INFRARED THERMOGRAPHY TO MAP THE UDDER HEALTH STATUS OF ZEBUINE DAIRY COWS †

[TERMOGRAFÍA INFRARROJA PARA MAPEAR EL ESTADO DE SALUD DE LAS UBRES DE LAS VACAS LECHERAS ZEBUÍNAS]

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SUMMARY

Background: Infrared thermography is a diagnostic imaging technique that detects the emission of heat by infrared radiation, indicating the temperature of a particular area. **Objective**: The objective of this study was to measure the magnitude of the udder temperature variation by infrared thermography for mastitis diagnosis of zebuine dairy cows in a tropical pasture-based system. **Methodology:** The udder temperature of Holstein-Gyr crossbred cows was measured with a FLIR® camera. Milk samples were collected for CMT and somatic cell count determination. The surface temperature of the mammary gland and rectal temperature were grouped according to udder health status of animals and were submitted to analysis of variance and correlation analysis. **Results:** The udder temperatures were higher for the clinical mastitis group (37.2±0.7 °C) than the subclinical mastitis (36.6±0.9 °C) and healthy udder (36.3 ±1.0 °C) groups. A weak positive correlation (r=0.18) was observed between somatic cell count (SCC) and udder temperature. A strong positive correlation (r=0.68) was observed between rectal temperature and udder temperature. **Implications:** The results of this work can be applied in the future in precision animal husbandry, generating more efficient strategies and practices for the diagnosis and control of mastitis, since it is a promising tool for easy and quick diagnosis of mastitis. **Conclusions:** Infrared thermography can be an effective method for diagnosing mastitis in cows. However, its use should be made with the complementary California mastitis test.
Keywords: infrared imaging; Girolando; mastitis diagnosis.

RESUMEN

Antecedentes: La termografía infrarroja es una técnica de diagnóstico por imagen que detecta la emisión de calor por radiación infrarroja, indicando la temperatura de un área en particular. **Objetivo:** El objetivo de este estudio fue medir la magnitud de la variación de la temperatura de la ubre mediante termografía infrarroja, para el diagnóstico de mastitis en vacas lecheras cebuínas en un sistema basado en pasturas tropicales. **Metodología:** La temperatura de la ubre de vacas cruzadas Holstein-Gyr se midió con una cámara FLIR®. Se recolectaron muestras de leche para CMT y determinación del recuento de células somáticas. La temperatura superficial de la glándula mamaria y la temperatura rectal se agruparon según el estado de salud de la ubre de los animales y se sometieron a análisis de varianza y análisis de correlación. **Resultados:** Las temperaturas de la ubre fueron más altas para el grupo de mastitis clínica (37.2 ± 0.7 °C) que los grupos de mastitis subclínica (36.6 ± 0.9 °C) y ubre sana (36.3 ± 1.0 °C). Se observó una correlación positiva baja (r = 0.18) entre el recuento de células somáticas (CCE) y la temperatura de la ubre. Se observó una fuerte correlación positiva (r = 0.68) entre la temperatura rectal y la temperatura de la ubre. **Implicaciones:** Los resultados de este trabajo pueden ser aplicados en el futuro en el área de la ganadería de

† S*ubmitted October 1, 2020 – Accepted September 27, 2021*.
ISSN: 1870-0462.

precisión, generando estrategias y prácticas más eficientes para el diagnóstico y control de mastitis, ya que es una herramienta prometedora para el diagnóstico fácil y rápido de mastitis. **Conclusiones:** La termografía infrarroja puede ser un método eficaz para diagnosticar mastitis en vacas. Sin embargo, su uso debe realizarse con la prueba complementaria de mastitis de California.

Palabras clave: imagen infrarroja; Girolando; diagnóstico de mastitis.

