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Cardiac activity in dairy goats whilst feeding side-by-side at two different distances and during social separation

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ABSTRACT

When loose-housed dairy goats feed in close proximity to each other, frequent social conflicts are often reported. The aim of this study was to investigate whether the cardiac activity of dairy goats is affected when they are obliged to feed side-by-side in close proximity. In five dyads of goats each stemming from eight groups (8×5 dyads) differing in terms of grouping age and presence of horns, heart rate (HR) and root mean square of successive beat-to-beat differences (RMSSD) as a parameter of heart-rate variability were measured in two experimental situations differing in distance during feeding. Dyads were allowed to feed for 5 min at two hayracks set-up side-by-side at a "far" or a "near" distance. Before goats were exposed to these test situations, baseline values of cardiac activity were measured. The differences of the baseline and test values (Δ) for HR and RMSSD were used for statistical analysis with linear mixed-effects models with crossed random effects. They were tested for dependence on feeding distance, rank within the group, dyadic rank relationship, grouping age, and presence of horns. In addition, cardiac activity was measured in a social-separation experiment conducted with the same goats.

Baseline cardiac activity depended on the goat's rank within the group, with higher/lower levels of RMSSD/HR found in high-ranking than in low-ranking goats. In the feeding experiment, a significant interaction (feeding distance*rank within group, $p = 0.01$) was found for Δ RMSSD: Low-ranking goats had lower Δ values at the far than at the near feeding distance. By contrast, high-ranking goats had lower Δ values at the near than at the far distance. In the separation experiment, Δ HR increased significantly compared to the feeding experiment ($p < 0.05$), whereas Δ RMSSD did not differ significantly. Our results show that cardiac response is context-specific in dairy goats (feeding vs. separation), and that the individual's rank within the group must be taken into account in any future studies of their cardiac activity.

1. Introduction

Social conflicts among dairy goats whilst feeding are common in loose housing. The strict dominance relationships of the goats [1] can negatively influence access to feeding places for low-ranking animals of a group [2,3]. As a result, individual differences in time spent feeding occur as a function on the animal's rank within the group [2], with high-ranking goats possibly claiming several feeding places at once whilst low-ranking goats must wait to feed [3]. When feeding side-by-side, there is a certain minimum distance which lower-ranking goats must observe to higher-ranking animals. If the lower-ranking goat comes closer than this distance, agonistic behaviour of the higher-ranking animal towards the lower-ranking one may be observed [4]. The feeding space usually recommended in farming practice is about 30–45 cm per goat [5–7]. As the median minimum tolerated distance between individuals in goat groups is usually greater than 40 cm and may reach up to 4.0 m [4], the forced proximity in dairy-goat housing during feeding is likely to be one reason for these frequent social conflicts.

It can be assumed that frequent social conflicts are associated with stress which might be reflected in performance, especially for low-ranking goats. In fact, Barroso et al. [8] found that the performance of low-ranking goats (milk and meat yield) was lower than that of middle- and high-ranking herd members. They also discovered, however, that performance did not increase linearly with the goats' rank within the group. Barroso et al. [8] concluded that aggressively maintaining one's rank within the group involves a high expenditure of energy for the animals, with the result that even high-ranking animals may suffer from the adverse effects of the rank relationships. The influence of rank relationships during side-by-side feeding in a barn is likely to be relevant in two ways. Firstly, a close feeding situation might be associated with stress for the higher-ranking and/or the lower-ranking animal in a pair of goats (= dyad) due to the dyad's specific rank relationship. Secondly, the degree of stress might depend on the two goats' ranks within the group, i.e. the lowest-

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ranking goat might experience greater stress when feeding near the highest-ranking conspecific than when feeding near a herd member with e.g. the second-lowest rank within the group.

Heart rate (HR) is often used as a physiological indicator of stress in studies with different animal species [e.g. [9–15]]. In recent years, however, the significance of HR as a stress parameter has been increasingly questioned, with the result that parameters of heart-rate variability (HRV) are now used as alternative indicators (for a review see [16]). There is, for example, growing evidence that the RMSSD (root mean square of successive differences in beat-to-beat intervals) is a suitable stress parameter, reflecting the activity of the parasympathetic nervous system [16]. In mammals, reduced parasympathetic nervous-system activity (i.e. low RMSSD values) indicates a stress response [17], and RMSSD has been used as an indicator of stress in several studies of different farm-animal species: horses [18], cattle [19,20], sheep [21], pigs [22], and dwarf goats [23].

The aim of this study was to investigate whether cardiac activity (HR and RMSSD) is affected by the distance between goats feeding side-by-side. HR and HRV were measured non-invasively by means of a portable ECG recorder while pairs of goats were feeding at two distances ("far" or "near"). For a better comparison of the cardiac values both between and within the animals in the two experimental situations, baseline values were measured before the goats were exposed to the feeding-experiment situations. In the statistical analysis, differences between the baseline and test values (Δ) were used to assess the influence of the feeding distance and dominance relationships on HR and RMSSD. We expected larger negative Δ HR and larger positive Δ RMSSD when two goats were feeding at near distances compared to far distances due to increased stress. Furthermore, we compared the Δ values measured in the feeding-experiment situations to those measured in a separation experiment conducted with the same individuals. Separation experiments are commonly used to induce stress in gregarious animals [24–26].

