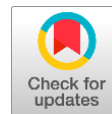


Do the ear and tail positions reflect the emotional state of calves?



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Abstract This study aimed to determine the associations between ear and tail position and stroking or umbrella stimuli in dairy calves at different stimulus application times (before the test, pretest, test, and posttest). Thirty-two mixedbred Holstein calves of different ages were subjected to stroking and an umbrella stimulus. For the stroking stimulation, a researcher stroked for 1 minute in the neck region with continuous and frequent movements (approximately 40 to 60 movements per minute) to simulate the speed with which an animal would receive allogrooming. For the umbrella stimulus, a researcher positioned himself one meter in front of the calf, with an umbrella in his hand, without making eye contact. Then, the umbrella was suddenly opened and remained open for one minute. The procedure was divided into six experimental attempts. The ear and tail positions were investigated. There was a significant effect of treatment on the ear and tail position variables. The "backward" ear posture and "arched" tail posture appear to be associated with the display of the stroking stimulus, while the "horizontal" ear and "between hind limbs" tail positions are associated with the umbrella stimulus. The data suggest that ear and tail positions are indicators of stroking or umbrella stimuli in dairy calves. There is great potential for using these methods to measure the emotional state of calves, and they can also be used as a tool to assess their welfare.

Keywords: body language, calf, emotion assessment

1. Introduction

Among other factors, animal behavior can be influenced by emotional state and individual experiences. Recently, there has been increased interest in investigating how changes and movements in different areas of the body can express the internal emotional state (Lambert et al., 2019), emphasizing the nonverbal language of the animals. The commitment to interpret emotional states through body indicators is justified by the great concern of the well-being and good treatment of animals, especially in important stages, such as the breeding of dairy calves, which will directly and indirectly influence the productive period.

In addition, there is a strong commitment of the private sector to developing software and technologies related to computer visualization through image analysis to evaluate animal body expression (Meunier et al., 2017). However, for these systems to operate properly, programs need to be well grounded in science. Therefore, to assess and promote good emotional states in animals, it is necessary to identify the ways in which emotions are expressed.

As emotions are accompanied by behavioral, physiological, and cognitive responses, it is possible to measure these responses and use them to interpret their

emotional state. Some studies in this area have sought to identify body postures (behavioral responses) in producing animals, such as sheep (Reefmann et al., 2009ab; Boissy et al., 2011; Briefer et al., 2015; Coulon et al., 2015), swine (Reimert et al., 2013) and cows (Proctor et al., 2014; de Oliveira et al., 2018). However, the interpretation of emotions displayed through body areas cannot be generalized among species and phases of life because there is great variation among them.

Ear posture and other body readings, such as tail position, are quite practical and less likely to be influenced by other factors, such as temperature, daytime variations, and physical activity (Boissy et al., 2011).

A better understanding of body posture variations in animals will have practical implications for assessing well-being in loco. This type of assessment is based on the human ability to interpret how an animal is feeling through visual variables.

The positive or negative effects of environmental stimuli on behavioral variables can be used to improve interactions between stockmen and animals. Humane handling and positive interactions between humans and dairy cows/heifers diminish the fear that animals feel like men (Kovács et al., 2015; Shahin 2018).

The use of stroking stimuli and the umbrella test have already been used in animal behavior studies to assess the emotional state of animals. However, for dairy calves, we know that this animal category does not exhibit changes in facial expression when subjected to stroking or umbrella stimuli (Machado et al., 2023). Based on these premises, we wondered whether dairy calves have different responses when subjected to stroking or umbrella stimuli. Is there standard behavioral body language related to tail and ear positioning in dairy calves? The hypothesis of this study is that the position of the ear and tail, different from the facial expression, is modified according to the stimuli of the caresses and umbrella. Due to the need to answer these questions and confirm our hypothesis, the objective of this study was to evaluate the responses of dairy calves to stroking or umbrella stimuli at different stimulus application times (before the test, pretest, test, and posttest) to the ear and tail positions of dairy calves.

