

**AUTOMATED TESTING AND ANALYSIS OF PARAMETERS
USED IN ASSESSING THE COMPOSITION AND HEALTH OF BUFFALO MILK**
TESTAREA AUTOMATĂ ȘI ANALIZA PARAMETRIILOR
UTILIZAȚI ÎN EVALUAREA COMPOZIȚIEI ȘI SĂNĂTĂȚII LAPTELUI DE BIVOLIȚĂ

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ABSTRACT | REZUMAT

The buffalo milk is characterized by a higher content in fat, protein, minerals and certain biologically active substances (free amino acids, taurine, vitamin A) than cow's milk.

The purpose of this study is to analyse the physico-chemical and hygienic parameters of buffalo milk using automated systems for monitoring mammary gland health and whole milk.

From a sample of clinically healthy buffaloes (n=16), milk was collected from each mammary quarter and pooled into a mean sample /animal, which was then tested using the COMBIFOSS automated system, comprised of a Milkoscan and a Fossomatic. With the Milkoscan system we determined an advanced set of physicochemical indicators with proven relevance for milk assessment, including the dry matter (DM), the non-fat dry matter (NDM), fat, total protein, B-casein, lactose, the cryoscopic point, urea, acetone and B-hydroxybutyrate (BHB). The main health and hygiene parameters, Total Bacterial Count (TBC), Somatic Cell Count (SCC), differentiated counting of somatic cells (DSSC), were also determined using the automated system BactoScan and the Fossomatic automated system, respectively.

We also tested the electrical conductivity, pH and temperature of the milk using a pH / Conductometer respectively and in order to measure the density we used a lactodensimeter.

The data obtained was statistically analyzed in the Windows operating system, using GraphPad, InStat and Microsoft Excel program, which allowed the calculation of the main statistical parameters.

The results revealed important oscillations of some compositional indicators and less important variations of markers used to monitor the health of the mammary gland or the biologically active potential of milk.

Laptele de bivoliță se caracterizează prin conținut mai bogat în grăsimi, proteină, minerale și anumite substanțe biologice active (aminoacizi liberi, taurină, vitamina A), decât cel de vacă. Scopul acestui studiu constă în analiza parametrilor fizico-chimici și igienico-sanitari ai laptelui de bivoliță, utilizând sisteme automate pentru monitorizarea sănătății glandei mamare și a laptelui integral.

De la un eșantion de bivolițe clinic sănătoase (n=16) au fost recoltate probe de lapte din fiecare sfert mamar și reunite în probe medii /animal, care au fost testate cu sistemul automat COMBIFOSS, compus dintr-un Milkoscan și un Fossomatic. Cu ajutorul sistemului Milkoscan am determinat un set avansat de indici fizico-chimici cu relevanță dovedită pentru evaluarea laptelui, incluzând substanța uscată (SU), substanța uscată negrasă (SUN), grăsimea, proteinele totale, B-cazeina, lactoza, punctul crioscopic, ureea, acetona și B-hidroxibutratul (BHB). Concomitent, au fost determinați și principalii indici igienico-sanitari, NTG cu sistemul automat BactoScan, respectiv NCS și DSSC cu sistemul automat Fossomatic. Suplimentar, am recurs și la testarea conductibilității electrice (CE), pH-ului și temperaturii laptelui cu un pH /conductometru, respectiv la măsurarea densității, cu ajutorul lactodensimetrului. Datele obținute au fost analizate statistic în sistemul de operare Windows, utilizând programele GraphPad, InStat și Microsoft Excel, care au permis calcularea principalilor parametri statistici.

Rezultatele analizelor efectuate au relevat oscilații importante ale unor indici compoziționali și mai puțin importante ale celor utilizați ca markeri în monitorizarea sănătății glandei mamare sau a potențialului biologic activ al laptelui. Conținutul total al SU a prezentat variații individuale importante (14,75 – 24,21%), date în principal de grăsime, care s-a dovedit cel mai variabil component al laptelui de bivoliță (7,05-10,82%). Variații mult mai strânse a prezentat însă densitatea (1.027-1.033g/cm³), SUN (10.97-12.51%), proteinele totale (4,08-5,67%) și lactoza (4,74-5,82%). Valorile indicilor cu impact major în monitorizarea sănătății glandei mamare și a laptelui, au indicat niveluri scăzute ale conductibilității electrice (2,55-3,07 mS/cm),

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The total DM content showed significant individual variations (14.75-24.21%), mainly due to the fat content, which proved to be the most variable component in the buffalo milk (7.05-10.82%). Other basic milk parameters had much more reduced variations: density ($1.027-1.033 \text{ g/cm}^3$) NDM (10.97-12.51%), total protein (4.08-5.67%) and lactose (4.74-5.82%).

