

# Efeito do Nível de Produção e Estação do Ano Sobre a Qualidade do Leite de Vacas Mestiças

## Production Level and Season of the Year on Quality of Milk from Crossbred Dairy Cows

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### Resumo

O estudo dos fatores que interferem na composição química e qualidade higiênico-sanitária do leite é importante, tendo em vista a heterogeneidade do sistema produtivo, e a necessidade de padronização da matéria prima. Objetivou-se avaliar o efeito do nível de produção e da estação do ano sobre a qualidade do leite de vacas mestiças. As coletas foram realizadas em nove propriedades, que foram classificadas em três níveis de produção, durante as quatro estações do ano. As amostras de leite foram analisadas quanto aos teores de gordura, proteína, caseína, nitrogênio ureico, lactose, sólidos totais e sólidos não gordurosos; contagem padrão em placas e contagem de células somáticas. Adotou-se o delineamento experimental inteiramente casualizado, sendo os dados coletados submetidos à análise de variância e as médias comparadas pelo teste de Tukey. Nenhum efeito significativo do nível de produção foi relacionado aos teores de gordura, proteína, caseína, lactose ou contagem de células somáticas. No entanto, os níveis de produção afetaram os teores de nitrogênio ureico no leite, cujo maior valor (15,18 mg / dL) foi observado nas propriedades com produção diária de leite superior a 500 L; e contagem padrão em placas, cuja maior média (5,73 log UFC/mL) foi encontrada em fazendas que produziam menos de 150 L de leite por dia. A menor contagem padrão em placas ocorreu no inverno (4,96 log UFC/mL). O nível de produção e a estação do ano influenciam o teor de nitrogênio ureico no leite e a contagem padrão em placas.

**Palavras-chave:** Bovinos. Composição do Leite. Período do Ano. Produtividade.

### Abstract

*The study of the factors that interfere with the chemical composition and quality of the hygienic-sanitary milk is important, considering the heterogeneity of the productive system, and the need for standardization of the raw material. The objective of this study was to evaluate the effect of production level and season of the year on the quality of milk from crossbred cows. Collections were performed on nine farms, which were classified into three production levels, during four seasons of the year. Milk samples were analyzed for the fat, protein, casein, urea nitrogen, lactose, total solids, and solids-not-fat contents; total bacterial count; and somatic cell count. A completely randomized experimental design was adopted, with the collected data subjected to analysis of variance and means compared by Tukey's test. No significant effect of production level was detected on fat, protein, casein, lactose, or somatic cell count. However, the production levels affected the milk urea nitrogen contents, whose highest value (15.18 mg/dL) was observed on the farms with a daily milk yield greater than 500 L; and total bacterial count, whose highest mean (5.73 log CFU/mL) was found on farms producing less than 150 L of milk daily. The lowest total bacterial count occurred in the winter (4.96 log CFU/mL). Production level and season of the year influence the milk urea nitrogen content and total bacterial count.*

**Keywords:** Cattle. Milk Composition. Period of the Year. Yield.

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### 1 Introduction

The milk composition is prone to variations, since several factors can modify its basic components, including hygienic-sanitary characteristics, which directly influence its quality (LUDOVICO; TRENTIN; RÊGO, 2019). Consequently, the income of producers, the industrial yield, and the quality of the end-product are affected (RIBAS et al., 2015).

The fat content is decisive to the standardization and control of the raw material. The protein, especially casein, is related to the industrial yield. Lactose, in turn, is a substrate for fermentation processes (SILVA; VELOSO, 2011). The non-protein nitrogen fractions present in the form of milk urea nitrogen, which mask the true protein, are linked to the

protein levels and may be associated with production and reproduction (DE SOUZA, 2019).

Somatic cell count levels are a reflection of the health of the mammary gland, while the total bacterial count indicates the hygiene in the process of obtaining and storing the milk, which makes it an important tool to check and adjust management strategies.

