

Full Length Research Paper

Procedure for collecting milk sample and the number of milkings in relation to chemical composition and somatic cells of the fresh milk

Jakeline Fernandes Cabral¹, Marco Antônio Pereira da Silva^{1*}, Thiago Soares Carvalho¹, Rafaella Belchior Brasil¹, Julliano Costa Garcia¹, Rodrigo Balduino Soares Neves², Edmar Soares Nicolau² and Moacir Evandro Lage²

¹Instituto Federal Goiano - Câmpus Rio Verde, Rodovia Sul Goiana, Km 01, P. O. Box 66, Zip Code 75901-970, Rio Verde, Goiás, Brazil.

²Centro de Pesquisa em Alimentos of Escola de Veterinária e Zootecnia of Universidade Federal de Goiás, Campus II da UFG, Rodovia GO-080 (Saída para Nova Veneza), Zip Code 749001-970, Goiânia, Goiás, Brazil.

Received 20 September, 2014; Accepted 10 September, 2015

This study was carried out to determine which system of milkings and sampling methodology presenting chemical composition results safer and lower variation of the results. In Properties I and II, the fresh milk samples were collected in 40 ml flasks containing bronopol in order to compare the chemical composition of the milk, as adopting two sample collection methods, that is two and three milkings. In order to compare the chemical composition of milk and SCC, between two sampling methodologies, in two daily milkings (Property I) and three daily milkings (Property II), fresh milk samples were collected randomly totaling 300 samples on the property I and 312 samples on the property II. The milk chemical components were determined through analytical principle that is based on differential absorption of infrared waves by components of the milk. By analyzing the treatments, experimental design was used entirely randomized and averages were compared by Tukey test using the statistical program SISVAR (statistical analysis system). When evaluating the data obtained through variation coefficient (VC), the occurrence of experimental was observed, since most results showed low variation. However the distinct procedures for sampling of the fresh milk may present varied levels of the chemical composition and SCC, therefore overestimating or underestimating the values of the milk composition, when samples are collected at different times of the day and different number of milkings.

Key words: Cows of high production, isolates samples, period, pool, sampling methodology.

INTRODUCTION

The samples of the fresh milk collected for analyses of the chemical composition and somatic cell count (SCC)

may suffer various kinds of changes before arriving at laboratory, since the collection method, time, storage

temperature, transportation and the milk homogenization for complete dissolution of the preservative may cause component changes.

The most common factors for variation of the milk components are: the animal individuality, breed, feed, lactation stage, age, environmental temperature, season, physiological factors (pregnancy, estrous cycle) and pathological ones (mastitis), persistency of lactation, cow size, cow mammary quarters, milking portion and interval between milkings (Weiss et al., 2002; Rangel et al., 2009). Although different factors may affect the composition of the milk, it is very important to repair the errors and to avoid any adulteration that may occur at the time of collection. The detection of the critical points that affect the quality and production of the milk is very important in controlling the herd.

In order to maintain the legitimacy of the samples in obtainment of the milk, they should be standardized, since the influence from milking type (mechanical or manual) and the method used to obtain the sample can alter the outcome of the chemical composition of milk (Reis et al., 2007). Taking into account some criteria such as the interval between milkings and the method for collecting milk samples in order to perform laboratorial analyses, it is possible to observe the occurrence of variation in composition of the milk (Cabral et al., 2013). According to Friggens et al. (2001), the milk component presenting more variability is fat, whereas protein and lactose do not differ according to criteria under evaluation. Therefore, only a single sample of milk is sufficient to obtain reliable results, when those variables are under evaluation.

When evaluating the quality of the milk, as taking into account some criteria such as fractioned collection of the milk sample in automatic milking systems, a high interference degree is observed in some milk components (Nielsen et al., 2005). However, the management practices adopted in rural properties and the milking type should be taken into account.

It is possible to assert the occurrence of variation in some components of the milk, according to the method used in obtaining the milk sample, the milking type adopted in the property, whether manual or mechanical, and the interval of eight or twelve hours between milkings.

In this context, this study was carried out to determine if two distinct procedures in collecting milk samples, such as pool of sample and independent samples, interfere with the chemical composition and somatic cell count of the fresh milk. If different number of milkings occasion

variation in the chemical composition of the milk, when samples are collected at different times of the day, and which sampling method provides security for laboratorial analyses.

MATERIALS AND METHODS

The research was conducted in two Dairy Properties located in Rio Verde municipality on Southwestern Goiás, according to ethical standards and approved by the Ethics and Biosafety Committee of the, Federal Institute of Education, Science and Technology of Goiano – Rio Verde, Campus – Go. The region has tropical climate, 740 m altitude, and average annual pluviometric index of 769 mm, with two distinct periods: rainy from October to April and dry from May to September. The research was conducted during the crop cycle period from May to October 2012.

