INFLUENCE OF SOMATIC CELL COUNT IN THE COMPOSITION OF GIROLANDO COW'S MILK IN TROPICAL ZONE

[INFLUENCIA DE RECUENTO DE CÉLULAS SOMÁTICAS EN LA COMPOSICIÓN DE LA LECHE DE VACAS GIROLANDO EN LA ZONA TROPICAL]

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SUMMARY

Bovine mastitis has been identified as the main disease affecting dairy cattle worldwide. Somatic Cell Count (SCC) in milk is one of the most important indicators to evaluate the udder health of cows due to the high direct correlation with the mammary gland's degree of infection. This study aimed to evaluate the different ranges of somatic cell count (SCC) on the composition of bovine milk as well as finding a correlation between somatic cell count and body condition score on milk production and composition of this species. The experiment was conducted on a commercial farm located in São José de Mipibu, Rio Grande do Norte, Brazil. The same cows were milked mechanically, obtaining a milk production record for the period of December 2011 to May 2012. For this, 24 Girolando breed cows (3/4 and 7/8) were used, being 50% primiparous and 50% multiparous with average production 7.51 ± 2.58 kg day^{-1} and 10.98 ± 2.49 kg day^{-1}, respectively. The cows were milked mechanically, obtaining a record of milk production over a period of five months, and milk samples were collected and sent for laboratory analysis. The levels of milk composition were evaluated. Lactose, non-fat solids and milk urea nitrogen were influenced by different intervals of somatic cell count of milk. In milk samples from primiparous and multiparous cows, positive correlations between somatic cell count and some components were found. As for body condition score, significant correlations were also found for milk production and composition. It was concluded the different levels of somatic cell count influenced the percentage of lactose, non-fat solids and milk urea nitrogen. Somatic cell count and body condition score also showed significant correlations with milk production and composition.

Keywords: lactose; mastitis; milk quality; production system.

RESUMEN

La mastitis bovina ha sido identificada como una de las principales enfermedades que afectan la producción de leche de vacuno en el mundo. El recuento de células somáticas (RCC) en la leche es uno de los indicadores más importantes para evaluar la salud de la ubre vacas debido a la alta correlación directa con el grado de infección de la glándula mamaria. Este estudio tuvo como objetivo evaluar los diferentes rangos de recuento de células somáticas (RCC) en la composición de la leche y la correlación entre el recuento de células somáticas y la condición corporal sobre la producción y composición de la leche. El experimento se llevó a cabo en una granja comercial ubicada en San José de Mipibu, Rio Grande do Norte, Brasil. Las mismas vacas fueron ordeñadas mecánicamente, obteniendo el registro de producción de leche a partir de diciembre / 2011 a mayo/2012. Con este fin, las 24 vacas mestizas Girolando (3/4 y 7/8) fueron usados, 50% y 50% multíparas primíparas, con una producción promedio de 7.51 ± 2.58 kg día^{-1} y 10.98 ± 2.49 kg día^{-1}, respectivamente. Las vacas fueron ordeñadas mecanicamente y se obtuvieron los registros de producción de leche durante un período de cinco meses em el cual se colectaron muestras de leche y para análisis de composición. La lactosa, sólidos no grasos y nitrógeno ureico en leche fueron influenciados por los diferentes rangos de recuento de células somáticas de la leche. Para todas las muestras de vacas primíparas y multíparas, se identificó una correlación positiva entre el número de células somáticas y algunos componentes de la leche. Para la condición corporal, la correlación significativa también se encontró para la producción de leche y su composición. Se concluye que los diferentes niveles de recuento de células somáticas influyeron en el porcentaje de lactosa, urea en leche, sólidos no grasos y nitrógeno. El recuento de células somáticas y la condición corporal también mostraron correlación significativa con la producción y composición de la leche.

Palabras clave: lactosa; mastitis; calidad de leche; sistemas de producción.

