Evaluation of the Milk Fatty Acid Profile from Mediterranean Buffalo Cows in the First Eight Weeks of Lactation

Lenita Camargo Verdurico, Jefferson Rodrigues Gandra*, Jose Esler de Freitas Júnior, Rafael Villela Barletta, Beatriz Conte Venturelli, Rodolfo Daniel Mingoti, Thiago Henrique Annibale Vendramini and Francisco Palma Rennó

Department of Animal Nutrition and Production – School of Veterinary Medicine And Animal Science, University of São Paulo -Av. Duque de Caxias Norte, 225- CEP: 13630-000- Pirassununga - São Paulo, Brazil

Abstract: The aim of this study was to evaluate the fatty acid composition of buffalo milk in the first eight weeks of lactation. It was used 18 cows multiparous Buffaloes of Mediterranean race, the data collection starting four weeks before of the calving provided by the 8th week of lactation. The animals were mechanically milked once daily in the morning. The milk samples used for composition analysis were collected weekly from parturition to eight weeks of lactation. There was effect of weeks during the transition period and early lactation for fat yield (kg/day) and for body weight of the buffalo cows in lactation (kg).There was effect of weeks of lactation on the fatty acid composition of milk fat.Variations in levels of unsaturated fatty acids of milk fat of buffaloes are similar to those found in dairy cows of the partum to eighth week of lactation.

Keywords: Body weight, fatty acids, lactation, metabolism, milk yield.

INTRODUCTION

Because of its rusticity, buffaloes are well adapted to low fertility soils, swampy areas, being able to convert roughage into high value protein (meat and milk), with longevity and possibility to occupy geographic areas not suitable to other species ruminants.

Among the most relevant factors that support the growth of world buffalo population can to detach the chemical characteristics of milk and meat from buffalo, the greater resistance to parasitic infections, at the development of diseases commonly observed in cattle, reproductive tract infections and mammary gland, indigestion and docility to the production and work [1, 2].

The importance of milk production and its quality aroused interest by the species in many countries such as Italy, Venezuela, Bulgaria, and Brazil. Furthermore, milk production opens a range of options for the preparation of derivatives, because their products are not known, except the mozzarella cheese, which has a unique flavor and has absorbed most of the production of milk in the market.

It is believed that the buffalo are subject to the same diseases that affect cattle in the transition period [3]. However, that appreciation purposes of the products derived from buffaloes can be reached and used in its fullness, is important to know the reference values of specific fatty acids in healthy animals, well as the factors causing variations [4].

Furthermore, the evaluation of the fatty acid composition in milk from buffalo cows is of extreme importance to human health since high intakes of specific fatty acids such as C 18:2 cis 9- trans - 11 and C18: 1 trans-11 (Vacenic) can help in the diseases prevention and help in maintaining and regulating of metabolism in humans. One reason for studying these fatty acids is that the production of vacenic acid seems to be a limiting step in the sequence of biohydrogenation in the rumen, because this fatty acid can be accumulated in the rumen, becoming more available for absorption [5]. After absorption, the vacenic acid is used as substrate for endogenous synthesis of CLA C18:2 cis-9, trans-11, which involves the $\Delta 9$ desaturase enzyme. According to [6, 7], the endogenous synthesis of CLA C18:2 cis-9, trans-11 is more important than the ruminal synthesis, being responsible by 80% of CLA C18:2 cis-9, trans-11 in humans.

Thus, the objective of this study was to evaluate the fatty acid composition of buffalo milk in the first eight weeks of lactation.

MATERIAL E METHODS

We used 18 multiparous Buffalo cows of Mediterranean race, the data collection starting four

*Address corresponding to this author at the Department of Animal Nutrition and Production – School of Veterinary Medicine And Animal Science, University of São Paulo -Av. Duque de Caxias Norte, 225- CEP: 13630-000- Pirassununga - São Paulo, Brazil; Tel: +55 19 3565-4368; Fax: +55 19 35654300; E-mail: jeffersongandra@usp.br

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weeks before of the calving provided by the 8th week of lactation. The animals were kept under extensive system in *Brachiaria Decumbens* and supplemented daily during milking with 2.0 kg / day of concentrate.