INTRODUCTION

Sensors for udder health management have been developed to help farmers reduce physical effort and labor costs through automation of processes. Automatic methods to detect mastitis are important to enable farmers to manage large herds. Milk electrical conductivity and gas sensors are practical devices to detect mastitis and predict milk composition (Lima *et al.*, 2018). Currently, measurement of udder skin temperature using infrared thermography is recommended to monitor udder health status. Infrared thermography is a diagnostic imaging technique that detects the emission of heat by infrared radiation, indicating the temperature of a particular area (Zaninelli *et al*. 2018). Therefore, the higher the temperature, the higher the molecular excitation and consequently the greater the intensity of the radiation emitted. This technology is unique and noninvasive. Each pixel generated in the picture represents a temperature point to generate the thermal profile (Zhang *et al.,* 2020).

Heat is one of the cardinal inflammation signs of mastitis, the most common disease in dairy production, characterized by inflammation in the mammary gland that alters blood flow and consequently temperature in the affected region, besides the quantity and quality of milk. Early diagnosis is crucial for successful mastitis treatment (Polat *et al*. 2010). As the disease progresses, local temperature increase is expected, since inflammatory processes increase blood flow and tissue metabolism, causing increased heat irradiation (Berry *et al*., 2003). In the inflammatory process of the mammary gland, there is an increase of the metabolic activity in the tissues because of the activation of the immunological responses against the microorganisms. With the evolution of the disease to the chronic stage, alveolar tissue is replaced by fibrous connective tissue, as a strategy to repair tissue damaged by microorganisms (Nogueira *et al*. 2013). Therefore, the observation of UST (udder surface temperature) in the affected area can be used to diagnose chronic infection or injury.

Infrared thermography technology is a non-invasive method used to detect thermal biometric changes in animal metabolism resulting from increased body temperature and changes in blood flow in response to environmental or physiological conditions.

The udder skin temperature has been found effective to monitor udder health status in Holstein cows using infrared thermography (Colak *et al*. 2008; Polat *et al*. 2010). In addition, udder temperature is useful to develop a predictive model for mastitis detection (Berry *et al*., 2003). However, this technology might work differently in cows of different breeds and environments.

The crossbreeding of zebu bulls with Holstein cows is widely used in tropical regions to improve milk composition and resistance to mastitis and other diseases, as well as to enhance body conformation, thermotolerance, feed efficiency, fertility and fitness compared with purebred Holstein cows (Galukande *et al*. 2013; Cardoso *et al*. 2015; Buckley *et al*. 2014). The udder health score can be measured by infrared thermography in different species (e.g., buffaloes, sheep, camels) and cattle breeds (Berry et al., 2003; Zhang *et al*. 2020). Studies with thermography been applied to zebu cattle in Brazilian tropical environments in experimental research (Cardoso et al., 2015) but not in animals breeds of cattle in the commercial farm. The objective of this study was to measure the magnitude of udder temperature variation using infrared thermography for mastitis diagnosis in zebuine dairy cattle in a pasture-based system under tropical environmental conditions.

MATERIAL AND METHODS

This study was approved by the Ethics Committee on Animal Experimentation of Norte Fluminense State University (UENF, Protocol no. 251), in accordance with the Brazilian Society of Laboratory Animal Science/Brazilian College of Animal Experimentation (SBCAL/COBEA).

Animals and Farms

The study involved 91 multiparous Holstein-Gyr crossbred cows kept at a commercial farm located in the northern region of the state of Rio de Janeiro (Brazil). The research was carried out in June, during the Southern Hemisphere winter. The weather during the study period was dry (total precipitation of 32.8 mm), with average nighttime temperature near 19°C and daytime temperature near 23°C (INMET, 2018). To avoid heat stress, the data collection was carried out during the morning, from 8:00 to 10:30 a.m. (Alfonzo *et al*. 2016).

The cows have an average milk yield between 15 to 20 liter/day, an average stage of lactation eight to 16 weeks and the average weight of 430 Kg.

