

## Beef lipids in relation to animal breed and nutrition in Argentina

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### Abstract

Fatty acid (FA) composition of intramuscular fat (IMF) in *M. Longissimus dorsi* (LD) was measured in 72 steers from Angus (A), Charolais × Angus (CHA×A) and Holstein Argentine (HA) breeds. The steers were allotted to four dietary treatments of six animals each: T1, steers grazed on pasture; T2, steers supplemented with cracked corn grain (0.7% of live-weight) daily and free access to pasture; T3, steers supplemented with cracked corn grain (1% of live-weight) daily and free access to pasture; and T4, feedlot (concentrate based on corn, alfalfa hay and soybean meal without access to pasture). At slaughter weight, samples of LD at the 11th rib were used for intramuscular lipid analysis. The diet was shown to be more important than breed in determining FA composition. Pasture beef had higher percentages of saturated fatty acids (SFA), *n*–3 polyunsaturated fatty acids (PUFA) and conjugated linoleic acid (CLA) and lower percentages of IMF, monounsaturated fatty acids (MUFA), *n*–6 PUFA and *n*–6/*n*–3 ratios than feedlot beef. HA beef presented lower percentages of SFA and more MUFA with a higher *n*–6/*n*–3 ratio than A and CHA×A. Comparing grass and feedlot beef the amounts of FA in muscle (mg/100 g) were, respectively 18:3 *n*–3 (44 vs. 11 mg), CLA (20 vs. 12 mg), 20:5 *n*–3 (20 vs. 11 mg), 22:5 *n*–5 (20 vs. 11 mg), 22:6 *n*–3 (12 vs. 6 mg) and *n*–3 PUFA (84 vs. 32 mg). Feedlot beef has more SFA (1372 vs. 1081 mg), MUFA (1574 vs. 1078 mg), PUFA (350 vs. 227 mg) and *n*–6 PUFA (318 vs. 143 mg).

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### 1. Introduction

In spite of being one of the few sources of dietary *n*–3 and *n*–6 highly polyunsaturated fatty acids (HPUFA), beef lipids are not generally regarded as a healthy component of the human diet. There are concerns about its relatively high concentrations of hypercholesterolemic, saturated fatty acids (SFA) and low concentration of hypocholesterolemic polyunsaturated fatty acids (PUFA). Consumption of saturated fatty acids (SFA) with 12–16 carbon atoms and cholesterol has been associated with increased serum low-density lipoprotein, a risk factor for coronary heart disease (CHD) (Keys, 1970; Ulbricht & Southgate, 1991). Recom-

mendations for *n*–6 and *n*–3 classes of PUFA are also important because scientists recognize differences in metabolism and physiological function between these fatty acid families (Scientific Review Committee, 1990). The *n*–6/*n*–3 fatty acids ratio is an important index to evaluate the nutritional value of a fat. According to the Department of Health (1994) it should be lower than 4 in human diet. Several researches (Enser et al., 1999; Mir, Rushfeldt, Mir, Paterson, & Weselake, 2000) have shown the positive effects of grass systems in the amounts of the conjugated isomer 9 *cis*, 11 *trans* 18:2 (CLA) in ruminant lipids. CLA refer to a mixture of positional and geometric isomers of conjugated dienoic derivatives of linoleic acid. The major dietary sources of CLA for humans are beef and dairy products. There is a great interest in CLA because of its anticarcinogenic and antiatherogenic properties and its ability to reduce body fat while enhancing lean body

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mass (Azain, Hausman, Sisk, Flatt, & Jewell, 2000; Park, Allen, & Cook, 2000; Tsuboyama-Kasaoka et al., 2000).