The indicators with major impact on monitoring the health of the mammary gland and raw milk, showed low levels of electrical conductivity (2.55-3.07 mS/cm), and average TBC (10,000-139,000 germs/mL), SCC (23,000-145,000 Cell/mL) and DSCC (24.33-78.4%) levels. Also relevant was the progression of the milk indicators for the metabolic profile (urea, BHB, acetone), which showed the health levels of the lactating buffaloes and lack of ketosis. The interpretations offered revealed that the tested samples were within the normal European standards for raw buffalo and bovine milk, except in one case that showed slightly elevated TBC values (139,000 germs/mL) with no pathological connotation. In conclusion, the compositional and hygienic parameters of buffalo milk rarely have oscillations with a major impact on the producer, the processor and the consumer.

Keywords: buffalo, milk, automated tests, physicochemical, hygienic-sanitary

respectiv niveluri medii ale NTG (10.000-139.000 germeni/mL), NCS (23.000-145.000 Cel/mL) și DSCC (24,33-78,4%). Relevantă s-a dovedit și evoluția indicilor lactici de profil metabolic (uree, BHB, acetonă), care a indicat un nivel bun al sănătății generale a bivolțelor lactante, respectiv lipsa cazurilor de cetoză. Ansamblul interpretărilor efectuate a relevat încadrarea probelor testate în standardele europene stabilite pentru laptele crud de bivolț și vacă, exceptând un singur caz care a prezentat valori ușor crescute ale NTG (139.000 germeni/mL), dar lipsite de conotație patologică. În concluzie, parametrii compoziționali și igienico-sanitari ai laptelui de bivolț prezintă rareori oscilații cu impact major asupra producătorului, procesatorului și consumatorului.

Cuvinte cheie: bubaline, lapte, testare automată, fizico-chimică, igienico-sanitară

The biochemical composition of buffalo milk is mainly characterized by its higher content in dry matter (over 18%), given mainly by the fat and casein content, which distinguishes it from cow milk and is bringing it closer to that of the sheep (1, 5, 23).

Another basic feature of buffalo milk is the much lower protein/fat ratio (0.54) than cow's milk (0.85-0.90), and the higher proportion of casein (77-78% of total protein). These are just a few particularities that confirm the superiority of using buffalo milk on its own or mixed with other types of milk in the manufacturing yoghurt, cheese and butter (18, 23).

According to statistical data provided by the International Dairy Federation (2009), 13% of the world's milk production is buffalo's milk, which currently has high quotas in many countries from the Southeast Asia, India and Pakistan. Buffaloes have enjoyed a great deal of attention in the past, being bred in many geographical areas, but they have also gradually adapted the intensive farming system. Although currently buffaloes are predominantly bred for milk and meat production, they were previously known for their ability to carry heavy workloads of various types but also working animals (rice production) (5, 25, 26).

According to the data accumulated over time, there

are two varieties of domestic buffaloes: the River buffalo, which is characterized by its black coat, curved horns or in the shape of an arch, and higher milk production and the Swamp buffalo, with a dark grey coat (24).

As Romania joined the European Community, the country has adopted the new microbiological and cytological standards for raw bovine milk, with values of total bacteria count (TBC) and somatic cell count (SCC) to be less than or equal to 100,000 germs/mL of milk, less than or equal to 400,000 Cell/mL, respectively. In the absence of specific rules for dairy buffaloes, these values have been extrapolated, by many hygienists and producers, to the buffalo milk. In this context, we mention that the compositional and hygienic parameters of buffalo milk must be rigorously monitored in correlation with the biochemical and technological practices during processing. This ensures the identification and prevention of potential risks that may arise during the production and exploitation of buffalo milk. We also mention that the adoption of these measures in the production and processing of buffalo milk is essential to ensure a high nutritional and biological value of the products obtained, as well as the food safety and traceability standards (3, 6, 14, 29).

