The Microorganism Contamination Affect the Physical and Chemical Composition of Milk

[1]Magnavita, A. P. A., [2]Ferrão S., [3]Malhado, C. H., [4]Silva E. R., [5]Matarazzo, S.V., Santana, V. S, *[6]Fernandes, S. A. de A

[1,2,3,4,6]Grupo de EstudosemLeite (GEL)/Department of Rural and Animal Technology – Campus Juvino Oliveira/State University of Southwest of Bahia.Itapetinga, CEP-ZIP Code 45-700-000. Bahia, Brazil.
[5]State University of Santa Cruz, Department of Agricultural and Environmental Sciences – UESC, Rod. Jorge Amado, s/n. CEP/ZIP Code: 45.662-900. Ilhéus, Bahia, Brazil.

Abstract—

Background

The changes that occurred in the Brazilian dairy industry since 1990 began the advances in milk production observed in recent years and the greatest reflection of this transformation was the implementation of the new legislations. The somatic cell count (SCC) and total bacterial count (TBC) in milk are among the most discussed factors due to their importance in Brazil and related to losses at farms and industry. Studies evaluating the effect of TBC on the milk components are scarce and generally assess the effects of confounding between SCC and TBC. The objective of this study was to evaluate the milk from Zebu contamination by somatic cell count (SCC) and total bacterial count (TBC), isolated.

Material, Methods and Results

The samples were collected monthly from 180 farms in the Territory of Identity of the Middle Southwest Bahia. The samples were collected in the farms after manual milking and before cooling in sanitized plastic containers with a volume of 1 L. The contents of crude protein, fat, and lactose, SCC and TBC were determined electronically. Were formed 4 classes of the SCC and TBC. The up limit of the SCC was200,000 cells/ml were selected in order to evaluate only the effect of TBC on milk components, being classified in the same categories mentioned above. As the SCC increased, the contents of fat, crude protein, and total solids increased, an inverse behavior in relation to the content of lactose, which decreased. The TBC presented an inverse behavior, and, as it increased, the contents of fat, protein, and total solids decreased, however with no significant effect between the first and the last class.

Discussion

The increase in the content of crude protein in milk with high SCC is caused by the increase in serum proteins, especially the immuno-globulins; however, the fraction which is the most important for the dairy industry, the casein, decreases. The TBC results indicate that the country will face difficulty to produce milk and derivatives for export.Still, the TBC indicate that there are effects on the macrocomponents of milk, without a very clear behavior. However, the most adverse effects are observed in the degradation process of these. Two processes interact to decrease the content of lactose in the milk, i) mastitis, which causes reduced production in the mammary gland, and; ii) increased consumption by contaminant microbiota, with the increase of that population. The action of the contaminant microbiota on the macrocomponents of milk does not make the adverse effects very clear. The analytical method used in this study determines the protein fraction as a whole, as well as the lipid fraction. However, the protein fraction is divided in: i) casein (CN): α-s1-CN and α-s2-CN; β-CN, γ-CN; ii) serum protein: α-lactalbumin and β-lactoglobulin; iii) nonprotein nitrogen. Among these, casein is the most important for the dairy industry, and the SCC exerts negative effect on it. The proteolytic action of the enzymes produced by the natural microbiota of milk exists; however, with the contamination of the milk (mastitis, or microbial contamination after milking), this exogenous flora increases this proteolysis.In the lipid fraction in milk, there are: i) phospholipids; ii) cholesterol; iii) triacylglycerols; iv) diacylglycerols; v) free fatty acids; vi) monoacylglycerols; vii) free cholesterol; and others. In addition to evaluating the effects on the content of fat, it is necessary to know the effect of SCC and TBC on the lipid fraction.As related, both (SCC and TBC) are the principal factors related to milk quality, especially, TBC.

Conclusion

The somatic cell count and the total bacterial count negatively affect the physical and chemical composition of milk from zebu animals. Few studies were found in the literature that evaluated the effects of contamination of milk from zebu cows, which demonstrates the importance of this study.

Keywords— cheese quality, dairy inefficiency, mastitis, milk contamination, zebu.

I. INTRODUCTION

The changes that occurred in the Brazilian dairy industry since 1990 began the advances in milk production observed in recent years [1], which indicate that Brazil is moving to self-sufficiency and may become an exporter. The greatest reflection of this transformation was the implementation of the new legislation, normative instruction (IN No. 51, from portuguese) in 2002, which regulates the production, identity, quality, collection and transport of milk in the country [2], amended in 2011 by the by another normative instruction (IN No. 62, from portuguese) [3].

