Introducing young dairy goats into the adult herd after parturition reduces social stress

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ABSTRACT

The aim of this experiment was to compare social stress, as measured by social behavior and adrenocortical activity, in young dairy goats during the first week after introduction into a herd of adult goats either during the dry period of the herd (i.e., all goats in the herd being pregnant or dry: PD) or shortly after parturition (i.e., all animals lactating or with their kids: LK). Thirty-two young goats that had had no contact with adult goats from the age of 7 wk were introduced into adult goat groups. Adult goats were kept in 2 groups of 36 animals each. Young goats were introduced (in groups of 4 animals each) into each of these 2 groups either during the PD period (2 repetitions) or during LK (2 repetitions); goats with different rearing experience were balanced over introduction periods. Young goats were more often receivers of agonistic social interactions when introduced during PD than during LK. Irrespective of the period of introduction, young goats had other young goats as neighbors more frequently than expected by chance alone, although this was even more distinct during PD. Cortisol metabolite levels increased markedly from baseline during PD, but not after parturition. Rearing showed an effect only on the nearest neighbors, with mother-reared young goats staying closer together. Our results indicate that young goats experience less social stress when being introduced into a herd of adult dairy goats shortly after parturition and with kids still present rather than during the dry period. Whether this effect is due to the period and lactational stage itself or to the presence of kids needs to be tested in future studies.

Key words: dairy goat, regrouping, social behavior, cortisol

INTRODUCTION

Wild or feral goats live in relatively stable herds based on female family groups (for review see Sembraus, 1978; O'Brien, 1988). That is, female offspring stay in the group of origin with their mother and siblings, whereas male goats leave their group. In dairy goats, however, young animals are generally reared separately from adult animals and introduced into the adult herd after several months of separation, usually when pregnant or after the first parturition. The social structure of goat herds (as in other social animal species) is characterized by an established (strict) dominance hierarchy (Keil and Sembraus, 1996; Barroso et al., 2000; Côté and Festa-Bianchet, 2001). Changes in group composition, either by mixing unfamiliar animals or by introducing a few unfamiliar animals into an already established larger herd, require the establishment of new dominance relationships. Consequently, increased levels of agonistic behavior are observed after mixing (e.g., cattle: Bøe and Færevik, 2003; goats: Alley and Fordham, 1994; Andersen et al., 2008), leading to an increased risk of injury, as demonstrated in dairy cows (Menke et al., 1999, 2000) and goats [E. Nordmann (Institute of Animal Husbandry and Welfare, University of Veterinary Medicine, Vienna, Austria), N. M. Keil (Agroscope Reckenholz-Tänikon ART, Ettenhausen, Switzerland), S. M. Waiblinger, unpublished data]. Physiological stress reactions (increase in cortisol: goats: Patt et al., 2012, cattle: Hasegawa et al., 1997) and a reduction in milk yield (cattle: Hasegawa et al., 1997; von Keyserlingk et al., 2008; goats: Fernández et al., 2007) also indicate the social stress experienced by animals during mixing, independent of species.

However, the level of stress experienced during integration may depend on different environmental and animal-related factors. For example, differences in the likelihood of becoming targets of aggression and in behavioral signs of stress are reported for the integration of single heifers compared with a group or pairs of heifers (Menke et al., 2000; O'Connell et al., 2008;

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Neisen et al., 2009). Regarding animal-related factors, the early social environment and previous social experiences may influence the way animals deal with situations of regrouping and encounters with unfamiliar animals (Le Neindre, 1991; Sachser et al., 1998; Wagner et al., 2012). Different reproduction stages (pregnancy, lactation) may also affect responses to challenging situations (Vierin and Bouissou, 2001). Hormones are known to be a major mechanism ensuring coordination between individuals (Adkins-Regan, 2005), influencing social behavior, among other factors (Hurnik et al., 1975). Some dairy goat farmers, as well as dairy cattle farmers, report fewer fights when animals are grouped shortly after parturition. Similarly, Schwarz and Samba (1997) found that in a dairy goat herd, social agonistic interactions were more frequent when one group of young goats was introduced into the herd during pregnancy rather than after parturition.

Therefore, the aim of this experiment was to compare social stress (as indicated by social behavior, injuries, and adrenocortical activity) in young dairy goats in the first week after introduction into a herd of adult goats either during the dry period of the herd (i.e., both young goats and adult goats in the herd were pregnant) or after parturition (i.e., all animals were lactating and with their kids). We hypothesized that introducing young goats after parturition and when kids are present would result in lower levels of social stress in young goats, indicated by less agonistic behavior, smaller increase in fecal cortisol metabolites (reflecting basal adrenocortical activity), and greater dispersion of young goats into the herd (i.e., not staying close together). Analyzing the neighbors of animals is a method of determining whether animals disperse at random or not (Sibbald et al., 2005). As cohesion within groups can vary with the degree of familiarity (Bouissou and Andrieu, 1978; Boissy and Dumont, 2002), we predicted that goats might stay in close proximity after their introduction, but to a lesser degree in the case of lower stress (i.e., after parturition).

MATERIALS AND METHODS

Animals, Housing, and Management

The experiment was performed with dairy goats (German Improved Fawn breed) of the Institute of Organic Farming in Trenthorst, northern Germany, between November 2008 and April 2009.