The purpose of the research, carried out in this study, is focused on the analysis of the physicochemical and hygienic parameters of buffalo milk obtained in traditional households, using the latest automated systems in monitoring the health of the mammary gland and that of the milk intended for public consumption.

MATERIALS AND METHODS

The investigations carried out in this study were conducted on samples of milk taken from a population of buffaloes, of indigenous breed, raised in individual households in the Rupea - Braşov area, with a long-lived tradition in breeding buffaloes. From this area we focused on the Hoghiz commune, consisting of 5 villages geographically located on the wold floor of Homorod, on the left side of the river Olt, adjacent to Baraolt and Făgăraş. Regarding the predominant vegetation in the area, we mention that it is rich in deciduous forests and pastures with abundant flora. Regarding the large ruminants population, it is noteworthy that Hoghiz currently has 118 buffaloes and 1461 cattle.

The research was initiated in March 2019, when during the first enquiry conducted in two of the villages from Hoghiz commune we selected a batch of 16 clinically healthy lactating buffaloes belonging to several traditional farms in these villages.

Thus, amongst the 28 buffaloes of Hoghiz (20 female buffaloes, one breeding male and 7 calves), we identified 11 lactating buffaloes, and in the 14 buffaloes from the Fantana village (8 females, a breeding male and 5 calves) we identified another 5 lactating buffaloes.

The buffaloes from these households are farmed, for both milk and meat. The milk is mostly pooled and marketed by collection centers and is less sold directly to the local consumers. Calves are slaughtered for family consumption and capitalizing on the local market.

The animals from these households are kept in closed shelters, in a tied system, during winter while during the summer the graze in pastures, along with cattle, horses or goats. Another local method of traditional farming is the "village sieve", where the buffaloes leave in the morning to graze in the pastures and return in the evening. Most households in the area have the necessary experience for obtaining good milk and meat production levels, which requires high fertility and vigorous calves, ensuring thus a proper diet, maintenance and care. In this regard, we mention that during the summer season the predominant feed is pasture grazing, to which 2-4 kg of fibrous feed and 1-1.5 kg of concentrate mixture are usually added.

According to tradition, during the winter the structure of the fodder is mostly comprised of fibrous feed, reaching 60-70% (hay and coarse feeds), followed by succulents (20-25%) and concentrates (10-15%).

We also remark that, in most of the households that were taken into study, buffaloes were naturally mating with local reproduction bulls, and the artificial insemination is practiced in a very small proportion. In addition, in these households manual milking is still practiced and still very popular amongst buffalo farmers in our country. As a technique, the milking is done using a full hand because it is better accepted by the animal and is less tiring for the farmer. Many households know and apply a proper hygiene procedure, ensuring the following sanitation measures: separating the first 3, 4 jets of milk per quarter and deeming the milk safe through boiling. Nowadays, due to the high effort and low productivity, we witness the gradual replacement of the manual milking with the automated one, as buffaloes are adapting well to this way of milking.

This study was conducted in the form of complex investigations that included a complete clinical examination based on the use of general and specific semiotic methods for the assessment of the mammary gland and a milk examination using the Contrast and Californian tests (CMT) (18, 23). We did not find any changes in the general health condition, or that of the mammary gland and milk, thus, appreciated that all buffaloes had a very good level of health and we included them in the clinically healthy category.

From the 16 lactating buffaloes, milk samples were collected, following the standard sanitation and hygienic-sanitary measures (18, 19). Initially, the milk samples were harvested separately from each quarter and then pooled in a mean / animal sample, resulting in the biological material for testing. After harvesting, the raw milk samples were refrigerated (4-6°C) and transported, without the addition of preservatives, and in the following 8 hours they underwent processing and testing using the latest automated systems. The milk samples were tested using the Combifoss automated system, which includes a Milkoscan and a Fossomatic, which operate in compliance with the European standards (30).

