Fatty Acids in the Meat of Buffaloes Supplemented with Fish Oil#

J.F. Cedres[1,*], E.M. Patiño[1], M.A. Judis[2], M. Sanchez Negrette[1], A.M. Romero[2], M.M. Doval[2], G. Rebak[1] and G.A. Crudeli[1]

[1]*Facultad de Ciencias Veterinarias, Universidad Nacional del Nordeste (UNNE), Sargento Cabral 2139, Corrientes (3400), Argentina*

[2]*Departamento de Ciencias Básicas y Aplicadas. Universidad Nacional del Chaco Austral (UNCAus). Presidencia Roque Sáenz Peña, Chaco, Argentina*

Abstract: The purpose of this study has been to investigate the influence of both a supplementary fish oil diet on conjugated linoleic acid (CLA) and n6 and n3 fatty acids on intramuscular fat in Mediterranean buffalo meat. Twenty animals were randomly divided into two groups and fed with *Brachiaria brizantha,* 3Kg rice bran, 500 g corn and 500 g sunflower pellets for 60 days. Group I received this diet only while in group II each animal received additional 100 ml fish oil daily. Results indicated a significant decrease of palmitic fatty acid in group II (232.67 mg/g fat) in relation to group I (254.73 mg/g fat). Among unsaturated acids (AGI), the 9c 11t CLA value of group II (21.23 mg/g fat) showed an increase in relation to group I (15.80 mg/g fat), while the linoleic acid content of group II (28,85 mg/g fat) decreased significantly in relation to group I (47,00 mg/g fat). However, the alpha linolenic acid showed no significant difference between the supplemented diet group and the control group (10.31 and 10.70 mg/g fat, respectively). Group II n6/n3 ratio was narrower (2.69:1) than that of group I (4.55:1). Summing up, group II diet, which included fish oil, increased the CLA content in intramuscular fat and decreased the n6 fatty acids, improving the n6/n3 ratio.

Keywords: Buffalo, meat, CLA, n 6 and n 3, fish oil.

INTRODUCTION

For some time to date, products including substances with healthy effects for the prevention and treatment of different diseases in their composition, or incorporating higher amounts than those naturally present, are increasingly being introduced in the food market. These products are called functional or nutraceutical food [1-12].

It is well-known that fat in bovine food products, both in milk and meat, is in many cases considered unhealthy due to their high content of saturated fat acids (SFA). But, in the last years, it has been found that the conjugated linoleic acid (CLA) has anti-carcinogenic properties [8] apart from its lipolytic and hypocholesterolemic activity, which prevent arteriosclerosis and diabetes [4]. Hence, ruminant food products are one of the major sources of CLA for human beings, with a potential significance in functional food production [13-19].

CLA is a natural substance typical of ruminant fat tissue formed in rumen as an intermediary, resulting from the linoleic acid bio-hydrogenation, by the isomerase linoleic acid, produced by *Butyrivibrio* anaerobic bacteria among others, which transforms said fat acid in 9-cis 11-trans isomer [10].

Milk and meat, which primarily contain 9-cis, 11-trans C18:2 (rumenic acid) and 9-trans, 11-cis C18:2, are the main sources of ruminant CLA, and 9-cis contributes with 60% of total CLA in muscle [7].

Current research in MERCOSUR determined CLA content and fatty acid profiles in Brazilian cattle buffaloes [9], while in Argentina only buffalo meat yield and nutritional value were studied [17].

In Argentina, buffalo population is estimated in 100,000 animals found mainly in the North-East (NEA) wet subtropical region, in the Provinces of Corrientes, Chaco, Formosa and Misiones, and Northern Santa Fe. Of all these provinces, Corrientes has the largest number of animals – approximately 45,000. In Tucumán, Buenos Aires, Entre Ríos, San Luis, La Pampa, Salta, Santiago del Estero and Mendoza, buffaloes are found in a smaller number. The main purpose for breeding buffaloes in Argentina is meat, and it is to point out the sale of buffalo calves at weaning or as heavy 30-month-old bubillo buffalo [15, 16].