Fatty acid composition of beef lipids from a specific production system represents the effects of breed, genotype, sex, age, nutrition and management (Marmer, Maxwell, & Williams, 1984). Meat animals are fed intensively with grains rich in  $n-6$  fatty acids but poor in  $n-3$  fatty acids and the consequences are meats with high concentrations of linoleic acid and with a higher than the recommended ratio  $n-6/n-3$  fatty acids. Researches from different countries are trying to improve the lipid profile of meats by decreasing the ratio  $n-6/n-3$  and increasing  $n-3$  fatty acids (Enser, 2000; Enser et al., 1998). Several studies have demonstrated in cattle that decreasing the proportion of concentrate in the diet and increasing grass intake, caused a decrease in the concentrations of intramuscular fat and in the  $n-6/n-3$  fatty acids ratio (French et al., 2000; García & Casal, 1993; García, Lunqvist, Pensel, & Margaría, 2000; García, Pensel, Margaría, Rosso, & Gómez, 1996; García, Pensel, Margaría, Rosso, & Machado, 1999). Besides ruminal biohydrogenation, beef shows important differences in the lipid profile due to animal diet. Grains are a source of linoleic acid ( $n-6$ ) and grasses are rich in linolenic acid ( $n-3$ ). Beef lipids are one of the few lipid natural sources poor in  $n-6$  but dietary grasses increase the amounts of linolenic and then the final result is an almost optimum  $n-6/n-3$  fatty acids ratio.

Breed also affects the fatty acid composition of muscle lipids (Choi, Enser, Wood, & Scollan, 2000). Malau-Aduli, Siebert, Bottema, and Pitchford (1998) found differences between Jersey and Limousin cattle in fatty acid composition of muscle phospholipids. Laborde, Mandell, Tosh, Wilton, and Buchanan-Smith (2001) found differences in fatty acid composition between Simmental and Red Angus although suggested that the biological and practical significance of these differences needs to be demonstrated. Unfortunately, most bovine fatty acid composition in the literature originates from breeds on diverse diets, ages, sexes, anatomical sites, etc. This makes it difficult to extrapolate the results and compare breeds because of the confounding effects of plane of nutrition, age, fatness, live-weight, developmental traits and other factors that affect lipid metabolism (Huerta-Leidenz et al., 1993).

The expression of stearoyl-CoA desaturase (SCD) is associated with adipocyte hypertrophy in a number of species (Martin, Luna, Britain, & Smith, 1999; Smith, Mersman, Smith, & Britain, 1999). Therefore, depressing SCD enzyme activity may decrease carcass adiposity. Myristate, palmitate and stearate are converted to their ( $n-9$ ) corresponding monounsaturated fatty acids by  $\Delta^9$  desaturase (Zembayashi, Nishimura, Lunt, & Smith, 1995) and palmitate is converted to stearate through chain elongation by elongase (Huerta-Leidenz et al., 1993). Tissues with less desaturase activity (ej. loin and round) are then more susceptible to differences in rumen outflow of fatty acids caused by changes in ruminal biohydrogenation (Griswold et al., 2003). The expression and activity of tissue  $\Delta^9$  desaturase or stearoyl-CoA desaturase (SCD) has been shown to be inhibited by PUFA. The depressive effect of PUFA on SCD increases with increase in carbon chain length and double bond number within the PUFA (Ntambi, 1999).

The aim of this study was to evaluate the effects of animal diet, pasture only, pasture plus 0.7 and 1.0% of live-weight of grain supplementation and feedlot on nutritional aspects related to the composition of intramuscular lipids from steers of three different breeds.

## 2. Materials and methods

### 2.1. Animals and management

Seventy two Angus (A), Charolais  $\times$  Angus (CHA $\times$ A) and Holstein Argentine (HA) steers were allotted from 5 to 7 months old to slaughter weight to the following four treatments of six animals each.

T1: Steers grazed on pasture, mainly alfalfa (*Medicago sativa*) and tall fescue (*Festuca arundinacea* Schreb).

T2: Steers supplemented with cracked corn grain (0.7% of live-weight) daily and free access to pasture.

T3: Steers supplemented with cracked corn grain (1% of live-weight) daily and free access to pasture.

