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Influence of fish oil in the concentration of conjugated linoleic acid and omega 6 and 3 in buffalo milk

[*Influência do óleo de peixe na concentração de ácido linoléico conjugado e Omega 6 e 3 no leite de búfalas*]

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ABSTRACT

The aim of this research work was to investigate the influence of fish oil supplementation on the concentration of conjugated linoleic acid (CLA) and omega 6 and 3 in samples of buffalo milk. A total of 24 female buffaloes separated at random into three groups were fed for 49 days with: natural pasture (group I), supplemented with 70mL of fish oil (group II) and 140mL of fish oil (group III). In the experiment the concentration of CLA showed differences (P<0.05) among the three groups, with a maximum of 7.14mg/g fat in group II. No significant differences were found in omega-6 among the three groups. The highest value of 3.82mg/g fat corresponded to group I, whicht had not been supplemented with fish oil. Significant differences were observed in omega 3 (P<0.05) in groups II and III with respect to group I. The highest average value of 2.42mg/g fat was obtained in group III. The closest relationship omega 6/3 (1.37:1) was observed in group III. As a result, the diets of groups II and III, which included fish oil, increased significantly the content of CLA and omega 3 with reductions in levels of omega 6.

Keywords: *Bubalus bubalis*, milk, CLA, Omega 6 and 3, fish oil

RESUMO

Estudou-se a influência da suplementação com óleo de peixe sobre a concentração dos ácidos graxos ômega 6 e 3, mostrado no leite de búfala. Foram utilizadas 24 búfalas aleatoriamente selecionadas e distribuídas em três grupos e alimentados com pastagem natural por 49 dias com: nenhum suplemento (grupo I), suplementado com 70mL de óleo de peixe (grupo II) e com 140mL de óleo de peixe (grupo III). A concentração de CLA diferiu (P<0,05) entre os três grupos, com valores máxima de até 7,14mg/g de gordura no grupo II. Não foram observadas diferenças significativas para os ácidos graxos omega 6, sendo o maior valor de 3,82mg/g de gordura no grupo I. Foi observada diferença significativa quanto aos ácidos graxos ômega 3 (P<0,05) nos grupos II e III em relação ao grupo I. O mais elevado valor, 2,42mg/g de gordura foi observado no grupo III. A relação ômega 6/3 mais estreita, 1,37:1, foi observada no grupo III. Os resultados mostram que os animais dos grupos II e III que receberam suplementação de óleo de peixe aumentou significativamente o teor de CLA e de omega 3, com diminuição de omega 6.

Palavras-chave: Bubalus bubalis, leite, CLA, ômega 6 e 3, óleo de peixe

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INTRODUCTION

Consumption of saturated fatty acids in milk and meat from ruminants is associated with an increased incidence of coronary heart disease in human beings (Menotti *et al*.,1999). The fat from ruminant products is frequently considered harmful to human health due to the high saturated fatty acid content. However, a fat component, the conjugated linoleic acid (CLA) contains anticancer and hypocholesterolemic properties and prevents arteriosclerosis and diabetes (National..., 1996). CLA is the term used to describe one or more positional and geometric isomers of linoleic acid (*cis-9, cis-12*, octadecadienoic acid), which contains double conjugated bonds. Such bonds are usually found in positions 9 and 11, or 10 and 12. They can be *cis or trans* configuration. The CLA biologically active form would be represented by the isomer *cis-9, trans-11* CLA (also called rumenic acid), representing 80 to 90% of total CLA in milk fat (Belury, 2002).

It has been shown that CLA inhibits the carcinogenesis onset in skin tumors, in tumors of the stomach and in mammary gland tumors, all of them induced experimentally with different carcinogens in mice (Ha *et al*., 1990; Ip *et al*., 1994). Hypocholesterolemic properties have been demonstrated in pilot studies with hamsters fed with a hypercholesterolemic diet and supplemented with CLA. This experiment showed a significant reduction in total cholesterol, LDL-cholesterol and triglycerides (Nicolosi *et al*., 1993). Hypocholesterolemic properties were also demonstrated in rabbits supplemented with CLA (Lee *et al*., 1994).

Linoleic and linolenic acids are essential fatty acids, synthesized by plants but not by mammals. These acids, therefore, must be supplied by food. They are the precursors for the synthesis of polyunsaturated fatty acids (PUFA) in the series omega 3 and omega 6, respectively (Gagliostro, 2004).