Adult Goats and Housing. Seventy-five adult dairy goats formed the lactating herd, with average milk yields of 570 kg/animal and year (3.35% fat, 3.01% protein) and an average age of 4.4 ± 1.64 yr (range: 2.7 to 7.7 yr). Three months (September 1, 2008) before

the start of the experiment, the goats were separated into 2 groups of 36 animals (3 of the 75 goats were sold before as only 36 feeding places per group were available). Groups were balanced for milk yield, age, and presence of horns (group 1: 2 polled animals; group 2: 1 polled animal; all others horned). The composition of the groups did not change until the end of the experiment. The 2 groups were housed in a deep litter system (group 1: 5.0 m² and group 2: 5.4 m²/adult goat) with a wooden palisade feed barrier with 36 feeding places (40 cm wide) and solid head partitions. Each group had access to a drinking trough, mineral blocks, and a brush. As soon as kidding started, an area with 2 hay racks that was accessible only to the kids was provided. All goats kidded in their groups and were then separated with their kids from the rest of the group by using metal elements. After about 5 d (5 to 7 d), the metal elements were removed and the goats were milked. The number of kids born was 63 in group 1 and 58 in group 2. Five goats lost their offspring in group 1 and 7 in group 2.

Young Goats and Rearing. The 32 young replacement goats were kept in one group before the start of the experiment (age at start: 1.8 yr). The rearing of these young goats differed during the first 45 d of life, with 17 (all horned) having been reared with their mothers and 15 (all horned except 1) reared artificially with automatic milk feeders from d 5 of life. From the age of 46 d, all young goats had no contact with adult goats until the start of the experiment and were kept as one group on pasture until the end of February 2009 or in a separate part of the barn thereafter. A calf shelter was provided on pasture. The animals had access to hay racks offering hay ad libitum and a drinking trough.

Mating and Kidding. Mating of adult goats took place in September and October; the adult goats were dried off at the end of November (1 d before the experiment started), and the kidding period lasted from February to the beginning of March 2009. To ensure constant group size and composition of adult goat groups 1 and 2 during introductions, the mating of young goats started after the mating of adult goats was completed and ended at the beginning of December. The kidding process for young replacement goats was equivalent to that for adult goats and took place between the middle of March and the beginning of April 2009.

Feeding and Milking. Goats were fed fresh hay at 0800 h in the dry period and at 0830 h during lactation; at 1230 and 1700 h, the remaining hay was pushed toward the feeding rack again. About 3 wk before expected birth, concentrate feed was added (2×250 g/d and animal). During LK, goats were milked twice daily in a side-by-side milking parlor with 10 milking places and 5 milking units in one row. The milking of both

groups lasted around 2 h for each milking (1 h per group). The waiting area provided a space of 0.9 m²/animal.

Experimental Design

Young goats were introduced in 8 groups of 4 animals each, following the experimental design described in Table 1. Half of the young goats (i.e., 4 groups, 2 in each of the adult goat groups) were introduced when all goats were pregnant and not being milked (pregnant and dry, introduction period **PD**; 2 repetitions per adult goat group) and the other half (4 groups, 2 groups per adult goat group) shortly after parturition, when all goats were lactating and their kids were still present (lactating with kids, introduction period **LK**; 2 repetitions per adult goat group). Young goats were balanced for rearing: artificial (**a**), mother-reared (**m**) within young goat groups.

Young goats were introduced at midday on d 1 and stayed in their respective adult groups for 7 d. After removal from the adult goat group on d 7, they were not returned to the original group of young goats but kept separately. The time between repetitions during PD and during LK was 1 wk (Table 1).

Young goats were allocated to the 2 groups of adult goats balanced for rearing (a and m) within young goat groups (Table 1). Young goat twins were separated, resulting in one animal having her mother present in the group of adult goats and the other one not. The allocation of young goats in the introduction period LK was influenced by the time they had given birth to their kids; therefore, twin and single goats were not as evenly distributed in LK as in PD. According to mating periods, the kidding of adult goats had to be finished before kidding of the young started to avoid changes in group composition in the experimental phase. Therefore, the number of days after parturition was greater for adult goats and their kids were older (median 52

± 8.74 d postpartum, range 26–62 d at the beginning of LK) than those of young goats (median 12 ± 4.06 d postpartum, range 6–19 d at the beginning of their respective introduction). To be able to introduce 4 young goats and their kids simultaneously into each of the adult goat groups (i.e., 8 young goats per repetition in LK), it was necessary to wait until 8 of them had given birth before the introduction of this repetition could begin. Consequently, for young goats, the number of days after parturition ranged between 6 and 19.

Space allowances in PD were 4.5 m²/animal in group 1 and 4.8 m²/animal in group 2. In introduction period LK, space allowances for the goat herd were smaller (because of the separated kid area, where kids could rest and feed undisturbed): 4.3 m²/goat (adult and young goats without kids) in group 1 and 4.5 m²/goat in group 2. In both groups, 0.90 feeding places/animal were offered. For observations at night, lighting was used in the barn, which was switched on for habituation 2 d before each observation.

Social Behavior

To be able to identify all goats individually, all animals (young and adult goats) were marked on their sides and backs with animal markers (young goats) or hair dye (adult goats) before introduction.

