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Can the emotional state of calves be noticed by their facial expression and heart rate?

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A R T I C L E I N F O

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A B S T R A C T

The aim of this study was to identify whether or not dairy calves change their facial expression and heart rate according to their emotional state when subjected to a stroking or umbrella stimulus. Thirty-two mixed-breed Holstein calves of different ages were submitted to stroking and umbrella stimulus. For the stroking stimulation, a researcher performed stroking for 1 min in the neck region with continuous and frequent movements (about 40–60 movements per minute) to simulate the speed with which an animal would receive allogrooming. As for 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 eyebrow position, mouth, eye white area, nostril area and heart rate were investigated. For the continuous variables, eye white area, nostril area and heart rate we used the linear mixed effects. For the binary variables, mouth (open or closed) and eyebrow (raised or normal). We used the generalized linear mixed model with logit link function. The variables eye white area, nostril area, mouth and eyebrow did not present significant effects ($P > 0.05$) as a result of the stimulus. However, the heart rate showed a significant difference ($F_{(1150)} = 15.264$, $P < 0.001$) according to the stimulus the animals received. Dairy calves do not express facial changes in their emotional state when subjected to stroking or umbrella stimuli, but there is a reduction in heart rate relates to the valence of a stimulus.

1. Introduction

Throughout evolution, facial expression has been was a widely used tool to display emotions as communicative means among animals and contributed to the evolution and adaptation of species (Hess and Thibault, 2009), considering the facial expression as a form of communication.

The perception of face-based emotion is a cognitive process which human beings are commonly able to recognize. Most researches in the field of facial expression in animals are focused on the expression of pain. For example, Borja (2008) evaluated the importance of this behavioral parameter in the clinical evaluation of pain in horses; Bloom and Friedman (2013) studied the ability of humans to identify facial expressions in dogs (*Canis familiaris*) through photographs taken under specific conditions, capable of stimulating defined emotions; Bellegarde et al. (2017) investigated the ability of goats to identify the expression of positive or negative emotions using images, and concluded that the animals showed different reactions to the images, besides being more attentive to the negative images.

Few studies have been conducted in cattle with the objective of evaluating the expression of good and bad emotions through facial expression as indicators of their welfare, with a few exceptions (Sandem et al., 2004). For example, Sandem et al. (2002) concluded that the calculated percentage of visible white in the eyes was greater than the baseline level in frustrated cows, while it was lower than the baseline level in cows receiving rewarding stimuli. Exposure of the white area of the eyeball (eye white) can be particularly useful for visualizing and identifying emotions in several species. Initially, the ability to extract emotional information through facial expression was attributed only to species with sophisticated orofacial motor system, such as primates and

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humans. However, studies with sheep (Tate et al., 2006, 2017) and cattle (Lambert and Carder 2017) displayed excellent ability for these animals to respond to emotions through facial changes.

This is because the proportion of visible white of the eye is controlled by the sympathetically controlled Musculus tarsalis, which lifts the eyelid. Some studies demonstrate a positive correlation between heart rate and relative eye opening and a negative correlation between heart rate variability and eye opening (Reefmann et al., 2009). Thus, a reduction in the visible white of the eye is presumed to be triggered by sympathetic deactivation or parasympathetic activation (Goméz et al., 2018). The visible white of the eye may therefore allow for reliable non-invasive assessment of stress responses in cattle.

Facial information and heart rate on pet and production animals have been used to measure their well-being, including the development of artificial intelligence (AI) software for image analysis and general behavior observation, as well as technologies capable of detecting patterns of feed intake and general health (Norton and Berckmans, 2017; Van Hertem et al., 2017). However, there are no published studies with dairy calves for this purpose.

This type of information is important to understand the emotion and behavior of dairy calves by non-invasive techniques. In addition, these studies are important to calibrate systems and software which are capable of analyzing, by artificial intelligence mechanisms, the general welfare state of the animals - which is a growing technological tendency. Therefore, the aim of this study was to identify whether or not dairy calves express facial and heart rate changes when subjected to a stroking or umbrella stimulus. It was hypothesized that (i) emotions are expressed through facial expression in calves when subjected to stroking or umbrella stimulus and (ii) heart rate relates to the valence of a stimulus.

2. Material and methods

2.1. Ethical note

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).