INTRODUCTION

Knowledge of factors that affect milk quality, as well as changes in physical, chemical or microbiological aspects of the product becomes extremely important (Araújo *et al*., 2012; Ribeiro Neto *et al*., 2012).

Among other factors, the colonization of the mammary gland by microorganisms is often a modifier of milk quality by altering the properties and decreasing the quality of the product (Langoni *et al*., 2011). Low quality milk causes great economic losses to the productive sector and represents a risk to public health, in addition to barring entry into more lucrative markets and undermining the credibility of the milk chain as a whole (Sant'anna and Paranhos Da Costa, 2011).

Bovine mastitis has been identified as the main disease affecting dairy cattle worldwide, causing serious economic losses to both producers of milk and the dairy industry (Philpot and Nickerson, 1991; Sharma *et al*., 2011). The colonization of the bovine mammary gland by pathogenic bacteria results in a series of events that lead to changes in milk composition (Nobrega and Langoni, 2011; Rhoda and Pantoja, 2012). According to Ballou (2012), the main factors related to the change of milk components are injuries to milk producing cells, which may result in changes in the concentration of lactose, protein, fat and increased vascular permeability, which determines the increase of nutrients passage from the blood to milk, such as electrolytes, immunoglobulins and other serum proteins.

Several methods have been developed seeking the diagnosis of mastitis. Somatic Cell Count (SCC) in milk is one of the most important indicators to evaluate the udder health of cows due to the high direct correlation with the mammary gland's degree of infection (Ruegg, 2003; Rangel *et al*., 2013).
The objective was to evaluate different intervals of somatic cell count (SCC) on the composition of bovine milk, as well as to obtain the correlations between somatic cell count and body condition score on the production and chemical composition of milk.

MATERIAL AND METHODS

Herd selection

The experiment was conducted on a commercial farm located in São José de Mipibu, 36 km south of the city Natal, Rio Grande do Norte (06 ° 04'30,0 "S and 35 ° 14'16,8" W, and 58 m altitude). The climate according to the Köppen classification is "As", meaning a tropical climate with a dry season. The rainy season runs from April to June with an average rainfall of 855 mm per year. The average temperature is 25.3°C and the average relative humidity is 79.0% (IDEMA, 2011.)

Twenty four cows of Girolando breed composed by ¾ Holstein + ¼ Gyr or 7/8 Holstein + 1/8 Gyr blood were used, being 50% primiparous and 50% multiparous with average production (7.51 ± 2.58 kg day^{-1} and 10.98 ± 2.49 kg day^{-1}), respectively. The same cows were milked mechanically, obtaining a milk production record for the period of December 2011 to May 2012. As a criterion for the selection of animals, we used animals that had recently given birth, thus decreasing the risk of the cows leaving their productive phase during the trial period. The animals were kept in a semi-intensive system and feeding management was done by rotational grazing with Tifton 85 (*Cynodon* spp.) and Tangola grasses (*Brachiaria* spp.), with a ration of corn bran, soybean meal and dehydrated cassava scraps. During January and February, there was supplementation with sugar cane and Tangola grass silage, respectively.

Milk Analyses

Milk samples were collected during the morning and afternoon milking, by a device coupled to the milking line. Subsequently, the milk samples were stored, mixed and properly identified with the number of animals in plastic bottles of 40 mL with Bronopol® preservative, kept in temperatures between 2 and 6° C, and sent to the Milk Clinic Lab of the "Luiz de Queiroz" School of Agriculture (ESALQ / USP) for analyses of milk components.

For the determination of protein, fat, lactose and total solids, the samples were analysed by infrared absorption. The milk urea nitrogen (MUN) concentrations were determined by spectrophotometric enzymatic method. The somatic cell count was determined using the Fourier transform infrared (FTIR) electron counting spectroscopy.

