SEX INFLUENCE ON INTRAMUSCULAR LIPID COMPOSITION AND NUTRITIONAL VALUE OF BARROSÃ-PDO VEAL

P. Costa[1], L.C. Roseiro[1], A. Partidário[1], V. Alves[2], R. J. B. Bessa[3], C. R. Calkins[4], C. Santos[1]

[1]*Instituto Nacional de Engenharia, Tecnologia e Inovação, DTIA, Estrada do Paço do Lumiar, 1649-038 Lisboa*
[2]*Universidade de Trás-os-Montes e Alto Douro, Quinta dos Prados-Apartado 202, 5001 Vila Real*
[3]*Estação Zootécnica Nacional - INIAP, Fonte Boa, 2005-048 Vale de Santarém, Portugal*
[4]*University of Nebraska, Department of Animal Science, Lincoln, Nebraska, USA.*
Paulo.Costa@mail2.ineti.pt

Introduction

Estimated in about 7500 dams, Barrosã effective is one of the most important among the autochthonous cattle breeds in Portugal. Produced by more than 2000 small farmers in the Norwest region of the country, these animals show medium/low growth rate and an appreciable propensity to deposit precociously intramuscular fat. The Barrosã-PDO (Protected Denomination of Origin) veal is obtained from non weaned and non castrated calves, raised close to their mothers, suckling and feeding on pasture according to a traditional grazing system and slaughtered from 5 to 9 months old in agreement with European Union regulation (Commission Regulation nº 1263/96, EC). Barrosã-PDO veal is highly appreciated by consumers due to its unique sensory quality and the natural production system applied.

Meat supplies high-quality and readily digested protein and energy and represents the main source of highly available iron in the diet (Lombardi-Boccia *et al.*, 2002). However, epidemiological and clinical studies have suggested that its consumption is related to certain diseases such as obesity, coronary heart diseases and cancer (Chizzolini *et al.*, 1999). On this concern, cholesterol level and fat composition of meat and their effects on human health have also been largely reported (Enser *et al.*, 1998; Laborde *et al*., 2001). Although the fatty acid profile in meat from ruminants is mostly dependent on rumen hydrogenation of the dietary fatty acids, some changes in this profile could be related to the diet composition. Beef from grass fed animals has been reported to contain higher proportions of *n-3* polyunsaturated fatty acids (PUFA) and total monounsaturated fatty acids (MUFA), a more favourable *n-3*/*n-6* ratio and higher levels of conjugated linoleic acid (CLA) (French *et al.* 2000) than meat from concentrated fed animals. All this issues have positive biological effects on human health (Simopoulos, 1999). On the other hand, intramuscular fat contributes to organoleptic characteristics (Laborde *et al*., 2001), being related to flavour, juiciness, tenderness and colour.

Although sex-related differences in fatty acid composition of beef cattle have been widely reported for older animals (Zembayashi *et al.*, 1995; Malau-Aduli *et al.*, 1998; Laborde *et al*., 2001; Deland *et al.*, 2001), the information about sex influence in calf fat composition is still scarce.

Objectives

The objective of the present study was to evaluate the influence of sex on the amount and composition of intramuscular lipid and nutritional value of Barrosã-PDO veal.

Methodology

Barrosã calves were reared on pasture with their dams, according to PDO specifications until slaughter at 7.4±0.9 months of age (carcass weight: 99.4±16.1 kg). One day after slaughter, about 200 grams of *Longissimus dorsi* (L4-L6) and *Supra spinatus* muscles were excised and stored at –20ºC until analysis.

Intramuscular total lipids (ITL) were extracted from duplicate 20 g samples of muscle, trimmed of visible adipose and connective tissues as described by Folch *et al.* (1957). Separation into neutral lipids (NL) and phospholipids (PL) was performed according to Juaneda & Rocquelin (1985). Lipid extracts were esterified with KOH (2N) in methanol (ISO 5509, 2000) and resulting fatty acid (FA) methyl esters were analysed by gas-liquid chromatography, using a HRGC 5160, Mega series from Carlo Erba instruments, equipped with a flame ionisation detector and a 60 m long DB 23 capillary column. Identification of FA was based on comparison of retention times with standard FA mixtures (Supelco and Nuchek GLC reference standard FAME mixture) and confirmed by GC-MS (Saturn 2200, Varian, Walnut Creek, CA, USA). FA were expressed as weight percentage.