The purpose of this paper has been to research whether the bubillo buffalo fed with *Brachiaria brizantha* strategically supplemented by a fish oil portion can increase the CLA content and modify the n 6/n 3 fatty acid ratio in intramuscular fat.

*Address correspondence to this author at the Facultad de Ciencias, Veterinarias, Universidad Nacional del Nordeste (UNNE), Sargento Cabral, 2139, Corrientes (3400), Argentina; Tel: 54-379-4425753;
E-mail: cedres@vet.unne.edu.ar

#Work sponsored by the National Agency for Scientific and Technological Promotion ANPCyT) PICTO-UNNE Nº 119 and the SGCyT of the UNNE.

ISSN: 1927-5196 / E-ISSN: 1927-520X/13

MATERIALS AND METHODS

Twenty bubillos from Florencia ranch, located at General Paz, Province of Corrientes, Argentina, were used. The animals were castrated at weaning (6 month old) and were 11- month-old average age at the time of the experiment. They were all Mediterranean race, identified in caravans and randomly distributed in two groups of 10 animals each. All the animals were fed with *Brachiaria brizantha* and a daily portion of 3Kg rice bran, 500 g corn and 500 g sunflower during the 60 days of the experiment. Animals in group I were fed with *Brachiaria brizantha* plus the daily portion of rice bran, corn and sunflower while animals in group II were similarly fed but received additional supplement of 100 ml fish oil. The liquid fish oil in the diet was partially accepted by the bubillos during the first days of the experiment, probably due to the limited adaptation period they had to this oil supplement.

The poly-unsaturated fatty acids (PUFA) composition of the fish oil used in the diet contained 85% Argentine hake (*Merluccis hubbsi*) and 15% anchovy (*Anchoa marinii*), as shown in Table **1**.

Table 1: Fish Oil Composition

Fatty acid composition	Proportion (%)
Alpha linoleic acid 18:3 n-3	1.10
Stearidonic acid 18:4 n-3	2.58
Eicosatrienoic acid 20:3 n-3 and 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
Docosapentanoic acid DPA 22:5 n-3	0.70
Docosahexaenoic acid DHA 22:6 n-3	16.47
Total fatty acids n-3	29.04
Total fatty acids n-6	3.52
Total free poly-unsaturated fatty acids	32.56

The bubillos received the diet portion and the fish oil in individual feeding boxes so as to give them independence.

Samples (n=20), one per animal, were taken from the *Longissimus dorsi* muscle, located between the 10th and 11th rib and were collected on day 60 of the experiment, between February and March 2011. The samples were stored in disposable containers, frozen at -20ºC, and kept in polyurethane boxes till they reached the laboratory.

To obtain the lipid profile each sample was processed twice. To extract the total lipids a mixture of chloroform and methanol was used, following the technique [14] and keeping the nitrogen atmosphere. Fatty acids conversion into methyl esters was done using NaOH and 14 % methanolic BF3, and boiling them for 8 minutes. The methyl esters were extracted with hexane and analyzed with a gas chromatograph. Methyl esters standards of 99% purity fatty acids (Lipid Standard 189-19 Sigma-Aldrich) were used. The fatty acid composition was obtained in an Agilent gas chromatograph equipped with a 60 mm long and 0.25 mm internal diameter capillary column (Supelco 2340), and a flame ionization detector. The gas chromatograph method used (GC-FID) was adjusted to ISO 15304 norm [3].

In order to make an evaluation of the sample estimators for each treatment (average, standard deviation, variation coefficient, and minimal and maximal range) a descriptive statistics was applied. Prior to the analysis the descriptive behavior of the sample was evaluated applying confidence intervals and box & whisker graphs. In addition, the basic assumptions of variance analysis - homogeneity and normality - were checked. In order to make differences upon the diet effects a randomized supplementary design, using the ($X_{ij} = \mu + \tau_j + e_{ij}$) additive lineal model, was carried out. Calculations were done using Infostat (2009) software of the School of Veterinary Sciences of the National University of the North East.

