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THE EFFECT OF THE SEASON ON THE PHYSICAL-CHEMICAL AND MICROBIOLOGICAL PARAMETERS OF MILK OBTAINED FROM BUFFALOES FROM THE FĂGĂRAŞ AREA

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Abstract

Currently, approximately 20,000 buffaloes are raised in Romania, of which 11% are found in the Făgăraş area. The present study was conducted to observe the effect of season on the components of raw milk obtained from buffaloes cows reared in this region. 320 milk samples were collected during the morning milking, during the grazing and stalling seasons, from 80 buffaloes cows in different stages of lactation. The fat, protein, lactose, non-fat dry matter (NFS), density and pH content were determined from the collected samples. Microbiological determinations mainly considered somatic cell count (NSC) and total germ count (NTG). The individual analysis of milk samples from buffaloes revealed significant differences in terms of the variation of these parameters, the researches carried out highlighted differences determined by the breeding system of the animals involved in the study, the feeding regime, as well as the reference season. Therefore, the results of the present research indicated that the season and stage of lactation influence the physicochemical and microbiological parameters of milk and could be minimized by better management practices.

Key words *: buffaloes, microbiological, milk, physico-chemical, season.*

INTRODUCTION

The Food and Agriculture Organization reports that worldwide buffalo milk in 2021 was 137 million tons, and represented 15% of total milk production obtained from domestic species (FAO, 2022). Interest and investment in obtaining buffalo milk in different countries has increased every year due to its unique taste and content in nutrients, being processed into many commercially important products such as butter, cream, assortments of hard and soft cheeses, yogurt and ice cream. The main physico-chemical characteristics of buffalo milk are defined by pH, density, fat content, protein, lactose and non-fat dry matter (NNF). Beside its physical and chemical properties, the microbiological quality is also quite important because in raw milk, microorganisms could multiply rapidly due to its rich nutrient content. High fat and calorie content, apart from dry matter, is considered the superior and distinctive property of buffalo milk. Changes in milk composition depend on many factors such as genetics, milking time, type of diet, age, udder hygiene and season. These factors greatly affect the quality and processability of milk into dairy products. Geographical area, climatic conditions and lactation period are known as seasonal changes and are listed as other factors affecting milk composition. The specialized literature is abundant regarding the composition of buffalo milk, but information about the influence of the animal breeding system, the feeding regime, as well as the reference season is limited. Currently, approximately 20,000 buffaloes are raised in Romania, of which 11% are found in the Făgăraş area. The total production of buffalo milk obtained in 2021 was 14,754 tons (INSSE, 2022). In this context, the aim of this study was to investigate the effect of the season on the physico-chemical and microbiological composition of milk obtained from buffaloes in the Făgăraş area , an important region with a dense population of buffaloes.

MATERIALS AND METHODS

The present study was carried out on 80 buffaloes in different stages of lactation, raised in 8 farms in the Făgăraș area, during 2022.
The buffaloes were kept on pasture between May and October, fed with discretionary green mass and concentrated fodder, in the amount of 2 kg/day/head, and between November and April in the shelter and fed with hay, in the amount of 8 kg/head/day, corn silage 20 kg/head/day and concentrated feed 2 kg/head/day. To ensure the necessary trace elements, the ration was supplemented with mineral salts in the form of lumps (briquettes for licking). The buffaloes were milked mechanically, with the help of the machine (installation) for mechanical milking at the drum. During the study, 4 milk samples were collected from each buffalo individually, during the morning milking, 2 samples during the grazing season (in May and August) and 2 samples during the stable season (November and December), in total 320 milk samples. Milk samples (250 ml/sample) were collected under aseptic conditions in sterile bottles, transferred to the laboratory and then analyzed. The analyzed parameters were represented by density, pH, fat, protein, lactose, ash, pH, nonfat dry matter content (SNF), somatic cell count (NCS) and total germ count (NTG).
Fat, protein, lactose, non-fat dry matter (NFS) and density were determined with an ultrasonic milk analyzer "EKOMILK-ULTRA". The pH values of the milk samples were determined by the portable pH-meter device "pH 3110".
Microbiological determinations mainly concerned: somatic cell count (NCS), total germ count (NTG). These parameters were determined using the following analysis methods: NCS through SR EN ISO 13366-2: $2007/AC/2011_{(NR)}$; NTG through SR EN ISO $4833\text{-}1/2014_{(AR)}$.
For the statistical evaluation of the milk parameters according to the season, the mean differences between the results of the collected samples were expressed according to the standard deviation (SD) and were interpreted using the ANOVA analysis of variance with the level of significance set at $P \leq 0.05$.

