Dynamic Evaluation of the Quality Parameters of Raw Buffalo Milk Using a High-Performance Equipment

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RESEARCH ARTICLE

Abstract
The aim of this study was to evaluate the quality parameters of raw buffalo milk, by determining the physicochemical parameters with major impact in terms of milk quality, number of somatic cells and total number of viable counts, at the level of a commercial farm. Over a period of 12 months, samples of raw buffalo milk were collected and analyzed from a farm authorized for the production of buffalo milk, resulting in a dynamic evaluation of quality parameters. The raw milk samples were analyzed using the CombiFossTM7 equipment, which analyzes all the parameters necessary for official controls of milk production and quality. The averages for the results of the physicochemical parameters fat, protein, lactose, total solids and urea were 8.821 g/100 g, 4.400 g/100 g, 4.291 g/100 g, 17.93 g/100g and 27.08 mg/dL, respectively. Even if the gross composition does not exceed the allowed limits, the TVC exceeded the European standards in the case of 58.3% of the analyzed samples, the annual average being 2059.65x10^00 cfu/ml. The value of the SCC average obtained was 304.85x1000 cells/ml, in accordance with the European standard. The values of SCC and TVC are increasing during the sheltering period, which means poor hygiene conditions.

Keywords: milk quality, dynamic evaluation, physical-chemical parameters, SCC, TVC, high performance equipment.

INTRODUCTION
Milk is one of the most complete sources of nutrition available. World milk production comes from cows, buffaloes, goats, sheep and camels. Buffalo milk is considered to be the second most consumed after cow’s milk, representing more than 12% of the world’s milk production (CNIEL, 2002, Ahmad et al., 2008, Khedkar et al., 2016). Buffaloes are widely distributed throughout Asia, the Middle East, Europe, China and South America. However, the largest number of buffaloes and buffalo breeds are found in India and Pakistan producing more than 90% of the world buffalo milk (IDF, 2010; Ariota et al., 2007, Hashmi and Saleem, 2015). This milk is used for making different dairy products, such as butter, butter oil (ghee), soft and hard cheeses, condensed and evaporated milk, ice cream and yoghurt, and due to its high nutritional value, is beneficial to the health of consumers (Napolitano et al., 2019; Ahmad et al., 2008). In terms of buffalo breeding, Romania is currently the second country in Europe after Italy, although the number of buffalo cows has decreased considerably in the last 30 years. In
products are also obtained from buffalo milk, such as

ghee, 1999; Hamad and Boiamy, 2010). Milk of different species contains the
same kind of constituents but in varying in amount (Salman et al, 2014). The milk composition depends on many
factors like genetic, lactation time, daily variations, alimentation type, age, udder cleaning and season. These factors
affect the quality and processing ability of milk. Geographic region, environmental conditions and stage of lactation
are known as seasonal changes and cited among factors affecting milk composition (Kanwal and Mirza, 2004). From
a compositional point of view, the buffalo milk is characterized by high levels of fat, total solids, proteins, casein,
lactose and ash than cow, goat, camel and human milk (Ahmad et al., 2013). The high fat content present in buffalo
cow milk gives it its unique flavor and makes it a particularly valuable commodity in India and other countries
where ghee, a type of clarified butter, is considered an important dairy product (Cockrill, 1974). Although similar,
proteins in bubaline milk are not identical to those in cattle. Buffalo cow milk is higher in casein and serum albumins.

