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# Physiological expression of emotional reactions in sheep

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ABSTRACT

With the aim of judging emotional valence from an animal's perspective, multiple physiological variables were recorded in sheep when they were exposed to situations likely to induce negative and positive emotional states. Fourteen sheep were conditioned for several weeks to anticipate the delivery of standard feed. In three experimental trials carried out thereafter, the animals' expectations regarding feed quality were either fulfilled by offering the familiar standard feed (control), frustrated by giving unpalatable wooden pellets (negative treatment) or surpassed by delivering enriched feed (positive treatment). Heart rate, root mean square successive difference (RMSSD), respiration rate, body-surface humidity, body-surface temperature and percentage of eye white were recorded prior to the delivery of feed (anticipation phase) and during the delivery (feeding phase) of either standard feed, wooden pellets or enriched feed. Data were analysed using linear mixed-effects models. Heart rate, respiration rate and variability of body-surface humidity were high during the delivery of wooden pellets and low during the feeding phases with standard and enriched feed; RMSSD showed an inverse pattern. In addition, heart rate was increased during the first feeding phase after the one with standard feed, independent of its presumed valence, whereas differential reactions were observed in the second feeding phase after standard feed. The results show that presumed negative and positive emotional states in sheep differ in their physiological reactions. Despite a need for validation in additional situations, the combination of heart rate, respiration rate, RMSSD and body-surface humidity appeared to be most useful for assessing physiological correlates of negative and positive emotional reactions in sheep.



## 1. Introduction

Emotions play a major role in the psychological well-being of animals [1,2]. The subjective nature of emotional states, however, makes their objective assessment difficult [3,4]. Despite years of efforts to judge situations from the animal's perspective, clear answers on how to measure, in particular, positive emotional states as objectively as possible remain a scientific challenge [5]. One classical approach to provoke emotional reactions in animals (in particular laboratory rodents) is to use so-called positive–negative contrasts [6]. In such studies, animals are trained to form expectations about future events, e.g. to anticipate a certain type of feed. With the delivery of the expected type of feed, animals should not show a specific emotional reaction, since anticipated and actual reward do not differ. Changing the familiar type of feed to lower or higher feed quality should, however, provoke negative and positive emotional reactions by frustrating or surpassing the animals' expectations [7].

Emotional reactions are accompanied by physiological changes [8] and decades of stress research gained knowledge of physiological reactions to physical and perceived negative emotional stressors [9–11]. Cardiac and respiratory measurements [11–13] and electrodermal activity as an indicator of perspiration [14,15] are well-known measures of stress and emotion from human psychophysiological research [16]. In animals, heart rate and, in particular, heart rate variability [17] have been used regularly to assess stress levels of animals in different housing systems [18,19] or various handling procedures [20,21]. Respiration rate may indicate the severity of stress, as found for cows [13]. Rats experiencing fear show increased back temperatures [22] and eye, nasal or ear temperature are on the way to becoming a convenient tool for the non-invasive assessment of stress in diverse animal species [23–26]. Finally, eye white is an additional measure that is controlled by the autonomic nervous system and involved in emotions [27]. Cows in a negative emotional state (e.g. frustrated or scared) were observed to have a higher percentage of visible eye white than cows in a non-negative state [28,29].

All these autonomic measures are useful for judging negative emotional states in animals. In respect of positive emotional states, however, comprehensive experimental knowledge of how physiological measures react to positive stimuli has mainly been elaborated in

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humans whereas knowledge from animal studies is scarce [5]. Recently, Schmied et al. [30] found reduced heart rates in cows when they were stroked at their preferred grooming sites. In cows, the percentage of eye white was lower during a state presumed to be satisfaction compared with a negative emotional state [31].

In response to both positive and negative emotional stimuli, human heart rate [32], electrodermal activity [33] or pupil size [34] can react in a similar way. Consequently, it has been hypothesised that no single variable may be sufficient to indicate the valence of emotional experiences [17]. Using a combination of several measurements is therefore recommended [35]. In men, anger, fear, happiness and sadness were distinguishable by a combination of heart rate and respiration in conjunction with the high frequency component of heart rate variability [36]. In another study, 15 specific emotions were discriminable using electrodermal activity combined with respiration rate [37]. However, there is a lack of animal studies that apply several physiological measurements and examine whether and how positive states can be differentiated from negative emotional states.

