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

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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.

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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 (Aschwanden et al., 2008a; Carbonaro et al., 1992; Price and Thos, 1980; 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). 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.

2. Methods

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 m^2 (approximately 3 m × 5 m), consisting of a deep-bedded straw area of 10.1 m^2 and an elevated feeding place (3.6 m^2) divided by a wooden partition into two compartments of equal size. A*d 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).

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).

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 (Figs. 1a and 1b). The space for the remaining six goats was reduced to 11.8 m^2 (8.2 m^2 deep-bedded area and 3.6 m^2 elevated feeding place), with the

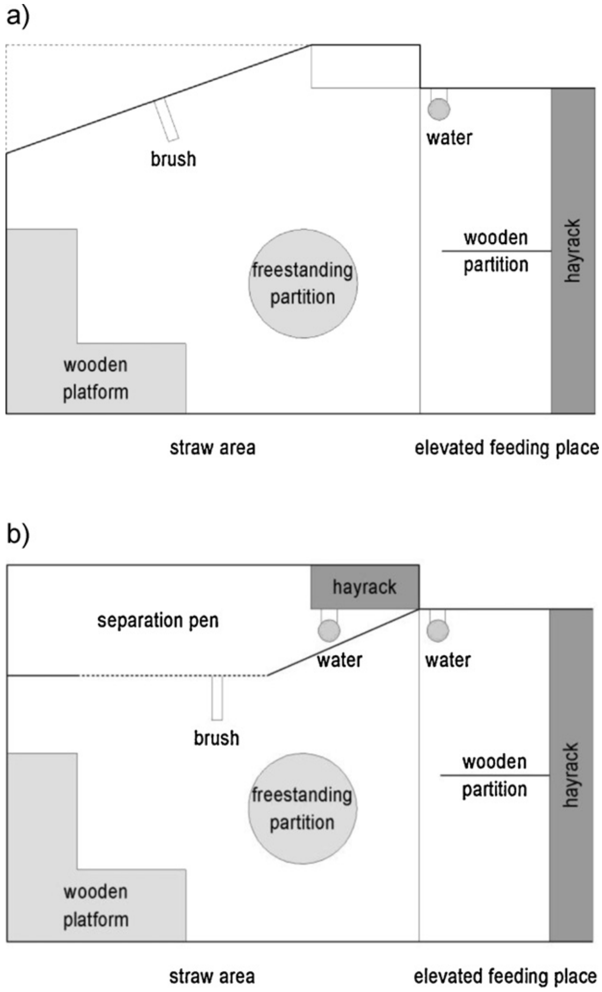

Fig. 1. Layout of pen (true to scale) during (a) reference and reintegration periods and (b) separation period. In (a) and (b), grey highlighting indicates that hayracks contain hay. In (b), the dotted section of the separation pen's partition indicates the bars through which visual and tactile contact between separated and resident goats was possible.

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 m^2. 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 m^2 and a 1.1 m^2 outdoor exercise area (3.5 m^2 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).

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 1, 2, 3 and 4 once. The order of regrouping corresponded to the order of separation, and regrouping was also performed at 15-minute intervals.

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 concentration 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 concentration 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.

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 threating 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). 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).

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). Owing to technical problems during data collection, seven days' worth of feeding data distributed among three different individuals was missing.

2.5.3. 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 concentration of cortisol metabolites was 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). 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%.

2.6. Statistical analysis

2.6.1. Fixed effects, 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.

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 Tables 1 and 2):

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 'treatment' for Lying S).