Data Analysis

For statistical analyses, the following were considered as sources of variation: Days in milk (DIM) = up to 60 days in milk (≤ 60), between 61 and 210 (61 ≤ x ≥ 210) days in milk and over 210 days in milk; Sampling month (December, January, February, March, April and May); Time = morning and evening milking; Calving order = primiparous or multiparous; and body condition score range = 1- 5, with a margin of 0.25. For the evaluation of the influence of the SCC on the other variables, the animals were grouped by SCC (0-400000 cells mL^{-1}, 400001-750000 cells mL^{-1}, 750001-1000000 cells mL^{-1} and above 1.000.000 cells mL^{-1}).

Data were subjected to variance analyses and mean comparison test. The effects of different treatments on each variable were compared by Tukey test at 5% probability. To study the relationships between production, chemical composition, somatic cell count and body condition score, correlation coefficients were obtained from Pearson. Statistical procedures were conducted adopting the PROC GLM and PROC CORR of SAS.

RESULTS AND DISCUSSION

Somatic cell count in bovine milk

The effects of sampling months, calving order, body condition score and days in milk were significant for somatic cell count (SCC) (P<0.05) (Table 1).

The month of the year influenced the somatic cell count, probably by variations in rainfall, temperature and relative humidity. Seasonal variation in SCC for dairy herds in North America and Europe are consistently reported (Ruegg and Tabone, 2000; Cicconi-Hogan *et al*., 2013). In general, due to increased exposure to pathogens (as a result of favourable climatic conditions for microbial growth) more intramammary infection occur in warmer, wetter seasons (such as summer) and reduced intramammary infection occur in cooler periods (such as winter or spring) (Ruegg and Tabone, 2000; Cicconi-Hogan *et al*., 2013).

These factors influence the survival and microbial colonization of the environment and the mammary gland (Bueno *et al*., 2005). There is also some evidence that heat stress can reduce the phagocytic ability of neutrophils, resulting in reduced capability of the cow to respond to intramammary infection (Amaral *et al*., 2011). Thus, during warm and wet seasons, cows experience increased exposure to mastitis pathogens while simultaneously having decreased ability to spontaneously clear pathogens, resulting in increased probability of persistent intramammary infection and increased SCC (Ruegg, 2011).

Table 1. Variance analysis for somatic cell count in bovine milk according to the effects of collection time, birthing order, time of milking, body condition score and days in milk.

Causes of variation	SCC	
	d.f.	Mean square
Month of collection	5	617342.242**
Calving order	1	6206725.101**
Time of milking	1	56912.250^{ns}
Body condition score	7	838144.797*
Days in milk	2	1364083.434*
Error	253	442874.4

*(P<0.05); **(P<0.01); ns = not significant.

The presence of dirt aggregated at the posterior of the of cows' udder, characteristic in periods of high rainfall, can also be a predisposing factor to changing the SCC over the months of the year (Sant'anna and Paranhos Da Costa, 2011; Ballou, 2012).

The calving order (primiparous or multiparous) also influenced the variation of milk SCC. Multiparous cows are usually exposed to a greater number of risk factors predisposed to mastitis according to Coentrão (2008), such as: udder near or below the hock, lack of training of the milkers, set of teats cups dipping in sanitizer solution between the milking of different animals, and full insertion of the antibiotic cannula into teats when drying off a cow. These factors combined with others can explain the variation in the SCC function of calving order.

Andrade *et al*. (2007) observed increasing trend in somatic cell score from Holstein cows with aging. Similar results were reported by Magalhães *et al*. (2006), who observed that cows from the 4^{th} lactation had higher levels of SCC in milk.

The body condition score (BCS) of cows influenced the somatic cell count of milk. In the present study, animals with lower BCS (2.5 and 2.75) had lower mean SCC than those animals with higher BCS (3.75 and 4). Innate immune mechanisms of the mammary gland are correlated with the nutritional status of cows (Bewley and Schutz, 2008; Carneiro *et al*., 2009). Thus, cows with higher BCS may have greater innate response and therefore higher SCC value in milk (Magalhães *et al*., 2006).

