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Chemical composition and somatic cells count of milk from Holstein x Braunvieh cows

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This study aimed to evaluate the chemical composition and somatic cells count (SCC) of milk from Holstein / Braunvieh (HB) cows of five different grade crossing (genetic groups): Seven ½ blood HB, 14 5/8 blood HB, 16 3/4 blood HB, 86 7/8 blood HB and 28 15/16 blood HB cows were used. Six samplings at seven-day intervals were performed. The quality of milk was assessed according to the fat, protein, lactose and defatted dry solids (DDE), urea and casein levels and somatic cells count. There was no significant difference (P>0.05) in fat content of milk among blood levels; however, the other nutrients in milk differed (P<0.05) among blood levels studied (lactose, DDE, protein, casein, urea). SCC was higher in milk produced by cows of 3/4 blood HB genetic composition. Variations in milk production among genetic groups were also verified, with 1/2 blood HB animals being more productive compared to other genetic compositions. Correlation of genetic composition, production volume, days in lactation, number of deliveries and SCC with the chemical components of milk was found. A positive correlation between genetic composition and DDE and negative with production volume and number of deliveries was found and the SCC value of 13.41% was above the recommended by Brazilian legislation for milk quality.

Key words: Casein, fat, genetics, mastitis, urea.

INTRODUCTION

In Brazil, Holstein breed is the most exploited due to the higher volume of milk production compared to other breeds; however, its breeding requires more care regarding thermal comfort and consequently larger investments with facilities to mitigate the effects of humidity and temperature imposed by the tropical climate.

Teodoro and Madalena (2005) highlight the importance of the Braunvieh breed in the genetic composition of crossbred dairy cattle due to the increased protein and fat content in the milk of these animals. Thus, milk from Braunvieh crossbred animals can be an alternative to

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Table 1. Chemical composition of forages offered to dairy cows.

Composition (%)	Mombaça	*B. brizantha*
Dry matter	21	22
Crude protein	24	20.2
Ether extract	1.5	1.6
Neutral detergent fiber	58	49.8
Total digestible nutrients	64.8	70.5

improve gains from dairy farming in the payment program for milk quality.

The chemical components of milk can be improved through the genetic selection that composes the herd; SCC can also be improved through the selection of animals with low SCC. According to Andrade et al. (2007), there is an additive genetic variation for SCC, which enables the selection of dairy cows with low SCC. The integration between farmers, industry, research centers and regulatory agencies is critical to the production of quality milk capable of competing on the international market (Nero et al., 2005). Studies on the causes of variation in milk production and composition in the primary milk production sector are important for the whole dairy chain, since they serve as tools for monitoring quality and increasing productivity. Given the important relationship between genetics and milk quality, this study aimed to evaluate the chemical composition and SCC of milk from Holstein/Braunvieh crossbred cows.

MATERIALS AND METHODS

The study was conducted during the rainy season, between February and March 2012 in a dairy farm located in the municipality of Rio Verde, GO. Milking was performed in a 2×10 herringbone parlor, high line milk pipeline, closed system, with individual feeder on each contention, central area with 10 vacuum milking sets and individual milk meters.

The herd consisted of approximately 150 lactating cows, with average production of 23.27 kg / cow / day. Animals were seven ½ blood HB, 14 5/8 blood HB, 16 3/4 blood HB, 86 7/8 blood HB and 28 15/16 blood HB cows. During the study, dairy cows fed on pasture consisting of *Panicum maximum* Jacq. cv. Mombaça and *Brachiaria brizantha* cv. Marandú, with chemical composition shown in Table 1, and individually supplemented with 4 kg of energy concentrate during milking.

The concentrate provided to cows was composed of 14% crude protein and 82% TDN. There was no difference in the amount of concentrate offered to cows, because cows under study consumed a total of 8 kg of concentrate per day. High proportion of forage was supplied to dairy cows, since it is a low-cost feeding source. Based on the chemical analysis, forage was a great source of available protein to animals, in addition to providing fiber, which is important to stimulate rumen motility, rumination, salivation and synthesis of milk fat.

