Semina: Ciências Agrárias
ISSN: 1676-546X
semina.agrarias@uel.br
Universidade Estadual de Londrina
Brasil

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Semina: Ciências Agrárias, vol. 37, núm. 4, julio-agosto, 2016, pp. 1963-1970
Universidade Estadual de Londrina
Londrina, Brasil

DOI: 10.5433/1679-0359.2016v37n4p1963

Physicochemical and sensory characteristics of milk from cows in different lactation stages and calving orders

Características físico-química e sensoriais do leite bovino em diferentes estágios de lactação e ordens de parto

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Abstract

Knowledge of the variation in milk composition is essential for determining milk quality, which is defined by several organoleptic properties. This study evaluated the effect of lactation stage and calving order on the physicochemical composition and sensory characteristics of milk from primiparous and multiparous lactating dairy cows. Milk samples were collected monthly from December 2011 to May 2012. Data were analyzed using ANOVA followed by Tukey's test ($p < 0.05$). Milk production (PROD), somatic cell count (SCC), and milk urea nitrogen (MUN) were significantly higher in multiparous cows, whereas the casein to total protein ratio (C/TP) was significantly higher in primiparous cows. All milk components except for non-fat dry solids (NDE) were significantly affected by lactation stage. Body condition score (BCS) was not affected by calving order or lactation stage. Color was the only sensory attribute affected by calving order. In conclusion, the physicochemical properties and sensory attributes of milk were affected by calving order and lactation stage.
Key words: Lactation stage. Milk quality. Primiparous. Multiparous.

Resumo

O conhecimento da variação na composição do leite é essencial para determinar sua qualidade, a qual é definida por várias propriedades organolépticas. Este estudo avaliou o efeito do estágio de lactação e ordem de parto sobre a composição físico-química e as características organolépticas do leite de vacas leiteiras primíparas e multíparas. As amostras de leite foram coletadas mensalmente no período de dezembro de 2011 a maio de 2012. Os dados foram analisados por meio de análise de variância (ANOVA), seguida pelo teste de Tukey ($p < 0,05$). A produção de leite (PROD), contagem de células somáticas (CCS) e nitrogênio ureico no leite (NUL) foram significativamente mais elevados em vacas multíparas, ao passo que a caseína e sua proporção na proteína total do leite (C/PT) foi significativamente mais elevada em vacas primíparas. Todos os componentes do leite foram significativamente afetados pela fase de lactação, exceto para os sólidos não gordurosos (SNG). Escores de condição corporal (ECC) não foram afetados pela ordem de parto ou estágio de lactação. A cor foi o único atributo sensorial afetado pela ordem de parto. Em conclusão, as propriedades físico-químicas e sensoriais do leite atributos foram afetadas pela ordem de parto e estágio de lactação.
Palavras-chave: Dias em leite. Primíparas. Multíparas.

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Recebido para publicação 15/09/15 Aprovado em 13/01/16

Introduction

Knowledge of the variation in milk composition is essential for evaluating milk quality, which is defined by several organoleptic properties (LANGONI et al., 2011; OLIVEIRA et al., 2011). According to Rangel et al. (2013), milk properties are affected by multiple genetic, environmental, and physiological factors. The main physiological factors affecting milk properties include age at first calving, lactation stage, calving order, and cow age, with milk production generally increasing as cows get older (RIBEIRO et al., 2009). Grande et al. (2009) argued that lactation stage is the main physiological factor associated with variations in milk composition.

Sensory analysis is an interdisciplinary science that utilizes panels of human assessors to evaluate the sensory characteristics and acceptability of food products (TEIXEIRA, 2009). Differences in dry matter composition of raw milk from cows in different lactation stages and calving orders may significantly affect the sensory characteristics of milk. Thus, this study aimed to evaluate the physicochemical composition (fat, protein, lactose, total solids, and urea nitrogen) and sensory characteristics (odor, flavor, residual taste, color, texture, and overall acceptability) of milk from cows in different stages of lactation and calving orders.

Materials and Methods

The study was conducted from December 2011 to May 2012 at a 550-ha commercial dairy farm in the coastal region of São José de Mipibu, 31 km south of Natal, the capital of the state of Rio Grande do Norte, Brazil. The region is characterized by a tropical rainy climate with well-defined dry and rainy seasons. The rainy season extends from April to June and has an average rainfall of 1.268,2 mm. The average temperature is 27.1 °C and average relative humidity is 76% (IDEMA, 2013).

