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Infrared thermography as a technique for detecting subclinical bovine mastitis

[*Termografia infravermelha como técnica para detecção de mastite bovina subclinical*]

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ABSTRACT

Infrared thermography is a non-invasive diagnostic tool for early detection of subclinical mastitis in pasture-raised dairy cows. The study was conducted on eight dairy farms, in Sena Madureira, Acre, in the Western Amazon, during the rainy season. One hundred and thirteen lactating crossbred cows were monitored, with measurements performed once a day before milking, from 02:00 to 06:00 in the morning. The California Mastitis Test (CMT) was subsequently applied. Data processing was performed in the R programming language and evaluated by linear models. Our results show that cow udder surface temperature by infrared thermography showed significant differences ($p<0.05$) between the months of the year. Cow udder temperatures of thermographic images were significantly different ($p<0.05$) from CMT results for animals with subclinical mastitis. CMT results showed that 45 cows (39.8%) in January, 52 cows (46%) in February and 57 cows (50.4%) in March had subclinical mastitis. Infrared thermography is a technique that can be used for the early detection of subclinical mastitis in dairy herds in a grazing system, as it detected an increase in the udder surface temperature of the same cows that were positive for CMT.

Keywords: once-a-day milking, udder health, pasture-based system, udder surface temperature, dairy cow

RESUMO

Avaliou-se a termografia infravermelha como ferramenta diagnóstica, não invasiva, para detecção precoce da mastite subclínica em vacas leiteiras criadas a pasto. O estudo foi conduzido em oito propriedades leiteiras, em Sena Madureira, Acre, na Amazônia Ocidental, durante o inverno amazônico. Foram monitoradas 113 vacas mestiças em lactação, com mensurações realizadas uma vez por dia, sempre antes da ordenha, realizadas das 2h às 6h da manhã. Em seguida, realizou-se o exame de Califórnia Mastitis Test-CMT. O processamento dos dados foi feito em linguagem de programação R, os quais foram avaliados por modelos lineares. Os resultados mostram que a temperatura da superfície do úbere das vacas avaliadas com a técnica da termografia infravermelha teve diferenças significativas ($P<0,05$) entre os meses do ano. Constatou-se alteração na temperatura do úbere das vacas, captada por meio das imagens termográficas, e foi significativo ($P<0,05$) o resultado do teste California Mastistis Test (CMT) para os animais que se encontravam com mastite subclínica. Com base nos resultados do CMT, foi possível constatar que 45 vacas (39,8%) em janeiro, 52 vacas (46%) em fevereiro e 57 vacas (50,4%) em março se encontravam com mastite subclínica. Conclui-se que a termografia infravermelha é uma técnica que pode ser utilizada para a detecção precoce de mastite subclínica em rebanho leiteiro em sistema de pastejo, pois foi capaz de detectar aumento na temperatura superficial do úbere das vacas, que resultaram positivas no teste de CMT.

Palavras-chave: ordenha uma vez ao dia, saúde do úbere, sistema baseado em pastagem, temperatura da superfície do úbere, vaca leiteira

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INTRODUCTION

Dairy cattle farming is one of the agricultural activities that provide food to the population, and Brazil is one of the world's largest cow milk producers, with 35.4 million liters of milk produced in 2020. The State of Acre contributed 42.6 thousand liters to that amount, with the municipality of Sena Madureira producing 2.6 thousand liters (Produção…, 2020).

Animal welfare has been increasingly recognized in importance by consumers and producers. Heat stress is easily recognized in dairy cows (Dunshea *et al*., 2013), as it significantly affects daily milk production (Ekine-Dzivenu *et al*., 2020).

Therefore, animal comfort is essential in current production systems for ethical reasons and higher productivities (Matarrese, 2013).

In this context, new precision dairy farming technologies have been adopted to improve animal welfare and zootechnical indices in different-sized farms.

However, producers should be aware of diseases that affect the mammary gland, such as bovine mastitis, causing high economic losses, as it leads to a reduction in production and changes in milk composition, in addition to promoting spending on antibiotics (Rodrigues *et al*., 2018).

In addition, mastitis contributes negatively to the industrial dairy process, especially at high herd infection rates, limiting milk production by up to 50% and restricting cow productivity (Martins *et al*., 2015).

Therefore, early mastitis detection is critical to minimize losses in milk volume, ensure higher productivity, improve milk quality, and reduce economic losses, in addition to being fundamental for the effectiveness of the intramammary infection treatment (Sathiyabarathi *et al*., 2016).

