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Skin surface temperature of the mammary gland, measured by infrared thermography, in primiparous Girolando cows fed diets containing different lipid sources

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Abstract

This research communication describes the influence of diet, mammary quarter position and milking process on the temperature of teats and udder of cows fed diets containing different lipid sources. Five primiparous cows were fed diets containing cottonseed, sunflower seed, soybeans or soybean oil as a source of lipids and a reference diet without the inclusion of lipid sources in a 5×5 Latin Square design. Milk yield was determined in the last five days of each period. Milk samples were collected for SCC analysis on the last two days of each experimental period. The images of the mammary gland were obtained using an infrared camera and were analyzed with appropriate computer software. Milk yield was 14.8% higher for cows fed soybeans as a source of lipids. Diets and somatic cell counts did not influence the temperature of teats and udder. The milking process reduced the temperature of teats and udder by 0.79°C. Rear teats and rear quarters had higher surface temperatures than front teats and fore quarters. Changes in temperature of teats and mammary quarters occurred as a function of the milking process and quarter position. However, the diet and the SCC did not influence the temperature of teats and mammary quarters in this experiment.

Dietary supplementation, aiming at increasing livestock productivity, leads to different physiological responses in animals as a function of factors such as lactation stage and genotype. Supplementing dairy cows with lipid sources provides better efficiency of energy use, due to the reduction of the caloric increment and increase of the productive efficiency of milk by the direct incorporation of dietary fat into milk. Increased milk production may result in increased temperature of the mammary gland because the larger volume of milk causes compression of the blood vessels and also higher producing cows tend to have higher blood flow in this region (Cunningham, 2014).

Lipid supplementation increases the energy density of the diet due to the content of the total dry extracts being favorable for food efficiency of animals in a state of limited consumption, as in caloric stress, late third of gestation or beginning of lactation, moments in which a nutritional energy deficit occurs. According to Hammami *et al.* (2015), cows under thermal stress remain lying down for longer time in order to control the temperature of the body. Contact of the teats with soil and dirt may increase susceptibility to intramammary diseases and, in addition, high temperatures of the mammary gland are favorable to the proliferation of pathogenic microorganisms (Das *et al.*, 2016). This analysis is expanded together with additional references in the online Supplementary File.

In this sense, the use of oilseed grains in dairy cows has an important role in controlling the temperature of the mammary gland. In addition to promoting changes in production that can change the temperature of the mammary gland by itself, they also reduce thermal stress, the need to dissipate heat through contact with the soil and consequently the risk of infection that may increase the temperature of the mammary gland.

For successful livestock production, physiological parameters must be monitored for detection of slight changes that might impact animal health and welfare. The quality of animal product depends on the farmer's ability to react to changes in physiological parameters in livestock. Among the physiological data, temperature is easy to measure, and correlates with several body functions such as nutrition, reproduction and stress. The thermal radiation emitted by the body would be expected to vary as a result of changes in blood flow and metabolism (Sellier *et al.*, 2014). Variation in body temperature may be related to productivity; lactating dairy cows are less able to dissipate heat because they require higher dry matter intake, increasing nutrient metabolism. There are several methods for measuring the body temperature of lactating cows. However, Leão *et al.* (2015) stated that non-destructive and non-invasive methods of temperature measurement can generate reliable data without the need for direct contact, thus avoiding stress. Therefore, infrared thermography becomes a potential tool in livestock production for diagnosis, prevention, and association with characteristics of clinical and economic interest.

Several studies have demonstrated the efficiency of using this tool in studies of production and well-being in dairy cattle and goats (Digiacomo *et al.*, 2014; Roberto *et al.*, 2014). Our hypothesis was that the addition of different lipid sources could alter the temperature of the mammary gland and its components. The aim of this research was to evaluate the influence of diet, mammary quarter position and milking process on the temperature of teats and udder of cows fed diets with different lipids sources.

Materials and methods

The experiment was carried out at the Experimental Farm of the Dom Bosco Catholic University, Campo Grande, Brazil, from August 14 to October 22, 2016. This study was approved by the Ethics Committee on Animal Use under the protocol no. 011/2016. A detailed account of methodologies is provided in the online Supplementary File.

