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SEASONAL VARIATIONS IN THE BIOCHEMICAL COMPOSITION OF BUFFALO MILK

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ABSTRACT. In this study the seasonal variations of buffalo milks' composition and fatty acids profile with emphasis on the percentage of Saturated Fatty Acids (SFA), Mono Unsaturated Fatty Acids (MUFA) and Poly Unsaturated Fatty Acids (PUFA) was investigated. In each season we gathered 30 samples of milk from the same farm located in North Transylvania. The mean data from the 120 samples gathered in all four season (September 2009 – August 2010) were compared using the ANOVA test. There were no significant differences in the amount of lactose, but the protein percent differed in a noticeable manner from winter to spring season. The significant differences were found also at the average percent of milk fat that ranged between 6.69% - 12% with a peak in the months of winter. The content of PUFA varied very little, the main fatty acid being the linoleic acid (C18:2). Our data show that the season influences the buffalo milk's biochemical composition and the fatty acids profile probably via the type and quality of forages consumed and of course the amount of supplementation used in the buffaloes' diet.

Keywords: *buffalo, fatty acids, season, protein, fat, lactose.*

INTRODUCTION

Although buffaloes hold the greatest promise and potential for milk production [1] and the Food and Agriculture Organization has rightly termed it „ an asset under evaluated", little attention has been paid to buffaloes' milk biochemical composition. The buffalo breeds are widely spread, but because of their wild behavior they are considered rogue animals, and no concerted efforts were made to grade them according to their productivity. Our study was conducted on the Murrah buffalo which is the most popular breed with a minimum milk yield of no less than 1,360 kg in 300 days [2]. Knowing the variation of the main biochemical parameters in the milk composition of this

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particular breed contributes to the development of the scientific data base necessary for the impact evaluation of the quality markers established in the traditional primary milk chain. The nutritional value of buffalo milk was analyzed by investigating the most common parameters like milk fat, protein, lactose, focusing mostly on the fatty acids composition, as they are the most important quality markers of a milk product.

Dairy fat is one of the most complex dietary fat and more than 400 different fatty acids have been detected in it. On account of higher content of saturated fatty acid, trans fatty acids, butyric acid and conjugated linoleic acid, the milk fat has been associated with human health, adversely or positively. Even though the milk fat content is high in saturated fatty acids, which have been claimed to contribute to heart disease [3], other components of milk are considered to be beneficial for human health [4]. Unsaturated fatty acids (UFA) particularly omega 3 – polyunsaturated fatty acids (PUFA), have been shown to have potential health effects, for example the prevention of mammary gland in skin tumors [5]. The variation of milk fatty acids has been shown to be influenced at bovines by the forage and concentrate ratio [6] and level of intake of saturated fatty acids, especially the ones high in oleic acid [7]. The numbers of studies concerning the seasonal influence on the biochemical composition of buffalo milk are too few to form an accurate statement that is why our study focuses on this matter.

RESULTS AND DISCUSSION

The results obtained after analyzing the main biochemical parameters investigated (fat, protein, lactose) are presented in figure 1.

Regarding the fat percent, the statistical analysis revealed that there were significant changes between the average value recorded in spring (8.88%) and the one recorded in winter (9.5%) ($P < 0,05$), but no significant changes among the rest of them (autumn: 8.34%, summer: 8.20%) ($P > 0.05$). 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 same results were obtained by Anderson and Pollak (1980) who have reported that the percentage of fat in bovine milk raised in countries with the same climate conditions has been influenced by the seasonal variations.

This 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%) ($P < 0.05$). 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.

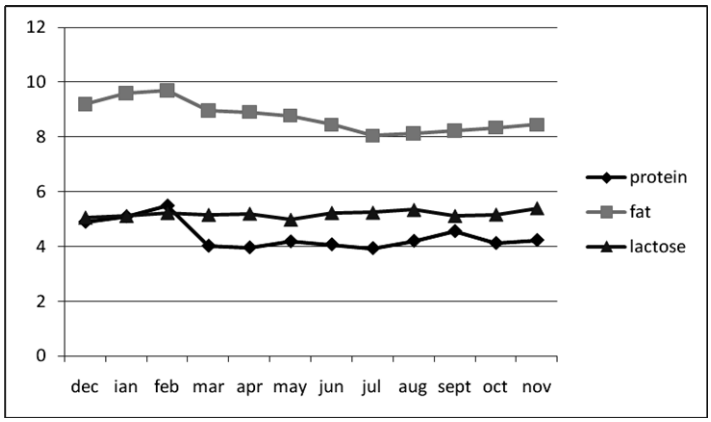


Figure 1. The average percent of the biochemical parameters in the months studied

