Scientific Papers. Series D. Animal Science. Vol. LXVII, No. 2, 2024
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE EFFECT OF LACTATION STAGE ON THE COMPONENTS OF MILK, DURING THE GRAZING PERIOD, IN BUFFALOES COWS FROM THE ROMANIAN BUFFALO BREED

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Abstract

Currently, approximately 20.000 buffaloes are raised in Romania. The present study was carried out to observe the effect of lactation stage on the components of raw milk obtained from buffaloes of the Romanian Bubaline breed. 120 milk samples were collected during the morning and evening milking, during the grazing period, from 60 buffaloes in different stages and ranks of lactation. From the collected samples, the content of fat, protein, lactose, fat-free dry matter (NFD), and total dry matter (TU) were determined. The individual analysis of milk samples from buffaloes revealed significant differences in terms of the variation of these parameters, the research carried out revealed differences determined by the stage of lactation in the same reference season. Therefore, the results of the present research indicated that the stage of lactation, during the grazing season, significantly influences the chemical parameters of milk, regardless of the lactation rank.

Key words*: buffaloes, fodder, milk, lactation, pasture.*

INTRODUCTION

In Romania, raising buffaloes is a traditional activity, they are raised and exploited for milk-meat, but also for their traction force. During the communist regime, the herd of buffaloes reached the historical peak of 228,000 heads, and in 2004, Romania ranked second in Europe in terms of buffalo growth with 100,000 heads. During the last decades, the number of buffalo herds has decreased significantly, reaching in 2022 17,818 heads with a production of 15,526 tons of milk (INS, 2023).

According to the Food and Agriculture Organization (FAO), the global production of buffalo milk in 2022 was 143 million tons, representing about 19% of the total milk production obtained from buffalo cow (FAO, 2024). The attraction and investment in buffalo milk has been steadily increasing every year due to its unique taste and nutritional value, including much lower cholesterol, being used in a wide range of commercial products. Buffalo milk is of a special quality and contains 40% less cholesterol than cow's milk, being preferable for patients with atherosclerosis, dyslipidemia and cardiovascular diseases.

The main composition of buffalo milk is defined by the content of fat, protein, lactose, non-fat dry matter (NF) and total dry matter (TS). The distinguishing feature of buffalo milk is its high fat and calorie content, along with its dry matter. Changes in milk composition are influenced by several factors such as genetics, milking time, type of diet, age, udder hygiene and season.

In this context, the aim of the research is to examine the effects of the season and stage of lactation on the chemical composition of milk obtained from female buffaloes regardless of the rank of lactation of the Romanian Buffalo breed. The research results can be practically applied to improve the management of buffalo farming and the quality of the milk produced, thus contributing to the revitalization of this important agricultural tradition. Besides the economic benefits, raising buffaloes also has ecological advantages, helping to maintain biodiversity and traditional landscapes. Moreover, modern technologies and innovative practices in buffalo raising play a crucial role in

increasing productivity and animal health. Buffalo milk, recognized for its nutritional benefits, rich in essential vitamins and minerals, is increasingly appreciated for its contribution to human health.

Support programs and public policies, such as SCZ in buffaloes cows, ANTZ (Transitional National Aid for Bovines), the state aid scheme for animal breeding and performance control, and the Agri-Environment and Climate Measure for Raising Animals from Local Breeds in Danger of Abandonment - the Romanian Buffalo, encourage farmers to maintain this tradition. These programs reflect the cultural and traditional importance of raising buffaloes in Romania and support farmers in their efforts to preserve and revitalize this essential agricultural sector.

MATERIALS AND METHODS

The study was carried out on a sample of 60 buffaloes from the Romanian Buffalo breed, in different stages of lactation. The Romanian buffalo is characterized by morphological type of milk, waist 132 cm, body weight 490 kg, age of first calving 43.92 months, duration of lactation 474.74 days, average amount of milk per lactation 1669.03 kg, average amount of milk at first lactation 1385.55 kg, fat 7.65% and protein 4.30%. They have a well-developed stature, large head and horns, thin neck, long and broad back, wide rump and short legs. The animals were grazed between July and September and were fed green mass at discretion and concentrated fodder, in the amount of 2 kg/day/head. The milking process was done mechanically, using a mechanical drum milking plant.

