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Analyses of the correlation between somatic cell count, centesimal composition and yield of rennet cheese type produced from milk of buffaloes of Autazes, Amazonas.

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Resumo

Análise da correlação entre contagem de células somáticas, composição centesimal e rendimento de queijo tipo coalho produzido do leite de búfalas em Autazes, Amazonas. A baixa contagem de células somáticas (CCS) é parâmetro importante para definir a qualidade do leite de búfalas, pois determina alto rendimento dos produtos obtidos dessa matéria-prima, garantindo a lucratividade a indústria de laticínios e ao produtor rural. O objetivo do trabalho foi analisar a correlação da quantidade de células somáticas presentes no leite de búfalas com a composição centesimal e o rendimento de queijo coalho. As amostras de leite foram coletadas de 73 búfalas de diferentes fazendas localizadas em Autazes, Amazonas, Brasil. A análise da composição, tal como a contagem de células somáticas foi realizada por espectrofotometria associado à citometria de fluxo. Os animais foram divididos em dois grupos, em função da CCS no leite produzido, sendo um grupo (n=67) com CCS inferior de 400.000 células mL^{-1} e outro grupo (n=6) com CCS superior a 400.000 células mL^{-1}. Na sequência, foi calculado o rendimento na produção do queijo tipo coalho para ambos os grupos. A contagem de células somáticas apresentou correlações significativamente positiva com o teor de gordura e significativamente negativa com a concentração de lactose no leite. O rendimento dos queijos tipo coalho produzidos, com CCS acima de 400.000 células mL^{-1}, foram 1,08% menor que o grupo com baixa CCS. De acordo com os dados observados nesse estudo é possível concluir que altas contagens de células somáticas no leite de búfalas foram relacionadas com elevação do teor de gordura e sólidos totais, redução da concentração de lactose presente no leite e redução do rendimento do queijo tipo coalho produzido.

Palavras-chave: bubalino, qualidade, queijo, coalho, Autazes.

Abstract

The low somatic cells count (SCC) is an important parameter to define the buffaloes milk quality, because it determines high yield of products obtained from the raw material, ensuring profitability for both the dairy industry and for the rural producer. The objective of this work was to analyze the correlation of amount of somatic cells present in milk of buffaloes with centesimal composition and yield of cheese curds. Milk samples were collected from 73 buffaloes of different farms located in the Autazes, Amazonas, Brazil. The analysis of the composition, such as somatic cell count was performed by spectrophotometry associated with flow cytometry. The animals were divided into two groups according to the SCC in milk produced, being a group (n=67) with SCC bottom of 400.000 cells mL^{-1} and another group (n=6) with SCC exceeding 400.000 cells mL^{-1}. As a result, income was calculated on rennet-type cheese production for both groups. The somatic cell count showed significantly positive correlations with fat and significantly negative with the concentration of lactose

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in milk. The income of rennet type cheeses produced with SCC above 400.000 cells mL^{-1}, 1.08% were less than the group with low SCC. According to the data reported in this study it can be concluded that high somatic cell counts in the milk of buffaloes were related to elevation of fat and total solids, reducing the concentration of lactose present in milk and reduction of the yield of the rennet-type cheese produced.

Keywords: Buffalo, quality, rennet-type cheese, Autazes.

1. Introduction.

The buffalo (Bubalus bubalis) is the second most important milk producing species in the world. Buffalo's milk represents 13% of the total milk produced globally. There were 1.261.922 buffaloes in Brazil in 2011, with 1,6 % growth rate greater than brazilian cattle herd, between 2011 and 2012 (BARROS et al., 2016).

The centesimal composition of milk is very important issue for the success of the dairy farm. The low quality of the raw material used for the manufacture of cheeses and others products derived from the milk of Buffalo, not only can to intervene on the production profitability, but also can create a public health problem (JORGE et al., 2005; MEDEIROS et al., 2011; BITTENCOURT et al., 2013; CIPOLAT-GOTET et al., 2015; GUIMARÃES et al, 2015; SEIXAS et al., 2015).