RESULTS AND DISCUSSION

Fatty acid compositions of intramuscular fat of buffalo's meat are shown in Table **2**. Regarding the palmitic acid content, although no differences were significant, the average value reached for buffaloes fed fish oil supplemented (group II) was lower (189.05 mg/g fat) with regard to the results of group I diet fed without fish oil (206.97 mg/g fat). This result is of a great significance since palmitic acid is believed to have hypercholesterolemic properties.

The content of fatty acids in buffaloes fed supplemented diet was lower to than that found in Brazil [9] (244.0 and 255.5 mg/g fat) when Murrah buffaloes (15-month-mean-age) when fed with diets including soybean oil and integral soybean grains, respectively. But the mean value for control group (I) of Corrientes was higher than that of buffaloes with no additional lipid diets in Brazil (250.4 mg/g fat).

Table 2: Fatty Acid Profile (mg/g fat) in Intramuscular Fat in both Groups

Fatty acids		Group I (fodder)			Group II (fish)			CV (%)
		n	mean	EE	n	mean	EE	
Saturated	(14:0) Myristic	10	15.48[a]	1.54	10	16.98[a]	1.54	27.56
	(15:0) Pentadecanoic	4	1.72[a]	0.38	6	2.23[a]	0.31	16.98
	(16:0) Palmitic	10	206.97[a]	11.20	10	189.05[a]	11.20	17.71
	(17:0) Heptadecanoic	10	13.82[a]	1.49	10	14.34[a]	1.49	27.73
	(18:0) Stearic	10	299.42[a]	32.83	10	293.83[a]	32.83	27.24
	(20:0) Arachidic	5	2.28[a]	0.50	7	3.32[a]	0.42	23.47
Unsaturated M	(16:1) Palmitoleic	10	17.67[a]	1.55	10	17.46[a]	1.55	23.74
M	(17:1) Heptadecenoic	10	7.59[a]	0.80	10	6.29[a]	0.80	32.38
M	(18:1) n9 t Vaccenic	9	9.14[a]	0.25	10	14.13[a]	0.25	49.27
M	(18:1) n9 c Oleic	10	366.22[a]	23.55	10	308.44[a]	23.55	21.57
P	(18:2) n6 c Linoleic	10	38.19[a]	4.72	10	23.45[b]	4.72	46.64
P	(18:3) n3 Alpha Linolenic	10	8.37[a]	0.94	10	8.69[a]	0.94	32.64
P	(18:2) 9 c,11t CLA	9	11.56[a]	2.23	10	17.09[a]	2.36	46.30

M: mono-unsaturated, P: poly-unsaturated, EE: standard error, CV: variation coefficient; difference: (different letters indicate significant differences among mean p <0.05.

Regarding 9c 11t CLA (18:2) content, the group II, which included fish oil in the diet showed an increase in CLA (17.09 mg / g fat) values compared to group I (11.56 mg / g fat). The high CLA content found in this study was greater than that obtained in Brazil [9], which presented a 2.20-4.60 mg/g fat variation, the highest value being that of the soybean oil supplemented diet. The 1.27 mg/g fat values found in Venezuela [5], who worked with Murrah × Mediterranean cross buffaloes, fed with unsupplemented *Brachiaria sp,* were also higher.

The CLA content increased in the *Longissimus dorsi* muscle fat in animals fed fish oil supplement is of great significance due to the beneficial effects its consumption may produce on human health for anti-obesity, anti-oxidizing and anti-carcinogenic properties among others [20].

At the end of the experiment the n6 fatty acid contents (linoleic acid 18:2 n6) for group II, supplemented with fish oil, were 23.45 mg/g fat, significantly lower ($p < 0.05$) than the average values found for group I (38.19 mg/g fat). These results were lower than those reported in Brazil [9] who found values between 40.7 and 51.5 mg / g fat where the highest value was obtained from diet with soybean oil.