The health of the mammary gland (ZIGO et al., 2021), genetic composition (breed) (MARTINEZ et al., 2021); feeding (BLAIR 2021); management practices during milking; calving order; production volume (GONZÁLEZ; NORO, 2011); and seasons of the year (WEBER et al., 2020) are factors that can influence the milk quality.

Several factors may exert influence. In Brazil, climatic

conditions have an expressive impact, since the tropical climate of the country renders the dairy herd exposed to high temperatures and consequently greater susceptibility to heat stress, which ultimately leads to reduced feed intake (NEVES et al., 2019) in addition fluctuations in the quantity and quality of feed available between seasons, causing losses in the composition and production of milk. The production level is also a relevant factor, given that a considerable share of producers work with low-technology systems, having a low daily yield and insufficient conditions to allow the production of high-quality milk (VALLEVA et al., 2005).

In view of the aforementioned scenario, the present study was proposed to evaluate the effects of production level and climatic seasonality on the physico-chemical, sanitary, and microbiological components of raw milk from crossbred cows.

## 2 Material and Methods

The experiment was developed in the microregion of São Luís de Montes Belos, GO, Brazil, on nine dairy farms: three with a milk yield of up to 150 L/day; three producing 151 to 500 L of milk per day; and another three with a milk yield larger than 500 L/day, all of which had crossbred herds. In the month prior to sampling, we determined the farms that would compose the sampling universe according to the production level.

The dairy farms fitting the different production levels had a similar feeding management. During the rainy period, of the nine properties, six performed rotational grazing on a *Panicum maximun* cv. Mombaça pasture. At the others, grazing performed extensively on a *Brachiaria brizantha* cv. Marandu pasture. In the dry period, corn silage was provided on all farms, and the animals were supplemented with a concentrate feed. The mechanical milking system was present in all cases; however, at Level-1 properties, the basic routine milking procedures were not adopted, e.g. establishment of a milking line, disinfection of the milker's hands, strip-cup test, pre/post-dipping, and drying of teats with paper towel. As for the sanitary aspects with the herd, all farms conformed to the prophylactic calendar established by the Brazilian Ministry of Agriculture and Livestock (MAPA).

Samples were harvested on four occasions, considering the astronomical seasons. As such, they were collected in March/summer, May/Fall, July/winter, and September/spring, thus comprising the four seasons of the year.

The thermoneutral zone for dairy cattle is between 5 and 25 °C; however, the maximum temperature values presented during the experimental period in all seasons were higher than recommended (Table 1). The summer was the season with highest rainfall.

**Table 1** - Temperature and precipitation according to the season of the year

Season	Temperature (°C)			Precipitation (mm)
	Maximum	Minimum	Mean	
Fall	31.31	18.27	24.50	11.10
Winter	31.70	17.25	24.34	79.3
Spring	30.01	20.32	24.84	563.7
Summer	30.25	21.19	24.91	763.4

Source: Meteorology and Hydrology System of Goiás State (SED/SIMEHGO, 2016).

Properties where cows are milked twice daily had 60% of the bottle corresponding to the morning milking and 40% from the individual afternoon milking of the animals. The composition, casein, milk-urea nitrogen (MUN), and somatic cell count (SCC) of these samples were analyzed. For the analysis of the milk from the tank, we collected one sample containing the preservative Bronopol and another containing the bacteriostatic preservative Azidol<sup>®</sup>, from each tank of the nine farms, totaling 72 samples during the four collections. The recommendations of the Brazilian Network of Milk Quality Laboratories (*Rede Brasileira de Laboratórios da Qualidade do Leite* - RBQL) adopted. After collection, bottles identified and the material was immediately homogenized to dissolve the pills. Subsequently, samples were placed in isothermal boxes containing recyclable ice in a sufficient quantity to maintain the internal temperature of the box at a maximum of 7 °C. The period between sample collection and arrival thereof at the laboratory did not exceed 96 h (DIAS, 2012).