During most of the day, the cows were kept in the pasture as part of the normal farm practice and were brought into handling yards twice a day for milking. After this period, the cows were fed a diet formulated according to their state of lactation.

Assessment of udder health status

Prior to milking, milk samples from 364 mammary quarters were collected using a black bottom container for clinical mastitis detection, a paddle for the California mastitis test (CMT) and bottles with bronopol for somatic cell count (SCC) analysis. Was each quarter considered an experimental unit. An animal was diagnosed with clinical mastitis when it was possible to observe clots, blood or other visible changes in the milk. For interpretation of the CMT, four scores were considered, according to the degree of gelatinization of the sample: negative (normal), weak positive reaction (+), positive reaction (++), and strongly positive reaction (+++). Milk samples were collected and sent to the laboratory for somatic cell counting (SCC). The mammary quarters were classified into three groups: healthy quarter ($<200x10^3$ cells/ml), subclinical mastitis (≥ 200 to $\leq 500x10^3$ cells/ml) and clinical mastitis ($>500x10^3$ cells/ml).

Udder thermal imaging and rectal temperature measurement

The udder surface temperature (UST) was measured by the infrared thermal camera between 8:00 and 10:30 a.m., from animals kept in the shade before milking. The average temperature in this time was 19 to 23°C. The animals were kept standing with the pelvic limbs slightly apart and the tail raised for lateral framing of the right udder, left udder, and posterior udder. The camera (FLIR® series i50; FLIR Systems Co. Ltd., Shatin, Hong Kong) was kept 1 meter away from the animal, with emissivity of 0.98 and rainbow palette (Freitas *et al*. 2018). The thermograms were evaluated using the FLIR Quickreport® software, where each pixel represents a temperature point. We assessed the right udder, left udder and posterior udder with the area tool.

The rectal temperature (RT - ºC) was obtained with a Med® digital thermometer (Cotronic Technology Ltd., Shatin, Hong Kong) inserted in the rectum of each animal to an approximate depth of 3.5 cm.

Statistical analysis

The design used was a completely randomized. Each quarter were considered an experimental unit. The data on udder surface temperature (UST) and rectal temperature of cows (RT) and somatic cell count (CCS) were submitted to analysis of variance that included the fixed effects of group (according to udder health status: healthy, subclinical and clinical) and the means were compared with the Tukey test using the package of R (Mendiburu, 2014). The Pearson correlation coefficients between rectal temperature and values of temperature of healthy and subclinical mastitis udder surface (USTc)were calculated using the cor () function of R. Results were considered statistically significant at $p \leq 0.05$.

RESULTS

We evaluated 364 mammary quarters and found that 13% had clinical mastitis, 46% had subclinical mastitis and 41% were healthy. Most cases of mastitis were diagnosed in the rear udder (62%), while the front udder was the healthiest. The environmental conditions during the data collection were moderate, with mean air temperature of 22.6 °C and relative humidity of 83.4% (THI = 70), in the range of thermal comfort for dairy cows (NRC,1971).

Thermographic images generate a spectrum of colors across a gradient of temperatures, where darker colors represent colder temperatures and lighter colors denote warmer temperatures. Figure 1, Figure 2 and Figure 3 present the temperature range from 20 to 37 °C, where 22 is the lowest color of the gradient and 37 is the highest.

Table 1 reports the descriptive statistics of udder surface temperature and rectal temperature, according to the health status of the mammary gland.

The average temperature of healthy udders was 36.3 °C (Figure 1), while for animals with subclinical mastitis it was 36.6 °C (Figure 2) and clinical mastitis was 37.2 °C (Figure 3). In general, the temperatures increased linearly according to the changes in udder health status (Table 1). The measurements of UST taken by infrared thermography revealed a statistical difference ($p<0.05$) between the healthy, subclinical and clinical mastitis udders. Comparison of temperatures between animals with different levels of subclinical mastitis revealed no significant difference ($p>0.05$). The change in somatic cell count in mammary quarters with subclinical mastitis was not accompanied by a significant increase in udder temperature. Temperature was significant only for clinical mastitis detection ($p<0.05$).