The lactose average percentage during the period studied did not show significant changes ($P > 0.05$), varying very little from one season to another (autumn: 5.4 g%, winter: 5.13 g%, spring: 5.11 g%, summer: 4.85 g%).

The composition of fatty acids (FAs) in buffalo milk throughout the four seasons of the year is presented in table 1. The FA composition was eminently influenced as the function of sampling season. The five most important fatty acids in quantitative terms (C10:0, C14:0, C16:0, C18:0 and C18:1) accounted for more than 80% of the total fatty acids in all the samples studied, no matter the season in which they were collected. Overall the concentration of short chain fatty acids (<14:0) in milk were highest in the winter season and the lowest in the spring season.

The content of palmitic acid (C16:0) was significantly higher in the autumn season compared with the summer season, and varied by 5 – 6 % throughout the year. In a study made by Talpur et al. (2008) the percent of palmitic acid in the milk of various ruminants (cow, ewe, goat, buffalo) was found to be the highest during the summer season, and varied very much 16 – 25% throughout the year. These changes can contribute to human health, as it is well known that only C12:0, C14:0 and C16:0 affect the plasma cholesterol levels LDL [10,11]. The C18:0 content varies along the entire year and being significantly lower in the summer period, a fact possible due to the changes in the food supplement of polyunsaturated fatty acids (PUFAs). The main poly unsaturated fatty acid was the linoleic acid which hasn't shown real differences in the values of each season.

Figure 1 and 2 illustrates the chromatograms of the most representative samples collected in autumn (September), respectively summer (August), in which the fatty acids are registered in the form of peaks separated from each other by increasing the length chain, and at the same length chain by increasing of unsaturated degree.

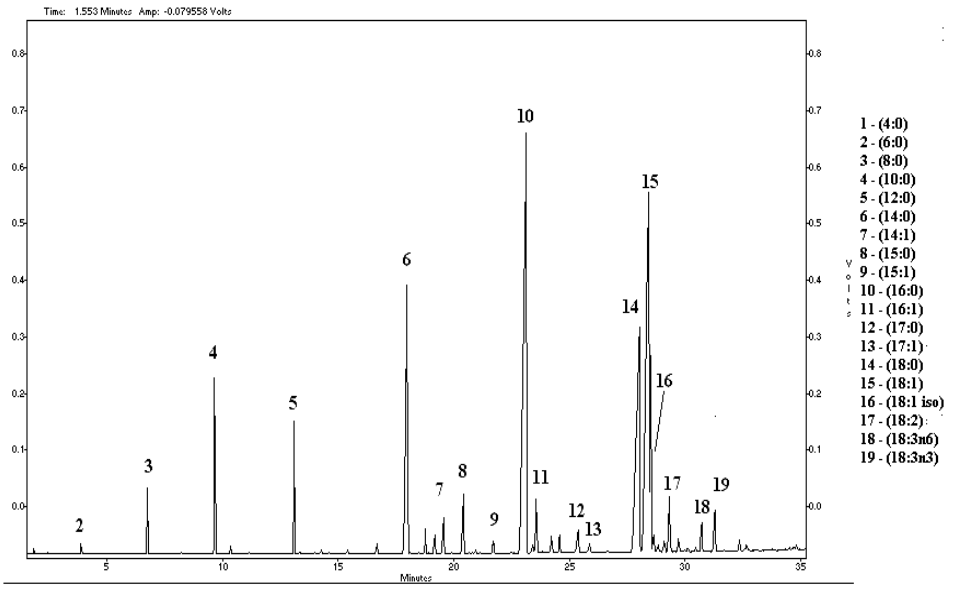


Figure 2. The cromatogram of a milk representative sample collected in autumn (September)

