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Positive and negative emotions in dairy cows: Can ear postures be used as a measure?

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ABSTRACT

Applying objective measures to assess the emotional states of animals is an important area of research and essential for improving animal welfare. In this study, we have built upon previous research to test whether ear postures can be used as an indicator of emotional states in dairy cows.

By using a positive and negative contrast paradigm, we elicited the emotional states of excitement and frustration in 22 dairy cows. Each cow was first conditioned to expect the delivery of standard feed when a bell was rung. Once they were familiar with the experimental set-up and the delivery of the feed, they were then given concentrates feed instead. As concentrates are highly desired, this was considered to elicit the emotional state of excitement. This was then repeated five times. On the following trial, the cows were given inedible woodchip, and the cows' unfulfilled expectations were considered to elicit a state of frustration.

We observed the cows' ear postures and mean heart rate (beats per minute) during 15-minute focal observations, each comprised of 5 min of pre-feeding (baseline), 5 min of feeding, and 5 min of post-feeding. Both the woodchip treatment and the concentrates elicited significantly higher mean heart rates compared with the standard feed, indicating that both treatments elicited a high arousal emotional state. The woodchip and concentrates treatments were also significantly associated with the increased performance of different ear postures, indicating that cows do perform certain ear postures in relation to both positive and negative high arousal emotional states.

Our results complement previous research performed with both cows and sheep, and indicate that with training and contextual knowledge, ear postures may be suitable as a reliable measure of emotional state in dairy cows.

1. Introduction

Understanding how animals communicate their emotional states is an important area of research, and it is necessary for implementing welfare improvements in practice (Descovich et al., 2017; Désiré et al., 2002). By understanding the emotional minds of animals, we can seek to improve their welfare by ensuring that negative emotions are minimised, and positive ones are promoted (Désiré and Veissier, 2004; Proctor, 2012). Finding practical and reliable non-invasive measures of emotional states is one area which has grown in interest in recent years (e.g. Briefer, 2012; Proctor and Carder, 2014, 2015a, 2015b;Reefmann et al., 2009a,b,c;Reimert et al., 2013; Vögeli et al., 2014). Despite their numbers in industry, we still know very little about the emotional lives of farm animal species, and there is a great need for valid, reliable, and objective behavioural measures that can be used in welfare assessments, but also in practice on a day to day basis by farmers and veterinarians (Sandem et al., 2002).

One potential tool that is growing in interest, is the use of facial expressions. In humans, facial expressions have been studied for hundreds of years as a measure of emotional experience (e.g. Darwin, 1872), but its use in non-human animals is still a burgeoning area of research. Facial expressions in animals vary widely across species, but there are species-specific patterns that can be used to explore the emotional state of the animal (Descovich et al., 2017). Primates have been shown to have limited voluntary control over their facial expressions, which suggests that they are a more reactive and honest portrayal of the animal's psychological state (Hopkins et al., 2011). Grimace scales now exist for different species. These scales, which are used in industry measure facial expressions to indicate the degree of pain an animal is in (e.g. Costa et al., 2014; Leach et al., 2012; Sotocinal et al.,

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2011). Recently, Gleerup et al. (2015) found that ear postures can be used along with other facial expressions and behaviours to assess pain levels in cattle.

Ear postures are categorised as facial expressions because the position of the ear is controlled by the animal's facial muscles. To date, research suggests that ear postures are an important indicator for both social communication and internal states in various species (Boissy et al., 2011; Wathan and McComb, 2014). For example, in horses, backward ear postures are associated with negative emotional experiences, such as fear (von Borstel et al., 2009). Whereas in sheep, backwards ears, and ears pointing up are considered to be associated with different negative emotional states, such as anger and frustration (Boissy et al., 2011). In 2014, we demonstrated the potential use of ear postures as a measure of a positive, low arousal emotional state in dairy cows (Proctor and Carder, 2014). We found that cattle spent longer in two types of ear postures when they were in a positive, low arousal emotional state, compared with the preceding neutral state. In this study however, we only looked at one type of emotion, whereas according to the dimensional theory of emotions, emotions vary both in terms of valence (the pleasantness or unpleasantness of the stimulus), and in arousal (the degree of excitement the stimulus elicits) (Mendl et al., 2010; Russell, 2009). Mendl et al., (2010) proposed a framework which is comprised of four quadrants of emotions; positive high arousal, positive low arousal, negative high arousal, and negative low arousal. Further research is therefore needed to determine the suitability of ear postures for assessing other types of emotional state, namely, negative high and low arousal states, and positive high arousal states. To address this, we have used negative and positive contrast paradigms in the current study to elicit high arousal, negative and positive emotional states in dairy cows.

1.1. Positive-negative contrasts

Positive-negative contrasts refer to the phenomenon observed when an animal is conditioned to expect a certain reward or event, and that reward or event is then shifted to one of either lesser perceived value, or one of greater perceived value (Flaherty and Rowan, 1986; Reefmann et al., 2009b). Flaherty (1982) described how this expectation can elicit an emotional response in the animal; either frustration or excitement, depending on whether their expectations have been surpassed or thwarted. Whereas, if the animal experiences no change in the expected event or reward, then there should be no recognisable effect on the animal's emotional state (Flaherty, 1982). This paradigm has been successfully used in many studies with rodents (e.g. Crespi, 1942; Mustaca et al., 2000; Pérez-Acosta et al., 2016). In the case of successive negative contrast, the animals work less hard for the lower value reward, or more typically in the runway tests, they will run more slowly towards the reward than they did for the previous, more highly valued reward (Flaherty, 1982; Flaherty and Rowan, 1986; Pérez-Acosta et al., 2016). Similarly, researchers have had success with eliciting a positive contrast effect when the reward value is increased. In this case, the animal rapidly increases in speed following an increase in reward value (Belke and Pierce, 2016; Crespi, 1942; Shanab and Spencer, 1978). However, many of these studies were previously hindered by what is now referred to as the ceiling effect, where an effect was not seen due to the fact that the study animals could not show a further increase in movement or motivation, as they were already running as fast as they could towards the reward (Flaherty, 1982).

