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Gas chromatographic quantification of fatty acids profile from administered forage to lactating buffaloes

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Abstract. The dairy buffalo feeding must supply their optimal economic and technical level and highlights their biological production potential output. This study aims to emphasize the results of the physical and chemical compounds analyze from the administered forage to lactating buffaloes, and profile of the fatty acids, using gas chromatography. The most representative fatty acids from the forage are: palmitic acid C16:0, linoleic acid C18:2 and linolenic acid C18:3. The dry matter of the administered forage to lactating buffaloes varies between the following average values: 88.65±0.02% and 93.59±0.01%.
Key Words: forage, fatty acids, gas chromatography.

Introduction. The fatty acids from diet are predominantly with long carbon chains (palmitic $C_{16:0}$, stearic $C_{18:0}$, oleic $C_{18:1}$, linoleic $C_{18:2}$ and linolenic $C_{18:3}$). Those fatty acids are bio-hydrogenated by the ruminal flora, so in both adipose tissue and milk are found more saturated fatty acids compared to fodder diets (Keenan & Patton 1995; Jensen & Clark 1988).

About one third of the palmitic acid and almost all oleic acid from total milk lipids originate from feed. Forages are the most important factor, which determines dairy production growth and sustainability. The necessary energy, protein, minerals, and water quantities must support the real production potential of the buffaloes (Chindriş 1998).

Normally the ruminants' diet contains between 2 and 4% lipids. The reduced lipid content in forages destined to ruminants is one of the reasons why research in the field of lipid digestion is still in development, in this species. The lipids are an important part of the administered diet to dairy cows and buffaloes, because they directly contribute with about 50% to milk fat, in the same time being the fodder most concentrate energy source.

During sub-nutrition period or in early lactation, the buffaloes take the necessary energy by mobilizing the stored fat in adipose tissues with the aim of obtaining the additional energy besides those obtained from fodder. The lipid composition and share are strongly influenced in rumen. The lipids metabolism has some particular, even unique, issues in ruminants, concerning: digestion and absorption, lipo-protein synthesis and lipid transport, lipid transfer in milk, lipo-genesis and use of the plasmatic volatile fatty acids (VFA) as metabolic indicator (Wattiaux & Grummer 2000).

Compared to cattle, the buffaloes have an enhanced capitalization capacity of the volume fodder, respectively are superior with 10-12% in case of forages with high content of cellulose, mentioning that the diet level of this component is most favorable at a 22-24% (Velea & Mărgineanu 2006; Velea & Zanc 2010).

Material and Method. In order to determine the fatty acids from forage, using gas chromatography are necessary the following steps: extraction, filtration, phase separation, drying, evaporation and trans-esterification.

The forage was grounded, and then homogenized with methanol and chloroform. 100 µL chloroformic lipid extract were dry evaporated (by blowing with methane) in trans-esterification Pyrex tubes. 1 mL benzene was added for lipid dissolution and 2 mL of 0.5M methanol solution of sodium methoxide and heated at 70ºC for 1h.

After cooling were added 100 µL of glacial acetic acid and 1 mL of distilled water. The methyl esters of the fatty acids were extracted with petroleum ether (2 × 3 mL) in a separation funnel and reunited ether extracts were anhydrified with anhydrous Na_2SO_4, filtered and dry evaporated. The residue was dissolved in 100 µL and chromatography was performed on 60F 254 (Merck) silica gel plate. Benzene was used as eluent and iodine for visualization. R_f=0.5 for methyl esters of fatty acids that finally were dissolved in 500 µL hexane, and than they were undergone to gas chromatography analyze.

The separation of the components from a complex mixture using gas chromatography can be put into practice based on different affinity for a mobile gaseous phase and a stationary liquid (CGL) or solid (CGS) phase.

The separation of the methyl esters of the fatty acids was performed with SHIMADZU GC-17A gas chromatograph equipped with a CHROMPACK capillary column, 25 cm length and 0.25 mm diameter.

The stationary phase (an ethylene glycol derivative) been filed into the column in the form of a 0.2 µm thin film. A FID detector was used, and mobile phase was represented by 99.90% purity helium. The Lactosan device served to analyze the physical and chemical milk parameters.

Results and Discussion. According results, the average percentage composition of forage (hay) in fatty acids is the following: $C_{16:0}$=27.18, $C_{18:0}$=3.21, $C_{18:1}$=13.13, $C_{18:2}$=26.70, $C_{18:3}$=22.95. By controlling and remodeling the diet, the ratio between fatty acids with short and long carbon chains produced by mammary gland may be substantially modified (Jensen 2002).