The region has slightly sloping relief and the soil is red latosol, which favors the planting of annual crops and the livestock.

Rural properties

In rural Property I, the herd consisted of 90 crossbred cows and the milkings were accomplished in two periods of the day at 5:30 and 16:30 h. The cows were divided in three lots and maintaining the criterion of production, as being the most productive (>20 L) medium production (15 to 20) and less productive (<15 L). In afternoon milking, during the experimental period, the lots of cows entered the contrary, that is, the less productive (<15 L) the average production (15 to 20 L) and the most productive (>25 L) in order to increase the interval between milkings of the highly productive cows.

The collection procedure followed the norms of good milking practices: teats were washed with water and the first three milk jets were discarded in a black bottom mug to verify the presence of lumps, then, with the aid of an applicator, teats were immersed in pre-milking solution based on sodium hypochlorite and waiting 25 s to obtain total production efficiency. After cleaning, teats were dried with paper towels for the coupling of teatcups. At the end of milking, post-milking solution was used, whose base was 0.25% glycerin iodine.

In rural Property II, the herd consisted of 180 crossbred cows and three daily milkings were accomplished. The first milking began at 4:00 h in the morning, the second at midday and the third at 18:00 h. The cows were divided in lots and milked according to production. The highly productive cows (>20 L) corresponded to first lot, whereas the medium-production cows (15 to 20 L) corresponded to second lot and the lowest productive cows (<15 L) corresponded to third lot. The property had the fish scale-type automatic milking machine, which worked in loop and had a milking line with twelve teatcup sets with individual collectors to measure daily milk production and sampling collections.

In preparing the cows for daily milking, the traditional method prevailed. So, the black-bottom bucket test was performed, by using the first three milk jets, pre-dipping with towels imbibed in bactericidal disinfectant solution based on 30% Di(aminopropyl)

*Corresponding author. E-mail: marcotonyrv@yahoo.com.br. Tel: +556436205637.

laurylamine solution. One tip of the towel was used for each teat. For drying the teat, the towel reverse corners were used. After sanitary procedure, the set of teatcups were coupled, and the milking was continuously performed.

After the milking process, the set of teatcups were automatically removed and the estimated milk production (in kg) was displayed on individual digital reader of the milking machine. Then, the post-dipping with 0.25% iodine was accomplished.

Feeding the cows

The experimental period occurred during crop cycle, as taking into account the dry season of six months. During this time interval, the cows were kept under feedlot system in both rural properties. On rural Property I, the cows were sheltered in collective feedlots under shady environment, whereas on Property II the animals were housed in free-stall.

In Property I, during the experimental period, the cows were given corn silage and cottonseed-based concentrate, soybean bran, corn germ, urea, vitamin and mineral premix and water ad libitum. In Property II, the diet consisted of corn silage and concentrate with corn, soybean meal, vitamin and mineral premix, urea and water ad libitum.

Collecting the samples

In Properties I and II, the samples of the fresh milk were collected in 40 ml flasks containing the conservative bronopol. In Property I, the milking was performed twice. Some months earlier, it was observed the variation in volume of the milk produced daily to obey the following proportion: 2/3 on morning and 1/3 on afternoon. So, the same proportions were adopted in pool sampling. In the first milking, the milk samples reached 2/3 of the flask and 1/3 in the second milking that represented the pool sample. The independent samples, corresponding to 100% milk of the flask, were collected together with the pool in each milking of the day (morning and afternoon). From each milked cow, three milk samples were obtained (one pool sample, one sample on morning and one sample on afternoon).

In Property II, a partial sample of the fresh milk was obtained in each milking (three daily milkings) that represented a pool, as following the proportion: 1/3 milk on morning, 1/3 milk on afternoon and 1/3 milk on evening added in the same 40 ml flask. Together with pool, an independent sample was collected in each milking, as totalizing 100% milk in the flask. For each milked cow, a total of four milk samples were obtained (a pool sample, a morning sample, an afternoon sample and an evening sample).

Electronic analysis of the milk chemical composition

The contents of fat, protein, lactose and nonfat dry extract (NDE) were determined, by adopting the analytical principle which is based on differential absorption of the infrared waves by milk components, as using the equipment Milkoscan 4000 (Foss Electric A/S, Hillerod, Denmark). The results were expressed as percentages (%).

Somatic cell count

The analysis of the somatic cells (SC), from which the analytical

principle is based on flow cytometry was performed through the equipment Fossomatic 5000 Basic (Foss Electric A/S, Hillerod, Denmark). The results were expressed in SC/ml.

Statistical analysis

With a view to comparing the chemical composition of the milk, between the sampling methodologies, in two daily milkings (Property I) and three daily milkings (Property II), the milk samples were collected from 50 cows in Property I. Cows were randomly allocated to three treatments with two replicates, as totaling 300 samples. In Property II, the milk samples were collected from 26 cows randomly allocated to four treatments with three replicates, as totaling 312 samples.