The somatic cell count (SCC) and total bacterial count (TBC) in milk are among the most discussed factors due to their importance in Brazil. The SCC is associated with mammary gland health, especially with mastitis. The TBC will vary with the initial contamination of milk. The milk secreted in the mammary gland is sterile. However, contamination can occur in the mammary gland (by mastitis-causing agents), during milking, storage, transportation, and processing [4, 5, 6].

Mastitis is an inflammation of the mammary gland and causes alterations in the composition of milk (fat, crude protein, and lactose) resulting in economic losses to the dairy industry [7, 8; 9]. Production losses can reach 18%, and the losses in fat and protein (casein) amount to 12% and 11%, respectively [9, 10]. These losses result in others, such as lower industrial output, lower shelf life, alterations in taste, costs with animal treatment, milk disposal, among others.

Considering the farms, losses are observed in costs with drugs, labor, and milk disposal, among others. Industry losses are related to decreased industrial output, the sensorial quality of the products, the return of cheeses from retailers (reverse logistics) [8, 11, 12] and the damage caused to the brand.

Healthy mammary glands must present SCC up to 200,000 cells/mL of milk; up from this value, production losses begin to be observed [13; 14]. Studies evaluating the effect of SCC are common [15]. On the other hand, studies evaluating the effect of TBC on the milk components are scarce and generally assess the effects of confounding between SCC and TBC.

Studies on the dynamics of the milk from bulk tanks help in creating strategies to solve potential problems in order to avoid losses that occur in the production chain [16, ;17].

This experiment aimed to evaluate the microbiological quality of raw milk from Zebu cow through the determination of somatic cell count and total bacterial count in association and the total bacterial count in isolation and the effects on the milk composition.

II. MATERIAL AND METHODS

The raw milk samples were collected monthly from 180 suppliers to dairy products industries in the territory of identity of the the Middle Southwest Bahia between January 2009 and December 2010. The samples were collected on farms after manual milking and before cooling; the milk was homogenized and collected in proportion to the production, in clean plastic containers with a volume of 1 L. Each sample was divided into three sub-samples of milk: i) 500 mL to determine the acidity (^{o}D), density, freezing point index [18] and pH; ii) 50 mL containing Bronopol (2-bromo-2-nitro-1.3-propanediol) to determine the content of crude protein, fat, lactose, total solids (TS), non-fat solids (NSF) and somatic cell count (SCC), and; iii) 50 mL with Azidiol pads to determine the total bacterial count (TBC).

The contents of crude protein, fat, and lactose were determined by reading the absorption of infrared light by using the equipment Bentley 2000®. The total solids and the solid non-fat (SNF) were determined by the sum of the total of solids, total solids minus fat, respectively. The SCC was performed by flow cytometry in the equipments Bentley 2000 [19] and Somacount 300 [20]; TBC was performed by using the Bactocount[21].

2.1 Somatic cell count classes

The classification of the SCC (cells/mL of milk) and TBC (colony forming units/mL of milk) was performed as follows: Class A: up to 100,000; Class B: greater than 100,000–200,000; Class C: greater than 200,000–600,000; Class D: greater than 600,000.

2.2 Statistical analysis

In order to analyze the effect of SCC on the variables (crude protein, fat, lactose, total solids, solid non-fat, density, acidity in Dornic degrees, and pH), the GLM procedure [22] was used under a model that considered as sources of variation the SCC, the TBC, and the interaction SCC versus TBC. The means were adjusted through the method of least squares and contrast of means at 5% significance.

After the first analyses, and depending on the interaction between SCC and TBC, a new classification was performed in order to assess exclusively the effect of TBC on the composition of milk. Thus, the up limit of the SCC was 200,000 cells/mL, the data were selected and subdivided into the following classes of TBC (colony forming units/mL of milk): Class A: up to 100,000; Class B: greater than 100,000–200,000; Class C: greater than 200,000–600,000; Class D: greater than 600,000.

III. RESULTS

The distribution of somatic cell count (SCC) and total bacterial count (TBC) by classes in the samples of milk indicates the need for adjustments in the studied farms concerning SCC and TBC (Figure 1).