The MilkoScan FT 6000 is a fully automated high capacity spectrophotometer (with IDO and FTIR technology conforming to AOAC standards), where FTIR technology has the potential to analyze most milk-specific components. On the other hand, the use of the Mid-Range Infrared (FTIR) technique allows the analysis of the main components, such as fat, protein, lactose and dry matter. Using this device, we have determined an

advanced set of physicochemical parameters distinctive for milk, including buffalo milk. These indicators are grouped as follows: compositional parameters (%), including dry matter (DM), non-fat dry matter (NDM), fat, total protein, B-casein (% of total protein), lactose and cryoscopic point ($^{\circ}\text{C}$), and the metabolic profile, such as the urea (mmol/L), acetone (mmol/L) and beta-hydroxybutyrate (BHB; mmol/L). In addition, we determined the main hygienic parameters using the Bacto ScanTM FC automated system to determine TBC ($\times 10^3$ germs/mL) and the Fossomatic automated system to determine SCC ($\times 10^3$ cells/mL) and differentiated counting of somatic cells (DSSC; %). Furthermore, we tested the electrical conductivity (EC; mS/cm), the pH and the temperature ($^{\circ}\text{C}$) of the milk using the pH meter Conductometer C 532, and in order to measure the density we used the lactometer. Finally, the data obtained was statistically analyzed in the Windows operating system with the GraphPad, InStat and Microsoft Excel programs, which allowed the calculation of the following parameters: the mean, standard deviation (St. Dev.), standard error (St. Error), median, minimum (Min) and maximum (Max).

The data obtained with the Bacto-ScanTMFC and Fossomatic automated systems were processed using the Foss Integrator software platform, creating a PHA chart that allowed a fast and accurate evaluation.

RESULTS AND DISCUSSIONS

The values obtained, in determining the main physical, compositional and hygienic parameters of the milk samples taken into study, are summarized in Table 1 and their individual distribution is shown in Figure 1. This data set, correlated with relevant bibliographic references, constituted the foundation of a detailed analysis of the indicators that are currently used in the assessment of the health of the mammary gland, and particularly the biologically active and nutritive value of buffalo milk.

Analysis of the values obtained in testing the physical parameters of milk

The results of the evaluation of the main physical indicators of milk revealed, according to the data presented in Table 1 and illustrated in Figure 1A, individual oscillating values but within the physiological ranges. Known and used in the case of bovine milk, the physiological values of these parameters can be extrapolated to buffalo milk, less researched in this regard (10, 11, 24). Thus, the density progression was cha-

racterized by relatively high oscillations of the individual values ($1.027\text{--}1.033\text{ g/cm}^3$) with the average value at 1.030 g/cm^3 (Table 1), relevant for a normal compositional balance of buffalo raw milk. It can also be noticed a correlation between the evolution of the density and other physical parameters of the milk, revealing several oscillations within the normal limits for physiological dairy secretions. These findings support the definition of milk density as being the ratio between the milk mass at $20\text{ }^{\circ}\text{C}$ and the mass of the same volume of water at $4\text{ }^{\circ}\text{C}$. Many studies in the field show that buffalo milk density varies in close proximity to bovine milk, usually with slightly higher values (11, 13). It is also considered that this slight increase in density can also be correlated with the increase of the viscosity, the decrease of the refractive index and the cryoscopic point (1, 5, 23). The data obtained in the pH evaluation indicated oscillations within the physiological ranges for raw milk with average values of 6.79 and individual values between 6.5 and 7.0 (Table 1). Regarding the cryoscopic point, we consider that the average value of $-0.54\text{ }^{\circ}\text{C}$ is very close to the lower limit of the normal range for milk in general (1, 16, 17). The individual values recorded by our study in this parameter were also at the lower limit, with some dropping below, between $-0.51\text{ }^{\circ}\text{C}$ and $-0.55\text{ }^{\circ}\text{C}$ (Table 1). According to standards, these results exclude the possibility of any added water in the milk (4, 9, 13). Regarding the freezing range of normal milk, we remark that it freezes at an average temperature of $-0.55\text{ }^{\circ}\text{C}$, with variations within $-0.53\text{ }^{\circ}\text{C}$ and $-0.58\text{ }^{\circ}\text{C}$, these values being similar to the cryoscopic point of blood serum (22, 23). Testing the electrical conductivity of the milk revealed minor and unimportant differences between the investigated samples, although they came from buffaloes with different lactation stages. This evolution is evidenced by the distribution of the data that we obtained, which reveals the placing of the average values at 2.401 mS/cm , with slight oscillations, between 2.16 and 3.07 mS/cm (Table 1).

Currently, the use of tests, such as the electrical conductivity of the milk, in monitoring the mammary gland, lactation progression and even the detection of heat in lactating cows, is universally accepted (19). Electrical conductivity is a physical parameter which, in the case of milk, is given by the correlation between the ion content (Na^+, K^+) and lactose. As a consequence, this parameter changes significantly in some physiological (the beginning and ending of lactation) or pathological (mastitis) states of mammary gland, increasing of the ionic content of the milk (1, 3, 19).

