

Full Length Research Paper

Impact of Lactation Stage, Calving Order, and Genetic Group on Milk Composition

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Milk production and quality are influenced by environmental factors such as nutrition, genetic factors such as breed and physiological. The aim of the present study was to evaluate the influence of lactation stage, delivery order and genetic group on the quality of milk from crossbred cows 3/4 Gyr, 7/8 Gyr and 15/16 Gyr. Fresh milk samples were collected from crossbred cows. Fat, protein, lactose, non fat dry extract (NDE) and somatic cell count (SCC) of milk according to lactation stage, delivery order of calving and genetic groups were evaluated. The experimental design was completely randomized. Tukey test at 5% probability was used. Animals at the final third of lactation (201 to 305 days) showed higher fat, protein, NDE and SCC levels when compared to animals of other lactation stages. First delivery animals produced milk with higher content of solids compared to animals with 2 deliveries and above three deliveries. First calving the animals produced milk with higher solids content compared to animals with two calving and above three calving. Fat, protein and NDE results of fresh milk were consistent with limits established by the Brazilian legislation for milk quality, regardless of lactation stage, delivery order of calving and genetic group. Milk SCC was higher in cows in advanced lactation stage, The results were however below limits required by law. 3/4 crossbred Gyr cows showed higher SCC, with average values above limit established by current legislation.

Key words: Somatic cell count (SCC), fat, non fat dry extract (NDE), fresh milk.

INTRODUCTION

Milk production and quality are influenced by environmental factors such as nutrition, genetic factors such as breed and physiological factors such as age at first delivery and delivery order of calving (Galvão Junior et al., 2010).

Animal age has a direct influence on milk quality and

production, that is, production increases from the first lactation until the cow reaches physiological maturity, slowly decreasing as the animal becomes older, and this effect is directly related to delivery order of calving (Soares et al., 2009). To Rennó et al. (2002), there are other factors influencing milk production such as season,

Table 1. Number of animals according to lactation stage, delivery order of calving and genetic groups.

| Lactation stage | n = number of animals |
|--|------------------------------|
| 0 to 100 days | 46 |
| 101 to 200 days | 79 |
| 201 to 305 days | 88 |
| Total | 213 |
| Delivery order of calving | |
| One delivery First calving | 124 |
| Two deliveries Second calving | 58 |
| Above 3 deliveries Above three calving | 31 |
| Total | 213 |
| Genetic group | |
| 3/4 Gyr | 34 |
| 7/8 Gyr | 38 |
| 15/16 Gyr | 76 |
| Total | 148 |

herd, and milking, age and lactation period.

The period between birth and cow drying is called lactation period (LP), with average duration of 305 days; thus the animal has a dry period of about 60 days and has one delivery per year (Rangel et al., 2009).

In early lactation, there is an increase in somatic cell count (SCC) of milk due to the presence of immunoglobulins and consequently defense cells. At the end of lactation, there is also an increase in SCC due to increased natural desquamation of the mammary gland epithelium (Harmon and Reneau, 1993).

SCC levels above those allowed by law are indicator that the dairy farm has a large number of animals affected with subclinical mastitis, and high SCC in milk influences the quality of the final product and may be a health risk for consumers. Alternative techniques and methods have been increasingly researched to prevent microorganisms from outside, which cause subclinical mastitis in cattle.

Knowledge of the productive potential of each breed and each crossing used in climate conditions in Brazil should be studied in order to have security when indicating a particular animal for the various production systems (Rennó et al., 2002).

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Animals with a higher proportion of Gyr had longer durations of lactation, which exceeds the average daily milk production and the total production of animal milk with higher composition of Holstein (Manus et al., 2008).

So, the aim of the present study was to evaluate the influence of lactation stage, delivery order of calving and

genetic group on the quality of milk from crossbred cows.