More recently, positive-negative contrasts have successfully been used in sheep to elicit different emotional states for testing potential behavioural and physiological measures of emotions (Reefmann et al., 2009a, 2009b). In their studies, Reefmann et al. (2009a, b) trained sheep to expect the delivery of feed pellets upon a signal. They then changed the feed to either an enriched high energy feed, or to inedible wooden pellets. The wooden pellets treatment resulted in a high arousal state, evidenced by increases in the sheep's heart rate, respiration rate, and variability of body-surface humidity, as well as an increase in ear posture changes, and a reduced performance of passive ear postures. The enriched feed treatment however, had a similar effect on the physiological measures to that of the standard feed, and both types of feed elicited a higher proportion of passive ear postures, and a lower number of ear posture changes, compared with the wooden pellets. The authors commented that the sheep mainly ate the feed in both feed treatments, and so they were clearly motivated to eat, and that this may be the reason for the lack of differences between the enriched and standard feed treatments.

1.2. Current study

Previously, we found that ear postures three (a backwards ear posture, see Fig. 3) and four (a passive posture where the ear hung loosely, see Fig. 4) were associated with a positive, low arousal emotional state in cows, and that ear posture four was almost exclusively performed during this state (Proctor and Carder, 2014). However, it was unclear what component of the stimuli had this effect (i.e. arousal, valence, or a combination of both). The objective of the current study therefore, is to explore the effects of both positive and negative high arousal stimuli upon cows' ear postures. By building upon our previous findings, we can draw comparisons between the two studies, and provide further insight into the effects of emotional state on ear postures in cattle.

2. Methods

2.1. Ethics

The study was performed in compliance with the ARRIVE guidelines, the UK Animals (Scientific Procedures) Act, 1986, and the Royal Veterinary College's ethical procedure. The study did not require a Home Office License.

2.2. Subjects and housing

We used 22 lactating Holstein dairy cows ranging in age from 3 to 7 years old, and randomly selected from a commercial dairy herd of 92 cows housed at Bolton's Park Farm, Hertfordshire, UK. The farm is part of the Royal Veterinary College's farm animal practical teaching facility. The study was conducted over 6 weeks from May to July 2015.

The cows used in the study were kept indoors for the experimental days in their usual housing system; a deep litter, free housing system, and were kept in the same group throughout. For the experiment, the focal cow was moved to the experimental pen and held in a stall. The same stall was used throughout the entire experiment and was located approximately 15 m from the home pen. The experimental set-up in the stall remained the same for all three treatments, including the continuous presence of all three types of feed, which were kept in identical, sealed buckets throughout the experiment.

One cow was used at a time, and she was used only twice a day with a minimum of 1.5 h between trials. As the cows were part of a teaching herd, prior to the study they were regularly moved and kept in these stalls singly and socially, and so it was not considered to be stressful for the cows to be moved and held in these stalls. In fact, a recent unpublished analysis into the cortisol levels of the cows when held in the stall, found no signs of increased cortisol levels compared with baseline measures (personal communication: Charlie Verity, 15 September 2014). Each cow was held for 25 min in the stall; 10 min of acclimatisation and equipment fitting, and 15 min for the focal observation. They were then returned to the home pen immediately after the focal observation ended. To avoid diluting the effects of the experimental procedure, the cows were not brought into the stalls at any other time during the study.

All of the study cows were habituated to wearing the heart rate monitor prior to the study. They were also already habituated to the presence of unfamiliar people during the regular teaching sessions they were exposed to, but the cows had no prior experience of the researchers or the experimental procedure.

2.3. Experimental procedure

2.3.1. Trial procedure

All of the study cows underwent the same procedure throughout a consecutive 5-day period; four standard feed trials, five concentrate feed trials, and one woodchip trial. To begin each trial, the focal cow was guided from the home pen into a stall, and then fitted with the physiological monitoring telemetry device (BioHarness 3.0, Telemetry System, Zephyr Technology Corporation). To ensure optimum conductivity, the area was shaved prior to the study, and the inbuilt electrodes were sprayed with saline before each fitting. The Bioharness was attached to an elasticated girth, and was placed and tightened around the cow's middle, just behind their front legs. Once the Bioharness was fitted, the focal cow was left to rest until a total of 10 min had passed since she had entered the stall.

During each focal observation, the cow's left ear was filmed using a Sony HDRXR160EB Handycam fitted on to a monopod. The ECG trace, recorded by the Bioharness, was transmitted and stored in real time via Bluetooth to a laptop using AcqKnowledge software version 4. As previous studies have shown no effect of lateralisation in cows' ears from changes in emotional states (Proctor and Carder, 2014), and in order to keep the experimental setup consistent for the cows entering the stall, only the left ear was filmed.

2.3.2. Treatment 1: standard feed

Each cow first underwent four standard feed trials, each comprised of three, 5-minute segments (pre-feeding, feeding, and post-feeding). Data was collected throughout the 15-minute focal observation. At 5:00 min the feeding segment began, and a researcher rang a bell, moved a feed trough in front of the cow, and then poured 500 g of standard feed into it. The standard feed was the same feed the cows had constant access to in their home pen (grass sileage). The feed trough was then left in the stall for 5 min (feeding segment). After this time, the trough was removed, and the data collection continued for a further 5 min (post-feeding segment). This standard feed procedure was performed four times per cow, twice on day one, and twice on day two.