The n3 acid contents found (18:3 n3) were 8.37 and 8.69 mg fat/ g for group I and group II, respectively, not showing significant differences ($p < 0.05$) between the groups. These values were higher than those reported in Brazil [9], who found 3.5 and 4.4 mg/g fat, where the highest value was obtained from diet free of lipid supplement.

In our experiment the reduction of n6 fatty acids concentration allowed for a much narrower ratio between n6 and n3 acids, which is more adequate for the prevention of certain degenerative diseases. The n6/n3 ratio for intramuscular fat of group II (2.49:1) was significantly increased than that obtained for group I (1.89:1), ($p < 0.05$); but was even lower than the ratio found in Brazil [9], who obtained 14.82: 1 to 19.03: 1 values.

The proportions of saturated, unsaturated, monounsaturated and polyunsaturated fatty acids for each group are illustrated in Table **3**.

Table 3: Fatty Acid Composition Percent in the Intramuscular Fat of Both Groups

Fatty acids	Group I (pasture)	Group II (fish)
Saturated	55.92	58.08
Unsaturated	44.08	41.92
Monounsaturated	39.29	36.43
Polyunsaturated	4.79	5.49

Regarding the unsaturated fatty acids (AGI) / saturated fatty acids (AGS) ratio, in group I was 0.87

while in group II was 0.81. These values were lower than those reported by other authors, who found values ranging from 0.1 and 1.00 [9], and 1.16 [11] in Brazil; while in Italy, a mean 1.27 value was obtained with Mediterranean buffaloes [2].

Since there is a direct correspondence between AGS content and blood cholesterol levels in human beings [18], this AGI /AGS ratio is of great interest for the health area. The great difference found in fatty acid profile in our study and the results found in Brazil [9] can be due to the fact that the bubillos of the Province of Corrientes (Argentina) were fed in fields with *Brachiaria brizantha* pasture supplemented by fish oil, while the Brazilian animals were kept within a limited area and fed a diet supplemented with soybean grains and oil.

CONCLUSSION

In our working conditions, the fish oil supplemented in the diet of bubillos increase the CLA levels in intramuscular fat and decreased n6 fatty acid values, hence decreasing the n6/n3 ratio. In our region, where animals are fed with nutritionally deficient natural pasture, the use of implanted pastures such as *Brachiaria brizantha* as well as fish oil supplement is an excellent strategy to increase fatty acid values.

Further research is needed in which the duration of the diet supplemented with fish oil should be lengthened. This would permit to detect the most appropriate period of time for the implementation of such diet. Besides, the challenge in feeding and nutritional technology is that it has to be economically profitable and easy to administer, without altering the organoleptic properties of meat fat.

ACKNOWLEDGEMENTS

"Florencia" farm in General Paz, Corrientes province, Argentina because they provided the animals and the infrastructure to carry out the experiment. Thanks to Omega Sur from Batan, Mar del Plata, Buenos Aires province, Argentina, who gave us the fish oil.

REFERENCES

[1] Hernandez BC, Serna SS. Alimentos Nutraceúticos el Futuro de Nuestra Alimentación. Revista Digital de Posgrado, Investigación y Extensión del Campus Monterrey, México 2003; 61: 1-5.

[2] Infascelli F, Gigli S, Campanile G. Buffalo meat production: performance infra vitam and quality of meat. Vet Res Commun 2004; 28: 143-48.

[3] ISO 15304. Animal and vegetable fats and oils-Determination of the content of trans fatty acid isomers of vegetable fats and oils – Gas chromatographic method 2002.

[4] Mcguire MA, Mcguire MK. Conjugate Linoleic Acid (CLA): A Ruminant Fatty Acid with Benefical Efects on Human Health. Proc Am Soc Anim Sci 1999; 1-8. Avaiable at: http://www.asas.org./jas/symposia/procedings

[5] Mendoza MG, Moreno LA, Huerta-Leidenz N, Uzcantegui-Bracho S, Beriain IN MJ, Smith GC. Ocurrence on conjugated linoleic acid in longissimus dorsi muscle of water buffalo (*Bubalus bubalis*) and zebu.type cattle raised under savannah conditions. Meat Sci 2005; 69: 93-100.