MATERIALS AND METHODS

The study was conducted at Fazenda Medalha located in the municipality of Rio Verde, southwestern State of Goiás in the period from July to August 2014, where fresh milk samples were collected from crossbred animals throughout the experimental period. Other breeds for variables lactation stage and order of calving were studied. While the variable genetic group were studied, only cows 3/4 Gyr, 7/8 Gyr and 15/16 Gyr data were grouped according to Table 1.

The dairy farm had a total area of 166 ha, 35 ha devoted to grazing and housing of animals for milk production, handling corrals, milking parlor with bulk tank with capacity of storing 4000 L of milk and shed for storage of inputs and shelter for agricultural machinery.

The milking parlor was 2x6 fishbone type with paved waiting corral, milk piping system, closed circuit with six sets of liners and individual milk meters.

The dairy farm had natural shaded and unpaved area are for the tract of animals, where diet composed of feed and corn silage directly produced on the farm were provided.

The milking parlor was 2x6 fishbone type with paved waiting corral, milk piping system, closed circuit with six sets of liners and individual milk meters. The dairy farm had natural shaded and unpaved area are for the tract of animals, where diet composed of feed and corn silage directly produced on the farm were provided. The cows regularly received all mandatory vaccines (Foot-and-mouth disease, Brucellosis and Carbuncle) according to recommendations from veterinarians accredited by the Regional Council of Veterinary Medicine and Animal Science of the State of Goiás and vaccine schedule requirements were stipulated by the Agricultural and Livestock Defense of the State of Goiás.

Fresh milk samples were collected at the end of the morning milking with the aid of individual collectors coupled to each set of liners, which had a valve at the bottom such that before collection of milk samples, it was set to stir for five seconds for milk homogenization, thereafter the valve was turned to emptying

Table 2. Mean values and standard deviation of fat, protein, lactose, non fat dry extract (NDE) and somatic cell count (SCC) and log SCC of crossbred cows at different lactation stages.

| Parameter | Lactation stage | | |
|-------------|------------------------------|------------------------------|------------------------------|
| | 0 - 100 days (n = 46) | 101 - 200 days (n = 79) | 201 - 305 days (n = 88) |
| Fat (%) | 3.60±0.52 ^a | 3.28±0.60 ^d | 3.71±0.89 ^a |
| Protein (%) | 3.15±0.25 ^d | 3.11±0.29 ^d | 3.65±0.40 ^a |
| Lactose (%) | 4.72±0.16 ^d | 4.81±0.25 ^a | 4.52±0.22 ^c |
| NDE (%) | 8.85±0.32 ^d | 8.92±0.48 ^d | 9.20±0.40 ^a |
| SCC (CS/mL) | 278.109±574.196 ^a | 304.848±761.966 ^a | 455.511±533.367 ^a |
| Log SCC | 6.10±0.49 ^d | 6.02±0.59 ^d | 6.44±0.45 ^a |

Different lowercase letters in line differ according to Tukey test at 5% probability.

position, transferring the milk from the meter to collection flasks. Milk samples were aseptically collected in plastic bottles containing approximately 40 mL of preservative Bronopol® for chemical composition and SCC analyses. Overall, six collections were performed throughout the experimental period at intervals of three days between collections.

Immediately after collection, milk samples were packed in isothermal boxes containing ice and transported to the Laboratory of Animal Products, Federal Institute of Education, Science and Technology of Goiás – Rio Verde Câmpus, and then sent to the Laboratory of Milk Quality (LQL), Research Center of Food of the Animal Science and Veterinary School, Federal University of Goiás, Goiânia, GO, for carrying out electronic analyses.

Fat, protein, and non fat dry extract (NDE) were evaluated through the analytical principle based on the differential absorption of infrared waves by the milk components using MilkoScan 4000 equipment (Foss Electric A / S. Hillerod Denmark). Samples were previously heated in water bath at temperature of 40°C for 15 min to dissolve fat. Results were expressed in percentage (International Dairy Federation, 2000).

SCC analysis was performed according to the analytical principle based on flow cytometry using Fossomatic 5000 Basic equipment (Foss Electric A / S. Hillerod, Denmark). Before analysis, samples were previously heated in water bath at a temperature of 40°C for 15 min to dissolve fat. Results were expressed in SC/mL (ISO 2006).