In analyzing the treatments, the entirely randomized experimental design was used and the averages were compared by Tukey test at ($P < 0.05$) significance level, as using the statistical program SISVAR (Statistical Analysis System) (Ferreira, 2003).

In order to verify the relationship between milk chemical composition and milk production and SCC, the simple correlation among variables was performed through ASSISTAT program, according to Silva and Azevedo (2006), by applying the t-Test at ($P < 0.05$) and ($P < 0.01$) significance levels.

RESULTS AND DISCUSSION

The average results of the chemical composition and somatic cell count (SCC) of the milk samples collected under different sampling and number of daily milkings are presented in Tables 1 and 2. According to the obtained data and the evaluation of the variation coefficient (VC), the experimental accuracy was confirmed, since 50% from the total results showed low variation, as demonstrating the variables that showed greater instability in Property I were the production of milk, fat and somatic cell count (SCC) with VC corresponding to 28.45, 23.76 and 22.89% respectively (Table 1) whereas, in Property II, the variables with greater variation were fat and SCC with VC corresponding to 31.84% and 24.40% respectively (Table 2), whereas the ideal VC to animals is lower than 20%.

During the experimental period, the milk production (milk kg/milking) of the highly productive cows, within the range of two and three milkings, showed average results with ($P < 0.05$) difference (Tables 1 and 2).

In Property I, the daily milk production representing the average pool showed the highest value, as differing ($P < 0.05$) from morning and afternoon milkings that did not differ from each other at ($P < 0.05$) significant level. In the morning, the milk volume was numerically higher than afternoon milking. This difference occurred because the pool average results from the daily average production of milk (Reis et al., 2007) (Table 1).

In Property II, the average values of the milk production differed ($P < 0.05$) (Table 2). The average of the pool samples presented the highest value, since it represents

Table 1. Average values of the chemical composition and SCC in samples of the fresh milk obtained in two daily milkings, in high production dairy systems.

Variable	Property I			VC (%)
	Two milkings			
	Pool	Morning	Afternoon	
Milk production (L)	28.62 ^a	14.35 ^b	14.27 ^b	28.45
Fat (%)	3.43 ^{ab}	3.38 ^b	3.68 ^a	23.76
Protein (%)	3.15 ^a	3.16 ^a	3.16 ^a	10.02
Lactose (%)	4.63 ^a	4.64 ^a	4.63 ^a	5.14
NDE (%)	8.77 ^a	8.80 ^a	8.80 ^a	5.00
SCC (x1000 SC/ml)	462 ^a	467 ^a	499 ^a	22.89

Lowercase letters in the line differ significantly at 5% probability. NDE: Nonfat dry extract. SCC: Somatic cells count. VC: Variation coefficient.

Table 2. Average values of the chemical composition and SCC in samples of the fresh milk obtained in three daily milkings, in high production dairy systems.

Variable	Property II				VC (%)
	Three milkings				
	Pool	Morning	Afternoon	Evening	
Milk production (L)	22.57 ^a	9.55 ^b	7.33 ^c	5.69 ^d	2.13
Fat (%)	3.79 ^{bc}	3.54 ^c	4.08 ^{ab}	4.48 ^a	31.84
Protein (%)	3.45 ^a	3.48 ^a	3.50 ^a	3.49 ^a	10.31
Lactose (%)	4.68 ^a	4.63 ^a	4.65 ^a	4.62 ^a	5.34
NDE (%)	9.08 ^a	9.04 ^a	9.10 ^a	9.06 ^a	4.81
SCC (x1000 SC/ml)	241 ^a	241 ^a	332 ^a	318 ^a	24.40

Lowercase letters in the line differ significantly at 5% probability. NDE: Nonfat dry extract. SCC: Somatic cells count. VC: Variation coefficient.

the daily milk production. In the sampling on morning, afternoon and evening, a gradual reduction occurred in production of the milk, which is probably due to interval between milkings. This occurrence was expected due to irregular schedules, that is, 8 h interval between first and second milking, 6 h between second and third milkings and 10 h between third to first milking of the subsequent day.

The averages of the variable fat, under different sampling methods and analyzed in two milkings, differed ($P < 0.05$) (Table 1). The fat content increased gradationally, as milkings were performed. The lowest fat average was observed on morning period, as compared with afternoon period. The fat average, obtained by sample pool method, differed from fat contents obtained in the other periods over which the samples were collected by using the independent sample method, respectively, since the pool represents the general average of the values in both periods (Table 1).

In Property II, the average results for fat content of the milk samples differed from each other ($P < 0.05$). The fat content increased on increasing order of the samples, according to accomplishment of the milkings from morning to night. In the morning period, the lowest average was observed. This one differed from samples obtained in other collection periods, besides presenting a result equal to the average of pool. In the afternoon period, the fat contents were equal to those obtained in the night period and the pool. In the night period, the fat content was equal to that obtained in the afternoon period and differentiated from other samples.

