

Methods for obtaining body temperature of infant dairy calves

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ABSTRACT

The aim of the present study was to evaluate noninvasive methods of body temperature determination as an alternative to the conventional method (rectal temperature) in lactating dairy calves. The study used rectal temperature measurements, obtained with digital contact thermometer, which were considered in the present study as a reference of body and physiological temperature in the comparison of the temperature of the forehead, pinna, back and vulva, obtained with the digital infrared thermometer with laser sight and with the thermographic camera. The main hypothesis of the study was that an image taken with a thermographic camera can be used as a noninvasive technique to replace the infrared thermometer (non-invasive) and the conventional digital contact thermometer (invasive) in recording the body temperature of infant Holstein calves. According to the results observed in the comparison of means, the highest temperature was the rectal temperature evaluated with the digital thermometer and significantly different from the others $(p<0.05)$ and the method that came closest to the physiological value, but with significant difference, was the thermographic camera (CT) in the evaluation of vulva temperature. In Pearson's linear correlation analysis, the rectal temperature data and the thermographic camera of the pinna were the ones that presented the highest linear correlation, that is, physiological changes were more responsive. It is concluded that less invasive alternative methods are promising techniques, however, they do not replace the obtaining of rectal temperature to diagnose deviations from the normal physiological condition of the calves.

Keywords: Thermographic camera, Non-invasive technique, Infrared thermography, Digital thermometer.

1 INTRODUCTION

Homeothermy is the ability of animals to keep their internal temperature controlled within the ideal limits, a condition that characterizes the maintenance of temperature at constant levels (little

variable), regardless of changes in ambient temperature and conditions the survival of homeothermic animals (AZEVEDO et al., 2005).

In general, temperature measurement is an important step in the clinical examinations of animals because there is an evident association between temperature and disease and, in addition, it represents a valuable tool for monitoring the physiological state, well-being and stress responses of animals (GIANNETTO et al., 2020). Rectal temperature measurement has traditionally been the most common method for assessing body temperature, however, obtaining it can be a stressful procedure, particularly if serial measurements are required (HALL et al., 2019). Invasive procedures, in reactive and stressful animals, can alter the measurement result, thus compromising its interpretation and the consequent diagnostic and therapeutic measures (SCHMID et al., 2021).

Stewart et al., (2008) recommend that non-invasive measurement techniques and remote sampling should be studied to improve animal welfare. In the same vein, Herron and Shreyeras (2014) point out that the guidelines for evidence-based practices recommend low-stress treatment procedures and reduce the use of invasive practices, replacing them with non-restrictive practices.

Modern measurement techniques are based on the use of diagnostic tools that reduce stress due to a rapid assessment of body temperature, without the need for containment or contact (SOERENSEN and PEDERSEN, 2015).

The temperature of the eyes evaluated with infrared thermography is a new tool that has already been proven as an adequate technique for the evaluation of animal stress, such as in horses (VALERA et al., 2012), cattle (STEWART et al., 2005), dogs (TRAVAIN et al., 2015) and rabbits (De LIMA et al., 2013). Infrared thermography was also used to assess stress by measuring body surface temperature in goats (Da SILVA et al., 2014).

Hoffmann et al., (2013) proposed that the eye and the back of the ear of calves proved to be practical regions for temperature monitoring on farms. In the first part of the study, the authors demonstrated that the regions of the body such as the eyes, the back of the ear and the vulva adjusted better to the core temperatures (rectal and vaginal) than the shoulder region (scapula) of the animals. The mean temperature of the head area of the calves (mean \pm SD: 37.5 \pm 0.4 °C) was higher (significant for rectal temperature ≤ 38.7 °C, not significant for rectal temperature ≥ 38.9 °C) than the mean body area temperatures (37.2 \pm 0.5 °C). A trend of increasing temperatures of thermographic imaging with increasing rectal temperature was also observed in calves, both in the body area and in the head area.

Respiratory rate, heart rate, or ear skin temperature may be more appropriate for estimating acute heat stress than rectal temperature in dairy calves, i.e., the associations between rectal temperatures and environmental heat stress indices are stronger the higher the thermal load (KOVÁCS et al., 2018).