Milk samples collected from the tank were analyzed for SCC, total bacterial count (TBC), composition, casein, and MUN. The SCC of the herds was quantified by flow cytometry, using automated instruments (Fossomatic 5000 Basic – FOSS and Somascope – Delta) according to ISO/IDF (International Organization for Standardization/International Dairy Federation). Analyses of TBC were performed using Bactoscan FC (FOSS) and Bactocount IBC (Bentley) analyzers, whose analytical principle is based on flow cytometry, as described in ISO/IDF. The milk composition (fat, protein, lactose, total solids) was determined by the analytical principle based on the differential absorption of infrared waves by the milk components, using a Milkoscan 4000<sup>®</sup> analyzer (Foss Electric A/S. Hillerod, Denmark). Urea (mg/dL) and casein (%) contents were determined by the analytical principle, which is based on the differential absorption of infrared waves, transformed by Fourier – FTIR, using a Lactoscope<sup>®</sup> analyzer (Delta Instruments).

A completely randomized experimental design was adopted in which the collected data were subjected to analysis of variance and means were compared by Tukey's test at the 5% significance level, including the effects of SCC, TBC, composition, and MUN contents and the interactions among these factors in the model. Somatic cell count ( $\times 10^3$  cells/mL)

and total bacterial count ( $\times 10^3$  CFU/mL) were transformed into natural logarithm (LSCC) by the equation  $\ln(\text{SCC}+1)$  and (LTBC) and  $\ln(\text{TBC}+1)$ , as they did not show normal distribution (MEYER et al., 2006). Results were analyzed using the SISVAR software version 5.6.

### 3 Results and Discussion

Production level and season of the year affected the quality standards of the refrigerated raw milk (Table 2). There were no problems regarding composition. Non-conformities were restricted to TBC and SCC, which were higher in the fall.

**Table 2** - Conformity of raw milk to Normative Instruction 76\*

Level	Season			
	Summer	Fall	Winter	Spring
$\leq 150$ L	33.33%	33.33%	66.66%	33.33%
$>150 \leq 500$ L	66.66%	0.00%	100.00%	33.33%
$>500$ L	33.33%	33.33%	33.33%	33.33%

Source: Brasil (2018).

The coefficients of variation for the physico-chemical components of milk (Table 3) indicated a certain homogeneity of the data. The lowest CV was observed for lactose, which was expected, since this is the component that varies the least, followed by SNF, protein, casein, total solids (TS), fat, and MUN, the last of which showed the highest variation.

**Table 3** - Effect of production level and season of the year on the physico-chemical parameters of raw milk from crossbred cows

Treatment	Season				× level	CV (%)	Significance
	Summer	Fall	Winter	Spring			
<b>Fat (%)</b>							
$\leq 150$ L	4.02	4.08	3.84	3.69	3.91a		
$>150 \leq 500$ L	3.86	3.95	4.14	4.15	4.02a	11.99	NS
$>500$ L	3.56	3.86	4.03	3.58	3.76a		
× season	3.81a	3.96a	4.00a	3.80a			
<b>Protein (%)</b>							
$\leq 150$ L	3.34	3.55	3.35	3.20	3.36a		
$>150 \leq 500$ L	3.38	3.53	3.50	3.39	3.45a	6.18	NS
$>500$ L	3.35	3.52	3.62	3.37	3.47a		
× season	3.36a	3.53a	3.49a	3.32a			
<b>Casein (%)</b>							
$\leq 150$ L	2.57	2.80	2.61	2.47	2.61a		
$>150 \leq 500$ L	2.65	2.77	2.67	2.60	2.67a	6.83	NS
$>500$ L	2.62	2.76	2.80	2.57	2.69a		
× season	2.61a	2.77a	2.69a	2.55a			
<b>Milk urea nitrogen (mg/dL)</b>							
$\leq 150$ L	14.88	7.62	6.62	7.76	9.22a		
$>150 \leq 500$ L	11.80	7.18	12.66	14.01	11.93ab	47.53	S
$>500$ L	20.22	13.77	12.70	16.08	15.17b		
× season	15.63a	9.52a	10.66a	12.62a			
<b>Lactose (%)</b>							
$\leq 150$ L	4.19	4.17	4.33	4.51	4.30a		
$>150 \leq 500$ L	4.51	4.49	4.47	4.53	4.50a	6.12	NS
$>500$ L	4.55	4.22	4.55	4.63	4.49a		
× season	4.42a	4.29a	4.45a	4.56a			
<b>Total solids (%)</b>							
$\leq 150$ L	12.84	12.79	12.77	12.63	12.76a		
$>150 \leq 500$ L	12.72	12.95	13.14	13.10	12.98a	11.31	NS
$>500$ L	12.98	12.85	13.25	12.61	12.92a		
× season	12.85a	12.86a	13.05a	12.78a			
<b>Solids-not-fat (%)</b>							
$\leq 150$ L	8.61	8.71	8.83	8.82	8.74a		
$>150 \leq 500$ L	8.88	8.99	8.99	8.95	8.95a	6.14	NS
$>500$ L	8.92	9.00	9.22	9.03	9.04a		
× season	8.80a	8.90a	9.01a	8.93a			