Social Interactions. The social behavior of the introduced young goats was recorded on d 1 (day of introduction) from 1200 to 1400 h, 1430 to 1550 h, and 1730 to 1930 h, and on d 2 to 7 from 0830 to 1030 h, 1300 to 1500 h, and 1730 to 1850 h by direct observation. The observer was located on an elevated seat in the feeding area between the 2 groups of adult goats. Observations on d 1 started immediately after the introduction of the goats.

Social interactions were observed by continuous focal sampling using Observer 5.0 software (Noldus, Wageningen, the Netherlands) on a netbook. On d 2 to 7,

Table 1. Timeline of the experiment and rearing history of young goats integrated into adult goat group 1 or 2 over introduction periods (pregnant and dry, PD; lactating with kids, LK) and repetitions (1, 2) within introduction periods

Timeline	Introduction period–repetition	Adult goat group 1		Adult goat group 2	
		Young goat group rearing ¹	Presence of mother ²	Young goat group rearing	Presence of mother
Week 1	PD–1	YG1: 2 a, 2 m	1	YG2: 2 a, 2 m	1
Week 2					
Week 3		YG3: 2 a, 2 m	0	YG4: 2 a, 2 m	1
Weeks 4 to 16	PD–2				
Week 17		YG5: 2 a, 2 m	1	YG6: 3 a, 1 m	1
Week 18					
Week 19	LK–2	YG7: 2 a, 2 m	2	YG8: 2 a, 2 m	2

¹For each group of young goats (YG1 to YG8), the number of young goats being reared artificially (a) or with their mothers (m) is indicated.

²Numbers of mother-reared young goats being in the same group as their mothers.

one person (the same individual throughout the observation period) observed both groups: the focal animal was changed every 4 min in the same order, alternating between the 2 groups of young goats that had been introduced simultaneously. On d 1, both groups were observed simultaneously; that is, 2 individuals observed in parallel. Thus, each young goat was observed for 64 min on d 1 and for 32 min on d 2 to 7. Each day's observations began with a different young goat (d 1 to 7) and adult herd (d 2 to 7). For each interaction, the actor (the goat initiating interaction) and receiver (goat being the target of interaction) were recorded. The ethogram is based on previous studies on social behavior in goats (e.g., Shank, 1972; Addison and Baker, 1982; Schino, 1998).

The following agonistic behaviors were recorded:

- **Butt:** A goat hits any part of the body of another goat, except the head, with her forehead/horn base, but without upward movement.
- **Horn kick:** A goat performs a quick upward movement with her head and hits another animal with the end of her horns or, in hornless goats, forehead. All extremities of the other goat stay on the ground.
- **Lift:** A goat performs a quick upward movement with her head and hits the body of another goat with the end of her horns or, in hornless goats, forehead and lifts up the receiver partly or totally. At least one extremity of the receiving goat loses contact with the ground.
- **Stroke:** When animals feed or stand close together, interactions of little intensity occur, such as sideways head movements toward neighboring goats. In horned animals, this is often limited to horn contact between the animals involved. Due to the proximity of the animals, this movement is similar to a butt or kick, but it is not as intense.
- **Bite:** A goat bites another one on any part of the body, except vulva or anus.
- **Push:** A goat pushes another one away from the feeding barrier using her shoulder or neck/head.
- **Threat:** A goat presents her horns, displays a similar threatening posture, indicates biting, or moves her head or body quickly toward another goat.
- **Avoid:** A goat retreats when another one approaches. In case of feeding, she leaves her feeding space without any visible agonistic behavior demonstrated by the approaching goat. The avoidance movement itself can be either slow or fast. In the case of avoiding, the animal walking away was recorded as receiver and the avoided goat as actor.
- **Clash:** Both goats face each other and strike forward, making contact either with their horns or, in hornless goats, with their foreheads; the animals may rear onto their hind legs before clashing.
- **Fight:** two or more goats face each other, either standing or circling each other; butts, horn kicks and lifting up may occur during fighting. The minimum duration of fighting bouts was 3 s, and when pauses lasted longer than 10 s, a new event was noted.

The following socio-positive interactions were recorded:

- **Lick, nibble:** A goat licks or nibbles on the body of another goat, except vulva or anus, using her tongue, teeth or lips.
- **Rub:** Slow, cautious rubbing of the head against any body part of another goat, except vulva or anus. The receiving goat does not withdraw.
- **Lean against each other:** A goat leans or rests her head/bottom jaw against any part of the body of another goat. The receiving goat does not withdraw.

Bites on vulva or anus were never observed and thus were not mentioned in the list as a separate category. For statistical analysis, agonistic interactions were grouped into the following 5 classes:

- agonistic interactions in which obvious physical contact was observed (**AgoPhysRec**); that is, sum of butt, horn kick, lift, stroke, bite, push, and clash, with the young goat as receiver.
- sum of avoid and threat, with the young goat as receiver (**AgoNonPhysRec**).
- agonistic interactions in which obvious physical contact was observed (**AgoPhysAct**); that is, sum of butt, horn kick, lift, stroke, bite, push, and clash, with the young goat as actor.
- sum of avoid and threat, with the young goat as actor (**AgoNonPhysAct**).
- sum of lick, nibble, rub, and lean against each other, with the young goat as either actor or receiver (**PositiveTotal**).

Fights were not analyzed further because of their rare occurrence (14 fights, total number of interactions 3,479) during the experimental period. **PositiveTotal** was only observed 28 times during the total experimental period and was therefore only analyzed descriptively.