Table 1

Statistical data obtained in testing the main physicochemical and hygienic-sanitary parameters of milk in a sample of buffaloes in the Rupea - Braşov area

Parameter	Statistical indices					
	Mean	St. Dev.	St. Err.	Median	Min.	Max.
Density (G/cm ³)	1.030	0.002	0.000	1.030	1.027	1.033
pH	6.798	0.162	0.041	6.780	6.590	7.080
Cryoscopic P (°C)	-0.540	0.012	0.003	-0.538	-0.511	-0.555
EC (mS /cm)	2.401	0.214	0.053	2.330	2.160	3.070
DM (%)	19.482	2.675	0.669	19.705	14.750	24.210
NDM (%)	11.760	0.497	0.124	11.795	10.970	12.510
Fat (%)	7.916	2.399	0.600	8.210	3.880	12.080
Protein (%)	5.109	0.483	0.121	5.305	4.080	5.670
Lactose (%)	5.259	0.260	0.065	5.245	4,74	5,82
TBC (x10 ³ germs /mL)	27.938	39.357	9.839	10.000	10.000	139.000
SCC (x10 ³ Cells /mL)	53.063	34.391	8.598	41.000	23.000	145.000
DSCC (%)	24.331	31.504	7.876	0	0	78.4
B-Casein (%)	4.213	0.407	0.102	4.38	3.41	4.66
Urea (mmol/L)	30.363	12.76	3.19	33.95	8.5	45.6
Acetone (mmol/L)	0.285	0.311	0.0777	0.175	0.04	1.33
BHB (mmol/L)	0.063	0.064	0.016	0.055	0	0.19

Legend: St. Dev. – Standard Deviation; St. Err. – Standard Error; Min.– Minimum; Max.– Maximum; Cryoscopic P – Cryoscopic point; EC – Electric conductivity; DM – Dry matter; NDM – Non-fat dry matter; TBC – Total bacteria Count; SCC – Somatic cells count; DSCC – Differentiated counting of somatic cells; BHB – B-hydroxybutyrate

Analysis of the values obtained in testing the biochemical parameters of the milk

Data obtained from the evaluation of the main biochemical indicators of the milk from the sample of buffaloes tested are shown in Table 1 and presented graphically in Figure 1B. The analysis of the evolution of the overall data revealed important oscillations in the individual values, but lacking statistical significance. Thus, for the total dry substance content, we found average values of 19.48%, with significant individual variations, drawing a large spectrum, in which the minimum values dropped to 14.75% and the maximum values were up to 24.21% (Table 1).

Less important, however were the oscillations of the values recorded in the determination of the non-fat dry matter content, where individual variations (10.97-12.51%) evolved much closer to the average of 11.76%. We consider the results obtained in the analysis of the fat content to have great importance,

as it is the compositional parameter with the most important impact for the marketing and processing of milk in general and of the buffalo milk in particular. Thus, the evolution of the fat content in the milk samples showed broad variations around the average value of 7.91%, the minimum being 3.88% and the maximum 12.08% (Table 1). The distribution of this data reveals that the average is particularly close to the maximum value, as only two samples were on the lower spectrum, with 3.88% and 5.4%, respectively.

These important oscillations of individual values confirm the unanimously accepted assertion, according to which, lipids are the most variable components of the milk, including buffalo milk, as evidenced by the results of our research. It is also unanimously accepted that the fat in the milk has a major impact on the physical, organoleptic and sensory characteristics of dairy products, these being particularly relevant for buffalo milk (15, 29). The analysis of the total milk

protein content in this sample of buffaloes revealed less significant individual variations, with oscillations around the average value, standing at 5.1%, results fluctuating within 4.08-5.67% (Table 1, Fig. 1B). In this context, it is worth mentioning the considerable increase in the contemporary concerns regarding the quantitative and qualitative analysis of the protein content of buffalo milk, because the available data still contain many controversies (7, 9). The interpretation of the data regarding the lactose content of the investigated milk samples completes the compositional table. In the case of this parameter, it was found to be relevant that the average value was very close to that recorded for the total protein content, with an average level of 5.25%, with unimportant individual oscillations ranging between 4.74 - 5.82% (Table 1; Fig. 1B).