[6] Milner JA. Funcional Foods and Health Promotion. J Nutr 1999; 129: 1395-97.

[7] Monson FD. Suplementación con Lípidos en Bovinos de Carne: Metabolismo, Efectos sobre la Calidad de la Canal, de la Carne y sobre la Salud Humana. Tesis Doctoral. Instituto Mediterráneo de Zaragoza. España 2004; p. 110.

[8] NATIONAL Research Council (NRC). Carcinogenesis and Anticarcinogenesis in the Human Diet. National Academy Press 1996; p. 242.

[9] Oliveira RL, Ladeira MM, Barbosa MAAF, Assuncao DMP, Matsushita M, Santos GT, Oilveira RL. Acido linoleico conjugado e perfil de ácidos graxos no musculo e na capa de gordura de novilhos bubalinos alimentados com diferentes fontes de lipídos. Arq Bras Med Zootec 2008; 60: 169-78.

[10] Parodi PW. Conjugated Octadecaienoic Acids of Milk Fat. J Dairy Sci 1977; 60: 1550-53.

[11] Rodrigues VC, Bressan MC, Cardoso MG, Freitas RT. Ácidos graxos na carne de búfalos e bovinos castrados e inteiros. Rev Bras Zootec 2004; 23: 434-43.

[12] Sanhuza JC, Nieto KS, Valenzuela AB. Acido Linoleico Conjugado: Un Acido Graso con Isomeria Tras Potencialmente Beneficioso. Revista Chilena de Nutrición 2002; p. 11.

[13] Bauman DE, Baumgard LH, Corl BA, Griinari JM. Biosynthesis of Conjugated Linoleic Acid in Rumiants. Proc Am Soc Anim Sci 1999; 4: 1-11.

[14] Bligh EG, Dyer WJ. A rapid method for total lipid extraction and purification. Can J Biochem Physiol 1959; 37: 911-17.

[15] Cedres JF, Crudeli GA, Patiño EM, Rebak GI, Bernardi A, Rivas PA, Barrientos GJ. Composición Química y Características Físicas de la Carne de Búfalos Criados en Forma Extensiva en la Provincia de Formosa. Actas Reunión de Comunicaciones Científicas y Tecnológicas. Secretaria General de Ciencia y Técnica. UNNE. Resistencia, Chaco. Argentina 2000a.

[16] Cedres JF, Bernrardis A, Rebak GI, Patiño EM, Crudeli, GA, Rivas PA. Composición Química y Valor Nutricional de la Carne Fresca de Búfalo (*Bubalus bubalis*) Criado en Forma Extensiva en el Nordeste Argentino. Actas XXIII Sesión de Comunicaciones Científicas. Fac. Cs. Veterinarias. UNNE. Corrientes. Argentina 2000b.

[17] Cedres JF. Rendimiento carnicero del búfalo. En el libro Búfalos en Argentina pags. 81 a 113. Editado por Moglia S.R.L. Corrientes, Argentina 2004; p. 230. ISBN Nº 987-43-7388-1.

[18] Farfan JA. Alimentos que influenciam os níveis de colesterol no organismo. In: Seminário Colesterol: análise, ocorrência, redução em alimentos e implicações na saúde, [s.n.]. Campinas. Anais. Campinas: ITAL 1996; pp. 35-44.

[19] Gagliostro GA. Manejo Nutricional para la Producción de Leches de Vaca y de Cabra con Alto Impacto sobre la Salud Humana. Area de Investigación en Producción Animal. INTA. Unidad Integrada Balcarce. Estación Experimental Agropecuaria. Fac.de Ciencias Agrarias / UNMdP 2004; p. 84.

[20] Park Y. Conjugated linoleic acid (CLA): Good or bad trans fat? J Food Composit Anal 2009; 225: 54-62.

Received on 20-02-2013 Accepted on 10-05-2013 Published on 18-06-2013

DOI: http://dx.doi.org/10.6000/1927-520X.2013.02.02.9