Nearest Neighbors. Combined with the observation of social interactions, the 2 nearest neighbors of all young goats were recorded on the 7 d of introduction using scan sampling (10-min intervals, 220 scans in total, after every second focal animal observation). The

heads of both animals were taken as point of reference. When young goats were feeding, the closest animals feeding to the right and to the left of the young goat were noted as neighbors.

For further analyses, the proportion of young goats as nearest or second-nearest neighbors was calculated (number of scans with young goat as nearest or second-nearest neighbors/total number of scans). The proportion of mothers as nearest or second-nearest neighbors of mother-reared young goats in relation to scans was also calculated; that is, the number of scans with mother as nearest or second-nearest neighbors of mother-reared young goats/total number of scans $\times$ 100).

Injuries

All young goats were examined for injuries in the hours immediately before being introduced into the adult herd (d 1) and on d 7. The inspection of young goats was divided into examinations of the abdominal side and udder (examination abdomen) and of the rest of the body, including head, back, and body sides (examination body). The abdominal side and udder were visually inspected by using a hand mirror and a flashlight; the rest of the body was visually inspected and manually scanned (palpation). In the case of injuries, the type (excoriation, lesion on the surface, deep lesion, scar, swelling, callus), shape (circular, horizontal, vertical, V- or L-shaped), size (<1 cm, 1–3 cm, >3 cm), and location of the injury were noted.

Adrenocortical Activity

The analysis was based on fecal samples of young goats taken between 1630 and 1730 h. About 1 g was taken rectally 2 and 1 d before introduction (baseline, d -2 and -1) and on d 3, 5, and 7 of introduction. Samples were put on ice immediately and stored at -20°C after samples of all goats had been collected. Samples were later thawed, and after the extraction procedure (0.5 g of feces with 5 mL of 80% methanol; Palme and Möstl, 1997), the concentration of cortisol metabolites was determined using a group-specific 11-oxoetiocholanolone enzyme immunoassay first described by Möstl et al. (2002). Enzyme immunoassay has been successfully validated for measuring adrenocortical activity in goats (Kleinsasser et al., 2010).

Statistical Analysis

Statistical analyses were performed using the program PASW Statistics 17.0 (SPSS Inc., Chicago, IL). Because the different rearing of the young goats might affect how young goats cope with their introduction,

we included this factor in the analysis. General linear mixed models (GLMM) were used to analyze social behavior (AgoPhysRec, AgoNonPhysRec, AgoPhysAct, AgoNonPhysAct) and cortisol metabolites. All outcome variables were analyzed, with introduction period (PD, LK), rearing (a, m) and, in the case of rearing with the mother, presence of the mother (yes and no) as fixed factors. For cortisol metabolites only, introduction period $\times$ day of sampling (d -2 , -1 , 3, 5, and 7 relative to introduction) was included as an additional fixed factor. Identity of the mother (young goats with the same mother were twins), group of adult goats (group 1 or 2), young goat group (each introduced group of young goats was numbered from 1 to 8), and young goat nested in the young goat group were chosen as random effects for all variables. Weight at the start of the introduction was included as a covariate factor for analyses of social behavior and cortisol metabolite.

For the analysis of social behavior and of cortisol metabolite, the day of introduction (1 to 7) and the day of sampling, respectively, were used as repeated measures. Covariance structure was heterogeneous autoregressive (social behavior) or autoregressive (cortisol metabolites). Nonsignificant random effects were excluded step by step (P -values of identity of the mother and group of adult goats were always excluded because $P > 0.05$), although young goat in the group of young goats was retained irrespective of P -values. Residuals were checked graphically for normal distribution, homogeneity of variance, and outliers. To fulfill model assumptions, logarithmic transformation was performed for 3 social behavior variables, whereas cortisol metabolite data were square root transformed.

To check for possible effects of repetition, all variables were analyzed separately for the introduction periods (PD, LK) using the same general linear model (GLM) and procedure as described above, except for replacing the fixed factor introduction period with repetition (1, 2). Agonistic behaviors (AgoPhysRec, AgoNonPhysRec, AgoPhysAct, AgoNonPhysAct) were also analyzed descriptively per day (d 1 to 7) for PD and LK. For cortisol metabolites, differences between mean base levels (day of sampling -2 , -1) and mean levels of the following sampling days (d 3, 5, and 7) were calculated for PD and LK and analyzed descriptively.

Data on injuries were analyzed descriptively because only 3 injuries (i.e., 3 animals) occurred in total in periods PD and LK.

For analysis of the nearest and second-nearest neighbors of young goats, a GLM was used. The number of scans in which young goats were recorded as the nearest and second-nearest neighbors and the number of scans in which the mother of a mother-reared young goat was recorded as neighbor were analyzed. Introduc-

tion period, rearing, presence of the mother (in the case of rearing with the mother), group of adult goats, and the interaction period of introduction $\times$ group of adult goats were included as factors. Furthermore, for each young goat, we tested whether she had other young goats as nearest and second-nearest neighbors more often than would be expected by chance. For those young goats that had been reared with their mothers and had their mother present in the group, we also tested whether they had their mothers as nearest and second-nearest neighbors more often than would be expected by chance. For these purposes, Z - and P -values were calculated:

$$Z\text{-value} = \sqrt{n} \times (\hat{p} - p_0) / \sqrt{p_0 \times (1 - p_0)},$$

$$P\text{-value} = 1 - \Phi(Z\text{-value}),$$

where $\hat{p}$ = proportion of young goats or mother as nearest or second-nearest neighbors; p_0 = proportion of young goats in the total group (0.1); n = total number of scans (220); Φ = the standard normal cumulative distribution function.

