

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

Alysson M Wanderley Luís CV Ítavo, Geraldo T dos Santos, Camila CBF Ítavo, Alexandre M Dias, Rodrigo G Mateus, Luiz C Pereira, Ariadne B Gonçalves, Endyara S Kohl and Celso RC Lima

### **5 INTRODUCTION**

6 The quality of milk in Brazil varies widely as result of different production systems, with a  
7 low number of qualified farmers and a large number of farmers with little or no expertise,  
8 mainly regarding the nutritional management of herds (Gabbi et al. 2013). Milk quality  
9 primarily comprises the centesimal evaluation of fat, protein, and total solids, but health-  
10 related factors such as the Somatic Cell Count (SCC) to detect mastitis are also evaluated  
11 (Vargas et al. 2014). Besides indicating sanitary problems, a high SCC also results in  
12 nutritional depreciation of the milk. Malic et al. (2018) reported a negative correlation  
13 between SCC and milk yield, as well as changes in lactose, fat, protein and total solids, which  
14 may in some cases render the milk unfit for human consumption.

15 According to Voltolini et al. (2001), the use of SCC is not enough for adequate  
16 detection of mastitis because SCC is based on leukocytes and epithelial cells, which makes it  
17 incapable of characterizing an infection of the mammary gland by itself. Hovien et al. (2008)  
18 demonstrate that the temperature measurement can be used as a method of mastitis detection  
19 as a result of the increased blood flow and redness caused by this infection. Milk quality can  
20 be improved by modifications in cow's diet (Pecka et al. 2013). For instance, the supply of  
21 oilseeds in the ruminant diet leads to better-quality milk fat, which can help prevent some  
22 types of cancer, obesity, diabetes and cardiovascular diseases (Kratz et al. 2013; Lahlou et al.  
23 2014).

24 Deitary supplementation, aiming at increasing livestock productivity, leads to  
25 different physiological responses in animals as a function of factors such as lactation stage  
26 and genotype (Horan et al. 2005). Supplementing dairy cows with lipid sources provides  
27 better efficiency of energy use, due to the reduction of the caloric increment and increase of  
28 the productive efficiency of milk by the direct incorporation of the fat of the diet into the milk  
29 (NRC, 2001; Palmquist e Mattos, 2011). This supplementation increases the energy density of  
30 the diet due to the content of the total dry extracts being favorable for food efficiency of  
31 animals in a state of limited consumption, as in caloric stress, late third of gestation or  
32 beginning of lactation, moments in which a nutritional energy deficit occurs (Paula et al.  
33 2012). In this sense, the use of oilseed grains in dairy cows has an important role in

34 controlling the temperature of the mammary gland. According to Hammami et al. (2015)

35 cows under thermal stress remain for longer time in order to control the temperature of the  
36 body and with this increase the contact of the ceilings with soil soils becoming more  
37 susceptible to intramammary diseases, in addition, high temperatures of the mammary gland  
38 are favorable to the proliferation of pathogenic microorganisms (Das et al., 2016).

39 For successful livestock production, physiological parameters must be monitored for  
40 detection of slight changes that impacts animal health and welfare. The quality of animal  
41 products depends on the farmer's ability to react to changes in physiological parameters in  
42 livestock. Among the physiological data, temperature is easy to measure, and it correlates  
43 with several body functions such as nutrition, reproduction, stress and sanity. Consequently,  
44 the thermal radiation emitted by the body varies as a result of changes in blood flow and  
45 metabolism (Roberto et al. 2014; Sellier et al. 2014).

46 The variation in body temperature may be related to the productivity of the cows;  
47 lactating dairy cows are less efficient in dissipating heat because they require higher dry  
48 matter intake, increasing nutrient metabolism (Kadzere et al. 2002). There are several  
49 methods for measuring the body temperature of lactating cows. However, Leão et al. (2015)  
50 stated that non-destructive and non-invasive methods of temperature measurement can  
51 generate reliable data without the need for direct contact, thus avoiding stress. Therefore,  
52 infrared thermography becomes a potential tool in livestock production for diagnosis,  
53 prevention, and association with characteristics of clinical and economic interest. Several  
54 studies have demonstrated the efficiency of using this tool in studies of production and well-  
55 being in beef cattle, dairy cattle, goats, and sheep (Digiacomio et al. 2014; Silva et al. 2004).  
56 The aim of this research was to evaluate the influence of diet, mammary quarter position and  
57 milking process on the temperature of teats and udder of cows fed diets with different lipids  
58 sources.

59

## 60 **MATERIALS AND METHODS**

61 The experiment was carried out at the Experimental Farm of the Dom Bosco Catholic  
62 University, Campo Grande, Brazil, from August to October 2016. This study was approved  
63 by the Ethics Committee on Animal Use under the protocol nº 011/2016. The analyses were  
64 performed at the Biotechnology Laboratory Applied to Animal Nutrition - Dom Bosco  
65 Catholic University, Laboratory of Applied Nutrition - Federal University of Mato Grosso do  
66 Sul and Laboratory of the Dairy Herd Improvement Association of Paraná - Paraná Holstein  
67 Breeders Association.