The aim of this study was to investigate how multiple physiological measurements related to the autonomic nervous system react to positive emotional states as compared with negative ones. Fourteen sheep were subjected to feed-related positive–negative contrasts as exemplary situations likely to induce states of negative and positive emotional valence. After conditioning the animals to the delivery of a standard feed, their expectations regarding feed quality were either fulfilled by offering the familiar standard feed (control), frustrated by offering unpalatable wooden pellets (negative valence) or surpassed by offering energy-enriched feed mixed with preferred feed items (positive valence). A variety of physiological measurements (heart rate, RMSSD, respiration rate, body-surface humidity, body-surface temperature, visible eye white) were recorded simultaneously and continuously in these experimental trials, both prior to (anticipation phase) and during (feeding phase) the delivery of feed.

## 2. Methods

### 2.1. Animals, housing and husbandry

Experimental subjects were nineteen female sheep (9 Swiss White Alpine and 10 Lacaune ewes, 1.5–2.5 years old, 53–92 kg). These were non-reproducing and non-lactating. As a consequence, it is unlikely that any relevant changes of sex steroid concentrations that might have influenced emotional reactions occurred during the few weeks of the experiment. All ewes had been acquired from the same shepherd, and were housed together as a group for approximately one year prior to the experiment at the Agroscope Reckenholz-Tänikon Research Station ART, Tänikon (Switzerland). The open-front pen (58 $m^2$) consisted of an area with deep litter straw bedding (42 $m^2$), a feeding area with a solid concrete floor (12 $m^2$) and a 7 m long hayrack. When weather conditions were dry, the animals were given access to an additional outdoor exercise yard (18 $m^2$). From spring to autumn, the sheep were on pasture at times when no experiments were being conducted. Water and hay were available ad libitum and the hayrack was refilled twice daily at around 8:30 a.m. and 4 p.m. Animals were not feed-deprived for the experiment and the study was approved and financed by the Federal Veterinary Office (Switzerland).

### 2.2. Experimental procedure

The sheep were subjected to feed-related positive–negative contrasts, in which their expectations regarding feed quality were either fulfilled by offering familiar standard feed (control), frustrated by offering unpalatable wooden pellets (negative) or surpassed by offering energy-enriched feed mixed with preferred feed items (positive). Data were collected during a six-minute period prior to feed delivery (anticipation phase) and a six-minute period with continuous feed delivery (feeding phase).

The experiment was carried out from May to June 2007 in a building near the sheep's home pen. During data recording, three sheep (one experimental animal and two companion sheep) were kept singly side-by-side in three partitions, each measuring 0.9 m × 2.40 m. Wire netting between the partitions enabled the animals to remain in visual contact with one another. At the front of each partition, a feed trough could be filled with different types of pellets by means of an automatic feeder.

Fourteen sheep were used as experimental animals, four as companion ewes, and one animal was not used at all due to an injury at the time of the experiment. Three of the fourteen sheep were initially used as experimental animals and later as companion animals. The pairs of companion ewes always comprised one Lacaune and one Swiss White Alpine sheep. Companion animals were chosen based on showing fewest spontaneous agonistic interactions with the experimental sheep during feeding in the home pen (data not presented).

All nineteen sheep were habituated to the experimental procedure. For two weeks, they were trained daily to voluntarily walk into the building with the three partitions, first in groups of several animals and later in triads (each sheep visiting the test room 4–10 times). Over the course of two additional weeks, triads were then conditioned to anticipate the feeding of standard feed (UFA 250 pellets, NEL 4.0 MJ/kg) in the test room with a delay of up to 6 min. To achieve this, each experimental sheep underwent training on seven working days during these two weeks, with three trials per day. For signalling the anticipation phase to the animals, a green light bulb at the height of the sheep's head lit up in each partition, and the engine of the automatic feeder began to run at the same time without dispensing pellets. The feeding phase started with a change from the green light to red and pellets were instantly and continuously dispensed into the troughs. During this period of training, the length of the anticipation and feeding phases was increased from one to 6 min in one-minute steps. During these last two weeks prior to the experiment, sheep were additionally habituated to wearing the physiological measurement equipment (for details see below) in the course of the training trials. All animals had been habituated to wear the devices for up to 1 h in a previous experiment.