Probably, this effect occurs because the greater volume of the milk produced by the animal during the morning period and, consequently, the chemical components will be lower. Therefore, corroborating with the results found by Nielsen et al. (2005) who showed the milk component which has a greater variation range, when applied to the milking interval to be the fat. This

result can be explained by the well-known dilution effect because with an increased volume of the milk, a reduction of the chemical components occurs (Mollenhorst et al., 2011).

Mendes et al. (2010) associated this effect with nutrition of the cows, that is, when a larger amount of the ration in relation to grass is supplied, an increase occurs in proportion of the propionic acid in relation to both acetic and butyric acids, therefore causing a reduction in fat by dilution. Different results were observed by Lammers et al. (1996). When relating the nutrition with fat contents, those authors affirmed the reduction of the fiber content in the diet to decrease the fat content in milk.

Contradicting the results obtained by the above mentioned author, Kargar et al. (2012) used different fat sources in the diet of Holstein cows and they observed the production and fat of the milk to be not affected by supplementation with high ration level in relation to forage levels, since the authors used a ration: grass proportion of 66:34.

The comparison between milk production and fat demonstrates that the greater is the production of the lactating cows, the lower is the percent fat. So, the lowest average of milk production occurred at night and, consequently, the highest percentage of fat (4.48%). The same result was observed by Friggens et al. (2001), who reported that the larger is the volume of milk the lower is the fat content.

With the results obtained in this study, it is possible to infer that in the collection of milk samples, as aiming at fat analysis, the sampling should be done by the method of pool. In this method, the collection of the milk samples is performed at ratio 2/3 and 1/3 of milk when in two milkings, or at ratio 1/3 of milk in each milking when performing three milkings.

Reis et al. (2007) evaluated the procedures for collecting milk. They reported that lower fat levels may be due to greater volume of milk accumulated inside the udder on the morning, as causing the dilution of fat. For validation of the results, however, the authors concluded that would be correct to accomplish a pool of the milk sample because the fat amount found (3.81%), which showed median value, when compared with the content observed in the morning period (3.44%) and in afternoon period (4.49%). However, it should be taken into account that the milk samples obtained by those researchers correspond to first jets of milk, which denotes the components were less concentrated.

According to Friggens et al. (2001), it is impossible to collect independent milk samples and provide daily estimates of the percent milk fat in conventional milking systems.

Wall and McFadden (2008) stated that milk samples used in studies to investigate the influence from milking

interval and herd management on milk composition and SCC should undergo standardization in order to make possible the collection of accurate data.

The average results of protein, lactose, NDE and SCC showed no difference ($P>0.05$) (Table 1 and Table 2). Therefore, it is possible to infer that only one milk sample obtained at any milking period is sufficient for reliable results issued for these variables.

Concerning to SCC, Brasil et al. (2012) found that, in mechanical and manual systems, there was a difference in SCC levels. The mechanical milking provided considerable increase in the SC number (545.000 SC/ml), whereas SCC obtained in the manual milking was much lower (253.000 SC/ml).

When evaluating the milk composition in two and three milkings, Österman and Bertilsson (2003) observed that differences in percent fat increased, whereas the milk protein content has a tendency to be more similar at the beginning and at the end of lactation. The results from analyses of the milk samples should be real and reliable because, as dairies pay for quality, the reliability of the results is fundamentally important to avoid frauds and errors in payments to producers and the demerit of the milk quality.

Figure 1 shows the averages of the production in the Properties respective. When comparing the trajectory of the milk production in Properties I and II, between the collection methods, it was observed the production index to be similar. This represents slight regression in liters, since it is observed that, when the practice of milking is constant, with less than eight-hour interval between milkings, the dairy capacity of the cows decreases.

Figure 1 shows that the production of milk, when milking was performed two times a day with 12 h interval, the average production showed a slight and not statistically significant reduction. However, when milking was performed three times a day at irregular intervals of eight, six and ten hours respectively, an abrupt drop in milk production occurred. This behavior is probably due to higher interval between the last milking of the day and the first one of the next day.

Results similar to those found in the present study were observed by Ouweltjes (1998), who demonstrated the relationship between milk production and the interval between milkings of dairy cows to occasion a greater milk production on the morning than on afternoon.

When comparing a milking system similar to the one adopted in the present study, that is two and three daily milkings, Wall and McFadden (2008) observed the lactation curve of the cows milked three times a day or more to maintain a prolonged lactation peak, which probably maintains the sanity of the mammary glands.