Because it is difficult to thoroughly check all model assumptions in view of the amount of available data, we analyzed the data by considering “young goat group” as the observational unit; that is, by averaging the individual goat values for social behaviors, nearest neighbor, and cortisol metabolites per young goat group, and then performing an ANOVA on the averaged data. This avoids any problem of dependence between individuals in the same group. In the GLMM, we included a compound symmetry dependence structure. Although this seems to be a natural assumption in our context, the alternative ANOVA model provides additional assurance, as no assumptions about a specific covariance structure are needed. We report the results for all variables at the end of the Results section.

RESULTS

Social Interactions

In total, 3,479 interactions were recorded: 2,079 of these during PD and 1,400 during LK; adult goats were involved in 64% of cases. The greatest numbers of observed social interactions were agonistic interactions without physical contact; that is, threats and avoidance, mostly with young goats as receivers (AgoNonPhysRec; Table 2) and rarely as actors (AgoNonPhysAct; Table 2). Agonistic behaviors with physical contact (AgoPhysRec; AgoPhysAct) were seen even less frequently (Table 2). Only 12 and 16 socio-positive interactions (PositiveTotal) were observed during PD and LK, respectively, accounting for 0.6 and 1.1% of all observed interactions.

During introduction period PD, young goats were more often targets of agonistic interactions without physical contact (AgoNonPhysRec) and tended to be so for agonistic interactions with physical contact (AgoPhysRec; Table 2, Figure 1). They also initiated interactions with physical contact (AgoPhysAct) more often when introduced in PD than in LK. According to the descriptive analysis of behavioral classes, higher incidences were found on almost all days in PD (Figure 1). Rearing and the presence of the mother for those animals reared with their mothers had no influence on agonistic behaviors (Table 2).

When introduction periods were analyzed separately, repetitions differed only in PD. Young goats received (AgoNonPhysRec; $P < 0.05$) more agonistic interactions without physical contact in repetition 1 than in repetition 2 (repetition 1: median 22.50 interactions/animal and hour; range 1.88–63.75; repetition 2: median 12.19 interactions/animal and hour; range 0.0–50.63) and tended to initiate (AgoNonPhysAct; $P = 0.090$) more in repetition 1 than in repetition 2 (repetition 1: median 0.0 interactions/animal and hour; range

Table 2. Results of general linear mixed model (GLMM) analysis for agonistic behavior (physical, Phys, and nonphysical, NonPhys) with young goats as receivers (AgoPhysRec, AgoNonPhysRec) and actors (AgoPhysAct, AgoNonPhysAct)¹

Factor	AgoPhysRec (log ₁₀)		AgoNonPhysRec		AgoPhysAct (log ₁₀)		AgoNonPhysAct (log ₁₀)	
	<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value
Introduction period	3.873	0.059	5.972	0.021	9.146	0.006	0.001	0.978
Rearing	0.039	0.845	1.153	0.292	1.423	0.244	0.406	0.529
Presence of mother	0.005	0.945	0.003	0.960	0.143	0.708	0.465	0.501
Item ²	PD	LK	PD	LK	PD	LK	PD	LK
Proportion (%)	10.1	7.7	71.8	68.0	5.4	4.5	11.9	18.5
Number	211	109	1,494	953	114	63	248	259

¹*P*-values and *F*-values are given for the fixed factors introduction period (pregnant/dry, PD; lactating with kids, LK), rearing (artificial or with mother) and presence of mother (yes, no). Transformations performed with response variables for statistical analysis are indicated in parentheses; $n = 32$.

²Proportion (%) and absolute numbers of the agonistic behavioral classes of all social interactions in introduction periods PD and LK.

Figure 1. Frequency of agonistic behavior (physical, Phys, and nonphysical, NonPhys) with young goats as receivers (AgoPhysRec, AgoNonPhysRec) and actors (AgoPhysAct, AgoNonPhysAct) for introduction periods PD (pregnant/dry) and LK (lactating with kids) in relation to days of introduction (d 1 = day of introduction) given in interactions/animal and hour, based on observed data ($n = 32$). Because of the much higher rate of occurrence, a different scale is used for AgoNonPhysRec. Data are presented as box-and-whiskers plots with boxes (25th and 75th percentiles, central bar = median), whiskers (range from minimum to maximum excluding outliers and extremes), dots (°) indicate outliers, and asterisks (*) indicate extremes.

0.0–30.00; repetition 2: 0.0 interactions/animal and hour; range 0.0–9.38).

Nearest Neighbors

On average, young goats were each other's nearest neighbors in 68% (± 0.161 , range 35–90) and second-nearest neighbors in 45% (± 0.139 , range 24–75) of the scans. Statistical analysis confirmed for all individuals that young goats were each other's nearest neighbors more often than would be expected by chance ($P < 0.001$ for 29 animals, $P < 0.05$ for 3 animals). In most cases, they also were each other's second-nearest neighbors ($P < 0.001$ for 23 young goats; $P < 0.05$ for 4 animals, $P > 0.05$ for 5 animals, all of which were introduced in LK).