Most bubaline casein (98.4%) is present in the form of large micelles (80 a 250 mm vs 70 a 110 mm in cattle).
Buffalo cow milk has higher lactofererin content. This glycoprotein plays a key role in infant protection by inhibiting
bacteria such as Escherichia coli sp, and also plays an important role in iron absorption (Kumar, 2008; Zava and
Sanseniena, 2017). Buffalo cow milk has been shown to be a healthy nutrient for humans. It has 58% more calcium,
35% more protein and 20% less cholesterol than cow milk. It is also a richer source of iron, phosphorus, vitamin A
and natural antioxidant tocopherol. Larger proportions of other bio protective compounds such as,
immunoglobulins, lysozyme, and lactoperoxidase make buffalo cow milk an excellent choice for a wider range of
healthy diets (Zava and Sanseniena, 2017). Milk quality is the most important factor for the success of its
industrialization and dairy products. This factor generates a significant increase in the price of milk, and benefits
for consumers who acquire better quality products (Figueiredo et al., 2010). The physicochemical composition (fat,
protein, lactose and total solids) and the microbiological analysis of raw milk samples are important indicators of
its quality. Measurement of the freezing point (FP) is used to detect any milk adulteration with water (Pesce et al.,
2016). Determining the bulk milk somatic cell count (SCC) is an internationally recognized method to establish milk
quality and the udder health status of the cows in the herd (Schukken et al., 2003). The analysis of the Total Viable
Count (TVC) determines the concentration of microorganisms present in the milk and is a suitable tool to test the
levels of hygiene of the milk production process, from initial management and storage to sampling (Gargouri et al.,
2013; Godinho et al., 2020). Because of the increasing of people awareness for food safety, knowing the chemical
composition of buffalo milk has a great significance for further development of hygienic processing into quality
products for the consumer (Mihaiu et al., 2010a).

The aim of this study was to evaluate the quality of buffalo milk at the level of a commercial farm, in order to
certify and consolidate the scientific information for the Romanian buffalo breed. The evolution of the conformity
parameters for buffalo milk was considered, especially the total number of somatic cells, this being a criterion for
assessing the quality of the milk and the payment of the milk according to it.

MATERIALS AND METHODS

Milk sampling
The material subjected to research was represented by twenty-four samples of fresh buffalo milk. The samples
were collected biweekly between March 2020 and February 2021 from a farm authorized for the production of
buffalo milk. The buffalo farm under study has a total number of 500 buffalo heads, of which 190 are lactating
females. Among lactating buffalo females, they can be grouped over several lactation periods, starting with the first
lactating females and going to the eighth lactation. Thus, 40 females are in the first lactation, 32 in the second
lactation, continuing with 25 in the third lactation, respectively 27 (lactation IV), 19 (lactation V), 22 (lactation VI),
17 (lactation VII) and 8 females (lactation VIII) from the fourth to the eighth lactation. In the warm season, the
animals are grazed on pasture, and during the winter, due to unfavorable weather conditions, the animals are
sheltered and fed with hay, concentrates and silage. Milking is realized using a mechanized canning system.
The samples were collected from the cooling tank of the farm after homogenizing the contents, in sterile plastic
containers with lids, appropriately labelled (farm registration number, date of harvest) and transported under
refrigerated conditions to the laboratory of «Inspection and food control of feed and animal origin products» in the
Veterinary Medicine Faculty from Cluj-Napoca, for the analysis. In the laboratory, the samples were transferred to
sterile containers compatible with the equipment of the working point.

CombiFoss™7 analyzer characteristics
To evaluate the quality parameters of buffalo milk was used the CombiFoss™7 from Fossanalytic. CombiFoss™7
is a device that has the ability to analyze all the parameters necessary for official controls of milk production and

Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Veterinary Medicine 19
quality (fat, protein, lactose, SU, pH, freezing point, urea, SCC and TVC).

The equipment is a combination of two devices, being composed of MilkoScanTMFT+, responsible for measuring compositional parameters, and FossomaticTMFC, which determines the number of somatic cells and their differentiation. This is an automated device consisting of a homogenization system, a sample transport system (working belt) and a scanner used to identify samples.

MilkoScanTMFT+ operates on the basis of the principle of FTIR (Fourier Transform InfraRed) interferometry, which measures the infrared region of the spectrum of electromagnetic radiation. Compared to the visible light, the longer wavelength and the lower frequency become measurable in a small sample. The basic theory is that the connections between different elements absorb light at different frequencies, and this light is measured with an infrared spectrometer. The MilkoScanTM FT + techniques comply with: ISO 9622/IDF 141:2013 (ISO 9622:2013) and the AOAC official method 972.16 (AOAC, 1996). The device analyzes parameters such as fat, total protein, casein, lactose, total and non-fatty dry matter, pH, urea, acetone and β-hydroxybutyrate.