Peng et al., (2019) conducted a study with the aim of better understanding the rectal temperature (RT) inflection point and body surface temperatures (TSC) of dairy cows under different temperature and humidity index (THI) conditions. The results showed that the TSC were affected by the THI conditions, and they observed high correlation coefficients of the mean and maximum temperatures of the forehead with the THI (0.808 and 0.740, respectively) and were 0.557 and 0.504 respectively, with the rectal temperature. The authors concluded that the animals' body surface temperatures are more sensitive to the thermal environment than RT, suggesting the variability of the TSC to reflect the core body temperature. In addition, they have shown that the forehead is a relatively reliable region to assess the heat stress that reflects RT, compared to the regions of the eyes, ears, cheeks, flank, rump, anterior udder and posterior udder.

In the evaluation of the anterior and posterior udder region, relatively higher temperatures were observed than the cheeks, flank, rump, except the eye, of lactating cows, which may be due to the thinner thickness of the skin and the lower density of hair (PENG et al., 2019). Due to the variation of the vasomotor activity of the skin (MONTANHOLI et al. 2008), the variations of the surface temperature of the skin can be evaluated by means of infrared thermography.

The thermographs present differentiated colors based on the temperature variation of the radiation emitted by any surface and that can be processed by means of software. Infrared thermography, as a tool for measuring body temperature, is considered better and more advantageous compared to other methods. In addition, manual determination of body temperature is time-consuming, virtually difficult for repeated or continuous observations, and involves the risk of disease transmission (McMANUS et al., 2016).

The purpose of the present study was to evaluate the correlation of the temperatures of different body regions of Holstein calves with rectal temperature, through the use of thermographic images and infrared thermometer with laser sight. The importance of this study is justified by the contribution of information that is being generated to evaluate the temperature intake of infant dairy calves, using practical, modern and less invasive tools.

2 INFRARED THERMOGRAPHY

Infrared thermography technology is based on detecting the infrared radiation emitted by objects to determine their temperature. Non-contact infrared thermometers use this principle to measure the surface temperature of the skin. This method of temperature measurement can provide an accurate reading in seconds and can be performed at a distance of several centimeters to meters (WANG et al., 2014).

Non-contact thermal screening, including the application of infrared thermography and noncontact infrared thermometers, is considered a safe tool for temperature screening (ZHAO; BERGMANN, 2023).

A factor for the safety of the veracity of the temperature, there is the characteristic of emissivity of the target body under evaluation. Emissivity is the ability of a surface to emit heat and is scored on a scale of 0 to 1. The emissivity of an object is higher (1) or lower (0), depending on the amount of heat being emitted (STELLETTA, et al., 2012).

The body surface of the cattle has very good emissivity ranging from 0.93 to 0.98 depending on the density and color of the hair and skin. Therefore, the surface of the animals' skin is compatible with the thermographic evaluation. Generally hairless areas of the body, such as the snout, eyes, and vulva, are preferred for thermographic measurements. With regard to skin coloration, it is believed that buffalo and black cattle have higher emissivity due to the ability of the black color to absorb and emit more infrared radiation compared to lighter colors (STELLETTA, et al., 2012).

In this context, the accuracy of temperature measurement can be affected by several factors. The surface of the body, which is not isothermal, is one of the factors of great significance in the process of taking temperatures, in addition to varying between different regions of the body. These variations are due to blood flow, thickness of the skin and underlying tissues, and contact between the parts.

Zhao and Bergmann (2023) refer in a systematic review on infrared thermometers that tympanic, axillary, cutaneous and oral temperatures are the most commonly used reference temperatures. For the authors, it is likely that there is a difference between the measured temperature of the reference body site and the true core body temperature in humans. Studies have shown that the rectum and gastrointestinal tract are closer to the actual body temperature of humans than other parts of the body.

No less important or significant, one has the environmental factors influencing the accuracy of the measurement, including air temperature, relative humidity, wind speed, and nearby heat sources, which influence the surface temperature of the body. Generally speaking, the far-infrared emissivity of the body's skin is 0.98. In the infrared range, skin emissivity decreases with increasing moisture (KHAN et al., 2021).

Another factor of importance is the operating distance, which can also influence the accuracy of body temperature. Accuracy tends to decrease as distance increases. In addition, certain body surface characteristics, such as skin and hair color, perspiration, hair, and clothing, can influence body heat radiation and provide variability in readings (HOLDER et al., 2023).

The differences between makes and models of equipment and the training of operators that can affect the accuracy and consistency of the results (COSTANZA; FLORES, 2020).

For Zhao and Bergmann (2023), to mitigate the challenges, protocols must be developed that take into account the environmental conditions, individual characteristics and possible distortions associated with non-contact temperature measuring devices and their operators.

3 METHODOLOGY

The procedures performed were approved by the Ethics Committee on Animal Experimentation, UB (Protocol No 2200016).