The GLM analysis showed that in both groups of adult goats, young goats were each other's neighbors more often during PD than during LK (Table 3). In LK, however, young goats were each other's neighbors more often in adult goat group 2 than in group 1 (Table 3), whereas in PD this variable did not differ between adult goat groups. Goats reared by their mothers had other young goats as neighbors more often than did artificially reared goats (Table 3).

In the introduction periods, social behavior did not differ consistently between repetitions. In PD, young goats were observed to be each other's neighbors more often in repetition 2 than in repetition 1 (nearest and second-nearest neighbors, $P < 0.05$; nearest neighbor: repetition 1: median 76%, range 67–90; repetition 2: 83%, range 60–90; second-nearest neighbor: repetition

Table 3. Results of general linear model (estimated means $\pm$ SE) analysis of nearest and second-nearest neighbors are given for introduction period, rearing, presence of mother, group of adult goats, and introduction period $\times$ group of adult goats¹

Item	Category	Nearest neighbor	Second-nearest neighbor
Introduction period	PD	79 $\pm$ 0.008	57 $\pm$ 0.010
	LK	55 $\pm$ 0.009	37 $\pm$ 0.008
	<i>P</i> -value	0.000	0.000
Rearing	Artificial	64 $\pm$ 0.014	45 $\pm$ 0.013
	With mother	72 $\pm$ 0.009	50 $\pm$ 0.009
	<i>P</i> -value	0.000	0.006
Presence of mother	Yes	66 $\pm$ 0.015	45 $\pm$ 0.015
	No	70 $\pm$ 0.008	49 $\pm$ 0.009
	<i>P</i> -value	0.096†	0.018
Group of adult goats	Group1	65 $\pm$ 0.010	45 $\pm$ 0.010
	Group2	71 $\pm$ 0.008	50 $\pm$ 0.009
	<i>P</i> -value	0.000	0.000
Introduction period $\times$ group of adult goats	PD group 1	79 $\pm$ 0.011	58 $\pm$ 0.014
	PD group 2	78 $\pm$ 0.011	57 $\pm$ 0.013
	LK group 1	48 $\pm$ 0.012	32 $\pm$ 0.011
	LK group 2	62 $\pm$ 0.012	43 $\pm$ 0.012
	<i>P</i> -value	0.000	0.000

¹Results (%; estimated means $\pm$ SE) indicate proportion of scans with young goats recorded as nearest and second-nearest neighbor ($n = 32$). PD = pregnant and dry; LK = lactating with kids.

†Tendency ($0.05 < P < 0.1$).

1: 52%, range 44–60; repetition 2: 66%, range 51–75). On the other hand, in LK, the ratio of young goats as neighbors was higher in repetition 1 than in repetition 2 (nearest and second-nearest neighbors, $P < 0.05$; nearest neighbor: repetition 1: 61%, range 47–74; repetition 2: 49%, range 35–66; second-nearest neighbor: repetition 1: 43%, range 32–49; repetition 2: 33%, range 24–43).

Injuries

In introduction period PD, no injuries of young goats were found. When young goats were introduced in LK, 3 animals were injured. Two artificially reared goats had a crust on the udder and on the ear, respectively (between 1 and 3 cm each); in 1 young goat reared by her mother, an area of the vulva (1–3 cm) was swollen. The udder and ear injuries occurred in the waiting area.

Adrenocortical Activity

Adrenocortical activity (i.e., concentrations of fecal cortisol metabolites) in young goats were affected by the introduction periods ($P < 0.001$, F -value 56.5), with higher levels in PD, and by the interaction of introduction period $\times$ day of sampling ($P = 0.001$, F -value 3.95). In PD, concentrations of fecal cortisol metabolites peaked on d 3 of sampling, and declined thereafter but remained higher on d 7 than before introduction (Figure 2). In LK, however, few, if any, changes in adrenocortical activity were seen throughout the days of sampling (Figure 2). Thus, irrespective of

higher baseline levels in PD compared with LK (Figure 2), differences between the mean baseline level and the means of the following sampling days were greater in PD than in LK (Figure 3, no model calculated with this variable); that is, levels of cortisol metabolites increased more in PD than in LK during introductions. Rearing ($P > 0.05$, F -value 0.03) and the presence of the mother ($P > 0.05$, F -value 0.02) did not influence concentrations of cortisol metabolites. When introduction periods were analyzed separately, we found no differences between repetitions.

Analysis with Group Averages

Differences between periods were largely confirmed when using the young goats group as the statistical unit. After averaging the data for each young goat group, the goats likewise initiated more interactions with physical contact (AgoPhysAct, $F = 14.72$, $P = 0.009$) and tended to be target of agonistic interaction more often (AgoNonPhysRec, $F = 4.083$, $P = 0.090$; AgoPhysRec, $F = 4.041$, $P = 0.091$) during PD. Young goats were each other's nearest neighbors ($F = 15.38$, $P = 0.008$) and second-nearest neighbor ($F = 9.998$, $P = 0.020$) more often during PD, and adrenocortical activity was higher during PD ($F = 48.91$, $P < 0.001$).