Results were submitted to analysis of variance with factors being compared: lactation stage (early: up to 100 days of lactation; intermediate: from 101 to 200 days of lactation and late: from 201 to 305 days of lactation), delivery order (one delivery; two deliveries; above three deliveries) and genetic group (3/4 Gyr; 7/8 Gyr; 15/16 Gyr).

The experimental design was completely randomized. Tukey test at 5% probability and the SISVAR software were used (Ferreira, 2003).

RESULTS AND DISCUSSION

The mean fat, protein, NDE and SCC values of crossbred cows at different lactation stages were consistent with values established by Normative Instruction 62/2011 (Brazil, 2011), which established minimum fat, protein and NDE levels of 3.00, 2.90 and 8.40%, respectively, and maximum somatic cells count of 500 thousand SC/mL (Table 2). Brazilian milk quality law does not establish minimum or mean lactose values.

According to the Table 2, fat values showed significant differences ($p < 0.05$) in relation to different lactation stages, and cows at intermediate stage (101 to 200 days) showed lower fat content (3.28%±0.60) compared to early (0 to 100 days) and late stages (201 to 305 days), which showed fat content of 3.60%±0.52 and 3.71%±0.89, respectively. Campos et al. (2006) evaluated milk composition in different periods of the first lactation stage in high-production cows and observed fat contents close to those of the present study, 3.62, 3.77, 3.59 and 3.55% in the second, fifth, eighth and eleventh lactation week, respectively.

Fat content is higher at the beginning and end of lactation and lower in milk production peak, considering standardized lactation of 305 days (Kolver et al., 2007). Fat is the milk component that varies the most along the lactation period of cows (Certódes et al., 2004).

At the late lactation stage, protein content was 3.65%±0.40, significantly differing ($p < 0.05$) from protein contents at the early lactation stage (3.15%±0.25) and at the intermediate stage, protein content was 3.11%±0.29. Noro et al. (2006) observed protein contents below those of the present study (2.99%) at the early lactation stage and 3.32% at the late lactation stage. Campos et al. (2006) observed protein content was below that established by legislation at 150 days and 240 days of lactation, with average values of 2.84 and 2.86% respectively.

According to Costa et al. (2009), when the animal advances in its lactation stage, there is a downward trend in the daily milk production and consequently the lactose content decreases, causing an increase in fat and protein levels.

The lactose content varied during lactation stages, which was higher at the intermediate stage (101 to 200 days), significantly differing ($p < 0.05$) from the early and late stages, which results were 4.81%±0.25, 4.72%±0.16 and 4.52%±0.22 lactose, respectively. Campos et al. (2006) evaluated cows up to eleven weeks of lactation, corresponding to 77 days of lactation and observed average lactose content of 4.65%, and these results are very close to those described in this study.

Table 3. Mean values and standard deviation of fat, protein, lactose, non fat dry extract (NDE) and somatic cell count (SCC) and log SCC of crossbred cows at different delivery orders calving.

| Parameter | Delivery order calving | | |
|-------------|------------------------------|------------------------------|------------------------------|
| | One delivery | Two deliveries | Above three deliveries |
| | First calving (n = 124) | Second calving (n = 58) | Above third calving (n = 31) |
| Fat (%) | 3.59±0.58 ^a | 3.55±0.67 ^{ab} | 3.22±1.25 ^b |
| Protein (%) | 3.31±0.35 ^b | 3.52±0.55 ^a | 3.15±0.27 ^b |
| Lactose (%) | 4.78±0.21 ^a | 4.52±0.27 ^c | 4.67±0.11 ^b |
| NDE (%) | 9.08±0.41 ^a | 9.01±0.52 ^a | 8.79±0.32 ^b |
| SCC (SC/mL) | 317.306±694.238 ^a | 501.310±637.146 ^a | 275.452±270.612 ^a |
| Log SCC | 6.11±0.55 ^b | 6.42±0.53 ^a | 6.23±0.46 ^{ab} |

Different lowercase letters in line differ according to Tukey test at 5% probability.