The experiment was conducted in a completely randomized design, with the covariate being the day of sampling. The data were submitted to Pearson's linear correction analysis and the results were presented in the form of a correlogram. It had 20 infant calves of the Holstein breed (repeats) that were submitted to the analysis of the temperature of different parts of the body (rectal, forehead, pinna, back and vulva), obtained with digital (rectal) and infrared thermometers and thermographic camera, which characterized 9 treatments.

The experimental period comprised 56 days, divided into periods of twenty-eight days for the evaluation of treatments (Figure 1), totaling 60 readings. The experiment was conducted in the warm season of the year, in the months of November and December (spring/summer). Climatological parameters (air temperature, radiation, relative humidity, wind speed and black globe temperature) were monitored at the time of taking the animals' temperatures.

We used 20 infant females of the Holstein breed, spotted black, with an average live weight of 54±5.9kg at the beginning of the experiment. Daily, 4 L of milk were provided distributed in the morning and afternoon. Water was available *ad libitum* from birth, and the initial pelletized concentrate (20% protein, 2.5% fat, 7.5% fiber) was provided from 1st week of age, offered about 0.3 kg, *ad libitum.* The calves were kept confined in a masonry bay (concrete blocks), with a roof covering of fiber-cement material. On the sides of the bay, the walls were 1.5 m high, with a span up to the right foot (2.2 m).

Figure 1 – Schedule of the experimental period, when thermographic images and temperature inspection were performed with infrared thermometer on the forehead, pinna, back and vulva of the calves, in addition to the rectal temperature (digital thermometer).

The rectal temperature measurement was performed with a digital thermometer, taking care to touch the side of the internal mucosa avoiding the introduction into the fecal mass, and the check was performed after the thermometer emitted a sound signal for the safe reading of the temperature (close to 1 minute) (Figure 2A). The temperature check was carried out in the afternoon, always at 2 pm.

In A) digital thermometer used for rectal temperature reading; in B) infrared thermometer used to read the temperatures of the body parts of the calves (forehead, pinna, back and vulva); in C) thermographic camera used to read the temperatures of the body parts of the calves (forehead, pinna, back and vulva). Source: own authorship.

A infrared thermometer with laser sight*,* brand BENETEC, model GM 300 (-50 to 380 ºC), with a resolution of 0.1 °C. This equipment has a lens, temperature sensor, signal amplifier, processing circuit and LCD screen. The lens captures the infrared energy emitted by the object and focuses on the sensor. The sensor then transforms the energy into an electrical signal, which will be shown on the

LCD screen after passing through the signal amplifier and processing circuit. The technique is noninvasive and non-destructive. It was positioned at a distance of 50 cm from the intended point in the calves (Figure 2B).

In the thermographic image, a FLIR T297 camera (FLIR Systems Inc.) was used, a device that detects and measures the infrared radiation emitted by an object or surface, and converts this information into a thermal image. This technology allows you to visualize temperature variations, making it possible to identify hot or cold areas that would not be noticeable to the naked eye. The technique is non-invasive and non-destructive.

Figure 3 – Thermographic images with the identification of the temperature (aim) in the evaluated parts of the body of the Holstein calves.

In A) thermographic image used to read the temperature of the forehead; in B) thermographic image used to read the temperature of the pinna; in C) thermographic image used to read the temperature of the side; in D) thermographic image used to read the temperature of the calf's vulva. Source: own authorship.

Infrared images were obtained at a distance of 50 cm from the area of interest (Figure 2C). An emissivity of 0.95 was adopted. The color palette, which corresponds to the graphic representation of the temperatures present, was configured in the "Rainbow" option, in which the lighter regions (green and blue) represent the milder temperatures and the darker regions (yellow, orange and red) represent the higher temperatures. The imaging procedure counted repetition of three images of each area of

taking was inspected for any external artifacts that could influence the surface temperature by adding cold spots to the thermogram, such as dirt, food particle, or manure.

The data were analyzed in relation to the heteroscedasticity of variances and normality of the error, excluding those that did not fit within the model. The data considered valid were submitted to analysis of variance.

Analysis of variance (ANOVA) was performed considering 1 factor (temperature measurements and a covariate sampling days to control the ambient temperature of the sampling day). In the comparison of means by the Tukey test, the significance of 5% was considered (GOMES, 1990). The data are submitted to Pearson's linear correction analysis.