The farm has 24 Girolando cows (3/4 and 7/8) that produce an average of 2500 L day^{-1}. The feeding system is semi-intensive rotational grazing of Tangola (*Cynodon* spp.) and Tifton 85 (*Brachiaria* spp.) grasses. Additionally, animals were fed a concentrate supplement consisting of corn meal, soybean, and cassava throughout the study period.

Cows in early lactation were selected to reduce the risk of animals finishing lactation before the end of the experiment. Twelve primiparous and 12 multiparous cows were selected, totaling 24 animals. However, because some animals had to be replaced, repeated monthly measurements were taken during the study.

Days in milk (DIM) was calculated from the calving data, and lactation was grouped into three phases: up to 60 days of lactation (DIM < 60), between 61 and 210 days of lactation (61 < DIM < 210), and DIM > 210 days. Cow body condition score (BCS) was determined on a 1–5 numerical scale with increments of 0.25, as proposed by Wildman et al. (1982) and Edmonson et al. (1989).

Milk samples from two milkings were collected monthly on the same day and transferred from the milk meter of an automated milking machine into 40-mL sterile plastic bottles containing Bronopol® preservative for analysis of milk composition and somatic cell count (SCC). Immediately after collection, samples were stored at 5 °C and sent to a laboratory accredited by the Brazilian Network of Milk Quality (BNMQ).

The concentrations of fat, protein, lactose, and total solids were determined by infrared Fourier transform spectroscopy analysis using a MilkoScan™ FT milk analyzer (Foss Electric A/S., Hillerød, Denmark). The concentration of milk urea nitrogen (MUN) was determined enzymatically using a ChemSpec 150® analyzer (Bentley Instruments Inc., Chaska, MN, USA) and results were expressed in mg/dL. Somatic cell counts (SCC) were determined by flow cytometry using an automated Somacount 300® flow cytometer (Bentley Instruments Inc.).

Milk samples used for sensory analysis were taken from each animal and mixed to obtain one 2-L composite sample. Samples were placed in sterile glass jars and cooled to temperatures below 5 °C.

Next, the milk was pasteurized by heating to 90 °C for 15 min followed by rapid cooling to 45 °C, as described by Medeiros et al. (2010).

Sensory analysis of an acceptable standard was done in the auditorium of the Technology Center at the Federal University of Rio Grande do Norte (UFRN), Natal, Brazil, by 50 untrained panelists aged 15-60 years and consisting of UFRN faculty and students. Milk from primiparous and multiparous cows was rated in separate trials. Each panelist received three plastic cups labeled with random three-digit codes containing 50 mL of milk cooled to 5 °C. Bottled water and toast were provided to clean the palate between samples. Sensory analysis tests were performed in individual booths away from noise and odors from 8:00 am–12:00 noon.

The following sensory attributes were evaluated: odor, flavor (odor and taste), residual taste, color, texture, and overall acceptability. Attribute intensities were scored on a structured 9-point hedonic scale where 1 represents "dislike extremely" and 9 represents "like extremely", as proposed by Chaves and Sproesser (2005).

The acceptability index (AI) was calculated for each attribute as described by Teixeira et al. (1987): AI = average score (AS)/highest score (HS) x 100. Mean scores represent the simple average of the values assigned by the panelists.

Data were analyzed using descriptive statistics, ANOVA, and Tukey's test for multiple comparisons of means. Wilks' multivariate analysis was used for the sensorial analysis in order to determine the main components and compare the milk from primiparous and multiparous cows. All analyses were performed using SAS®. Differences between means were considered significant at $p < 0.05$.

Results and Discussion

Mean values of milk production and composition variables are presented in Table 1. Most variables were not affected by calving order. However, some variables, including milk production (PROD), somatic cell count (SCC), milk urea nitrogen (MUN), and the casein to total protein ratio (C/TP), were significantly affected by calving order.

Milk production was significantly higher in multiparous cows than in primiparous cows. A similar result was also reported by Souza et al. (2010), who evaluated the effect of calving order on milk production in cows and found higher milk production in 3rd and 4th lactation cows, followed by 2nd lactation cows, whereas primiparous cows and 5th lactation cows were less productive. Similar findings were reported by Noro et al. (2006), Andrade et al. (2007), and Ribeiro et al. (2009).

Table 1. Analysis of variance and comparisons of means and significance test to variables according to the calving order.