In the study, two milk samples were collected from each buffalo, at morning and evening milking, resulting in a total of 120 milk samples. Buffaloes were divided into three distinct groups based on lactation stage: early lactation (1-2 months), mid-lactation (3-6 months) and late lactation (7-9 months).

Milk samples (250 ml/sample) were collected under aseptic conditions in sterile bottles and then transferred to the laboratory for detailed analysis. The analysed parameters included the content of fat, protein, lactose, dry matter without fat (SNF) and dry matter (SU).

The determination of the content of fat, protein, lactose, dry matter without fat (SNF) was carried out with the help of an ultrasonic milk analyser "EKOMILK-ULTRA". The data obtained were subjected to a statistical analysis to evaluate the average differences between the results of the determinations, expressed according to the standard deviation, using the ANOVA analysis of variance, with a level of significance set at $P>0.05$.

In addition, each milk sample collection procedure followed strict sanitation and handling protocols to ensure the integrity and validity of the data obtained.

These methods were applied to ensure accurate and relevant results were obtained in the study carried out on Romanian buffaloes.

Figure 1. Values obtained from a milk sample (own souce)

RESULTS AND DISCUSSIONS

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

Table 1. Results of chemical analysis of buffalo milk according to stage of lactation

Parameter/Stage of Lactation (N)	Fat (%)	Protein (%)	Lactose (%)	SNF (%)	SU (%)
The beginning of lactation N = 20	7.11± 0.21	4.59± 0.26	4.51± 0.22	9.36 ± 0.14	17.19 ±0.07
Mid lactation N = 20	7.42± 0.25	4.46± 0.24	4.39± 0.27	8.75 ±0.14	17.36 ±0.11
End of lactation N = 20	7.53± 0.23	4.28± 0.16	4.25± 0.33	8.96 ± 0.12	17.62 ±0.21

(%) = (g/100 ml); n = number of determinations

Fat

During the grazing period, the fat content of buffalo milk can be influenced by the quality and type of forage available. Grazing on fresh, nutrient-rich grass can contribute to higher milk fat content compared to feeding nutrient-poor

forage. One of the most important components of buffalo milk is fat. The fat content not only directly affects the nutritional and economic value of milk, but also has an effect on the organoleptic properties. The fat content of raw milk is so important that milk processors tend to set the price based on the fat content. The quality of dairy products such as cheese, cream and butter depends largely on the quantity and quality of milk fat. In particular, buffalo mozzarella, famous for its soft and elastic texture, owes its superior qualities to the fat of buffalo milk. The fat in buffalo milk is a valuable source of fat-soluble vitamins such as vitamins A, D, E and K, which are essential for healthy bones, skin and the immune system. Regular consumption of buffalo milk can help improve your overall health due to its nutrient-rich profile and beneficial fatty acids. The amount of milk fat during the grazing period recorded an average of 7.35%. The highest amount of fat was recorded at the end of lactation, 7.52%, and the lowest at the beginning of lactation, 7.11%, the differences being statistically significant. Compared to cow milk, buffalo milk has a significantly higher fat content, averaging 7.42% in mid-lactation and reaching up to 7.53% in late lactation during the grazing period. In comparison, cow's milk generally has a lower fat content, around 3.5%. Correct management of the fat content of buffalo milk is essential to ensure high quality dairy products appreciated by both consumers and processors.

Protein

Buffalo milk protein is a component that influences the processing of milk into cheeses and attracts attention due to its role in the final quality of dairy products. Proteins influence coagulation time and syneresis, essential factors in the cheese-making process (Bota A., 2023). Casein, the main milk protein, accounts for about 80% of the total protein content and is responsible for the texture and elasticity of cheeses. Protein content varies according to the lactation period, with significant values recorded at different stages of this cycle. During the grazing period, the average protein content is about 4.44%. The highest protein concentration, 4.58%, is observed in early lactation, reflecting a period of maximum milk production and nutrients. In contrast, towards the end of lactation, the protein content decreases slightly to 4.28%, but significant statistical differences underline the importance of this variation in the context of milk production and optimal use of buffalo resources. These fluctuations in protein content demonstrate the complexity and dynamics of the lactation process in buffaloes, directly influencing the quality and yield of the final dairy products.