The animals were mechanically milked once daily in the morning, by using the bucket system by foot and milk yield was recorded daily throughout the experimental period. The animals were weighed weekly from parturition to eight weeks of lactation.

The milk samples used for composition analysis were collected weekly from parturition to eight weeks of lactation. The analyses of fat content of milk were performed according to the method of separation of sulfuric acid in the Laboratory of Technology of Animal Products, Department of Nutrition and Animal Production.

Analyses of the fatty acid profile of milk fat were performed in the Laboratory of Animal Physiology and Biochemistry, Department of Nutrition and Animal Production of the Faculty of Veterinary Medicine and Animal Science. The milk samples used to evaluate the fatty acid profiles were obtained from a daily milking. For the extraction process, the samples were centrifuged at 17.800× g for 30 min at 4^oC and around 19.300 × g for 20 minutes at 4^oC, according to Feng *et al*. [8]. The separated fat (300-400 mg) was methylated and the methyl esters were formed according to [9]. Two internal standards C18:0 and C19:0 were used to correct the losses during the methylation process.

Fatty acids were quantified by gas chromatography (GC Shimatzu 2010 with automatic injection) using a capillary column SP-2560 (100 m × 0.25 mm of diameter with 0,02 mm of thickness, Supelco, Bellefonte, PA). The initial temperature was 70°C for 4 minutes (13°C/minute) until reaching 175ºC, holding for 27 minutes. Then a further increase of 4^oC/minute was started until 215°C, holding for 31 minutes. Hydrogen (H_2) was used as carrier gas with a flow of 40 cm/s. During the identification process were used four patterns: standard C4-C24 of fatty acids (Supelco ® TM 37), vacenic acid C18: 1 trans-11 (V038-1G, Sigma ®), CLA C18: 2 trans-10, cis - 12 (UC-61M 100mg), CLA and C18: 2 cis-9, trans-11 (UC-60M 100mg) (NU-CHEK-PREP ® USA).

Data were analyzed using PROC MIXED of SAS 9.1, (2004). Fixed effect in the model of weeks of lactation was considered. The means were extracted using the LSMEANS. The data of the concentrations of fatty acids in milk were also analyzed by means of polynomial regression, where levels were considered the first 8 weeks of lactation of the animals through the PROC REG of SAS 9.1, (2004).

RESULTS

There was effect of weeks during the transition period and early lactation for fat yield (kg/day) and for body weight of the buffalo cows in lactation (kg) ($P<0.05$; Table **1**). However, the values of MY, BWC and the percentage of milk fat were not affected by weeks of lactation.

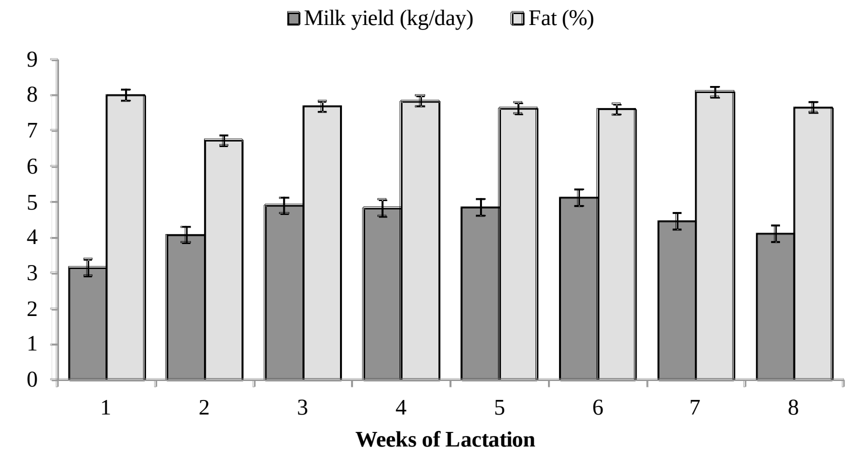
The higher values of milk fat yield (kg/day) were observed from the second week of lactation, and remained stable until the seventh week of lactation (Table **1**, Figure **1b**). When evaluating the values of C16 observed in this study it is possible verify that the concentrations of this fatty acid in the buffalos are approximately 20% larger as compared to the cow's milk (Figure **1a**).