Animals, housing and feeding

Five Girolando primiparous cows were used, with an average body weight of 422 kg at the beginning of lactation with an average of 35 ± 8 d of lactation at the beginning of the experiment and 105 at the end of the total experiment period. The cows used were certified free of mastitis before the beginning of the experiment. They were kept in individual pens outdoors with artificial shading, without artificial temperature and humidity control and with free access to water and mineral supplementation.

The cows were weighed after 16 h of fasting on the first day of each period. The diets were supplied as total mixed ration after milking at 6 am and 4 pm. The diets consisted of 400 g/kg of corn silage and 600 g/kg of concentrate (being partially replaced by the lipid source until reaching the desired ether extract content). The amount offered was estimated on an intake of 3.5% of the body weight and adjusted to allow 5% of leftovers. Diets were formulated according to meet recommendations for a dairy cow producing 18 kg of milk per day, with 180 g/kg of crude protein and 70 g/kg of ether extract in diets added with lipid sources (NRC, 2001).

Experimental design

The cows were distributed in a 5 × 5 Latin Square experimental design with five animals and five treatments (diets) for five periods of 14 d, totaling 70 d. The cows went through a pre-experimental period of 14 d for adaptation to the diet and management. The treatments consisted of diets containing different oilseeds (sunflower, soybeans, and cottonseed), a diet containing soybean oil and a reference diet (without additional lipid source).

The cows were milked twice a day at 5 am and 3 pm using a mechanical bucket-type milking machine with a vacuum set at 40 kPa. The time spent for each milking, with the milking machine attached to the teats, was approximately five minutes per cow. Milk yield measurements were performed in the last three days of each period. The values obtained in the morning and afternoon milk weighing were summed and considered as the daily production. A 25 ml aliquot of milk from each milking on the last two days of each period was collected and stored at 4°C with Bronopol-B2 until the time of analysis of SCC, protein, fat, lactose, urea and total solids of milk.

The thermographic images were obtained on the last day of each experimental period at the morning milking, in a masonry milking room covered with clay tiles, without temperature and humidity control, using the infrared camera Flir SC 620®. This camera converts the radiation emitted by the animal's skin to a wavelength of 8–12 mm in an electrical signal that is then processed to a pattern of skin temperature variation, with accuracy of ±1°C. Infrared photographs were taken 1 meter away from the mammary gland, before and after milking, on both sides of the cow to get an image of the four udder quarters. The camera was calibrated each day of sampling for room temperature and relative humidity. The images were analyzed with FLIR QuickReport 1.2 software to demarcate the largest possible area of the udder and teats to obtain the average temperature. The average ambient temperature of the periods varied between 19.5 and 20.8°C.

Statistical analysis

Data are presented as means ± SEM. Milk yield, somatic cell count (SCC), mean udder and teat temperatures were analyzed using the ExpDes.pt package from the software R. The Shapiro–Wilk's test was used to determine the normality of the data and Bartlett's test to verify the homogeneity. After meeting assumptions, an analysis of variance of a 5 × 5 Latin square was performed. The Dunnett's multiple comparison test was applied to compare means at a significance level of 5% according to the mathematical model

$$Y_{ijk} = \mu + A_i + P_j + T_k(i, j) + e_{ijk},$$

where Y_{ijk} = value of the dependent variable; μ overall average; A_i = effect of the animal; P_j = period effect; $T_k(i, j)$ = effect of treatment within each animal and period; e_{ijk} = experimental error.

The surface skin temperatures of the fore quarters, rear quarters, front teats, rear teats, udder before milking, udder after milking, teats before milking and teats after milking were analyzed using the ExpDes.pt package from the software R. An ANOVA was performed for a 2 × 5 factorial design in a randomized block design, in which the blocks were the periods and the cows were the replicates. The same was performed to evaluate the position of the teat and mammary quarter. For the comparison of means, the Tukey's test was used at 5% significance using the mathematical model

$$Y_{ijk} = \mu + B_i + T_j + V_k + T_{jx}V_k + e_{ijk},$$

where Y_{ijk} = value of the dependent variable; μ overall average; B_i = block; T_j = effect of diet; V_k = effect of place (front and rear quarters) or milking (pre and post); $T_{jx}V_k$ = interaction between diet and local or milking; e_{ijk} = experimental error.