2. Methods

2.1. Animals, housing conditions and the evaluation of rank

The experiments were conducted with eight groups of nine non-lactating female goats kept in eight identically equipped loose-housing pens. Belonging to various Swiss milking breeds (Saanen, Toggenburger, Appenzeller, Chamois Coloured, St Gallen Booted, Grisons Striped, Valais Blackneck) and their crossbreeds, the goats had been bought on different Swiss farms and grouped in May 2005. At grouping, the breeds were randomly distributed among the eight groups. Four of the groups were grouped as juveniles at an age of about three months (almost one year before the start of the study), whilst the other four groups were grouped as adults (average age per group at grouping: between two and three years). Of the four juvenile and adult goat groups, two had horns, whilst the other two were hornless (unknown whether naturally hornless or dehorned) according to a 2×2 factorial design.

The loose-housing home pens were divided into a deep-bedded straw area of 11.7 m^2 (ca. 3 m×4 m) and a 0.5 m elevated feeding place (3.6 m^2). Hay was fed ad libitum and the animal/feeding-place ratio in the pens was 1:1 with a calculated feeding space of 45 cm per goat. The dominance relationships of the goats in each group were evaluated by direct observation during the morning and evening feeding times in the barn. Indicators for dominance/subordination were displacements effected by a dominant goat and avoidance behaviour by subordinate animals. Displacement behaviour was defined as one goat forcing another to leave her current position, either by a threat or a butt. Avoidance behaviour was defined as a goat leaving her current position after being approached by another goat which neither butted it nor constituted an obvious threat. In order for rank relationship within each dyad to be deemed clear-cut, at least three agonistic interactions with the same goat in the dominant role had to be observed. If at least one outcome of such interactions was contradictory, a dyad was observed until one goat was twice as often clearly dominant over the other. For each goat, the rank within the group (between 0=omega and 1=alpha) was calculated as follows: number of dominated group members divided by the number of possible rank relationships (i.e. eight for a group of nine).

2.2. Animal selection, experimental periods and experimental rooms

Five goats were selected from each group for the experiments (average weight 57.0 kg, +/− SD 13.0 kg). From these five individuals of a group, it was possible to form ten combinations of dyads with each individual involved in four dyads. From these ten potential dyads of each group, we chose of each group five dyads (in total 40 dyads) in a way that a given goat was not involved in more than three dyads. From the findings of a previous study [4], we had learned that the quality of a social relationship between two goats (i.e. negative/positive) can influence the distance tolerated between them. The tolerated distance, however, is not related to the difference in rank within the group of the two goats in a dyad. Thus, for all dyads, we selected goats that had a neutral (i.e. neither negative nor positive) social relationship, defined by dyads lying close to each other in the pen but never with bodily contact (see [4] for detailed information).

The feeding experiment was carried out in August–September 2006 in a separate experimental room (air temperature between 15 and 22 °C) away from the home pens. One complete goat group was always taken to this room together. The experimental room consisted of a waiting area (2.7 m×8 m) in which all goats were loosely tethered at the neckband in order to avoid agonistic interactions in the group, as well as a test area (4 m×8 m). Goats involved in the experiment had constant visual and acoustic contact with their herd. To enhance feeding motivation for the experiment, goats were given straw only instead of hay the evening and morning before the tests. Owing to prior experiments, all goats were familiar with being tethered in the waiting area, with the test area, and with the procedure of being tested in dyads.

The separation experiment with 40 goats in total followed in January–February 2007 (air temperature between −5 and 5 °C). As with the feeding experiment, a complete goat group was tethered in the waiting area of the experimental room. From there, individual goats were brought once into the separation pen. Except for four goats, the goats used in the separation experiment were the same individuals which were used to form the dyads in the feeding experiment. The four excluded goats were known to have unusable ECG signals from the feeding experiment and were therefore replaced by goats of similar rank within the group. The separation pen was located in a shelter about 20 m from the experimental room. Within this shelter, the goats were kept in the corner, with a wooden panel (ca. 1.70×1.70 m) restricting their view. The separated goats could not see their group, but had acoustic contact, if they vocalised loudly enough.

2.3. Heart-rate measurements

Heart rate was recorded non-invasively with a three-channel digital Holter Lifecard CF® (DelMar Reynolds). The three electrodes (Lead-Lok, Procamed AG) were positioned on the left shoulder, the left armpit within the area of the infrasternal angle, and the left side of the sacral bone (Fig. 1). At least one day before the start of an experiment, the goats were shorn in the appropriate spots. At 8 am on the day of the experiment, the shorn spots were swabbed with alcohol and the electrodes were pasted in place. A harness consisting of an elastic bandage wrapped around the goat's torso directly behind the forelegs and a second elastic bandage wrapped around the middle of the body was used to hold in place and protect the cables running from