There was effect of weeks of lactation on the fatty acid composition of milk fat for the C6:0,C8:0,C10:0,C12:0,C14:0,C15:0,C16:0,C17:0,C18:0, C18:3, >C16, <C16, satC18, unsatC18 and total of fatty acids satured and unsatured ($P>0.05$; Table **2**).

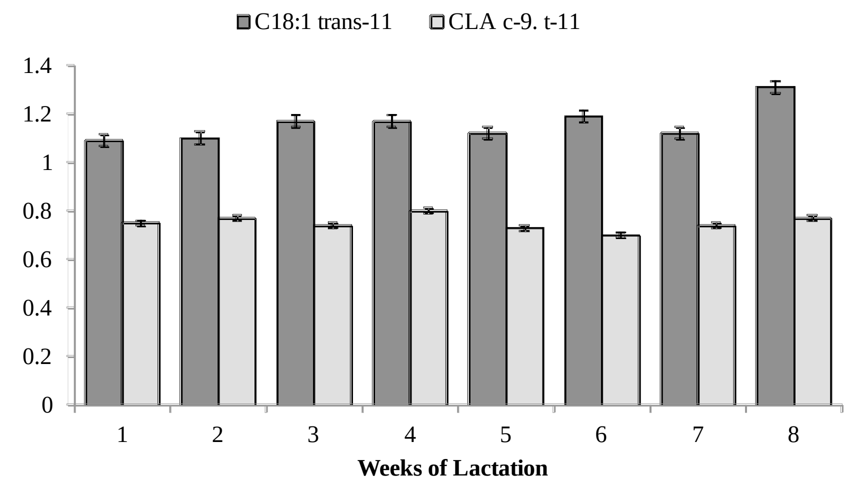
Table 1: Weekly Means for Milk Yield and Composition

Item	Weeks Post-partum								Means	P^2
	1	2	3	4	5	6	7	8		Time
MY(kg/d)	3,16	4,08	4,90	4,83	4,85	5,12	4,46	4,11	4.44	0,508
F (kg/d)	0,21	0,21	0,38	0,40	0,37	0,40	0,36	0,29	0.33	0,042
BW	711,38	704,13	692,06	687,69	685,69	688,94	683,13	685,07	692.26	<0,001
BWC	2,81	2,19	4,13	4,63	5,63	3,00	4,56	3,00	3.74	1,000
Fat %	8.00	6.73	7.68	7.83	7.63	7.61	8.09	7.65	7.65	0,341

[2]Probability of time fixed effect adjusted by PROC MIXED.



(**1a**)



(**1b**)

Figure 1: Milk yield and fat content (%) (**1a**). Fatty acid concentration C18:1 trans-11 and CLA c-9 t-11 (**1b**).

Table 2: Fatty Acid Profile of Milk Fat According to Weeks of Lactation

Item	Weeks Post-partum								P^2
	1	2	3	4	5	6	7	8	Time
g/100g of Total FA									
C4	1.68	1.60	1.74	1.69	1.69	1.65	1.65	1.78	0.235
C6	1.29	1.19	1.27	1.23	1.21	1.18	1.16	1.21	0.093
C8	0.73	0.67	0.71	0.67	0.65	0.64	0.63	0.66	0.037
C10	1.55	1.41	1.48	1.40	1.37	1.34	1.32	1.37	0.071
C12	2.14	1.98	2.06	1.96	1.89	1.89	1.87	1.95	0.030
C14	10.72	10.54	10.71	10.47	10.30	10.29	10.11	10.37	0.034
C15	0.92	1.00	1.02	1.04	1.04	1.10	1.09	1.06	0.004
C16	35.65	36.38	36.23	36.17	35.68	35.20	35.07	32.57	0.015
C16:1*cis*	1.96	1.95	1.99	2.08	2.05	2.14	2.04	1.93	0.531
C17	0.66	0.67	0.66	0.65	0.69	0.71	0.71	0.67	0.065
C18	9.71	9.89	9.85	10.44	10.50	10.61	10.85	10.32	0.005
C18:2 cis	0.60	0.61	0.58	0.58	0.62	0.60	0.66	0.60	0.733