Muscle cholesterol content (mg/g muscle) was quantified according to Roseiro *et al.* (2002), using a HPLC (Spectra-Physics Model Spectra 100) set at 206 nm and equipped with a Spherisorb S5W silica cartridge, 5μm, 4.0x125 mm (Waters PSS 845549). The mobile phase was hexane/isopropanol (97:3) at a flow rate of 1.0 mL/min.

α−Tocopherol (mg/100 g muscle) was determined according to European Standard-EN 12822 with minor modifications, using a HPLC (Spectra-Physics, model Spectra 100) equipped with a Spherisorb S5W silica cartridge (Waters PSS 845549), set at 292 nm. The mobile phase was hexane/1,4-dioxane (99:1) at a flow rate of 0.8 mL/min. Recovery of α-tocopherol from meat was determined by the addition of an internal standard to samples before saponification.

Heme iron was quantified following the analytical procedure of Lombardi-Boccia (2002). Briefly, samples were analysed for heme pigment content according to Hornsey (1956) and the heme iron concentration was calculated from the standard curve as follows: heme iron (mg/100g) = hematin content (mg/100g)×AW/MW, where AW is the atomic weight of iron and MW the molecular weight of hematin.

Protein content (g/100g) was performed using the Bicinchonic acid protein assay kit (Sigma, Saint Louis, USA).

The influence of sex on the results was evaluated in Ld and Ss muscles by analysis of variance (ANOVA) according to a general linear model procedure of SAS (2000). Analysis of means was performed by the LSD test for 95% of probability.

Results & Discussion

The effect of sex on intramuscular total lipids (ITL), neutral lipids (NL), polar lipids (PL), cholesterol, α-tocopherol, heme iron and protein contents in Ld and Ss muscles are depicted in Table 1. Although results were not affected by sex on both muscles, males and females had higher ITL, NL and heme iron mean values in Ld and Ss muscles, respectively. Irrespective of muscle considered, males showed higher cholesterol and PL and lower protein contents than females ($P>0.05$). Cholesterol level found in the present study was similar to that reported by Prates *et al* (2005a) for Ld muscle. In contrast, α-tocopherol content was lower. This difference could be attributed to diet, slaughter season effects and also to the size of the analysed populations.

The amount of intramuscular lipid fractions is referred to be dependent on the metabolic properties of muscle, with those presenting a more oxidative profile having higher amounts of total and neutral lipids, phospholipids and cholesterol than the predominantly glycolytic ones (Alasnier *et al.* 1996). Our results did not totally agree with those findings since Ss muscle, with much more pronounced oxidative status than Ld muscle (Roseiro *et al.*, 2004), showed lower total and neutral lipid contents.

Iron plays an essential role in many metabolic processes including oxygen transport, oxidative metabolism and cellular growth (Lynch, 1997). Beef consumption improves iron status in the body both by supplying highly available heme iron and by improving absorption of the dietary nonheme iron (Cook and Monsen, 1976). Heme iron content in Barrosã-PDO veal ranged from 1.35 to 1.76 mg/100 g which is considerably higher than the value reported by Lombardi-Boccia for veal (0.71 mg/100 g). Based on consumption of a 100 g steak, Barrosã-PDO veal provides 15-20 % of daily iron recommended requirements for adults (Guéguen, 2004).

Working on Semitendinosus muscle of Limousin males slaughtered between 6 and 12 months of age, Jurie *et al.* (1995) observed that protein content remained close to 18 g/100 g. A similar muscle protein level was observed in the present study, except in Ld muscle from female calves which presented a higher content (20.93 g/100 g).

In both sexes and muscles, the most abundant FA was C18:1 *cis-9*, followed by C16:0 and C18:0, which together comprised more than 70% of total FA in NL fraction. The same trend was obtained by Webb *et al.* (1998) in triacylglycerol fraction of *Longissimus thoracis* from Belgian Blue breed. In PL, the most abundant FA were C18:1 *cis-9*, C16:0 and C18:2 *n-6*, representing together more than 50% of this fraction for both sexes and muscles.

Regarding NL fraction from Ld muscle, females contained higher C16:1 *cis*, C17:1, C18:1 *cis-9* and total MUFA contents than males which in turn, showed higher C18:0 and total SFA contents (Table 2). Zembayashi *et al.* (1995) also found differences among sexes in NL from Ld muscle, with heifers presenting higher C18:1 and total MUFA and lower total SFA proportions than steers.

Concerning PL composition of Ld muscle, females presented higher *anteiso*-C17:0 and C18:2 *cis-9, trans-11* and lower C18:2 *n-6* contents than males.