Based on the considerable levels recorded in the case of fat and protein content in the milk samples considered, we appreciate that the buffaloes ensure a superior usage of the predominant pastures and fodder feeds in the central area of Transylvania (5, 25, 26).

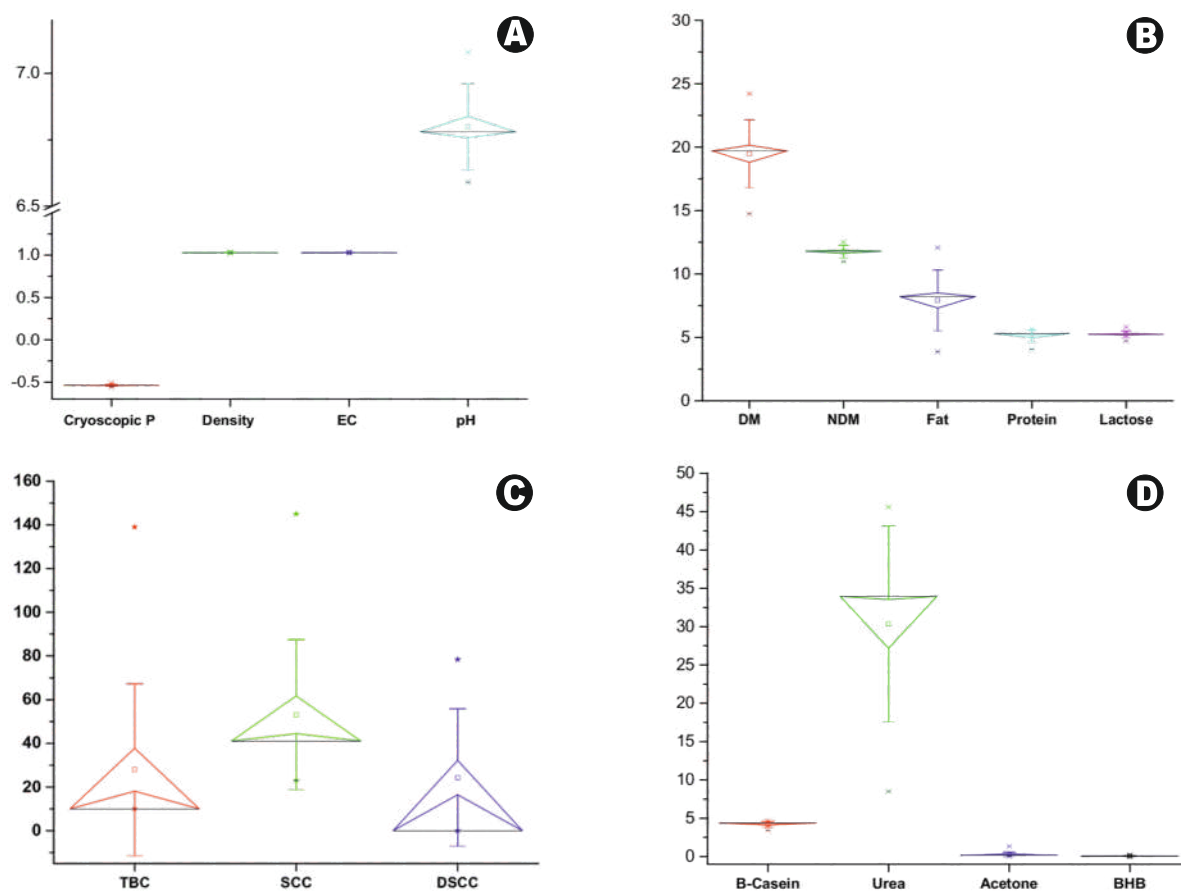
Analysis of the values obtained in the testing of some hygienic-sanitary indices and the metabolic profile of the milk