2. Materials and Methods

2.1. Ethical note

The experiment was approved by the Animal Ethics Committee of the University of São Paulo (Protocol No. 2017.5.2380.11.6 and CEUA N^o. 2017-35).

2.2. Animals and management

The study was conducted from January to March of 2018 at the Animal Science Department of the Luiz de Queiroz College of Agriculture—ESALQ/USP, located in

Piracicaba-SP (554 meters altitude, 22°42'30" latitude, and 47°38'01" longitude). Thirty-two male and female crossbred Holstein calves (24 males and 8 females) aged 1 to 8 weeks (mean - 4 weeks of age) and clinically healthy were studied.

The calves were randomly grouped according to sex and age, considering the formation of homogeneous groups in terms of age and sex. Sixteen calves were subjected to an umbrella stimulus, and 16 calves were subjected to a stroking stimulus. Information on the birth of calves, health and nutritional management and facilities can be found in Machado et al. (2023).

2.3. Adaptation period

The animals were subjected to treatments for one week. Each animal was handled once a day, and at the same time, it was exposed to equipment to measure its behavior (two camcorders positioned on a tripod), test chamber, stopwatch, table, paper and clipboard. The calves were also familiarized with the presence of three people: researchers 1, 2 and 3. Each animal was subjected to six different phases (Table 1) of the test according to its treatment. All the experimental procedures took 18 minutes per animal.

2.4. Experimental procedure

The test chamber consisted of a shelter with dimensions of 1.40 m × 0.80 m × 1.25 m in an iron structure with open sides and a galvanized aluminum roof tile painted white on the upper side (Figure 1).

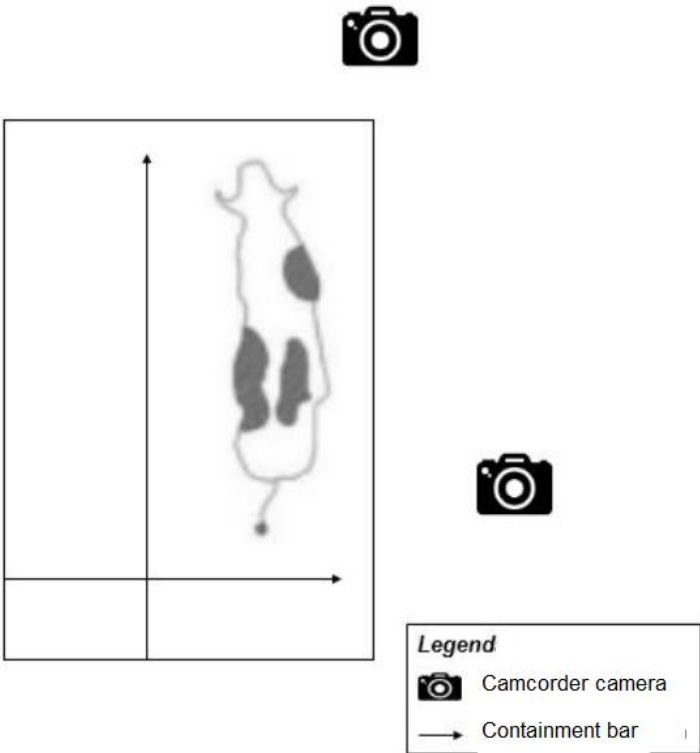


Figure 1 Schematic drawing of the test cameras used during the experiment

Table 1 Experimental evaluation procedures showing the phases, duration of each phase and the experimental activities/procedures in each phase.

Phase	Duration	Activity
1 – natural environment I	1 minute	Video recording of the animal in its natural environment (tropical shelter) before it goes to the test chamber.
2 – pre test	5 minutes	Video recording of the animal kept within the test chamber without receiving any intervention.
3 - test	1 minute	Researcher 1 approached the test chamber to apply the stroking or umbrella stimulus to the animal. This procedure was video recorded.
4 – post test I	5 minutes	Video recording of the animal kept within the test chamber without receiving any intervention.
5 – post test II	5 minutes	Video recording of the animal kept within the test chamber without receiving any intervention.
6 – natural environment II	1 minute	Video recording of the animal in its natural environment (tropical shelter) after exiting the test chamber.