CLA precursors are the PUFAs present in the forage feed for ruminant as linoleic acid (*cis-9,trans-12* C18: 2) and α-linolenic acid. The former abounds in corn silage, cereals and various oilseeds such as sunflower and soybean. α-linolenic acid is present in greater percentage in green pasture and flax. (Gagliostro, 2004). The transformation of unsaturated fatty acids occurs in the rumen by bacteria of the genus *Butyrivibrio* through ruminal biohydrogenation, which can be improved by a strategic diet rich in unsaturated fatty acids. This improves the salubrity of milk and meat from ruminants due to higher CLA and omega 3 concentrations (Menotti *et al*., 1999; Chilliard *et al*., 2000).

Studies have shown that omega 3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic (DHA) have hypocholesterolemic, antitrombic and antiinflammatory properties in human beings (Williams, 2000). Moreover, experimental studies with laboratory animals indicate that the incidence of colon tumors is lower in animals fed with diets enriched with fish oil than in animals fed with a diet rich in saturated fat or vegetable oil (Reddy and Sugie, 1988; Rao *et al*., 2001).

Other research works have shown that diets enriched with fish oil decreased the development of colon tumors implanted in mice (Cannizzo and Broitman, 1989; Tisdale and Dhesi, 1990) and colon tumors in nude mice (Calder *et al*., 1998).

In human health the concept of the relationship omega 6/omega 3 is convenient to be used (Gagliostro, 2004). A lower ratio of omega-6/omega-3 fatty acids is more desirable in reducing the risk of many of chronic diseases of high prevalence in Western societies, as well as in developing countries (Simopoulos, 2002).

The main sources of CLA in human diets are milk and dairy products, which contain mainly *cis-9, trans-11* C18:2 (rumenic acid) and *trans-9, cis-11* C18:2. (Gagliostro, 2004).

Many factors can influence the increase of CLA in buffalo milk: seasonal factors (Talpur *et al*., 2008) and the number of lactations (Van Nieuwenhove *et al*., 2004) but in reality the animal's diet is what has greatest emphasis in increasing the CLA (Bergamo *et al.,* 2003; Secchiari *et al*., 2005; Patiño *et al*., 2010).

The research works carried out in buffaloes that determined CLA in milk in countries such as Italy (Fedele *et al*., 2001), Pakistan (Sechiari *et al*., 2005; Talpur *et al*., 2007; 2008), India (Tyagi *et al*., 2007), Brazil (Medeiros 2002; Oliveira *et al*., 2009; Caldeira *et al*., 2010) and Argentina

(Van Nieuwenhove *et al*., 2004; 2007; Patiño *et al*., 2008; 2010) obtained different values due to the different diets used.

This work aimed at investigate whether fish oil supplementation in the diet of buffaloes bred in the Province of Corrientes and fed on natural pastures modifies the CLA concentrations, the omega-3 and 6 fatty acids and whether that supplementation improves the omega 6/3 relationship in the milk.

MATERIAL AND METHODS

The animals used belong to a herd from a farm located in the town of San Cosme 35km from the city of Corrientes, Province of Corrientes, Argentina. 24 multiparous buffaloes of Murrah breed and Murrah x Mediterranean crossbred were used, identified with alphanumeric caravans, distributed in three groups, consisting of eight animals each. All animals were fed in natural pastures during the 49-day experiment. Control group I was fed only with natural pastures, group II was fed daily with 70mL of fish oil and group III with 140mL of fish oil. The animals supplemented with fish oil, made of 85% argentine haddock *(Merluccis hubbsi)* and 15% anchovy *(Anchoa marinii),* (Table 1) received the ration at milking time in an individual feeder to meet the assumption of independence.

Table 1. Fish oil composition

Fatty acids composition	(%)
Alfa linoleic 18:3 n-3	1.10
Estearidonic acid 18:4 n-3	2.58
Eicosatrienoic acid 20:3 n-3 y n-6	0.16
Arachidonic acid 20:4 n-3	0.62
Eicosapentaenoic acid EPA 20:5 n-3	7.18
Eneicosapentaenoic acid 21:5 n-3	0.23
Docosapentaenoic acid DPA 22:5 n-3	0.70
Docosahexaenoic acid DHA 22:6 n-3	16.47
Fatty acids n-3	29.04
Fatty acids n-6	3.52
PUFAs	32.56
Free fatty acids	0.43
Other	5.41

The natural pasture was composed mainly by species such as *Andropogon lateralis, A. sellononaus, Cynodon dactylon, Elionorus sp., Paspalum notatum, P. Almuna chase, Sorghastrum agrostoides, Desmodium canum* and *Shylosanthes macrosonics.*

The buffaloes were milked by hand in the morning. The samples (n= 24) were obtained in the beginning and in the end of day 49 in the experiment carried out between May and July 2009 during the second stage of lactation of the experimental herd. During the milking routine and after discarding the first streams, samples of 200mL of milk were taken from each animal. The samples were packed in disposable containers, frozen at -20°C and kept in polyurethane boxes until they were taken to the laboratory.