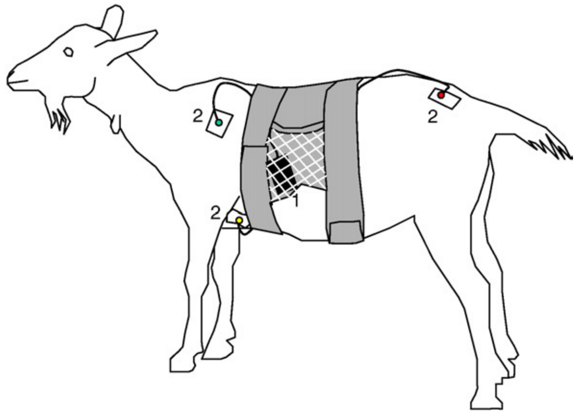

Fig. 1. Picture of a goat wearing the ECG harness with the positions of the three electrodes. 1 = Holter recorder in the pocket, 2 = electrodes.

the electrodes to the recorder. The two bandages were connected with a textile mesh that covered the back of the goat and contained the pocket for the Holter recorder. Prior to the experiment, each goat was thoroughly trained to wear the ECG harness by having it applied and removed several times per day (5–6 times) over a period of several days, first in the home pen and afterwards in the experimental room.

2.4. Feeding experiment

In order to test the effect of the feeding distance between two goats on cardiac activity, two hayracks which could be fixed at different distances from each other were mounted on the wall of the test area of the experimental room. For data collection, each dyad fed for 5 min (= one feeding test), once at a "far" distance and once at a "near" distance. The distance categories "far" and "near" were chosen individually for each dyad based on the findings of an earlier study [4]. "Far" was the average freely chosen distance of a given dyad measured while the goats fed simultaneously side-by-side at a 6-m-long hayrack. The average freely chosen distance of the dyads selected for this study was 1.8 m (±SD 0.7 m). The "near" distance was 20 cm more than the minimum distance at which a dyad was able to feed simultaneously side-by-side at two hayracks without aggressive interactions (= threats and attacks). The average minimum distance of the dyads selected for this study was 0.8 m (±SD 0.3 m).

Both goats of a dyad were equipped with a Holter recorder whilst feeding. To take into account intra-individual variability of HR and HRV levels, the difference between the HR values measured during a test and the baseline HR values (Δ HR) one the one hand, as well as the difference between the baseline RMSSD values and the RMSSD values measured during a test (Δ RMSSD) on the other hand, were used to compare the cardiac activity of a given goat in the two test situations. Baseline measurements were recorded in the waiting area of the experimental room, immediately before the start of a feeding test. During these measurements, goats were loosely tethered at the neckband. We used the ECG recordings as soon as the goats had calmed down after the fixation of the ECG harness and showed one of the following behaviours: lying idle, lying with rumination, standing idle, standing with rumination, standing with gnawing at barn equipment. The baseline measurement was stopped when the bout of one of those behaviours lasted at least 3 min. In total we recorded two baseline ECGs of the two individuals in each of the 40 dyads (=160 baseline ECGs) and two ECGs of the two individuals whilst feeding in each of the 40 dyads (=160 feeding test ECGs).

On a given day, the same dyad was tested for one of the distances in the morning (between 9.00 and 12.00 h) and for the other distance in the afternoon (between 13.00 and 16.00 h). The sequence of the two conditions was randomised across dyads. Goats participating in more than one dyad had a break of at least 30 min between subsequent tests. During the feeding tests, the behaviour of the goats (feeding at the hayrack, feeding from the ground, change of feeding place, threat, attack) was also recorded continuously with ETHO computer software (non-commercial). To calculate a rate of the general activity of a goat during a feeding test, the numbers of changes of feeding place, threats and attacks observed over the 5 min of a test were summed up and divided by five. On average, the goats exhibited 0.6 (SD ± 1.0) general activity elements per minute. The range was between 0 (in 79 of 143 tests with usable ECGs, cf. 2.6) and 4.5 (in two of 143 tests). As the distribution of these frequencies was unbalanced, four classes of general activity with the categories 0, 1, 2 and ≥3 activity elements per minute were used in statistical analysis. With this approach, we were able to include a potential effect of general activity on HR and RMSSD data.

2.5. Separation experiment

The separation experiment was conducted once with each of the 40 selected individuals in the morning between 9.00 and 12.00. Before each goat was actually separated from the group, the same procedure as if feeding tests were being repeated was performed three times with it. A goat fitted with the ECG harness (as described above, cf. 2.3) was left for at least 5 min in the waiting area and was then allowed to feed for 5 min alone at the hayrack. The goat was then brought back into the waiting area and the ECG harness was removed. Following a break of about 15 min, the ECG harness was fitted a second time, and after at least 5 min in the waiting area the goat was again allowed to feed. During the third repetition, the baseline ECG was recorded while the goat was in the waiting area. Afterwards, instead of being allowed to feed, the goat was lead to the separation pen, where it was loosely tethered at the neckband and left alone for 7 min at most. The behaviour of the goats was observed by video camera. The behavioural elements of "moving more than one step" and "standing up alongside the wall" were summed up for each goat in order to measure general activity. On average, goats exhibited 2.0 (SD ± 1.0) behavioural elements of general activity per minute: the range was between 0 in one and 3.75 in two goats out of 23 (=data of goats with analysable ECGs, cf. 2.6).