The classes of days in milk influenced the somatic cell count of milk. The mean SCC of animals in the second class of days in milk (between 61 and 210 days) showed significantly higher values than the other classes (P<0.05).

The dynamics of somatic cell counts along the lactation is a complex and variable matter. Cows in early and peak lactation have a negative energy balance and impaired innate immune response, often with higher values of SCC. Furthermore, cows in peak lactation produce more milk and dilute components including defense cells present in milk. Cows heading toward the end of lactation typically present higher SCC mainly by: physiological desquamation of the mammary secretory epithelium, concentration of milk components by reducing the volume produced and acquired an infection in early and mid-lactation (Sharma *et al*., 2011; Magalhães *et al*., 2006; Carneiro *et al*., 2009; Rangel *et al*., 2011).

Chemical composition X Different class of somatic cell count

Lactose, non-fat solids dry extract and milk urea nitrogen were influenced by different intervals of somatic cell count in milk (Table 2).

The decrease in levels of lactose with increasing levels of milk SCC (P<0.05) can be explained by a decreased capacity for synthesis of the disaccharide by the mammary gland, or an increase in the NaCl concentration in the milk, resulting in osmotic disruption in the gland and also sugar deprivation by bacteria (Bueno *et al*., 2005; Rysanek and Babak, 2005).

The total solids did not suffer influence of SCC intervals, however, the levels of non-fat solids (NFS) decreased significantly due to the increase in SCC (P <0.05), which can be explained due to lower levels of lactose observed in milk with higher SCC. Following the same line of reasoning, it was expected that the total solids also suffer this influence, however probably due to the increasing levels in the percentage of fat, although not significant, this fact did not materialize.

Levels of milk urea nitrogen (MUN) suffered influences for different ranges of SCC (P <0.05). The averages presented are increasing in intervals up to 1 million cells mL^{-1}, and then decreased. Arunvipas *et al*. (2003) and Meyer *et al*. (2006) observed a decrease in MUN with increased SCC. However, Rysanek and Babak (2005) argue that mastitis is responsible for increased concentrations of non-protein nitrogen, in particular the levels of milk urea nitrogen as a result of changes in the permeability of cell membranes, which may explain the variations in the concentrations of MUN in this work.

The results in the literature for the influence of SCC in the concentrations of fat and protein are very different due to the large number of variables that affect them, however it is important that the mean values observed in this study are in accordance with the standards required by 62 Normative Instruction (NI) Brazil (2011), which requires averages above 3% fat and 2.9% protein.

Table 2. Effect of somatic cell count (SCC) in bovine milk chemical composition.

Variable	SCC (1000 cells/mL)				SD
	< 400	> 400 < 750	>750 < 1.000	> 1.000	
Fat (%)	3.31^{a}	3.16^{a}	4.08^{a}	3.58^{a}	1.03
Protein (%)	3.25^{a}	3.34^{a}	3.33^{a}	3.37^{a}	0.36
Lactose (%)	4.61^{a}	4.36^{b}	4.36^{b}	4.07^{c}	1.09
Total Solids (%)	12.07^{a}	11.74^{a}	12.70^{a}	11.94^{a}	1.16
NFS (%)	8.76^{a}	8.58^{ab}	8.62^{ab}	8.35^{b}	3.18
Casein (%)	2.59^{a}	2.62^{a}	2.53^{a}	2.70^{a}	9.93
MUN (mg/dL)	13.14^{c}	16.09^{ab}	19.92^{a}	14.52^{bc}	4.37

Different letters in the same row indicate significant differences (P <0.05); NFS = non-fat solids; MUN = milk urea nitrogen; SD = standard deviation.

Correlation coefficients to somatic cell count

Milk production, correlated positively with the SCC (correlation = 0.22) in the analysis of general correlation which agrees with Pösö and Mäntysaari (1996), and states that milk production is positively correlated with the occurrence of clinical mastitis and to SCC (Table 3).