Each sample was processed in duplicate to obtain the lipid profile. To obtain the total lipids, a mixture of chloroform and methanol according to the Bligh and Dyer technique (1959) was used. The conversion of fatty acids in methyl esters was carried out with methanol NaOH and BF_3 at 14% boiling for 8 minutes. The methyl esters were obtained with hexane and analyzed with a gas chromatograph. Standards of methyl esters of 99% pure fatty acids (Lipid Standard 189-19 Sigma-Aldrich) were used. The fatty acid composition was determined in an Agilent gas chromatograph equipped with a capillary column 60mm long and 0.25mm of internal diameter (Supelco 2340) and a flame ionization detector. The gas chromatography method used (GC-FID) met the ISO 15304 standard (2010).

The dependent variables studied were: conjugated linoleic acid (CLA) rises from quantifying the conjugated linoleic acid isomers with double bonds: CLA: 18:2, 9c, 11t; CLA: 18:2, 12c, 10t ; CLA: 18:2, 10c, 12c.

Omega 6, composed by the following fatty acids: C 18:2 n-6 t linolelaidic acid; C 18:2 n-6 c; linoleic acid; C 18:3 n-6 gama linolenic acid; C 20:2 n-6 eicosadienoic acid; C 22:2 n-6 docosadienoic acid; C 20:3 n-6 eicosatrienoic acid; C 20:4 n-6 arachidonic acid.

Omega 3 composed by the following fatty acids: C 18:3 n-3 alfa linolenic acid; C 20:3 n-3 eicosatrienoic acid; C 20:5 n-3 eicosapentaenoic acid; C 22:6 n-3 docosahexaenoic acid.

Descriptive statistics was applied to assess the sample estimates for each treatment (mean, standard deviation, coefficient of variation and minimum and maximum ranges). Prior to analysis, the performance description of the

sample using confidence intervals and box & whisker charts was used. The basic assumptions of the analysis of variance, consistency and standardization, were also checked. In order to infer the effects of the diet, a randomized design with the linear model was carried out. The calculations were performed with the help of the Infostat software (Infostat..., 2009), owned by the School of Veterinary Sciences of the *Universidad Nacional del Nordeste.*

RESULTS AND DISCUSSION

The CLA milk content of buffaloes fed with the three diets is shown in Table 2. On day 49 there were significant differences (P<0.05) among the three groups (Table 2). The highest average value of CLA (7.14mg/g fat) was obtained from the buffaloes belonging to group II. Group II had an increase of 86.42% compared to Group I. In contrast group III, which received more oil supplementation, had a significant increase of 59.8% compared to group I. However, it was not higher than group II fed with less than half of the fish oil.

It has been shown that fish oil in the diet of ruminants is very effective to increase CLA fatty acids and omega 3 in milk fat.

Table 2. CLA content (mg /g fat) in milk of buffaloes of different groups on day 49

Groups	n	Mean	SD	CV	Min	Max	Dif
I	8	3.83	1.14	29.28	2.27	5.13	a
II	8	7.14	1.78	24.97	4.97	10.23	b
III	8	6.12	1.67	27.32	3.55	7.54	c

SD: standard deviation, CV: coefficient of variation; Min and Max: ranges; Dif: difference (different letters indicate significant differences between mean, P<0.05).

In the present experiment it was observed that the increase of the CLA fatty acids and Omega 3 was related to the incorporation of the fish oil in the diet provided. However, a higher increase of these fatty acids was obtained when using low amounts of fish oil in the diet. This is due to the process of biohydrogenation in the rumen, which causes a toxic effect when the amount of fish oil is exceeded. The amount of unsaturated fatty acids provided in the diet has a toxic effect on the microbial metabolic activity (CLA producer), mainly on the *Butyrivibrio fibrisolvens* microorganism with the consequent decrease in the production of CLA fatty acid (Maia *et al*., 2010).