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### **69 *Animals, Housing, and Feeding***

70 Five Girolando primiparous cows with an average body weight of 422 kg were tagged,  
71 drenched and vaccinated at the beginning of lactation and were kept in individual pens with  
72 free access to water and mineral supplementation. Cows were fed corn silage, concentrate and  
73 a source of lipid. The offered was estimated on an intake of 3.5% of the body weight and  
74 adjusted to allow 5% of leftovers. The cows were distributed in a 5 × 5 Latin Square  
75 experimental design with five animals and five treatments (diets) for five periods of 12 days,  
76 totaling 60 days. The cows went through a pre-experimental period of 12 days for adaptation  
77 to the diet and management. The treatments consisted of diets containing different oilseeds  
78 (sunflower, soybeans, and cottonseed), a diet containing soybean oil and a reference diet  
79 (without additional lipid source) (Table 1).

80 Diets were formulated according to meet NRC (2001) recommendations for a dairy  
81 cow producing 18 kg of milk per day. The experimental diets were isonitrogenous and  
82 isolipidic, targeting an ether extract content of 70 g/kg of dry matter (DM). The cows were  
83 weighed after 16 hours of fasting on the first day of each period. The diets were supplied as  
84 total mixed ration after milking at 6 and 16 h.

85

### **86 *Milk yield and Milk Somatic Cell Count***

87 Cows were milked twice a day at 5 and 16 h using a mechanical milking machine set at 40  
88 kPa. Milk yield measurements were performed in the last five days of each period. These  
89 values were obtained by weighing the milk produced by the cow in each milking (morning  
90 and afternoon). Then, the daily milking was calculated. Sampling for milk analysis was  
91 performed on the last two days of each experimental period. The milk samples were sent to  
92 the Central Laboratory of the Dairy Herd Improvement Association of Paraná - Paraná  
93 Holstein Breeders Association for analyzing the composition and somatic cell count (SCC).

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### **95 *Image Collection***

96 The thermographic images were obtained on the last day of each experimental period using  
97 the infrared camera Flir SC 620. This camera converts the radiation emitted by the animal's  
98 skin to a wavelength of 8-12 mm in an electrical signal that is then processed to a pattern of  
99 skin temperature variation, with accuracy up to  $\pm 1^{\circ}\text{C}$ . The camera was calibrated each day of  
100 sampling for room temperature and relative humidity. The emissivity used was 0.98 as  
101 recommended by the manufacturer for biological tissues. Five images were taken per cow and  
102 the image with the best focus and exposure of the mammary gland was chosen. The distance

103 between the camera and the point where the image was captured was standardized at one  
 104 meter. The images were analyzed with FLIR QuickReport 1.2 software to demarcate the  
 105 largest possible area of the udder and teats to obtain the average temperature.

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### 107 ***Variables and Statistical Analysis***

108 Milk yield, somatic cell count (SCC), mean udder and teat temperatures were analyzed using  
 109 the ExpDes.pt package from the software R. The Shapiro-Wilk test was used to determine the  
 110 normality of the data and Bartlett's test to verify the homogeneity. After meeting assumptions,  
 111 an analysis of variance of a  $5 \times 5$  Latin square was performed. The Dunnett's multiple  
 112 comparison test was applied to compare means at a significance level of 5% according to the  
 113 mathematical model  $Y_{ijk} = \mu + A_i + P_j + T_{k(i,j)} + e_{ijk}$ , where:  $Y_{ijk}$  = value of the dependent variable;  $\mu$   
 114 = overall mean;  $A_i$  = effect of the cow;  $P_j$  = effect of the period;  $T_{k(i,j)}$  = effect of the treatment  
 115 nested within each cow and period;  $e_{ijk}$  = experimental error.

116 The surface skin temperatures of the fore quarter, rear quarter, front teat, rear teat,  
 117 udder before milking, udder after milking, teat before milking and teat after milking were  
 118 analyzed using the ExpDes.pt package from the software R. An ANOVA was performed for a  
 119  $2 \times 5$  factorial design in a randomized block design (2 sites - fore or rear quarters and 5 diets),  
 120 in which the blocks were the periods and the cows were the replicates. The same was  
 121 performed to evaluate the position of the teat and mammary quarter. For the comparison of  
 122 means, the Tukey's test was used at 5% significance according to the mathematical model  
 123  $Y_{ijk} = \mu + B_i + T_j + V_k + T_{j \times V_k} + e_{ijk}$ , where  $Y_{ijk}$  = value of the dependent variable;  $\mu$  = overall mean;  
 124  $B_i$  = effect of block;  $T_j$  = effect of diet;  $V_k$  = effect of the position (fore and rear quarters) or  
 125 milking (before and after);  $T_{j \times V_k}$  = interaction between diet and position or milking;  $e_{ijk}$  =  
 126 experimental error.

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