DISCUSSION

The results of this study confirm our hypothesis that social stress is lower when nonfamiliar young goats are introduced into a herd of adult goats after parturition,

Figure 2. Concentration of cortisol metabolites in feces (ng/g) of young goats by day of sampling (–1, –2, 3, 5, and 7 d relative to introduction) and introduction period (PD and LK). Figure is based on observed data, $n = 32$. PD = pregnant and dry; LK = lactating with kids.

when kids are present (LK), compared with introduction during pregnancy, when the adult goats are dry (PD). This is expressed in both behavioral and physiological variables. Young goats in PD were subjected to aggression more often and initiated aggression more often, dispersed less into the adult herd, and, in contrast to young goats in LK, showed marked physiological stress reactions (increase in fecal cortisol metabolites), which is in agreement with our predictions.

Introduced animals were targets of agonistic interactions (AgoPhysRec, AgoNonPhysRec) rather than initiators (AgoPhysAct, AgoNonPhysAct) in all introductions, coinciding with the results reported by Mench et al. (1990) in cattle and Patt et al. (2012) in goats, but the incidence was lower during LK. Especially during PD, the highest levels of agonistic interactions directed against young goats were observed on d 1 (AgoPhysRec) and on d 1 to 3 (AgoNonPhysRec), in line with other studies and species (e.g., Addison and Baker, 1982; Andersen et al., 2008; von Keyserlingk et al., 2008). The less pronounced increase for LK may indicate an easier integration of nonfamiliar animals through the easier establishment of dominance relationships during that period. The lower frequency of agonistic interactions during LK compared with PD agrees with the findings of Schwarz and Sambras (1997) for one goat herd and confirms our predictions.

All young goats were each other's neighbors more often than would be expected by chance, irrespective of introduction period. Interindividual associations depend on the quality of the relationship: animals associate more closely with familiar than with unfamiliar

Figure 3. Change of concentration of cortisol metabolites in feces (ng/g) of young goats after introduction (mean of samples of d 3, 5, and 7) as a percentage of base level (mean of samples of d –2 and –1) for introduction periods PD (pregnant/dry) and LK (lactating with kids). Figure is based on observed data, $n = 32$.

individuals (Boissy and Dumont, 2002) and with individuals with whom they have an “amicable” relationship (Aschwanden et al., 2008), which explains the formation of subgroups of young goats. In LK, however, this subgroup formation was less pronounced and young goats were more dispersed into the adult goat herd, suggesting lower subgroup cohesion, possibly because of the lower level of aggressive behavior directed against young goats by adult goats during that period. Young goats probably experienced lower levels of social stress and thus had a lower need for social support (Boissy and Dumont, 2002) during LK.

The notion of less social stress during PD is evidenced by hypothalamic-pituitary-adrenal (HPA) axis activity. Concentrations of fecal cortisol metabolites increased markedly in reaction to introduction in all but 2 young goats in PD. In LK, an increase was measured in only half of the animals and the size of the increase was smaller. Baseline values and cortisol increases in response to an acute stressor (a dog) did not differ between pregnant and lactating goats (Olsson and Hydbring-Sandberg, 2011), indicating no general difference in HPA axis reactivity due to reproductive stage and supporting the interpretation that the smaller increase in cortisol in LK indeed reflects lower levels of stress during introduction.

The results in PD are in line with results in cattle where the mixing of nonfamiliar animals affected HPA

axis activity in the short and long term (Friend et al., 1977; Hasegawa et al., 1997; Gupta et al., 2005) and a recent study in goats where fecal cortisol metabolite concentrations increased in the goat introduced singly into a small established group (Patt et al., 2012). Andersen et al. (2008) did not record any differences in plasma cortisol levels between stable and unstable groups of goats on the day of regrouping and 5 d after. However, Andersen et al. (2008) took only one blood sample per day, so that the problem of short-term fluctuations arises (Möstl and Palme, 2002), which is likely to have obscured possible effects. In fecal samples, minor short-term fluctuations are dampened, diurnal variation in cortisol secretion is smoothed out, and adrenocortical responses to potential chronic stressors can be detected without the need for frequent sampling (Palme, 2012). Additionally, fecal cortisol metabolite concentrations reflect adrenocortical activity within specific periods before sampling (11 to 13 h in goats: Kleinsasser et al., 2010), and thus the sampling procedure itself (handling of goats) does not affect the values.

Mixing with nonfamiliar animals as well as the separation from bonding partners may have contributed to stress experienced by young goats. Established dominance hierarchies result in a predictable behavior in stable social systems (Sachser et al., 1998), whereas social behavior in a new group is less predictable and thus causes stress, especially in newly introduced goats, because of their low status in the hierarchy (Mench et al., 1990; Kjæstad, 1999). The levels of cortisol metabolites during introduction in this study, being highest on d 3 and declining thereafter, were in accordance with the process of dominance hierarchy establishment discussed earlier. The levels on d 7 (higher than before introduction), however, may indicate that the dominance hierarchy within the group had not yet been fully established (Hasegawa et al., 1997).

The smaller or absent increase in adrenocortical activity in LK is in agreement with behavioral variables of less aggression toward young goats, lower cohesion between them, and higher levels of feeding in this period. Several factors that characterize the 2 introduction periods may have contributed to reduced social stress in LK.