Tyagi et al, 2008 performed a study concerning the fatty acids and CLA content in fodder administered to cows and buffaloes. The fatty acids composition in concentrate fodder and mixture of straw and wheat indicated the values of 14.98 mg/g for C18:3 and 14.22 mg/g for C18:2.

The (Figure 1 and 2) represent the chromatograms resulted during quantification of fatty acids from forage using two temperature programs, 70 and 150ºC, when gas chromatography was performed.

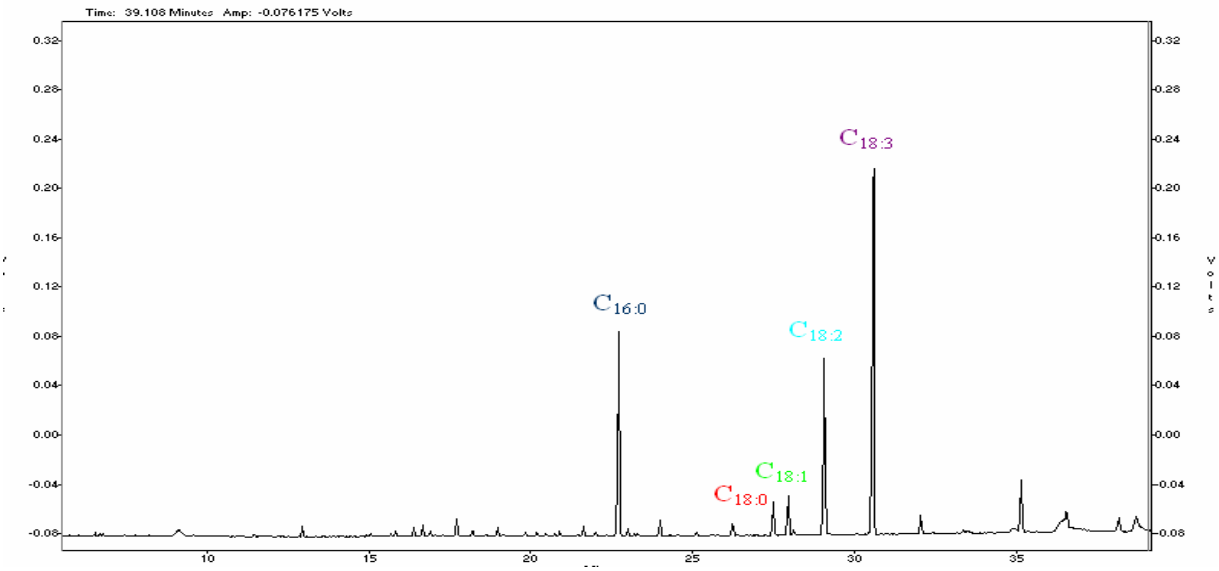

Figure 1. Analyzed fodder fatty acids chromatogram, 70°C program.

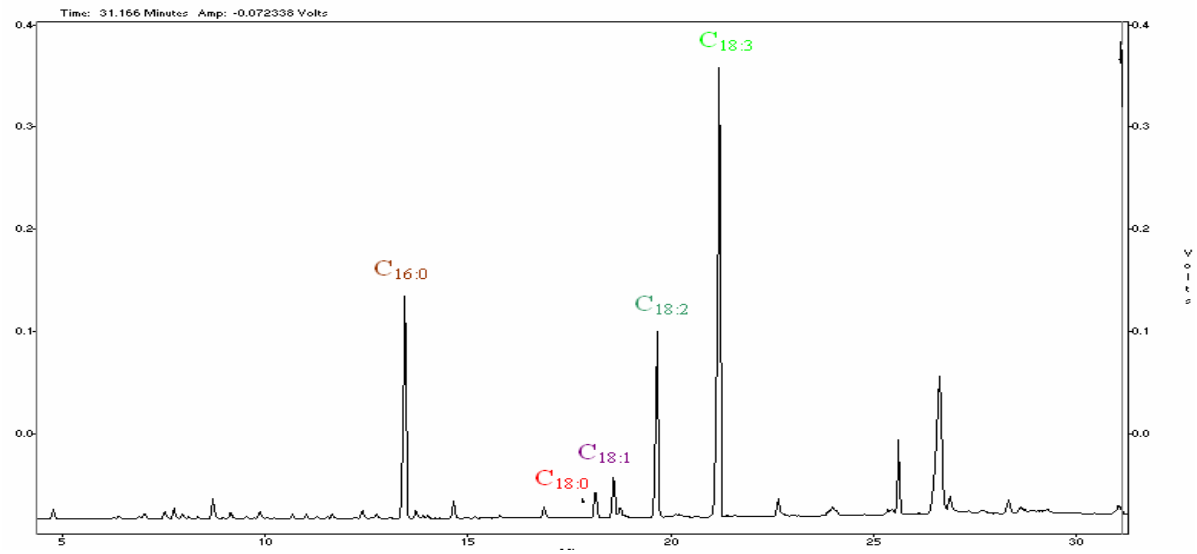

Figure 2. Analyzed fodder fatty acids chromatogram, 150°C program.

