 6), it was also possible to detect a reduced feeding duration compared with reference values from the 'mildest' of the applied treatments, i.e. the separation with remaining visual and tactile contact to the group. This was surprising because especially low-ranking separated goats, given that they had undisturbed access to food during the separation, were expected to feed longer during separation than during the reference period. Thus, the extent to which feeding duration is reduced might allow inferring the extent of the welfare impairment.

When assessing the effects of introducing individual goats into established groups (chapter 4) and of separating and reintegrating individual goats (chapter 6), **concentrations of faecal cortisol metabolites** were increased by the second day of

the applied treatments but not on the first day. As the first sample was taken 12 hours after the onset of the potential stressor, a time delay considered to be at the lower limit necessary for cortisol to be metabolised and to be excreted in the faeces (Kleinsasser et al. 2010), it is likely that the time lag was too short for detecting changes on the first day of sampling. Thus, the time interval should be increased in future studies, but it seems that analysing cortisol metabolites in faeces is a promising tool for assessing long-term stressors in goats. When measuring concentrations of cortisol metabolites to monitor short-term stressors, such as the social confrontations described in chapter 5, it is necessary to sample several times in succession to ensure that potential peaks are not missed. The fact that levels of cortisol metabolites did not increase considerably and that levels differed only slightly between goats that were confronted alone and goats that were accompanied by two peers might indicate that being confronted with unfamiliar goats did indeed not lead to a strong activation of the hypothalamic-pituitary-adrenal axis. However, it is also possible that peaks were missed between the successive samples and that intervals need to be shorter when monitoring stressors during a short, one-hour time period.

Level of activity, which was measured in the second study when goats were confronted with unfamiliar goats (chapter 5), did not indicate treatment differences between goats confronted alone and goats accompanied by two familiar conspecifics. This was surprising, as confrontations were characterised by many short intervals of high activity during which unfamiliar goats directed many agonistic behaviours towards confrontees and/or their peers and chased them around. As the numbers of agonistic interactions varied clearly between the two treatments, it was expected that this would also be reflected in the confrontees' level of activity. However, between intervals of high activity, longer intervals with only little activity were observed, and it is possible that subtle differences were masked by summarising activity values over 15 minute intervals. Therefore, this outcome variable remains to be tested further to conclusively assess its suitability.

Apart from **sniffing**, behaviours that were categorised to be affiliative (scratching, licking, and mock fighting) occurred too rarely to be analysed. Furthermore, goats mainly sniffed at the base of the other goats' horns where scent glands are located. Consequently, sniffing might rather reflect olfactory inspection and thus a more neutral behaviour than represent a true affiliative behaviour. This assumption is supported by the results described in the third study (chapter 6), where the probability of goats sniffing at each other was generally higher during the treatment that allowed only acoustic contact. Further, more group members were sniffed at by the reintegrated goat on the first day of the reintegration period than on any other day. Therefore, it remains to be tested whether goats use sniffing behaviour at the horn base to discriminate between individual goats by means of olfactory cues.

Among all variables measured, the informative value of **agonistic interactions**, a variable most frequently recorded when investigating regrouping in farm animal species, was the only one that proved to be ambiguous. Only considering agonistic interactions between the introduced goat and the group members (chapter 4) might have led to the erroneous conclusion that adverse effects on welfare were limited to only the first day of the introduction period, thus underestimating the negative welfare effects. In contrast, when assessing the effects of the presence of familiar conspecifics during social confrontations (chapter 5), agonistic interactions allowed differentiating between the two treatments more explicitly than other measured outcome variables. This stresses the importance of research investigating context dependency of welfare indicators and of employing a combination of different outcome variables to make reliable inferences.

7.3 Establishment of dominance relationships and group formation

In the context of these ambiguous results concerning the informative value of agonistic interactions as an indicator of animal welfare, one should consider the presumed underlying motivation responsible for the agonistic interactions displayed during the experiments described in chapters 4 and 5. Agonistic

interactions following an introduction of unfamiliar animals into a group are thought to reflect the establishment of dominance relationships (Barash 1977). Hence, after introducing unfamiliar individual goats into established groups and after confronting goats with unfamiliar groups of goats, a higher level of agonistic interactions was expected. To be indicative of the establishment of dominance relationships, these interactions were predicted to be reciprocal, i.e. to be initiated and won by both group members and introduced or confronted goats. Further, a high proportion of the displayed agonistic interactions was expected to involve physical contact. Especially fights were thought to occur, as fights seem to be an efficient way of establishing dominance relationships in goats (Shank 1972). With the establishment of dominance relationships between each pair of goats, the level of agonistic interactions and the proportion of interactions with physical contact were thought to return to their initial baseline levels. Contrary to these assumptions, introduced goats rather isolated themselves by hiding in lying niches and did not get involved in agonistic interactions (chapter 4). Further, agonistic interactions were mainly initiated and won by the members of the groups that goats were introduced into (chapter 4) or confronted with (chapter 5). Additionally, fights did only rarely occur. When trying to interpret these unexpected patterns of agonistic behaviour, one could conclude that the group members intended to drive away the unfamiliar animals, i.e. the introduced goat (chapter 4) or the confrontees (chapter 5), rather than being motivated to establish dominance relationships.

