UDDER THERMOGRAPHY OF GYR COWS FOR SUBCLINICAL MASTITIS DETECTION

TERMOGRAFIA DO ÚBERE DE VACAS GIR PARA DETECÇÃO DE MASTITE SUBCLÍNICA

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ABSTRACT

Aiming to evaluate the infrared thermography technique for early detection of subclinical mastitis in Gyr cows, 70 animals were selected in 2nd or 3rd lactation, with an average production of 7.2 kg / d between 90 and 200 days in milking. Images of two quarters (front and backside) of the same udder side were monthly performed during three months using a thermal camera to evaluate the surface temperature at three different heights (upper, median and lower), totalizing 420 measurements. Milk samples from each quarter were also collected to evaluate the SCC and microbiological culture. The SCC did not influence udder temperature at different heights ($p>0.05$). However, temperatures among the regions of the quarter were different, the upper area had higher values than the median and lower regions ($p<0.05$). There was no difference in udder temperatures in relation to the type of microorganisms isolated in milk ($p>0.05$). The use of thermal camera allowed the identification of temperature variations of skin surface at different udder regions of Gyr cows. However, this technique was not effective in the detection of subclinical mastitis.

Keywords: Gyr, Subclinical mastitis, Thermal camera, Dairy cows.

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RESUMO

Com o objetivo de avaliar a técnica de termografia infravermelho para detecção precoce de mastite subclínica em vacas da raça Gir, foram selecionadas 70 vacas de 2ª ou 3ª lactação, com produção média de 7,2 kg/dia, entre 90 e 200 dias de lactação. Imagens de dois quartos colaterais (anterior e posterior) do úbere, foram realizadas mensalmente, durante três meses utilizando uma câmera termográfica para avaliação da temperatura superficial em três alturas distintas (superior, medial e inferior), totalizando 420 avaliações. Também foram colhidas amostras de leite de cada quarto para avaliação da CCS e cultura microbiana. A CCS não influenciou a temperatura do úbere nas alturas estudadas (p>0,05). Porém, entre as regiões dos quartos houve diferença de temperatura, com valores mais altos na área superior (p<0,05) do que nas regiões medial e inferior. Não houve diferença de temperaturas no úbere em relação ao tipo de microrganismo isolado no leite (p>0,05). O uso da câmera termográfica permitiu identificar as variações de temperatura superficial da pele em diferentes regiões do úbere de vacas Gir. Porém, essa técnica não foi eficiente no diagnóstico da mastite subclínica.

Palavras-chaves: Câmera termográfica, Gir, Mastite subclínica, Vacas leiteiras.

INTRODUCTION

The use of Infrared Thermography has proved to be an interesting and non-invasive tool in veterinary research, since it is highly sensitive for detecting temperature changes of skin surface (SCOOT et al., 2000; HOVINEN et al., 2008). This technique involves the relationship between the variation in subcutaneous blood flow and the heat emission from the studied area, detected by the thermograph in a colorimetric scale correlated with changes in temperature (in Celsius degree).

This technique has been adopted for animal experimentation in order to evaluate the efficiency of the facilities in animal's welfare, the metabolic responses to heat stress, and also for the diagnosis of inflammation in body tissues (NIKKHAH et al., 2005; SCHAEFER et al., 2007).

It has also been successfully employed in the evaluation of meat quality in pigs (SCHAEFER et al., 1989), feathers cover in chickens (COOK et al., 2006), estrus detection (HURNIK et al., 1985) and the stress assessment in dairy cattle (STEWART et al., 2007).

Furthermore, it is extensively applied in human medicine used as a parameter for prevention of diseases and inflammation, as the organisms metabolically respond with temperature changes (SCOTT et al., 2000) and thus may reduce the cost of long-term treatments.

Mastitis is one of these infectious diseases that affect dairy herds and causes great damage to the milk productive chain. Mastitis is an udder inflammation that typically results in temperature rises of the affected area, followed by a reduction in milk secretion and changing in permeability of the membrane which separates the milk from the blood (BRAMLEY et al., 1996). HOVINEN et al. (2008) showed the capacity of the infrared thermography in the identification of temperature increases (> 1°C) in cow's udder with the clinical mastitis.