According to Table 1 the average content of fatty acids in forage are the following: $C_{16:0}$ = 27.69±0.34, $C_{18:0}$ = 3.26±0.03, $C_{18:1}$ = 13.29±0.21, $C_{18:2}$ = 26.83±0.02, $C_{18:3}$ = 22.97±0.04.

Table 1

Average values and variability percentage of analyzed fodder fatty acids

Fatty acid	*Abbreviation*	*n = 8*		*V %*
		$\overline{X} \pm s_{\overline{X}}$	*s*	
Palmitic	16:0	27.69 ± 0.34	0.91	3.28
Stearic	18:0	3.26± 0.03	0.08	2.34
Oleic	18:1	13.29 ± 0.21	0.55	4.17
Linoleic	18:2	26.83 ± 0.02	0.04	0.15
Linolenic	18:3	22.97± 0.04	0.11	0.46

The diets administered to dairy buffaloes must supply animals with complete and equilibrated feeding, adapted to the specific evolutionary character of lactation period and curve. The lipid excess in feeding (>8%) may have negative effects on dairy production and fat percent in buffaloes.

Unsaturated lipids have a higher negative impact compared to saturated lipids. However, lipids may be protected with the aim to slow hydrolyse rate. The covered seeds tent to protect the lipids from inside, making them less accessible for ruminal hydrolysis, compared to free oil. In figure 3 are graphically represented the main fatty acids from the forage administered to buffaloes during lactation.

About 50% of dairy fat quantity derives from fatty acids absorbed by mammary gland. These fatty acids are derived primarily from lipids rich in TG formed during intestinal lipid absorption. An increase of the quantity of long chain fatty acids (>16 carbon atoms) in feed determine the increase of their secretion in milk, but also inhibits the synthesis of the short and medium fatty acid chains in mammary tissue.

Such a significant decrease of milk fat because of low fiber diet may be only partially offset by increasing the amount feed fat (Wattiaux & Grummer 2000).

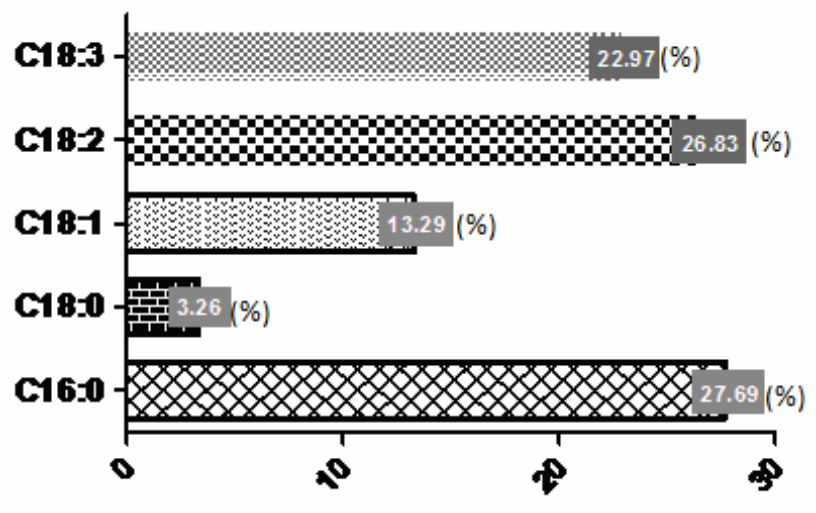

Figure 3. Average values of analyzed fodder fatty acids.

