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Composition of milk and mammary gland health in Criollo breed mares during lactation

[*Composição de leite e saúde de glândulas mamárias em éguas da raça Crioula durante lactação*]

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ABSTRACT

The aim of this study was to evaluate the components of milk and mammary gland health of Criollo mares. A total of 12 mares coming from a farm in southern Brazil were used. Samples of milk were taken from each mare every two weeks up to 180 days of lactation. The characteristics analyzed were fat, protein, lactose, total solids, somatic cell count (SCC) and total bacterial count (TBC). In relation to the health of the udder and the milk of the mares, the SCC was 24.1 x 10^3cells / ml and TBC was 44 x 10^3CFU / ml, while the average of milk components was 0.57% fat, 1.95% protein, 6.71% lactose and 9.24% total solids. Stage of lactation and individual characteristics may influence the level of milk components. The low SCC and TBC found in the Criollo breed mares' milk ensure the quality of their milk compared to other species.

Keywords: mare, lactose, protein, fat, somatic cells

RESUMO

O objetivo deste estudo foi avaliar os componentes do leite e a saúde das glândulas mamárias de éguas da raça Crioula. Foi utilizado um total de 12 éguas, localizadas em uma fazenda no sul do Brasil. Foram retiradas amostras de leite de cada égua quinzenalmente até 180 dias de lactação. As características analisadas foram gordura, proteína, lactose, sólidos totais, contagem de células somáticas (CCS) e contagem bacteriana total (CBT). Em relação à saúde do úbere e ao leite das éguas, a CCS foi 24,1 x 10^3 células/mL e a CBT foi 44 x 10^3UFC/mL, enquanto a média dos componentes do leite foi 0,57% de gordura, 1,95% de proteína, 6,71% de lactose e 9,24% de sólidos totais. O estágio da lactação e as características individuais podem influenciar o nível de componentes do leite. As baixas taxas de CCS e CBT encontradas no leite de éguas da raça Crioula asseguram a qualidade desse leite comparado ao de outras espécies.

Palavras-chave: égua, lactose, proteína, gordura, células somáticas

INTRODUCTION

Criollo breed horses has its origin in horses brought by the conquerors to America four centuries ago. Some of these horses have been lost or abandoned and reproduced freely in the South America, becoming extremely well adapted to the region (Pons, 1993; Teixeira, 2011). Nowadays, after the natural selection that occurred over the centuries, the Criollo horse is known for its resistance, longevity and fertility. The growth of the Criollo breed in Brazil in 2015 reached 6.4 % with 402,341 animals registered in 2015 (Anuário…, 2016). The breed is present in all states of the country and the earnings per year are > U$ 400 million.

In equine breeding, the most important product is the foal. There is great concern about the health and growth of the newborn foal, therefore, for a better understanding of the nutritional needs of

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mares and their offspring, knowledge of the production and composition of mares' milk is essential (Malacarne *et al*., 2002; Doreau and Boulot, 1989; Oftedal *et al*., 1983). The health and quality of the milk and mammary gland is vital to foal growth and future mammary performance (Wells *et al*., 2012). The industry is facing a growing need for research and additives for use to fed foals; thereby the study of composition of mare milk is becoming increasingly important (Pietrzak- Fiećko *et al*., 2009). There are few studies on quality and composition of equine milk, and it is unprecedented in Criollo breed.

Based on the fact that several factors, such as, breed, age, and time of year affect milk production (Medhammar *et al*., 2012; Reis *et al*., 2007) and taking into account the importance of meeting the nutritional needs of foals during the first weeks of life for its proper development, as well as the lack of publication on the theme in the Criollo breed, the aim of this study was to evaluate the health of the mammary gland, quality and the main constituents of the milk of Criollo mares.

MATERIAL AND METHODS

All the procedures performed in this study were approved and carried out according to the ethics committee from Universidade Federal de Pelotas (CEEA-UFPel). A total of 12 multiparous Criollo mares belonging to a farm located in southern Brazil were used during the one reproductive season (September to July). The average age of the animals was 8.16±2.97 years and average weight was 441.88±15,42kg. Animals were kept under natural daylight in a 20 ha pasture with grass fork (*Paspalum notatum*) and had free access to a 4 ha paddock of season forage (ryegrass - *Lolium multiflorum* and oats - *Avena strigosa*), water and trace-mineralized salt).