Dairy cows had access to pasture for a period of 12 h, that is, after each milking, cows grazed in a new paddock. The shorter time spent in paddocks with higher stocking pressure was established due to the rapid growth of forage favored by high temperatures and relative humidity in the region where the study was conducted.

Cows were milked twice daily, with the first milking being held at 04:00 am and the second at 04:00 pm. The criterion used for the formation of lots to be milked was the body scores of cows, where light and heavy animals were kept in separate lots. The dairy herd had intermediate body scores ranging from 3.25 to 4.0.

At the time of milking, the first three jets of milk were collected in the mug of black bottom for identification of clinical mastitis, and positive animals were not milked. Then, vacuum milking devices were immersed in a 5% iodine solution (pre-dipping) with thorough drying using paper towel, and after pre-dipping, the vacuum milking devices were coupled. Upon complete and uninterrupted milking, the vacuum milking devices were removed and immersed in 5% iodine solution (pre-dipping), and then animals were release for grazing.

Milk samples were obtained at the end of milking with the aid of individual milk meters, transferring the contents of the milk meter into the collection bottle containing Bronopol ®, with capacity of 40 ml, previously identified with barcode for each animal. For the measurement of milk production (kg), three consecutive measurements were performed, with an interval of seven days. The milk sampling was performed in two daily steps, one in the morning milking and another in the afternoon milking, accounting for 2/3 and 1/3 of the collection bottle capacity, respectively. After collection, milk samples were stored in isothermal boxes containing ice and sent to the Laboratório de Produtos de Origem Animal of Instituto Federal Goiano – Câmpus Rio Verde, Goiás to perform the electronic analysis and issuance of the final report with results.

Fat, protein, lactose and defatted dry extract (DDE) were determined by differential absorption of infrared waves for milk components using a MilkoScan 4000 equipment (Foss Electric A/S, Hillerod, Denmark), and results were expressed as percentage (%). Urea (mg/dL) and casein (%) contents were determined by differential Fourier transform infrared spectroscopy - FTIR using Lactoscope equipment (Delta Instruments). The results were expressed as mg/dL. Somatic cells count (SCC) was performed by flow cytometry using Fossomatic 5000 Basic equipment (Foss Electric A/S, Hillerod, Denmark) and results were expressed as SC/mL.

To compare the quality of milk between genetic compositions, the study was set up in a completely randomized design (CRD). Data of variables produced milk volume, fat, protein, lactose contents, DDE, urea, casein and SCC were submitted to analysis of variance using the Tukey test at 5 % probability for comparison of means. Data collection from the three milk measurements was consecutively made with an interval of seven days to measure the average milk volume from each group studied. Analyses were performed using the SISVAR software version 5.3. For all analyses, SCC data were transformed into $\log^{10}$, so data were normally distributed. To verify the existence of associations between quality, production and genetic composition, simple correlation was performed between variables through the ASSISTAT software, applying the t test for significance levels of 5 and 1%.

RESULTS AND DISCUSSION

According to Table 2, there was no significant difference ($p>0.05$) in the milk fat content between genetic groups, and the coefficient of variation (CV) was 18.02%. These results indicated uniform forage and concentrate intake during the experimental period, confirming the results reported by Rennó et al. (2006), who also observed no such variation when offering the same diet to cows of different genetic groups. Grandisson and Ford (1986)

Table 2. Variation in the chemical composition and SCC of milk from Holstein/Braunvieh crossbred cows.

Parameters	Genetic composition Holstein / Braunvieh					CV (%)	p-value
	½ (n=40)	5/8 (n=80)	¾ (n=71)	7/8 (n=442)	15/16 (n=150)		
Fat (%)	3.35[a]	3.49[a]	3.60[a]	3.48[a]	3.62[a]	18.02	0.0555
Lactose (%)	4.51[b]	4.73[a]	4.53[b]	4.68[a]	4.74[a]	4.10	0.0000
DDE (%)	8.57[c]	9.06[a]	8.79[b]	8.98[a]	9.13[a]	4.76	0.0000
Protein (%)	3.12[b]	3.35[a]	3.29[a]	3.32[a]	3.39[a]	9.74	0.0001
Casein (%)	2.44[b]	2.62[a]	2.56[ab]	2.59[a]	2.64[a]	12.14	0.0048
Urea (mg/dL)	12.56[ab]	13.34[a]	11.79[b]	12.96[ab]	13.69[a]	27.30	0.0043
SCC (log^{10})	2.19[b]	2.13[b]	2.51[a]	2.28[b]	2.12[b]	21.07	0.0000
SCC (x1000 SC/mL)	229	420	585	403	236	-	-