For each of three experimental trials, one experimental and its two companion sheep were herded in the feeding area of the home pen. Here, physiological equipment was attached to the experimental animal. Afterwards, the passage to the test room was opened and the animals voluntarily entered the building. The experimental sheep was gently guided into the middle partition, flanked by two companion sheep in the partitions on both sides. The animals were then allowed to calm down from walking during a five-minute rest period. The experiment proper began with the anticipation phase: the green light bulb lit up and the engine of the automatic feeder began to run for 6 min without dispensing pellets. The feeding phase started with a change from the green light to red and pellets were instantly and continuously dispensed into the troughs for 6 min. On three consecutive days and at the same time of the day, each experimental sheep was exposed to feeding treatments assumed to differ in terms of emotional valence. On the first day of testing, the standard feed (400 g) was dispensed into the troughs (standard feed treatment). Data collected during the anticipation phase of this day served as a baseline for describing the behaviour of a sheep expecting nothing but delivery of the standard feed. On the second and third days of testing, balanced for breed and age, half of the animals were first tested in a treatment of presumed negative valence and the other half in a treatment assumed to have positive valence for the sheep. In order to provoke a negative emotional state, sheep were given 400 g unpalatable wooden pellets instead of the standard feed (wooden pellet treatment). To induce a positive emotional state, a mixture of higher energy value feed (UFA 864 pellets, NEL 7.0 MJ/kg), raisins and

small pieces of dry bread (400 g in total) was dispensed into the troughs (enriched feed treatment). All animals had gained experience with wooden pellets, enriched pellets, raisins and pieces of dry bread in their home pen before the current experiment, but they had never been given these types of feed from the automatic feeder in the test room.

### 2.3. Physiological measurements

In order to record correlates of emotional reactions in the experimental animals, heart rate, heart rate variability, respiration rate, body-surface humidity, body-surface temperature and percentage of eye white were recorded non-invasively (see Fig. 1 for positions of measurement devices on a sheep). All devices had been synchronised in time before the experiment.

The electrocardiogram of a sheep was continuously measured with a Holter recorder commonly used in humans (Modular Digital Holter Recorder, Lifecard CF, DelMar Reynolds GmbH, 96 mm × 57 mm × 18 mm, 130 g). The device was fixed to a leather belt around the abdomen of the sheep behind its fore legs. Three electrodes (Red Dot 2560, 3 M, 35 mm × 40 mm) were fixed on shorn skin on the caudal part of the left scapula (shoulder blade), on the sternum, and on the loin to the left of the spine. Mean heart rate (beats per minute) and heart rate variability (root mean square of successive difference, RMSSD) were calculated based on the first 5 min of data recording within each experimental phase using Pathfinder software (DelMar Reynolds GmbH).

A commercial logger (MSR145 W, Modular Signal Recorder Electronics GmbH; 60 mm × 16 mm × 14 mm, 16 g) detected the relative body-surface humidity [%] as a measure of perspiration and the body-surface temperature [°C] of the sheep. This logger was fixed on the depilated skin with a breathable adhesive tape (50 mm × 65 mm Fixomull stretch, BSNmedical GmbH) on the sheep's mid-side. Previous validation had shown that a microclimate developed beneath the tape within 10 min so that the measured local relative humidity and temperature reflected the values of the sheep's skin. The logger recorded the humidity and temperature values once per second. An average value of body-surface humidity and body-surface temperature was calculated for each sheep and for a given experimental phase.