4 RESULTS AND DISCUSSION

The mean values of the temperatures recorded in the calves, according to the treatments evaluated, can be seen in Table 1.

The results indicate that there was a significant influence of the method for the acquisition of temperature and of the body parts evaluated in the calves. In the comparison between the methods, a significant difference was detected in the mean rectal temperature obtained by the digital thermometer (mean of 39.46ºC), which is considered a standard conventional evaluation of the physiological temperature of the animal. In the comparison with rectal temperature (digital thermometer), the method that was closest to the physiological value, but with a significant difference, was the thermographic camera (CT) in the evaluation of vulva temperature (mean of 37.92 °C) (Table 1).

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Treatments		Temperature $(^{\circ}C)$						
Rectal	60	39,46	A					
Vulva CT	60	37,92		В				
Vulva IV	60	35,24			C			
P. Auricular IV	60	35,12			\mathcal{C}			
P. Auricular CT	60	35,00			C			
CT Side	60	33,57				D		
Front CT	60	32,53					And	
Side IV	60	31,48						
Front IV	60	31,40						

Table 1. Information on the grouping of treatments evaluated in infant dairy calves of the Holstein breed.

Averages that do not share a letter are significantly different, by Tukey's method and 95% Confidence. Rectal (rectal temperature by digital thermometer); Vulva CT (temperature of the vulva by the thermographic camera); Vulva IV (temperature of the vulva by infrared thermometer); P.Auricular IV (pinna temperature by infrared thermometer); P.Auricular CT (pinna temperature by thermographic camera); Costado CT (temperature of the back by the thermographic camera); Costado IV (temperature of the back by the infrared thermometer with laser sight); Forehead CT (forehead temperature by thermographic camera); Fronte IR (forehead temperature by infrared thermometer). Source: own authorship.

The calves evaluated inside the individual stalls of the calf, where they were kept, presented average temperature considered slightly above the upper limit of normal standards, according to

Dirksen; Gründer; Stöber (1993) indicate standard values of 38.0 to 39.3º C for the evaluation of animals kept in hot environments.

The use of a digital thermometer to measure rectal temperature is the most widely used method (BURFEIND et al., 2010), as it can provide the best approximation value of a cow's core temperature. However, this method is time-consuming and requires animals to be apprehended to obtain their rectal temperatures, which can cause stress responses in cows (NAYLOR et al., 2012).

Table 1 shows that the mean values of vulva temperatures obtained with the infrared (IR) thermometer, the pinna by the IV and the TC were similar to each other ($p \ge 0.05$).

In dogs, the auricular temperature measured with infrared thermometer was similar to the body temperature obtained with rectal thermometer (ZANGHI, 2016). Kunkle et al. (2004) reported highly variable values using an infrared thermometer for body temperature measurements (a mean difference of 0.07 °C and limits of agreement of 1.43 °C and -1.36 °C), which they considered unacceptable for clinical purposes in cats.

Ocular surface temperature is highly correlated with core body temperature (NG and KAW, 2006; GEORGE et al., 2014). The use of thermographic imaging to measure eye temperature is fast, relatively easy, and less invasive compared to alternative methods of measuring body temperature, such as rectal thermometers, tympanic infrared thermometers, thermal microchips, and ruminal boluses (JOHNSON et al., 2011, TIMSIT et al., 2011). In the present study, the ocular surface was not an option for the body region to be evaluated, since the infrared thermometer used contained a laser sight, which could cause damage to the animals' eyes.

In the calves, the temperature values of the CT side differed from the others, which was also observed in the temperature of the CT forehead (Table 1). The lowest temperature values recorded were observed for costate IV and forehead IV, being similar to each other (Figure 4).

The comparison of skin temperature measurements by the types of thermometers examined showed that the infrared thermometer recorded lower mean temperature values in the region of the side and forehead, and the temperature of these regions was significantly different from that recorded by the thermographic camera (Figure 4). As expected, the areas closer to the center of the body (pinna) present less difference in relation to rectal temperature and present a narrower range (less variation), since more distal regions (costate) present a wider range. Body surface temperatures in calves are more related to room temperature than rectal temperature.

Figure 4. Dispersion of the mean temperature values obtained according to the treatments evaluated in infant dairy calves of the Holstein breed.

Rectal (rectal temperature by digital thermometer); Vulva CT (temperature of the vulva by the thermographic camera); Vulva IV (temperature of the vulva by infrared thermometer); P.Auricular IV (pinna temperature by infrared thermometer); P.Auricular CT (pinna temperature by thermographic camera); Costado CT (temperature of the back by the thermographic camera); Costado IV (temperature of the back by the infrared thermometer with laser sight); Forehead CT (forehead temperature by thermographic camera); Fronte IR (forehead temperature by infrared thermometer). Source: own authorship.