Different results were observed by Marnet and Komara (2008), when evaluating the amount of milking performed

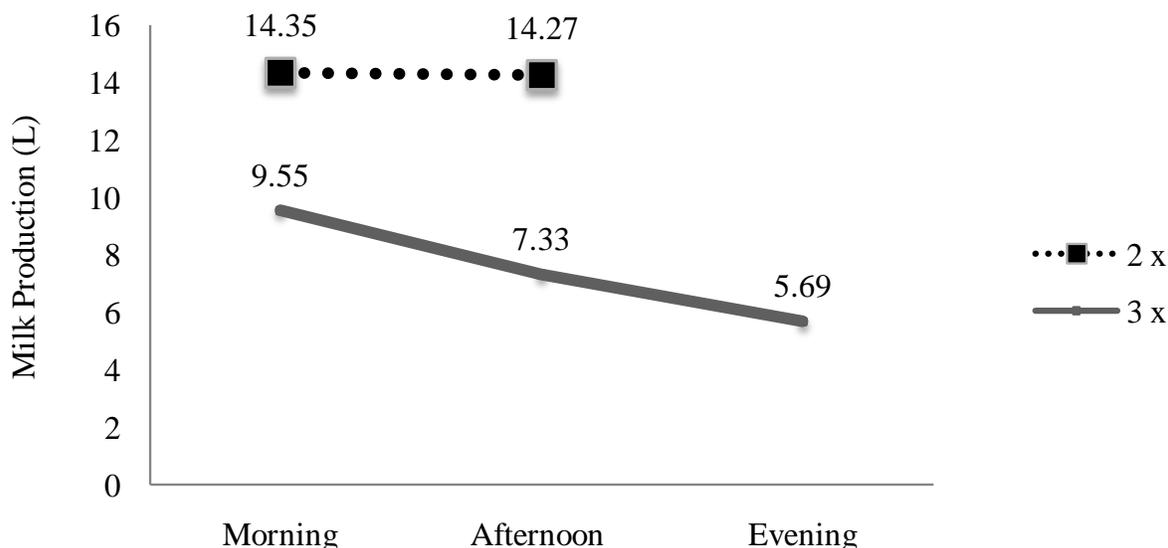


Figure 1. Average results of the milk production in Dairy Farms with two and three milkings.

Table 3. Simple correlation among milk production and SCC with chemical composition of the milk samples obtained in two and three milkings.

Correlation	Milk production		SCC	
	2 milkings	3 milkings	2 milkings	3 milkings
Fat	-0.2811**	-0.2412**	0.0642 ^{ns}	0.2061**
Protein	-0.3132**	-0.0061 ^{ns}	0.2535**	0.3102**
Lactose	-0.0903 ^{ns}	0.0124 ^{ns}	-0.4202**	-0.4374**
NDE	-0.2941**	-0.0158 ^{ns}	-0.0446 ^{ns}	-0.0570 ^{ns}
SCC	-0.0676 ^{ns}	-0.0922 ^{ns}	-	-

** Significant at 1% probability level (P<0.01). ns = non-significant (P>0.05).

daily and the profitability. They observed the system of milking only once a day in order to save time and flexibility to be not the best for cows, since only one daily milking causes greater impact upon physiology of the udders and udder health, therefore limiting the use of this system. Table 3 shows the results from simple correlation among variables of the chemical composition in dairy systems with two and three milkings.

In dairy farms adopting two and three daily milkings, the fat content was negatively correlated with the volume of milk production, which is related to an increase in milk volume and a decrease in milk fat concentration, as presenting highly significant values at (P<0.01) probability level.

The results of simple correlation in the range between two and three milkings with twelve hours apart (Property

I) and 8, 6 and 12 h (Property II), respectively, corroborate with Nielsen et al. (2005) who found that fat content varies when milk is milked at intervals of six and twelve hours, since higher results were observed at six hours interval, as compared to the result observed in the milking interval of twelve hours.

When the protein values are compared between two daily milkings, the correlation with milk production was negative at 1% significant probability level; when samples were obtained at three milkings, the correlation between production and protein was negative, however not significantly different at (P<0.05). According to Dürr (2004), the changes in the milk protein content are less significant than the changes in fat, although the fat and protein influence the milk production; however, the protein content has lower variation in the results than fat.

The results from simple correlation of fat and protein, observed in two daily milkings were negative at ($P < 0.01$) probability level. Similar results were reported by Friggens et al. (2001), who also found negative correlation of the variables fat and protein with milk production. According to Santos et al. (2001), the increase of the milk production in relation to percent fat and decreased percent protein is related to the use of supplementary fats in the diet, which is due to substitution of the rumen-available carbohydrates by lipids, as causing toxic effects to microorganisms of the rumen, which causes the reduction in microbial growth and consequently affecting the transport of amino acids to mammary glands. Thus, the concentration of the milk protein may decrease because the deficiency of one or more amino acids.