2.6. Analysis of HRV

The software Pathfinder (DelMar Reynolds) was used to analyse ECG data. The software marks all the valid single heartbeats in the ECG recording, which it then uses to calculate RMSSD and HR. Artefacts in the ECG recording, as well as two successive heartbeat intervals following an artefact were excluded from the calculation of RMSSD and HR by the analyser software itself. All ECG recordings were first checked visually to see whether the software specified heartbeats and artefacts correctly. Ill-detected heart beats were manually classified as artefacts, and were then excluded from analysis by the software. Two consecutive minutes of each recorded ECG were chosen to calculate both mean HR and RMSSD as a parameter for HRV. From the 3 min baseline measurements, we analysed 2 min that started at least 30 s after the goats had calmed down. Two criteria were applied to the selection of these 2 min in the feeding and the separation experiment. Firstly, goats had to have been standing still for at least 20 s before the start of timing. Secondly, if several two-minute intervals met the first condition, the 2 min during which the goat exhibited the smallest number of behavioural elements of general activity (see Section 2.4 and 2.5) were chosen. Sometimes, a behavioural element of general activity caused an artefact in the ECG signal during the chosen two-minute interval. Such artefacts (duration 8–16 s) were excluded, and an analysable ECG piece of the same length following after the end of the chosen interval was used to complete the two-minute interval.

Not all of the ECG measurements could be analysed. In the feeding experiment, 143 of 160 baseline ECG measurements stemming from

Table 1
Fixed effects, interactions and the random effects which were included in the full models for the statistical analysis of the feeding experiment

Outcome variables	Baseline models		Δ value models	
	Baseline RMSSD, baseline HR		Δ RMSSD, Δ HR	
	Explanatory variables	Levels	Explanatory variables	Levels
Fixed effects	Gnawing	Yes/no (factor)	Feeding distance	Near/far (factor)
	Rumination	Yes/no (factor)	Dyadic rank relationship	Higher-ranking/lower- ranking (factor)
	Position	Lying/standing (factor)	Number of behavioural elements of general activity	0, 1, 2, ≥3 (ordered factor)
	Rank within group	0.0−1.0 (continuous)	Rank within group	0.0–1.0 (continuous)
	Grouping age	Adult/juvenile (factor)	Grouping age	Adult/juvenile (factor)
	Presence of horns	Yes/no (factor)	Presence of horns	Yes/no (factor)
	Time of day	Morning/afternoon (factor)	Time of day	Morning/afternoon (factor)
	Number of measurements	1, 2, 3, 4, ≥5 (continuous)	Number of measurements	1, 2, 3, 4, ≥5 (continuous)
Interactions	Grouping age	With all other fixed effects	Feeding distance	With all other fixed effects
	Presence of horns	With all other fixed effects	Grouping age	With time of day
	Rank within group	With all other fixed effects	Dyadic rank relationship	With time of day
			Presence of horns	With time of day
			Presence of horns	With grouping age
Random effects	Individual within group		Row and column individual nested within group (partly crossed)	

Baseline RMSSD, baseline HR, Δ RMSSD und Δ HR were used separately as outcome variables in a linear mixed-effects model with crossed random effects.

40 dyads were usable: the remaining 17 had to be excluded because the software was unable to detect the heartbeats correctly (amplitude of the T-waves was as high as the R-peaks or even higher). Of the 80 ECG measurements made during the feeding experiment at each of the two feeding distances, 72 were successfully analysed for the "near" distance and 71 for the "far". Sixteen ECGs had to be excluded for the same reasons as described for the baseline measurements, and one ECG could not be analysed because of loose electrodes. Baseline values were available for all of the 143 ECGs recorded during a feeding test. In the separation experiment, 37 out of 40 baseline ECGs could be analysed, but only 23 of the 40 ECGs recorded during separation were analysable: the goats had been trembling heavily (whether from the cold or from fear was unknown), resulting in massive muscle potentials which disturbed the ECG signals. Baseline ECGs were available for each of these 23 ECG recordings during separation.

2.7. Statistical analysis

Due to the repeated occurrence of individuals in dyads with other animals out of their own group, the analysis of social interaction matrices poses a special problem [27]. Hemelrijk [27] proposed an approach for calculating correlations between two social matrices from the same group, correcting for the dependence in the data of the same individual. Here, we needed an extension allowing the inclusion of data from several groups and several explanatory variables. Such a data structure may be viewed as a special case of a linear mixed-effects model with crossed random effects [28,29].

As the selected goats stemmed from groups differing in terms of grouping age and presence of horns, these two factors were considered in all statistical models calculated for the feeding experiment. The goats' rank within the group was also included in each model. In addition, the dyadic rank relationship (i.e. one goat being the higher-/lower-ranking within a dyad) was included when analysing the effect of distance on cardiac activity in the feeding tests. As some goats were teamed up in several dyads of a group (three at most), a maximum of six baseline ECG measurements and six ECG measurements whilst feeding in a test (3×near and 3×far feeding distance) were recorded per goat. Since we expected an influence of the number of repetitions of ECG measurements on the cardiac activity of a goat, we included the variable "number of measurements" in statistical analysis, separately numbering from 1 to 6 the baseline ECG measurements as well as the ECG measurements conducted during the feeding tests. Because of the low number of goats participating in three dyads (n=9), we combined the classes 5 and 6 and categorised the number of measurements as follows: 1, 2, 3, 4 and ≥5 measurements.