Milk samples were collected fortnightly from each mare, during the lactation period (15,30,45,60,75,90,105,120,135,150,165,190,205 ,220,235,250, 265 and 280 days) nine months), the first gathering held within 15 days after delivery. The foals were separated from the mares two hours before sampling. Before each sampling hand hygiene of the milker was performed. The teats of the mares were cleaned with 70% alcohol and paper towel. The foremilk was discarded and the remainder collected in an aluminum container with capacity of one liter in which the homogenization was carried out. Samples were composed, i.e., obtained from two teats, and milking of each teat was complete, since the amount of fat is greater at the end of milking. After homogenizing, the samples were transferred to a plastic bottle with 45ml capacity containing 8mg of bronopol as preservative for the analysis of TBC and to other sterilized vial of the same size containing 0.1ml of azidiol to 30ml milk to evaluate the milk composition and SCC (Reis *et al*., 2009). All samples properly identified were stored in cool boxes, containing ice and sent the same day of collection for Milk Analysis Laboratory (SARLE) at University of Passo Fundo (UPF) in Passo Fundo, RS. For analysis of the composition of milk (fat, protein, lactose, and total solids), the infrared device Bentley 2000 was used (Reis *et al*., 2007) (Bentley Instruments, Inc.), and for SCC, a cell counter flow cytometric Somacount 300 (Bentley Instruments, Inc). The statistical analysis was performed using Statistix 9.0. The variables were analyzed by frequency distribution and descriptive statistics. The effect of the stage of lactation, the number of foals and age of the mare on changes in the composition of Criollo mares' milk was investigated using Kruskall-wallys one-way AOV. Mares were divided in two groups according to their age. Mares with less than 10 years were classified as young mares (n= 5) and mares with more than 10 years were classified as old mares (n= 7). The test used for comparison of means was LSD, differences were considered significant when $P< 0.05$.

RESULTS

The mean and standard deviation of the milk compounds, SCC and TBC evaluated in 216 samples from 12 mares during 9 months (18 collections) are shown in Table 1 There was a increase in the fat content during lactation (P= 0.0001) as seen in Figure 1. A higher standard deviation was observed in fat and protein samples.

Table 1. Mean (±SD) of fat, protein, lactose total solids, SCC and TBC found in milk of Criollo mares

Components	Mean (±SD)	Minimum	Maximum	Time of collection	Age of mares
Fat (%)	0.57±0.38	0,00	1,57	P= 0.0219	P= 0.9731
Lactose (%)	6.71±0.96	5,52	10,71	P= 0.0249	P= 0.6828
Protein (%)	1.95±0.96	0,16	3,45	P= 0.0337	P= 0.6172
Total Solids (%)	9.24±0.85	7,85	11,53	P= 0.0001	P= 0.3812
SCC(x10^3 cells/mL)	23.53±79.30	0	675	P= 0.0650	P= 0.0016
TBC (x10^3 CUF/mL)	37.64±68.07	1	775	P= 0.0045	P= 0.3687

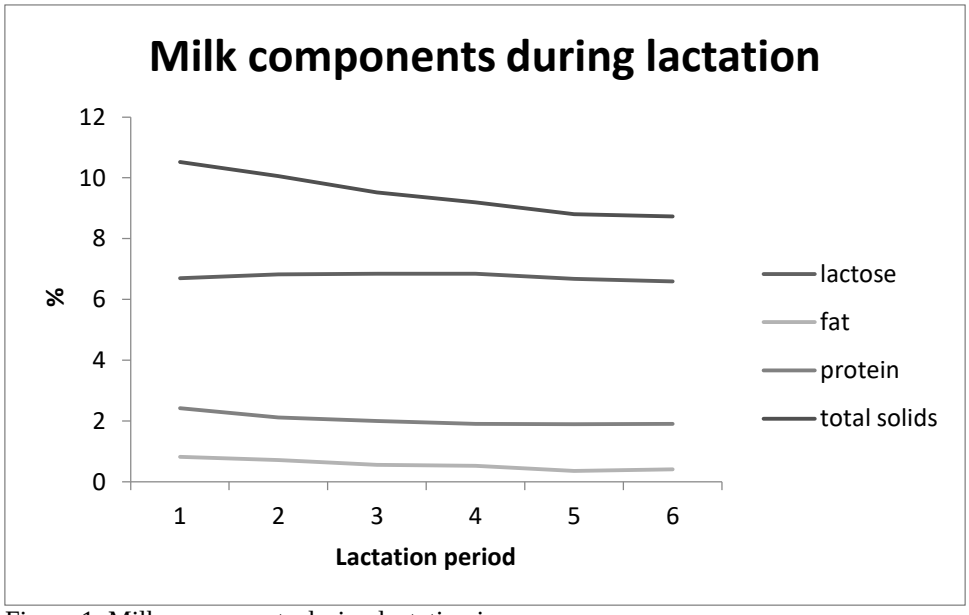

Figure 1. Milk components during lactation in mares.