The somatic cells count (SCC) are consider the standard measure of quality, because it is relate to the composition, performance and security of industrial milk. In works with milk of buffaloes, high CCS indicated many changes in the composition and in the milk clotting time influencing negatively the industrial yield and the quality of the final product (MEDEIROS et al., 2011).

Somatic cells are composed of defense cells in mammary gland secretory tissue, especially leukocytes. The somatic cells counts of buffaloes milk can indicate quantitatively the degree of mastitis (HARMON, 1994). MEDEIROS et al., (2011) conclude that CCS values above 280.000 cells/mL is indicative of infection of the mammary gland.

Given the importance of somatic cells count, the European Union and Australia have established a legal limit of 400.000 cells mL-1 for the bovine milk be market. In Canada and in the United States, the standards are of 500.000 cells mL-1 and 750,000 cells mL-1, respectively. The Ministry of Agriculture, Livestock, and Supply (Ministério da Agricultura, Pecuária e Abastecimento, abbreviated MAPA) is a federal department in Brazil that regulate, by means of normative instruction nº 62, of December 29, 2011, minimum quality requirements for raw bovine milk in rural properties. This establishing the legal limit of 750.000 cells mL-1, July 2010 until December 2012, of 600.000 cells mL-1 from January until June 2013, 2015 to 500.000 cells mL-1, July 2015 until June 2017 and 400.000 cells mL-1, from July 2017 to the north and northeast regions (BRAZIL, 2011).

However, despite the economic importance there is a lack of specific legislation for regulated the Brazilian quality of milk buffalos. This may be due to the reduced research that represent important technical parameters, like centesimal composition and somatic cells count (SCC) (MEDEIROS et al., 2011; CIPOLAT-GOTET et al., 2015).

The municipality of Autazes stands out for being the biggest dairy basin in Amazonas State. Has the largest production of buffalo milk from Brazil (24.300 liters produced each day), with 1.7 million liters produced in 2006 (IBGE, 2006). The production of buffaloes in the Amazon are perform in extensive system, in the same way, as the cattle are breed in this region being 24,000 buffalos animals

The objective of this research was to analyze the relationship between somatic cell count on milk composition of buffaloes (Bubalus bubalis) and the yield of the rennet cheese produced in the town of Autazes, AM.

2. Material and methods.

2.1 Sampling

Seventy-three buffaloes, mestizo, created in rural properties located in right bank of Autaz-Açu River, municipality of Autazes (Amazonas, Brazil), were select according to the following inclusion criteria: stage of lactation more than ten days and not be in treatment by antibiotics, respecting the grace period of the

therapeutic base employee. The buffaloes were milked by hand, with average per animal being 4,5 liters, due lack of mechanical milking machines on site, in the morning, being 20 mL milk collected and stored for later analysis.

Milk samples were place in plastic sterile vial containing a tablet of Bronopol ® (D and F Control Systems, CA, USA). After the dissolution of the samples were transported, under isothermal conditions, in boxes containing ice, until the milk Lab of the UNIVATES University Center for performing the analysis of the composition and CCS of milk obtained.

2.2 Composition analysis and somatic cell count

The analysis of the composition, such as somatic cell count was performed by spectrophotometry associated with flow cytometry, using the equipment Bentley Combi System ® 2300 (Bentley Instruments Incorporated ®, Chaska, USA), composed of a 2000 Bentley ® equipment unit and a Somacount 300 ® equipment, calibrated to analyze milk veal (COELHO et al., 2004).