The highest average value of CLA obtained in this experiment, was higher than those recorded in Brazil by Oliveira *et al*. (2009) with diets based on corn and soybean (5.08 to 6.91mg/g fat). However, this value was lower in diets with soybean oil, (10.80mg/g fat) and in buffaloes fed only with Brachiaria grass *(Brachiaria decumbens)* the value was 11.0mg/g fat (Caldeira *et al*., 2010). Values were also lower than those obtained in India by Tyagi *et al*. (2007) in Murrah buffaloes with concentrated diets (7.7mg/g fat), concentrates plus clover *(Trifolium alexandrium)* (13.4mg/g fat) and entirely clover (17.0mg/g fat).

The omega-6 fatty acid content in the milk of buffaloes fed with the three diets is shown in Table 3. There were no significant differences among the three groups. The highest value was 3.82mg/g fat in group I, a group not supplemented with fish oil. The highest average value of omega-6 found in this research work (3.82mg/g fat) is lower than the average value quoted in Brazil (7.2mg/g fat) by Caldeira *et al*. (2010) and below the average value recorded in India (16.4mg/g fat) by Tyagi *et al*. (2007) with a diet based on clover.

Table 3. Omega 6 (mg/g fat) in milk of buffaloes from different groups on day 49

Groups	N	Mean	SD	CV	Min	Max	Dif
I	8	3.82	0.80	20.21	2.34	4.45	a
II	8	3.28	1.08	32.84	1.21	4.43	b
III	8	3.31	0.78	23.72	1.96	4.28	c

SD: standard deviation, CV: coefficient of variation; Min and Max: ranges, dif: difference (different letters indicate significant differences between mean, P<0.05).

The omega-3 fatty acid content of the three diets is shown in Table 4. Significant differences (P<0.05) between groups II and III when compared to group I were found. The highest average value (2.42mg/g fat) was obtained in Group III with an increase of 56.1% compared to Group I. Group II (2.30mg/g fat) showed an increase of 48.4% compared to Group I. The omega-3 highest average value found in our experiment (2.42mg/g fat) was below the average obtained in India (14.2mg/g fat) by Tyagi *et al*. (2007) with the diet based on clover. This shows the importance of green pasture in the increase of fatty acid concentration. Due to the results obtained in our experiment and when compared with other authors we can infer that the best values are obtained with implanted pastures, but acceptable results are also achieved with green natural pastures.

The omega -6/-3 relationship in buffalo milk is shown in Table 5. Diets supplemented with fish oil improved the omega -6/-3 relationship. The closest relationship was that of group III (1.37:1) at the end of the experiment. In India, Tyagi *et al*. (2007) achieved an omega -6/-3 relationship of 1:1 with the diet based on clover.

Table 4. Omega 3 (mg/g fat) in milk of buffaloes from different groups on day 49

Groups	Nº	Mean	SD	CV	Min	Max	Dif
I	8	1.55	0.73	20.93	0.99	1.87	a
II	8	2.30	0.43	18.57	1.60	2.98	b
III	8	2.42	0.73	30.12	1.19	3.09	b

SD: standard deviation, CV: coefficient of variation, Min and Max: ranges, Dif: difference (different letters indicate significant differences between mean, p <0.05).

Table 5. Ratio of omega 6/3 (mg/g fat) in milk from buffaloes of different groups

Groups	Day 0			Day 49		
	6 Ω	3 Ω	Relation: Ω 6/3	6 Ω	3 Ω	Relation: Ω 6/3
I	1.83±1.01	0.78±0.32	2.35:1	3.82±0.80	1.55±0.34	2.46:1
II	4.23±0.68	2.63±0.23	1.61:1	3.28±1.08	2.30±0.43	1.43:1
III	1.93±0.71	0.95±0.33	2.03:1	3.31±0.78	2.42±0.73	1.37:1

CONCLUSIONS

The fish oil used in the present experiment increased significantly (P<0.05) the CLA and omega -3 content in milk of buffaloes and improved the omega -6/-3 relationship. In our region, where the animals are fed with nutritionally deficient natural pastures, supplementation with fish oil is an excellent strategy to increase the values of fatty acids. The possibility of increasing the concentration of these fatty acids through strategic supplementation, which is cost-effective, easily implemented and managed and does not alter the organoleptic properties of milk, is the challenge of research in the area of nutrition and technology of food.

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