2.1.1. Animals and management

The research was conducted from January to March of 2018 in Piracicaba, São Paulo, Brazil (554 m altitude, 22º42’30″ latitude, and 47º38’01″ longitude). Thirty-two male and female crossbred Holstein calves, aged 1–8 weeks and clinically healthy were studied.

For the inclusion of animals in the study, calves had to be born from assisted births and with no evidence of dystocia. All animals included in the study had high vigor and no night-born calves were included. The animals were weighed and the navel was disinfected with 7% iodine.

The calves were randomly grouped according to sex and age, considering the formation of homogeneous groups in age and sex. Finally, 16 calves were submitted to umbrella stimulus and 16 calves to stroking stimulus. All the animals were kept in individual tropical shelters (Fig. 1), measuring 1.00 × 0.80 × 1.25 m, where water and concentrated feed were available 24 h a day.

After the colostrum feeding period, the calves were fed in a bucket with 6 L/day of milk replacer (Sprayfo Azul, Nutreco Brasil Nutrição Animal LTDA, SP, Brazil) diluted to 14% solids, divided into two meals (7:00 and 17:00). The initial concentrate (Agroceres Multimix, Rio Claro, SP, Brazil) was provided daily in the morning for ad libitum consumption. The calves were milk fed for eight weeks. Chemical-bromatological information on the concentrate and replacer is provided in Table 1.

2.1.2. Adaptation period

The adaptation period consisted of a period of habituation due to the change of environment with the introduction of equipment, collection of biological data, researchers and stimuli (stroking and umbrella). The animals were randomly submitted to tests for one week. Each animal was handled once a day, and at the same time, it was exposed to equipment to measure their behavior (two cameras positioned on tripods) and heart rate (stethoscope and the person who did the measurement), the test chamber, stopwatch, a table, paper and clipboard. The calves were also habituated to the presence of three duly trained researchers with skills in handling dairy calves. Each animal was analyzed in six different phases, as shown in Table 1. The inclusion of different phases is justified by the need for the evaluation of the animal in different experimental situations; specifically, we want to know if there would be changes or not in facial expression and heart rate between these phases.

2.1.3. Stroking stimulus

To apply the body stroking stimulus, researcher 1 approached the animal and stood by its side. The stroking was performed in the region

Table 1
Chemical-bromatological composition of the concentrate and substitute.

	Concentrate	Replacer[a]
Dry matter %	89.27	96.06
Mineral matter % DM	9.66	8.80
Crude protein DM	24.67	22.46
Ethereal extract % DM	5.22	16.20
Neutral detergent fiber. % DM	13.89	0.06
Acird detergent fiber. % DM	5.52	-
Non-fibrous carbohydrates.% DM	46.57	52.20

[a] Diluted replacer for 14% solids

Fig. 1. Tropical shelters: individual (A) and overview (B).

comprising the neck (thoracic insertion to the atlas / axial region and atrial insertion), always on the left side of the animal, by the hands of the researcher 1, with continuous and frequent movements (about 40–60 movements per minute) to simulate the speed at which an animal would receive "allogrooming" (Schmied et al., 2008). The stroking 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 (Schmied et al., 2008; Westerath et al., 2014; Shahin, 2018). At the end of 1 min, 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.

2.1.4. Umbrella stimulus

In order to apply this treatment, researcher 1 positioned himself 1 m away ahead from the calf, with an umbrella in hand, without making eye contact. Then the umbrella was suddenly opened and so it remained open for 1 min.

2.1.5. Experimental procedure

The animals were tested for four consecutive days, two days after the end of the adaptation period. No calves reacted negatively to the procedures when they received the stroking stimulus. Each animal was evaluated twice a day and individually, in the test chamber. The experimental procedure consisted of the same 6 phases (Table 2). Altogether, the experimental procedure took 18 min per animal.

The test chamber consisted of a shelter measuring 1.40 m x 0.80 m x 1.25 m, an iron structure with open sides and tile in galvanized aluminum sheet painted white on top (Fig. 2). All animals were monitored by video camera during the procedures. A Sony Cybershot digital camera, model Dsc-H300 20.1MP, positioned by researcher 1 at the best angle to view the face of the animal, 1.5 m away, using a tripod.

