Influence of Fish Oil Supplementation on the Concentration of Conjugated Linoleic Acid (CLA) and Omega 6 and 3 in Buffalo Milk

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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 70 ml 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 7.14 mg/g fat in group II. No significant differences were found in omega-6 among the three groups. The highest value of 3.82 mg/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*, CLA, fish oil, milk, omega 6 and 3

INTRODUCTION

The CLA is the term used to describe one or more positional and geometric isomers of linoleic acid (*cis-9, cis-12*, octadecadienoic acid) that 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

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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 must be therefore 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, that can be improved by a strategic diet rich in unsaturated fatty acids. This improves the salubrity of milk and meat from ruminants due to a higher CLA and omega 3 concentration (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). In human health the concept of 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 source of CLA in human diet are milk and dairy products, that contain mainly *cis-9, trans-11* C18:2 (rumenic acid) and *trans-9, cis-11* C18:2. (Gagliostro, 2004). This work aimed at investigating 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. This paper is a summary of an earlier publication in a specialized journal.

MATERIALS AND METHODS

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

The buffaloes were milked by hand in the morning. The samples (n = 24) were obtained at the beginning and at the 49-day of the experience 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 200 ml 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 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 60 mm long and 0.25 mm of internal diameter (Supelco 2340) and a flame ionization detector. The gas chromatography method used (GC-FID) met the ISO 15304 standard (2010).

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

RESULTS AND DISCUSSIONS

In the experiment the concentration of CLA showed differences ($P<0.05$) among the three groups, with a maximum of 7.14 mg/g fat in group II. No significant differences were found in omega-6 among the three groups. The highest value of 3.82 mg/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 2.42 mg/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.

In our experience we have observed that the increase of the CLA fatty acids and omega 3 is related to the incorporation of the fish oil in the diet provided. However, a greater 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 that 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).

CONCLUSIONS

Taking into account our work conditions, the fish oil used in the present experience 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 animals are fed with nutritionally deficient natural pastures, supplementation with fish oil is an excellent strategy to increase the values of fatty acids. Milk and its derivatives with higher CLA and omega -3 concentrations together with a close relationship between omega -6 and -3, give us a product that has nutritional, anticancer, hypocholesterolemic as well as antitrombotic properties having a big in impact human health. The possibility of increasing the concentration of these fatty

acids through strategic supplementation, that 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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