In the feeding experiment, we first tested whether the baseline of RMSSD and HR differed depending on behaviour during the measurement (i.e. lying/standing, rumination, gnawing at barn equipment), on characteristics of the animals (rank within group, grouping age, presence of horns), on the number of measurements, and time of day. For each outcome variable, baseline RMSSD and baseline HR (both log-transformed), a separate linear mixed-effects model with individual nested in group as a random effect was set-up. Details on the variables included in the full models are listed in Table 1.

Next, in order to see how the far and near feeding distance influenced the goats' cardiac activity, we analysed the Δ values of both RMSSD (difference of the baseline value minus the value obtained in the test) and HR (difference of the value obtained in the test minus the baseline value). Each Δ value served as an outcome variable in a separate linear mixed-effects model with crossed random effects. In both models, we tested for the influence of the feeding distance (near/far), the dyadic rank relationship, the number of behavioural elements of general activity during feeding (i.e. categories 0, 1, 2 and ≥3), characteristics of the animals (rank within group, grouping age, presence of horns), and factors linked to the experimental procedure (time of day, number of measurements). The crossed random effects of the row individual of a dyad and the column individual within the same dyad nested within group were included in both models. These random effects reflect the variability attributed to the general sociability of the row individual, as well as the general effect of the column individual on its dyadic partners.

All linear mixed-effects models were calculated via the "lmer" method [30] in R 2.3.1 [31]. Residuals were checked graphically for normal distribution, homoscedascity and outliers. The models were set-up as full models and then reduced via a stepwise backwards method (threshold $p<0.05$). The number of measurements was treated in all models as a continuous variable. To allow for some curvature in the dependence of the response variable on number of measurements which was visible in the residual plots, the square of the number of measurements was included as an additional variable. Because this variable and its interactions did not reach significance, however, the quadratic terms were dropped from the model in the stepwise backwards method. The calculation of the p-values of such a model is non-trivial [32], and we followed the recommendations of Bates [32] and Bates et al. [33] and used a Markov Chain Monte Carlo method to resample the posterior distribution of the parameter estimates (a method borrowed from Bayesian statistics, e.g. [34]) to provide confidence intervals for the model parameters. By calculating the percentile X at which the confidence interval borders on the value zero (e.g. the 99% confidence interval), we attributed a p-value to the parameter as $p=1-(X/100)$ (e.g. $1-(99/100)=0.01$).

In a third step, we compared the Δ values of cardiac activity of the goats from the separation experiment with those of the same goats from the feeding experiment. To do this, we first had to obtain a comparable subset of data. Both the time of day and the number of measurements influenced the RMSSD baseline values in the feeding experiment. Because of this, the only values of the goats from the feeding experiment which were used were first-time morning measurements. Since the first measurement in the morning was made either in the near or in the far feeding situation, half the values that we compared with the separation values stemmed from the near and the other half from the far feeding-distance situation.

Due to the low number of ECGs (values of only 23 goats analysable, see above) a multivariate approach was not possible, and Wilcoxon signed rank tests were carried out instead. The values of the twelve goats that had been measured in the feeding experiment at the near distance and the eleven that had been measured at the far distance were compared separately. Categories of rank within group were balanced over the groups. Five of the goats measured at the near distance had a rank within group of <0.5 and seven of ≥0.5, whilst six of the goats measured at the far distance had a rank within group of <0.5 and five of ≥0.5.

3. Results

3.1. Feeding experiment

3.1.1. Baseline of RMSSD and HR

The RMSSD baseline values differed as a function of the goats' rank within the group, (Fig. 2a), significantly interacting with the behaviours no gnawing/gnawing ($p=0.01$) and rumination/no rumination ($p=0.05$), as well as with the time of day ($p=0.05$).

The estimated effects for the interactions influencing RMSSD baseline are listed in Table 2. In all three interactions, the RMSSD baseline increased with the goats' ascending rank within the group. With low-ranking animals, baseline RMSSD was on a similar level regardless of whether they showed gnawing or not, whether they showed rumination or not, or whether it was morning or afternoon. By contrast, baseline RMSSD levels in high-ranking animals were higher when they exhibited gnawing behaviour than when they did not, higher when they exhibited rumination behaviour than when they did not, and higher in the morning than in the afternoon. The RMSSD baseline was also influenced by the significant interaction of the grouping age and the number of measurements ($p=0.05$). The baseline RMSSD of goats grouped as adults remained on the same level at each measurement, whereas in goats grouped as juveniles, the level decreased slightly with each measurement repetition. No effects could be found for the position during the measurement (i.e. lying or standing), for the presence of horns, and for all other remaining interactions, which we therefore excluded by the stepwise backwards method.