Body stroking was performed as described above. The umbrella test was performed according to the protocol proposed by Sandem et. al. (2004) in which, at 4:30 min of the phase 2 – pre-test, researcher 1 holding a black umbrella with 80 cm of diameter, positioned himself 1 m away ahead of the animal. The umbrella, still closed, was pointing towards the calf in the eye direction. At the very beginning of phase 3 - test (after 30 s) the umbrella was opened quickly and kept open for 1 min. At that time, the animal had the possibility to move away from the umbrella - up to 1.5 m. At the end of phase 3 - test, the umbrella was closed slowly and researcher 1 stepped away from the animal's line of sight.

The heart rate was measured by researcher 1 through auscultation between the third and fifth intercostal space, in the left ventrolateral position, using a stethoscope, as the methodology proposed by Frondelius et al. (2015). Heart rate measurement was performed at all experimental phases, with the exception of phase 3 - test, because it was not possible to interrupt the stimulus to measure this variable.

Table 2
Experimental evaluation procedures showing the phases, duration of each phase and the experimental activities/procedures in each phase.

Phase	Experimental evaluation	
	Duration	Activity
1 – home pen I	1 min	Video recording of the animal in its home penn (tropical shelter) before it goes to the test chamberr. Heart rate was measured.
2 – pre test	5 min	Video recording of the animal kept within the test chamber without receiving any intervention. Heart rate was measured.
3 - test	1 min	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 min	Video recording of the animal kept within the test chamber without receiving any intervention. Heart rate was measured.
5 – post test II	5 min	Video recording of the animal kept within the test chamber without receiving any intervention. Heart rate was measured.
6 – home pen II	1 min	Video recording of the animal in its home pen (tropical shelter) after exiting the test chamber. Heart rate was measured.

2.1.6. Behavioral indicators

To evaluate the facial expression of calves, behavioral information was based on facial action units (AU) (Müller et. al, 2015). The images captured by the digital camera were evaluated at all phases. Images of the eyebrow, mouth, eye white and nostril were analyzed. The experimental ethogram with the facial variables eyebrow and mouth analyzed are presented in Table 3. These variables were analyzed visually by the focal animal method (Bosholn and Anciães, 2018) with momentary recording.

The variables eye white and nostril were analyzed according to the area. To calculate the areas of the eye white and the nostril, the images were analyzed by ImageJ software (Fig. 3), developed by Wayne Rasband, National Institute of Health (1997).

2.1.7. Statistical analyses

For the emotional state of calves experiment, statistical analyses were performed using R software, version 3.5.2 (R Core Team, 2018). For the quantitative variables area of the eye white, area of the nostril and heart rate we used the linear mixed model. An analysis of variance (ANOVA) was performed and, subsequently, a Tukey's test was performed to make paired comparisons of means when significant. For the binary variables mouth (open or closed) and eyebrow (raised or normal) we used the generalized linear mixed model with binomial distribution and logit link function.

For the area of the eye white and nostril variables we used the linear mixed model considering: phases (six phases), stimulus (stroking and umbrella stimulus) and interaction between phases and stimulus as fixed effects. Animal was included as random effects.

For the heart rate variable, we used the linear mixed model considering: phases (five phases), stimulus (stroking and umbrella stimulus) and interaction between phases and stimulus as fixed effects. Animal was included as random effects.

For the dichotomous variable mouth (open or closed) and eyebrow (normal or raised) we used the generalized linear mixed model for binary data with logistic link function considering: phases (six phases), stimulus (stroking and umbrella stimulus) and interaction between phase and stimulus as fixed effects. Animal was included as random effects. The term Z, in our results, represents the value of a statistic which has a standard normal distribution.

3. Results

There was no significant interaction effect between phases and stimulus ($F_{(5,\ 180)} = 0.794$, $P = 0.554$) and for the stimulus ($F_{(1180)} = 0.033$, $P = 0.854$) for the eye white variable. A phase effect ($F_{(5.180)} = 17.630$, $P < 0.001$; Table 4) is observed.

There was no significant interaction effect between phases and stimulus ($F_{(5,\ 180)} = 0.108$, $P = 0.990$) for the nostril area variable. However, there was a significant main effect for phases ($F_{(5180)} = 7.237$, $P < 0.001$). Furthermore, there was no significant main effect for stimulus ($F_{(1180)} = 0.366$ $P = 0.545$; Table 5).