Lactose is related to regulation of the mammary gland osmotic pressure, so that increased lactose production leads to higher milk production (Peres, 2001). According to Rangel et al. (2009b), increased SCC values cause reduction in lactose and NDE percentage.

The reduction in the lactose percentage may be explained by the loss of lactose from the mammary gland to blood due to changes in the permeability of the separation membrane. Thus, in healthy mammary gland, the more lactose is secreted, the more liters of milk are produced (Reis et al., 2012).

SCC evaluation according to count in absolute numbers showed no significant difference ($p>0.05$) in the different lactation stages; however, to reduce the variation range, the results (SC/mL) were transformed into Log SCC for statistical evaluation, revealing that there was an increase of somatic cell count at the end of lactation (201 to 305 days).

Voltoini et al. (2001) observed that in relation to the lactation stage, SCC showed a numerical increase at the beginning and end of lactation; however, these values do not show significant differences. The lactation stages could exert greater influence on SCC in herds with high counts. Moreover, the beginning and end of lactation are stressful stages for the cow, which will naturally increase SCC.

The mean values and standard deviation of fat, protein and NDE of crossbred cows at different delivery orders calving are described in Table 3. Fat, protein and NDE values are within standards set by Normative Instruction 62 of December 29, 2011.

Higher fat contents were observed in cows of first and second deliveries calving, differing ($p<0.05$) from animals of one delivery calving and animals above three deliveries calving, with 3.55%±0.67, 3.59%±0.58 and 3.22%±1.25 fat, respectively. From the third delivery calving, there is a tendency of fat to decrease. Due to its mechanism of synthesis, fat is the milk component of greatest variation, where the delivery order of calving can influence milk fat content, among other factors (Galvão Junior et al., 2010).

Second delivery calving cows showed higher protein content compared to first delivery calving cows and those above three deliveries calving, significantly differing from each other ($p<0.05$), with 3.52%±0.55, 3.31%±0.35 and 3.15%±0.27 protein, respectively. Different results were found by Noro et al. (2006), who observed lower protein content in milk from first delivery calving cows, and Souza et al. (2010) who found no difference in milk protein content of cows up to the fifth delivery calving.

According to Santos and Fonseca (2006), first delivery calving cows are still in body growth phase and development of the mammary gland and therefore have lower milk production capacity.

Milk lactose contents varied among delivery orders of calving, and first lactation showed higher lactose percentage, followed by cows with more than three deliveries calving and cows with two deliveries calving. According to Cunha et al. (2008), lactose is the milk component with the highest osmotic capacity.

Different results were observed by Corrêa (2010), who reported that the lactose percentage in milk was higher in the first and second lactation, keeping constant from the third to the seventh lactation, and decreasing with increasing delivery orders of calving and the lowest point is in the ninth lactation.

Second delivery cows showed SCC above values established by law. According to Magalhães et al. (2006), first delivery animals primiparous cows have less contact with mastitis-causing pathogens. As lactations increase, which coincides with increasing age, animals become more susceptible and are more often exposed to infection.

Animals with higher number of lactations have higher SCC, that is, animals with SCC over 100,000 SC/mL produce less milk. SCC indicates inflammation of the mammary gland, in the majority of cases resulting from bacterial infection (Cunha et al., 2008).