Table 1. Minimum, mean, maximum and standard deviation values of somatic cell count (x10[3]cells/ml), udder surface temperature (°C) and rectal temperature (°C) for each udder's health status of the Girolando cows.

Udder Health Status (n= number of observations)	SCC[a] Mean[d] ±SD[e] (max– min)	UST[b] Mean[d] ± SD[e] (max– min)	RT[c] Mean[d] ± SD[e] (max – min)
Healthy (n=148)	134±71[b] (28 – 265)	36.3±1.0[b] (33.6 - 38.1)	38±0.4[b] (37.2 – 39.2)
Subclinical (n=168)	883±1015[b] (307– 4825)	36.6±0.9[ab] (34- 38.6)	38.1±0.5[b] (37.0 – 39.4)
Clinical (n=48)	8755±3506[a] (5264– 9500)	37.2±0.7[a] (35.9 - 38.6)	38.4±0.6[a] (37.6 - 39.4)

[a]SCC= somatic cell count (x1000 cells/ml); [b]UST= udder surface temperature; [c]RT= rectal temperature; [d]Mean; [e]SD=standard deviation of the mean.
Different letters in the same column indicate statistical difference through the Tukey test ($P<0.05$).

Table 2. Pearson correlation was performed between somatic cell counting (SCC), values of temperature of healthy and subclinical mastitis udder surface (USTc) and rectal temperature of the Girolando cows.

Traits	SCC	USTc	RT
SCC	-		
USTc	.18	-	
RT	.32	.68	-

The mean rectal temperature of cows with healthy udders was 38 °C, while for animals with subclinical mastitis it was 38.1 °C and for those with clinical mastitis it was 38.4 °C. The rectal temperature data revealed a statistical difference ($p<0.05$) in the means of animals with clinical mastitis in relation to the other animals (Table 1). About 10% of the animals had rectal temperature above normal, which in healthy cattle ranges from around 36.7 °C to 39.1 °C (Reece and Rowe 2017).

A Pearson correlation was performed between SCC and USTc was weak positive and low. The correlation between RT and USD was a strong positive and high and there was a positive correlation between SCC and RT (Table 2).

DISCUSSION

This study evaluated the temperature magnitude variation to determine udder health status and mastitis of Holstein-Gyr crossbred cows (*Bos indicus x Bos taurus*) under tropical conditions of Brazilian commercial farms. The udder temperatures were higher for the clinical mastitis group (37.2±0.7 °C) in relation to the subclinical mastitis (36.6±0.9 °C) and healthy udder (36.3 ±1.0 °C) groups.

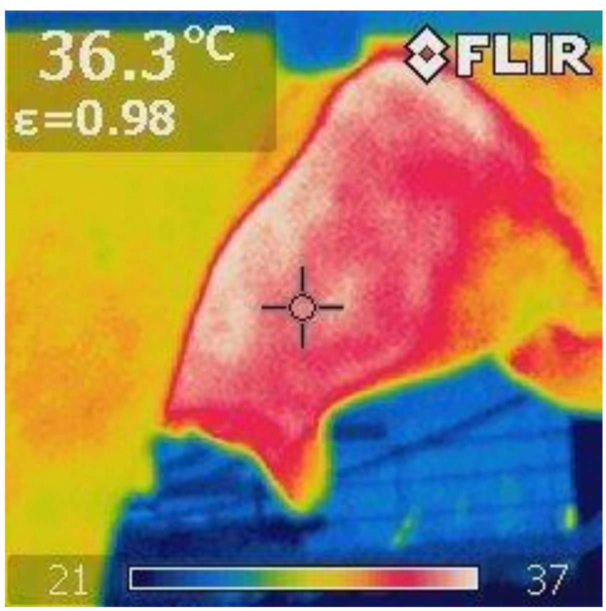

Figure 1. Thermographic image of the udder surface of a cow and the respective temperatures. Darker colors represent colder temperatures (at the left of the palette) and lighter colors denote warmer temperatures (at the right of the palette).