2.3.3. Treatment 2: concentrates

Once each cow had undergone four standard feed trials, we moved on to the concentrates treatment. The procedure was as follows. After the bell was rung at 5:00 min, the trough was again placed in front of the cow, but this time the researcher poured 500 g of concentrates feed in. Concentrates is known to be highly desirable to dairy cows, and they only have access to it during milking, twice a day. Each of the focal cows were motivated to eat the feed, but they all took different lengths of time to consume it. To ensure consistency, we kept the amount of feed the same for all cows, and then ended the feeding segment once the cow finished the feed, as this was always less than 5 min. Each cow underwent the concentrates procedure five times; twice on days three and four, and once on day five. The feed trough was removed once the cow finished feeding, and the observations continued for a further 5 min.

2.3.4. Treatment 3: woodchip

On day five, after each cow underwent the fifth and final concentrates treatment, we began the woodchip treatment. For this, we applied the same procedure as during the standard and concentrates feed treatments; the cows were first observed for 5 min (pre-feeding segment), but this time when we rang the bell, we gave the cows 350 g of inedible woodchip (same volume as the standard and concentrates feed). The cows were exposed to the woodchip for 5 min, after which, the trough was removed, and observations continued for a further 5 min (post-feeding segment). Each cow underwent this procedure once.

Fig. 1. Ear posture 1.

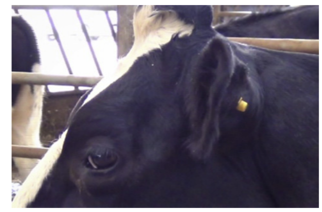

Fig. 2. Ear posture 2.

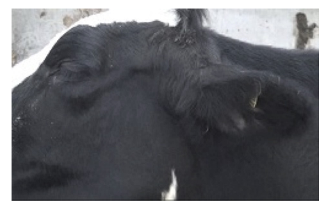

Fig. 3. Ear posture 3.

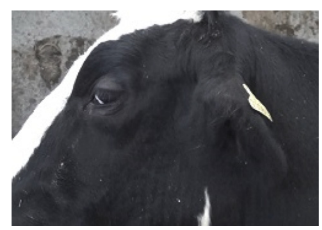

Fig. 4. Ear posture 4.

2.4. Ear postures; identification

Four unique ear postures (see Figs. 1–4) were identified in a prior study (Proctor and Carder, 2014), and preliminary observations with different cows from the herd deemed them to be appropriate for this study too. Ear posture one (EP1) referred to when the cow's ear was held upright on their head, with the ear pinna either facing forwards or to the side. Ear posture two (EP2) was a forward-facing posture where the ear pinna faced forwards, in front of the cow, whilst the ear was held on the horizontal plane. Ear posture three (EP3) was a backwards ear posture, characterised by the ear being held back on the cow's neck, but not drooping or flopping downwards, yet not held vertically as in EP1. Finally, ear posture four (EP4) occurred when the ear hung loosely from the cow's head, without being held backwards, and naturally falling perpendicular to the head.

2.5. Ear postures; video analysis

We analysed 216 videoed focal observations to determine the number of ear posture changes performed in each focal observation, and the duration of time each cow spent in each of the four ear postures. Four videos were unavailable for viewing due to technical faults.

The videos were randomised prior to analysis, and were analysed blind to the treatment and subject, although sometimes the type of treatment and cow number were apparent from the video. The ear postures were recorded continuously, and the data was entered into a spreadsheet (i.e. the start and stop time of each ear posture for the entire 15-minute focal observation). The timings were then used to calculate the number of ear posture changes, and the total duration of time spent in each of the ear postures for each segment.

2.5.1. Inter-observer reliability tests

Three researchers analysed the ear postures, and they were fully trained for the task. The lead researcher's (Helen Lambert) analyses served as the silver standard throughout training and for all comparisons. To ensure consistency among the researchers, all three researchers conducted three inter-observer reliability tests prior to starting data analysis. For this, all three of the researchers analysed the same three 15 min focal observations, and then recorded the duration of each new ear posture and the total number of ear posture changes. The researchers' responses were compared to one another, and a percent agreement was calculated by dividing the number of agreement scores by the total number of scores. For the durations of ear postures, each individual duration score was treated as a separate data point, and then an overall percent agreement was calculated for all four ear postures. We also examined each researcher's calculations for any durations that were more than 2 s different from the silver standard, but we did not find any for any of the tests. The percent agreement for test video 1 was 96.42% for the ear posture changes, and 95.3% for the ear posture durations. For test video 2, it was 95.58% for the ear posture changes, and then 96.43% for the durations. For test video 3, it was 98.3% for the ear posture changes, and then 96.98% for the durations. The video analysis took 6 weeks to complete, and a new inter-observer reliability test was performed after 3 weeks, and another at the end of the 6 weeks to ensure consistency throughout. The researchers scored 96.05% agreement in test video 4 for the ear posture changes, and 97.12% agreement for the ear posture durations. They then scored 96.67% agreement in test video 5 for the number of posture changes, and 98.58% agreement for the ear posture durations. Each test used a new focal observation.

2.6. Heart rate analysis

We used AcqKnowledge (version 4) to analyse the ECG trace collected in each focal observation. When a clear, unbroken ECG was visible, we selected six focus areas of 10 s at the following times; 00:00, 4:50, 5:00, 9:50, 10:00 and 14:50. If a clear ECG was not visible at these times, then the nearest 10 s within that segment was selected within a 30 s window. However, this did not apply to the ECG analysed at 5:00 min, as in order to identify any effects from the bell, the ECG was only analysed at that specific time point. If a clear ECG was not available at the 5:00 min point, then it was recorded as a missing data point. The beats per minute (bpm) were extracted from each of the six focus areas.