Differences between sexes in NL composition of Ss muscle were significant for *anteiso*-C15:0, C15:0, C15:1, C18:0, C20:0 and total SFA with higher contents in males, whereas females showed higher C16:1 *cis*, C17:1, C18:1 *cis-9* and total MUFA and h/H (hypocholesterolaemic/hypercholesterolaemic ratio) levels than males (Table 3).

Concerning PL of Ss muscle, sex variations were limited to C20:3 *n-3*, C24:1 and C22:6 *n-3* FA.

Terrel *et al.* (1968) reported that sex effects in cattle were associated with the NL fraction rather than the PL fraction FA. The same tendency was observed in the present study, with sex mostly affecting the NL fraction of both muscles. In contrast Malau-aduli (1998) reported extensive sex differences in PL composition among yearlings and postulate that these variations were due to hormonal differences between steers and heifers. According to those authors, steers had more C14:0, C16:1, C18:0, C18:1 *n-9*, C18:1 *n-7*, C22:1 and total MUFA than heifers whereas the latter shown more C18:2, C20:4, C20:5, total PUFA, *n-3* PUFA, *n-6* PUFA and a higher P/S (polyunsaturated fatty acids/saturated fatty acids) than steers.

A minimum value of 0.45 and a maximum value of 4.0 for P/S and *n-6*/*n-3* PUFA, respectively, have been recommended in dietary fat for humans (Department of health, 1994). The indexes P/S and *n-6*/*n-3* PUFA were similar between females and males on ITL of Ld (0.35 vs. 0.34 and 3.94 vs. 4.09, respectively) and Ss muscle (0.52 vs. 0.56 and 3.46 vs. 3.92, respectively) (data not shown). The P/S value in Ld muscle was not in line with nutritional demands, due to its higher NL content, which has a lower P/S value than PL. Males showed a higher *n-6*/*n-3* PUFA proportion than females for both muscles, probably due to higher C18:2 *n-6* content. The h/H index, considered nowadays a better approach to evaluate the nutritional value of dietary fat, was higher in females than in males (2.28 vs. 2.25 and 1.91 vs. 1.83, in Ss and Ld muscle, respectively). These values were slightly lower than those reported by Prates *et al.* (2005b) for Ld muscle (T1-T3) from Carnalentejana-PDO beef slaughtered at autumn and at spring (2.03 and 2.38, respectively).

Conclusions

Sex had a significant effect on the nutritional value of intramuscular fat, particularly on NL FA composition. In general, females presented higher levels of C18:1 *cis-9*, total MUFA and h/H and lower *n-6*/*n-3* PUFA content than males. These results suggest that meat from females is more nutritionally desirable than meat from males.

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Table 1. Effects of sex on intramuscular total lipids (ITL, g/100g), neutral lipids (NL, g/100g), polar lipids (PL, g/100g), cholesterol (mg/g), α-tocopherol (mg/100g), heme iron (mg/100g) and protein contents (g/100g) in *Longissimus dorsi* and *Supra spinatus* muscles of Barrosã calves

Trait	Ld				Ss			
	Female	Male	SE	P	Female	Male	SE	P
n	46	46			46	46		
ITL	2.94	3.01	0.16	ns	2.38	2.15	0.11	ns
NL	2.40	2.46	0.17	ns	1.57	1.40	0.10	ns
PL	0.66	0.65	0.01	ns	0.73	0.75	0.02	ns
Cholesterol	0.50	0.54	0.02	ns	0.55	0.58	0.02	ns
α-Tocopherol	0.23	0.23	0.02	ns	0.25	0.25	0.02	ns
Heme Iron	1.35	1.43	0.05	ns	1.76	1.65	0.05	ns
Protein	20.93	17.98	1.61	ns	18.62	18.52	0.99	ns

ns = not statistically significant (P>0.05)

SE = standard error of the analysis of variance

Table 2. Effect of sex on fatty acid composition (w/w %) of neutral lipids and polar lipids in *Longissimus dorsi* muscle