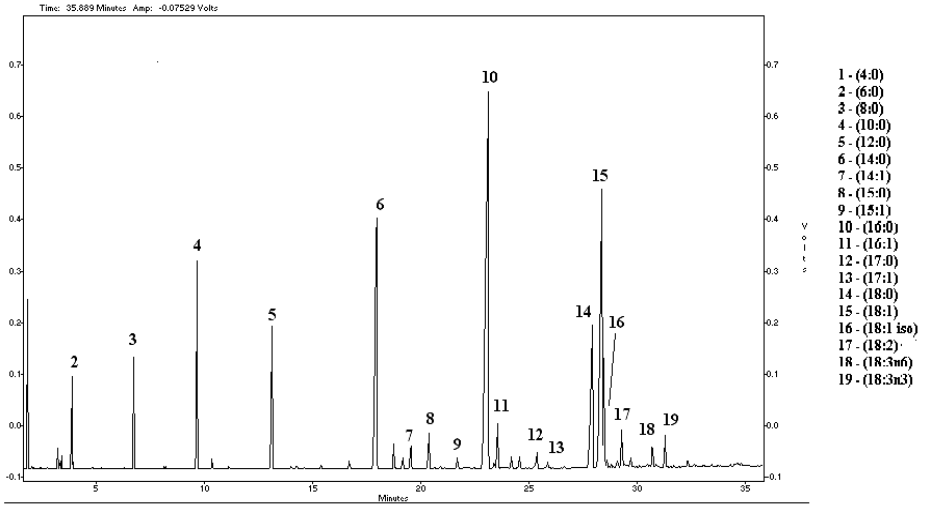
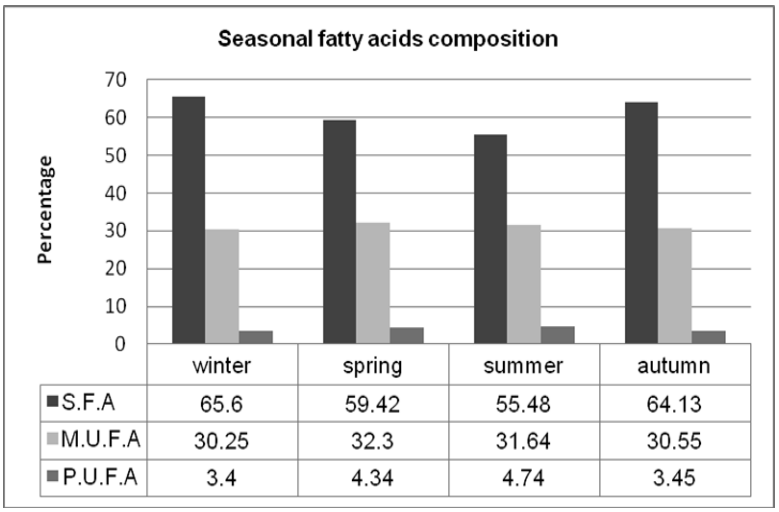


Figure 3. The cromatogram of a representative sample collected in summer (August)

Table 1. Seasonal changes in the fatty acid profile of buffalo milk taken from the semi-subsistence dairy farm

Fatty acid		Winter	Spring	Summer	Autumn	Average	SEM	ANOVA
Butyric	C4:0	n.d	n.d	n.d	n.d			
Caproic	C6:0	1.28	1.47	1.35	1.86	1.48	0.25	***
Caprilic	C8:0	1.16	1.82	1.13	1.17	1.32	0.33	***
Capric	C10:0	2.36	4.06	2.21	2.50	2.78	0.85	***
Undecanoic	C11:0	n.d	n.d	n.d	n.d	N.D		
Lauric	C12:0	5.30	2.74	4.49	2.69	3.80	1.30	***
Tridecanoic	C13:0	n.d	n.d	n.d	n.d	N.D		
Miristic	C14:0	13.36	9.46	9.43	11.62	10.96	1.89	***
Miristoleic	C14:1	0.55	0.77	0.98	0.44	0.68	0.23	**
Pentadecanoic	C15:0	1.21	1.22	1.13	1.54	1.27	0.18	*
Cis –10 -Pentadecanoic	C15:1	0.30	0.27	0.30	0.34	0.30	0.02	NS
Palmitic	C16:0	25.38	23.65	22.79	26.71	24.63	1.75	***
Palmitoleic	C16:1	1.42	1.36	1.60	1.41	1.44	0.10	NS
Heptadecanoic	C17:0	0.64	0.82	0.86	1.02	0.83	0.15	**
Cis-10-Heptadecanoic	C17:1	0.28	0.35	0.27	0.30	0.30	0.03	NS
Stearic	C18:0	14.91	14.18	12.09	15.02	14.05	1.35	***
Oleic	C18:1	22.80	26.78	24.99	23.35	24.48	1.79	***
Elidic	C18:1iso	4.90	2.77	3.50	4.71	3.97	1.01	***
Linoleic	C18:2	1.59	1.90	2.13	1.71	1.83	0.23	**
Linolenic	C18:3n3	0.75	1.21	0.93	0.84	0.93	0.19	**
γ – linolenic	C18:3n6	1.10	1.23	1.68	0.90	1.22	0.33	*