The results obtained in the evaluation of the main hygienic-sanitary indices, given by the evolution of the cellular and microbial content of the milk, and the parameters regarding the metabolic profile, used in the detection of ketosis, are summarized in Table 1 and graphically represented in Fig. 1C and 1D. The analysis of the TBC dynamics was characterized by the fact that the values recorded were within the accepted standards for bovine milk, which are also valid for buffalo milk, reaching a maximum of 100,000 germs/mL of milk (11, 17). In the sample we investigated, the TBC mean values were well below the maximum admitted level of 27,938 germs/mL of milk. In assessing the distribution of the individual values, which ranged between 10,000 and 139,000 germs /mL of milk, it appeared that only one case was slightly over the maximum admitted level. We also believe that the health of the mammary gland in this sample of buffaloes is relevant, conveyed by the predominant inclusion of TBC values in normal ranges. With regard to potential risks, it should be noted that raw milk produced in the extensive system can be frequently contaminated with coliform bacteria (13, 17). It is also known that the normal microflora of the buffalo milk is quite diverse and rich, given mainly by microorganisms that reach the mammary gland by exogenous

and, less frequently, by endogenous ways (8, 23, 28). In this context, we can also situate the slightly increased TBC value in one of the buffaloes considered in the sample (139,000 germs/mL of milk). The progression of the SCC also revealed that the values recorded were within the accepted standards for bovine milk, which are also valid for buffalo milk, the maximum admitted value being 400,000 Cell/mL of milk (11, 12, 20, 21). The overall assessment of the data obtained during the SCC determination for the whole buffalo sample revealed predominantly low values for this parameter. The SCC oscillations, less important (23,000-145,000 Cell/mL), were around the average of 91,429 Cell/ mL of milk, with slight increases of 100,000 Cell/ mL in two buffaloes only, the latter ones far below the accepted upper limit. This result confirms the health of the mammary gland, also established by the distribution of the values obtained for the DSCC parameter. This newly introduced parameter refers to the polymorphonuclear monocyte / leukocyte ratio, which is calculated from the SCC absolute value. We believe that the mean (24.33%) and maximum (78.4%) values of the DSCC recorded in our investigations correspond to a good level of health of the mammary gland and, implicitly, of the milk produced.

Correlating the data obtained with the information recorded in the consulted references, we consider the following characterization of the buffalo milk composition to be appropriate. This inevitably starts with the fat content oscillations, which are extremely important (6.4-13.4%), and give the high caloric and nutritive value specific to buffalo milk as well as its superior yield in the preparation of butter and cheeses (4, 6, 7, 29). On the other hand, it should be mentioned that although it has a higher fat content, buffalo milk is considered a healthy food because it contains less cholesterol than the bovine milk (17). The protein concentration of buffalo milk is also higher than cow's milk and is given by casein (over 80%) and whey proteins (3, 24). As far as the structure goes, buffalo milk proteins are very similar to those of bovine milk (1, 2, 3). Regarding the lactose concentration, it is well-known that in buffalo milk its level is higher in the first four months, reaching 4.9%, after which it gradually decreases to 4.7% (10). A distinct type of analysis is required to determine the importance of whether freezing the milk influences its quality, an aspect which is less studied, but it seems to be associated with the increase in fragments of β -casein resulting from freeze-dried casein hydrolysis (15). Starting from this, some studies have used rapid freezing to reveal that the

Fig. 1. Values obtained in the testing of physical (A), biochemical (B), hygienic-sanitary (C) parameters and the metabolic profile (D) of milk from a sample of buffaloes bred in a traditional system



Legend: Cryoscopic P – Cryoscopic point; EC – Electric conductivity; DM – Dry matter; NDM – Non-fat dry matter; TBC – Total bacteria Count; SCC – Somatic cell count; DSCC – Differentiated counting of somatic cells; BHB – B-hydroxybutyrate.

quality of the milk is lessened through such methods (23). For the same purpose, the use of ultrasound waves was also reported to create small ice crystals during freezing and other various effects (14). The widespread expansion of automated systems for the rapid assessment of the number of milk cells has improved the number of networks that can be monitored when it comes to the production and processing buffalo milk. In this context, the results of our researches, suggest the necessity of introducing automated monitoring procedures for TBC and SCC, for assessing the health of the milk and mammary gland where the buffalo is concerned. The risks of microbial contamination of raw buffalo milk are essentially the same as those encountered in cows, from either primary (direct contact between animals) or secondary (e.g., milking equipment, feed, soil, faeces, vegetation) sources (11, 12, 13). On the other hand, determination of TBC and SCC is also well-known for diagnosing mastitis, but also for its prophylaxis and control (8,20,21).

Regarding the metabolic profile indicators, it should be mentioned that subclinical ketosis, the main metabolic disorder that occurs during lactation, has a much lower incidence in buffalo than in cows, probably correlated with a lower level of milk production (11, 16). The very low values of urea, BHB and acetone, obtained in this study (Table 1 and Figure 1D), confirm the sustained findings, that there is significant lower in the risk of ketosis and other metabolic disorders associated with lactation in buffalo.