The respiration rate was measured with an extendable belt (1132 Pneumotrace II, UFI, 280 mm × 25 mm × 3 mm) that was fixed with a Velcro strap around the abdomen in front of the sheep's hind legs. It generated a continuous signal for the relative extension of the belt during inspiration–exhalation cycles. The signal was saved at a rate of 10 Hz by the logger used for body-surface humidity and temperature recordings (see above). The respiration rate was determined based on the strongest frequency in a smoothed spectrogram of the signals' time series (in S-PLUS, Version 7.0 for Windows). Since the respiration rate varied greatly over the 6 min of an experimental phase, only the third minute of each phase was evaluated.

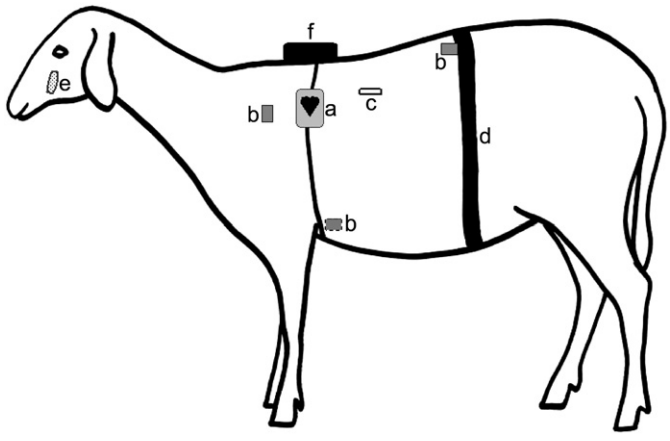


**Fig. 1.** Sheep with physiological measurement devices: positions of the electrocardiogram-Holter recorder (a) with its three electrodes (b), the body-surface humidity and temperature logger (c), the respiratory belt (d), the eye-cameras (e) and the battery pack (f).

The visible eye white was determined using pictures from video recordings of the sheep's eyes. These were made by two finger cameras (DV-2000B Weatherproof, CCD B&W finger camera, Conrad Electronics, Ø 20 mm, 69 mm long, 32 g with 6 infrared LEDs (875 nm) for better illumination) that were fixed to the head with a halter. Via radio frequency, image data were transferred from the camera to a stationary receiver (15-1200VR, CL-Electronics GmbH), alternately for 1 s for the right and 1 s for the left eye. These sequences were recorded with a tape recorder (Time Lapse Recorder AG-6040 E, Panasonic) and later digitised (V-Mate, SanDisk). For analysis, one image within each second of video recordings of the first and the third minute of each experimental phase was used, and on each of these digitised images, the area covered by the eye white and the area of the complete visible eye were digitally measured by superimposing a polygon on the picture. These polygons were built up by adding their corners one after the other using on-screen mouse clicks (R-package rpanel, [38]). The percentage of eye white was then calculated by dividing the area of the eye white by the area of the complete visible eye. Due to the sheep's head movements, the preset focus of the cameras was altered in several cases, resulting in out-of-focus images. Usable images remained for three, two and one of the three experimental phases in two, five and four sheep, respectively. Twenty-eight images (median) were available per experimental phase (range 8–50).

### 2.4. Statistical analysis

Statistical analysis was performed in R (version 2.6.1; [39]). One data point was calculated per individual, trial (standard feed, wooden pellets, enriched feed) and phase (anticipation, feeding; six values per individual). Due to software failure, only data on 12 animals were available for respiratory data. Owing to technical problems resulting in loss of data, one sheep was tested twice in all three experimental trials. The animal was conditioned to standard feed again in three trials before the second series of trials, and only the second set of data was analysed. All variables except for the percentage of eye white were modelled separately as response variables using linear mixed-effects models [40]. These included the explanatory variables experimental trial (factor with 3 levels: wooden pellets, standard feed, enriched feed), phase (factor with 2 levels: anticipation, feeding) and sequence of testing (factor with 2 levels: standard feed–wooden pellets–enriched feed, standard feed–enriched feed–wooden pellets) and all possible two-way interactions and the one three-way interaction. Except for the main effects of experimental trial, phase and their interaction, the terms were reduced in a step-wise backwards procedure and will only be mentioned if significance was reached ($\alpha \leq 0.05$). The homogeneity of variance for body-surface humidity appeared to differ in respect of valence. Therefore, an additional model was calculated using the variability of body-surface humidity as a response variable, measured by the variance of the lagged differences of all humidity measurements of a phase. The data for the model of the percentage of eye white were restricted to the feeding phase and the model thus included experimental treatment, sequence of testing and minute of treatment (min 1 or min 3), and the interaction of treatment and minute as explanatory variables. In order to reflect the experimental design, a random effect for experimental trial nested within individual sheep was included in all models. The assumptions of normal distribution and homoscedasticity of the errors of the models were checked by a graphical analysis of the residuals. To satisfy assumptions, log transformations were used for RMSSD, respiration rate and the variability of body-surface humidity, and a logit transformation for the percentage of eye white. Heart rate