Outcome variable	Trans-formation	Error distribution	Type of effect[1]	Absolute values 'no-contact'		Treatment		F-/χ^2-value	p-value
						'no-contact'	'contact'		
						Estimated effects			
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 (h/day)	–	Normal	a	3.42		0.00	−0.12	$F_{1,35} = 0.59$	0.448
Feeding S (h/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 (h/day)	–	Normal	a	12.92		0.00	0.22	$F_{1,35} = 0.52$	0.474
				Day −7 × 'no-contact'					
					Day −7	0.00	0.13		
					Day −6	0.80	0.55		
					Day 0	−4.43	0.72		
Lying S (h/day)	–	Normal	a	12.10	Day 1	−0.40	1.16	$F_{6,131} = 4.44$	< 0.001
					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	Trans- formation	Error distribution	Type of effect[2]	Absolute values Day 'a'	Reference[1]		Separation		Reintegration				F-/χ^2-value	p-value
					Day 'a'	Day 'b'	Day 0	Day 1	Day 2	Day 3	Day 4	Day 11		
					Estimated effects									
Agonistic R-R[3] (no./animal/day)	–	Binomial	OR	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)	log	Normal	m	3.62	1.00	0.96	–	–	1.06	1.13	1.05	–	$F_{4,220} = 0.57$	0.682
Agonistic S-R (yes/no)	–	Binomial	OR	0.22	1.00	0.82	–	–	1.94	1.42	1.00	–	$\chi^2_6 = 10.92$	0.028
Sniffing R-R (yes/no)	–	Binomial	OR	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)	–	Binomial	OR	0.46	1.00	1.76	–	–	2.87	1.76	1.46	–	$\chi^2_6 = 5.97$	0.202
Sniffing S-R (yes/no)	–	Binomial	OR	0.04	1.00	1.54	–	–	5.20	0.66	1.36	–	$\chi^2_6 = 27.37$	<0.001
Feeding R (h/day)	–	Normal	a	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 (h/day)	–	Normal	a	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)	log	Normal	m	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)	log	Normal	m	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 (h/day)	–	Normal	a	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 (h/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] a = additive, m = multiplicative, OR = odds ratio: additive = add the quoted estimated effect size to the value of reference day 'a'; multiplicative = multiply the estimated effect by the value of reference day 'a'; odds ratio <1 = decreased probability, >1 = increased probability in relation to day 'a'.

[3] 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.

[4] See Table 1.

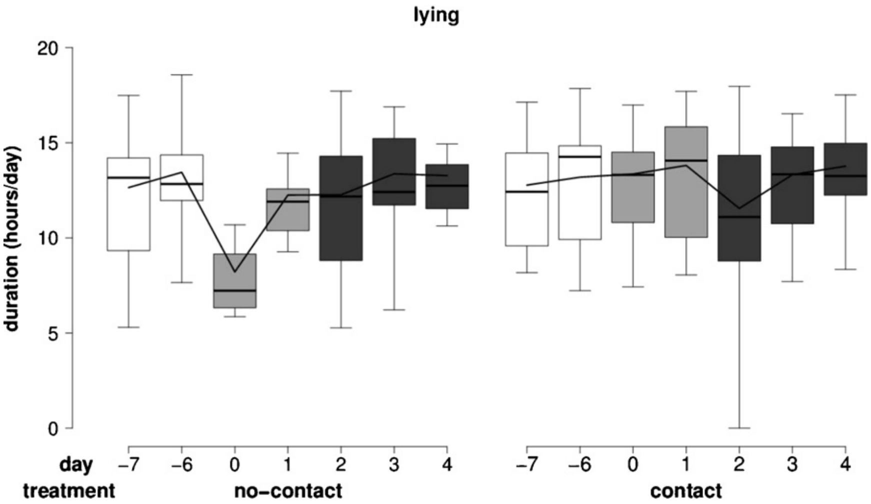

Fig. 2. Lying duration (h/day) for separated goats with respect to the 'no-contact' and 'contact' treatments. Box-and-whiskers plot: boxes = 1st and 3rd quartile, thick line = median, whiskers = range from minimum to maximum value. White boxes = reference situation before separation (days −7 and −6), light grey boxes = separation period (days 0 and 1), dark grey boxes = reintegration period (days 2, 3 and 4). Solid line = model estimate for medium-ranking goats.