After the animal entered the test chamber, two video cameras (Sony Cybershot, model Dsc-H300 20.1 MP) were placed 1.5 meters from the animal on a tripod to capture images of the ears and the tail. Then, the experimental phases were counted, and the recording of the animals started at the same time as the application of the treatments.

2.5. Stroking stimulus

Stroking was performed on the calf's neck by the researcher's hands and was always standardized on the animal's left side. This stimulus was chosen because this body region was identified as the preferred area of dairy cows in previous studies (Shahin, 2018; Schmied et al., 2008; Westerath et al., 2014). The stroke speed was approximately 40 to 60 strokes/minute to simulate the speed at which an animal would receive "allogrooming" (Schmied et al., 2008). Striking was performed on the neck because this body region was identified as the preferred area of dairy cows in previous studies to receive this kind of stimulus (Shahin, 2018; Schmied et al., 2008; Westerath et al., 2014). At the end of 1 minute, researcher 1 discontinued the stroking and stepped away from the animal's line of sight. During the stroking stimulus, no animals rejected the stroking or moved away from researcher 1.

2.6. Umbrella stimulus

The umbrella test (negative stimulus) was performed according to the protocol proposed by Sandem et al. (2004), where at 4:30 minutes of the pretest phase, researcher 1 held a black umbrella 80 cm in diameter and positioned himself 1 meter in front of the animal. The umbrella, still closed, was pointed at the calf at eye level. At the exact time of the start of the test phase, the umbrella was quickly opened and kept open without movement for 1 minute. At that time, the animal had the freedom to move away from the umbrella by up to 1.5 meters or approach the umbrella by 0.8 meters. At the end of the test phase, the umbrella was slowly closed, and researcher 1 stepped away from the animal's line of sight.

2.7. Behavioral indicators

To evaluate whether the body expression of calves is influenced by the treatments, behavioral information about

the position of the ear and tail was collected. An experimental ethogram with the behavioral variables "ear position" and "tail position" evaluated is presented in Figure 2.

The ethogram for ear position was adapted from Proctor and Carder (2014), who worked with cows. An adapted ethogram was constructed for calves from preliminary observations, and in this case, it was adopted for this study. The ethogram for tail positions was constructed from preliminary observations of calves in daily activities, such as feeding, weighing, veterinary care and other handling activities.

The ear positions were analyzed by means of visual observation from the videos produced during all the experimental phases, by the focal animal sampling method with continuous recording, during the 18 minutes of testing for each animal. Three trained surgeons analyzed the videos with interobserver reliability > 95%. The data were tabulated in a specific worksheet and transformed into observation ratios for each PO (ear position) category.

The tail position (PC) analysis was also performed through the visual observation of the videos of the animals produced during the experiment by the focal animal sampling method with continuous recording. The data were tabulated and transformed into observation ratios for each PC category.

2.8. Data analysis

Statistical analysis was performed in the free software R, version 3.5.2 (R Core Team, 2018). For the ear and tail position variables, Pearson's chi-square test was performed to test whether the proportions in several groups were the same. For post hoc comparisons, we used a t test with Bonferroni correction.

3. Results

3.1. Ear position

The position of the ears varied according to the test phases, mainly in response to the caress stimulus (Table 2). For example, for the caress stimulus, PO1 and PO2 had lower frequencies ($P < 0.05$) at the time of the test. However, PO3 showed higher frequencies at the time of the test and lower frequencies at other times of the test (natural environments 1 and 2). PO4 did not differ ($P > 0.05$) between

the experimental phases. In the umbrella test, all ear positions were similar ($P>0.05$) between the experimental

phases, except for PO4, which was lower ($P<0.05$) in the pretest, test and posttest phases.