Dominance relationships have been defined as 'a pattern of repeated agonistic interactions between two individuals, characterised by a consistent outcome in favour of the same dyad member' (Langbein and Puppe 2004). Thereby, dominance relationships regulate the access to resources without the need for explicit agonistic interactions (Kaufmann 1983). Especially under intensive housing conditions, where space availability and access to resources are limited, the 'function' of dominance relationships to reduce agonistic interactions is likely to be relevant (chapter 3.3). Consequently, it can be hypothesised that having

established dominance relationships is a precondition for a group of goats when housed together under intensive housing conditions. Nevertheless, it has to be noted that not only dominance relationships but also other social relationships, such as positive social bonds, influence social behaviour between group members. Thus, established dominance relationships, although representing an important criterion in defining housed animals as a 'group', do not inform about the quality of a group.

In the context of introducing unfamiliar goats, this means that a group formation can be considered as given as soon as dominance relationships can be evaluated between all pairs of goats. For group formation to occur, however, it is conditional that the goats are motivated to establish dominance relationships. Based on the results described in chapter 4, it seems that when dominance relationships have been established, negative welfare effects associated with introducing unfamiliar goats should be mitigated effectively. Therefore, to find ways of integrating goats into unfamiliar groups, it has to be investigated how group formation can be facilitated.

7.4 Current knowledge of group formation in goats and in other farm animal species

As summarised in chapter 3.4, introducing unfamiliar animals into established groups has been associated with negative welfare effects not only in goats but in most farm animal species. Assuming that associated negative welfare effects can be effectively mitigated only when animals are motivated to form a group, it is useful to investigate whether regrouping approaches tested so far facilitated group formation.

Even if the level of agonistic interactions per se does not allow concluding that a group has been formed, information on whether group members or introduced animals initiate and win agonistic interactions and on whether agonistic interactions with or without physical contact predominate might help to assess whether group formation occurred (chapter 7.3). Thus, previously tested

regrouping approaches might indicate how group formation can be facilitated. For example, it has been hypothesised that **repeatedly regrouping or confronting animals** will habituate the animals to the procedure and that due to the repeated experience of establishing dominance relationships they will establish them more quickly with an increasing number of regroupings and/or confrontations (Raussi et al. 2005, Christensen et al. 2011). In these studies, it was expected that with an increasing number of regroupings, the number of agonistic behaviours would decrease. Based on what has been discussed above, it could not only be of interest to consider the number of agonistic interactions but also to assess whether repeated regrouping affects who participates in and wins agonistic interactions as well as the proportion of agonistic behaviours involving physical contact. Results from studies investigating the effects of repeated regrouping on goats with intervals of one and two weeks did not indicate that this is a promising approach to facilitate group formation. Although blood cortisol concentration did not increase after regroupings (Andersen et al. 2008) and milk yield decreased only after the first of three regroupings (Fernández et al. 2007), the level of agonistic interactions after the initial regrouping did not decrease with an increasing number of regroupings (Fernández et al. 2007, Andersen et al. 2008). As repeated regrouping was not effective in changing the number and quality of agonistic interactions or decreasing stress response in horses, pigs, and sheep (Giersing and Andersson 1998, Sevi et al. 2001, Coutellier et al. 2007, Christensen et al. 2011), it also did not seem to facilitate group formation in these species. The results for heifers/cows are ambiguous as to whether the animals habituate to repeated regroupings. Whereas regrouped heifers reacted less in novel and potentially fearful situations and showed a lower hypothalamic-pituitary-adrenal axis reactivity compared with heifers that were not regrouped (Raussi et al. 2006), regrouping persistently evoked agonistic interactions, and no differences were detectable between regrouped and control animals in a social confrontation test (Raussi et al. 2005). This corresponds to the results described in chapter 5. Although agonistic interactions decreased with each additional confrontation, the

quality of interactions remained unchanged: A high proportion of agonistic interactions involved physical contact, most of the agonistic interactions were initiated by unfamiliar goats, and fights were hardly recorded. Thus, for the studies presented here as well as for the studies cited above, it can be concluded that repeated regrouping does not facilitate group formation and that the agonistic interactions observed during regrouping are triggered rather by unfamiliarity than by the motivation to establish dominance relationships.