Milk production losses due to increased SCC are absolute, that is, it is independent of the animal production level. They begin to occur from SCC of 17,000 SC/mL and are different for primiparous and multiparous

Table 4. Mean values and standard deviation of fat, protein, lactose, non fat dry extract (NDE) and somatic cell count (SCC) and log SCC of cows of different genetic groups.

| Parameter | 3/4 Gyr (n = 34) | 7/8 Gyr (n = 38) | 15/16 Gyr (n = 76) |
|-------------|-------------------------------|------------------------------|------------------------------|
| Fat (%) | 3.74±0.59 ^a | 3.99±1.09 ^a | 3.25±0.58 ^b |
| Protein (%) | 3.73±0.36 ^a | 3.58±0.50 ^a | 3.01±0.25 ^b |
| Lactose (%) | 4.60±0.12 ^b | 4.55±0.25 ^b | 4.71±0.21 ^a |
| NDE (%) | 9.28±0.39 ^a | 9.09±0.40 ^a | 8.71±0.38 ^b |
| SCC (CS/mL) | 508.118±432.1312 ^a | 358.605±598.596 ^a | 402.697±774.769 ^a |
| Log SCC | 6.54±0.43 ^a | 6.29±0.47 ^b | 6.28±0.49 ^b |

Different lowercase letters in line differ according to Tukey test at 5% probability.

(Coldebella et al., 2004). The mean values and standard deviation of fat, protein, NDE and SCC of crossbred cows of different genetic groups are presented in Table 4.

The milk fat content differed ($p < 0.05$) among genetic groups, and 15/16 Gyr cows showed less fat when compared to 3/4 and 7/8 Gyr cows, which fat contents were 3.25%±0.58, 3.74%±0.59 and 3.99%±1.09, respectively.

Reis et al. (2012) evaluated the effect of breed on milk yield and composition and concluded that Holstein cows were more productive, however, milk had lower concentrations of lipids and proteins than milk from Gyr cows and crossbred animals, and the average fat values were different when compared with the present study, 3.64, 3.49 and 3.68%, respectively, for Gyr, Holstein and crossbred cows.

Protein levels were higher in 3/4 and 7/8 Gyr cows, being significant ($p < 0.05$) when compared with 15/16 Gyr cows, which values were 3.73%±0.59, 3.58%±0.50 and 3.01%±0.25, respectively. The average milk protein levels of crossbred cows were higher than those of Holstein cows, averaging 3.28 and 3.22%, respectively, for animals in extensive production system.

Both are affected by the technological improvement of the production system with a decrease in milk protein (Gonzalez et al., 2003). Oliveira et al. (1999) pointed out that NDE is the cause of variation of protein content, since the main solid represented in the NDE assessment is protein.

The lactose content of milk from cows of different genetic groups showed significant difference ($p < 0.05$) and for 3/4 and 7/8 Gyr cows, the lactose content was lower when compared with 15/16 Gyr cows, with lactose contents of 4.60%±0.12, 4.55%±0.25 and 4.71%±0.21 respectively. Botaro et al. (2011) evaluated the composition of milk from Holstein, Jersey and Gyr cows and observed lower average lactose contents of 4.42, 4.30 and 4.45% respectively. The reduction in the lactose content can be as a result of reduced synthesis of this component in cows with mastitis.

When actual data of milk SCC were transformed into Log CCS, significant differences ($p < 0.05$) among results were observed, and 3/4 Gyr cows were more susceptible

to mastitis compared with 7/8 and 15/16 Gyr cows, which results were 6.54±0.43, 6.29±0.47 and 6.28±0.49, respectively.

Reis et al. (2012) evaluated Gyr, Holstein and Crossbred cows and found values lower than those of the present study, whose parameters are within standards required by NI/62 2011 (Brazil, 2011), with average values of 395,000 SC/mL, 472,000 SC/mL and 423,000 SC/mL, respectively.

SCC quantitatively indicates the degree of infection of the mammary gland. This infection compromises milk composition, resulting in damage of the mammary gland secretory cells, leading to a reduction of casein, fat, lactose synthesis and increase in total protein concentration (Deitos et al., 2010).

Conclusion

The fat, protein and NDE results of fresh milk were consistent with limits established by the Brazilian legislation for milk quality, regardless of lactation stages, delivery order and genetic group. Milk SCC was higher in cows with advanced lactation stage; however, with results below limits required by law. 3/4 Gyr cows showed higher SCC, with average values above limits established by the Brazilian legislation.

Conflict of Interests

The author have not declared any conflict of interest

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