Figure 1. Mechanically milking buffalo in the household of the population

RESULTS AND DISCUSSIONS

The results of the physical analyzes performed on the milk samples taken during the grazing and stable period are presented in Table 1.

Table 1. Results of physical analyzes of buffalo milk (Mean with standard deviation X ± SD)

Season (n = 320)/Month of observation		Density (g/cm^3)	pH
Pasture (n = 160)	May - August (n = 160)	1.032 ± 0.01 ^{a}	6.74 ± 0.03 ^{a}
Stabilization (n = 160)	November - December (n = 160)	1,030 ± 0.01^{b}	6.83 ± 0.02^{a}

n = number of determinations
^{ab} the difference between mean values with different letters in the same column is statistically significant $P \leq 0.05$.

The density of milk reflects its composition. The extraction of fat causes the density to increase and the addition of water to decrease it. It is a characteristic of the species and lies in the range of 1.026-1.034 g/cm^3. The density of buffalo milk normally varies between 1.027 - 1.033 g/cm^3. The current research produced results that support the conclusions of much of the previous work on buffalo milk, with milk density in the grazing season averaging 1.032 g/cm^3, and in the stalling season 1.030 g/cm^3, showing statistically significant difference ($p \leq 0.05$). The results of the present investigation are in agreement with the findings of some researchers who indicated that the season and stage of lactation influence the density of buffalo milk.

The density of buffalo milk collected from buffaloes in different stages of lactation from the Research and Development Station for Buffalo Breeding has statistical relevance between seasons ($p<0.05$), the lowest density value is recorded in the autumn season (1.027 g/cm^3), and the highest value is recorded in the summer of 1.033 g/cm^3 (Tătaru et al., 2019). Pece et al. (2009) stated that milk densities in the Romanian Buffalo breed were 1.034, 1.032, 1.032 and 1.033 g/cm^3, for the winter, spring, summer and autumn seasons. Romanian food regulations report that the density of raw buffalo milk must be equal to or greater than 1.028 g/cm^3.

Immediately after milking, the pH of the milk is neutral (due to the amphoteric characteristics of the proteins) or has a slightly acidic tinge, due to the preponderance of carboxylic groups or due to the presence of some acidic substances (citrates and carbonic acid). The metabolism of lactose causes the appearance of lactic acid and the decrease of pH. This acidification of milk can occur even within the mammary in the case of the development of some infections of the mammary gland determined by lactozofermentative bacteria (*Staphylococcus* sp., *Streptococcus* sp.), the milk immediately after milking has an alkaline tinge. Several studies have reported the pH of fresh buffalo milk, with large variations between individuals. In India, fresh milk obtained from Murrah buffaloes had pH values of 6.74 ± 0.08. In Italy a mean of 6.78 ± 0.03 was reported for fresh milk from Italian Mediterranean buffaloes, with marked seasonal fluctuations of 6.73 in August (summer) and 6.85 in December (winter) (Cockrill, 1974, Zava, 2011, Zava & Sanseniena, 2017, cited by Nikolau et al., 2022). In our study, the pH values varied between 6.74, during the grazing period and 6.83, during the stalling period, indicating that there is no infection in the udder and statistically, between the seasons, there were no significant differences in the values recorded ($p<0.05$). In a study carried out on the milk obtained on a farm with 190 Romanian buffalo, in different stages of lactation, between March 2020 and February 2021 (Nikolau et al., 2022), the highest pH value it was recorded in the spring season (6.64), and the lowest value (6.54) was recorded in the winter.

Table 2. Results of chemical analyzes of buffalo milk (Mean with standard deviation X ± SD)

Season (n = 320)/ Month of observation		Fat (%)	Protein (%)	Lactose (%)	SNF (%)
Pasture (n = 160)	May - August (n = 160)	7.14 ± 0.78^b	4.62 ± 0.22^a	4.54 ± 0.23^a	9.37 ± 0.35^a
Stabilization (n = 160)	November-December (n = 160)	7.53 ± 0.53^a	4.29 ± 0.27^b	4.26 ± 0.17^b	8.98 ± 0.27^b

(%) = (g/100 ml) ; n = number of determinations;
^{ab}the difference between mean values with different letters in the same column is statistically significant $P \leq 0.05$.