Table 2
Estimated effects for the significant interactions influencing the baseline RMSSD and HR in the feeding experiment

			Baseline RMSSD (ms)		Baseline HR (beats/min)	
Rank within group			0.0	1.0	0.0	1.0
	Gnawing	Yes	18.4	131.3	102.6	67.5
		No	24.5	62.7	90.0	81.9
	Rumination	Yes	24.5	62.7	90.0	81.9
		No	31.6	38.7	82.8	92.7
	Time of day	Morning	24.5	62.7	n.s.	n.s.
		Afternoon	26.7	41.4	n.s.	n.s.
Grouping age			Juvenile	Adult	Juvenile	Adult
	Number of measurements	1	24.5	29.9	90.0	85.5
		≥5	18.4	29.4	121.5	83.7

n.s.= not significant.

The HR baseline was also influenced by the significant interaction of the goats' rank within the group (Fig. 2b) with the behaviours gnawing/no gnawing ($p=0.001$) and rumination/no rumination ($p=0.05$). In low-ranking goats, the baseline HR level was higher during gnawing or rumination behaviour than when these two behaviours were not exhibited. In high-ranking goats the pattern was reversed: the HR baseline level was higher when no gnawing or no rumination took place than when they did. Furthermore, the interaction of grouping age with the number of measurements

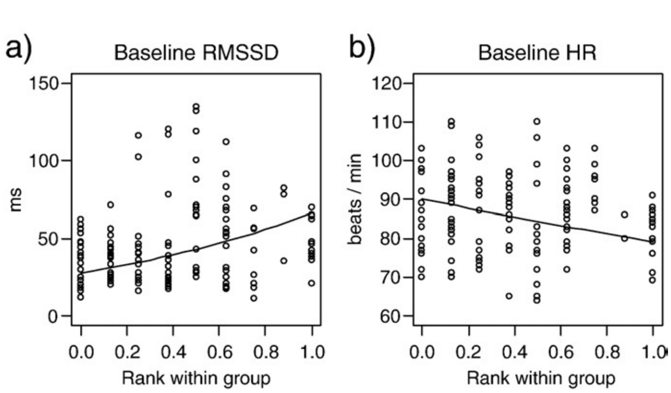

Fig. 2. Baseline RMSSD (a) and baseline HR (b) measured prior to the feeding tests with respect to rank within group. Raw data are shown together with the model fits. $N=143$ measurements from a total of 36 dairy goats in each case.

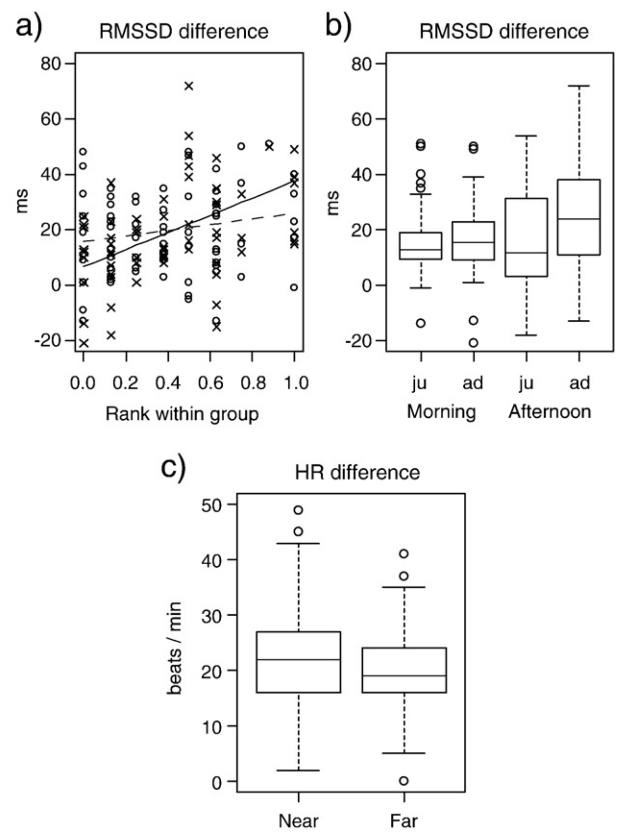

Fig. 3. Feeding experiment: Values of Δ RMSSD in relation to (a) the goat's rank within the group and feeding distance (open dots = near distance, crosses = far distance) (interaction $p=0.01$); (b) grouping age (ju = juvenile, ad = adult) and time of day of the measurement (interaction $p=0.01$); and (c) values of Δ HR in relation to feeding distance ($p=0.05$). Raw data are shown, as well as (in (a)) the model fits (dashed line = near distance, continuous line = far distance).

($p=0.05$) significantly affected the HR baseline. In goats grouped as juveniles, baseline HR increased with higher numbers of measurements, whilst the baseline HR of goats grouped as adults remained on the same level. As no effect on baseline HR was detectable for the position during the measurement (i.e. lying or standing), presence of horns, time of day, or any other remaining interaction, these variables were excluded from the model.