CV - coefficient of variation; NS - not significant; S - significant. Means followed by common letters in the rows and columns do not differ significantly by Tukey's test ( $P < 0.05$ ).

Source: resource data.

Production level and season of the year did not significantly affect the fat, protein, casein, lactose, TS, and solids-not-fat

(SNF) contents ( $P > 0.05$ ). However, production level had a significant influence of the on MUN ( $P < 0.05$ ), with the highest

concentrations obtained on Level-3 farms (15.18 mg/dL); i.e. farms with a daily yield larger than 500 L, which demonstrates that MUN was strongly influenced by production.

The fat content was lower on Level-3 farms (<500 L); i.e. those with largest milk production. It is recurrently reported in the literature that the fat content is inversely proportional to the milk yield, which is related to nutrition aspects, since high-yield dairy cows receive a total diet with low contents of effective fiber and high levels of concentrate, which results in a lower fat content. This is explained by the fact that fat is positively influenced by higher concentrations of acetic and butyric acids, which originate from the ruminal fermentation of the fiber; moreover, these volatile fatty acids are primary precursors of the milk fat (NRC, 2001).

In the present study, the protein and fat contents were inversely proportional, with Level-3 farms (>500 L) exhibiting a higher percentage of protein and simultaneously a lower percentage of fat. Noro et al. (2006) observed that, in general, as the protein content of milk was increased, milk yield also increased, which was not true for fat. According to Teixeira et al. (2010), protein is the second component that most varied as a function of environmental factors, especially nutritional aspects. As stated in the NRC (2001), of the nutritional factors, the amount of dietary protein and its amino acid profile are those that most directly influence the milk fat content.

We emphasize that the results obtained for casein relative to production level were similar to those of protein. This is explained by the fact that casein constitutes a larger portion of the milk protein; on average, 95% of the CP is true protein, 80% of which corresponds to casein.

Lactose values were practically constant, which was a consequence of the small natural variation of this component in milk and its participation as a regulating agent in production, considering that this element is not susceptible to alterations of nutritional nature (Fagan et al., 2010).

Addressing TS, Peres (2001) stressed that variations in their content depend on variations in the milk fat. Because there was no significant difference for the fat content between the different production levels, TS also did not change.

In the analysis of the milk components as a function of the seasons of the year, no significant influence was detected ( $P>0.05$ ). Silva and Veloso (2011) stressed that fat and protein are the components that vary the most in milk, whereas the lactose concentrations remain practically always constant. In contrast to the present findings, Noro et al. (2006), Pérez (2011), and Henrichs et al. (2014) found that the seasons of the year exerted an influence on the fat, protein, lactose, and TS contents.