Table 1. Mean and standard error of the mean (SEM) of the milk yield, milk Somatic Cell Count (SCC) and temperature of teats and udder of cows fed diets with different lipids sources

	Diets					
	Reference diet	Cottonseed	Sunflower seed	Soybeans	Soybean oil	*P* Value
Milk (kg/d)	12.70 ± 0.28	11.40 ± 1.21	13.42 ± 0.29	14.58 ± 2.24*	12.70 ± 0.28	0.029
SCC (×1000/ml)	21.15 ± 2.76	34.00 ± 5.48	51.90 ± 12.75*	23.38 ± 4.76	33.40 ± 7.96	0.050
Udder (°C)	33.25 ± 0.41	33.87 ± 0.47	33.77 ± 0.25	33.98 ± 0.59	33.89 ± 0.63	0.835
Teats (°C)	32.90 ± 0.32	32.27 ± 1.00	32.58 ± 0.61	32.78 ± 0.32	32.25 ± 0.46	0.512

*Value within a row with different superscripts differ significantly of reference diet by the Dunnett's test ($P < 0.05$).

Table 2. Mean and standard error of the mean (SEM) of the temperature of skin surface of teats udder and mammary quarters as a function of diets milking process and mammary quarter position of cows fed diets with different lipids sources

	Milking			
	Teats		Udder	
Diets	Before	After	Before	After
Reference diet	32.98 ± 0.58	32.78 ± 0.47	33.91 ± 0.56	33.13 ± 0.48
Cottonseed	32.75 ± 0.90	31.75 ± 1.28	34.03 ± 0.34	33.72 ± 0.61
Sunflower seed	33.56 ± 0.35	31.74 ± 1.02	34.37 ± 0.33	33.16 ± 0.46
Soybeans	33.08 ± 0.23	32.44 ± 0.59	34.23 ± 0.66	33.74 ± 0.61
Soybean oil	32.39 ± 0.54	32.06 ± 0.50	34.52 ± 0.73	33.26 ± 0.56
Mean	32.95 ± 0.24[a]	32.16 ± 0.35[b]	34.21 ± 0.23[a]	33.40 ± 0.23[b]
P Value				
Diets	0.579		0.777	
Milking	0.016		0.002	
Interaction	0.506		0.653	
	Position			
	Teats		Mammary Quarters	
Diets	Fore	Rear	Fore	Rear
Reference diet	32.64 ± 0.42	33.16 ± 0.45	32.64 ± 0.42	33.16 ± 0.45
Cottonseed	31.68 ± 1.07	32.86 ± 0.97	31.68 ± 1.07	32.86 ± 0.97
Sunflower seed	32.32 ± 0.64	32.84 ± 0.61	32.32 ± 0.64	32.84 ± 0.61
Soybeans	32.57 ± 0.37	32.99 ± 0.30	32.57 ± 0.37	32.99 ± 0.30
Soybean oil	31.96 ± 0.65	32.53 ± 0.42	31.96 ± 0.65	32.53 ± 0.42
Mean	32.23 ± 0.29[b]	32.88 ± 0.25[a]	33.27 ± 0.20[b]	34.34 ± 0.24[a]
P Value				
Diets	0.219		0.836	
Position	0.005		<0.001	
Interaction	0.800		0.748	

[a,b]The value in a line with different letters differs significantly between treatments by the Tukey's test ($P < 0.05$).