For the variables mouth and eyebrow, there was no significant interaction effect between phases and stimulus ($Z = -1.376$, $P = 0.168$; $Z = -\ 1.355$, $P = 0.175$), phases ($Z = 1.404$, $P = 0.160$; $Z = 0.119$, $P = 0.905$) and stimulus ($Z = 0.620$, $P = 0.535$; $Z = -0.827$, $P = 0.408$), respectively (Data not shown in Table).

There was no significant interaction effect between phases and stimuli ($F_{(4,\ 150)} = 0.987$, $P = 0.416$) and effect for phases ($F_{(4150)} = 0.375$, $P = 0.825$). However, the animals which received the stroking stimulus had lower heart rate than the animals which received umbrella

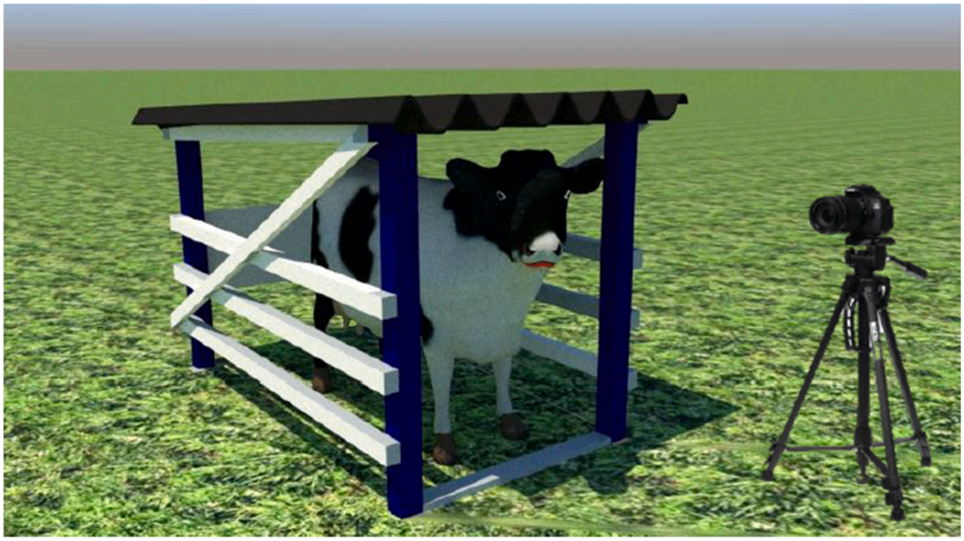

Fig. 2. Schematic drawing of the test camera used and positioning of the video cameras.

Table 3
Experimental ethogram containing the eyebrow and mouth facial action units analyzed by visual observation.

Facial action units	Categories	Description
Eyebrow	Normal	No muscle tension above the eyes
	Raised	Muscle tension above the eyes
Mouth		
	Closed	Upper and lower lips together
	Open	Upper and lower lips separated

stimulus ($F_{(1150)} = 15.264$, $P < 0.001$; Table 6).

4. Discussion

The variables studied did not confirm the hypothesis that emotions are externalized through facial expression in calves. Our objective was to identify whether or not the emotions caused by external stimuli (stroking or umbrella) could be externalized and identified through facial movements. The results showed that the facial action units studied were not good indicators of emotional states in calves, considering facial expression, since they did not present significantly different responses to the applied stimuli (body stroking or umbrella test).

It seems likely that recognition of face-based emotions may be widespread among mammals. But we found no significant difference between the responses of the eye white area and the type of stimulus applied to the animals. Agreeing with the study by Gómez et al. (2018), who reported that no differences were detected in the visible white of the eye, although a higher cortisol concentration was found during claw clipping in dairy cows exposed in a positive feeding situation and in a negative clipping stress situation. We recommend carrying out more studies with newborn calves because, as far as we know, this is the first study carried out with calves which evaluated the facial expression in animals submitted to different stimuli.