These factors suggest that the thermograph could assess the health status of the mammary gland by skin surface temperature. In mammary gland and in other organs in activity, there is heat transfer associated with the bloodstream (BHATTACHARYA & MAHAJAN, 2003). When there is pain (hypersensitivity), swelling, and hyperthermia in the initial phase of an inflammation and infection, the skin surface temperature may reflect the underlying metabolism of the bloodstream and target tissue (BERRY et al., 2003; PAULRUD et al. 2005).

Early diagnosis of subclinical mastitis could improve the quality and milk yield efficiency due to a reduction in costs with laboratory tests and more effective treatments in the early phase of infection (SANTOS & FONSECA, 2007). Besides the infrared thermography does not cause damage to animal's health, the technique is able to evaluate the welfare in different environmental conditions (COLAK et al., 2008). The use of thermal camera allowed the evaluation of changes in the skin surface temperature in different regions of Gir cows, similar to results described in others studies with different breeds (COLAK et al., 2008; HOVINEN et al., 2008). COLAK et al. (2008) using thermographs observed differences in udder temperatures in Brown Swiss and Holstein cows with subclinical mastitis in temperate climate. However, in a tropical climate and with the use of more adapted breeds, research with udder thermography is still scarce. Thus, the present study aimed to evaluate the infrared thermograph technique for early detection of subclinical mastitis in Gyr cattle.

Animals and Experimental protocol

Seventy Gir cows were used as experimental animals, in second or third lactation and average milk yield of 7.2 kg/d (SEM ± 0.3 kg) in post-peak (90 to 200 days in milking), which were sampled three times during a period of three months in two quarters of the same udder side (front and backside), totalizing 420 measurements.

Cows were handled in tropical pastures with free access to water and fed concentrate according to their state of lactation. Milkings were carried out at 6h00 and 16h00 following the standard sequence of procedures: clinical mastitis diagnosis, pre-dipping, dry of teats with paper towel, teat cups attachment, withdrawal after milking and post-dipping. Before the onset of milkings, cows were kept in a shaded waiting area with natural ventilation.

A tandem parlor with six stalls each side of the pit was used. The following machine parameters were constantly set throughout the experiment: vacuum = 45 kPa, pulsation ratio = 60:40% and pulsation rate = 60 c/min. The temperature and relative humidity were monitored on days of measurements, remaining in the range considered of thermal comfort for dairy cows (THI < 72) according to SILVA et al. (2009).

Individual milk yield was monthly measured and milk samples were collected for somatic cell counts (SCC) for three months. After, the thermal images were recorded by placing the camera about 50 cm distance from each quarter on the same side of the udder.

The experiment was performed according to the approved experimental protocol, under the permission given by the Ethics Committee for animal experiments of the University of São Paulo.

Milk sampling and Thermal Imaging

Milk samples and thermal images were taken only in two quarters of the same udder side (front and backside) according to the position of cows in the milking parlor (right or left). In order to determine the subclinical mastitis incidence, milk samples were collected during the early milking and stored in plastic bottles containing the preservative bronopol (Broad Spectrum Microtabs II, D & F *Control Systems Inc., Chaska*, MN, USA), and analyzed for SCC determination by the cell count fluoro-opto-electronic method (Somacount 300, Bentley, *Instrument Inc., Chaska*, MN, USA).

The SCC was then used in an indirect way to characterize subclinical mastitis. Cows with subclinical mastitis were considered when the CCS was above 200,000 cells / mL and with isolation of the pathogen. Clinical cases of mastitis were defined by the presence of abnormalities in milk suggestive of inflammation of the mammary gland, such as flakes, clots, or other unusual aspect.

These samples were not used for thermography analysis. Samples for microbiological culture were aseptically collected in the early milking and bacteriological tests performed according the National Mastitis Council (NMC, 1999). Cultures with more than one species of bacteria isolated were excluded from data analysis.

Thermal images were taken at the early milking, immediately after the milk samples, using an infrared thermographic camera (FLUKE TI 20™, *Fluke Corporation, Washington, EUA*), positioned 50 cm from the lateral side of the udder. After, in a colorimetric thermal mapping program, the elements (pixels) were interpreted resulting in the surface temperatures (^{o}C). The reading of pixels consists in a complex set of algorithms, designated by colors that exactly match the value of a temperature specified in the coordinates.

In Figure 1, it is possible to observe three areas designated according to the height of the udder in the dorsal-ventral direction in which the skin surface temperatures were measured.