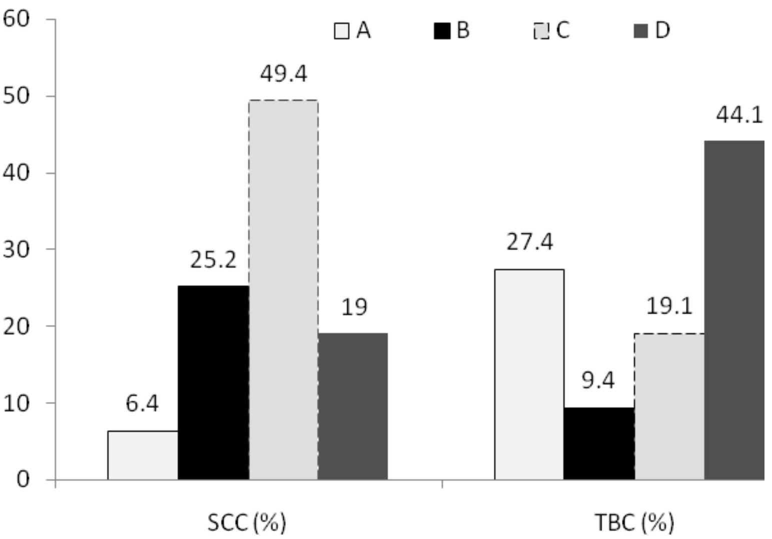

FIGURE 1.PERCENTAGE DISTRIBUTION OF THE SOMATIC CELL COUNT (SCC) AND TOTAL BACTERIAL COUNT (TBC), BY SAMPLES CLASSES OF RAW MILK.

[SCC Classes (x 1,000 cells/mL) andTBC Classes (x 1.000 colony forming units/mL): A = up to 100; B = greater than 100–200; C = greater than 200–600; D = greater than 600]

The IN No. 51, amended by the IN No. 62 [3], establishes a maximum limit of 600,000 cells/mL of milk for SCC in Northeast Brazil. Only 19% of the samples are in non-compliance with Brazilian legislation, a good response from the producers.

Regarding SCC, only 19% (Figure 1) are in non-compliance with the IN No. 62 [3], which apparently is positive. However, two premises must be evaluated: i) SCC above 200,000 cells/ml is indicative of mastitis [13], and, based on this report; ii) the legal limit (600,000 cells/mL) is not ideal from the point of view of animal health and characteristics of milk and its derivatives [14, 23]. There is a report of lack of influence of SCC on the fat content [23], as well as there are records of increase [24, 25].

Currently, the IN No. 62 [3] indicates maximum legal limit for TBC of 600,000 CFU/ml (colony forming units/mL); however, upon achieving its full implementation, it will be of 100,000 CFU/mL. This indicates that the optimal milk must have a maximum TBC of 100,000 CFU/mL. The percentage of samples in accordance with the current schedule is 56.9% (maximum of up to 600,000 CFU/mL) and 44.1% in non-compliance (Figure 1). These data demonstrate the consumption of milk with high contamination, probably associated with the milking environment, especially considering that these farmers milking their cows manually and in corrals.

All evaluated variables underwent effect of increase of SCC in milk (Table 1). The lower fat content was observed at A class, that was similar to B, when increasing, with the highest value observed at D class. There is a report of lack of influence of SCC on the content of fat [23], as well as there are records of increase [24, 25].

TABLE 1
EFFECT OF SOMATIC CELL COUNT (SCC/mL^{-1}) IN MILK FROM ZEBU COW ON THE CONTENTS OF FAT (F), CRUDE PROTEIN (CP), LACTOSE (L) AND TOTAL SOLIDS (TS)

SCC classes	F (n=1380)	CP (n=1380)	L (n=1380)	TS (n=1380)
A	3.58±0.083^{c}	3.29±0.032^{b}	4.66±0.033^{a}	12.49±0.111^{b}
B	3.65±0.041^{c}	3.32±0.016^{ab}	4.65±0.016^{a}	12.55±0.055^{b}
C	3.88±0.031^{b}	3.35±0.012^{ab}	4.57±0.013^{b}	12.75±0.042^{a}
D	4.08±0.059^{a}	3.37±0.023^{a}	4.50±0.024^{c}	12.90±0.079^{a}

[SCC Classes (x 1,000 cells/mL): A = up to 100; B = greater than 100–200; C = greater than 200–600; D = greater than 600
Different letters in the same column indicate significant difference (P<0.05)]

The protein content increased with the increase of SCC, reaching its highest level in D, although differing significantly (p<0.05) only from the lowest class (A), as noted in literature [24, 25].

No significant effect was observed (P>0.05) for lactose between classes A and B, which represent the healthy mammary glands. However, in milk with SCC higher than 200, the content of lactose decreased significantly (p<0.05), probably, in function, of the mastitis and of the ambiental milk contamination. Thus, classes A and B differed from C, which differed from D.