A significant difference was observed during the phases of observation, a reduction on the area of the eye white in the pre-test, test and posttest I and II was observed. Sandem et. al. (2004) found an increase in the white area exposed in the eye during the application of umbrella stimuli in adult cows. However, the same authors found the same result when the animals received a positive stimulus by obtaining concentrated feed (Sandem et al., 2006), which does not corroborate the results obtained in this study, as the two types of stimulus (positive and negative) caused a reduction in the white area exposed in the eye. This reduction in the umbrella test is justified because opening an umbrella

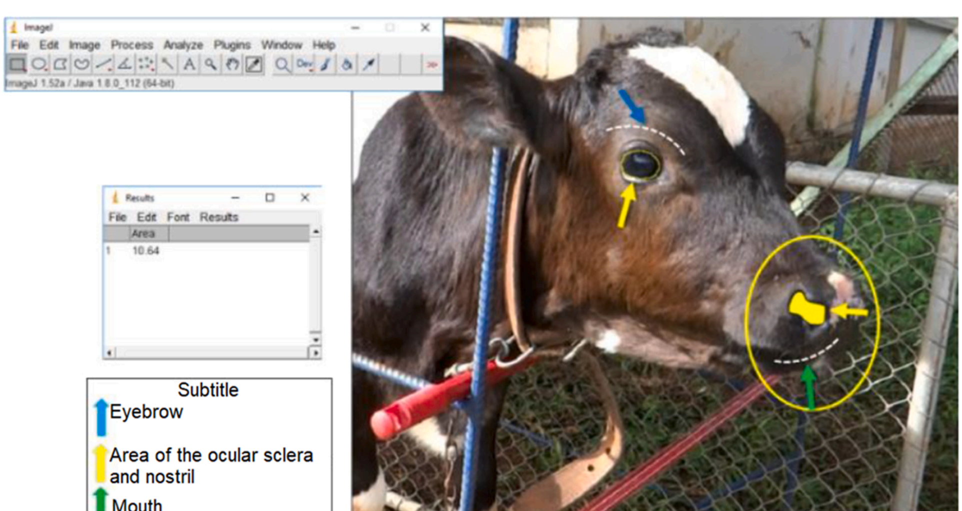

Fig. 3. Analysis of facial expression units.

Table 4
Pairwise comparisons among phases using the Tukey's test for the area of the ocular sclera.

Phases	Averages
1 – home pen I	3.189[a]
6 – home pen II	2.839[a]
3 - test	1.228[b]
4 – post test I	1.224[b]
2 – pre test	1.188[b]
5 – post test II	0.940[b]

[ab] lowercase letters in the same column differ by Tukey's test ($P < 0.05$)

Table 5
Pairwise comparisons among phases using the Tukey's test for the nostril area.

Phases	Averages
1 – Home pen I	8.478[a]
6 – Home pen II	7.288[ab]
2 – Pre test	5.717[bc]
4 – Post test I	5.598[bc]
3 - Test	5.247[bc]
5 – Post test II	5.052[c]

[ab] Lowercase letters in the same column differ by Tukey's test ($P < 0.05$)

Table 6
Comparison between stimulus means using the Tukey's test for heart rate.

Stimulus	Averages
Umbrella stimuli	125.125[a]
Stroking stimuli	111.887[b]

[ab] Lowercase letters in the same column differ by Tukey's test ($P < 0.05$).

close to the calf's eyes leads to eye closure as the corneal reflex overcomes widening. In addition, to the sound of opening the umbrella that caused an increase in the cornea of the eyes, reducing the eye white area.

The dilation or contraction of the existing muscles in the region of the nostril causes variation in its opening area. This variation has been studied as a unit of facial action associated to pain in several species. According to Muller et al. (2015) and Gleerup et al. (2015) nostrils dilation is associated with acute pain in beef and dairy cattle.

This facial action unit has been studied in other species and proven as an indicator of pain (Langford et al., 2010; Keating et al., 2012; Dalla Costa et al., 2014). We observed that the area of the nostril narrowed when the animals received the body stroking and umbrella test, and it was not possible to identify any differences among the stimuli received by the animal.

According to the results found by McLennan et. al. (2016), the area of the nostril may be associated with the expression of emotions. However, it was not possible to differentiate good or bad emotions through this element of facial action, because there was no significant difference in the response of the animals in both stimulus.

In this study, up to the time those animals were evaluated in the test chamber to identify possible treatment effects (post stimulus II), it was not possible to detect a significative variation in the eye white and nostril areas as a function of the stimulus.