Means followed by same letters in the same row do not differ ($p > 0.05$) by the Tukey test. n = number of samples collected. CV = coefficient of variation. SCC = somatic cells count expressed in thousand SC/mL.

refers to the bulky as the most important food in the diet of dairy cows, which can influence the physicochemical characteristics of milk, and according to Oliveira et al. (2007), the proportion of forage offered to lactating cows as source of bulky is related to the production of acetate, which is the primary precursor of milk fat synthesis.

The average results of the fat content of milk from HB cows ranged from 3.35 to 3.62% , and are in accordance with minimum standards established by legislation (Brasil, 2011), which recommends minimum fat content of 3.0%. Mean fat values higher than those observed in the present study were reported by Santos et al. (2008), who evaluated the quality of refrigerated milk and found average fat contents ranging from 3.79 to 4.07%; however, the dairy systems evaluated by these researchers had low technological level.

In assessing the composition of milk from Holstein cows kept in elephant grass pasture, Voltolini et al. (2010) reported mean fat values of 3.98%; however, the milk production observed by these authors was lower (16.72 kg) than the average of 23.27 kg found in this study.

Table 2 shows that the milk produced by HB cows resulted in a significant difference ($p<0.05$) in the lactose contents, especially in the genetic groups 5/8 blood HB, 7/8 blood HB and 15/16 blood HB, compared to ½ blood HB and ¾ blood HB cows, which produced milk with lower lactose content. The lactose content of milk ranged from 4.51 to 4.74%, which is the parameter that less varied among chemical components in bovine milk.

Average lactose results slightly smaller than those of this study were described by Oliveira et al. (2007), who observed values ranging from 4.35 to 4.42%. Silva et al. (2010) found intermediate values compared to those of the present study, with average of 4.57% in animals manually milked on dairy farms in southwestern state of Goiás.

The association of pathogens with external factors results in a wide range of damage to the animal; however, milk production may decrease and the secretory tissue can also be damaged, in addition to variations in milk components such as reduced lactose content, which can occur due to the increase in SCC, related to the scaling of the secretory tissue (Silva et al. 2000).

The DDE contents of milk from genetic groups differed significantly ($p < 0.05$), and the results demonstrated that 5/8 blood HB, 7/8 blood HB and 15/16 blood HB animals had, respectively, mean values of 9.06, 8.98 and 9.13%, whose results are higher than those obtained for 3/4 blood HB cows (8.79%), followed by ½ blood HB cows (8.57 %).

The mean DDE values found in this study were higher than those recommended by Brasil (2011), which establishes minimum value of 8.4%, and similar to those reported by Santos et al (2008), who found DDE values from 8.4 to 8.8% with 7/8 Holstein/Zebu cows. According to Reis et al. (2007), DDE is positively correlated with protein and lactose, which was also observed in the present study, explaining the superiority of 5/8 blood HB, 7/8 blood HB and 15/16 blood HB animals, which produced milk with higher DDE concentrations.

The protein contents of milk did not differ significantly ($p > 0.05$) among 5/8 blood HB (3.35%), 3/4 blood HB (3.29%), 7/8 blood HB (3.32%) and 15/16 blood HB (3.39%) groups, but were higher ($p < 0.05$) than ½ blood HB group, which showed mean protein content of 3.12%. The lowest mean protein value found in milk from ½ blood HB cows was due to the higher milk volume produced by these animals, causing dilution of this component, corroborating the study by Schutz et al. (1990), who reported that variations in protein percentages are negatively related to milk production. The mean protein values of milk from different genetic groups were higher than those recommended by Brasil (2011), which is at least 2.9%.