3.1.2. Δ values of RMSSD and HR

The effect of near or far feeding distance on Δ RMSSD was dependent on rank within group, as we found a significant interaction of the feeding distance with the goats' rank within group ($p=0.01$). In general, Δ RMSSD increased along with the goats' ascending rank within the group in both feeding situations (Fig. 3a), although in goats with a low rank within the group it was higher with the near feeding distance than with the far distance. By contrast, goats with a high rank within group had a higher Δ RMSSD with the far feeding distance than with the near one. Furthermore, a significant interaction effect was found for the variables time of day and grouping age ($p=0.01$; Fig. 3b): in the morning, Δ RMSSD values were similar for animals grouped as adults and as juveniles, whereas in the afternoon, Δ RMSSD values increased for goats grouped as adults whilst remaining on the same level for those grouped as juveniles. No significant effect on Δ RMSSD was found for dyadic rank relationship, number of measurements, or interaction of dyadic rank relationship with feeding distance. Using the stepwise backwards method, the number of behavioural elements during feeding, the presence of horns, the number of measurements and all other remaining interactions were excluded from the model.

The only significant effect on Δ HR was from feeding distance ($p=0.05$, Fig. 3c), with the near distance producing a slightly higher Δ HR than the far distance. All other effects were excluded by the stepwise backwards method.

3.2. Separation experiment

Besides moving a few steps and trying to stand up along the walls, the behaviour of the goats in the separation pen consisted of intense vocalisation, trembling, and jerky head movements when looking around and listening to surrounding noises.

With regard to Δ RMSSD, no significant difference was found either for the Δ RMSSD of the near feeding distance compared to that of the same goats in the separation experiment (Fig. 4a), or for the Δ RMSSD of the far feeding distance compared to that of the same goats in the separation experiment. The median of the baseline of RMSSD before separation was 26 ms for the goats from the group tested at the far feeding distance, decreasing to 25 ms during separation (median values of RMSSD of the feeding experiment for the same animals: decrease from 33 ms before to 20 ms during the feeding tests). For the goats from the group measured at the near feeding distance, the decrease of the median of RMSSD was from 38 before to 25 ms during separation (median values of RMSSD for the same animals: decrease from 39 before to 25 ms during the feeding tests).

By contrast, the same comparisons for the Δ HR values were significant: goats tested at both the far and near distance exhibited significantly higher Δ HR values in the separation experiment (far: $V=3$, $p<0.01$; near: $V=2$, $p<0.01$; Fig. 4b). The median of the HR baseline was 103 beats/min before separation for the goats tested at the far feeding distance, increasing to 167 during separation (median HR values of the feeding experiment for the same animals: increase from 89 beats/min before to 106 beats/min during a feeding test). For the goats measured at the near feeding distance, increase in median HR was from 105 before to 157 beats/min during separation (median values of HR of the feeding experiment: increase from 91 beats/min before to 107 beats/min during a feeding test).

4. Discussion

The cardiac activity (HR and RMSSD) of dairy goats varied significantly with feeding distance, and was also affected by rank within group. In line with our expectations, Δ RMSSD values of goats with a low rank within group were higher for the near than for the far feeding distance. By contrast, Δ RMSSD values of goats with a high rank within group were greater with the far than with the near feeding distance. Cardiac activity of the same individuals was also measured during social separation. No difference was found for Δ RMSSD values of both experiments. By contrast, Δ HR values recorded in the separation experiment were significantly higher than those measured in the feeding experiment.

Regarding the baseline values measured prior to the feeding tests, the effect of rank within group produced a uniform pattern regardless of the goat's actual behaviour (gnawing, rumination) or whether it was morning or afternoon. Thus, goats of high rank within the group seemed to be generally in a more relaxed basic status than goats of low rank within the same group. Chronic psychological stress in low-ranking goats, resulting from the dominance structure accompanied by agonistic interactions within their groups, might account for this effect. Such chronic stress could well have a long-term impact on basic parasympathetic activity, as reflected in the low RMSSD values of the low-ranking goats. Although there is a lack of information in the literature on the influence of rank within group on HRV, there are

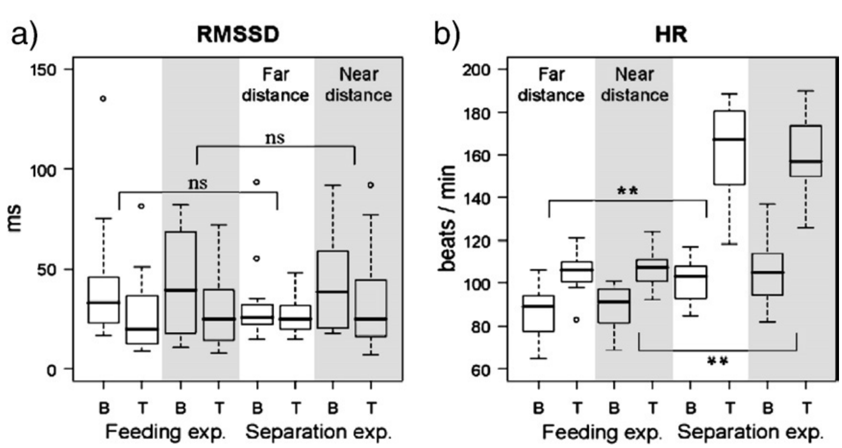

Fig. 4. Comparison of RMSSD (a) and HR values (b) of the feeding experiment with the values of the separation experiment. For each situation, baseline values (B) and values recorded during the test situation (T) are shown. The respective white and grey sections indicate measurements stemming from the same individuals (e.g. the white box plots of the separation experiment refer to the same individuals tested in the "far" situation in the feeding test). (ns = not significant, ** = $p<0.05$).

several studies investigating HR as a function of the rank status of an animal. All of these found higher HR levels in subordinate animals in the long-term (wild rabbits: [10]; rats: [35]; treeshrews: [36]) and ascribed their findings to the chronic stress experienced by subordinates. Our findings, therefore – at least in relation to HR – do not contradict the results of these studies.