2.7. Data analysis

We used IBM SPSS Statistics Version 23 to statistically analyse the data. We used a repeated measures ANOVA test to compare the time spent in each ear posture, and the number of posture changes performed across the three treatments (fixed factor; standard, concentrates, and woodchip), comparing each segment (pre-feeding, feeding, and post-feeding). We also compared the durations and number of posture changes within each treatment by comparing the pre-feeding, feeding and post-feeding segments as the fixed factor for each treatment (standard, concentrates and woodchip). The Bonferroni correction was applied to correct for multiple tests. A repeated measures ANOVA test was also used to identify any effects from the cows' increasing familiarity with the experimental treatment, and we analysed the durations and number of ear posture changes throughout the course of each treatment. We also used a repeated measures ANOVA to analyse the differences in heart rate across the three treatments, and for within each treatment, to determine when the cows were most, and least aroused. We performed Post-Hoc Pairwise Comparisons (Hochberg's GT2) to identify the significant differences between the ear posture durations, changes, and the mean heart rate. We performed the paired-samples *t*-test to compare the ear posture durations from the fifth concentrates trial and the woodchip trial for each ear posture, and for each segment. When the Mauchly's test indicated that the assumption of sphericity had been violated, the degrees of freedom and p-values were corrected using the Greenhouse-Geisser estimates of sphericity. All other assumptions for the One-Way ANOVA and the paired-samples *t*-test analyses were met.

3. Results

3.1. Ear postures

All four ear postures were performed across the study period. EP3 and EP4 however, were rarely performed, and the cows spent most of their time in EP1 and EP2. EP1 was the most commonly performed ear postures across all three of the treatments (standard, concentrates and woodchip), and almost all of the segments (pre-feeding, feeding and post-feeding). Whereas, EP4 was rarely performed during any of the treatments.

3.1.1. Treatment effects: pre-feeding

During the pre-feeding segment, the three treatments had no significant effect on the duration of time the cows spent in each of the ear postures (EP1, $p = 0.85$; EP2, $p = 0.09$; EP3, $p = 0.71$; and EP4,

Table 1
The mean duration (mm:ss) spent in ear postures 1–4 during the three treatments.

		Pre-feeding				Feeding				Post-feeding			
		EP1	EP2	EP3	EP4	EP1	EP2	EP3	EP4	EP1	EP2	EP3	EP4
Standard	M	03:49	00:54	00:07	00:01	03:41	01:05	00:10	00:01	03:41	00:08	00:59	00:02
	SD	00:51	00:43	00:16	00:04	00:55	00:48	00:19	00:00	00:57	00:22	00:48	00:07
Concentrates	M	03:19	01:28	00:06	00:00	04:00[a,c,**]	00:48	00:10	00:00	03:09	00:10	01:33	00:00
	SD	01:14	01:11	00:19	00:02	00:50	00:49	00:32	00:02	01:11	00:33	01:04	00:00
Woodchip	M	03:35	01:2	00:02	00:01	02:20	02:35 [a,b,**]	00:05	00:00	03:01	00:02	01:38	00:14
	SD	00:29	00:29	00:02	00:01	00:38	00:38	00:07	00:00	00:36	00:03	00:32	00:27

[a] Significantly higher than in the standard feed treatment.
[b] Significantly higher than in the concentrates treatment.
[c] Significantly higher than in the woodchip treatment.
** $p < 0.001$.

Table 2
The mean number of ear posture changes performed in each treatment.

		Pre-feeding	Feeding	Post-feeding
Standard	*M*	14.67	14.15 **,b	13.52
	SD	09.25	07.91	07.42
Concentrates	*M*	14.55	05.35	15.71
	SD	07.87	04.72	07.20
Woodchip	*M*	16.50	16.59 *,a,*,b	14.95
	SD	04.22	03.77	04.52

[a] Significantly higher than in the standard feed treatment.
[b] Significantly higher than in the concentrates feed treatment.
** $p < 0.001$.
* $p < 0.05$.

$p = 0.15$) (Table 1). There was also no significant difference between the numbers of ear posture changes performed during the three experimental treatments ($p = 0.06$) (Table 2).

3.1.2. Treatment effects: feeding

During the feeding segment, the mean duration of time the cows spent in EP1 ($F_{(2, 40)} = 19.75$, $p < 0.001$) and EP2 ($F_{(2, 36)} = 16.07$, $p < 0.001$) was significantly affected by the experimental treatments (Table 1). The post-hoc analyses showed that EP1 was performed for significantly longer in the concentrates treatment, compared with during both the standard feed ($p < 0.001$), and the woodchip treatments ($p < 0.001$). There was no significant difference however, between the duration of time spent in EP1 in the standard feed and woodchip treatments ($p = 0.09$). EP2 was performed for significantly longer in the woodchip treatment, compared with during both the concentrates ($p < 0.001$), and the standard feed treatments ($p < 0.05$). The durations of EP3 and EP4 did not significantly differ between the experimental treatments (EP3 $p = 0.45$; EP4 $p = 0.14$).

We found that the treatments had a significant effect on the number of ear posture changes performed during the feeding segments ($F_{(1.68, 182.73)} = 129.34$, $p < 0.001$). The post-hoc analysis showed that the cows changed their ear postures significantly more during the woodchip treatment, compared with during both the standard feed ($p < 0.05$), and the concentrates treatments ($p < 0.001$). The number of changes was also significantly higher during the standard feed treatment, compared with during the concentrates treatment ($p < 0.001$).