Factors affecting comfort of dairy cattle under grazing systems and effects on animal performance, milk quality, and animal health play a key role in mastitis control and prevention. Such information allows farmers to choose the best control strategies to reduce cow udder infections and production costs, besides maintaining animal welfare. Thus, using tools that can detect it early is essential, given that mastitis is one of the main challenges for milk producers.

Thus, infrared thermography arises as an alternative to assess the impact of environmental factors on animal production, supporting decisions, promoting animal health and welfare, in addition to being able to perceive changes in the surface temperature of the mammary gland in response to different degrees of infection severity, similar to CMT (California Mastitis Test) (Leão *et al*., 2015).

In this sense, this study aimed to evaluate infrared thermography as a non-invasive diagnostic tool for early detection of subclinical mastitis in dairy cows raised on pasture in the Western Amazon.

MATERIAL AND METHODS

All animal management practices were approved by the Ethics Committee on the Use of Animals/UFAC (CEUA) (Process No. 23107.016410/2019-06). The study was carried out during the rainy Amazon winter period, from January to March 2020, in Sena Madureira, State of Acre, in the Western Amazon. Eight farms with an average area of 39 ha for the dairy production system were used to collect the data. Sena Madureira is located at latitude 09°03′57″ S and longitude 68°39′25″ W and 150 m above sea level. The climate is tropical, according to the Köppen classification. Throughout the year, temperatures range from 20 to 33 °C, rarely below 13 °C or above 37 °C (Condições…, 2021).

One hundred and thirteen crossbred lactating cows with the genetic profile Holstein x Girolando, Holstein x Nellore, and Holstein x Zebu and an average daily production of 4.8 L/day were evaluated on the eight farms. Data were collected three times (January, February, and March) 30 days apart. Milking is carried out manually on seven farms (87.5%) and mechanically on one (12.5%). Milking management had no modifications for this research to avoid stressful conditions. The cows were raised on pasture and brought to the pen only at milking time.

Production system consisted of extensive grazing, and the following forages were grown: koronivia grass (*Brachiaria humidicola*), spreading liverseed grass (*Brachiaria decumbens*), palisade grass (*Brachiaria brizantha* cv. Marandu and *Brachiaria brizantha* cv. MG-5), and scattered paspalum (*Paspalum conspersum*). The animals had access to mineral supplementation and water *ad libitum* throughout the experiment.

Three thermogram evaluations of the udder of 113 cows (*Bos taurus taurus* x *Bos taurus indicus)* were performed in the pen in the morning before milking (between 2:00 am and 6:00 am according to the farm routine). On each farm, the data were collected once a day (one day in January, another in February, and the last day in March 2020). Images were obtained using a FLIR E63900 T198547 infrared camera, with an emissivity of 0.95, thermal resolution of 160 x 120, temperature range from 20 to 250 °C, and accuracy of ±2% or 2 °C. Captures were made at one meter from the right lateral view of the entire body, forehead, eye, and hind leg and 0.6 m from the udder, both from the right and left antimere.

The images were captured before starting the milking process to avoid a possible change in the surface temperature of the animal's body after milking. The camera operator acquired at least ten images, thus ensuring a clear image, without any movement of the animal that could partially hide the udder. The images were analyzed by the FLIR Tools® software, which allowed importing, editing, and analyzing the images using polygons to assess the temperatures of desired areas.

A total of 452 mammary quarters of crossbred lactating cows (n=113) were performed using the California Mastitis Test (CMT) for the diagnosis of subclinical mastitis, following the methodology recommended by Simões *et al*. (2016). An appropriate tray was used, 2 mL of milk was milked from each mammary quarter, and about 2 mL of reagent was immediately added, mixing with circular movements, and visualizing the result by gelatinization.

Scores were used in the quantitative analysis to characterize the inflammatory reaction and in the qualitative analysis from the crosses according to the intensity to measure the degree of mastitis severity in the mammary quarters through CMT: score 0, no precipitation was formed (healthy); one teat = 0.25; two teats = 0.50; three teats = 0.75; four teats = 1.0. To evaluate the degree of gel formation was performed through the quantification of crosses. The numerical classification was performed aiming at the statistical analysis.

All processing was performed in the R programming language (R Core Team, 2019), in which the parameters were evaluated by generalized linear models (GLM) with a Gaussian distribution. Month- and farm-influenced parameters were evaluated by the Tukey's test at 5% significance level. Month-influenced parameters were individually evaluated on each farm.

RESULTS AND DISCUSSION

The udder surface temperature of the cows (*Bos taurus taurus* x *Bos taurus indicus*) evaluated with the infrared thermography technique had significant differences ($p<0.05$) between months of the year (January, February, and March 2020). The cows (*Bos taurus taurus* x *Bos taurus indicus*) that had an increase in udder temperature over the months were individually evaluated on each farm.