DISCUSSION

Lactation is the phase in which growth of foals is faster and the nutritional requirements are very high, being one of the most important periods of life of mammals (Anderson, 1992; Malacarne *et al*., 2002; Santos and Zanine, 2006). Therefore, it is very important to know the composition of equine milk, because the existing literature on this subject is scarce, making it difficult to establish a nutritional program for lactating mares and their foals in growth. Today, the farms seek nutritional programs for the mares to be in good body condition until delivery and during lactation, aiming a good milk supply with components suitable for growth of offspring (Oftedal, 1983; Prestes *et al*., 1999).

In this study the milking of each teat was complete, because the fat is the most affected component changing, and 10 to 20 times greater at the end than at the beginning of milking (Csapó *et al*., 1995; Salamon *et al*., 2009). The fat in the milk of mares is low compared to bovine milk (Csapó *et al*., 1995; Pikul and Wójtowski, 2008; Pietrazk-Fiecko *et al*., 2009; Salamon *et al*., 2009; Gonzáles and Noro 2011). It is known that there may be variation from 0 to 7.9% equine milk fat (Gibbs *et al*., 1982; Oftedal *et al*., 1983; Schryver *et al*., 1986; Csapó *et al*., 1995; Santos *et al*., 2005; Reis *et al*., 2007). The variation in fat content is related to factors such as food, environment and stage of lactation (Oftedal *et al*., 1983; Schryver *et al*., 1986; Csapó *et al*., 1995; Santos *et al*., 2005; Reis *et al*., 2007). In this study, there was a range of results regarding fat, being 0% the milk that had the lowest content and 1.57% the highest concentration of fat obtained. This fact is explained due to the different stages of lactation, in which mares were by the time the collection had been conducted, since there was an increase in fat from the first to the ninth month of lactation (P= 0.001). Also, one must consider

individual characteristics, because it was found that some mares showed higher fat content (P= 0.001) than other mares during lactation (Csapó *et al*., 1995).

Furthermore, there may be variation in fat even during the actual milking, the cow's milk fat can be used as an example, which has a lower concentration at the start of milking and increases gradually until the end of milking, due to this it is necessary to perform a complete milking of two teats (Gonzáles *et al*., 2001). Due to the manipulation of hand milking during the collection, some mares presented milk retention and so a complete milking could not be performed, suggesting lower fat concentration of the sample.

The average protein level in this study was 1.95% (Table 1) and it was similar to the average of 1.96% obtained by Schryver *et al*. (1986) and less than 2.3% in the study of Holmes *et al*. (1947) and Csapó *et al*. (2009) from the 8th to the 45th day of lactation of 29 mares. But the variation of 0.16 to 3.45% showed a slight difference in values reported by others (Gibbs *et al*., 1982; Oftedal *et al*., 1983; Santos *et al*., 2005) In Criollo mares, a difference in the amount of protein (P= 0.001) among the mares in the study was observed and, furthermore, it was found that the stage of lactation influences (P= 0.03) the protein content, agreeing with the literature (Santos *et al*., 2005; Santos and Zanine, 2006; Reis *et al*., 2007). According to Csapó *et al*. (2009), stage of lactation is the factor that most influences the protein content of milk.

Lactose presented an average of 6.71%, confirming the high rate of sugar in the horse milk, for it is its main energy substrate (Spers *et al*., 2006). The average lactose content in Criollo mares was slightly higher than the average obtained by Reis *et al*. (2007). In this study, lactose showed variation from 5.52 to 10.71%, differing from the results found by Santos *et al*. (2005) that observed that variation from 5.8 to 7 in Mangalarga Marchador mares, and Oftedal *et al*. (1983) found 6.70 to 7.12% of lactose in the milk of equines of various breeds. The high rate of lactose increases the palatability of the milk and stimulates intestinal calcium absorption, with important role in the formation of bone structure of foals. Also, lactose contains a factor that improves the quality of the intestinal flora, since it causes the death of pathogenic microorganisms in the intestine (Santos and Zanine, 2006; Reis *et al*., 2007).

It was found that all components of milk are influenced by lactation stage, except lactose (P= 0.2880). Also, according to the literature, it shows no variation according to the diet except that the animal receives a very poor glucose feeding (Santos and Zanine, 2006), but an individual difference was observed (P= 0.000). Lactose is an important factor in controlling the osmotic pressure of the milk, which also contributes to filling of the udder (Doreau *et al*., 1991).

In this study of Criollo mares, total solids had an average of 9.24% (Table 1), being 10.4% lower than the average found by Reis *et al*. (2007) in Mangalarga Marchador mares. Oftedal *et al*. (1983) 10.4 to 11.1% and Gibbs *et al*. (1982) observed an average of 10.5% total solids in Quarterhorse mares. It was observed that the total solids also vary depending on the lactation stage (P= 0.0113) and 7.85% the minimum value and maximum value found was 10.53%. There were also differences in the values of total solids between mares, showing that this variable is also influenced by individual characteristics. The total solids represent the sum of all solid components present in milk and translate their true concentration thereof, eliminating the influence of water content.