Regarding susceptibility to mammary infections, buffaloes are generally more resilient than cows, except for the first 4 months of lactation and the weaning period (months 10-12) when they are predisposed to mastitis (13). For buffaloes, as for other species, SCC is an indicator used in monitoring the health of the mammary gland but also for identifying mastitis, and it is estimated that it shouldn't exceed the limit of 400,000 Cell/mL milk (20). Mastitis in buffaloes is characterized by an increased pH and electrical conductivity of

the milk associated with elevated levels of minerals (sodium, potassium, phosphorus, calcium, magnesium, zinc, iron) and enzymes (lactate dehydrogenase, aspartate aminotransferase and alkaline phosphatase), by a decrease the fat, protein, lactose and NDM content of the milk (12, 27).

Regarding Differential Counting of Somatic Cells in Milk, Damm et al. (2017) show that although for mastitis screening there are other relevant indicators, such as SCC or the milk electrical conductivity, their management implies a continuous renewal of the biomarkers and tests used in their determination. In this context, the DSCC is determined mostly through the flow cytometry technique. This indicator is measured in percentage and it represents the combined proportion of polymorphonuclear leukocytes (PMN) and lymphocytes, while calculating the proportion of macrophages, by subtracting DSCC from 100%. Therefore, the DSCC parameter reflects the antidromic tendency of PMN and macrophages. In this regard, we only mention the results of the extensive study conducted by Damm et al. (2017) which attribute particular relevance to this indicator, with values ranging from 34-79% for samples with less than 400,000 Cell/mL of milk and values in the range of 53-89% for those with over 400,000 Cell/mL of milk. Also noteworthy is the low cost of DSCC tests, which require however further investigation to achieve the expected level for implementation.

As is well known, in ketosis and diabetic ketoacidosis, beta-hydroxybutyrate is the best represented (about 75%) of the three types of ketones (beta-hydroxybutyrate, acetoacetic acid and acetone) produced in excess in these disorders. It is also recognized that ketosis increases the β -hydroxybutyrate level more than acetoacetate and acetone, being a relevant marker for the detection of ketoacidosis and subclinical ketoacidosis. Knowing that in subclinical ketosis BHB concentration in the blood, urine and milk is increased, the paraclinical tests available on the market are mainly based on the detection of ketone bodies in the urine and milk, as well as the BHB from the blood. The diagnostic value of these tests increases, when combined with the assessment of other metabolic indicators relevant in ketosis, such as those with an energetic profile (hypoglycemia, hyperlipidemia and hyperketonemia), sometimes a decreased alkaline reserve) or liver specific. In the absence of quantitative detection tests for the ketone bodies in the expired air, whose emanation can only be subjectively appreciated based on the odor, this assessment is currently done only on a qualitative level.

CONCLUSIONS

The analysis of physicochemical and hygienic-sanitary parameters of milk from a sample of buffaloes bred in the traditional system revealed important oscillations of important compositional parameters and less important variations for the markers used in the monitoring of the health of lactating females or the biologically active potential of milk. Thus, the total content in the DM showed significant individual variations (14.75 - 24.21%), mainly due to the fat content, which proved to be the most variable component and in the case of buffalo milk, ranging between 7.05 - 10.82%. In contrast, the variation ranges were more intense for density (1.027-1.033 g/cm³) and NDM concentrations (10.97-12.51%), total protein (4.08-5.67%) and lactose (4.74-5.82%). Regarding the evolution of the parameters with major impact in monitoring the mammary gland and milk, it is worth mentioning the low levels recorded in the case of electric conductivity (2.55 - 3.07 mS/cm), the average levels in TBC testing (10,000 - 139,000 germs / mL milk), SCC (23,000-145,000 Cell./mL) and DSCC (24.33-78.4%) respectively. We also attribute great importance to the physiological limits of metabolic profile indicators (urea, BHB, acetone), signifying a good level of general health of the lactating buffaloes considered, especially associated with lack of ketosis and mastitis. From this set of interpretations it is apparent that the tested parameters were within the European standards for the compositional parameters for buffalo milk and hygienic parameters for raw bovine milk, except in one case that showed slightly increased values of TBC (139,000 germs / mL), but lacking any pathological connotation.

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