T4: Feedlot (concentrate based on corn, alfalfa hay and soybean meal without access to pasture).

The fatty acid composition of main diet ingredients is presented in Table 1. Differences in pasture fatty acid composition were detected among seasons and this could be

Table 1  
Fatty acid composition of pastures and other diet components during the trial (g/100 g of identified fatty acids)

Fatty acid	Summer	Fall	Winter	Spring	Corn	Alfalfa hay	Soybean meal
<i>Pasture</i>							
16:0	30.2	22.3	29.3	22.8	14.7	39.6	15.8
16:1	3.1	3.1	3.3	3.0	0.4	0.4	0.4
18:0	5.7	3.3	6.1	5.4	3.3	7.2	4.8
18:1	7.3	4.4	10.17	11.8	32.1	12.6	19.7
18:2	22.8	18.5	15.1	18.0	46.7	18.7	51.3
18:3	30.9	47.6	36.0	38.9	1.8	21.8	8.0
%EE (MS) <sup>a</sup>	2.1	3.0	2.0	2.3	5.41	1.4	4.1
18:3/18:2	1.35	2.57	2.38	2.14	0.04	1.16	0.16

<sup>a</sup> Ether extract (dry matter).

important because it affects the intake of  $n-6$  and  $n-3$  PUFA and CLA precursors. T1, T2 and T3 steers were slaughtered during the fall. All steers were conventionally slaughtered in a commercial abattoir at similar degree of finishing: T1 (390, 457 and 507 kg), T2 (412, 472 and 509 kg), T3 (429, 503 and 541 kg) and T4 (385, 381 and 367 kg), respectively for A, CHA×A and HA breeds.

The diets and breeds studied are representatives of Argentine beef production system. All the animals were fed on the same pasture, under the same management conditions, and the muscle samples were taken from the same anatomical site. Having eliminated most of the obvious phenotypic sources of variation, the breeds were compared for differences in fatty acid composition of total intramuscular fat.

## 2.2. Lipid analysis

After 24 h at 4 °C steaks of Longissimus dorsi muscle at the 11th rib were taken from each treatment, carefully dissected and used for chemical analysis. All samples were stored at -20 °C until analysis were performed. Aliquot samples of 10 g each, trimmed of external fat, minced carefully, dried and extracted in a Tekator apparatus using hexane as the extraction solvent according to official methods (AOAC, 1992), were used to determine total intramuscular fat (IMF). Aliquot samples of 5 g each were extracted using the Folch, Lees, and Sloane-Stanley (1957) method. The chloroform extract was used for fatty acid analyses and for total cholesterol determinations, after saponification with 4% KOH in ethanol absolute, with an enzymatic and colorimetric reactive (BioSystem S. A.). Fatty acid methyl esters (FAME) were prepared according to the method of Pariza, Park, and Cook (2001) and measured using a Chrompack CP 900 equipment fitted with a flame ionization detector. Fatty acid methyl esters were separated with a fused silica capillary column CP-Sil 88 (100 m × 0.25 mm i.d.); Chrompack Inc., Middleburg, The Netherlands, with nitrogen as the carrier gas. The oven temperature was programmed at 70 °C for 4 min, increased from 70 to 170 °C at a rate of 13 °C/min and then increases from 170 to 200 °C at 1 °C/min. Individual fatty acids were identified by comparing relative retention times with individual fatty acids standard (PUFA-2, animal source, Supelco). Fatty acid compositions of diet components were determined in the same conditions. Mathematical indices for calculating the activities of stearoyl-CoA desaturase were determined according to Malau-Aduli, Siebert, Bottema, and Pitchford (1997). Analytic results were expressed as percentages of total fatty acids. On the basis of the content of fat in the muscle and the fatty acid profile the content of the different fatty acids were calculated by 100 g of meat.