Increasing **weight heterogeneity** between confronted individuals has also been assumed to facilitate the establishment of dominance relationships. It is hypothesised that a large weight difference allows the animals to assess each other's fighting ability more easily during confrontations (Rushen 1988). A large weight difference has indeed been shown to reduce fighting duration in pigs (Rushen 1987, 1988), especially when the same individuals were confronted again (Rushen 1988). However, the likelihood to fight was not reduced in the studies of Rushen (1987, 1988) nor was the proportion of pairs of pigs that showed overt fighting reduced in confrontations between pigs with a large weight difference (Jensen and Yngvesson 1998). Moreover, fight participation and fight success (Andersen et al. 2000) as well as the nature and duration of agonistic interactions (D'Eath 2006) occurring after regrouping unfamiliar pigs could be explained to only some extent by differences in weight. Finally, if differences in physical characteristics, such as age, size, and weight, which have been related to a goat's fighting ability and consequently its success in dominance interactions (chapter 3.3), indeed facilitated the establishment of dominance relationships, this should have been reflected in the data of this thesis. Based on the assumptions tested in pigs and hypothesised for goats (Alley and Fordham 1994), most agonistic interactions should be observed between group members with a social status similar to that of the introduced, reintegrated, or confronted goat. In the three studies presented here, however, no such pattern was observable, and high-ranking introduced goats were not more successful than low-ranking introduced goats. Consequently, physical characteristics such as weight might

affect an animal's fighting ability to some extent and influence the outcome of agonistic interactions. However, this effect is of significance only when animals establish dominance relationships, which neither the animals in our studies nor those in the cited studies seemed to do.

Another approach to facilitate group formation has been to **pre-expose animals prior to the actual encounter**. It is hypothesised that a pre-exposure, e.g. through bars or a small opening, would facilitate the establishment of dominance relationships because the animals can assess each other's fighting ability beforehand (Rushen 1988, Hartmann et al. 2011). However, pre-exposure could not reduce the occurrence of explicit agonistic interactions or the duration of fighting in young pigs (Rushen 1988, Jensen and Yngvesson 1998). In horses, results were contradictory as to whether pre-exposure led to a slightly reduced proportion of agonistic interactions involving physical contact during subsequent confrontations (Hartmann et al. 2009, 2011). This goes along with the results described in chapters 4 and 5: In both studies, goats were housed in the same building and had acoustic and visual contact to the goats they were later introduced to (chapter 4) or confronted with (chapter 5). Thus, they were not completely unfamiliar. Despite this form of pre exposure, negative effects on animal welfare were considerable for the regrouped goats in both regrouping procedures. Although we do not know if effects would be even more adverse when confronting completely unfamiliar animals, at least this form of pre-exposure did not result in goats establishing dominance relationships quickly after introductions or confrontations.

Taken together, it appears that not being motivated to establish dominance relationships in situations of regrouping might not only be a particular trait of goats but also occur in other farm animal species. So far, none of the applied regrouping approaches facilitated group formation after introducing unfamiliar animals. Assuming that negative welfare effects associated with the introduction of unfamiliar animals can be effectively mitigated only when the animals have

fulfilled the minimal criterion for group formation by establishing dominance relationships, new approaches are necessary.

7.5 Prospects for future research: facilitation of group formation

From what is discussed above, it seems necessary for future research to take a more general approach at how to prepare goats to deal with regrouping and to facilitate group formation and thus the establishment of dominance relationships instead of focusing on variations in the introduction procedure itself. In the following, three approaches are presented. The first focuses on the information needed about goats living under natural conditions in order to assess whether introducing unfamiliar animals might overtax the goats' adaptive capacities. The second approach takes into account social characteristics of goats and addresses how these might help to facilitate group formation under farm housing conditions. The third approach suggests that these housing conditions affect the development of social behaviour and might contribute to the goats' inability to accept new group members.

7.5.1 Social organisation of goats living under natural conditions

To generate new, testable hypotheses on how to facilitate group formation in goats, a first step is to obtain more information about the social organisation of goats living under natural conditions. As this organisation represents the social environment in which goats evolved, it can be expected to reflect their adaptive capacities. The available observations suggest that neither the introduction of (individual) goats into established groups nor the separation from the group comply with the social organisation of goats living under natural conditions (chapter 3.2). However, to reliably infer whether the adaptive capacities of goats might be overtaxed by regroupings, more quantitative data are needed. In the context of group formation, information on whether the family group is a closed social unit or whether goats are socially organised outside the family group would be especially important. Although feral female goats were shown to form very

stable groups based on behavioural data, nothing is known about the genetic relatedness between the associated goats (Stanley and Dunbar 2013). However, such knowledge is essential to find out whether and how goats of different matriline are organised socially.