Henrichs et al. (2014) observed that the mean percentages of total solids in milk from dairy herds in Paraná State (Brazil) were higher in the winter (12.4%) and lower in the summer (12.07). Noro et al. (2006) obtained similar results evaluating the effect of environmental factors on the composition of milk from herds in the state of Rio Grande do Sul, where the fall

and the winter were the most favorable seasons to the milk chemical quality. The highest concentrations of fat (3.7%), protein (3.18%), lactose (4.6%), and total solids in milk were observed during that period. Andrade et al. (2014), on the other hand, did not find influences of the seasons on the composition of milk in the same evaluation of herds from the state of Rio Grande do Norte, which might have been a result of climatic differences between the two states.

Henrichs et al. (2014) explained that the possible cause of the reduction in TS contents in temperate zones during the summer was the reduced feed intake of the animals, in an attempt to reduce body heat production resulting from the digestive processes, which also led to production losses.

Fagan et al. (2010) also made similar remarks, declaring that differences in chemical composition between the seasons were not related to the climatic conditions, but rather to the individual quality of the total diet provided in each season. The lack of significant differences in the milk physico-chemical components in the different seasons of the year can be explained by the level of feed control and the nutrition of the herd regardless of the season (GARZON et al., 2021)

Data obtained from the samples for MUN revealed the following results for minimum, maximum, and average contents (mg/dL): Level 1 farms - 6.62, 14.88, and 9.22; Level-2 farms - 7.18, 14.01, and 11.93; and Level-3 farms - 12.70, 20.22, and 15.17. Normal MUN values should be between 10 and 16 mg/dL, and these concentrations may vary across herds, lots, and cows within the same herd (KANANUB et al., 2021). Of the three production levels, only one showed a mean below the recommended range, while the others showed a satisfactory mean MUN. However, when the amplitude is taken into account, the minimum values of Level-1 and -2 farms and the maximum value of Level-3 farms are out of the range.

Mean values for MUN differed significantly between production levels ( $P<0.05$ ), with the highest mean found in Level-3 farms, indicating that this variable followed the increase in production level. Meyer et al. (2006) observed that among the non-nutritional factors, that which most affected the MUN concentration was the average daily milk yield. Every additional kilogram in daily milk yield led to a 0.1054 mg/dL increase in MUN. Kananub et al. (2021) found a positive relationship between the MUN concentration and milk yield.

Mutsvangwa et al. (2016) found a similar relationship, which was attributed to increases in the dietary protein content rather than to the effect of milk production. Aguilar et al. (2012) found that alterations in every percentage unit of dietary protein led to a change of 1.1 mg/dL in MUN.

Broderick and Clayton (1997) concluded that the effect of milk yield on MUN is a result of the correlation between yield and dietary protein, since an increase in dietary protein associated with adequate amounts of rumen-degradable protein improves the efficiency of use of the absorbed nutrients, resulting in a larger milk yield (NRC, 2001).

With respect to the seasons of the year, the average MUN

contents did not differ significantly from each other ( $P>0.05$ ). These results disagree with those obtained by Doska et al. (2012), who reported that MUN concentrations differed significantly from each other ( $P<0.05$ ), with higher values in the winter as compared with the other seasons.

Fatehi et al. (2012) also found a significant influence of the season of the year on the MUN concentration. Godden et al. (2001) observed higher levels in the months of July and September; Fatehi et al. (2012) observed higher levels in spring and summer, with maximum and minimum concentrations achieved in July and in the fall-winter period, respectively.

As can be seen in Table 4, the hygienic-sanitary characteristics of the milk were the variables with the highest coefficients of variation, indicating the great variability of these components in the cooling tanks, mainly for TBC. Mean TBC values differed significantly for the production levels ( $P<0.05$ ), with the highest values obtained in Level-1 farms (5.73 log CFU/mL); in terms of season of the year ( $P<0.05$ ), the lowest means were observed in the winter (4.96 log CFU/mL). Mean values for SCC did not manifest a significant variation ( $P>0.05$ ) as a function of production level or season.