FossomaticTMFC performs a precise analysis of the milk in terms of the number of somatic cells. The device is based on the flow cytometry method that counts somatic cells in accordance with ISO/IDF and FDA/NCIMS standards. Flow cytometry is a popular technique of cell biology that uses laser-based technology to count, sort and profile cells in a mixture of heterogeneous fluids.

Flow cytometry uses the principles of light scattering, light excitation and the emission of fluorochrome molecules to generate data with specific parameters and multiples from cells.

For the counting of somatic cells, the device uses the nuclear DNA of the cells, which is stained with fluorescent dyes (e.g., ethidium bromide, propidium iodide or syto13), and depending on the colorant properties of the dye used, we can determine the number of cells. Fossomatic technology complies with: AOAC official method and ISO 13366-2/IDF 148-2:2006 (ISO 13366-2:2006).

**Preparation of milk samples and working method**

The milk samples are placed in 60 ml sterile containers, which are homogenized and then placed on the appliance belt with the lids of the containers open. The sample temperature can vary between 2 and 42°C. To record the evidence for this paper, our own numbering was used. The device automatically analyzes each sample. It contains an active stirrer which first homogenizes the samples and the component which extracts approximately 4.5 ml of the milk sample for analysis. After about 10 minutes, the results are displayed on a monitor computer connected to the device.

**Statistics**

The statistical analyses were realized with Origin 8.5 software (OriginLab Corporation, Northampton, MA 01060, USA). Mean differences between the monthly samples were analyzed using analysis of variance ANOVA. The results were expressed according to the standard deviation (SD), with significance level established at P < 0.05. Post-hoc test comparation using Bonferoni, Tukey’s and Scheffe’s were performed.

**RESULTS AND DISCUSSIONS**

In Table 1 are presented the mean values of compositional and hygienic parameters (± SD) of buffalo milk during the period of this study.

The mean fat measurements for all analysed buffalo milk samples from the period of this study was 8.82 ± 1.0 g/100 g, and the fat content ranged from 7.7 g/100 g to 9.63 g/100 g. In a study made by Asker et al. (1974), the content of fat from the buffalo milk samples were between 6.9% and 8.5%. Varriichio et al. (2007) reported the fact that the fat content has an average value of 8.3% but can also reach 15% under normal conditions. In another research conducted by Zava and Sansienena (2017), the fat content varied between 7-9.6 g/100 g.

In Romania, Mihaiu et al. (2010b) conducted a study on seasonal variations in the biochemical composition of buffalo milk, finding that the percentage of fat resulting from statistical analyzes showed that there were significant variations between the average value recorded in the spring (8.88%) and that recorded in winter (9.5%), but without significant differences in the rest of the seasons (autumn 8.34%, summer 8.20%). This difference in the fat content is probably due to the feeding frequency of low fiber, high grain diets which increase milk fat levels during the winter and autumn period and the lack of herbage, which was not available, only in the spring and summer seasons. The authors also mention in their study that the seasonal effect was also significant in regard to the average protein content of milk (winter: 5.16 g%, spring: 4.05 g%, summer 4.06 g%, autumn: 4.30 g%). In the spring and summer season, again probably due to the diet based mainly on green forages, the protein content decreased, showing a slightly increase in autumn and then reaching the peak in winter months (Mihaiu et al., 2010b). The overall median for protein was calculated at 4.40 g/100 g with values ranging from 4,28±0.13 g/100 g to 4,58±0.2 g/100 g. The mean fat and protein contents were closest than those reported in studies conducted in Italy (Pesce et al., 2016; Pasquini et al., 2018). In another study conducted by Godinho (2020) in southern Brazil, the average...
measurements of fat and protein were much lower than those obtained in our study, which were 5.5 g/100 g for fat and 4.06 g/100 g for protein. Fat content ranged from 4.26 g/100 g to 9.57 g/100 g, and for proteins the values were between 3.42 g/100 g to 4.60 g/100 g.