3.1.3. Treatment effects: post-feeding

During the post-feeding segment, the treatment had no significant effect on the amount of time the cows spent in each of the ear postures (EP1, $p = 0.62$; EP2, $p = 0.19$; EP3, $p = 0.19$; and EP4, $p = 0.50$) (Table 1). The number of ear posture changes did differ significantly across the treatments ($F_{(2,218)} = 3.52$, $p < 0.05$), and the concentrates had the highest number, and the standard feed had the lowest number of changes (Table 2). Although the post-hoc analysis found no significant individual differences between the treatments ($p > 0.05$).

3.1.4. Within treatment effects

We compared the ear posture durations and ear posture changes within each of the treatments across the three segments (pre-feeding, feeding and post-feeding).

3.1.4.1. Standard feed. There were no significant differences between the duration of ear postures performed in the three segments during the standard feed treatment for; EP1 ($p = 0.43$), EP2 ($p = 0.19$), EP3 ($p = 0.41$), or EP4 ($p = 0.31$). There was also no significant difference between the number of ear posture changes across the three segments during the standard feed treatment ($p = 0.53$).

3.1.4.2. Concentrates. EP1 significantly differed in duration between segments in the concentrates trials ($F_{(2, 212)} = 27.12$, $p < 0.001$). Post-hoc analyses showed that the cows spent significantly longer in EP1 in the feeding segment, compared with during both the pre-feeding ($p < 0.001$) and the post-feeding ($p < 0.001$) segments. There was no significant difference between the pre-feeding and post-feeding segments ($p = 0.32$).

EP2 also significantly differed in duration between the three segments during the concentrates treatment ($F_{(2, 212)} = 1.72$, $p < 0.001$). Post-hoc analyses showed that the cows spent significantly longer in EP2 during the pre-feeding ($p < 0.001$) and post-feeding ($p < 0.001$) segments, compared with during the feeding segment. There was no significant difference between the pre-feeding and post-feeding segments ($p = 0.85$). There was also no significant difference in the time spent in EP3 ($p = 0.36$) or EP4 ($p = 0.7$) across the three segments during the concentrates treatment.

The number of ear posture changes did significantly differ between the three segments ($F_{(2, 212)} = 104.91$, $p < 0.001$). The post-hoc analyses showed that there were significantly fewer posture changes in the feeding segment, compared with during the pre-feeding ($p < 0.001$) and post-feeding ($p < 0.001$) segments.

3.1.4.3. Woodchip. EP1 significantly differed in duration between segments in the woodchip treatment ($F_{(2, 40)} = 10.01$, $p < 0.001$). Post-hoc analyses showed that the cows spent significantly longer in EP1 in the pre-feeding ($p < 0.01$) and post-feeding ($p < 0.05$) segments, compared with during the feeding segment. There was no significant difference between the pre-feeding and post-feeding segments ($p = 0.38$).

There was a significant difference in the duration spent in EP2 across the three segments of the woodchip treatment ($F_{(2, 40)} = 11.08$, $p < 0.001$). Post-hoc analyses showed that the cows spent significantly longer in EP2 during the feeding segment, compared with during both the pre-feeding ($p < 0.05$) and post-feeding ($p < 0.05$) segments. There was no significant difference between the pre-feeding and post-feeding segments ($p = 1$).

There was no significant difference in the time spent in EP3 ($p = 0.35$) or EP4 ($p = 0.35$) across the three segments. There was also no significant difference in the number of ear posture changes between the three segments ($p = 0.37$).

3.1.4.4. Experience effects. To determine whether the increased experience of the experimental treatment had any effect on the ear postures, we compared the ear postures across the trials for the standard feed (4 trials) and concentrate (5 trials) treatments.

3.1.4.5. Standard feed. During the feeding segments of the standard feed treatment, EP1 showed significant differences across the trials ($F_{(3, 63)} = 8.45$, $p < 0.001$), and it was performed for significantly longer in trials 3 ($M = 4{:}04$, $SD = 0{:}38$) and 4 ($M = 4{:}08$, $SD = 0{:}45$), compared with trial 1 ($M = 3{:}00$, $SD = 0{:}49$, $p < 0.001$). EP2 was also performed significantly differently in the feeding segment across the four trials ($F_{(3, 63)} = 13.45$), $p < 0.001$). Post-hoc analyses showed that EP2 was performed for significantly longer in trial 1 ($M = 1{:}52$, SD, 0:48), compared with during trials 2 ($M = 0{:}46$, $SD = 0.36$, $p < 0.001$), 3 ($M = 0.59$, $SD = 0{:}56$, $p < 0.01$), and 4 ($M = 0{:}46$, $SD = 0{:}39$, $p < 0.001$).

There were no significant differences found in the pre-feeding segment (EP1: $p = 0.82$; EP2: $p = 0.27$; EP3: $p = 0.06$; EP4: $p = 0.23$), or in the post-feeding segment across the trials (EP1: $p = 0.35$; EP2: $p = 0.82$; EP3: $p = 0.2$; EP4: $p = 0.45$). There were also no significant differences in ear posture changes across the trials for the pre-feeding: ($p = 0.54$), feeding ($p = 0.13$), or post-feeding ($p = 0.51$) segments.

3.1.4.6. Concentrates. During the pre-feeding segment, EP1 showed a significant difference across the five concentrates trials ($F_{(4, 84)} = 5.94$,

$p < 0.001$). The post-hoc analysis revealed that EP1 was performed for significantly longer in trial 1 (*M* = 3:43, *SD* = 0:58) and 2 (*M* = 3:38, *SD* = 1:12), compared with in trial 5 (*M* = 2:38, *SD* = 1.22, $p < 0.001$).