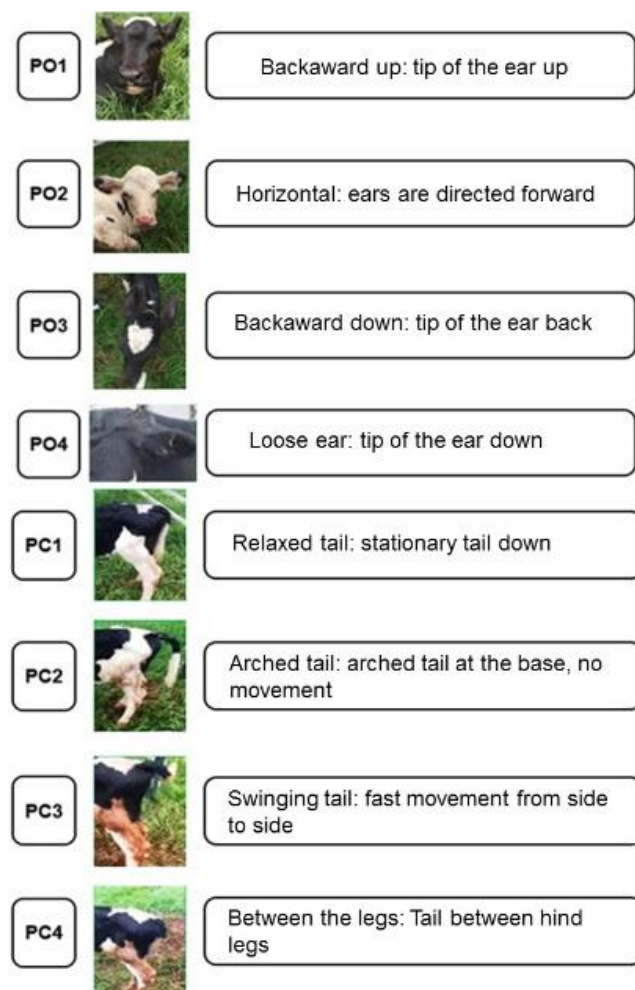


Figure 2 Experimental ethograms constructed specifically for the ear (top) and tail (below) positions of dairy calves. PO = ear position; PC = tail position.

Table 2 Ear position observations (time) according to applied treatment and between experimental phases (n=32 calves).

Ear position	Treatment	Phase					
		Natural environment 1	Pretest	Test	Posttest 1	Posttest 2	Natural environment 2
PO1 – Backward up	Stroking	0.12 ^b	0.62 ^a	0.25 ^b	0.44 ^{ab}	0.44 ^{ab}	0.31 ^{ab}
PO2 - Horizontal		0.688 ^a	0.375 ^b _c	0.062 ^{bc}	0.438 ^a	0.500 ^a	0.500 ^a
PO3 – Backward down		0.062 ^a	-	0.438 ^b	-	-	0.188 ^a
PO4 – Loose ear		0.125 ^a	-	0.250 ^a	0.125 ^a	0.062 ^a	-
PO1 – Backward up	Umbrella	-	0.38 ^a	0.19 ^a	0.44 ^a	0.50 ^a	0.25 ^a
PO2 - Horizontal		0.38 ^a	0.50 ^a	0.75 ^a	0.50 ^a	0.31 ^a	0.44 ^a
PO3 – Backward down		0.188 ^a	0.062 ^a	-	-	-	0.062 ^a
PO4 – Loose ear		0.438 ^a	0.062 ^c	0.062 ^c	0.062 ^c	0.188 ^b	0.250 ^a

^{ac}Frequencies within a row with different superscripts differ ($P < 0.05$) according to Pearson's Chi-Square analysis, with the P-value adjusted by the Bonferroni method.

3.2. Tail position

The tail position varied ($P<0.05$) according to the experimental phase (Table 3). With respect to the caressing stimulus, the relaxed ear was smaller ($P<0.05$) at the time of

the test and pretest than at the time of natural environment I and II and posttest I and II. The opposite was observed for the arched ear ($P<0.05$) at the time of the test compared to the other experimental phases. The tail between the legs

did not vary ($P>0.05$) according to the experimental phases of the caising stimulus.