2.3 Production of white cheese of buffalo milk

Depending on the somatic cells count, the animals were divided into two groups, being: 1 = Range (CCS < 400.000 cells mL-1) and 2 = Interval (> 400.000 cells mL CCS-1), being used 16 liters of milk for each group, for the manufacture of cheese. Seven days after the first collection, the buffaloes chosen were submit to hand milking in the morning, and each group was milk separately. The milk of each group of buffaloes was collected from gallons of polypropylene sanitized, with a capacity of 50 liters, where it was removed a sample (20 mL), which was wrapped in plastic sterile vial containing a tablet of Bronopol ®, to the analysis of the composition and CCS. The gallons were transport immediately to the Autalac dairy factory, located in Autazes. This procedure was perform during three consecutive days for obtaining two cheeses a day, being a cheese of each group of buffaloes. Rennet cheeses were produced, according to the technique described by Chan (2007), which consisted of weighing the milk, pasteurization, addition of calcium chloride solution 50%, adding rennet powder solution as recommended by the manufacturer, coagulation, cutting of the curd, salting, forming, pressing, weighing, packing and storage.

2.4 Obtaining the cheese yield

The gross income of the obtaining of the cheeses in the different treatments was determined according to Yunes & BENEDET (2000), by means of the formula: R (%) = (Pq/Pf) x 100, where: R = yield; PQ = weight of the finished cheese; PF = weight of the formulation (plus milk ingredients).

2.5 Statistical analyses

Correlations were evaluated between the somatic cell count (SCC) and the content of fat, protein, lactose and solids (ST) using the Statistical Analysis System (RANGEL et al. 2009).

3. Results and discussion.

The average percentage of fat, protein, lactose and total solids of milk, according to the range of the CCS, are in table 1. In 73 samples analyzed, 67 samples was CCS ranged from 4.000 to 311.000 cells mL-1. Observing medium levels of fat in different ranges of CCS, it was noted that 6 samples was in the range 2, with fat content was significantly higher than in the range 1, not corroborating with the observed in work, which in bovine milk reported the occurrence of reduced-fat as increased CCS (HARMON, 1994). This result also differs from other works made with milk of buffaloes, where there was no correlation between the CCS and the fat content of milk (JORGE et al., 2005; BARRETO et al., 2010). However, the difference resembles the results of jobs that have higher concentration of fat in milk from cows with mastitis (COELHO et al., 2012).

High CCS changes the permeability of the blood vessels of mammary gland and reduces the secretion of milk components (protein, fat and lactose). However, when the decrease in production occurs in a manner more pronounced that the synthesis, the fat content suffers a proportional increase, which might have occurred in the present study (MACHADO et al.,

2000). The results of the works that focus on the relationship between somatic cell count and protein content are contradictory. Pereira et al. (1999) observed that the protein content was greater in milk samples from tanks with CCS more than 283.000 cells mL-1, while other authors observed that from 500.000 cells mL-1 occurred a reduction of protein content (MACHADO et al., 2000).

Table 1 - Average percentages of fat, protein, lactose and solids of buffaloes milk, in accordance with the range of the somatic cells count (SCC) of 73 samples analyzed, from animals raised in the city of Autazes, Amazonas, Brazil.

CCS intervals	n	Fat	SD	Protein	SD	Lactose	SD	ST	SD
1 (< 400.000 cells mL $^{-1}$)	67	5,78^{b}	1,25	4,26^{a}	0,36	5,19^{a}	0,26	16,22^{a}	2,86
2 (> 400.000 cells mL $^{-1}$)	6	7,33^{a}	0,92	4,56^{a}	0,25	4,63^{b}	0,40	17,44^{a}	0,79

a, b - Medium followed by lowercase letters, in the same column, do not differ statistically between themselves. SD = standard deviation. ST = Total solids;

As demonstrated in table 1, that to the average total protein content there was no significant difference between the ranges of somatic cells. For the buffaloes ' milk, there is no correlation between the CCS and the levels of crude protein in milk (RANGEL et al., 2009; BARRETO et al., 2010).