EP2 also showed a significant difference across the trials during pre-feeding ($F_{(4,\ 84)} = 7.04$, $p < 0.001$), and the post-hoc analysis indicated that EP2 was performed for significantly longer during trial 5 (*M* = 2:13, *SD* = 1:23), compared with during trials 1 (*M* = 1:03, *SD* = 0:53, $p < 0.005$) and 2 (*M* = 1:08, *SD* = 1:01, $p < 0.005$).

There were no significant differences found during the pre-feeding segment for EP3 ($p = 0.59$) or EP4 ($p = 0.13$). There were also no significant differences found during the feeding segment (EP1: $p = 0.52$; EP2: $p = 0.13$; EP3: $p = 0.32$; EP4: $p = 0.47$), or during the post-feeding segment (EP1: $p = 0.4$; EP2: $p = 0.11$; EP3: $p = 0.34$; EP4: $p = 0.41$), across the concentrate trials.

There was a significant difference in the number of ear posture changes across the trials during the post-feeding segment ($F_{(4,\ 76)} = 21.62$, $p < 0.001$). The post-hoc analysis showed that the cows changed ear posture significantly more in trial 1 (*M* = 14.6, *SD* = 5.96), compared with trials 4 (*M* = 6.15, *SD* = 5.28, $p < 0.001$) and 5 (*M* = 3.8, *SD* = 2.87, $p < 0.001$); significantly more in trial 2 (*M* = 14.4, *SD* = 7.5), compared with trials 4 ($p < 0.01$) and 5 ($p < 0.001$); and significantly more in trial 3 (*M* = 16.85, *SD* = 1.86), compared with trials 4 ($p < 0.001$) and 5 ($p < 0.001$). There were no significant differences in ear posture changes across the trials for the pre-feeding ($p = 0.15$) or feeding ($p = 0.6$) segments.

3.1.4.7. Concentrates versus woodchip. As the total duration of time the cows spent in EP1 and EP2 changed with experience during the concentrate treatment (i.e. they spent longer in EP2 and less time in EP1 as the trials continued), we compared the fifth concentrates trial with the one woodchip trial to determine whether the woodchip stimulus had a significant effect on the cows' ear postures.

During the pre-feeding segment, the cows spent significantly longer in EP1 during the woodchip trial compared with during the final concentrates trial ($t_{(21)} = -3.47$, $p < 0.005$). The cows spent significantly longer in EP2 during the final concentrates trial, compared with during the woodchip trial ($t_{(21)} = 3.37$, $p < 0.005$). There were no significant differences between the time spent in EP3 ($p = 0.35$) or EP4 ($p = 0.24$) between the two trials.

During the feeding segment, the cows spent significantly longer in EP1 in the final concentrates trial, compared with the woodchip trial ($t_{(21)} = 4.69$, $p < 0.001$). The cows spent significantly longer in EP2 during the woodchip trial, compared with the fifth concentrates trial ($t_{(21)} = -5.36$, $p < 0.001$). There were no significant differences between the time spent in EP3 ($p = 0.73$) or EP4 ($p = 0.39$) between the two trials.

There were no significant differences between any of the ear posture durations in the post-feeding segment (EP1: $p = 0.57$; EP2: $p = 0.26$; EP3: $p = 0.18$; EP4: $p = 0.45$).

3.2. Heart rate

The treatments had a significant effect on the cows' mean heart rate (beats per minute) in all three segments; pre-feeding, ($F_{(1.88,\ 355.34)} = 88.36$, $p < 0.001$), feeding, ($F_{(1.89,\ 357.29)} = 125.70$, $p < 0.001$), and post-feeding ($F_{(1.90,\ 358.93} = 62.58$, $p < 0.001$) (Table 3). The post-hoc analyses showed that the woodchip treatment elicited the highest mean heart rate in all three segments; pre-feeding ($p < 0.001$), feeding ($p < 0.001$), and post-feeding ($p < 0.001$). The heart rate in the concentrates treatment was significantly higher than in the standard feed treatment in both the feeding ($p < 0.001$), and the post-feeding segments ($p < 0.05$), but not in the pre-feeding segment, where the standard feed heart rate was non-significantly higher ($p = 0.77$).

Table 3
Mean heart rate (bpm) recorded for each segment and during each treatment.

		Pre-feeding	Feeding	Post-feeding
Standard	*M*	77.63	79.88	78.64
	SD	5.12	5.23	5.41
Concentrates	*M*	77.05	83.06 [*,a]	88.95 [**,b]
	SD	7.03	7.58	7.17
Woodchip	*M*	83.01 [*,a,*,b]	88.85 [*,a,*,b]	84.51 [*,a,*,b]
	SD	3.90	5.10	4.24

[a] Significantly higher than in the standard feed treatment.
[b] Significantly higher than in the concentrates feed treatment.
* $p < 0.001$.
** $p < 0.05$.

4. Discussion

Our results show a clear difference in the performance of ear postures for what we considered to be positive and negative high arousal states. The cows spent longer in EP1 when they were exposed to the concentrates stimulus, thought to elicit excitement, and longer in EP2 when exposed to the woodchip stimulus, expected to elicit frustration, compared with the other treatments. Both the concentrates and woodchip treatments elicited significantly higher levels of heart rate in the cows, compared with the standard feed treatment. The findings suggest that cows perform certain ear postures in response to different emotional states.