For the umbrella stimulus, the relaxed tail was also smaller ($P<0.05$) at the test and pretest time points. Tail

arching and swinging behavior was not observed in response to the umbrella stimulus. Finally, the distance between the tails of the legs was greater ($P<0.05$) at the time of the umbrella test.

Table 3 Tail position observations (time) according to the applied treatment and between experimental times.

Tail position	Treatment	Time					
		Natural environment 1	Pretest	Test	Posttest 1	Posttest 2	Natural environment 2
PC1 - Relaxed	Stroking	0.94a	0.62b	0.44c	0.94a	0.94a	0.88a
PC2 – Arched tail		0.062c	0.250b	0.562a	0.062c	-	0.062c
PC3 – Swinging tail		-	-	-	-	-	-
PC4 – Between legs		-	0.125a	-	-	0.062a	0.062a
PC1 - Relaxed	Umbrella	0.88a	0.88a	0.50c	0.81ab	0.81ab	1.0a
PC2 – Arched tail		-	-	-	-	-	-
PC3 – Swinging tail		-	-	-	-	-	-
PC4 – Between legs		0.12b	0.12b	0.50a	0.19b	0.19b	-

^{ac}Frequencies within a row with different superscripts differ ($P < 0.05$) according to Pearson's Chi-Square analysis, with the P-value adjusted by the Bonferroni method.

4. Discussion

This is the first study carried out to evaluate the position of the tail and ear of dairy calves subjected to different stimuli. Our main findings are that the horizontal position of the ear occurred mainly during the test phase when the animals received the umbrella stimulus. This position seems to indicate animals that are frightened and/or in a state of consciousness and attention, which may be associated with low activity in negative valence emotional states. However, the loose position of the ear does not seem to be related to negative emotional states. For tail behavior, the arched position is more often observed during positive stimuli and seems to be an indicator of positive emotions. On the other hand, the observation of the position between legs decreases in positive situations and increases in negative situations. The relaxed position of the tail seems to be more constant, regardless of the stimulus that the animal receives; therefore, it does not seem to present a defined emotional state.

4.1. Ear position

The treatments had significant effects on the ear position. In the groups of calves that received the stroking stimuli, there was a clear change in the position of the ear between the evaluation phases, and the backward down ear posture (PO3) was recorded in a greater proportion of these groups. This increase in the proportion of participants in the stroking treatment group indicates that this category of ear posture is related to the manifestation of positive emotions. When the animals were stroked, they exhibited an emotional state of tranquility, which was demonstrated through the posture of the backward down ears.

The results presented in this study corroborate the work performed with dairy cows by Proctor and Carder et al. (2014), who observed that the backward down ear position (PO3) and loose ear position (PO4) are maintained during stroking events. These authors further state that these ear

positions are related to positive low-arousal emotional states.

Similar to the findings of Proctor and Carder et al. (2014), we also did not find a significant difference in loose ear posture (PO4) during the evaluation tests, except between the pretest, test and posttest 1 in the umbrella treatment, in which this classification of ear posture was observed in a smaller proportion.

According to our data, it was clear that the backward up (PO1) and horizontal ear (PO2) positions are quite frequent in situations without stimuli, such as in the natural environment before the test, in the pretest, in the posttest and in the natural environment after the test.

In groups of calves that received the umbrella treatment, a different pattern of ear position was observed. During the test, there was a greater proportion of individuals with a horizontal ear posture (PO2).

Backward up ear (PO1) and horizontal ear (PO2) positions appear to be associated with two types of situations: situations of environmental exploration (not indicating a good or bad emotional state) and frightened animals and/or aware and attentive ones. According to Lambert and Carder (2019), the increase in PO2 observed may be attributed to an umbrella stimulus.

The results show an increase in the proportion of individuals with a horizontal ear posture (PO2) in negative situations, which corroborates the findings of Proctor and Carder (2014) and Lambert and Carder (2019).

There was a reduction in the ratio of backward down (PO3) and loose ear (PO4) positions in umbrella situations, which reinforces the relation of these postures to positive emotions.