(Table 2). Continued

Item	Weeks Post-partum								P^2
	1	2	3	4	5	6	7	8	Time
C18:3	0.23	0.20	0.20	0.18	0.18	0.17	0.17	0.19	0.005
C18:1*trans*-11	1.09	1.10	1.17	1.17	1.12	1.19	1.12	1.31	0.982
C18:1 *cis*-9	14.99	14.99	16.55	16.11	16.38	16.95	16.40	15.37	0.803
C20:0	0.07	0.08	0.08	0.08	0.09	0.09	0.09	0.07	0.188
CLA *c-9. t-11*	0.75	0.77	0.74	0.80	0.73	0.70	0.74	0.77	0.107
>C16	24.44	24.59	27.00	26.53	26.73	27.79	26.82	25.36	0.003
<C16	13.15	14.03	14.60	14.45	14.07	14.43	13.41	13.92	0.017
Total C18									
Sat. C18:0	6.51	6.66	7.32	7.28	7.27	7.66	7.35	6.81	0.024
Unsat. C18:0	16.63	16.61	18.32	17.86	18.09	18.72	18.10	17.24	0.013
Uns/Sat C18:0	0.40	0.41	0.41	0.41	0.41	0.41	0.41	0.36	0.957
Total									
Saturated	53.69	55.33	56.28	55.84	55.09	55.22	53.99	51.38	0.047
Unsaturated	19.51	19.64	21.50	21.27	21.36	22.16	21.28	20.43	0.035
Rel.Uns/Sat	2.79	2.88	2.70	2.66	2.62	2.52	2.58	2.31	0.195

[2]Probability of time fixed effect adjusted by PROC MIXED.

Analyzing the concentrations of fatty acids in milk fat over the weeks of lactation was observed decreasing linear effect to C6:0, C8:0, C10:0, C12:0, C14:0, C15:0 and C18:0. There was observed quadratic effect to C16:0, >C16, <C16, satC18, unsatC18 and total of fatty acids satured and unsatured (P>0.05; Table **3**).

DISCUSSION

Data of fat yield during lactation were presented of manner different of the results obtained in dairy cows, because in cows the milk yield is inversely correlated with fat content of milk reason explained by the greater dilution of the milk solids.

Table 3: Polynomial Regression of Fatty Acid Profile of Milk Fat According to Weeks of Lactation

Item	Equation	R^2	Critical values		Value of P	
			week(x)	fatty acid(y)	Linear	Quadratic
C6	Y=1.236-0.005X	0.60	-	-	0.034	0.234
C8	Y=0.689-0.005X	0.67	-	-	0.012	0.874
C10	Y=1.441-0.012X	0.66	-	-	0.029	0.670
C12	Y=1.999-0.010X	0.67	-	-	0.033	0.118
C14	Y=10.561-0.038X	0.71	-	-	0.001	0.654
C15	$Y=0.84+0.08X-0.005X^2$	0.74	8th	1.16	0.039	0.019
C16	$Y=34.87+0.94X-0.14X^2$	0.87	3th	36.43	0.567	0.006
C18	Y=9.723-0.126X	0.65	-	-	0.002	0.888
>c16	$Y=22.34+1.88X-0.18X^2$	0.68	5th	27.24	0.097	0.011
<c16	$Y=12.79+0.70X-0.08X^2$	0.35	4th	14.31	0.080	0.029
Sat. C18:0	$Y=5.82+0.64X-0.06X^2$	0.74	5th	7.52	0.613	0.008
Unsat. C18:0	$Y=15.28+1.17X-0.11X^2$	0.64	5th	18.38	0.346	0.001
Saturated	$Y=17.88+1.46X-0.14X^2$	0.73	5th	21.68	0.551	0.008
Unsaturated	$Y=52.08+2.05X-0.27X^2$	0.56	4th	55.96	0.123	0.017

This behavior occurred because in buffalos the fat content of milk always remains above 5.5%, which is a characteristic of particular species, regardless of experimental conditions. In Brazil the average levels ranging from 5.5% to 10.4%, with values around of 6.0% [10-13] similar to those observed in study abroad (6.6% to 8.4%) according to Nutritional Research Coincil [14] and Bovera *et al*. [15].