Another variable which is important for the quality of the milk is the total bacterial count (TBC). There are few studies on the effects of TBC on the macronutrients in milk, especially on zebu cow milk.

The TBC affected significantly (p<0.05) all the studied variables (Table 2). The most evident behavior was the lack of statistical difference (P<0.05) between the first three classes (A, B, and C) for the contents of fat, crude protein, lactose, and total solids. Among these, only the behavior of lactose (L) followed the expected, decreasing concentration with increasing TBC, although significant difference was observed (P<0.05) only between classes A and D. The contents of fat, crude protein, and total solids presented the same behavior, with class B differing significantly only from D. Interestingly, classes A and D did not differ (P>0.05) for both fat and crude protein content. A possible cause of these results is the high standard error of the mean, especially for class C.

TABLE 2
EFFECT OF TOTAL BACTERIAL COUNT (CFU/mL^{-1}) IN ZEBU COW MILK ON THE CONTENT OF FAT (F), CRUDE PROTEIN (CP), LACTOSE (L) AND TOTAL SOLIDS (TS).

TBC classes	F (n=481)	CP (n=481)	L (n=481)	TS (n=481)
A	3.63±0.061^{ab}	3.30±0.032^{ab}	4.69±0.035^{a}	12.53±0.107^{ab}
B	3.78±0.075^{a}	3.37±0.039^{a}	4.68±0.044^{ab}	12.78±0.131^{a}
C	3.64±0.099^{ab}	3.32±0.052^{ab}	4.62±0.058^{ab}	12.51±0.174^{ab}
D	3.50±0.055^{b}	3.26±0.029^{b}	4.58±0.032^{b}	12.28±0.097^{b}

[TBC Classes (x 1.000 colony forming units/mL): A = up to 100; B = greater than 100–200; C = greater than 200–600; D = greater than 600
Different letters in the same column indicate significant difference (P<0.05)]

The density and the freezing point were not influenced (P>0.05) by SCC. The SCC, in turn, influenced (P<0.05) the titratable acidity (Table 3). The highest acidity was observed in class B, which did not differ significantly from A. On the other hand, the acidity observed at A, C, and D classes, did not differ statistically (P>0.05) from each other.

TABLE 3
EFFECT OF SOMATIC CELL COUNT (SCC/mL^{-1}) IN ZEBU COW MILK ON DENSITY (D), FREEZING POINT (FP) AND TITRATABLE ACIDITY (TA) IN DORNIC DEGREES.

SCC classes	D (n = 686)	FP (n = 685)	TA °D (n = 632)
A	1,0310±0,001[a]	-0,5502±0,004[a]	17,82±0,430[ab]
B	1,0307±0,001[a]	-0,5503±0,002[a]	18,13±0,201[a]
C	1,0308±0,000[a]	-0,5462±0,001[a]	17,29±0,154[b]
D	1,0302±0,001[a]	-0,5454±0,002[a]	17,29±0,268[b]

[SCC Classes (x 1,000 cells/mL): A = up to 100; B = greater than 100–200; C = greater than 200–600; D = greater than 600
Different letters in the same column indicate significant difference (P<0.05)]

TABLE 4
EFFECT OF TOTAL BACTERIAL COUNT (TBC, as CFU/mL^{-1}) IN MILK FROM ZEBU COW ON THE DENSITY (D), FREEZING POINT (FP) AND TITRATABLE ACIDITY (TA).

TBC classes	D (n = 686)	FP (n = 685)	TA °D (n = 632)
A	1.0304±0.000[a]	-0.5470±0.003[a]	17.29±0.402[b]
B	1.0306±0.000[a]	-0.5485±0.003[a]	18.39±0.458[ab]
C	1.0308±0.000[a]	-0.5502±0.004[a]	17.71±0.581[ab]
D	1.0311±0.000[a]	-0.5538±0.002[a]	18.28±0.290[a]

[TBC Classes (x 1.000 colony forming units/mL): A = up to 100; B = greater than 100–200; C = greater than 200–600; D = greater than 600
Different letters in the same column indicate significant difference (P<0.05)]

The mastitis caused an increase of the passage of some minerals from the bloodstream into the milk, due to the increased permeability of blood vessels, especially for maintaining osmorality[26, 13]. A decrease of titratable acidity was observed, although there was no difference (P>0.05) between Classes A, C, and D, in absolute values. The previously reported movement is the possible explanation for this behavior, since the pH of milk, with a high SCC, increases [13].