The position that most seems to indicate a negative state is the horizontal one (PO2), which is more often recorded. However, according to Lambert and Carder (2014), this position may also be related to a situation of agitation or exploration of a new element. In this research, the umbrella may have represented a new object.

According to Reefmann et al. (2009b), it is possible to use the proportion of horizontal ear (PO2) observations to compare the valence of emotional situations so that a higher proportion of PO2 indicates a more negative emotional state. Bellegarde et al. (2015) reported that sheep presented a greater proportion of horizontal ear (PO2) when they visualized an image of another sheep under umbrella conditions. Moreover, these authors found that a greater percentage of time in the horizontal ear (PO2) is spent by ewes in situations of negative valence, such as social isolation, or in situations in which a high level of attention is needed, such as exposure to an unfamiliar environment.

This information corroborates the data from this study, which relates the horizontal ear posture (PO2) to the expression of negative valence emotions. Oliveira and Keeling (2018) related the horizontal ear (PO2) to negative situations since their frequency of observation decreased when adult cows were stroked and when they were being fed (positive situations).

4.2. Tail position

The relaxed tail position (PC1) was the most frequently observed position in almost all the phases in this study. Oliveira and Keeling (2010) also reported that this position is more frequent in adult cows.

A large proportion of the arched tail (PC2) was also observed in the groups subjected to stroking stimuli during practice. According to our results, PC2 differed significantly in the test phase when the animals were stroked, which seems to be related to the display of positive emotions. Arched tails are observed in calves at pleasurable and known positive moments, such as feeding time (2004).

According to Oliveira and Keeling (2010), regarding the tail position, there is a gap in our knowledge and still little information on the expression of emotions in cattle. However, the authors state based on their results, that most tail movements in cattle may be associated with positive emotions. Other studies with goats (2015), swine (2013), and dogs (2007) have shown that a greater tail positioning repertoire is observed in positive situations. In this study, greater variation in tail position was also observed in the groups that received the stroking treatment.

In the group of animals that received the stroking stimulus, the between legs tail position (PC4) did not differ significantly between the evaluating phases and was observed in a low proportion. However, in the groups that received the umbrella treatment, this tail position differed significantly only during the test phase, in which the stimulus was applied. At that time, the proportion of PC4 observed was greater.

In dog studies (Stellato et al., 2020; Flint et al., 2018), a tail between the hind limbs was associated with negative emotions, such as the approach of a stranger. These studies indicate that this tail position is indicative of negative valence emotions in dogs.

The results of this research suggest that the tail between hind legs (PC4) is also associated with negative valence emotions in calves, as this position was observed in the negative treatment groups and differed significantly during the application of the umbrella stimulus.

5. Practical Application and Future Research

Using the ear and tail position of dairy calves as a measure of emotional state has potential for use on farms, unlike the facial expression of calves reported by Machado et al. (2023). In addition, these behaviors can be recorded remotely without the need for physical contact or getting close to the animal, unlike physiological variables, which are mainly measured by invasive methods. In addition, human interference can affect the measurement of these parameters. Another advantage of using the evaluation of the position of the ears and tails in the evaluation of animal welfare is that it does not generate any cost to the producer. However, further studies are encouraged to develop big data for the use of artificial intelligence tools, such as deep learning, aiming to apply algorithms to identify the position of the tail and ears as accurate measures to evaluate the welfare of dairy calves.

6. Conclusions

Behavioral indicators suggest that calves present differentiated responses when subjected to stroking and umbrella stimuli. In relation to the ear and tail position, it can be concluded that the "backward down" ear position and the "arched" tail position are associated with the expression of stroking emotions. The "horizontal" ear position and the "between legs" tail position are related to umbrella emotions. There is great potential for using these methods to measure the emotional state of calves, and they can also be used as tools for assessing their welfare condition, in addition to generating and calibrating technological resources for this purpose.

Ethical considerations

The experiment was approved by the Animal Ethics Committee of the University of São Paulo (Protocol N°. 2017.5.2380.11.6 and CEUA N°. 2017-35).

Conflict of interest

The authors declare no conflicts of interest.

Funding

The authors declare no conflicts of interest.

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