Table 1. Mean values of the compositional parameters (±SD) of buffalo milk in the period of March 2020-February 2021

<table>
<thead>
<tr>
<th>Date of sampling</th>
<th>Fat (g/100g)</th>
<th>Protein (g/100g)</th>
<th>Lactose (g/100g)</th>
<th>Total solids (g/100g)</th>
<th>pH</th>
<th>Freezing point (°C)</th>
<th>Urea (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mar-20</td>
<td>8.97±0.07</td>
<td>4.50±0.05</td>
<td>4.28±0.08</td>
<td>18.21±0.007</td>
<td>6.72±0.02</td>
<td>-0.516±0.07</td>
<td>32.55±3.5</td>
</tr>
<tr>
<td>Apr-20</td>
<td>9.0±0.35</td>
<td>4.48±0.05</td>
<td>4.11±0.08</td>
<td>18.05±0.56</td>
<td>6.68±0.02</td>
<td>-0.516±0.0</td>
<td>29.85±1.48</td>
</tr>
<tr>
<td>May-20</td>
<td>9.01±0.5</td>
<td>4.58±0.2</td>
<td>4.31±0.21</td>
<td>18.72±0.12</td>
<td>6.72±0.09</td>
<td>-0.528±0.16</td>
<td>29.9±6.73</td>
</tr>
<tr>
<td>Jun-20</td>
<td>8.38±0.14</td>
<td>4.36±0.07</td>
<td>4.22±0.01</td>
<td>17.22±0.27</td>
<td>6.71±0.02</td>
<td>-0.506±0.12</td>
<td>15.8±8.20</td>
</tr>
<tr>
<td>Jul-20</td>
<td>8.24±0.03a</td>
<td>4.30±0.05</td>
<td>4.21±0.05</td>
<td>16.98±0.06a</td>
<td>6.72±0.007</td>
<td>-0.496±0.07</td>
<td>17.8±0.14a</td>
</tr>
<tr>
<td>Aug-20</td>
<td>8.83±1.00</td>
<td>4.45±0.16</td>
<td>4.37±0.25</td>
<td>18.08±1.77</td>
<td>6.69±0.03</td>
<td>-0.522±0.34</td>
<td>33.5±9.19</td>
</tr>
<tr>
<td>Sep-20</td>
<td>8.09±0.46</td>
<td>4.43±0.08</td>
<td>4.35±0.05</td>
<td>17.02±0.71</td>
<td>6.71±0.0</td>
<td>-0.498±0.09</td>
<td>19.2±4.52</td>
</tr>
<tr>
<td>Oct-20</td>
<td>9.44±0.26</td>
<td>4.44±0.06</td>
<td>4.33±0.38</td>
<td>18.81±0.69</td>
<td>6.70±0.09</td>
<td>-0.535±0.007</td>
<td>29.35±3.22</td>
</tr>
<tr>
<td>Nov-20</td>
<td>8.93±0.03</td>
<td>4.42±0.08</td>
<td>4.39±0.03</td>
<td>18.1±0.18</td>
<td>6.73±0.07</td>
<td>-0.513±0.006a</td>
<td>31.2±2.82</td>
</tr>
<tr>
<td>Dec-20</td>
<td>8.94±0.18</td>
<td>4.28±0.13</td>
<td>4.22±0.00</td>
<td>17.87±0.31</td>
<td>6.73±0.10</td>
<td>-0.515±0.01a</td>
<td>29.4±2.4</td>
</tr>
<tr>
<td>Jan-21</td>
<td>8.88±0.08</td>
<td>4.45±0.02</td>
<td>4.35±0.10</td>
<td>18.02±0.06</td>
<td>6.87±0.04</td>
<td>-0.514±0.003</td>
<td>36.65±3.6</td>
</tr>
<tr>
<td>Feb-21</td>
<td>9.04±0.10</td>
<td>4.41±0.007</td>
<td>4.03±0.05</td>
<td>18.11±0.13</td>
<td>6.68±0.06</td>
<td>-0.508±0.003a</td>
<td>19.85±2.5a</td>
</tr>
</tbody>
</table>