7.5.2 Facilitating group formation under farm housing conditions

Even if information about the social organisation of goats living under natural conditions would reveal that regrouping goats is likely to overtax their adaptive capacities, there might be measures to facilitate group formation at least to some extent under farm housing conditions. These measures could use available knowledge of the quality of social behaviours in goats. Despite the importance of dominance relationships for regulating access to resources without the need for explicit agonistic interactions, positive social relationships exist between goats and have been shown to reduce the level of agonistic interactions in established groups. In previous studies, the presence of positive social bonds was independent of the dominance relationships of the bonded animals (Aschwanden et al. 2008b). It was presumed that the likelihood to develop such positive social relationships is positively correlated with the amount of time goats spend together (Aschwanden et al. 2008b). Thus, depending on the number of positive social relationships, groups could differ greatly in the number of agonistic interactions. If the **likelihood to form positive social relationships** between a given pair of goats could be linked to certain characteristics of these animals, groups could be formed more effectively. In goats living in small groups under intensive housing conditions, it appeared that groups varying in both grouping age and presence of horns differ in their social characteristics (Zweifel 2008). Further, persistent social preferences have been identified in feral female goats (Stanley and Dunbar 2013), but so far, it is not known which characteristics might determine why certain individuals are more likely to associate with each other. In other species, first attempts have been undertaken to investigate group structures in relation to characteristics of the group members. For example, the social organisation of sealions was partly

explained by characteristics such as sex, age class, and site fidelity (Wolf et al. 2007), and in fish, individuals were found to assort by behavioural type (Croft et al. 2009). In terms of facilitating group formation, such studies ultimately aim to investigate whether goats that are more likely to form positive social relationships would also be more likely to accept new group members and thus be more tolerant when unfamiliar animals are introduced.

Another aspect relating to an animal's sociality could also be worth investigating: Within a given group, some animals have more social contacts than others (Wey et al. 2008, Stanley and Dunbar 2013). In primate groups, animals with many social contacts have been shown to be especially important for the stability of their group (Flack et al. 2005, 2006). Thus, it would be interesting to test whether goats can be differentiated based on the **number of their social contacts** within a group and whether highly social goats would be more tolerant towards unfamiliar goats. By using goats that differ in their sociality, this hypothesis could be tested experimentally with the aim to mitigate negative welfare effects associated with introducing unfamiliar goats.

7.5.3 The effect of the social environment on the development of adequate social behaviour

The fact that goats did not establish dominance relationships and thus did not form a group might also reflect deficits in the goats' social behaviour. An animal's behaviour can be profoundly influenced by its social environment during ontogeny (Sachser et al. 2011). It would thus be important to investigate whether and how the behavioural development of goats might be affected by housing conditions and the social environment during both the prenatal and the rearing periods. Deficits in the behavioural development might lead to inadequate social behaviour and thus contribute to the goats' reluctance to accept new group members. As early as during the **prenatal period**, environmental influences can affect the offspring. These influences during the prenatal period are thought to prepare the offspring for future environmental conditions, making use of an animal's

phenotypic plasticity (Welberg and Seckl 2001). In guinea pigs and other laboratory rodents (reviewed by Kaiser and Sachser 2005), strong indications were found that the female offspring of mothers living in socially instable environments showed masculinisation, which included their endocrine state, brain development, and behaviour. These traits were absent in offspring whose mothers lived in a stable social environment. As common management procedures in farming practice involve regroupings, an increasing number of researchers begin to investigate potential consequences of social instability experienced by mothers on their offspring (e.g. goats and pigs: Jarvis et al. 2006, Andersen et al. 2008, Otten et al. 2010). As masculinisation in female guinea pigs was hypothesised to be associated with being more competitive, potential changes of social behaviour due to prenatal social stress could be an interesting focus for investigating the reactions towards unfamiliar individuals in farm animals. However, much more basic research is necessary before effects of social stress during pregnancy on the offspring can be studied in an applied setting, i.e. in the context of housing conditions and management procedures. In goats, potential differences in the offspring's phenotype have to be identified and their causal relationships to applied prenatal treatments have to be determined before any further experiments can be conducted.