Significance, NS, not significant P>0,5; * P< 0,5; ** P<0.01; *** P<0.001;
Data is presented as least square mean.SEM, standard error of the mean.



	winter	spring	summer	autumn
S.F.A	65.6	59.42	55.48	64.13
M.U.F.A	30.25	32.3	31.64	30.55
P.U.F.A	3.4	4.34	4.74	3.45

Figure 4. The seasonal average fatty acids composition in the buffalo milk samples studied

The FA composition was eminently influenced as the function of sampling season. As it is presented in figure 4 the seasonal average of the fatty acids composition varied in the year studied. In general the saturated fatty acids (SFA) were higher in winter and lowest during the summer, there being 5–10% less SFAs present in milk fat in summer compared with winter. The mono saturated fatty acids (MUFA) showed a lower discrepancy in comparison to the SFAs, varying only with 1 -3% throughout the year. The lowest percentage of MUFA was registered in winter (30.25%) and the highest in spring (32.3%) but then again the differences were not significant. A high concentration of PUFA (polyunsaturated fatty acids) was noticed during the summer season reaching an average of 4.75% in comparison to the autumn season when the lowest value was registered (4.34%).

CONCLUSIONS

The main biochemical parameters evaluated in the milk samples gathered from Murrah buffaloes are strongly influenced by the season variations. The average fat and protein percent recorded at the samples collected in the warm seasons (spring, summer) is lower than the one registered in the cold ones (winter and autumn). The lactose percent varied very little according to the seasonal changes. The five most important fatty acids in quantitative terms in the buffalo milk are C10:0, C14:0, C16:0, C18:0 and C18:1 which counted for more than 80% in all the seasons in which the samples were taken and analyzed. The hypercholesterolemic average values

(C12:0, C14:0 and C16:0) obtained in this study, varied from 35.85% in spring to 45.59% in winter. This shows a significant increase due to the feeding particularities of these seasons. The highest amount of monounsaturated fatty acids was found in the samples collected in spring season (32.3%) and the polyunsaturated fatty acids average percent (4.74%) was the highest in summer, in this respect the milk's nutritional quality being the highest during the warm seasons. The quantification of the seasonal variations in the main biochemical parameters with an emphasis on the fatty acids profile from milk certifies them as quality and traceability markers at the Murrah buffaloes' milk primary production.

EXPERIMENTAL SECTION

Samples

The samples were gathered from a semi–subsistence buffalo dairy farm situated in north Transylvania, monthly starting from September 2009 and finishing in August 2010. Each month 30 samples were collected from buffaloes fed on the same dietary regimen throughout the year, including green forages (constitutes 70% of dietary intake during spring and summer) and a mixture of crop residues.

The analysis of the main biochemical parameters

During milk collection and preparation, concerted efforts were made to avoid microbial contamination. The raw, unpreserved samples were stored overnight at +4ºC and analyzed on the following day. Milk was analyzed for protein, lactose and fat content on a Milkoscan 134 (Foss-Electric A/C, Hillord, Denmark) (IDF standard 141 B:1996) which applies the well-known infrared spectrophotometry, providing a simple, rapid and accurate analysis of the key control parameters.