Carlén *et al*. (2004) found that positive values may indicate that cows with high genetic values for milk production tend to be more susceptible to mastitis and the incidence of mastitis may increase in response to selection for milk production. When analyzing sorting order of lactation (primiparous and multiparous) there was no correlation (r = -0.04 and r = -0.07, respectively). According to Magalhães *et al*. (2006), whether the correlation is positive or negative, most estimates of genetic correlation between milk production and the SCC is of small magnitude, which could indicate the principle that selection for production has little or no influence on resistance to mastitis.

There was a positive correlation between SCC and the fat, protein, and milk urea nitrogen, and the correlation was negative for the lactose constituents and non-fat solids, (Table 3). Rangel *et al* (2009) and Ventura *et al*. (2006) found similar correlations to the present study, noting that when there was an increase in SCC values, there was a minimum increase in the percentage of fat and protein.

When correlations were performed separating the animals by order of calving, primiparous cows showed positive correlations between SCC and fat and total solids and negative correlations to levels of lactose and non-fat solids, while multiparous cows showed positive correlations for protein, casein and MUN content, and negative correlation to lactose (Table 3).

Correlation coefficients to body condition score

The variable milk production was negatively correlated with the body condition score (BCS) of primiparous cows, whereas for multiparous cows this correlation was positive (P <0.05) (Table 4). According to Bewley and Schutz (2008), cows with higher BCS tend to have higher milk production due to the large contribution of body reserves in providing nutrients for the synthesis of milk and its components.

Table 3. Pearson correlation coefficients between milk production, milk composition, and milk urea nitrogen (MUN) to somatic cell count (SCC).

Variable	General	Primiparous	Multiparous
Production of milk (kg)	0.22**	-0.04^{ns}	-0,07^{ns}
Fat (%)	0.12*	0.26**	-0.03^{ns}
Protein (%)	0.17**	0.15^{ns}	0.30**
Casein (%)	0.11^{ns}	0.12^{ns}	0.26**
Lactose (%)	-0.53**	-0.39**	-0.51**
Total Solids (%)	0.03^{ns}	0.17*	-0.06^{ns}
NFS (%)	-0.23**	-0.12^{ns}	-0.11^{ns}
MUN (mg/dL)	0.27**	0.00^{ns}	0.26**

*(P <0.05); **(P <0.01); ns = not significant; NFS = non-fat solids; MUN = milk urea nitrogen; SCC = Somatic cell count.

Table 4. Pearson correlation coefficients between body condition score (BCS) and the production and milk composition of primiparous and multiparous cows.

Variable	General	Primiparous	Multiparous
Production (kg)	-0.08*	-0.32**	0.15*
Fat (%)	0.04^{ns}	0.09^{ns}	-0.02^{ns}
Protein (%)	0.20**	0.30**	0.01^{ns}
Casein (%)	0.22**	0.32**	0.04^{ns}
Lactose (%)	-0.01^{ns}	-0.03^{ns}	0.06^{ns}
Total Solids (%)	0.11^{ns}	0.21*	0.00^{ns}
NFS (%)	0.17**	0.26**	0.09^{ns}
MUN (mg/dL)	0.08^{ns}	0.10^{ns}	0.05^{ns}

*(P <0.05); **(P <0.01); ns = not significant; NFS = non-fat solids; MUN = milk urea nitrogen;; BCS = Body Condition Score.

There was no effect of BCS on milk composition in multiparous cows (P >0.05). However, primiparous cows had positive correlations to the content of protein, casein, total solids and non-fat solids. Rennó *et al*. (2006) studied the effect of BCS at calving on milk composition and found higher production of milk and its components in cows with 3.25 BCS at calving.

CONCLUSION

The different levels of somatic cell count influenced the composition of bovine milk on such factors as the percentage of lactose, non-fat solids and milk urea nitrogen. Significant correlations between somatic cell count, milk production and composition (percentage of fat, protein, urea nitrogen, lactose, solids dry extract were found. As for body condition score, significant correlations were also found for production and composition (percentages of protein, casein, non-fat dry solids).

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