

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

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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 concentration 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.

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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 (Sondergaard 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 h, 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., 2008), small herd sizes (Gaudernack Tonnesen et al., 2008), unstructured pens (Aschwanden et al., 2009a,b) and restricted resources (Loretz et al., 2004; Meisfjord Jorgensen 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.

2. Methods

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 (approx. 3 m × 5 m), consisting of a deep-bedded straw area of 11.7 m² (approx. 3 m × 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 × 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 × 0.65 m, 0.55 m high) providing climbing opportunities and both elevated (above) and protected (below) lying areas, as well as a free-standing partition in the centre of the pen (approx. 1 m in diameter and 0.8 m in height) also serving as a platform.

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).

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. (2008)* 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

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 (<i>Shank, 1972</i>); 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 (<i>Addison and Baker, 1982</i>)
Explicit displacement	Using physical contact (with biting, butting and fighting not considered), a goat pushes another goat out of the way (<i>Addison and Baker, 1982</i>)
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 (<i>Tölä and Savas, 2007</i>)
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 to 2 m distance – possibly rearing up – the two goats clash heads (<i>Shank, 1972</i>)
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

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).

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 min 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 (5×24 h). 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).

2.4.1. Agonistic and affiliative behaviour

Agonistic and affiliative behaviour was directly observed between 8.30 and 11.30 am and again between 4 and 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 min 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 min time slots. On day 0 (introduction day), the first observation slot for each experimental group included the 15 min following the introduction of the unknown individual, thereby ensuring that the first 15 min 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.

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 min 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).

2.4.3. Lying behaviour

Lying behaviour was recorded using a commercial 3D acceleration logger (MSR145WA, Modular Signal Recorder Electronics GmbH; 33 mm \times 15 mm \times 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-h period.

2.4.4. Feeding and rumination behaviour

Feeding behaviour was recorded using a commercial logger (MSR145WS, Modular Signal Recorder Electronics GmbH, 20 mm \times 15 mm \times 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 h, data had to be transferred daily. To enable this, daily feeding behaviour was only recorded for 21.75 h a day. For purposes of analysis, data were extrapolated to the duration (no. of hours) of feeding and rumination per 24 h. Loggers were attached to the newly introduced goats as well as to three of the resident goats in each experimental group.

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 h 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 h 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 5β - 3α -hydroxy-11-oxo structure. This EIA has been successfully validated for monitoring adrenocortical activity in goats (Kleinsasser et al., 2010).

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.

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 and Maechler, 2010) 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 an 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.

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.

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.

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.

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.

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.

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 spent lying (no. of hours) per goat per 24 h. 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.

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 h (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.

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'.

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.

3. Results

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, butting was 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 butting 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 (butting) towards the introduced goat.

3.2. Agonistic and sniffing behaviour

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; Fig. 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)

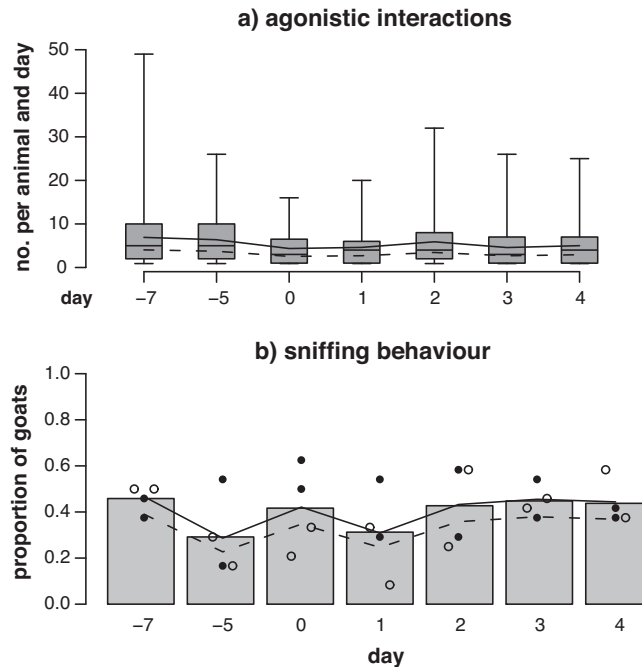


Fig. 1. (a) Number of agonistic interactions directed against resident goats from other resident goats per animal and day (box-and-whiskers-plot, boxes = 1st and 3rd quartile, thick line = median, whiskers = range of data). (b) Proportion of resident goats directing sniffing behaviour at other resident goats. Circles: proportions in the four groups; open circles = horned goats, filled circles = hornless goats. Solid line = model estimate for hornless medium-ranking goats, dashed line = model estimate for horned medium-ranking goats.

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; Fig. 1b).