Mastitis affects 5-10% of the herd of breeding mares, usually in the period of involution of the mammary gland, and it may cause impairment in weight gain of suckling foals, especially after a clinical mastitis (Prestes *et al*., 1999; Albrecht, 2007; Reis *et al*., 2009).

The TBC is the counting the number of colonies of bacteria in the milk sample. Therefore, it is measured in colony forming unit (CFU). Total microbial population varies according to the initial contamination, which can be obtained from the inside of the mammary gland, outside the udder and teats, milking and the contact surfaces. The storage temperature exerts influence on the bacterial count, so the milk must be stored at temperatures below 4.5°C (Pales *et al*., 2005. Bueno *et al*., 2008). The fresh milk examined directly from individual cows contains small amounts of bacteria (Gonzáles *et al*.,

2001). Samples of milk from Criollo mares came to the laboratory for analysis at temperatures between 3 and 4°C. TBC is used to determine microbial contamination of milk in the mammary gland itself, or health of the herd in terms of mastitis, and also to check the milking hygiene (Pales *et al*., 2005).

There is limited literature on the subject on the equine species. The mean value of TBC was found 37.64 x 1000CFU / ml (Figure 2) being higher than 33.3 x 1000CFU / ml, the result obtained by Reis *et al*. (2009) in Mangalarga Marchador mares. However, the value was within the range 35-54 x 1000CFU / mL, found by Danków *et al*. (2006) in mares at different stages of lactation. In Criollo, the influence of individual characteristics (P= 0.0368) and stage of lactation (P= 0.0174) were observed. The equine milk has the highest concentration of lysozyme, an enzyme with antibacterial properties, so this factor may be responsible for fewer TBC in mares than in cows (Danków *et al*., 2006). The low number of microorganisms allows drinking the equine milk without pasteurization, Danków *et al*. (2006) maintains that the total bacterial count in milk of mares corresponds to bovine milk pasteurized.

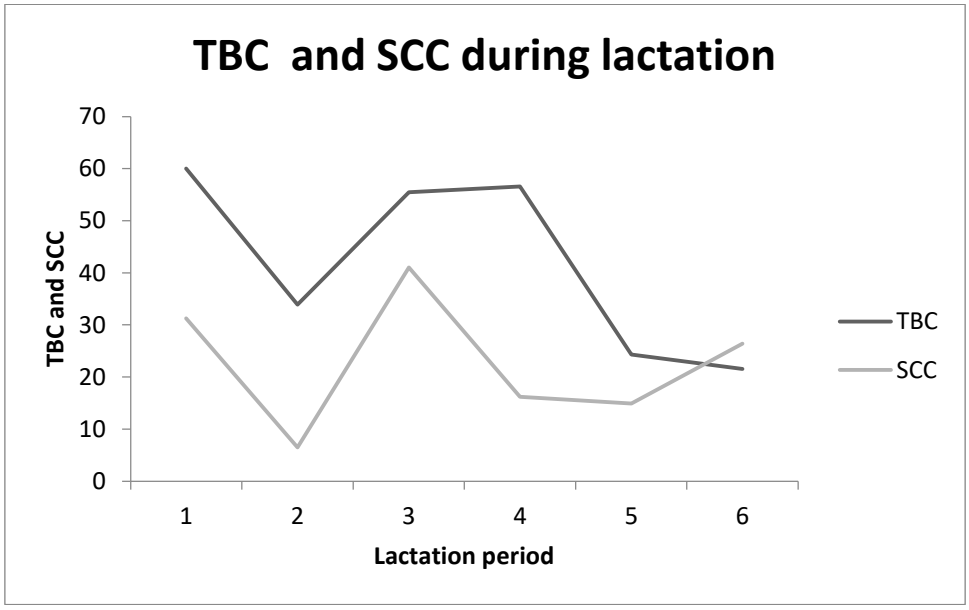

Figure 2. Somatic cells count and total bacterial count during lactation in mares.

During the study, a mare produced a table of clinical mastitis in the 5th month of lactation and was removed from the trial that month. Two days before presentation of symptoms, milk samples were collected, which revealed that 5051 X 1000 cells / mL SCC and 3112 x 1000CFU / mL TBC. It is important to note that this collection was removed from all analyzes. The animal was treated with antibiotics, nonsteroidal anti-inflammatory and local hydrotherapy. On the second day of treatment, the animal demonstrated an improvement of clinical signs.

CONCLUSIONS

The average of the mean components of Criollo mare's milk were described and found that these components can vary depending on the animal and the stage of lactation. This finding may help farmers in developing diets for growth of foals. The milk of Criollo mares has lower values in the characteristics related to quality of milk (SCC and TBC) compared to bovine milk, which can be used as a marketing strategy for the use of this milk for human consumption.

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