The time effect observed for the values of body weight of buffalo cows can be most clearly seen in the period between 1 and 8 weeks of lactation (Table **1**). In this period can also be observed higher milk yield, making clearer the partition of nutrients, especially energy for milk synthesis, demonstrating that there was mobilization of body reserves.

The fatty acids of milk fat have two origins: the synthesis de novo and the fatty acids from the bloodstream (dietary origin. 88%; or endogenous, 12%). The FA with until 14 carbons are formed from the synthesis de novo, and the palmitic acid is derived from two mechanisms, each representing approximately 50% [16]. According to Palmquist *et al*. [17], the long-chain fatty acids in the diet of ruminants have an effect on the synthesis de novo of the milk fat. The values of short-chain fatty acids and <C16.>C16 and total C18 unsaturated did not show marked variations. This result contradicts the physiological behavior observed in the fatty acid composition along of the weeks of lactation in dairy cows. According to Palmquist and Jenkins [18], the stage of lactation, and the seasonal effect represent the two major factors that alter the fatty acid profile in milk from dairy herds along of the lactation. Physiologically, in early lactation, dairy cows especially high-production remained in negative energy balance (NEB) for long periods, causing mobilization of long-chain fatty acids of the body reserves (adipose tissue). The long chain fatty acids mobilized present in the bloodstream in the form of non-esterified fatty acids (NEFA) and very low density lipoproteins (VLDL) are captured by the mammary gland and used for formation of milk fat.

Furthermore, the use of these compounds for synthesis of milk fat is directly related to its blood concentration [19]. Concomitantly, high blood concentrations of LCFA inhibit the synthesis de novo of SCFA in the mammary gland. Palmquist and Jenkins [18] evaluated the concentrations of the fatty acids C4. C6. C8. C10. C12. C14, C16 and C18 in the first twelve weeks of lactation in dairy cows. According to this author. two typical responses are expected when these physiological changes occur during early lactation: (1) the concentrations of SCFA in milk, except for C4 are low in early lactation, (2) inhibition of SCFA synthesis occurs through different variations related to the concentrations of fatty acids C:6 and C:12.

Palmquist and Jenkins [18] describes that the variations in fat content of milk reflect the concentrations of fatty acids of the milk fat. This result was clearly similar to that observed in this study, and can be analyzed through the proportions of SCFA and LCFA (<C16/C18) (Table **2**).

Polidori *et al.* [20] found that the buffalo milk contains about 33% of unsaturated fatty acids (UFA), or 67% of SFA. Similar results were observed by Oliveira *et al*. [21] for lauric and myristic acid, in cow milk. However, for palmitic acid (C16), the values were respectively 32 and 26%. i.e., the buffalo milk showed six percentage points more than the cow's milk.

The fat of buffalo milk differs of the cow's milk because it has higher values in the long chain unsaturated fatty acids as palmitoleic (C16:1) and linoleic (C18:2), but less fatty acid content myristoleic (C14:1) and oleic (C18:1). Furthermore there is a higher concentration of long-chain saturated fatty acids as palmitic (C16:0) and stearic (C18:0) [22, 23]. For content of short chain fatty acids in the fat of buffalo milk, the butyric acid (C4:0) presents lower value in relation to the fatty acids caproic (C6:0), caprylic (C8:0), capric (C10:0), lauric (C12:0) and myristic (C14: 0) compared to cow's milk fat. For levels of saturated and unsaturated fatty acids, except linoleic acid, the results are similar to those reported by Fonseca and Gutierrez [24].

CONCLUSION

Variations in levels of unsaturated fatty acids of milk fat of buffaloes are similar to those found in dairy cows of the partum to eighth week of lactation. However, higher concentrations of CLA in milk of buffalo cows bring greater benefits to human health due to higher amounts of fat in milk of these animals.

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