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 1–4 ($F_{4,60} = 15.09$, $p < 0.001$; Fig. 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 ('day': $F_{4,60} = 170.84$, $p < 0.001$; Fig. 2b).

The proportion of agonistic interactions involving physical contact decreased over the course of the introduction period ($F_{4,60} = 2.77$, $p = 0.035$; Fig. 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: 0.18) throughout the introduction period ($F_{4,60} = 2.02$; $p = 0.10$).

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 1–4 (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).

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

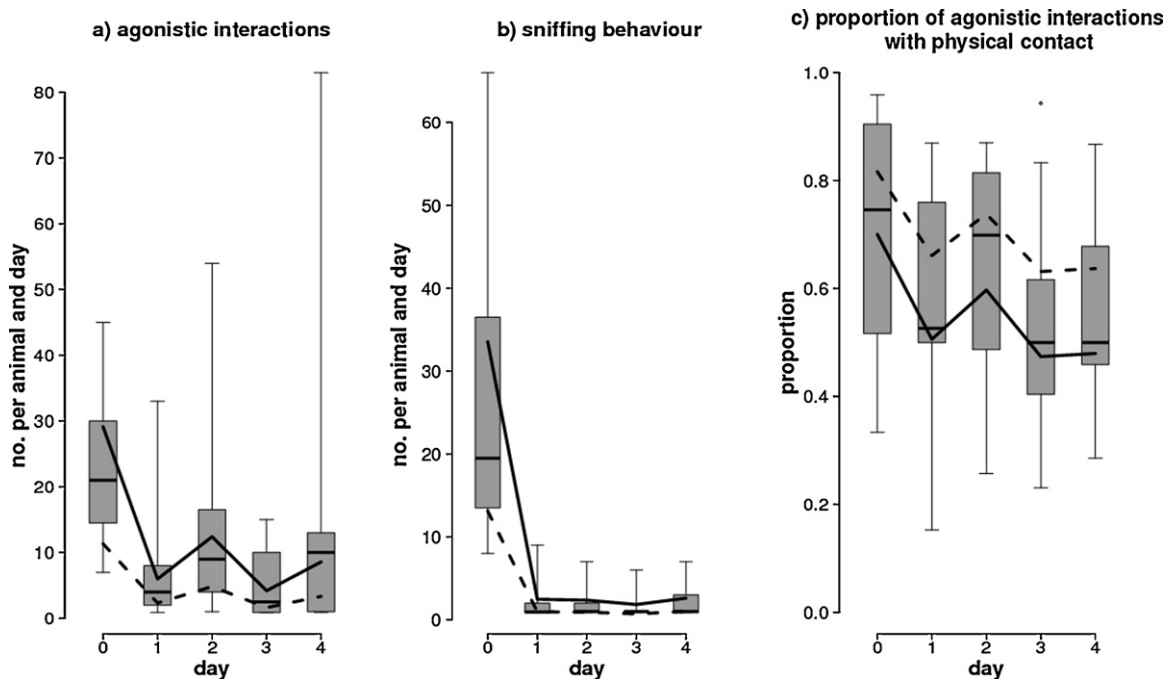


Fig. 2. (a) Number of agonistic interactions directed against introduced goats by resident goats per animal and day. (b) Number of sniffing behaviours directed against introduced goats by resident goats per animal and day. Box-and-whiskers-plots, boxes = 1st and 3rd quartile, thick line = median, whiskers = range of data. (c) Proportion of agonistic interactions with physical contact out of all agonistic interactions directed against introduced goats by resident goats. Solid line = model estimate for hornless medium-ranking goats, dashed line = model estimate for horned medium-ranking goats.

of scans spent by introduced goats in the niche remaining the same throughout the introduction period ($F_{4,59} = 1.76$, $p = 0.15$).

3.4. Lying behaviour

All hornless resident goats spent less time lying on day 0 than on the control days and on days 1–4 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 h was observed in horned and hornless medium-ranking resident goats (Fig. 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 and 23.3 h per 24 h. 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 (Fig. 3b).