In relation to dairy, observed significantly lower concentration ($p < 0.05$) for the interval 2. The values found were of 4.95% and 4.63 for the interval 1 interval 2 and, respectively. The results of this study were similar to those jobs that observed negative significant correlation of the concentration of lactose in milk of buffaloes as the increase of CCS (CERON-MUÑOZ et al., 2002). Data presented in Table 1.

In relation to the total solids, it is observed that the concentration of total solids has increased as the elevation of the CCS, however the difference was not significant. The concentration of total solids may be influenced by the significant increase in the fat content in milk as the increase of CCS, because lipids represent a fraction of total solids of milk (RIBAS et al., 2004). This possibly explains the positive correlation shown in table 1, different from what occurred in the work that found no difference in the content of total solids as the increase of CCS (COELHO et al, 2012).

In Table 2, the coefficient of correlation between the CCS and the lactose content (n = 73) was significant (-0.45). The reduction in the percentage of lactose can be explained by the loss of dairy of the mammary gland into the blood, due to changes in membrane permeability of mammary gland (MACHADO et al., 2000).

Table 2 - Analysis of correlation (R), probability (P) and coefficient of determination (r^2) between somatic cells count (SCC) and content of fat, protein, lactose and total solids in buffaloes milk samples from animals raised in the city of Autazes, Amazonas, Brazil.

Associação	P	R	r^2
SCC x fat	0.0202*	0.2713	0,0736
SCC x protein	0.0802	0.2061	0,0424
SCC x lactose	0.0001*	-0.4475	0,2002
SCC x total solids	0.0183*	0.2755	0,0759

* Significant correlation ($p < 0.05$).

In relation to the yield of cheese 4.446 grams of milk were used, with CCS exceeding 400.000 cells mL-1, for the production of one kilogram of cheese curds. This yield is lower

when compared to the milk containing CCS less than 400.000 cells mL-1, where they were used 4.241 g of milk per kilogram of cheese curds. Minas Frescal cheeses made with milk containing more than 400.000 cells mL-1 also showed reduced performance when compared to the cheeses made with milk containing up to 200.000 cells mL-1 (ANDREATTA et al., 2009). The data are show in Table 3.

Table 3 - Rennet cheese yield in different ranges of somatic cell count (SCC) in buffaloes milk samples from animals raised in the city of Autazes, Amazonas, Brazil.

SCC	Income
< 400.000 cells mL -1	23,41%
> 400.000 cells mL -1	22,33%
Variation	1,08%

In the present work, the income of rennet type cheeses produced with CCS above 400.000 cells mL-1 were 1.08% less than the group with low CCS. This loss is less than described by Coelho et al. (2012). This may be explained by the significant increase in the fat content in milk with CCS exceeding 400.000 cells mL-1, because the fat globules to be retained in the dough are contributing for increasing the yield of cheese manufactured with milk of buffaloes (AMARAL et al., 2005).

It is worth noting that, according to the data described in the literature, this paper presents important for being the first study of the technology of milk buffaloes performed with animals reared in Amazonas State, under conditions other than those other regions of Brazil, such as high temperature and relative humidity throughout the year.

4. Conclusions.

According to the data reported in this study it can conclude that high somatic cell counts in the milk of buffaloes were relate to elevation of fat and total solids, reducing the concentration of lactose present in milk and reduction of the yield of the rennet-type cheese produced.

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Statement of the authors:

Nós autores do artigo intitulado "Análise da correlação entre a contagem de células somáticas, a composição centesimal e o rendimento de queijo tipo coalho produzido a partir do leite de búfalas (*Bubalus bubalis*), no município de Autazes, Amazonas, Brasil." declaramos, para os devidos fins, que o projeto que deu origem aos dados do mesmo não foi submetido para avaliação ao Comitê de Ética da Universidade Federal do Amazonas, mas estamos cientes do conteúdo das resoluções do Conselho Nacional de Controle de Experimentação Animal - CONCEA "http://www.mct.gov.br/index.php/content/view/310553.html" caso envolva animais.

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