Buffalo milk has an average protein content of 4.46% in mid-lactation and peaks at 4.59% in early lactation during the grazing period, while cow milk has an average protein content of about 3.2-3.4%.

The table below provides a comparative picture of the composition of buffalo milk from different countries and breeds as reported in various studies.

Table 2. The chemical composition of buffalo in different countries (%) (according to M.H. Adh El-Salam, S. El Shibiny, 2011)

Fat	SU	SNF	Protein	Lactose	Country/ Author
7.0± 1.3	16.6± 2.6	-	3.73± 0.82	4.57± 0.23	Argentine/ (Patino s.a., 2003) *Murrah
7.6± 1.8	16.8± 2.6	-	3.73± 0.88	4.51± 0.21	Argentine/ (Patino s.a., 2003) *Mediterranean MurrahxR
8.8± 0.3	18.4± 0.2	-	5.20± 0.14	4.55± 0.01	Argentine/ (Patino & Stefani, 2005)
8.4± 9.0	-	-	4.24± 4.45	4.90± 5.05	Azerbaijan/ (Akhundov & Farzalieva, 1979)
8.4± 0.3	17.7± 0.4	9.5± 0.2	3.97± 0.06	4.80± 0.1	Bangladesh (Khan s.a., 2007) *Swamp buffalo
7.34 ±0.5	16.7± 0.1	9.2± 0.2	3.77± 0.26	4.76± 0.18	Bangladesh (Khan s.a., 2007) *River buffaloes

Argentina (Patino & Stefani, 2005) - The study on Murrah buffaloes from Argentina reported a protein content of 5.20%, the highest value seen in the table. This high value can be attributed to efficient management practices and optimal feeding conditions, which contribute to high quality milk production.

Azerbaijan (Akhundov & Farzalieva, 1979) - The Azerbaijan study reported variable protein values, with a content of 4.24% and 4.45%. These differences may reflect variability in the breeds studied and in environmental and feeding conditions.
Bangladesh (Khan et al., 2007) - The Bangladesh study observed a protein content of 3.97% for Swamp buffaloes and 3.77% for river buffaloes. These values are lower compared to those in other studies, indicating possible differences in diet and animal management.

Lactose

There is only one carbohydrate in milk, lactose, which influences its sensory properties. This disaccharide, composed of glucose and galactose, gives milk a slightly sweet taste and contributes to its distinctive flavor. Lactose comes in three forms: hydrated alpha, anhydrous beta and an amorphous form, which consists of a mixture of alpha and beta lactose in a ratio of 1:5 (Coroian, 2009). Lactose partially decomposes at temperatures of 70-80ºC with the formation of organic acids in particular, formic and lactic acid. Lactose plays an important role in the fermentation processes needed to make yogurt and other fermented products. Lactic acid bacteria use lactose as an energy source, producing lactic acid and thus contributing to the texture and taste of the final product. The results of the determinations showed that there is a significant difference ($p \leq 0.05$) in terms of lactose content between the milk obtained at the beginning of lactation (4.50%) and that at the end of lactation (4.24%), this being in protein balance. The average lactose in milk obtained from buffaloes at different stages of lactation is 4.04%.
Buffalo milk has an average lactose content of 4.39%, reaching a maximum of 4.51% in early lactation during the grazing period, while cow milk has an average lactose content of about 4.8-5%.
Lactose has numerous health benefits, including providing a quick source of energy and aiding the absorption of calcium and other minerals. Lactose intolerance is caused by a deficiency of the enzyme lactase, which is needed to digest lactose. Buffalo milk, although high in lactose, can be processed to produce lactose-free dairy products so that it is also accessible to those with intolerances.
Pece et al. (2009) stated that the lactose content of the Romanian Buffalo breed milk was 4.8%, for all seasons.
To better understand the variability in the composition of buffalo milk in different regions of the world, data from various studies are presented below. This information illustrates the differences in lactose, fat and protein content by country and the conditions specific to each study.