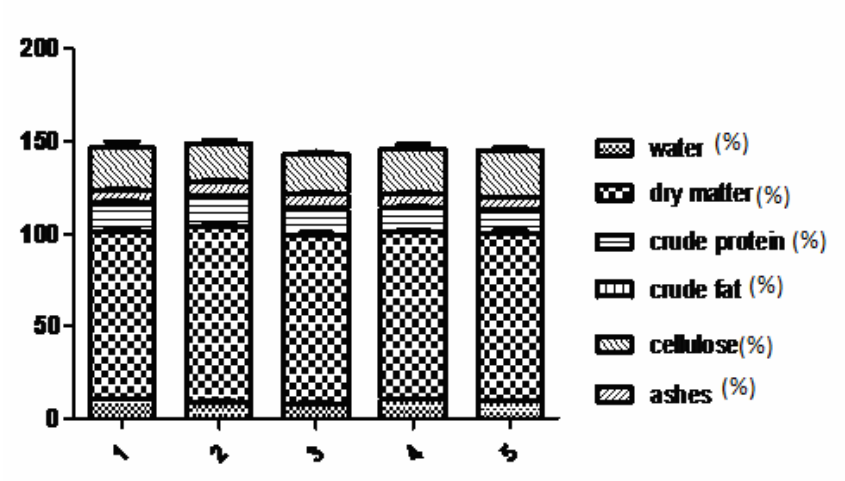

Figure 4. Composition of the fodder administered to lactating buffaloes (1-5 lactation).

Research performed in USA emphasize that in milk produced by animals fed on pasture, especially on those cultivated with *Lollium*, the level of conjugated linoleic fatty acids (CLA) is bigger compared to those found in animals fed with silage; but quantity of conjugated linoleic acid also increases when animals are fed with flax and soy oil, in a proportion of 2–4%. In this case, the level of linoleic acid is found in the same proportions as in the case where buffaloes are fed on pasture.

Experimentally, it was demonstrated that the diet rich in conjugated linoleic acid inhibits the development of the carcinogen tumors in stomach, mammary gland and skin (Palmaquist et al 1993).

The energy rich diets may confer superior coagulation properties to buffalo milk. When energetic concentration of administered feed is low, the quantity of fatty acids with multiple bonds increases in milk (Chindriş 1998). Figure 4 emphasizes the chemical composition of the fodder administered to studied buffaloes.

The average minimal and maximal values (%) of the main components of the fodder are the following: dry matter 88.65±0.02 and 93.59±0.05; water: 7.43±0.01 and 10.15±0.03; crude protein 10.24±0.01 and 17.74±0.03; crude fat 0.56±0.01 and 1.12±0.02; ash 4.98±0.02 and 7.65±0.03; cellulose 17.56±0.04 and 28.16±0.02. Figure 5 presents the chemical composition of milk according to lactation.

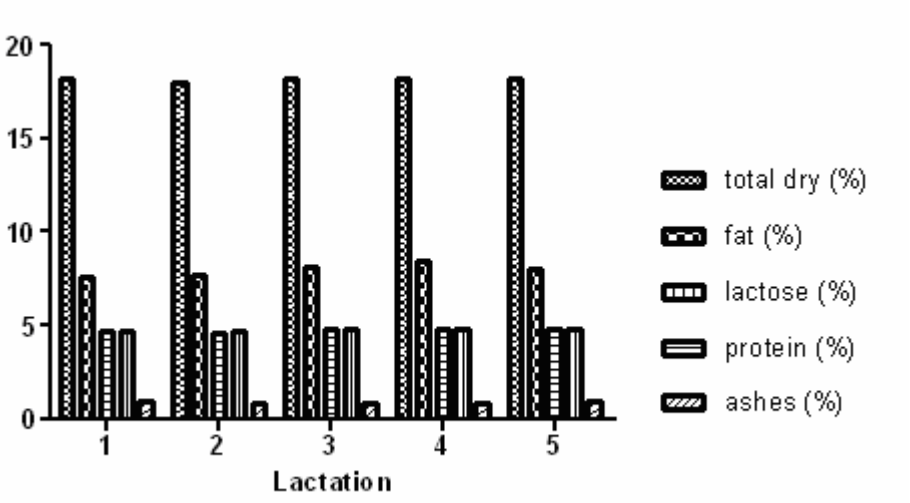

Figure 5. Chemical composition of milk depending on lactation.

Conclusions. The buffalo feeding has profound consequences on dairy production, also having special particularities determined by the feeding specific and season. To obtaining high dairy production imposes the practice of a balanced feeding regime concerning all nutritional components. Obtaining high quantitative and qualitative indices for dairy production during lactation is influenced by lots of factors of which, feeding level and technique are determinant. When rational feeding and correct techniques of forage production are practiced, the vitamin requirements of lactating buffaloes with moderate production performances may be satisfied by using vegetal forages, but when intensive technologies are practiced, they must be supplemented.

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