Increases in cow udder surface temperature may be correlated with the possibility of mastitis. According to Sathiyabarathi *et al*. (2016), the inflammatory response in mastitis is initially associated with an increase in udder temperature due to inflammatory processes in the mammary gland, characterized by pathological changes in the glandular tissue (Peres Neto and Zappa, 2011).

Similarly, Sathiyabarathi *et al*. (2016) carried out a study on a farm under subtropical climate conditions where the temperature rises to 36°C in the summer and reaches 15°C during the winter and found that the average udder surface temperatures of the affected quarters were 37.9 and 38.2°C, respectively. In the current research, the udder temperature obtained through thermographic imaging for cows (*Bos taurus taurus* x *Bos taurus indicus*) affected with subclinical mastitis ranged from 38 to 40°C (Fig. 1).

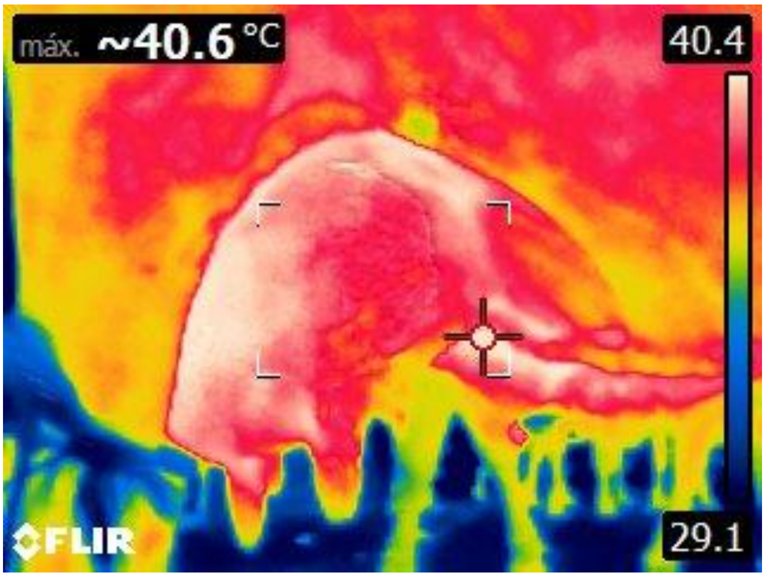

Figure 1. Measurement of cow's udder temperature (*Bos taurus taurus* x *Bos taurus indicus*) using thermographic imaging

The change in udder temperature of cows (*Bos taurus taurus* x *Bos taurus indicus*) captured through thermographic images was significant (p<0.05) by the California Mastitis Test (CMT) for cows that had subclinical mastitis. It evidences that the analysis of thermographic images can show changes in the udder surface temperature of dairy cows from extensive grazing systems diagnosed with subclinical mastitis through CMT (Table 1). Therefore, thermography can be applied to detect subclinical mastitis.

Table 1. Udder surface temperature obtained through thermographic images of 113 lactating crossbred cows (*Bos taurus taurus* x *Bos taurus indicus*) on eight farms from January to March in the municipality of Sena Madureira, State of Acre, Brazil

Month	Farm								Mean
	A	B	C	D	E	F	G	H	
January	38.4b	38.8b	38.8a	38.8a	38.5b	38.8a	38.8b	38.8a	38.7b
February	38.4b	39.3ab	38.3a	39.0a	38.7ab	38.9a	39.4ab	39.2a	38.9ab
March	39.5a	39.5a	38.6a	38.7a	39.2a	38.5a	39.6a	39.1a	39.0a
Mean	38.8A	39.2A	38.2A	38.9A	38.8A	38.7A	39.2A	39.0A	

Means followed by different lowercase letters in the column and uppercase letters in the row differ statistically by the Tukey test at a 5% probability (P<0.05).

The CMT results showed that 45 cows (39.8%) in January, 52 cows (46%) in February and 57 cows (50.4%) in March had subclinical mastitis. Farm H presented 29 cows with subclinical mastitis (25.7%) (Table 2). The low technological level of the studied farms and the excess of mud in the environment may be responsible for the higher prevalence of mastitis in lactating cows (*Bos taurus taurus* x *Bos taurus indicus*).

The high rate of mastitis found in this study corroborates that in Linhares *et al*. (2021). These authors found high incidence of mastitis of a clinical nature in eight farms (44%) when carrying out a study to analyze the applicability of some management tools, such as SWOT matrix, GUT matrix, brainstorming, PDCA, Ishikawa diagram, and 5W2H, in improving milk quality in 18 family-run farms in the city of Senador Guiomard in the State of Acre.