and body-surface temperature remained non-transformed. Raw data are presented as boxplots indicating observed median, first and third quartile and absolute range of data. All mean values given in the text are based on model estimates.

## 3. Results

The anticipation phases of the three experimental trials did not differ significantly for any of the response variables (Fig. 2). The sheep's heart rate (beats per minute, bpm) increased from the anticipation phase to the delivery of wooden pellets (estimated mean: +4–5 bpm) and decreased when standard feed was delivered (by −3 bpm). In the trial with the enriched feed, heart rate slightly increased (by +1 bpm) but remained at a lower level than during the delivery of wooden pellets (Fig. 2a; interaction of experimental trial and phase: $F_{2,35}=5.39$; $p=0.009$).

Moreover, heart rate was influenced by the sequence of testing of the different treatments (Fig. 2b; interaction of experimental trial and sequence: $F_{2,23}=9.96$; $p<0.001$). Irrespective of whether wooden pellets or enriched feed were offered, heart rate was higher during the first treatment after the standard feed treatment (light grey boxes in Fig. 2b). The enriched feed trial, in particular, was influenced by the sequence of testing since heart rate was much lower if the enriched feed treatment was tested last (dark grey box) compared to being delivered directly after the standard feed treatment.

RMSSD increased most from anticipation to the standard feed treatment (+16 ms). The increase was lower for the enriched feed