The TBC also exerted no significant effect (P>0.05) on the density and freezing point of zebu cow milk; however, it influenced the titratable acidity (°Dornic). As might be expected, the increase in colony forming units (CFU/mL) causes the consumption, by the microorganisms present in milk, of the lactose (Tables 1 and 2), increasing the titratable acidity (°Dornic).

IV. DISCUSSION

The increase in the content of crude protein in milk with high SCC is caused by the increase in serum proteins, especially the immuno-globulins [24, 25]; however, the fraction which is the most important for the dairy industry, the casein, decreases [23], in addition to an increase in proteolysis, degrading casein [24], adversely affecting industrial productivity [27, 15].

In addition to the animal health aspect, another consideration should be directed associated to SCC. When it is around 500,000 cells/ml, losses are caused in milk production of approximately 6% [14], and high SCC also causes an increase in the coagulation time of milk, resulting in increased production costs, as well as increasing proteolysis, which affects the sensorial quality of cheese [23].

The TBC results indicate that the country will face difficulty to produce milk and derivatives for export. Despite being one of the largest producers of milk in the world, the general characteristics of this raw material need substantial improvement, because even if the level of quality required for export is reached in certain regions of the country, if this level is not the standard throughout all the territory, buyers will have difficulty in making purchases.

Still, the TBC indicate that there are effects on the macrocomponents of milk, without a very clear behavior. However, the most adverse effects are observed in the degradation process of these. Thus, the lipolysis and proteolysis of milk must be evaluated, because the level of these exerts a marked effect on the quality of dairy products.

Two processes interact to decrease the content of lactose in the milk, i) mastitis, which causes reduced production in the mammary gland, and; ii) increased consumption by contaminant microbiota, with the increase of that population [24].

Therefore, these results indicate the need to evaluate the influence of SCC and TBC: i) at the time of milking; ii) arrival at the cooler and in different periods; iii) before collection in the trucks, and; iv) delivery to the dairy, for understanding of the dynamics of contamination of milk; as well as to assess the effects thereof, seeking to understand the pattern of alteration in the fractions of milk and not only in its macrocomponents.

The sampling occurred at the sites of delivery for milk cooling, with the milking usually starting at approximately 5 in the morning and lasting until 9. In this period, the milk is kept in cans, with no refrigeration. After completion of the milking, the milk is transported to the locations of cooling, which takes no longer than two hours. Thus, between the start of milking and sampling, there was a long time, which may have facilitated bacterial growth, in particular psychrotrophic microorganisms, which have optimal growth temperature between 0 and 40°C [28]

The action of the contaminant microbiota on the macrocomponents of milk does not make the adverse effects very clear. The analytical method used in this study determines the protein fraction as a whole, as well as the lipid fraction. However, the protein fraction is divided in: i) casein (CN): α-s1-CN and α-s2-CN; β-CN, γ-CN; ii) serum protein: α-lactalbumin and β-lactoglobulin; iii) nonprotein nitrogen [29, 30]. Among these, casein is the most important for the dairy industry, and the SCC exerts negative effect on it [31]. The proteolytic action of the enzymes produced by the natural microbiota of milk exists; however, with the contamination of the milk (mastitis, or microbial contamination after milking), this exogenous flora increases this proteolysis [24].

In the lipid fraction in milk, there are: i) phospholipids; ii) cholesterol; iii) triacylglycerols; iv) diacylglycerols; v) free fatty acids; vi) monoacylglycerols; vii) free cholesterol; and others [32]. In addition to evaluating the effects on the content of fat, it is necessary to know the effect of SCC and TBC on the lipid fraction.

The aim of this study was to evaluate the quality of milk at the time of delivery to the location of cooling (cooling tanks) or directly at the plant, i.e., without cooling. However, monitoring is necessary at all stages, from farm to processing, in order to evaluate the effects of the contamination of milk and its dynamics. The contamination of milk can occur in the many models of milking, from the most modern [5] to the simplest ones.

As related, both (SCC and TBC) are the principal factors related to milk quality, especially, TBC [33, 34], that affect milk composition negatively and increasing enzyme activity that will deteriorate milk and dairy products during storage [35]. Changes in the milk production, specially a hygiene procedure, could be used to improve the milk bulk tank [17].

V. CONCLUSION

The somatic cell count and the total bacterial count negatively affect the physical and chemical composition of milk from zebu cow

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CONFLICT OF INTEREST

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