First, the presence of kids may influence the social behavior of their mothers (young and adult goats) by attracting their attention and thus distracting them from (aggressive) interactions with other goats, leading to less social strain for young goats and thus lower HPA activation and lower cohesion. Second, the presence of kids is very likely to alter their mothers' behavior and cortisol levels via suckling-related physiological pathways. In mammals, oxytocin is released during suckling. Both in humans and in other species, oxytocin

has multiple physiological and behavioral effects, which can be summarized as a relaxing, "anti-stress," and pro-social effect (Uvnäs-Moberg and Eriksson, 1996; Uvnäs-Moberg, 1996). Lactating rats and mice suckling their offspring are found to be less responsive to certain stressful stimuli and show reduced anxiety and fearfulness compared with nonlactating animals (Lonstein et al., 1998; Lonstein, 2005). In humans and other species, oxytocin increases socio-positive behaviors and social skills (recognition of social communication signals) and leads to lower blood pressure and lower cortisol levels (Uvnäs-Moberg, 1998; Uvnäs-Moberg and Petersson, 2005). These effects of suckling are likely to explain lower aggression levels, easier integration, less need for social support from their peers and thus easier dispersion of young goats into the herd, as well as lower cortisol baseline levels and the lack of increase or small increase in cortisol after introduction in LK. Third, hormonal status differs between pregnant and nonpregnant (lactating) animals (Gall, 2001) and thus may have effects that are also independent of the presence of offspring. Hormonal changes in pregnancy were shown to reduce fear reactions in pregnant compared with non-pregnant ewes (Viérin and Bouissou, 2001). It is not clear, however, how this might affect reactions during grouping. Because kids were present, it was not possible to disentangle the effects of lactation from those due to the presence of kids. Oxytocin is also released during milking, even though levels are lower than during suckling (Tancin et al., 2001). Therefore, responses to the introduction of nonfamiliar animals may be similar in the absence of kids.

Space allowance was another factor differing between PD and LK, with lower space allowances in LK than in PD. According to the literature, space allowance is negatively associated with agonistic interactions, especially with body contact (goats: Jørgensen et al. 2007; Szabó, 2008; ewes: Bøe et al., 2006; dairy cows: Menke et al., 1999; Fregonesi and Leaver, 2002), and therefore does not contribute to reduced aggression levels, as observed in LK. Space allowances in studies with ewes and dairy cows, however, were rather low (e.g., 0.5, 0.75, or 1.00 m²/ewe in Bøe et al., 2006) compared with this study (4.5 and 4.8 m²/animal in PD, 4.3 and 4.5 m²/animal in LK). Even though the space for goats was reduced in LK because of the presence of kids and a separated kid area, our results suggest that space allowance remained above the critical level that would influence social behavior adversely.

The relatively generous space allowance is also likely to have contributed to the lack of injuries from horn butts during PD, as the risk of injuries increases with lower space allowance (in horned dairy cattle: Menke et al., 1999; Waiblinger et al., 2001). Three injuries were

recorded during LK. Two of these injuries (udder and ear) occurred in the waiting area (the first author was present during that time and observed an agonistic interaction and thereafter the fresh udder injury and, on another occasion, a discharge of blood from the ear) and one (vulva) in the barn. The waiting area is a critical area with respect to injuries, especially in horned goats, as space is much more limited ($0.9 \text{ m}^2/\text{animal}$) than in the barn (4.3 to $4.8 \text{ m}^2/\text{animal}$). More space allowance in the waiting area can decrease the frequency of agonistic behavior and, consequently, the occurrence of injuries (Szabó, 2008). Goats were not milked during the introduction period PD, and therefore animals were not confined in the waiting area. Additionally, the larger and firmer udders of lactating goats may increase the risk of injuries in the barn. We suggest the lower incidence of injuries in LK was due to an expected lower level of aggression, overlooking the different shapes of the udder and the use of the waiting area only during this period.

In this study, adult goats could have been habituated to introductions, which would have led to a decrease in their levels of aggression and thus affected the behaviors observed in introduced animals. However, we observed no signs of habituation (i.e., a constant decrease or increase) in the recorded variables. According to Mackintosh (1987), the habituation process includes a decline in responses to a neutral stimulus that is repeatedly presented. Regrouping, however, which involves aggressive behaviors, can hardly be seen as neutral, to young or to resident adult animals. In other studies, aggression remained at almost constant high levels after repeated regroupings of goats (Fernández et al., 2007; Andersen et al., 2008).

The early social environment of young goats (reared artificially or by their mothers) did not affect agonistic behavior or adrenocortical activity. Mother-reared goats, however, showed greater cohesion to their peers (i.e., other young goats) by having them as neighbors more often than artificially reared ones. Because no effect of rearing was seen on adrenocortical activity or agonistic interactions, greater cohesion does not seem to reflect higher stress, but may suggest higher sociality in goats reared with their mothers compared with artificially reared ones. This aspect merits further investigations with a greater number of animals.

CONCLUSIONS

The introduction of unfamiliar goats into a herd of adult goats is less stressful for introduced animals when done shortly after parturition, when kids are still present, compared with the period when goats are pregnant and dry. Therefore, introductions should take place

shortly after parturition and with kids present. Further research is needed to disentangle the effects of the presence of kids from those of the stage of reproduction (i.e., lactation vs. pregnancy). In this experiment, early social environment had no effect on the ability to cope with introduction but may have affected sociality.

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