Milk fat extraction

Milk fat was extracted by using the following protocol: About 2ml of milk samples were mixed with 0,6 ml ammonia 25%, 2ml EtOH, 4ml Ethyl ether and 4 ml hexane and then agitated for 2-3min. After this process the lower layer (the ammonia layer) was discarded. Following this step the mixture was passed through a cellulose filter with Na_2SO_4 and then brought to dryness.

Transesterification

Fatty acids were converted to methyl esters by reaction with boron trifluoride/methanol at 80ºC for two hours in a closed Pyrex glass tube. The content was transfered into a separatory funell.

The methyl ester extraction

The extraction was made using 10 ml hexane. The hexanic fractions collected were dried using anhydrous sodium sulfate, filtered, concentrated under a nitrogen stream and finally re-eluted in 1 mL hexane. Fatty acids were analyzed by gas chromatography (GC) with flame ionization detection (FID). A 1µL sample was injected into the Shimadzu GC-17A series gas-chromatograph, equipped with a 30m polyethylene glycol coated column (Alltech AT-WAX, 0.25mm I.D., 0.25µm film thickness). Helium was used as the carrier gas at a pressure of 147 kPa. The injector and detector temperatures were set at 260ºC. For the oven temperature the following program was used: 70ºC for 2 min. then raised to 150ºC at 10ºC/ min. rate and held at 150ºC for 3min., then further raised up to 235ºC at a 4ºC/min.

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REFERENCES

1. W.R. Cockrill, “Present and future of buffalo production in the world”, *Proceedings of the Fifth World Buffalo Congress*, 27 - 30 June, **1994**, Sao Paolo, Brazil.
2. M.M. Appannavar, S. Kumar, T. Shashidara, "Note on production traits in a herd of Surti buffaloes", *Indian J.Dairy Sci.,* **1995**, *48:*480-481.
3. A. Chisholm, J. Mann, D.W. Sutherland, A. Duncan, M. Skeaff, C. Frampton, “Effect on lipoprotein profile of replacing butter with margarine in a low-fat diet: randomised cross-over study with hypercholesterolaemic subjects”, *Medical British Journal*,**1996**, *312*, 931-934.
4. P.W. Parodi, “Milk fat in human nutrition”, *Dairy Technology Journal*, **2004**, *59*, 3-59.
5. M.A. Belury, “Dietary conjugated linoleic acid in health: Physiological effects and mechanism of action", *Annual Revising Nutrition*, **2002**, *22*, 505 - 531.
6. J.M. Griinari, D.A. Dwyer, M.A. McGuire, D.E. Bauman, D.L. Palmquist, K.V.V. Nurmela, “Trans-octadecenoic acids and milk fat depression in lactating dairy cows”, *Journal of Dairy Science*, **1998**, *81*, 1251 - 1261.
7. T.R. Dhiman, G.R. Anand, L.D. Satter, M.W. Pariza, “Conjugated linoleic acid content of milk from cows fed different diets”, *Journal of Dairy Science*, **1999**, *82*, 2146 - 2156.
8. G. Anderson, M. Pollack, “Genetic variation in the yields and contents of milk constituents”, *International Bulletin Dairy Federation*, **1980**, *125*, 73 - 82.

9. F.N. Talpur, M.I. Bhanger, A.A. Khooharo, G. Zuhra Memon, “Seasonal variation in fatty acid composition of milk from ruminants reared under the traditional feeding system of Sindh, Pakistan”, *Livestock Science*, Elsevier, **2008**, *118*, 166 - 172.
10. E.H. Maniapane, A.M. Salter, Diet, Lipoproteins and Coronary Heart Disease: A Biochemical Prospective. Nottingham University Press, Nottingham,**1999**.
11. K. Maijala, “Cow milk and human development and well being”, *Livestock Production Science*, **2000**, *65*, 1 -18.
12. C.G. Harfoot, G.P. Hazlewood, “Lipid metabolism in the rumen”, The Rumenmicrobial Ecosystem, Blackie Pp. London, **1997**, 382 - 426.
13. M. Doreau, Y. Chilliard, “Digestion and utilization of fatty acids by ruminants”, *Animal Feed Science and Technology*, **1997**, *45*, 379 - 396.