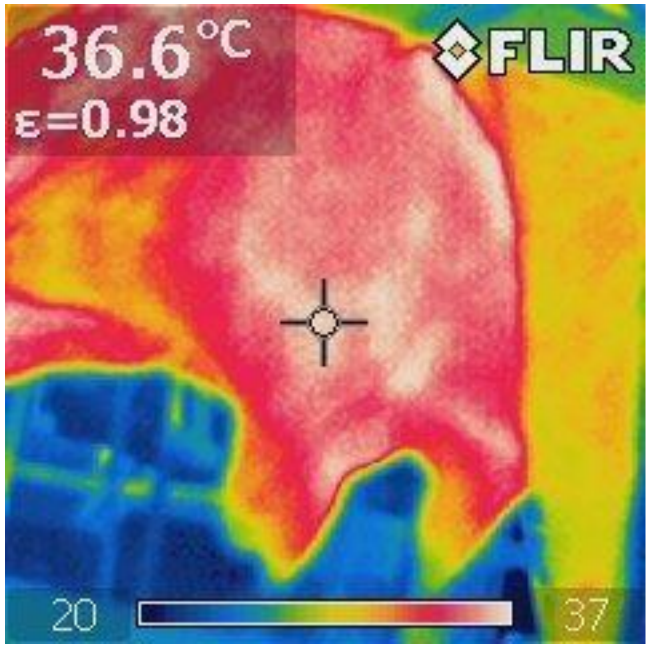

Figure 2. Thermographic images of the udders of cows with subclinical mastitis and the respective temperature. Darker colors represent colder temperatures (at the left of the palette) and lighter colors denote warmer temperatures (at the right of the palette).

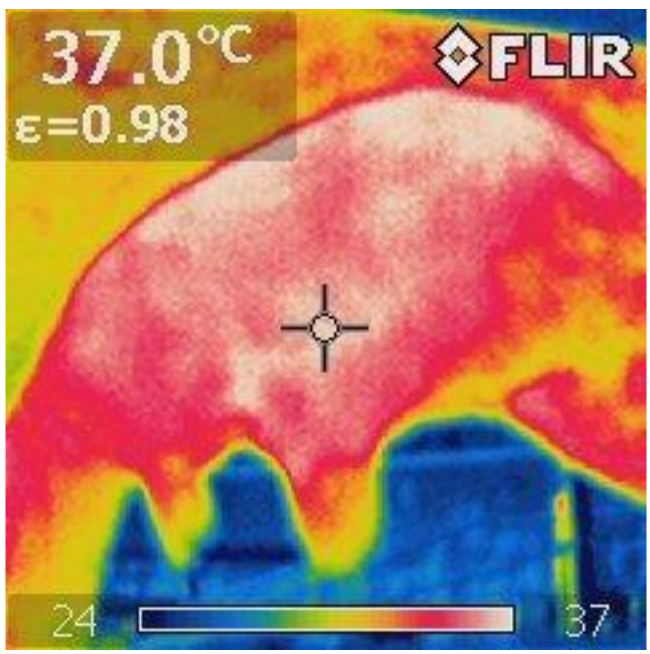

Figure 3. Thermographic images of udders of cows with clinical mastitis and the respective temperature. Darker colors represent colder temperatures (at the left of the palette) and lighter colors represent warmer temperatures (at the right of the palette).