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 (Fig. 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 h/24 h (SD: 0.68) for resident goats. With the exception of horned low-ranking resident goats, which ruminated for

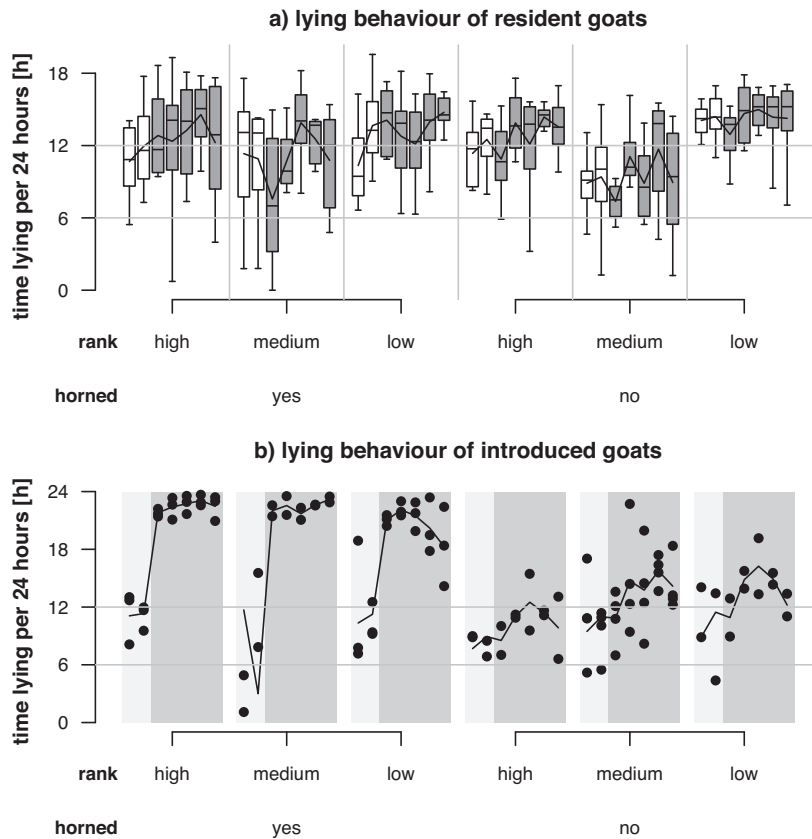


Fig. 3. (a) Time (in hours) spent lying per 24 h for (a) resident goats (box-and-whiskers-plots, boxes=1st and 3rd quartile, thick line=median, whiskers=range of data) and (b) introduced goats (filled circles=data of the individual goats of the indicated horn-status and rank category per day before/after 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.

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 ($F_{6,91} = 13.64$, $p < 0.001$, Fig. 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 h/24 h (SD: 1.69) during the control measurements and 3.03 h/24 h (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 h/24 h (SD: 0.82) during the control measurement and 3.68 h/24 h (SD: 1.58) during the introduction period (decrease of around 35%), whereas low-ranking goats spent

5.09 h/24 h (SD: 1.01) ruminating during control measurement and 4.32 h/24 h (SD: 1.89) during the introduction period (decrease of around 15%).

3.6. Cortisol metabolites

High-ranking resident goats exhibited higher cortisol metabolite concentrations than medium- and low-ranking animals (Fig. 5a). Despite this, the introduction of unknown individuals had no influence on the concentration 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 metabolite concentration returned to baseline concentrations by day 11 (maximum increase on day 2 compared to day –4: +63%; Fig. 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 metabolite 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