The casein contents in milk, which are related to cheese production yield were higher ($p<0.05$) in milk produced by 5/8 blood HB, 7/8 blood HB and 15/16 blood HB genetic groups, with mean values of 2.62, 2.59 and 2.64%, respectively. ½ blood HB cows produced milk with lower casein content (2.44 %) and the casein content of milk from 3/4 blood HB cows did not differ significantly between groups. Mean casein content of 2.21% was reported by Freitas Júnior et al. (2010), which is lower than results of this study.

As Hermansen et al. (1994), the casein contents have a positive correlation with the protein content of milk, since casein represents approximately 80% of total proteins of milk; therefore, in this study, the genetic groups that showed the highest protein levels consequently showed the highest casein levels. Zanela et al. (2006) reported that the percentages of casein and solids are affected mainly by nutrition; however, as there was no difference in the diet in the present survey, it could be inferred that there is a close relationship between increased blood levels and increased casein content in milk.

The urea concentrations in milk produced by 5/8 blood HB and 15/16 blood HB genetic groups were higher compared to 3/4 blood HB genetic group and no significant difference (p>0.05) in the urea levels between groups of animal previously mentioned and ½ blood HB and 7/8 blood HB groups. The urea in milk is an important source in the nutritional monitoring of the herd, as it is related to protein metabolism of dairy cows (Jonker et al., 1998). The cause of variation in the urea content of milk in the present study may be related to the metabolism of the different blood levels studied, since metabolism is related to the degree of adaptation of animals to the environment.

The average urea results in this study were below normal levels, and according to Gonzales and Campos (2003), it should be between 24.10 and 34.2 mg/dL and that the milk nitrogen urea concentration (NUL), where 1 mol NUL is equivalent to 2.14 mol of urea, is directly related to the blood nitrogen urea concentration, which can be changed depending on the levels of degradable protein in the rumen and the protein: Energy ratio of diets offered to dairy cows, and reported that urea concentrations in milk below 19 mg/dL indicate protein deficiency in the diet offered, thus explaining the low urea levels found in the present study.

The average urea levels ranged from 11.79 to 13.69 mg/dL, and these values are smaller than those found by Oliveira et al. (2001), which ranged from 47.41 to 58.82 mg/dL, when evaluating the increasing levels of non-protein nitrogen in the diet of Holstein cows.

The statistical analysis of SCC (Table 2) in this study was performed as log^{10}, but the discussion will be based on the unit recommended by Brazilian legislation for milk quality, which establishes maximum count of 600,000 SC/mL for the period 01 January 2012 to June 30, 2014, for the region comprised in this study, located in central Brazil.

The SCC values was significant influenced (p<0.05) by the blood level, where 3/4 blood HB cows produced milk with higher SCC (585,000 SC/mL) compared to other blood levels, which showed no significant difference (p>0.05) from one another. The mean SCC value of 229,000 SC/mL and maximum value of 585,000 SC/mL reported in this study were similar to SCC values found by Moreira et al. (2003), of 289,000 SC/mL and 465,000 SC/mL when compared to the SCC value found in the milk of dairy cows fed high forage percentages. According to Souza et al. (2009), variations in SCC have been assigned to the pathogen species present in the mammary gland, and *Streptococcus agalactiae* is the main responsible for higher SCC in the milk of dairy cows. Although the present study has shown variations in SCC values, the mean values were below 600,000 SC/mL, corresponding to the maximum allowed by IN 62/2011, which from 2014 will be reduced to 500,000 SC/mL.

The numerical SCC values of 5/8, 3/4 and 7/8 blood HB cows were higher (403,000 SC/mL to 585,000 SC/mL) when compared to the average values of ½ and 15/16 blood HB cows, respectively, resulting in SCC values of 229,000 and 236,000 SC/mL; however, the SCC results are in accordance with Brazilian legislation for milk quality.

Variations in milk production (Table 3) were significantly influenced (p < 0.05) by genetic groups, and the group of ½ blood HB animals was more productive (28.95 kg) compared to 5/8 blood HB (23.15 kg), 3/4 blood HB (22.79 kg), 7/8 blood HB (23.87 kg) and 15/16 blood HB (20.30 kg) animals. The average milk production results of multiparous Holstein cows reported by Silva et al. (2011) were higher (30.56 to 31.07 kg / milk / day) compared to the average of 23.27 of the different genetic groups of this study.