Results

The data referring to the averages of the five periods for each treatment are shown in Table 1. A difference in milk yield of plus 14.8% ($P < 0.05$) was observed for cows fed soybeans in relation to cows fed the diet without lipid inclusion. The SCC values were higher ($P = 0.050$) in the milk of cows fed sunflower seed when compared to cows fed the diet without lipid inclusion. Diets did not affect ($P > 0.05$) udder or teat temperatures when

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they were evaluated for milking process (before and after) (Table 2). The temperature of teats was 0.8°C higher ($P = 0.01$) before milking and the same increase was observed for the skin surface temperature of the udder ($P < 0.01$).

Diets did not affect ($P > 0.05$) udder or teat temperatures when they were evaluated at different sites in fore and rear quarters. The position of teats and mammary quarters influenced their temperature. The temperatures of the rear teats and rear quarters were 0.64°C ($P < 0.01$) and 1.07°C ($P < 0.001$) higher, respectively, when compared to front teats and fore quarters.

Discussion

Cows fed soybeans as the lipid source had higher milk yield. The average milk yield is similar to the reported by Canaza-Cayo *et al.* (2017) of 11.00 l/d for Girolando cows in the first lactation. This milk yield associated with proper handling and husbandry kept the SCC within accepted standards. The diet influenced the SCC, but the counting remained low throughout the experiment. The highest SCC milk count in cows fed sunflower seeds as a source of lipid was below 200 000. The appearance of mastitis in a single cow receiving a diet with sunflower grain was responsible for the increase in the average SCC of this treatment According to Pantoja *et al.* (2009), visibly normal mammary quarters producing milk containing over 200 000 SCC are considered carriers of subclinical mastitis. The SCC did not interfere with udder and teat temperatures. This result corroborates that presented by Nakagawa *et al.* (2016), who evaluated the surface temperature of the udder of dairy cows and found no changes in temperature as a function of SCC.

The teat temperature decreased from 32.95 to 32.16°C after milking. The use of lower vacuum pressure (40 kPa) in the present experiment may have been one of the causes of no increase in temperature of the teats after milking. Hanusová *et al.* (2016) reported an increase in the temperature of teats after milking, of approximately 2.0°C, even at 40 kPa pressure. This result may be related to the difference in breed and age of cows evaluated (primiparous Girolando vs. multiparous Holstein cows).

Udder skin temperature was higher before milking; this result can be justified by the compression of the blood vessels as a result of milk accumulation in the mammary gland cistern and, possibly, by increased blood flow in that period (Cunningham, 2014). However, it is important to recognize that blood supply to the functional mammary gland (which can be measured) is distinct from the peripheral supply to the skin of the udder (which cannot easily be measured). Another possible cause is the locomotion of the cows from the pens to the milking parlor, generating heat through movement and friction between the hind legs and the mammary gland. This result differs from those found by Daltro *et al.* (2017), who stated that there were no changes in udder temperature as a result of milking. The lower milking time and the reduction of blood flow in teats in relation to high-yielding cows are factors that may have contributed to the reduction of teat temperature after milking, in addition to the emptying of the mammary gland, low vacuum of the milking machine and a longer interval between the movement and exposure to solar radiation at the time of temperature measurement.

The higher temperature in the rear quarters of the mammary gland observed in this experiment agrees with Daltro *et al.* (2017). The authors reported that the position of the mammary quarters correlates with skin surface temperature and it is considered the second most relevant factor for its alteration. The rear teats and mammary quarters had higher average temperature when compared to the front teats and fore quarters, this temperature variation is associated with greater exposure of the fore mammary quarters to the environment, allowing greater temperature change. Other factors may contribute to a higher temperature in the rear quarters, such as higher volume of milk storage and production, as well as greater contact with the hind legs.

Milk yield did not influence the skin surface temperature of the mammary gland. It is possible to state that the higher temperature observed in the rear quarters of the mammary gland is associated with less exposure to the environment, greater contact with venous irrigation in the hind legs and friction between them during the locomotion.

In conclusion, the inclusion of lipid source in the diet did not change the temperature of the mammary gland showing that these sources can be used in the nutrition of dairy cows without causing thermal discomfort in the mammary region of cows. The changes observed in this study are related to the process of milking and positioning of the udder quarters in relation to the body of the cow and not to the sources of lipids used.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029920000333.

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