Variables[1]	Calving order		Value of p(<0.05)
	Primiparous	Multiparous	
PROD (kg)	$7.51^b \pm 2.58$	$10.98^a \pm 2.49$	0.000
FAT (%)	$3.21^a \pm 0.91$	$3.45^a \pm 1.15$	0.569
PROT (%)	$3.28^a \pm 0.39$	$3.27^a \pm 0.26$	0.742
LACT (%)	$4.63^a \pm 0.30$	$4.44^a \pm 0.26$	0.908
TS (%)	$12.04^a \pm 1.03$	$12.03^a \pm 1.16$	0.424
NFS (%)	$8.83^a \pm 0.49$	$8.58^a \pm 0.32$	0.357
SCC* ($x10^3$cel mL^{-1})	$169.40^b \pm 501.90$	$509.40^a \pm 730.60$	0.000
MUN (mg dL^{-1})	$12.51^b \pm 3.35$	$14.91^a \pm 4.52$	0.001
CAS (%)	$2.55^a \pm 0.33$	$2.50^a \pm 0.22$	0.491
PCTMP (%)	$77.74^a \pm 1.72$	$76.60^b \pm 1.39$	0.000

[1] Production (PROD), fat (FAT), protein (PROT), lactose (LACT), total solids (TS), non-fat solids (NFS), somatic cell count (SCC), milk urea nitrogen (MUN), casein (CAS) and percentage of casein in total protein (PCTMP).
* The original data were transformed using log10
[a, b] Averages with lowercase letters in the same row differ (P<0.05) by t test.

Milk production correlates positively with body size (AKERS et al., 2006; DANIELS et al., 2009). Thus, the higher milk production observed in multiparous cows may be associated with mammary gland development (i.e., increased number of milk-secreting cells) and body growth (i.e., higher feed intake capacity). Conversely, primiparous cows have not reached physiological maturity and their mammary system does not support a large volume of milk.

Somatic cell counts (SCC) are generally used as an indicator of udder health, especially to monitor mastitis in dairy herds. In this study, SCC was significantly higher in multiparous cows than in primiparous cows. In fact, SCC are usually higher in cows with a greater number of lactations. Older cows with more lactations are more likely to have intramammary infections, as they have been at risk of being exposed to causative pathogens of mastitis for a longer period (ZAFALON et al., 2005; De VLIEGHER et al., 2012). Differences in SCC may also be explained by differences in the mammary epithelium of old cows and heifers, since the more developed mammary epithelium of older cows has more secretory tissue and more epithelial desquamation.

Milk urea nitrogen (MUN) also was significantly higher in multiparous than in primiparous cows. It has been suggested that normal MUN values should range between 10 and 16 mg dL^{-1} (ROY et al., 2011). Galvão Júnior et al. (2010) evaluated the effect of calving order on the physicochemical composition of Zebu milk and found a positive correlation between MUN and calving order, which the authors attributed to differences in nitrogen metabolism between cows and heifers.

The casein to total protein ratio (C/TP) was significantly higher in primiparous cows than in multiparous cows. The higher casein concentration may be related to the lower milk production in primiparous cows.

All milk composition variables were significantly affected by lactation stage except for non-fat dry extract (NDE) (Table 2). The variation in milk composition may be explained by changes in the mammary gland secretory epithelium of lactating cows and the higher feed intake during lactation.

Table 2. Analysis of variance and comparisons of means and significance test to production (PROD), fat (FAT), protein (PROT), lactose (LACT), total solids (TS), non-fat solids (NFS), somatic cell count (SCC), milk urea nitrogen (MUN), casein (CAS) and percentage of casein in total protein (PCTMP) according to the lactation stage.