Also, it was not possible to identify a significant effect of the stimulus on the mouth. In other studies, authors have observed a connection between this variable and the facial expression of pain in cattle, sheep and seals (*Phoca vitulina*) (Tate et al., 2006; Müller, 2015; Barros et al., 2017; Amelia et al., 2018). However, this variable does not appear to be indicative of calves' emotional expression, according to the results of this experiment. Therefore, it is recommended that the facial action unit mouth should not be used for evaluating the expression of emotions in calves.

Gleerup et. al. (2015) developed a scale of pain identification in cattle through facial expressions. This scale includes the eyebrow as a variable indicator, since the same authors detected a significant effect of a painful stimulus on the muscles tension responsible for raising the eyebrows. In this study, we did not identify a significant effect of the treatments on the eyebrow.

It was observed that the animals in the stroking treatment had a reduction of the heart rate when receiving the body stroking stimulus, and the animals of the umbrella treatment presented an increase of this variable when they received the shock caused by the umbrella test. This decrease in HR has also been observed in other studies on human grooming of farm animals (e.g. in horses (Feh et al., 1993) and cattle (Schmied et al., 2008) and used as an indicator of positive emotion. A limitation of heart rate measurement is human presence may have altered heart rate measurements, i.e, calves may react to human presence itself and this reaction may depend on the most recent exposure (stroking versus umbrella)

Dairy calves do not appear to have a wide variety of facial expressions; however, the results of this research indicate that the eye white area and the nostril area are influenced by emotions provoked by external stimuli, but it was not possible to identify whether these changes were caused by emotions provoked by stroking or umbrella stimuli.

5. Conclusion

Dairy calves do not express facial changes in their emotional state when subjected to stroking or umbrella stimuli, but there is a difference in heart rate and this can be considered a biomarker of valence of a stimulus.

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Declarations

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Code availability

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

References

Amelia, A., Macrae, M., Makowska, I.J., Macrae, A.M., Makowska, I.J., Fraser, D., 2018. Initial evaluation of facial expressions and behaviours of harbour seal pups (Phoca vitulina) in response to tagging and microchipping. Appl. Anim. Behav. Sci. https://doi.org/S0168159118302235.

Barros, J., Maria, R., Pires, L., Ambrósio, L.A., Oliveira, A., De, Trevisan, G., 2017. Facial Expr., vocalization Abnorm. Postures Cattle Submitt. Conv. eletroejaculation Expr. Facial, vocalización Y. Post. 1085–1097 https://doi.org/H T T P: / /DX. DOI. ORG/ 1 0. 2 2 2 5 6 / PUBV E T. V1 1N1 1. 1085 - 1 0 97.

Bellegarde, L.G.A., Haskell, M.J., Duvaux-ponter, C., Weiss, A., Boissy, A., Erhard, H.W., 2017. Face-based perception of emotions in dairy goats. Appl. Anim. Behav. Sci. 193, 51–59. https://doi.org/10.1016/j.applanim.2017.03.014.

Bloom, T., Friedman, H., 2013. Classifying dogs' (Canis familiaris) facial expressions from photographs. Behav. Process. 96, 1–10. https://doi.org/10.1016/j.beproc.2013.02.010.

Bosholn, M., Anciães, M., 2018. Focal animal sampling. In: Vonk, J., Shackelford, T. (Eds.), Encyclopedia of Animal Cognition and Behavior. Springer, Cham. https://doi.org/10.1007/978-3-319-47829-6_262-1.

Briefer, E.F., Tettamanti, F., Mcelligott, A.G., 2015. Emotions in goats: mapping physiological, behavioural and vocal pro fi les. Anim. Behav. 99, 131–143. https://doi.org/10.1016/j.anbehav.2014.11.002.

Dalla Costa, E., Minero, M., Lebelt, D., Stucke, D., Canali, E., Leach, M.C., 2014. Development of the Horse Grimace Scale (HGS) as a pain assessment tool in horses undergoing routine castration. PLoS One 9, 1–10. https://doi.org/10.1371/journal.pone.0092281.

Feh, C., De Mazières, J., 1993. Grooming at a preferred site reduces heart rate in horses. Anim. Behav. 46, 1191–1194.

Frondelius, L., Järvenranta, K., Koponen, T., Mononen, J., 2015. The effects of body posture and temperament on heart rate variability in dairy cows. Physiol. Behav. 139, 437–441. https://doi.org/10.1016/j.physbeh.2014.12.002.