The fat content of the milk decreases with increasing volume of the milk obtained in the milking procedure, regardless of the number of milkings accomplished per day. Concerning to protein content and NDE, however, the correlation was significant only in dairy system with two daily milkings.

The lactose content was not significantly different between dairy systems of two and three milkings in relation to milk production. Therefore, it should infer that milk production varies as a function of the variable lactose. These results differ from those reported by Meyer et al. (2006), who affirmed the levels of lactose to be highly correlated to milk production.

The study carried out by Silva et al. (2000) on mastitic milk and non-mastitic milk revealed a decrease of the lactose contents in the mastitic milk. However, such a relation was already expected because the infection leads to destruction of the secretory tissue. So, there is a reduction in the synthesis of the mammary glands, which leads to reduction of the lactose contents and consequently the decrease in milk production.

According to Santos and Fonseca (2007), milk that has mastitis presents several alterations mainly in the physical and chemical composition. Mendes et al. (2010) reported that the components undergoing more changes are proteins, that is, the casein decreases and the serum proteins increase, whereas 10% reduction occurs in fat and lactose.

According to Eifert et al. (2006), the lactose is the primary and most important osmotic component of the milk, since it is associated with secretion of water and the volume of the milk produced. It is a component depending on glucose in its synthesis and, when it occurs at lower proportion in milk, may suggest insufficiency of glucose in the animal, as a resulting tendency to lower milk production.

For NDE in two-milkings dairy systems, the correlation is negative and the results were significant at ($P < 0.01$) in

three-milkings, the correlation presented no significant differences at ($P < 0.05$).

The correlation of SCC with milk production was not different at ($P < 0.05$) level in two and three daily milkings. Numerically, SCC of the cows in lactation had a gradual increase as milkings were accomplished, and SCC levels increased with decreased milk production.

This performance of SCC increase was different from that found by Mollenhorst et al. (2011), who observed that the parameters under study, such as milk production, calving order and interval between milkings, showed low relationship, since a very low SCC variation was observed when the intervals between milkings were lower than six hours.

The results found by Takahashi et al. (2012) did not differ from those found in the present study. No effects of the production upon variation of SCC occurred. However, when SCC was compared as function of the seasons, the effect was with changes mainly during the summer. The author relates this effect with the thermal stress that affects the animals, as making them more susceptible to infections in the mammary glands. However, the author also states that, in numerous herd, the animal SCC affects just slightly the count of the tank as compared to a small herd, which can probably be related to technological level of the Property (Zanela et al., 2006).

According to Coldebella et al. (2004), the SC increase occasion absolute losses in milk production, however it is independent from production level of the animal, where those losses occur from the count of 17.000 SC/ml. When comparing the reduction of the milk production with SCC/ml, the authors observed that, in the first lactation, there was a reduction of 0.30 kg milk per 100,000 SC/ml and 0.61 kg per 200.000 SC/ml. In the second lactation, the cows with SCC of 200.000 cells/ml had a reduction of 0.63 kg in milk production at 50th day postpartum, 0.92 kg at day 150th and 1.77 kg at day 250th postpartum. The cows at 3rd lactation or higher, with this same SCC showed a reduction of 0.60 kg, 1.09 kg and 1.85 kg on days 50, 150 and 250 postpartum, respectively.

Heuven et al. (1988) evaluated the inheritance of SCC and its genetic relationship with milk production and order of births. They found the inheritance of the somatic cell count to be low in early lactation and the variation of SCC is likely to alter from the second delivery, since it is correlated with milk production.

When observing the correlation between SCC and fat, it is observed that no differences ($P < 0.05$) occurred in dairy systems adopting two milkings. In the systems adopting three milkings, however, the relationship between SCC and fat was highly ($P < 0.01$) as showing a positive correlation. This suggests that, with the increased SC contents, an increase in the fat levels occurs.

The SCC results, in relation to protein, were highly significant ($P < 0.01$) and presented positive correlation in two and three milkings. Results differing from the present study were reported by Bueno et al. (2005), who observed a reduction in protein level of the refrigerated raw milk, when SC levels increased. Lacerda et al. (2010) obtained similar results to the present study, since the relationship between protein and SCC were correspondingly higher between properties with SCC increased.

The values of the lactose in milk correlated negatively with SCC, since they were highly ($P < 0.01$) in two and three milkings. Those values also proved the increase in somatic cells to cause reduction in the synthesis of lactose. Results similar to the present study were found by Bueno et al. (2005), who observed a reduction in lactose content as SCC increased. The same was observed by Auldust (1995), when verifying the reduction in concentration of lactose to be related with increased SCC.

The reduction of the lactose contents would result from either lower synthesis of this milk component in infected mammary glands and the loss of lactose from gland to bloodstream, due to increased permeability of the membrane separating the milk from blood, as leading to its excretion in urine (Pereira, 2000). Lacerda et al. (2010) reported different results for relationship between lactose and SCC, when presenting results of increase in lactose content.