	Neutral lipids				Polar lipids			
Fatty acids (%)	Female	Male	SE	P	Female	Male	SE	P
n	20	20			20	20		
C12:0	0.22	0.24	0.01	ns	0.03	0.04	0.01	ns
C14:0	5.11	5.17	0.22	ns	0.52	0.42	0.06	ns
C14:1	0.87	0.78	0.06	ns	0.08	0.06	0.01	ns
iso-C15:0	0.22	0.21	0.01	ns	0.06	0.03	0.01	ns
anteiso-C15:0	0.24	0.29	0.01	ns	0.06	0.05	0.01	ns
C15:0	0.66	0.71	0.03	ns	0.26	0.25	0.01	ns
C15:1	0.25	0.26	0.01	ns	0.13	0.13	0.03	ns
C16:0	24.20	24.79	0.32	ns	19.77	18.95	0.44	ns
C16:1 *cis*	4.00	3.57	0.13	*	1.18	0.99	0.07	ns
iso-C17:0	0.72	0.72	0.01	ns	0.54	0.51	0.03	ns
anteiso-C17:0	0.64	0.63	0.01	ns	0.21	0.16	0.01	*
C17:0	1.01	1.06	0.03	ns	0.51	0.51	0.03	ns
C17:1	0.82	0.74	0.01	*	0.55	0.57	0.03	ns
C18:0	12.77	14.54	0.42	**	10.06	11.09	0.40	ns
C18:1 *trans*	0.53	0.44	0.21	ns	0.33	0.39	0.06	ns
C18:1 *cis-9*	41.44	39.47	0.64	*	19.69	19.22	0.66	ns
C18:1 *cis-11*	0.00	0.03	0.03	ns	2.09	2.28	0.18	ns
C18:2 *n-6*	2.55	2.79	0.18	ns	16.25	17.97	0.50	*
C18:2 *cis-9, trans-11*	0.90	0.80	0.04	ns	0.37	0.29	0.02	*
C18:3 *n-6*	0.12	0.09	0.03	ns	0.16	0.12	0.03	ns
C18:3 *n-3*	0.58	0.62	0.03	ns	2.33	2.36	0.16	ns
C20:0	0.11	0.13	0.01	ns	0.12	0.13	0.03	ns
C20:1	0.12	0.10	0.01	ns	0.08	0.10	0.01	ns
C20:3 *n-6*	0.06	0.05	0.01	ns	0.39	0.43	0.04	ns
C20:3 *n-3*	0.07	0.08	0.03	ns	1.73	1.69	0.08	ns
C20:4 *n-6*	0.20	0.19	0.06	ns	10.64	9.54	0.64	ns
C20:5 *n-3*	0.12	0.07	0.03	ns	5.31	4.79	0.31	ns
C22:4 *n-6*	0.09	0.09	0.03	ns	0.01	0.01	0.00	ns
C24:1	0.13	0.09	0.03	ns	3.12	3.27	0.18	ns
C22:6 *n-3*	0.01	0.01	0.01	ns	0.96	0.86	0.08	ns
SFA	46.04	48.60	0.57	**	32.72	32.67	0.68	ns
MUFA	48.17	45.50	0.59	**	27.42	27.11	0.64	ns
PUFA	4.73	4.80	0.29	ns	38.55	38.46	1.15	ns
P/S	0.10	0.10	0.01	ns	1.20	1.20	0.06	ns
n-6 PUFA	3.14	3.21	0.24	ns	27.53	28.15	1.00	ns
n-3 PUFA	0.79	0.79	0.07	ns	10.66	10.02	0.47	ns
n-6/*n-3* PUFA	4.09	4.22	0.25	ns	2.66	2.98	0.18	ns
h/H	1.54	1.45	0.04	ns	2.90	3.00	0.24	ns

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids

n-3 PUFA = C18:3 *n-3* + C20:3 *n-3* + C20:5 *n-3* + C22:6 *n-3*

n-6 PUFA = C18:2 *n-6* + C18:3 *n-6* + C20:3 *n-6* + C20:4 *n-6* + C22:4 *n-6*

h/H = hypocholesterolaemic/hypercholesterolaemic ratio = [(sum of C18:1 *cis-9*, C18:2 *n-6*, C18:3 *n-6*, C18:3 *n-3*, C20:3 *n-6*, C20:3 *n-3*, C20:4 *n-6*, C20:5 *n-3*, C22:4 *n-6* and C22:6 *n-3*)/(sum of C12:0, C14:0 and C16:0)]

ns = not statistically significant; * = P<0.05; ** = P<0.01; *** = P<0.001

SE = standard error of the analysis of variance

Table 3. Effect of sex on fatty acid composition (w/w %) of neutral lipids and polar lipids in *Supra spinatus* muscle