4.1. Arousal levels

To confirm both the woodchip and the concentrates treatments elicited high arousal states in the cows, we analysed the focal cows' heart rates (bpm). Both the concentrates and woodchip treatments caused a significant increase in the mean heart rate in the feeding segment, compared with during the standard feed treatment. This indicates that the cows experienced an increase in arousal during the woodchip and concentrates treatments. Furthermore, the mean heart rate in the post-feeding segment was higher in both the concentrates and the woodchip treatments, compared with during the standard feed treatment, which suggests that the increased level of emotional arousal continued for at least 5-minutes following the feeding segment.

Our findings are similar to those found with sheep (Reefmann et al., 2009b). In their study, Reefmann and colleagues found that the sheep's heart rate increased when they received wooden pellets, compared with during the anticipation phase beforehand when they expected standard feed to be delivered, and compared with when they received enriched feed. They also found that the enriched feed increased the sheep's heart rate, but not as much as the wooden pellets (Reefmann et al., 2009b) In our study, we also found that the woodchip treatment elicited the highest heart rate during the feeding segment, and that this was also significantly higher than during the concentrates treatment.

4.2. Treatment effects

4.2.1. Within-treatments

We assumed that the delivery of standard feed would serve as a neutral stimulus as the cows had continuous access to it. The concentrates however, were expected to elicit excitement, as they were a positive reward, and the woodchip was thought to elicit frustration, as it thwarted the cows' expectations. Our results showed that the delivery of the standard feed (feeding segment) had no significant effect on the duration spent in each of the four ear postures, compared with the pre-feeding and post-feeding segments. Whereas the delivery of the concentrates resulted in an increase in EP1 and a decrease in EP2 during the feeding segment, compared with during the pre-feeding and post-feeding segments. Furthermore, the arrival of the woodchip resulted in a decrease in EP1, and an increase in EP2, compared with the pre-feeding and post-feeding segments. These results suggest that the standard feed did not elicit a measurable emotional response, whereas both the concentrates and the woodchip elicited different types of emotional responses, as evidenced by the difference in performance of the ear postures upon feed delivery, and the differences in heart rate.

4.2.2. Between treatments

4.2.2.1. Pre-feeding. The three treatments had no significant effects on the ear posture durations, or on the number of ear posture changes during the pre-feeding segments. The lack of significant difference between the three treatments could suggest that the cows were not anticipating the concentrates, or that the anticipation was not strong enough to significantly change the ear posture durations. In addition, the mean heart rate was significantly higher in the pre-feeding segment of the woodchip treatment, compared with in the pre-feeding segments of the concentrate treatment, indicating increased arousal levels in the former. Given that at this point the experimental set-up was identical to the concentrates treatment, it is possible that the focal cows had learnt to anticipate the concentrates, and their anticipation significantly affected which ear postures they performed, and further increased their arousal levels. This suggests that it may have taken the cows more than five trials to anticipate the concentrates feed. Future research would benefit from additional standard feed and concentrates feed trials to explore this further.

4.2.2.2. Feeding. Regardless of the presence or lack of anticipation effects, both the woodchip and concentrates treatments appeared to have significant effects on the ear posture durations in the feeding segment. The increased duration of time spent in EP2 during the feeding segment of the woodchip treatment, suggests that EP2 is more likely to be performed as a result of a negative, high arousal emotional state, than it is for other emotional states. Similarly, because EP1 was performed for longer during the feeding segment of the concentrates treatment, compared with during the woodchip or standard feed treatment, this suggests that EP1 is likely to be performed in response to a positive high arousal state, such as excitement. In addition, there was no significant difference between the time spent in EP1 during the woodchip or standard feed treatments, suggesting that EP1 was unaffected by the negative treatment. When feeding, cows will perform all four types of ear posture, regardless of the position of their head. The effect seen here, is therefore unlikely to be the result of feeding postures in the concentrates treatment, and the absence of feeding in the woodchip treatment, although further research should explore whether this is a confounding effect. Further research would also be required to determine whether the effects seen are only seen in response to the specific stimuli used in our study, or whether ear postures are a reliable measure of emotional state in cows.

As both the positive and negative emotional states were considered to be high arousal, these results suggest that the ear postures were not solely affected by arousal levels, but by valence too. The cows spent significantly longer in certain ear postures during each of the experimental treatments, which means that EP1 and EP2 are potentially useful in determining the valence of the cows' emotional state. Furthermore, the near absence of EP3 and EP4 suggests that these postures are more common in low arousal, positive emotional states, as seen in our previous study (Proctor and Carder, 2014), than in high arousal states. Whilst EP1 and EP2 are more likely to be performed in high arousal states as seen in the current study.

The total numbers of ear posture changes were also significantly affected by the treatment, with the woodchip treatment eliciting the highest number of changes, and the concentrates treatment eliciting the lowest. This suggests that ear posture changes could also indicate emotional valence, and not arousal, as both treatments induced a similarly high state of arousal. Similar findings were found with sheep, where the number of ear posture changes was lowest when the sheep received the anticipated standard feed, or the unexpected but positive, enriched feed. Whereas, the sheep changed ear postures more frequently when they received the negative wooden pellets (Reefmann et al., 2009c). In our previous study however, we found that the number of ear posture changes increased during the positive, low arousal emotional state, and so it is unclear whether the number of ear posture changes is a useful indicator in dairy cows, and further research is required to explore this.

4.2.2.3. Post feeding. In the post-feeding segment, we found no significant differences between the ear posture durations, or in the number of ear posture changes across the treatments. This suggests that although the woodchip and concentrates treatments appeared to elicit the frustrated and excited emotional states, their effects on the cows' emotional state was not long-lasting, despite the fact that the cows' arousal levels remained significantly high in both the woodchip and concentrates treatments.