For the comparison of the two feeding distances, the individual Δ values of the baseline and the test values were used. Interestingly, the parasympathetic response (Δ RMSSD) to the two feeding distances varied according to the rank within group of each goat, but was not significantly influenced by the dyadic rank relationships. At both feeding distances, goats of high rank within the group had a higher Δ RMSSD than goats of low rank within the group, indicating a stronger reaction to the test situation. With low-ranking goats, however, Δ RMSSD values were – as expected – higher for the near feeding distance than for the far one. Hence, the low-ranking goats were more relaxed whilst feeding at the far than at the near distance. By contrast, Δ RMSSD values in high-ranking goats were greater at the far distance than at the near distance. Depending on social characteristics (i.e. aggressive/dominant individuals vs. less aggressive/subordinated ones), differences in cardiovascular stress responsivity to a social stressor are also apparent in rats (for a review see [37]). However, the comparability of these studies with ours is only marginal. Since no comparable studies have been conducted with dairy goats or other animal species, we can only speculate on the reasons for the observed pattern. It might be that, for the high-ranking goats, feeding at a greater distance (1.8 m ± SD 0.7 m) represented a kind of loss of the control that they were used having in their home pens.

Usually, increasing physical activity leads to an increase in HR (i.e. sympathetic activation) accompanied by a decrease in parasympathetic activity [38]. Different states of physical activity (e.g. walking, running) imply different dimensions of HR values, and thus different degrees of influence on parasympathetic activity [39]. It is therefore recommended that only RMSSD values measured during the same physical states be compared. In our feeding experiment, all goats were standing while feeding at the hayrack, and the number of behavioural elements of general activity included in the analysis did not influence cardiac activity. The estimated Δ HR at the near feeding distance was significantly greater than the estimated Δ HR at the far feeding distance. It must be questioned, however, whether this small difference (2.5 beats/min) is biologically relevant; rather, it might confirm the goats' similar physical state in both feeding situations and allow the comparison of Δ RMSSD values in the two test situations.

In the separation experiment, we expected the RMSSD to be much lower than for the feeding experiment, resulting in a higher Δ RMSSD for the former than for the latter. For a gregarious animal, separation from the group represents a substantial stressor, and separation experiments have also been used to induce stress in goats [24,25]. The qualitative behavioural observation of the goats during the separation experiment showed them to be extremely tense and vigilant rather than calm and relaxed, indicating that the situation was indeed associated with stress for the animals. Nevertheless, we did not find a significant difference between Δ RMSSD values in the separation experiment and those in the feeding experiment. By contrast, Δ HR differed significantly between the separation and the feeding experiments, with the HR values during separation being much higher. As mentioned above, physical activity usually causes increased HR [39]. During the measurement of cardiac activity whilst separated, however, all goats were standing as in the feeding experiment and were tethered at the neckband, to which the taking of the baseline measurements had accustomed them. Although the goats exhibited more behavioural elements of general activity per minute (2.0, SD ± 1.0) during separation than in the feeding tests (0.6, SD ± 1.0), in our opinion these movements would be incapable of causing the remarkable increase in HR in the separation pen compared to the baseline measurements. According to this notion, we assume that the observed increase in heart rate was due to the activation of the sympathetic branch of the autonomous nervous system whilst the activity of the parasympathetic branch remained on the same level. The absence of a decrease in parasympathetic activity despite sympathetic activation has previously been found in rats [40] and lambs [41]. The effect occurred when the animals were experimentally exposed to a sudden stimulus (acoustic in the case of the rats, visual in the case of the lambs) and was interpreted as a sort of startle response or a protective response to predation. We can only speculate as to whether these findings are comparable to ours. Clearly, however, the cardiac response in terms of HR and HRV in the separation experiment was completely different to the response in the feeding experiment. If this is a reflection of the different context of stress in the two experiments, it might rule out the direct comparison of their RMSSD values. This highlights the need for further studies on the interdependence between HR and RMSSD, particularly in relation to the context of a (n) (experimental) stress situation.

To conclude, our results show that the cardiac response of dairy goats, measured in terms of HR and RMSSD, is context-specific (feeding vs. separation). Furthermore, cardiac response can be influenced by group composition factors (i.e. such as grouping age in our study) and factors relating to study design (in our case, the time of day, and the number of measurements). Consequently, cardiac-activity values may be influenced by various factors, and should be interpreted cautiously. Finally, our study demonstrates that HR and RMSSD baseline values seem to depend on the goats' rank within the group, and that a(n) (experimental) feeding situation can produce the opposite effect on RMSSD values in high-ranking animals than in low-ranking ones. Rank within group should therefore be considered in the experimental set-up of subsequent studies on cardiac response in dairy goats, and might well also be of importance in studies of other social (farm) animal species.

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