	Neutral lipids				Polar lipids			
Fatty acids (%)	Female	Male	SE	S	Female	Male	SE	S
n	20	20			20	20		
C12:0	0.18	0.21	0.01	ns	0.03	0.02	0.01	ns
C14:0	3.99	4.48	0.25	ns	0.41	0.40	0.01	ns
C14:1	0.79	0.67	0.06	ns	0.03	0.04	0.01	ns
iso-C15:0	0.18	0.18	0.01	ns	0.05	0.05	0.00	ns
anteiso-C15:0	0.20	0.26	0.01	**	0.05	0.05	0.01	ns
C15:0	0.50	0.61	0.03	*	0.22	0.23	0.01	ns
C15:1	0.19	0.24	0.01	*	0.12	0.11	0.01	ns
C16:0	22.15	22.83	0.39	ns	17.11	17.56	0.44	ns
C16:1 *cis*	4.04	3.28	0.14	**	0.95	0.96	0.04	ns
iso-C17:0	0.72	0.68	0.03	ns	0.47	0.47	0.01	ns
anteiso-C17:0	0.60	0.60	0.01	ns	0.19	0.17	0.01	ns
C17:0	0.91	1.01	0.06	ns	0.47	0.47	0.01	ns
C17:1	0.80	0.72	0.02	*	0.53	0.54	0.04	ns
C18:0	12.58	15.72	0.47	***	10.94	11.28	0.44	ns
C18:1 *trans*	0.59	0.56	0.22	ns	0.36	0.43	0.06	ns
C18:1 *cis-9*	42.23	38.60	0.75	**	18.82	19.20	0.59	ns
C18:1 *cis-11*	0.18	0.03	0.07	ns	2.17	2.30	0.10	ns
C18:2 *n-6*	3.68	4.07	0.28	ns	17.36	18.40	0.56	ns
C18:2 *cis-9, trans-11*	0.85	0.75	0.06	ns	0.38	0.38	0.01	ns
C18:3 *n-6*	0.11	0.09	0.03	ns	0.12	0.10	0.01	ns
C18:3 *n-3*	0.69	0.71	0.06	ns	2.10	2.18	0.13	ns
C20:0	0.07	0.11	0.01	**	0.12	0.12	0.01	ns
C20:1	0.08	0.09	0.01	ns	0.11	0.08	0.01	ns
C20:3 *n-6*	0.07	0.09	0.01	ns	0.53	0.46	0.06	ns
C20:3 *n-3*	0.15	0.16	0.03	ns	2.07	1.86	0.06	*
C20:4 *n-6*	0.75	0.82	0.19	ns	12.15	11.11	0.52	ns
C20:5 *n-3*	0.32	0.33	0.07	ns	5.17	4.70	0.33	ns
C22:4 *n-6*	0.23	0.24	0.07	ns	0.01	0.01	0.00	ns
C24:1	0.38	0.36	0.08	ns	3.63	3.21	0.13	*
C22:6 *n-3*	0.07	0.09	0.03	ns	1.12	0.79	0.07	**
SFA	42.39	46.83	0.56	***	30.69	31.30	0.47	ns
MUFA	49.61	44.60	0.67	***	26.84	26.98	0.60	ns
PUFA	6.83	7.37	0.61	ns	41.41	40.38	0.80	ns
P/S	0.16	0.16	0.03	ns	1.36	1.30	0.04	ns
n-6 PUFA	4.75	5.31	0.49	ns	30.22	30.15	0.77	ns
n-3 PUFA	1.25	1.32	0.47	ns	10.81	9.84	0.42	ns
n-6/*n-3* PUFA	3.92	4.29	0.25	ns	2.87	3.22	0.17	ns
h/H	1.85	1.66	0.06	*	3.46	3.35	0.10	ns

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids

n-3 PUFA = C18:3 *n-3* + C20:3 *n-3* + C20:5 *n-3* + C22:6 *n-3*

n-6 PUFA = C18:2 *n-6* + C18:3 *n-6* + C20:3 *n-6* + C20:4 *n-6* + C22:4 *n-6*

h/H = hypocholesterolaemic/hypercholesterolaemic ratio = [(sum of C18:1 *cis-9*, C18:2 *n-6*, C18:3 *n-6*, C18:3 *n-3*, C20:3 *n-6*, C20:3 *n-3*, C20:4 *n-6*, C20:5 *n-3*, C22:4 *n-6* and C22:6 *n-3*)/(sum of C12:0, C14:0 and C16:0)]

ns = not statistically significant; * = P<0.05; ** = P<0.01; *** = P<0.001

SE = standard error of the analysis of variance