Table 3. The chemical composition of buffalo milk in different countries (%) (according to M.H. Adb El-Salam, S. El Shibiny, 2011)

Fat	SU	SNF	Protein	Lactose	Country/Author
8.1± 1.9	-	-	4.65± 0.48	-	Italy (Tufarelli s.a. 2008)
7.6± 0.1	-	9.8± 0.1	4.11± 0.02	-	Pakistan (Imran s.a.2008)
7.0± 0.6	-	-	4.35± 0.02	5.21± 0.11	Pakistan (Arian s.a., 2008)
7.64	18.16	-	4.69	4.85	Romania (Velea C s.a., 2006)
7.8	17.5	-	4.0	4.9	Romania (Vidu Livia, 2007)
7.1± 1.4	16.6± 1.6	9.6± 0.8	4.40± 0.51	-	Turkey (Sekerden s.a., 1999)

Italy (Tufarelli et al., 2008): Buffalo milk has a high content of fat (8.1±1.9%) and protein (4.65±0.48%), reflecting the superior nutritional qualities of buffalo milk.
Pakistan (Imran et al., 2008): Buffalo milk has a protein content of 4.11±0.02% and a SNF (fat free solids) level of 9.8±0.1%, indicating a balanced nutritional profile.
Pakistan (Arian et al., 2008): Lactose content reaches 5.21±0.11%, a relatively high level, suggesting a more pronounced sweetness.
Romania (Velea et al., 2006): Lactose content is 4.85%, indicating slight seasonal and regional variability in Romanian buffalo milk.
Romania (Vidu, 2007): In this study, the lactose content was 4.9%, reinforcing the idea of a consistent lactose profile in Romanian buffalo milk.
Turkey (Sekerden et al., 1999): This study revealed a protein content of 4.40±0.51%, with a variability in SNF content of 9.6±0.8%, indicating the influence of local conditions and feed on composition of buffalo milk.

Solid non-fat

One of the important parameters when evaluating the quality of milk is the content of solid non-fat (SNF). The SNF in buffalo milk includes protein, lactose, vitamins, calcium, and trace minerals. These components significantly contribute to the nutritional value of the milk, influencing both its physico-chemical and organoleptic properties. A high SNF content contributes to the density and texture of dairy products such as yogurt, cheese, and milk powder. It has been found that the lactation stage affects the fat-free solids, with a tendency to decrease from early to late lactation (9.36-8.95%). Standardizing the ratio of fat to non-fat solids in raw milk is essential for dairy production.

The study revealed a significant difference in the dry matter content in different stages of lactation, with an increasing trend from the beginning of lactation towards its end (17.36±0.11% in the peak period of lactation). Buffalo milk has an average SNF content of 8.75% in mid-lactation, reaching a maximum of 9.36% in early lactation during the grazing period, while cow milk has an average SNF content of about 8.5-9%.

CONCLUSIONS

The results of the present study indicate that lactation stage affects certain components of milk obtained from buffaloes during the grazing season. Milk obtained from buffaloes with a stage of lactation towards the end, recorded values of the fat content of 7.53±0.23%, significantly higher compared to milk obtained from buffaloes in the beginning and middle stages of lactation (7.11±0.21%, respectively 7.42±0.25%). On the other hand, the protein and lactose content of milk had significantly higher values in buffaloes with early lactation stage (4.59±0.26% protein and 4.51±0.22% lactose), compared to those in the end stage of lactation (4.28 ±0.16% protein and 4.25±0.33% lactose). Regarding the percentage of dry matter in milk, significantly higher values are noted in buffaloes in the stage of advanced lactation, compared to those with early and middle lactation. The stage of buffaloes, being a physiological process, cannot be changed, instead good management practices such as proper feeding and maintenance during the grazing period could be the physico-chemical properties of the milk obtained. Adjusting feed composition and quality to meet the nutritional needs at different lactation stages could further enhance milk quality.

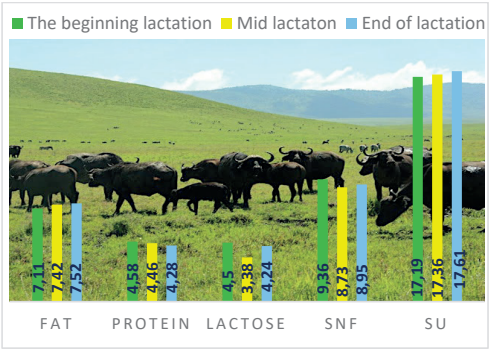

Figure 2. Composition of buffalo milk at different stages of lactation during the grazing period

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