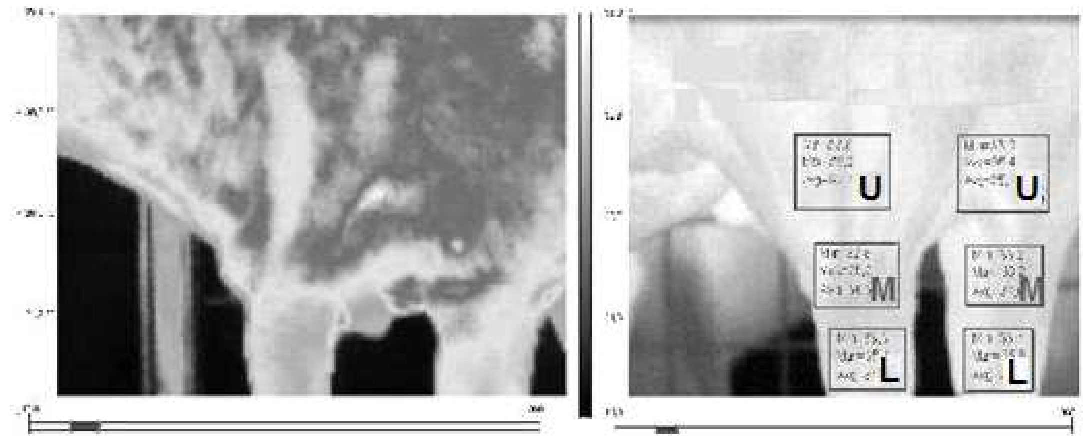

FIGURE 1 – Thermal images of the udder and skin surface temperature at three different heights: upper (U), median (M) and lower (L)

The thermal camera is able to perform measurements at a focal distance of at least 15 cm and presents objective lens of 20° in horizontal, 15° in vertical and its measurement capacity is from -10 to 350 ºC. The infrared spectrum has a wavelength between 1 mm to 700 ´m and frequencies between 300 GHz to 400 THz.

Statistical analyses

The design used was a completely randomized. It was evaluated the effect of udder skin surface temperature in different heights udder (upper, median and lower), the effect of SCC in four classes (< 10^5; 10^5 to $2x10^5$; $2x10^5$ to $3x10^5$ and > $3x10^5$ cells/mL) and the effect of pathogen type on the skin temperature on mammary quarter. The MINITAB™ software package (MINITAB™ *Statistical Software*, 2000) was used for analysis of data by means of ANOVA, based on GLM models Effects were separated by Tukey test. All values are given as the mean ± one standard error of the mean (SEM). The relationship between SCC measured in milk samples and udder skin surface temperatures was evaluated by Pearson correlation coefficients. Significance level was declared at $p<0.05$.

RESULTS AND DISCUSSION

The SCC means (LogSCC) and udder skin surface temperatures in three different heights are shown in Table 1.

TABLE 1 – Means (± SEM) of udder skin surface temperatures (°C) in three different heights in Gyr cows

SCC (cells/mL)	N	Udder skin surface temperature (°C)		
		Upper	**Median**	**Lower**
< 10^5	53	33.98 ± 0.02 aA	32.65 ± 0.05 aB	32.23 ± 0.06 aB
10^5 to $2x10^5$	32	34.00 ± 0.04 aA	32.36 ± 0.08 aB	32.52 ± 0.09 aB
$2x10^5$ to $3x10^5$	21	33.65 ± 0.12 aA	32.90 ± 0.15 aB	31.95 ± 0.05 aB
> $3x10^5$	104	34.11 ± 0.02 aA	32.59 ± 0.05 aB	31.57 ± 0.04 aB

Means followed by different letters within a column (small letter) and lines (capital letter) differ by Tukey test ($p<0.05$)

The log SCC did not influence udder skin surface temperatures in all heights (p>0.05). However, there was difference of temperature between the heights, with higher values in the upper area (p <0.05) than in the regions of the teats (median and lower). The skin surface temperature of the mammary gland may be influenced by several external factors such as humidity, temperature, ventilation and dirt on the udder (KUNC et al., 2007). In this case, the difference observed may be due to greater teat surface area exposed to the environment, thus allowing the exchange of heat by convection currents.