* significant difference between the mean (p<0.05); ** Significant differences between the mean (p<0.01)

For lactose, the overall median was calculated at 4.291 g/100 g with values between 4.00 g/100 g and 4.62 g/100 g. A study conducted in the USA by Han et al. (2012), revealed that the average content of total solids, fats, lactose and proteins varied between 16.39-18.48%, 6.57-7.97%, 4.49-4.73%, respectively 4.59-5.37%. Based on the statistical analysis no significant differences were recorded between proteins, lactose, freezing point and pH. In the case of fats and total solids significant differences were recorded in the case of samples from July 2020.

In our study, lower values of urea were recorded during June, July, September and January 2021 (p<0.05). Approximate values to those reported in the study by Becskei et al. (2020), in which urea ranged between 33.90-36.70 mg/dl were obtained in the other months of the year, when urea values ranged between 29.35 ± 3.22 and 36.65 ± 3.6 mg/dL. Milk urea measured at the group level can be used to monitor the efficiency of nitrogen utilization in commercial buffalo herds and as an indicator of the protein feeding situation in buffaloes (Di Francia et al., 2003). Compared to the study by Francia et al., (2003), the values of our study were lower than those obtained from his findings (40.8 mg/dL).

Several studies have reported pH of fresh bubaline milk, with great variation among individuals. In India it was obtained fresh milk from Murrah buffalo cows and reported pH values of 6.74 ± 0.08. In Italy it was reported an average 6.78 ± 0.03 for fresh milk from Italian Mediterranean buffalo cows, with marked seasonal fluctuations of 6.73 in August (summer) and 6.85 in December (winter) (Cockrill, 1974; Zava, 2011; Zava and Sanseniena, 2017). Bubaline milk tends to lose continuously carbon dioxide and to slightly increase its pH overtime. Subsequently, pH drops due to acid lactic produced by bacterial activity. A mastitis-infected animal will exhibit elevated pH values (>7.0) in the infected quarter. The pH of fresh bubaline milk does not appear to be linked to daily output, stage or number of lactations (Cockrill, 1974; Zava, 2011). In our study the pH values ranged between 6.61 and 6.9, which indicates that there is no infection in the udder.

In Figure 1 and Figure 2 are presented the mean values of hygienic parameters of buffalo milk during the period of our study. Through our study, we evaluated the somatic cell count over a year, to see the changes that occur from month to month, this being a parameter used for the sanitary control of milk, and also for the diagnosis of subclinical mastitis. The value of the annual SCC average obtained was 304.85x1000 cells/ml, in accordance with the European standard, which allows 400,000 somatic cells/ml for buffalo milk.

The evolution during a year is represented in Figure 1. From March to April there is an increase, and from April to May there is a new decrease of SCC. From May, the value gradually increases until July. From July to September the value of the SCC decreases to a minimum (170.5±3.53x1000 cells/ml), following that during the other months of study, it will increase until it reaches the maximum analyzed value, this being in February, with a value of
484.5±104.65x1000 cells/ml, thus exceeding the European standard.

For SCC the values obtained exceeded the maximum limit for the period of two months, in April where the SCC is 407±90.5x1000 cells/ml, respectively 484±104.65x1000 cells/ml in February. A possible increase in SCC in these months is the onset of lactation. Singh and Ludri (2000) reported that the lowest value of SCC in buffalo milk is reached during 90 to 150 days of lactation. A significant negative correlation was found between milk yield and SCC during different stages of lactation (Singh and Ludri, 2000).

![Figure 1](image1.png)

**Figure 1.** Mean values of somatic cell count (±SD x1000) of buffalo milk during March 2020 -February 2021

The total viable count plays an important role in determining the degree of milk hygiene and implicitly the quality of the milk. In the case of products made with raw milk, without heat treatment the total germ load at 30 °C must be ≤500 000 cfu/ml. (Mihaiu et al., 2014)

![Figure 2](image2.png)

**Figure 2.** Mean values of total viable count (±SD x 1000) of buffalo milk during March 2020-February 2021
The TVC was in an increasing number, the annual average being 2059.65x1000 cfu/ml. This is largely due to poor hygiene conditions.