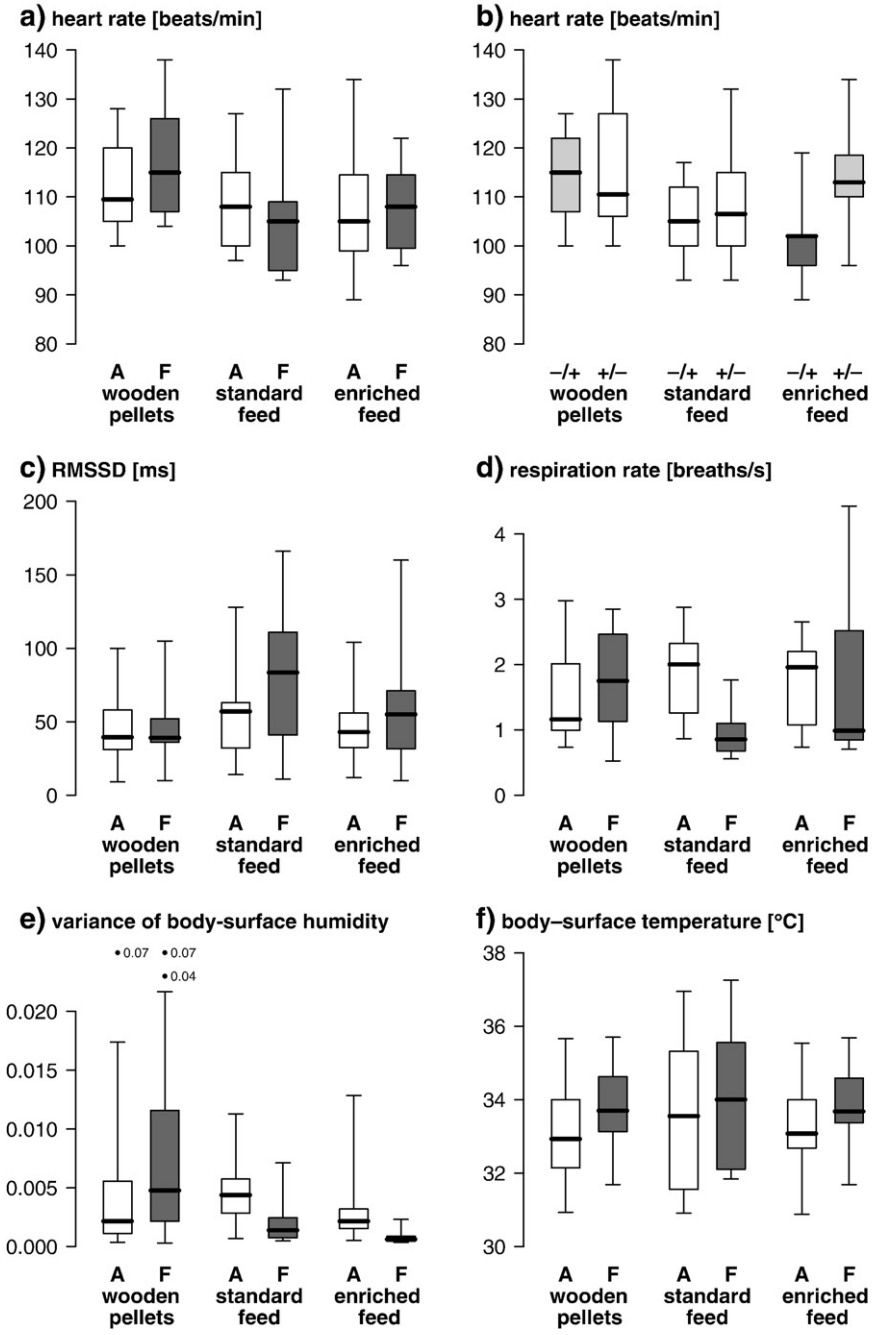


**Fig. 2.** Heart rate (a), RMSSD (c), respiration rate (d), variance of body-surface humidity (e) and mean body-surface temperature (f) recorded in sheep ($n=14$, respiration $n=12$) during the anticipation phase (A, in white) and the delivery of different types of pellets (feeding phase F, in grey) when offered wooden pellets (negative treatment), standard feed (control treatment) or enriched feed (positive treatment). In addition, heart rate is shown in respect of the sequence of testing (b; −/+ wooden pellets offered before enriched feed; +/− enriched feed offered before wooden pellets; first treatment after control in light grey, enriched feed tested last in dark grey).

(+5 ms) and smallest for the wooden pellet treatment (+2 ms), but these changes missed statistical significance (Fig. 2c; interaction of experimental trial and phase: $F_{2,35}=2.40$; $p=0.11$).

The respiration rate of the sheep was lowest with 0.9 breaths/s during the delivery of standard feed, which was almost half the value observed in the anticipation phase (−0.81 breaths/s). From the anticipation phase to the delivery of enriched feed, breathing rate decreased less markedly (−0.21 breaths/s) and in the wooden pellet treatment, respiration rate increased from anticipation to the delivery of pellets (+0.19 breaths/s; Fig. 2d; interaction of experimental trial and phase: $F_{2,33}=5.82$; $p=0.007$).

The change in the values of the mean body-surface humidity between anticipation and feeding phase did not differ between treatments (interaction of experimental trial and phase: $F_{2,39}=1.98$; $p=0.15$). However, variance of body-surface humidity showed a similar pattern to respiration rate. The variance increased from anticipation to the delivery of wooden pellets ($+2.6*10^{-3}$) and decreased from anticipation to both the standard feed ($-2.2*10^{-3}$) and the enriched feed treatment ($-0.001*10^{-3}$; Fig. 2e; interaction of experimental trial and phase: $F_{2,39}=16.37$; $p<0.001$).