**Table 4** - Effect of production level and season of the year on hygienic-sanitary characteristics of raw milk from crossbred cows

Treatment	Season				× level	CV (%)	Significance
	Summer	Fall	Winter	Spring			
TBC (log CFU/mL)							
≤150L	5.84	5.10	6.27	5.72	5.73a		
>150≤500 L	4.42	4.67	4.29	4.42	4.45b	145.96	NS
>500 L	4.88	5.80	4.32	4.83	4.96b		
× season	5.05a	5.19a	4.96b	4.99a			
SCC (log cell/mL)							
≤150L	5.82	5.87	5.61	5.52	5.70a		
>150≤500 L	5.76	5.82	5.56	5.70	5.71a	51.09	NS
>500 L	5.79	5.77	5.77	5.74	5.77a		
× season	5.79a	5.82a	5.65a	5.65a			

CV - coefficient of variation; TBC - total bacterial count; SCC - somatic cell count; NS - not significant.

Means followed by common letters in the rows and columns do not differ significantly by Tukey's test ( $P<0.05$ ).

Source: resource data.

Andrade et al. (2014) observed that the average SCC and TBC did not change expressively across the seasons. In an evaluation of production levels emphasized that the most specialized systems, with higher yields, had milk with lower SCC. Galvão Júnior et al. (2010), however, did not observe significant variations in SCC relative to the average production levels. In turn, stated that the elements that most influence TBC and SCC are factors related to the milking management rather than production level and season.

The literature features recurrent reports of increases in SCC and TBC values in the seasons with greater incidence of rainfall because of an increase in sources of contamination and greater difficulty disinfecting the teats, thereby predisposing the udder and the milk to contamination (FONSECA; SANTOS, 2019). This fact was evidenced for TBC, whose lowest means were observed in the winter (06/21 to 09/22) — the period of rain scarcity in the sampled region.

For the milk produced in the Central-West, South, and Southeast regions of Brazil, the maximum limits acceptable as of July 1st, 2006 were postponed for two years, remaining at 300,000 CFU/mL instead of 100,000 UFC/mL and 500,000 cells/mL instead of 400,000 cells/mL. It is noteworthy that the mean values for physico-chemical components found here meet the minimum requirements established by Normative Instruction no. 62 (Brazil) for refrigerated raw milk: 3.0% fat, 2.9% protein, 11.5% TS, and 8.4% SNF. However, only 33.89% of the samples conformed to the maximum TBC

(300,000 CFU/mL) and SCC (500,000 cells/mL) values established by the current legislation.

Studies conducted by Pérez (2011) revealed that more-intensive production systems, with a higher yield, showed lower TS values. By contrast, their milk presented superior sanitary quality than on farms with less-intensive systems and a consequent lower production, which the present findings corroborate.

Overall, the non-manifestation of a significant difference of the physico-chemical characteristics of milk, except for MUN and SCC, in response to the different production levels and seasons of the year might have been influenced by breed-related characteristics, since the present study evaluated crossbred cows while most experiments are conducted with purebred cows, which are more susceptible to climatic and management-related variations. Azevedo et al. (2005) and McManus et al. (2014) stressed that crossbred animals are more resistant to heat stress and other environmental stressors, as they adapt better to adverse conditions and hot climates.

#### 4 Conclusions

Of the milk physico-chemical components, urea nitrogen is the only variable influenced by the production level, with highest means observed on farms with a larger milk yield (over 500 L of milk per day). The production level also influences the hygienic-sanitary characteristics of milk, with higher total bacterial counts detected on farms with lower production

and a lower technological level. The lowest mean values for somatic cell count are found in the winter — the period of little incidence of rainfall. Fat, protein, casein, lactose, total solids, solids not-fat, and somatic cell count are not influenced by the production level or by the season.

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