Gleerup, K.B., Andersen, P.H., Munksgaard, L., Forkman, B., 2015. Pain evaluation in dairy cattle. Appl. Anim. Behav. Sci. 171, 25–32. https://doi.org/10.1016/j.applanim.2015.08.023.

Hess, U., Thibault, P., 2009. Darwin and emotion expression. Am. Psychol. 64, 120–128. https://doi.org/10.1037/a0013386.

Keating, S.C.J., Thomas, A.A., Flecknell, P.A., Leach, M.C., 2012. Evaluation of EMLA cream for preventing pain during tattooing of rabbits: changes in physiological, behavioural and facial expression responses. PLoS One 7, 1–11. https://doi.org/10.1371/journal.pone.0044437.

Langford, D.J., Bailey, A.L., Chanda, M.L., Clarke, S.E., Drummond, T.E., Echols, S., Glick, S., Ingrao, J., Klassen-Ross, T., Lacroix-Fralish, M.L., Matsumiya, L., Sorge, R.E., Sotocinal, S.G., Tabaka, J.M., Wong, D., Van Den Maagdenberg, A.M.J.M., Ferrari, M.D., Craig, K.D., Mogil, J.S., 2010. Coding of facial expressions of pain in the laboratory mouse. Nat. Methods 7, 447–449. https://doi.org/10.1038/nmeth.1455.

Müller, B.R., 2015. Dor em bovinos na marcação a ferro quente: expressão facial, outros indicadores comportamentais e fisiológicos e a percepção dos produtores 1–77. Available at: ⟨http://hdl.handle.net/1884/38047⟩. Accessed 05/22/2018.

Norton, T., Berckmans, D., 2017. Developing precision livestock farming tools for precision dairy farming. Anim. Front 7, 18. https://doi.org/10.2527/af.2017.0104.

Sandem, A.I., Braastad, B.O., Bøe, K.E., 2002. Eye white may indicate emotional state on a frustration-contentedness axis in dairy cows. Appl. Anim. Behav. Sci. 79, 1–10. https://doi.org/10.1016/S0168-1591(02)00029-1.

Sandem, A.I., Janczak, A.M., Braastad, B.O., 2004. A short note on effects of exposure to a novel stimulus (umbrella) on behaviour and percentage of eye-white in cows. Appl. Anim. Behav. Sci. 89, 309–314. https://doi.org/10.1016/j.applanim.2004.06.011.

Sandem, A.I., Braastad, B.O., Bakken, M., 2006. Behaviour and percentage eye-white in cows waiting to be fed concentrate-a brief report. Appl. Anim. Behav. Sci. 97, 145–151. https://doi.org/10.1016/j.applanim.2005.08.003.

Schmied, C., Waiblinger, S., Scharl, T., Leisch, F., Boivin, X., 2008. Stroking of different body regions by a human: effects on behaviour and heart rate of dairy cows. Appl. Anim. Behav. Sci. 109, 25–38. https://doi.org/10.1016/j.applanim.2007.01.013.

Shahin, M., 2018. The effects of positive human contact by tactile stimulation on dairy cows with different personalities. Appl. Anim. Behav. Sci. 204, 23–28. https://doi.org/10.1016/j.applanim.2018.04.004.

Tate, A.J., Fischer, H., Leigh, A.E., Kendrick, K.M., 2006. Behavioural and neurophysiological evidence for face identity and face emotion processing in animals. Philos. Trans. R. Soc. https://doi.org/10.1098/rstb.2006.1937.

Van Hertem, T., Rooijakkers, L., Berckmans, D., Peña Fernández, A., Norton, T., Berckmans, D., Vranken, E., 2017. Appropriate data visualisation is key to precision livestock farming acceptance. Comput. Electron. Agric. 138, 1–10. https://doi.org/10.1016/j.compag.2017.04.003.

Wayne Rasband, National Institute of Health. 1997. Image J - An Open Platform for Scientific Image Analyses. Available at: ⟨https://imagej.net/Welcome⟩. Accessed 02/03/2018.

Westerath, H.S., Gygax, L., Hillmann, E., 2014. Are special feed and being brushed judged as positive by calves. Appl. Anim. Behav. Sci. 156, 12–21. https://doi.org/10.1016/j.applanim.2014.04.003.