4.3. Experience effects

The duration of time spent in the ear postures changed over the course of the trials. For instance, the duration of EP1 decreased with repeated trials of the concentrates treatment, whereas, EP2 increased in duration over the repeated trials. Therefore, both EP1 and EP2 showed a significant effect from the repetition of the concentrates treatment.

In order to determine whether the effects seen in the woodchip trial were the result of familiarity with the procedure, or whether they were indeed an effect from the woodchip stimulus, we compared the final concentrates trial with the woodchip trial. We found that both EP1 and EP2 differed significantly in the pre-feeding and feeding segments. The effects seen in the pre-feeding segment may have been the result of increased experience as discussed earlier, as nothing had changed about the experimental set-up. However, the differences in the feeding segment could be the result of the woodchip stimulus, although further research with more trials would be able to explore this further.

4.4. Previous findings

In our previous study, we found that EP1 and EP2 were performed for significantly less time when the cows were experiencing a positive, and low arousal emotional state elicited by stroking, and that EP3 and EP4 were performed for significantly longer during the stroking stimulus, compared with before and after (Proctor and Carder, 2014). In the current study, the cows rarely performed EP3 and EP4, which further confirms our previous conclusions that the performance of EP3 and EP4 are indicative of a positive, low arousal emotional state, as at no point were the cows expected to be in a low arousal, positive emotional state during the current study (Proctor and Carder, 2014). Interestingly, sheep were also found to rarely perform passive and backward ear postures, comparable to EP4 and EP3 in our study, when they were exposed to negative stimuli. Instead, they primarily performed postures similar to EP1 and EP2 when given positive and frustrating stimuli (Reefmann et al., 2009c). Similarly, when they were socially isolated, a negative experience for sheep, they spent more time in the forward ear posture, similar to EP2 in our study, and were less likely to perform the backwards ear posture (comparable to EP3) (Reefmann et al., 2009a).

EP1 was the most performed posture in the absence of the influencing stimulus (woodchip or feed). Therefore, although EP1 is associated with the emotional state of excitement in this current study, it is important to note that the performance of this posture is not purely attributable to this emotional state. Instead, it is the increased duration of time the cow spends in this posture that is indicative of this emotional state. Similarly, EP2 is also performed when there is no stimulus, albeit for less time than EP1. It is therefore easier to spot a change in duration for this ear posture, and a significant increase in its performance may be attributable to a negative, high arousal emotional state, such as frustration. However, like EP1, it is not necessarily only performed in response to this emotional state, and so comparisons of durations and contextual information are necessary to interpret the reason for its performance. For example, is the cow spending longer in this ear posture than before, following the introduction of a new herd member or husbandry procedure?

4.5. Practical application

Using ear postures as a measure of emotional state in dairy cows has a number of advantages. They are less likely to be affected by the cows' activity levels than some physiological measures (e.g. cardiac measures), and they can be recorded remotely without having to touch or approach the animal. Whereas physiological measures, such as heart rate and heart rate variability, are highly influenced by such interventions, as well as by diurnal effects (Reefmann et al., 2009c). In addition, observers can be trained to measure ear postures, and it incurs no additional cost as no equipment is necessary. However, it is important to note that often the changes in durations can be subtle, as seen in our results, and cows vary from one another. Therefore, although the differences were significant, the effects can be moderate. Further research is required to develop an extensive bank of ear posture data which encompasses data from numerous contexts and breeds, and to create reliable averages and parameters which can be drawn upon for comparisons.

4.6. Limitations and future research

In future studies, it would be worth exploring the effects of an increased number of trials for the standard feed and concentrates feed treatments, to ensure that the cows were conditioned to the procedure. In addition, our study involved humans giving the signal and providing the feed. It would have been useful to have an automated device, as this would have eliminated the possible human effects on the cows.

Due to the nature of the videos, blind analysis of the videos was not always possible as the researcher could often identify the cow and the treatment from the content of the video. This is a limitation of the study, as there is potential for observer bias. Future research should seek to address this by selecting appropriate stimuli.

Future research may also benefit from measuring the postures of both ears, rather than focussing on just one, as done in the current study. Although we previously found no effects of lateralisation upon ear postures (Proctor and Carder, 2014), asymmetry in ear postures has been found in other species to be indicative of emotional state (e.g. goats; Briefer, 2012), and so future research should explore this further.

Testing the suitability of ear postures as a measure of emotions still needs further work with different stimuli in a range of environments, such as outside and during transportation. In this study, the cows were singly housed for the trials, whereas in our previous study we tested the measure on unrestrained, group housed cows. For the measure to be practical, further research should test this measure in a variety of contexts to test its reliability in a range of situations. In addition, in this study we have only explored the effects of one positive and one negative stimulus on the cows. Further research is required to explore whether the effects seen are specific responses to these stimuli, or whether they are reliable representations of the cow's emotional experience. Our research has built upon our previous findings to further explore the potential of ear postures to be used as a measure of emotional state in cows, but further research is required to test more stimuli, of varying degrees of arousal and valence.

5. Conclusion

This research has built upon our previous work on ear postures as a measure of emotional state in dairy cows. We have shown that the types of ear postures cows perform are indicative of both positive and negative emotional states. Because there were significant differences between the effects of the positive and negative high arousal emotional states, our results suggest that ear posture types are indeed sensitive to differences in valence, as well as to arousal. These results show that there is potential to use ear postures as a measure of emotional state in cows.

Understanding how animals communicate their emotional state will help farmers and welfare assessors improve animal welfare by promoting positive emotional states and reducing negative ones. With further research, the findings from this study, and our previous study (Proctor and Carder, 2014), have the potential to develop a valuable tool in cattle welfare assessments, and provide considerable insight into a practical measure of animal emotion.

Declarations of interest

None.

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