According to Figure 2, the total number of viable in milk exceeded European standards in the case of 58.3% of the samples analyzed, so TVC falls within European limits in only 4 months out of 12. These months are represented by March, where TVC has a value of 380±131.5x1000 ucf/ml, in April with an TVC of 324±67.9x1000 cfu/ml, in July with a value of 347.5±217x1000 cfu/ml and in September with an TVC value of 419±185x1000 cfu/ml.

We also notice that in May, the germ load is the highest, being 5599±4843x1000 cfu/ml. This value far exceeds the European standard, being correlated with poor hygiene.

The figure shows significant variations from month to month, observing a considerable increase from April where the TVC value is the lowest in the whole study in May, where the TVC value is the highest during the study months. Then there is a gradual decrease, reaching a value within the European limit, in July, and then increasing in August, September will fall within the limits. Since September, there have been increases in the bacterial load, which persists during the last 5 months of the study.

In the two figures it can be seen that in most of the autumn-winter months, when the animals are introduced to the stable, both the SCC and TVC values are increasing, which can mean conditions of hygiene and poor animal care.

Following an analysis of several studies conducted in several countries, El-Salam and El-Shibiny (2011) mention that variations in buffalo milk composition were observed. In addition to the distinct methods of analysis, these differences reflect a variability between the number of lactations, the stage of lactation, management, environmental conditions, feeding and seasonality. Compared to cow’s milk, buffalo’s milk is less susceptible to intravital contamination, but there are various possibilities for extravital contamination; inadequate hygiene of the mammary gland, milking, shelter; lack of milk cooling conditions; failure to ensure hygienic transport conditions; non-compliance with hygiene conditions during milk processing, from receipt to delivery of the finished product (Chindris and Stetca, 2010).

Also, even if the animals are not infected, the number of somatic cells may increase as the number of bacteria in the milk increases (contamination is possible extravitally). We can correlate this type of contamination with SCC, observing according to Figure 2, that in February the SCC is extremely high, far exceeding the imposed limits, being 4759.5 x 1000 cfu/ml. Extravital contamination is the culprit for the increase in TVC on the farm on which this study was conducted. According to Hashmi and Saleem, (2015) the provision of quality microbiological parameters of raw milk and dairy products plays an important role in quality control. The important rule in food processing is the good quality of the raw material. Good quality of the final product cannot be obtained from a raw material with poor hygiene quality. The process of damaging the milk begins with milking. Due to unhealthy conditions in milk production and other sources of contamination in milk processes, milk can be a carrier of pathogens that threaten public health.

CONCLUSIONS

The amount of fat in the analyzed milk was generally higher than the values usually found in buffalo milk of 7-8% g/100 g.

The averages for the results of the physicochemical parameters fat, protein, lactose, total solids and urea were 8.821 g/100 g, 4.400 g/100 g, 4.291 g/100 g, 17.93 g/100g and 27.08 mg/dL, respectively. There is a directly proportional correlation of TVC and SCC depending on the season, attesting to lower levels for both parameters during the grazing period.

Determining the number of somatic cells is an important parameter that must be considered for the control of the quality of raw milk and as a control parameter regarding the mastitis in milk buffaloes.

Regarding the total number of germs, its average exceeded the maximum value allowed by European legislation in seven months during the study. The other parameters analyzed do not confirm the existence of an infection in the udder, so the main reason for these higher counts of TVC should be ascribed to poor hygiene conditions during milking, collection and transport. It is recommended to improve the hygiene conditions in order to obtain a qualitative milk from a compositional and hygienic point of view.

Author Contributions: M.M. Conceived and designed the analysis; N.F.G., N.A., I.A. Collected the data; M.M., D.S.D., R.M. Contributed data and analysis tools; D.S.D., N.F.G., F.A.I. Performed the analysis; D.S.D., F.A.I. Wrote the paper. All authors read and approved the final manuscript.

Conflicts of Interest

None of the authors has any potential conflict of interest related to this manuscript.
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