The relationship between SCC and NDE showed no significant difference ($P > 0.05$), as demonstrating that SCC probably does not interfere in NDE contents. Lacerda et al. (2010) obtained different results for relationship between SCC and NDE, since they stated that the average NDE increased when SCC increased during summer and winter.

The sanity of the lactating cows may cause alteration in chemical composition of the milk. However, Brasil et al. (2012) reported the incidence of mastitis in the herd to result in increased SCC, which is one of the main parameters used for evaluation of the milk quality, since it is related to either decreased concentrations of the milk components and changes in sensory characteristics of the dairy products.

The results of this study, conducted in Properties I and II, showed values of the centesimal composition and SCC/ml according to the parameters required by legislation (IN/62 2001) (Brazil, 2011). Because being according to legislation, the milk produced in both rural properties under different milking and management systems, as one property being high-tech and the other one low-tech but presenting result according to legislation, the quality of the product commercialized in the region is adequate to consumption.

Conclusion

The distinct procedures for sampling of the fresh milk may present varied levels of the chemical composition and SCC, therefore overestimating or underestimating the values of the milk composition, when samples are collected at different times of the day and different number of milkings.

So, the pool sample collection would be the procedure indicated, since the result for composition presents a daily relative average, therefore a homogenous and legitimate result. The sampling for issuing reports concerning to milk quality should be judiciously performed in order to representatively express the average values of the milk chemical constituents.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The Fapeg, Capes and CNPq for financial support for research.

REFERENCES

- Auldust MJ, Coats S, Rogers GL, McDowell GH (1995). Changes in the composition of milk from healthy and mastitic dairy cows during the lactation cycle. *Aust. J. Exp. Agric.* 35:427-436.
- Brasil (2011). Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa. Instrução Normativa nº 62, de 29 de dezembro de 2011. Regulamento Técnico de Produção, Identidade e Qualidade do Leite tipo A, o Regulamento Técnico de Identidade e Qualidade de Leite Cru Refrigerado, o Regulamento Técnico de Identidade e Qualidade de Leite Pasteurizado e o Regulamento Técnico da Coleta de Leite Cru Refrigerado e seu Transporte a Granel. *Diário Oficial da República Federativa do Brasil*, Brasília, 30 de dezembro de 2011. Seção 1:1-24.
- Brasil RB, Silva MAP, Carvalho TS, Cabral JF, Nicolau ES, Neves RBS (2012). Avaliação da qualidade do leite cru em função do tipo de ordenha e das condições de transporte e armazenamento. *Rev. Inst. Latic. Cândido Tostes* 389(67):34-42.
- Bueno VFF, Mesquita AJ, Nicolau ES, Oliveira AN, Oliveira JP, Neves RBS, Mansur JRG, Thomaz LW (2005). Contagem celular somática: Relação com a composição centesimal do leite e período do ano no Estado de Goiás. *Cienc. Rur.* 35(4):848-854.
- Cabral JF, Silva MAP, Brasil RB, Carvalho TS, Giovaninni CI, Nicolau ES (2013). Effect of different sample collection methods on the results of raw milk analysis. *Rev. Inst. Latic. Cândido Tostes* 394(68):40-44.
- Coldebella A, Machado PF, Demétrio CGB, Júnior Ribeiro PJ, Meyer PM, Corassin CH, Cassoli LD (2004). Contagem de células somáticas e produção de leite em vacas holandesas confinadas. *Rev. Bras. Zootec.* 33(3):623-634.
- Dürr JW (2004). Programa nacional de melhoria da qualidade do leite: Uma oportunidade única. In: Dürr JW, Carvalho MP, Santos MV. O compromisso com a qualidade do leite no Brasil. Passo Fundo: Editora Universidade de Passo Fundo, pp. 38-55.