Variables[1]	Lactation stage			Value of p
	DIM<60	61< DIM >210	DIM ≥210	(<0.05)
PROD (kg)	$9.52^{a} \pm 3.15$	$9.28^{a} \pm 2.99$	$7.00^{b} \pm 3.32$	0.000
FAT (%)	$3.04^{b} \pm 0.65$	$3.37^{b} \pm 1.10$	$4.09^{a} \pm 1.27$	0.003
PROT (%)	$3.13^{c} \pm 0.25$	$3.30^{b} \pm 0.34$	$3.54^{a} \pm 0.13$	0.000
LACT (%)	$4.69^{a} \pm 0.19$	$4.50^{b} \pm 0.31$	$4.32^{c} \pm 0.29$	0.000
TS (%)	$11.76^{b} \pm 0.70$	$12.06^{b} \pm 1.16$	$12.91^{a} \pm 1.10$	0.000
NFS (%)	$8.71^{a} \pm 0.34$	$8.69^{a} \pm 0.46$	$8.82^{a} \pm 0.38$	0.669
SCC* ($x10^3$cel mL^{-1})	$149.10^{b} \pm 198.40$	$414.10^{a} \pm 742.30$	$187.90^{ab} \pm 167.30$	0.005
MUN (mg dL^{-1})	$12.13^{b} \pm 3.39$	$14.32^{a} \pm 4.35$	$12.40^{ab} \pm 1.99$	0.001
CAS* (%)	$2.43^{c} \pm 0.21$	$2.54^{b} \pm 0.29$	$2.79^{a} \pm 0.10$	0.000
PCTMP* (%)	$77.50^{b} \pm 1.68$	$76.94^{b} \pm 1.61$	$78.75^{a} \pm 1.11$	0.000

* The original data were transformed using log10

[a, b] Averages with lowercase letters in the same row differ (P <0.05) by t test.

Milk production (PROD) was higher in early to mid-lactation but was significantly lower at the end of lactation, following the standard lactation curve of cows: milk yield peaks in early lactation, becomes stable at mid-lactation, and decreases at the end of lactation.

Fat (FAT) and total solids (TS) contents were higher in the final third of lactation. This result can be explained by the increased concentration of milk components caused by the reduction in milk production at the end of lactation. In addition, Mendes et al. (2010) ascribed the production of more dilute milk to cow nutrition, as high concentrations of propionic and butyric acids in concentrates fed to lactating cows have been shown to reduce milk fat content (MENDES et al., 2010).

Protein (PROT) and casein (CAS) levels and the casein to total protein ratio (C/TP) were significantly different across lactation stages, with the highest averages observed in the final third of lactation. Because milk production and, consequently, nutritional requirements are lower at the end of lactation, the energy/protein ratio in milk is generally more balanced then.

Milk urea nitrogen (MUN) values were highest at mid-lactation, when milk yield was highest. According to Obitsu and Taniguchi (2009), optimal MUN values vary with milk production, but levels below 12 mg/dl and above 18 mg/dL may indicate inadequate nutrition.

In light of the role of lactose (LACT) in the osmotic regulation of milk, the variation in lactose content observed in this study may be explained by lactation stage. In fact, lactose concentrations decline at the end of lactation when milk yield is lower. According to Pollott et al. (2014), increased desquamation (greater shedding of the epithelium) of the mammary gland secretory epithelium in the final third of lactation increases SCC, resulting in a decline in the concentration of lactose, which passes from the mammary gland into the blood due to changes in the permeability of cell membranes.

Somatic cell count (SCC) was highest in mid-lactation. SCC increases gradually toward the end of lactation because of shedding of the mammary gland secretory epithelium. Moreover, the decline in milk yield in late lactation causes an apparent increase in the number of cells because of the higher concentration of cells in a lower volume of milk (RANGEL et al., 2011, 2013). Moreover, epithelial renewal in the mammary gland via apoptosis may also increase the number of defense cells such as somatic cells at the end of lactation. According to Capuco et al. (2001), epithelial cells undergo apoptosis after the peak of lactation because of the decline in secretory activity in the absence of mammary growth.

SCC and milk component values for all of the calving orders and lactation stages met the minimum requirements in Normative Instruction 62 (IN # 62/2011) (BRASIL, 2011) for northeastern Brazil, including those for protein (2.9%), fat (3.0%), non-fat dry extract (8.4%), and the upper threshold for SCC (7.5 x 10^5 cells mL^{-1}).

Body condition score (BCS) was not affected by calving order or lactation stage (Table 3), indicating that cows were fed adequate diets in the different lactation stages, during each of which animals have specific requirements.

Table 3. Indices of body condition score for the three (03) stages of lactation and the two (02) calving orders.

Variables		BCS
Lactation phases (p=0.20)	Stage 1	3.46[a]
	Stage 2	3.38[a]
	Stage 3	3.50[a]
Calving order (p=0.19)	Primiparous	3.38[a]
	Multiparous	3.42[a]

[a, b] Averages with lowercase letters in the same row differ (P<0.05) by t test.