According to Ramey, Bachmann and Lee (2011), the differences in skin temperature may be due to the relative power of different regions of the body to emit heat by radiation (emissivity), linked to the length, color, diameter and density of the coat, in addition to the corresponding muscle mass. In general, coat is a major factor in the difficulty of detecting infrared emissions in some places on the body, which would explain why lower temperature readings are associated with greater hair length (MEISFJORD JØRGENSEN et al., 2020). The different arterial blood flows in each portion of the body represent another factor that may play a role in the variability of the data (NUTT; LEVY; Tucker, 2016).

Since the environment is an extrinsic factor that influences the surface temperature of the animals' bodies, some climatological parameters were raised. The mean results of each of the parameters in the days of temperature sampling of the animals can be seen in Figure 5. It was observed that the air temperature (AT) was similar in both environments (bay and sun) with an average of 29.2 °C; the relative humidity recorded in the stalls and in the sun environment was 36.8 and 40.7% respectively; the temperature of the black globe was 31.7 and 37.1°C, respectively. The average wind speed was the same in both environments, of 0.65 m/s and the radiation recorded in the bays was at 80.1 W/m2 and in the environment at sun was at 465.1 W/m^2 . It should be noted that the environment of the stalls provided a certain barrier against solar radiation on the calves, providing protection from direct solar radiation.

Regarding the Pearson correlation index of the variables evaluated, the values obtained can be seen in Figure 6, in which bluish tones are representing positive correlations and, reddish, negative correlations. The variables represent the body temperatures (ºC) recorded as a function of the method of obtainment (digital contact thermometer, thermographic image and infrared radiation) evaluated in different body regions (forehead, pinna, rectal, vulva and rib region).

In the horizontal axis of the correlogram (Figure 6) the variables are presented in the following order: rectal temperature (RETAL), infrared temperature of the forehead (FRONTE IV), infrared temperature of the backside (COSTADO IV), infrared of the pinna (P.AURIC IV), infrared of the vulva (VULVA IV), thermographic image of the forehead (FRONTE CT), thermographic costado (COSTADO CT), thermographic pinna auricular (PAURIC CT). In view of the objectives of the present study and, despite the multiple comparisons presented in the correlogram, the discussion was held only about the correlations obtained with rectal temperature with a digital thermometer, considered as a standard quantitative physiological procedure.

Figure 6. Pearson's correlogram with correlation coefficient values. Bluish tones are representing positive correlations and, reddish, negative correlations.

Rectal (rectal temperature by digital thermometer); Vulva CT (temperature of the vulva by the thermographic camera); Vulva IV (temperature of the vulva by infrared thermometer); P.Auric IV (pinna temperature by infrared thermometer); P.Auric CT (pinna temperature by thermographic camera); Costado CT (temperature of the back by the thermographic camera); Costado IV (temperature of the back by the infrared thermometer with laser sight); Forehead CT (forehead temperature by thermographic camera); Fronte IR (forehead temperature by infrared thermometer). Source: own authorship.

Although the vulva temperature obtained with the use of a thermographic camera approaches the rectal temperature obtained with the digital contact thermometer (physiological pattern), this vulva temperature presented a linear correlation with the rectal temperature of 0.42 considered as a weak correlation according to Kirch (2008), indicating that physiological changes recorded in the rectal temperature are not so noticeable in the thermographic image taken on the vulva.

In the present study, the use of thermographic imaging of the pinna (APD) presented Pearson's correlation coefficient of 0.61 considered as moderate according to Kirch (2008), that is, with this parameter it is possible to identify physiological temperature changes, even presenting lower mean values of temperature of 4.5ºC when compared with rectal temperature. The obtainment of body temperature by infrared in the pinna presented a correlation coefficient of 0.53, also considered as moderate.

5 CONCLUSIONS

There is a direct influence of the method of obtaining the temperatures and temperatures between the body parts of Holstein calves, so it is necessary to continue adopting the standard method (rectal temperature) for the verification of physiological disorders reflected on the body temperature of these animals.

The numerical proximity of the body temperature obtained from non-contact and non-invasive techniques in relation to the rectal temperature intake (physiological pattern) should not be the only parameter to be used in the choice of the technique, being perhaps of greater importance the ability to respond numerically to physiological changes.

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