Physiological factors, animal conformation and breed can affect skin temperatures measured by thermography (Cardoso *et al*., 2015). In a study conducted by Poikalainen *et al*. (2012), the temperature of healthy udders on both sides was assessed by infrared thermography before milking (left quarter 32.4 °C, right quarter 32.4 °C) and afterward (left quarter 32.9 °C, right quarter 33.2 °C). However, no significant differences were found between left and right-side temperatures, either before or after milking (Poikalainen *et al*., 2012). According to Berry *et al*. (2003), the anterior and posterior udders of Holstein Friesian cows can have a different temperature because the posterior surface of the udder is more exposed to the environment than the anterior surface. However, in our study we found no differences between temperature of the right and left sides. This could possibly be explained by the fact that it works with a different genetic group of cows, which are adapted to the hot tropical climate.

In another study, Porcionato *et al*. (2009) evaluated the surface at three different heights (upper, medial and lower) of 70 Gyr cows and observed that the SCC did not influence the udder temperature. Mammary glands of Brown Swiss cows suffering from subclinical mastitis were evaluated by thermography: the average temperature of healthy mammary glands was 33.23 °C, while for subclinical mastitis levels 1 (+), 2 (++) and 3 (+++), the average temperatures were 34.64 °C, 35.73 °C and 36.27 °C, respectively (Polat *et al*. 2010). Daltro *et al*. (2017) measured udder surface temperature in Holstein and Girolando cows (*Bos indicus x Bos taurus*) without evaluating udder health. These authors related that temperature of lateral region and posterior region of the udder measured with infrared thermography in the morning, for Holstein cows were 39.05°C and 40°C and for Girolando cows was 38.42°C and 38.43°C, respectively. In our study with Girolando cows, but of the tropical region, in the morning measures of all quarter udder surface temperature were in average 36.3°C in healthy cows and 37.2°C in cows with clinical mastitis.

A Pearson correlation test was performed between SCC and UST. A weak positive correlation (r=0.18) was observed between them, indicating that the temperature increased according to disease development, such as clinical mastitis. However, in the early phase of subclinical mastitis, the subcutaneous blood flow in the capillaries is not required to promote changes in udder surface temperature. That is in agrees with Zaninelli *et al*. (2018) in a study carried out with 155 dairy cows in the Lombardy region. They reported that when the SCC levels increased, the maximum temperature values followed the same trend.

High body temperature is generally used as a sign of disease and promotes malaise in the animal. However, the temperature of most of the animals in this study was within the range considered normal. Cardoso *et al*. (2015) reported that animals of the Girolando breed showed lower rectal temperatures (38.65 °C) compared to other zebu dairy breeds, e.g., Gyr (39.05 °C) and Sindhi (38.86 °C). Dikmen *et al*. (2013) also found the temperatures of Holstein cows to be around 38.8 °C.

According to the Pearson correlation, there was a strong positive correlation between RT and USTc (r= 0.68) and a weak positive correlation between SCC and RT (r = 0.32), showing that animals with rectal temperature above normal also presented clinical mastitis. This indicates that the mammary gland and body use the same mechanisms to regulate the temperature (Bitman *et al*., 1984). Metzner *et al*. (2014) suggested that udder thermography offers the possibility to monitor the body temperature of cows. Likewise, Daltro *et al*. (2017) observed a high correlation (r = 0.74) between the udder and rectal temperature. The same authors identified udder as an adequate location to measure thermal comfort in dairy cows using thermography, because even in a thermoneutral environment, heat has to be transferred to the extremities with round surface shapes, which have good heat transfer properties to the environment (Kräuchi, 2002). Due to occlusion of the rectum by the tail, the measurement of temperature of this region can hinder the practical application of infrared thermography on farms. On the other hand, forehead temperature had the strongest association with rectal temperature in Jersey cows (Salles *et al*., 2016), indicating an alternative site to measure body temperatures than visually obstructed regions.