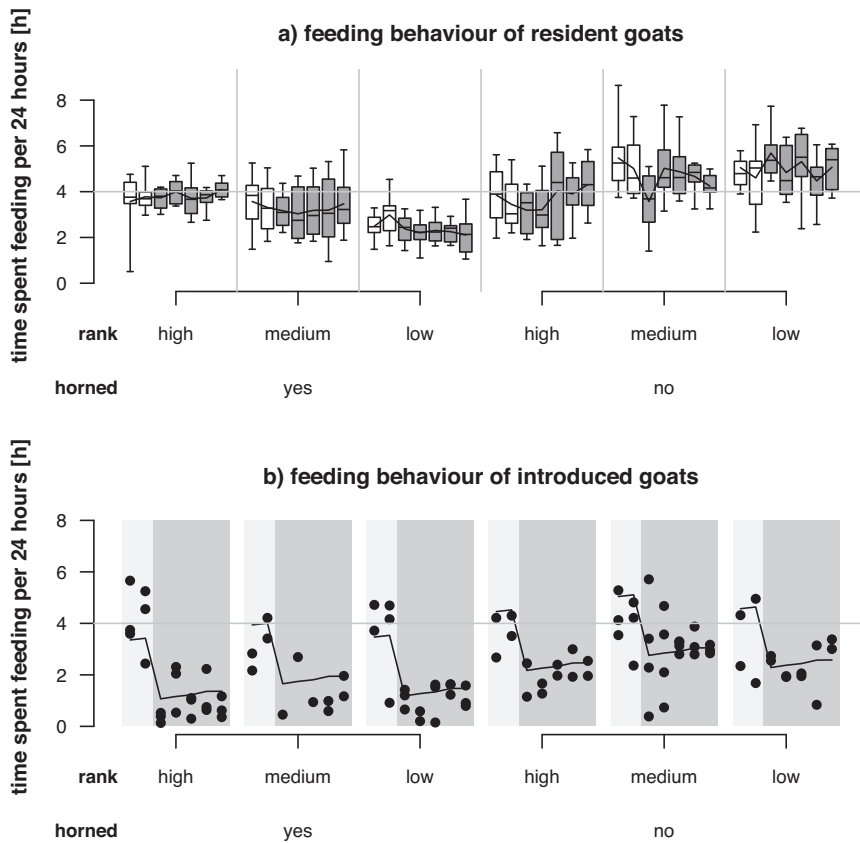


Fig. 4. Length of time (in hours) spent feeding per 24 h for (a) resident goats (box-and-whiskers-plots, boxes = 1st and 3rd quartile, thick line = median, whiskers = range of data) and (b) introduced goats (filled circles = data of the individual goats of the indicated horn-status and rank category per day before/after introduction). White boxes (resident goats/a) or light-grey background (introduced goats/b) = control situation before introduction (days -6 and -5), grey boxes (resident goats/a) or grey background (introduced goats/b) = days during introduction period (days 0–4). Solid lines indicate model estimates.

horned medium- and low-ranking goats and hornless goats (Fig. 5b).

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. 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

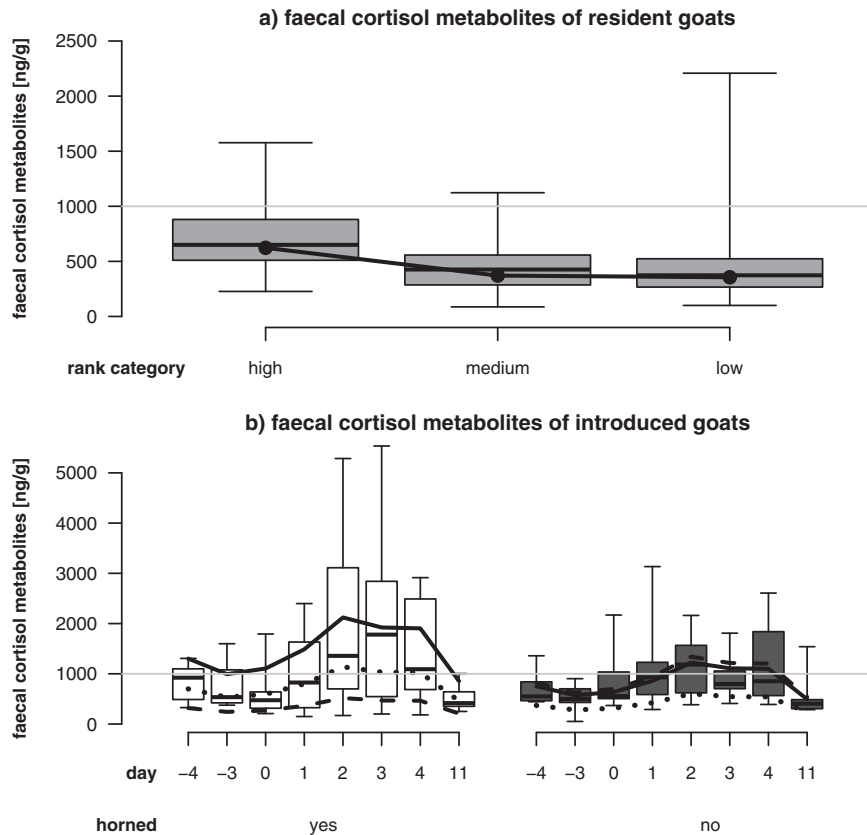


Fig. 5. (a) Concentrations of faecal cortisol metabolites (ng/g) of resident goats with respect to 'rank category'. Solid line = model estimate averaged for horned and hornless goats. (b) Concentrations of faecal cortisol metabolites (ng/g) of introduced horned goats (white boxes) and hornless goats (black boxes) with respect to 'day' and 'presence of horns'. Solid lines = model estimates for high-ranking goats; dashed line = model estimates for medium-ranking goats; dotted line = model estimates for low-ranking horned and hornless goats. Box-and-whiskers-plots, boxes = 1st and 3rd quartile, thick line = median, whiskers = range of data.

introduced goat. Furthermore, injuries sustained by the resident goats were negligible, and the introduction of the individual goat had no effect on cortisol metabolite concentration 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 Sambras (1997), throughout a six-week

observation period, the feeding durations of a group of introduced young goats never equaled those of the resident goats, but time spent feeding did increase consistently, reaching approximately 40% of the resident goats' times within 1 h 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 concentration 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 12–15 h 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 h 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., 2008). 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 metabolite concentration 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 concentration 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.

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