- Eifert EC, Lana RP, Lanna DPD, Leopoldino WM, Oliveira MVM, Arcuri PB, Campos JMS, Leão MI, Valadares Filho S (2006). Consumo, produção e composição do leite de vacas alimentadas com óleo de soja e diferentes fontes de carboidratos na dieta. *Rev. Bras. Zootec.* 35(1):211-218.
- Ferreira DF (2003). Sistema de análise estatística para dados balanceados (SISVAR). Lavras: UFLA/DEX.
- Friggens NC, Rasmussen MD (2001). Milk quality assessment in automatic milking systems: accounting for the effects of variable intervals between milkings on milk composition. *Livest. Prod. Sci.* 73:45-54.
- Heuven HCM, Bovenhuis H, Politiek RD (1988). Inheritance of somatic cell count and its genetic relationship with milk yield in different parities. *Livest. Prod. Sci.* 18:115-127.
- Kargar S, Ghorbani GR, Alikhani M, Khorvash M, Rashidi L, Schingoethe DJ (2012). Lactational performance and milk fatty acid profile of Holstein cows in response to dietary fat supplements and forage: Concentrate ratio. *Livest. Sci.* 150(1-3):274-283.
- Lacerda LM, Mota RA, Sena MJ (2010). Contagem de células somáticas, composição e contagem bacteriana total do leite de propriedades leiteiras nos municípios de Miranda do Norte, Itapecuru - Mirim e Santa Rita, Maranhão. *Arq. Inst. Biol.* 77(2):209-215.
- Lammers BP, Buckmaster DR, Heinrichs AJ (1996). Simple method for the analysis of particle sizes of forage and total mixed rations. *J. Dairy Sci.* 79(5):922-928.
- Marnet PG, Komara M (2008). Management systems with extended milking intervals in ruminants: Regulation of production and quality of milk. *J. Anim. Sci.* 86:47-56. <http://dx.doi.org/10.2527/jas.2007-0285>
- Mendes CG, Sakamoto SM, Silva JBA, Jácome CGM, Leite AI (2010). Análises físico-químicas e pesquisa de fraude no leite informal comercializado no município de Mossoró, RN. *Cienc. Anim. Bras.* 11(2):349-356.
- Meyer PM, Machado PF, Coldebella A, Cassoli LD, Coelho KO, Rodrigues PHM (2006). Fatores não-nutricionais e concentração de nitrogênio uréico no leite de vacas da raça holandesa. *Rev. Bras. Zootec.* 35(3):1114-1121.
- Mollenhorst H, Hidayat MM, van den Broek J, Neijenhuis F, Hogeveen H (2011). The relationship between milking interval and somatic cell count in automatic milking systems. *J. Dairy Sci.* 94(9):4531-4537.
- Nielsen NI, Larsen T, Bjerring M, Ingvarsen KL (2005). Quarter health, milking interval, and sampling time during milking affect the concentration of milk constituents. *J. Dairy Sci.* 88(9):3186-3200.
- Österman S, Bertilsson J (2003). Extended calving interval in combination with milking two or three times per day: Effects on milk production and milk composition. *Livest. Prod. Sci.* 82(2):139-149.
- Ouweltjes W (1998). The relationship between milk yield and milking interval in dairy cows. *Livest. Prod. Sci.* 56:193-201.
- Pereira JC (2000). Vacas leiteiras: aspectos práticos da alimentação. Viçosa, MG: Aprenda Fácil. Ed. UFV. P. 198.
- Rangel AHN, Guedes PLC, Albuquerque RPF, Novais LP, Lima Júnior DM (2009). Desempenho produtivo leiteiro de vacas guzerá. *Rev. Verde Agroecol. Desenvolvimento Sustentável* 4:85-89.
- Reis GL, Alves AA, Lana AMQ, Coelho SG, Souza MR, Cerqueira MMOP, Penna CFAM, Mendes EDM (2007). Procedimentos de coleta de leite cru individual e sua relação com a composição físico-química e a contagem de células somáticas. *Cienc. Rur.* 37(4):1134-1138.
- Santos FL, Lana RP, Silva MTC, Brandão SCC, Vargas LH (2001). Produção e composição do leite de vacas submetidas a dietas contendo diferentes níveis e formas de suplementação de lipídios. *Rev. Bras. Zootec.* 30(4):1376-1380.
- Santos MV, Fonseca LFL (2007). Estratégias para controle de mastite e melhoria da qualidade do leite. Barueri, SP: Manole, P 328.
- Silva LFP, Pereira AR, Machado PF, Sarriés GA (2000). Efeito do nível de células somáticas sobre os constituintes do leite II-lactose e sólidos totais. *Brazi J. Vet. Res. Anim. Sci.* 37(4).
- Silva FAS, Azevedo CAVA (2006). New version of the Assistat - Statistical Assistance Software. In: World Congress on Computers in Agriculture, 4, 2006, Orlando. Proceedings... Reno, RV: Am. Soc. Agric. Biol. Eng. pp. 393-396.
- Takahashi FH, Cassoli LD, Zampar A, Machado PF (2012). Variação e monitoramento da qualidade do leite através do controle estatístico de processos. *Cienc. Anim. Bras.* 13(1):99-107.
- Wall EH, Mcfadden TB (2008). Use it or lose it: Enhancing milk production efficiency by frequent milking of dairy cows. *J. Anim. Sci.* 86:27-36.
- Weiss D (2002). Variable milking intervals and Milk composition. *Milchwissenschaft* 57(5):246-249.
- Zanela MB, Fischer V, Ribeiro MER, Junior WS, Zanela C, Marques LT, Martins PRG (2006). Qualidade do leite em sistemas de produção na região Sul do Rio Grande do Sul. *Pesq. Agropec. Bras.* 41(1):153-159.