High levels of humidity and temperature in the milking parlor can increase the panting of animals and influence the temperature of external parts of the body (PORCIONATO et al., 2009). However, during the experimental period the THI values remained below 72, characterizing the thermoneutral conditions for dairy cows (SILVA et al., 2009).The minimum temperature recorded in the upper area of the udder was 31.17°C and the maximum 35.83°C. At the end of the teat, the minimum temperature recorded was 29.00°C and the maximum 34.80°C. Despite this high variation (Fig. 4), these results showed no significant correlation with SCC.

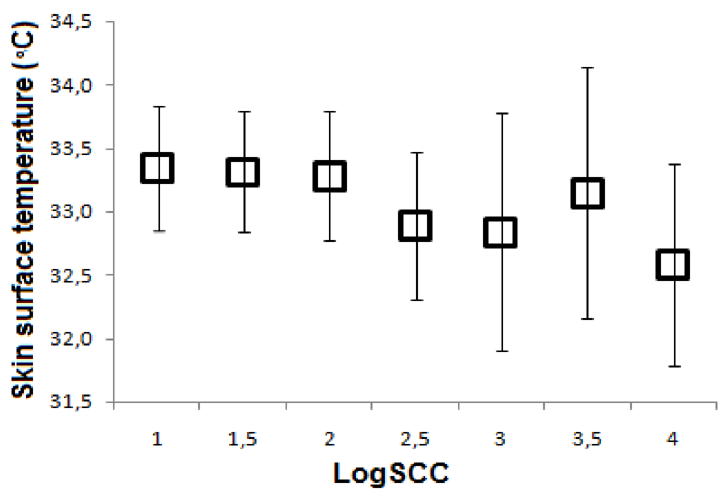

FIGURE 1 – Means of the skin surface temperature (℃) in relat ion to LogSCC

COLAK et al. (2008), studied the temperature of Holstein and Brown Swiss cows, using a thermal camera, and observed a high correlation (R^2 = 0.93, p<0.0001) between the SCC and the udder skin surface temperature with increases exceeding 1ºC as an indicator of mastitis, proposing the use of this technique as a complementary tool in early diagnosis of subclinical mastitis.

However, in this study there was no significant (p>0.05) correlation between log SCC and udder skin surface temperatures measured, or between the type of pathogens and udder skin temperatures in three different heights in Gyr cows (Table 2).

TABLE 2 – Correlation between LogSCC or mastitis pathogens and udder skin surface temperatures (°C) in three different heights in Gyr cows

LogSCC	Heights of the udder		
	Upper	**Median**	**Lower**
R^2	0.02	0.02	-0.14
P-value	*0.75*	*0.79*	*0.12*
Pathogens			
R^2	0.01	0.07	-0.01
P-value	*0.26*	*0.46*	*0.94*

HOVINEN et al. (2008) evaluated the variation in udder skin surface temperature after induction of experimental mastitis and suggested further studies under field conditions, where the systemic inflammatory reaction might be less pronounced and had no signs of swelling. Thus, in the present study, it was identified the prevalence of mastitis-causing pathogens in milk samples and related to the udder skin surface temperatures in Gyr cows (Table 3).

TABLE 3 – Means (±SEM) of udder skin surface temperatures in Gyr cows and the prevalence of mastitis pathogens (%) in the microbiological culture

Microbiological culture	Prevalence (%)	Udder skin surface temperature (ºC)
Negative	37.93	32.82 ± 0.02 a
Corynebacterium sp.	6.90	33.41 ± 0.12 a
Staphylococcus coagulase negative	20.39	33.53 ± 0.04 a
Staphylococcus aureus	34.48	33.95 ± 0.14 a
Streptococcus dysgalactiae	0.30	32.68 ± 0.06 a

Means followed by different letters within a column differ by Tukey test ($p<0.05$)

There was no difference between the udder skin surface temperatures in relation to the type of mastitis pathogen isolated in the microbiological culture of milk ($p> 0.05$). The cases studied refer to subclinical mastitis, suggestive of inflammatory reactions in early phase or not much pronounced, unable to influence the subcutaneous blood flow in the capillaries and therefore reflect changes in skin temperature of the udder (HOVINEN et al., 2008).

CONCLUSION

The use of thermal camera allowed the identification of variations in skin surface temperature in different heights of the udder of Gyr cows. However, this technique was not effective in the diagnosis of subclinical mastitis.

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