Cows are endothermic; they use metabolic heat to keep their internal temperature constant despite differences in ambient temperature (Bitman *et al*., 1984). Some factors that influence heat production in cattle are the net energy needs for maintenance, growth, milk production, reproduction and body reserves (Blaxter, 1961). For nutrition assessment, energy is commonly expressed in terms of kilocalories (Kcal), defined as the amount of heat needed to raise the temperature of 1 kilogram of water by 1 °C (Hargrove, 2006). Besides this, the genetic background and the environment also influence heat production in animals (Berman, 2011). In a study conducted by Stone *et al*. (2017), the authors observed significant difference of reticulorumen temperatures among Holstein (38.9 ± 0.1 °C), Jersey (38.7 ± 0.1 °C) and crossbred cows (Jersey × Holstein, 38.8 ± 0.1 °C). Berry *et al*. (2003) reported that measurement of changes in udder skin temperature should consider daily variations under various environmental conditions. Vilela *et al*. (2013) complemented that the adverse environmental conditions in tropical climates, such as high temperature, humidity, air movement, radiation and precipitation, combine to lower body heat loss in animals.

To maintain thermoneutrality, several physiological mechanisms, such as cutaneous vasodilation, increased respiratory rate and perspiration, are activated by the organism. The blood vessels help to dissipate heat by radiation from the body surface into the atmosphere. The humid and hot air in the lungs is vaporized by convection and heat is lost through evaporation. The wet skin dissipates heat by evaporation (Vilela *et al*., 2013). The respiratory rate of crossbreeds such as Girolando cattle appears to be efficient to control body temperature, so that it does not cause an increase in rectal temperature (McManus *et al*., 2014). This breed has a thinner and darker skin and greater hair density (1283 cm^2) and length (0.99 cm) than other breeds, attributes which facilitate heat loss (Cardoso *et al*., 2015). These advantages are some reasons for the widespread crossbreeding of cattle in Brazil.

Other factors attributed to cattle bred for tropical climates are lower maintenance requirements, higher pasture grazing ability, better resistance to parasites and good fertility under heat stress (Berman, 2011). It is recommended to use thermography at times that avoid thermal stress so as not to disturb the accuracy of the diagnosis. Since the udder region is highly vascularized, in situations of thermal discomfort the loss of heat in this region increases, leading to false positive results. In order to avoid the false positives results, one diagnostic test make be done is the California Mastitis Test, that evaluate the cow's milk. Mastitis may result in changes in the milk, composition and udder the cow, flecks, and changes in color or consistency can be seen when milk is stripped on a dark surface.

We obtained basic data of udder temperature to evaluate mastitis using infrared thermography on Girolando dairy cows in a tropical climate. The results of this work can be applied in the future in the area of precision animal husbandry, generating more efficient strategies and practices for the diagnosis and control of mastitis, since it is a promising tool for easy and quick diagnosis of mastitis. In addition, the temperature of the mammary gland can be considered in the future as a new phenotype chosen for the selection of mastitis resistant animals.

CONCLUSIONS

It was possible to verify the health of the udder by evaluating the temperature variation by thermography. The aggravation of the clinical condition of the udder causes an increase in the local and body temperature of the animal. Thus, infrared thermography can be an effective method for diagnosing mastitis in cows. However, its use should be made with the complementary California mastitis test.

Acknowledgements
The authors are grateful to the farmer Mila de Carvalho Laurindo e Campos of Recreio farm.

Funding. This study was financed in part by fellowship of the Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior- Brazil (CAPES), finance code 001. We are also thankful for the support of fellowship the Brazilian National Council for Scientific and Technological Development – CNPq.

Declaration of interest. The authors declare that there are no known conflicts of interest associated with this publication.

Ethics statement. This study was approved by the Ethics Committee on Animal Experimentation of Norte Fluminense State University (UENF, Protocol no. 251), in accordance with the Brazilian Society of Laboratory Animal Science/Brazilian College of Animal Experimentation (SBCAL/COBEA).

Data availability. Data are available with the corresponding author at: *crq@uenf.br,* upon reasonable request.

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