Color, which was the only sensory attribute affected by calving order (Table 4; $p < 0.05$), may have been affected by the higher casein to total protein ratio (C/TP) in primiparous cows (Table 1). Casein micelles are the primary contributors to the light scattering in milk that is responsible for its characteristic yellowish-opaque color. Further studies on milk color are needed, as it is an organoleptic property with a large influence on acceptance, not only of raw milk, but also of other dairy products such as cheese (OLIVEIRA et al., 2011).

Table 4. Mean and standard deviation of the sensory attributes according to the calving order.

Variables	Calving order		Value of p (<0.05)
	Primiparous	Multiparous	
Odor	$7{,}18^{a} \pm 1{,}48$	$6{,}89^{a} \pm 1{,}80$	0,29
Flavor	$7{,}03^{a} \pm 1{,}42$	$6{,}98^{a} \pm 1{,}68$	0,32
Taste	$6{,}93^{a} \pm 1{,}63$	$6{,}98^{a} \pm 1{,}92$	0,45
After taste	$6{,}33^{a} \pm 1{,}68$	$6{,}36^{a} \pm 1{,}90$	0,27
Color	$7{,}11^{b} \pm 1{,}63$	$6{,}11^{a} \pm 2{,}13$	0,00
Texture	$6{,}75^{a} \pm 1{,}78$	$6{,}27^{a} \pm 2{,}18$	0,08
Overall Acceptance	$7{,}12^{a} \pm 1{,}48$	$6{,}85^{a} \pm 1{,}92$	0,26

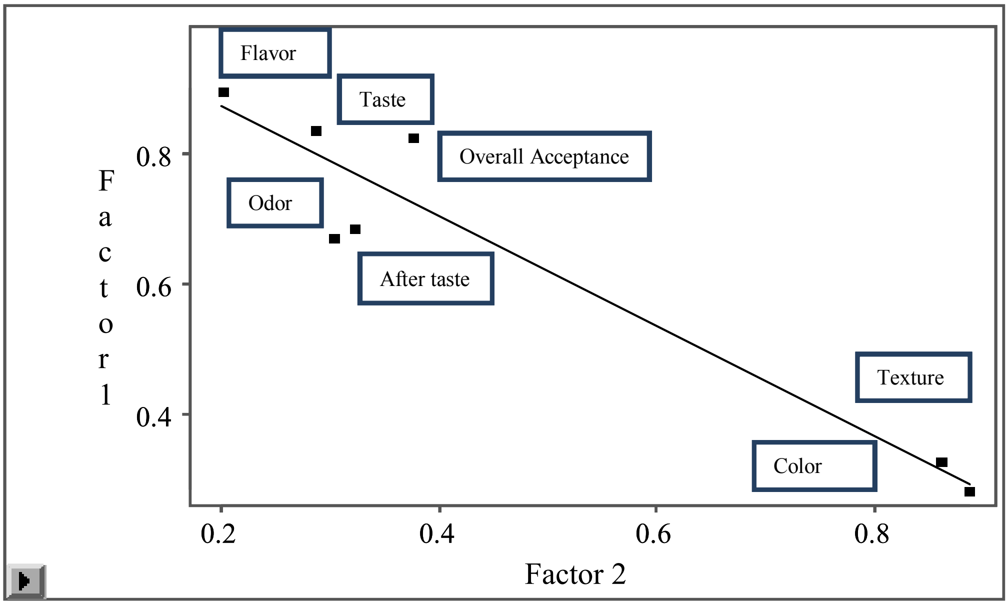
$^{a,\,b}$ Averages with lowercase letters in the same row differ (P<0.05) by t test.

Mean panelist ratings for sensory attributes are listed in Table 5, and the distribution of sensory attributes in the PCA plot is shown in Figure 1. Panelist ratings for the color of milk from multiparous cows did not achieve the 70% minimum required for sensory approval (ALVES et al., 2009), indicating that it should be improved. Although texture was also rated low, it achieved the minimum required for acceptance.

Table 5. Acceptance indices of the sensory attributes according to the calving order.

	Primiparous	Multiparous
Odor	79.78a	76.56a
Flavor	78.11a	77.56a
Taste	77.00a	77.56a
After taste	70.33a	70.67a
Color	79.00a	67.89b
Texture	75.00a	69.67a
Overall Acceptance	79.11a	76.11a

Figure 1. Principal component analysis of cow's milk from primiparous and multiparous lactating.



Conclusion

Milk production, composition, and sensory attributes were affected by calving order and lactation stage of cows.

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