The higher milk production attributed to 1/2 blood HB genetic group can be explained by the greater capacity to adapt to the milk production system under study. Similar to that observed in the present study, Facó et al. (2002) reported that there was no benefit in increasing the proportion of Holstein genes in the most hostile environmental conditions; however, for more favorable conditions, such increase seemed to be interesting to increase milk production.

The HB blood levels were negatively correlated (Table 4) with milk volume (r = -0.1184), indicating that the higher the Holstein/Braunvieh blood level, the lower the milk production. With increasing Holstein blood level, there was a decrease in milk production, indicating lower adaptability of these animals the study conditions. Cows with higher Holstein blood level showed lower milk production capacity in environments that do not meet minimum comfort conditions required by these animals.

There was a negative correlation (r = -0.2741) between genetic composition and the number of deliveries, that is, the greater the HB blood level, the lower the number of deliveries. Thus, it appears that animals with highly specialized genetic composition for milk production have difficulty to impregnate.

Table 3. Variation in milk production of Holstein / Braunvieh cows (HB).

Parameters	Genetic composition Holstein / Braunvieh					CV (%)	p-value
	1/2 blood HB	5/8 blood HB	3/4 blood HB	7/8 blood HB	15/16 blood HB		
n	20	41	34	224	77		
Milk production (Kg)	28.95 [a]	23.15 [b]	22.79 [b]	23.87 [b]	20.30 [b]	26.86	0.0000
Average milk production (Kg)			23.27				

Means followed by the same letters in the same row do not differ (p>0.05) by the Tukey test. n = number of samples collected. CV = coefficient of variation.

Table 4. Linear correlation between production and quality of milk from Holstein/Braunvieh crossbred dairy cows.

	Volume	DL	Deliveries	SCC[1]	Fat	Protein	Lactose	DDE	Urea	Casein
GC	-0.1184*	0.0673[ns]	-0.2741**	0.0183[ns]	0.0204[ns]	0.0838[ns]	0.0913[ns]	0.1382**	0.0382[ns]	0.0424[ns]
Volume	-	-0.5184**	0.4590**	-0.0927[ns]	-0.3727**	-0.4952**	-0.1185*	-0.5275**	-0.2272**	-0.3874**
DL	-	-	-0.3036**	0.0703[ns]	0.3018**	0.5944**	0.0361ns	0.5553**	0.3261**	0.5096**
Deliveries	-	-	-	0.1591[ns]	-0.0635ns	-0.2422**	-0.2924**	-0.3936**	-0.2598**	-0.1930**
SCC[1]	-	-	-	-	0.1657**	0.1461**	-0.3347**	-0.0569[ns]	-0.0742[ns]	0.1971**

** Significant at 1 % probability level (p < 0.01). *Significant at 5% probability (0.01≤p <0.05). Not significant (ns) (p≥0.05). The t test was applied at levels of 5 and 1%. Genetic composition (GC); days in lactation (DL), total dry extract (TDE); defatted dry extract (DDE), somatic cells count (SCC). [1]SCC values were transformed into $\log^{10}$ for data standardization.

Blood level was positively correlated (r = 0.1382) with the defatted dry extract content, demonstrating that the higher the HB blood level, the greater the DDE content in milk. There was no significant correlation (p>0.05) between blood level, days in lactation, SCC and fat, protein, lactose, urea and casein contents.

The correlation between milk volume and days in lactation (r = -0.5184), fat (r = -0.3727), protein (r = -0.4952), lactose (r = -0.1185), DDE (r = -0.5275), urea (r = -0.2272) and casein (r = -0.3874) contents was negative. The higher the milk volume produced by HB crossbred cows, the lower the days in lactation, so that cows in the early stage of lactation produced higher milk volume.

In the conditions of the present study, the higher the volume of milk produced, the lower the fat, protein, lactose, DDE, urea and casein contents, indicating that there is a dilution of milk components with the increase in the volume of milk produced by cows, which agrees with the results by Trevaskis and Fulkerson (1999), who observed that high milk production cows fed with forage produced milk with lower urea concentration, possibly due to the dilution effect of increased production.