The change in mean body-surface temperature was not influenced by treatment (Fig. 2f; model estimates: anticipation 33.1–33.5 °C, wooden pellets 33.8 °C, standard feed 34.0 °C, enriched feed 33.8 °C; interaction of experimental trial and phase $F_{2,39}=0.64$; $p=0.53$). The same was true for the percentage of eye white (wooden pellets 3.2%, standard feed 2.4%, enriched feed 3.1%; $F_{2,3}=0.94$; $p=0.48$).

## 4. Discussion

In this experiment, the negative situation (wooden pellet treatment) was clearly differentiable from the control (standard feed) and the positive treatment (enriched feed) due to the sheep's higher heart rate, faster breathing and higher variance of perspiration (body-surface humidity). These differences are in line with the results of previous studies in non-human animals [13,41,42] and humans [43,44] showing that cardiovascular measurements, respiration rate, electrodermal activity and core as well as skin temperature increase in emotionally negative compared to control situations. Often, such physiological indicators of emotional states are also accompanied by behavioural reactions. Investigating the animals' facial expression, we observed differences in the sheep's ear postures with respect to experimental trial [45]. In that study, frequent ear-posture changes and high proportions of forward ear postures occurred during presumed negative emotional situations, and a high proportion of passive ear postures with situations likely to induce positive emotional states. The patterns visible in these ear postures across situations of different emotional valence were similar to some of our physiological data presented here (respiration rate, variance in body-surface humidity and inverse heart rate variability), and may be interpreted as increased and decreased attention in response to negative and positive emotional stimuli, respectively.

Based on the theories of positive–negative contrasts [6,7], the standard feed treatment was not expected to provoke a specific contrast reaction since anticipated and actual reward did not differ. Surprisingly, however, the physiological reactions of the experimental animals to the standard feed and the enriched feed were rather similar. With regard to feeding behaviour during these two treatments, animals were mainly feeding throughout the 6 min when offered standard and enriched feed. By contrast, the experimental animals were not feeding (with one exception) during the delivery of wooden pellets, but spitting out the pellets after taking them into their mouths. It is likely that the animals experienced a positive contrast from the anticipation phase – during which no feed was available – to the delivery of any type of feed. An example from rats shows that animals in an experiment have no preference for fructose or glucose when these are offered sequentially, but they prefer glucose when both sugars are offered simultaneously [46]. So despite the fact that lambs are known to prefer several palatable items to one type of feed [47], the comparison of hedonic values of two different types of feed across two days may have been of minor importance to the sheep's perceptions. Hence, assuming that sheep interpreted both treatments as positive, the similar reactions of the animals to the standard feed and the enriched feed treatment can be explained. Thus, positive emotional states would be characterised by lower heart rate and respiration rate, increased RMSSD and reduced perspiration variability when compared to negative emotional states. These data are in line with human research in which specific positive emotional states such as happiness coincided with lower heart and respiration rates than excitement or anger [43].

Knowing that RMSSD reflects parasympathetic input to heart rate generation [48–50] and that its values only approached a statistical trend for being influenced by treatment in our study, we conclude that the parasympathetic nervous system played a minor role in the control of heart rate. Hence, reactions of sheep to the different feeding situations were mainly driven by reduced sympathetic activation in the standard feed and the enriched feed treatment, and increased sympathetic activation in the wooden pellet treatment. This hypothesis is supported by our data on perspiration variability that is likely to reflect activation of the sympathetic nervous system on eccrine sweat glands of the skin [35,51,52]. With each sympathetic activation, sweat is released on to the skin surface, causing a measurable wave of increased skin conductance [53]. Hence, data of the present experiment suggest that negative emotional reactions in sheep may coincide with sympathetic activation, and positive emotional reactions with reduced sympathetic input, partly influenced by additional parasympathetic activation.