Table 2. Evaluation of the prevalence and incidence of subclinical mastitis using the California Mastitis Test (CMT) relative to the number of lactating crossbred cows (*Bos taurus taurus* x *Bos taurus indicus*) evaluated in 2020 in Sena Madureira, State of Acre, Brazil

Farm	January				February				March			
	Negative		Positive		Negative		Positive		Negative		Positive	
	Cow	%	Cow	%	Cow	%	Cow	%	Cow	%	Cow	%
A	7	70	3	30	6	60	4	40	5	50	5	50
B	5	50	5	50	5	50	5	50	5	50	5	50
C	13	100	0	0	13	100	0	0	12	92.3	1	7.7
D	3	30	7	70	6	60	4	40	5	50	5	50
E	13	92.8	1	7.2	10	71.4	4	28.6	10	71.4	4	28.6
F	8	72.7	3	27.3	7	63.6	4	36.4	5	45.5	6	54.5
G	7	77.8	2	22.2	7	77.8	2	22.2	7	77.8	2	22.2
H	12	33.3	24	66.7	7	19.4	29	80.6	7	19.4	29	80.6
Total (cows)	68	60.2	45	39.8	61	54	52	46	56	49.6	57	50.4

The high rate of mastitis (25.7%) on farm H may be due to its ineffective management, especially in the drainage system in the milking parlor. Water accumulation may generate mud with the trampling of the cows. Besides, this farm had a mechanical milking machine in its production system. Its malfunction and improper use can increase the risk of new mastitis cases by increasing bacterial transfers between cows while milking, increasing lesions on the skin and tip of the teats, and facilitating the introduction of bacteria through the teat canal (Gentilini and Santos, 2019).

Environmental hygiene may be one of the main factors contributing to the prevalence of mastitis in lactating cows on this property. According to Mariano *et al*. (2015) the microorganisms involved in this disease remain in the environment and infections usually occur between milking practices. According to Simões *et al*. (2016), the proliferation of bacteria in the udder by pathogens leads to an immediate inflammatory response and the cells migrate, causing an increase in somatic cells in the milk, negatively affecting milk quality.

In general, good milking practices for all farms (100%) are still a great challenge to become a reality in the region. In this region, culturally, the calf is released first, and only then does the cow have its legs tied when performing manual milking. In addition, producers claim that the process of carrying out prophylactic measures and mastitis detection takes time. Thus, producers do not perform the dark bottom cup test or post-dipping for the diagnosis of clinical mastitis, nor do they perform CMT for diagnosing subclinical mastitis, which are mastitis prevention and diagnosis practices.

This high mastitis rate probably occurred due to ineffective management because of the high rainfall in the Amazon winter (about 1500 mm) (Franca and Mendonça, 2016). Water accumulation leads to soil waterlogging and animal trampling turns it into mud, a preponderant factor for the occurrence of environmental mastitis. There may be a direct relationship between udder health and high air humidity. It may reflect on the survival and proliferation of pathogens due to the high environmental temperature in the region, with an average air temperature of 29.6°C at the milking time and 33 °C over the course of the day (Condições…, 2021).

Silva *et al*. (2020) conducted a study using principal component analysis for physiological and environmental thermohydrometric data correlated with the detection of subclinical and clinical mastitis in Capoeiras in the State of Pernambuco. The authors found temperature differences between cows with healthy udders (28.79°C) and those with subclinical (32.66°C) and clinical (37.82°C) mastitis, showing that animals with mastitis have the udder surface temperature affected by the disease.

Polat *et al*. (2010) compared infrared thermography and somatic cell count with CMT to identify subclinical mastitis. The authors reported that infrared thermography can be used to detect subclinical mastitis by measuring the udder surface temperature with a diagnosis similar to that of CMT because the CMT scores increased as the udder skin surface temperature (USST) increased linearly (USST, °C = 33.45+1.08 × CMT score; R^2=0.75 P<0.0001), as shown by infrared thermography.

CONCLUSION

During the experiment, the highest number of cows (*Bos taurus* x *Bos taurus indicus*) affected by subclinical mastitis was 57 and was observed in March. Among the farms evaluated, farm H had the highest number of affected cows. The animals were diagnosed using the California Mastitis Test – CMT.

The udder temperature of cows affected by mastitis captured through thermographic images ranged from 38 to 40 ºC. Infrared thermography is a technique that can be used for early detection of subclinical mastitis in dairy herds in a grazing system, as IRT detected an increase in udder surface temperature in the same cows that CMT was positive for subclinical mastitis.

Knowledge about the factors that affect dairy cattle raised in grazing systems and the effects that can cause physiological changes plays an essential role to prevent and control mastitis. Aware producers will be able to adopt management and prophylactic measures for decision-making.

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