Oltner et al. (1985) observed a positive correlation between milk production and urea concentration in milk. Kaufmann (1982) found a similar relationship, which was attributed to increases in protein content in the diet and in milk production, and not only to the milk production effect. The volume of milk produced was positively correlated (p<0.01) with the number of deliveries (r = 0.4590), therefore, it is desirable that lactating cows are kept for longer time along with the herd in order to significantly express the productive potential.

There was no significant correlation (p>0.05) between milk volume and SCC and therefore the incidence of mastitis would be related to handling and environment and not to productive factors.

Days in lactation resulted in negative correlation (r = -0.3036) with the number of deliveries, indicating that cows with long periods of lactation had fewer offspring. The explanation is the involuntary persistence of the exploitation of cows with high milk production potential.

Days in lactation were positively correlated with body fat (r = 0.3018), protein (r = 0.5944), DDE

Table 5. Incidence of milk samples from high milk production cows outside the standards for somatic cells count, according to the Brazilian legislation for milk quality.

SCC	Holstein blood levels				
	1/2 blood HB n = 40	5/8 blood HB n = 80	3/4 blood HB n = 71	7/8 blood HB n = 442	15/16 blood HB n = 150
Percentage (%)	5.00	16.25	29.58	13.12	7.33
Mean			13.41%		

Standard SCC = below 600,000 SC/mL according to Brasil (2011).

(r = 0.5553), urea (r = 0.3261) and casein (r = 0.5096) contents. Cows with longer lactation produced milk with higher fat, protein, DDE, urea and casein contents. This fact is due to a reduction in the volume of milk produced by animals at the final stage of lactation and corresponding increased concentration of milk chemical components.

SCC and lactose were not significantly correlated (p>0.05) with days in lactation. The mean protein, lactose, DDE, urea and casein contents were negatively correlated with the number of deliveries (p<0.01). The greater the number of deliveries, the lower the concentration of these components in milk.

SCC and fat were not significantly correlated (p>0.05) with the number of deliveries. SCC showed negative correlation (p<0.01) with lactose (r = -0.3347), so, the higher the SCC values, the lower the lactose content in milk. Lactose is related to larger volumes of milk produced, and for this reason, cows with high SCC produce smaller milk volumes, since high SCC correspond to inflammation of the mammary gland, resulting in decreased milk production and consequently decreased lactose content, and as Auldist et al. (1995), this reduction was probably due to tissue injury and also to the passage of carbohydrates from the alveolar lumen into the bloodstream.

When SCC values are correlated with milk fat, protein and casein contents, a positive correlation was found, proving that high SCC values influence the fat, protein and casein levels of milk, so, high SCC levels lead to decreasing volume of milk produced, promoting an increase in milk fat, protein and casein concentrations. SCC values were not significantly correlated (p>0.05) with DDE and urea.

The average percentage of samples with SCC above 600,000 SC / mL was 13.41%, ranging from 5.00 to 29.58% (Table 5). The group of ½ blood HB animals showed the lowest percentage (5%) of samples above 600,000 SC/mL and the group of ¾ blood HB animals showed the highest percentage (29.58%) of samples outside standard set by IN 62/2011.

The results shown in Table 5 indicate that ½ blood HB animals had lower pre-disposition to infection in the mammary gland. Values greater than those of this study were reported by Cunha et al. (2008), who identified a progressive reduction in the percentage of animals with subclinical mastitis between the years 2000 (43.9%) and 2003 (38.7%). This result can be attributed to greater strictness in obtaining milk aimed at exports and increased demand from the domestic market, indicating that the southwestern state of Goiás will likely meet the requirements of Brazilian legislation for milk quality for the current period and from 2014.

Conclusion

The milk produced by 15/16 blood HB cows showed higher fat, lactose, DDE, protein, casein and urea contents and lower SCC values. Thus, it can provide higher profitability to farmers when inserted in the payment program for milk quality. All genetic groups produced quality milk, meeting the requirements previously established by IN 62/2011. ½ blood HB cows are the best options for the dairy production systems in the Cerrado region of Goiás; however, the lowest milk production was attributed to 15/16 blood HB genetic composition due to the higher requirements of this breed composition regarding environmental conditions.

Conflict of Interests

The authors have not declared any conflict of interests.

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