Another effect observed in this study was the influence of the sequence of testing of the different types of feed on heart rate. Animals were habituated to receiving standard feed during the experiment and that expectation was not fulfilled when they were offered a different type of feed in the first treatment after the standard feed, irrespective of whether wooden pellets or enriched feed were delivered. The type of feed was unexpected in the specific situation, and hence unfamiliar. Novel stimuli can lead to an activation of the autonomous nervous system, irrespective of negative or positive valence [41,54], and this may have been responsible for the increase in heart rate observed in the first treatment following the control treatment. Interestingly, the effect of the unexpected enriched feed on heart rate was much more pronounced in individuals that were exposed to the enriched feed treatment first compared to individuals that were offered enriched feed after the wooden pellet treatment. Such an effect was also observed in the sheep's behaviour (proportion of asymmetric and axial ear postures [45]). This difference in the response depending on the sequence of testing may have been caused by several combined issues. Firstly, sheep might have been prepared to be offered something else but standard feed in the third test situation, and this could have coincided with reduced tachycardia [54,55]. Similar to heart rate, unexpected types of feed coincided with e.g. increased proportions of asymmetric ear postures shown by the sheep, and may be interpreted as increased attention coinciding with unexpected situations [45]. Secondly, the enriched feed was still feed, and hence, no specific response to deal with the situation was demanded compared to the wooden pellet treatment. Thirdly, enriched feed was more attractive to the sheep than wooden pellets, on which they had been tested the day before, and this potentially provoked a greater positive contrast reaction. Thus, our data show that positive situations combined with physiological activation, potentially provoked by unfamiliarity, are similar to both novel and familiar negative emotional states. Familiar positive situations, in contrast, were characterised by much lower heart rate. In the wooden pellet treatment, the effect of increased heart rate towards unfamiliarity was superimposed on the increased heart rate elicited by the wooden pellets as a negative emotional situation. This may have additionally coincided with a greater negative contrast reaction due to testing after

enriched feed. Interestingly, a similar effect was observed in behavioural measurements, in which ear postures were also influenced by the sequence of testing of the different types of pellets, and e.g. asymmetric ear postures were more frequent during the first exposure to either the wooden pellets or the enriched feed [45].

Body-surface temperature did not differ by treatment but increased from the anticipation to the feeding phase, and was not particularly high during the negative treatment as expected from literature [22]. In this experiment, the negative situation provoked by frustrated expectations may not have been intense enough to result in changes in body-surface temperature.

The percentage of eye white in relation to the open eye did not differ by treatment. This contrasts with studies in cows that clearly showed an increased percentage of visible eye white in a negative situation such as frustration [28,31]. However, loss of data was high in this experiment, which reduced the potential for identifying treatment effects. Measurement techniques will have to be improved in order to establish eye white as a parameter for the assessment of emotional reactions in animal species that have smaller eyes than cows.

For practical purposes, it would be desirable in future investigations to reduce the wide array of physiological measurements used in this study as indicators of emotional reactions in sheep. At first glance, this seems permissible because similar patterns were observed for heart rate, respiration rate and perspiration variability, and an inverse pattern for RMSSD. However, the activity of the sympathetic and parasympathetic branch of the autonomous nervous system may change without observable changes in heart rate [56] and multiple measurements may be necessary to detect all physiological changes provoked by emotional stimuli. For a minimal set of variables, we would thus suggest RMSSD as a measure of parasympathetic input and electrodermal activity as an indicator of sympathetic activity in addition to heart and respiration rate. Heart rate seems to be an important component for assessing subjective states since it was able to detect differences in cognitive appraisal of a given environmental stimulus (i.e. exposure to unexpected feed in the first treatment after the standard feed treatment), as shown by others before [57,58]. It remains to be clarified if the reactions found for the negative situation of frustrated expectations and the positive one of fulfilled or surpassed expectations can be generalised to other specific emotions of negative or positive valence.

## 5. Conclusion

This experiment showed that presumed negative emotional states in sheep can be distinguished by means of physiological variables from presumed positive situations. Results indicated that sheep may have perceived the standard feed treatment as positive. For the investigated situations, the combination of heart rate, RMSSD, respiration rate and body-surface humidity appears to be most useful for describing the physiological reactions of the autonomous nervous system towards experimentally induced negative and positive emotional states in sheep.

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