One of the most important components of buffalo milk is fat. Fat content not only directly affects nutritional value and economy of milk, but also has an effect regarding the organoleptic properties. The fat content of raw milk is so important that milk processors tend to price it based on fat content. Also, the quality of dairy products such as cheese, cream and butter largely depend on the amount and quality of milk fat. The amount of fat in the milk, during the grazing period, recorded an average of 7.14% and during the stable period it was 7.53% statistically significantly higher than during the grazing period ($p\leq0.05$). This increase in fat content during the stall period can be explained by the change in feed, the late period of lactation and the decrease in the amount of milk milked. The determined values are lower than the findings of Pece et al. in 2009, who stated that the fat in buffalo milk was 8%, 7.5%, 7.4%, and 7.8%, respectively, for winter, spring, summer, and autumn seasons. In another study carried out on buffaloes of the Romanian Buffalo breed (Nikolau et al., 2022), maintained under similar conditions as in the present study, the average fat measurements for all buffalo milk samples analyzed was 8.821 g/100 ml and ranged from 7.7 g/100 ml to 9.63 g/100 ml, with significant differences between the months of the year. The effect of season on milk fat percentage, which varied between 6.69-12% with a peak in the winter months, was found in a study of 120 samples collected from buffaloes in all four seasons (September 2009 - August 2010), from a farm located in Northern Transylvania (Mihaiu et al., 2010).

Another component of buffalo milk is the protein, which is responsible for the yield of milk processing into cheeses, internationally the amount of protein received compared to that of fat. Protein content of milk in grazing season (4.62%) and stable season (4.29%) showed statistically significant difference ($p \leq 0.05$). In a similar study (Tătaru et al., 2019), buffalo milk protein was significantly higher during summer (4.36%) and significantly lower during spring (4.00%) compared to the of autumn and winter and no significance was found for protein during the winter and autumn season. Pece et al. (2009) determined the protein in buffalo milk was 8%, 7.5%, 7.4%, and 7.8%, for winter, spring, summer, and autumn seasons, who stated that fat from buffalo milk, with the following mean values of 4.6%, 4.7%, 4.6%, and 4.6%, for the winter, spring, summer, and fall seasons. In our study, lactation stage was also a significant source of variation in protein percentage with a decreasing trend from early to late lactation (4.62-4.29%).

There is only one carbohydrate in milk, lactose, which influences its sensory properties, such as aroma and flavor, giving it a pleasant taste. In this study there was a significant difference ($p \leq 0.05$) in terms of lactose content between the milk obtained during the grazing period (4.54%) and that during the stall period (4.26%), this being being in balance with the protein. Other authors, in the qualitative evaluation of raw buffalo milk, (Tătaru et al., 2019), determined the highest values of lactose in spring (4.16%) and autumn (4.09%), and the lowest in summer (3.85%) and winter (3.94%). Pece et al. (2009), stated that the lactose content of the Romanian Buffalo breed milk was 4.8%, for all seasons. For lactose content, the global median was calculated to be 4.291 g/100 ml with values ranging from 4.00 g/ 100 ml to 4.62 g/100 ml. The average lactose in milk obtained from buffaloes in different stages of lactation, in the period March 2020 - February 2021, was calculated at 4.291 g/ 100 ml with values between 4.00 g/100 ml and 4.62 g/100 ml (Nikolau et al., 2022).

One of the important parameters when evaluating milk quality is the non-fat dry matter (NFS) content. The SNF in buffalo milk includes protein, lactose, vitamins, calcium and trace minerals. They contribute significantly to the nutritional value of milk. The results of the statistical analysis of the present study ($p \leq 0.05$) suggest that the milk obtained during the grazing period has a significantly higher SNF content (9.37%) than the milk obtained during the stalling season (8.98%). Other authors (Tătaru et al., 2019), showed that the solid substance without fat in buffalo milk, presents statistical significance ($p<0.05$) in winter compared to spring, autumn and summer, the lowest value of it is recorded in the winter season (8.86%), and the highest value is recorded in the summer (9.02%). The low content of SNF in milk, during the stalling period, is due to the lack of protein and nutrients in the diet, but also to heat stress. Standardizing the ratio of fat to non-fat solids in raw milk is essential for dairy production.