- 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 form the previously separated goat,
- the lying duration (h/day) of focal residents (Lying R) and of separated goats (Lying S),
- the feeding duration (h/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).

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.

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 (Fig. 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); Tab. 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; Tab. 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; Tab. 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.

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 (Fig. 3). During separation, the concentration 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 (Fig. 4). On the first day of reintegration,

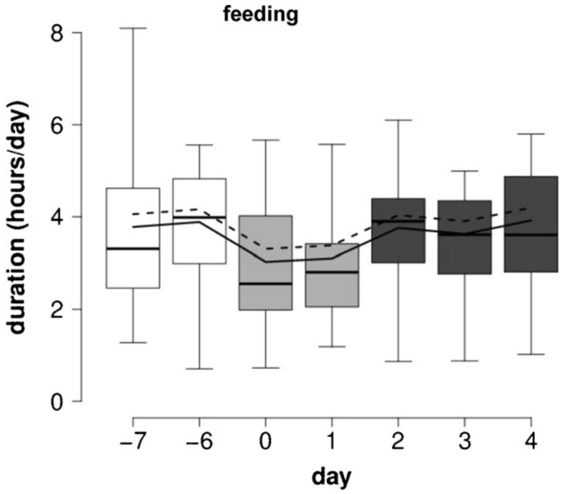

Fig. 3. Duration (h/day) separated goats spent feeding. Box-and-whiskers plot: boxes = 1st and 3rd quartile, thick line = median, whiskers = range from minimum to maximum value. White boxes = reference situation before separation (days −7 and −6), light grey boxes = separation period (days 0 and 1), 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.

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).

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 = 8.19) generally increased as the rank of the individual in question decreased. The concentration 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.

4. Discussion

In the present study, we tested the effects of two different methods of separation and a subsequent reintegration

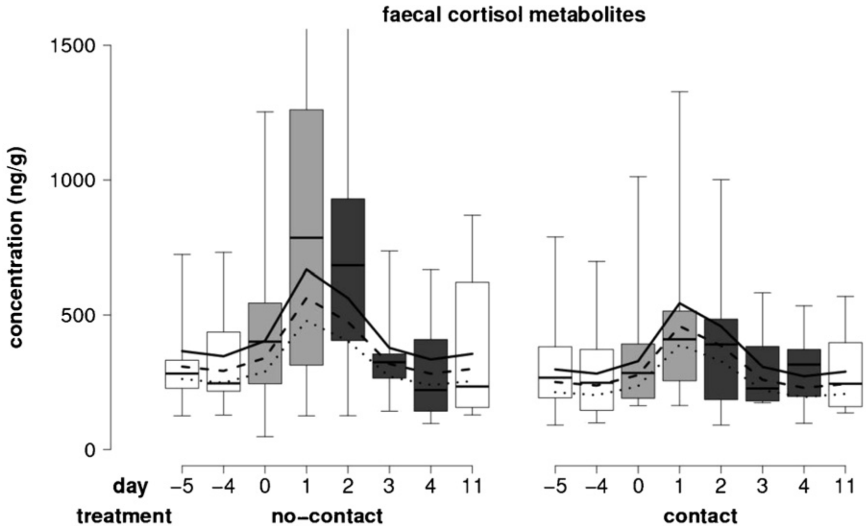

Fig. 4. Concentrations of faecal cortisol metabolites (ng/g) of separated goats with respect to the 'no-contact' and 'contact' treatments. Box-and-whiskers plot: boxes = 1st and 3rd quartile, thick line = median, whiskers = range from minimum to maximum value. White boxes = reference situation before separation and one week after reintegration (days −5, −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 line = model estimate for medium-ranking goats, dotted lines = model estimates for low-ranking goats.

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.

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 metabolite 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., 2012). 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 (Kay and Hall, 2009; Parrott et al., 1988; Piller et al., 1999).

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; Meisfjord Jorgensen 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 (Carr, 2002; Heinrichs and Richard, 1999). 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.

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).

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.

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