After birth, **conspecifics** are important for the development of an animal's species-specific social behaviour. Not allowing animals to interact with conspecifics has been shown to result in deficits in social behaviour, for example in singly housed calves compared with group-housed calves (Veissier et al. 1994, Jensen et al. 1999). In farming practice, the importance of conspecifics is generally acknowledged, and (dairy) goats, like dairy sheep and dairy cattle, are commonly housed together with **same-aged peers** after the separation from their mothers shortly after birth. Apart from allowing young goats to interact with conspecifics, this management procedure has the advantage of reducing the individual distances between goats (Aschwanden et al. 2008b). Similarly, it has been hypothesised that group housing of calves after separation from their mother

shortly after birth can strengthen the social bonds between calves (Veissier and Le Neindre 1989). Thus, at first sight, rearing ungulates in same-aged peer groups appears to be sufficient for the development of adequate social behaviour. However, the importance of the influences of the **mother** for behavioural development might have been underestimated. In rodents and primates, even temporary maternal separation has been shown to modify neurobiological development and thereby behaviour in a way that has been associated with psychopathologies (Sánchez et al. 2001). Whereas not being able to suckle has been linked to the development of abnormal behaviours (i.e. cross-sucking) in calves (Fröberg and Lidfors 2009, Roth et al. 2009), the impact of motherless-rearing on the development of social behaviour is less investigated in other farm animal species. With cattle, there are indications that the presence of the mother influences the social behaviour of calves/heifers independent of the opportunity to suckle, i.e. in the context of feeding (Veissier and Le Neindre 1989, Krohn et al. 1999). In rhesus monkeys, the link between rearing environment and the development of social behaviour was established because peer-reared individuals showed more excessive aggression, less impulse control, and less competent social behaviour later in life than their mother-reared analogues (e.g. Higley et al. 1996). These behavioural differences were persistent and could be linked to the development of the monkeys' central serotonergic system (Ichise et al. 2006), which is involved in brain development (Hansson et al. 1998, 1999) and the regulation of aggression (Lesch and Merschdorf 2000, Lesch et al. 2012). Recently, Wagner et al. (2012) investigated potential long-term effects of mother rearing on the behaviour of heifers during introductions. Although both mother and artificially reared heifers were negatively affected by the introduction, as shown by social and lying behaviour as well as concentrations of faecal cortisol metabolites, subtle differences in social behaviour indicated that more extensive research could be promising. Similar to investigating the effects of prenatal social stress, such research would have to focus first on identifying phenotypic

differences between mother- and peer-reared goats and the underlying mechanisms before applied studies can be conducted.

Furthermore, it is conceivable that not only the mother but also the **social composition of a group**, e.g. its size and age structure, could be important for the development of adequate social behaviour. Thereby, a group composition more similar to the one found under natural conditions might be beneficial for the development of species-specific social behaviour. In addition to potential beneficial effects on the establishment of dominance relationships (chapter 3.3), groups with a heterogeneous age structure might also influence the behavioural development, as adults might be an important source of social stimulation and necessary for the development of social skills in young animals. Although this aspect has rarely been investigated in farm animals, there is a study in horses that supports this idea. As a consequence of just temporarily introducing adult horses into homogeneous groups of young horses, the frequency of agonistic interactions between young horses decreased and young horses expressed new behaviours (Bourjade et al. 2008). Hence, a heterogeneous age structure might indeed be important for the behavioural development in young animals. This questions the rearing of subadults with only same aged peers and should be worth investigating in goats.

These considerations lead to the question if apart from the adequate social partners, **distinct periods in ontogeny**, for example the early postnatal period or adolescence, are also essential for the development of adequate social skills. In guinea pigs, it has been shown that males introduced to unfamiliar colonies integrated into the social group only when already housed in colonies during adolescence. Individuals that had spent their adolescence with only another female, however, were involved in many agonistic interactions and did not integrate. It also has been hypothesised that the different social environments and thus social experiences made during adolescence influence the development of the hypothalamic-pituitary-adrenal axis and, in turn, result in differences in the ability to adapt later in life (Sachser et al. 2011). In goats, two main aspects would

have to be investigated: first, whether and how the social composition of a group can affect the development of the social behaviour of kids and second, whether these effects are more pronounced during certain phases of ontogeny, e.g. adolescence. This information might help to find ways to ensure that goats develop the social skills needed for group formation.

To conclude, to be able to deal more effectively with welfare issues related to the regrouping of goats, specifically the introduction of unfamiliar individuals into established groups, research should take a more general approach instead of focusing on the introduction procedure itself. By gaining more knowledge of the complexity and quality of social interactions between individuals, groups could be formed based on the compatibility of their members. As a consequence, investigating how to ensure the development of adequate social behaviour might help to find efficient methods of introducing unfamiliar goats successfully.

8 References

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