Table 3. Results of microbiological analyzes of buffalo milk (Mean with standard deviation X ± SD)

Season (n = 320) / Month of observation		NTG (cfu/ml)	NCS (no. cells/ml milk)
Pasture (n = 160)	May - August (n = 160)	140600 ± 68970[b]	82988 ± 3260[a]
Stabilization (n = 160)	November-December (n = 160)	164600 ± 170080[a]	84752 ± 4255[a]

n = number of determinations; ufc = Number of colony-forming units; [ab]the difference between mean values with different letters in the same column is statistically significant $P \leq 0.05$.

The presence of pathogens and microorganisms in raw milk and products made from inadequately heat-treated milk could pose a threat to public health. Table 3 shows the results of the microbiological examination of buffalo milk. We can thus describe that the microbiological analyzes show a significant increase ($p \leq 0.05$) of the total number of germs in the milk during the stall period ($164.85 \times 10^3 \pm 170.08 \times 10^3$ cfu/ml) compared to the grazing period where it was recorded an average of $140.60 \times 10^3 \pm 68.97 \times 10^3$ cfu/ml, the microbial load of raw milk being directly dependent on the hygienic conditions in the shelters during stalling. In other research conducted on buffalo milk, microbiological analyzes revealed an increase in total germ count (NTG) in winter (3.4×10^4 cfu/ml) compared to autumn, when the lowest NTG

was recorded (1.1 x 10^4 cfu/ml), in 120 samples studied between August 2017 and May 2018 (Tătaru et al., 2019). Nicolau et al. (2022), in their study states that from the buffalo milk samples analyzed over the course of a year, NTG falls within European limits in only 4 months out of 12. These months are represented by March, where NTG has a value of 380 ± 131.5 x 1000 cfu/ml, in April with an NTG of 324 ± 67.9 x 1000 cfu/ml, in July with a value of 347.5 ± 217 x 1000 cfu/ml and in September with an NTG value of 419 ± 185 x 1000 cfu/ml.

Somatic cell count (SCC) is an internationally recognized parameter for evaluating milk quality and udder health. Determination of somatic cell count is an important parameter to be considered for quality control of raw buffalo milk and as a control parameter for udder health. In the case of somatic cells in buffalo milk, the result of the study (Table 3) has no significant difference between seasons (84752 ± 4.25 x 10^3 cells/ml in the stable period and 82988 ± 3.26 x 10^3 cells/ml in the grazing period).

In another similar study on 120 raw buffalo milk samples, the average somatic cell count was 1026.5 x 10^3, the lowest value was 9 x 10^3 cells/ml and the highest was 2044 x 10^3 cells/ml (Tătaru et al., 2019). Regarding the evolution of NCS during one year, the average value obtained was 304.85 x 1000 cells/ml, and it fluctuates, in September having the minimum value (170.5 ± 3.53 x 1000 cells/ml), following as in the other months of study to increase until reaching the maximum analyzed value, which is in February of 484 ± 104.65 x 1000 cells/ml (Nikolau et al., 2022).

CONCLUSIONS

The results of the present study indicate that the season and stage of lactation affect certain components of milk obtained from buffaloes in the Făgăraș area. The milk obtained during the grazing period presented a better quality both in terms of density (in the grazing season being on average 1.032 g/cm^3, and in the stable season of 1.030 g/cm^3), nutrients such as protein (4.62% in the grazing season and 4.29% in the stable period), lactose (4.54% in the grazing season and 4.26% in the stable period), SNF (9.37% in the grazing and 8.98% in the stable period), as well as the microbial load (140.60 x 10^3 cfu/ml, in the grazing period and 164.85 x 10^3 cfu/ml in the stable).

Seasonal variations result in a varied composition of buffalo milk, mainly due to the feeding of the animals. In the summer, the buffaloes feed on grass in the pasture, and in the winter they are fed with canned fodder. As mentioned above, feeding green fodder not only has a direct effect on the physico-chemical composition of milk, but can lead to a reduction in its microbial load.

The stage of lactation in buffaloes, being a physiological process, cannot be changed, instead good management practices such as adequate nutrition and maintenance during the stall period would improve the physico-chemical and microbiological properties of the milk obtained.

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