## 2.3. Statistical analysis

Statistical analysis were performed by means of the statistical software SAS 6.1, 1996 using the GLM procedure.

If there was a significant treatment effect by  $F$ -test, the Tukey's Studentized Range (HSD) was used for follow-up comparisons of treatment means. The data are shown as mean ± standard error.

In order to determine which variables discriminate between the four diets a discriminant analysis employing a forward stepwise procedure was applied to the fatty composition data, using the SPSS 10:0 program.

## 3. Results and discussion

### 3.1. Changes in fatty acid composition according to diet

All saturated fatty acids with exception of myristic acid (14:0) were affected significantly by diet. Myristic acid, a hypercholesterolemic and thrombogenic fatty acid increased with the amounts of grain but not statistically significant (Table 2). Duckett, Wagner, Yates, Dolezal, and May (1993) found that 112-days on grain were required to significantly change concentrations of 14:0 in Longissimus dorsi muscle relative to pasture-fed cattle. Pentadecanoic (15:0) acid was affected significantly ( $p < 0.05$ ) by diet but the contamination with some myristoleic acid (14:1) isomers made difficult the interpretation of these differences. Palmitic acid (16:0) another hypercholesterolemic and thrombogenic fatty acids was only slightly affected by the diet. Similar results were found by Laborde et al. (2001). Margaric acid (17:0) showed higher percentages ( $p < 0.01$ ) in T4 compared to T1, T2 and T3. Stearic acid (18:0) decreased with the amounts of dietary grain, 13.10 vs. 10.82% for T1 and T4, respectively.

Monounsaturated fatty acid myristoleic (14:1) and palmitoleic acid (16:1) were affected by diet following, as expected, an inverse trend that 18:0. The higher values were found in T4 and the lower ones in T1.

Heptadecanoic acid (17:1) was affected by diet with significant ( $p < 0.01$ ) higher values for T4 compared to T1, T2 and T3 (1.63 vs. 0.96, 0.87 and 0.86% for T1, T2 and T3, respectively). 18:1 *trans* percentages were affected by diet being lower in T1, T2 and T3 compared with T4 (3.22, 2.73, 2.25 and 4.35% for T1, T2, T3 and T4, respectively). The 18:1 *trans* isomers are reported as one value. As the column temperature program used incompletely resolves them, we cannot exclude some minor contamination with the other isomers of *trans* 18:1. One of them could be vaccenic acid (VA) (*trans* 18:1  $n-11$ ) that is also produced by ruminal biohydrogenation of 18:2  $n-6$  (Harfoot & Hazelwood, 1997). Ruminant CLA and VA are dependent on dietary factors including the source and levels of dietary lipids (Grinari & Bauman, 1999) and ruminal production of these fatty acids will therefore impact yields of CLA in meat lipids.

Diet effects on oleic acid (18:1  $n-9$ ) were not, as expected, higher in T4 compared with T1. The increased oleic acid content of tissue in response to concentrate-rich diets is associated with an increase in stearoyl-CoA desaturase gene expression (Daniel, Wynn, Salter, & Buttery, 2004).

Table 2

Breed, diet and interaction effects on fatty acid composition of Longissimus dorsi of steers from the three breeds fed different diets (% by weight of total fatty acids)

Fatty acid	Diet				Breed			S.E.	Diet	Breed	Diet × Breed
	Pasture	Pasture + 0.7%	P + 1%	Feedlot	A	CHA×A	HA				
14:0	2.19	2.14	2.26	2.44	2.46a	2.24ab	2.07b	0.15	NS	***	NS
14:1	0.48b	0.47b	0.59ab	0.67a	0.56	0.51	0.59	0.06	***	NS	NS
15:0	0.56a	0.45ab	0.37b	0.49a	0.46	0.45	0.45	0.05	***	NS	NS
16:0	23.12ab	23.42a	23.50a	22.08b	23.40a	23.27a	22.42a	0.59	**	**	NS
16:1	3.35b	3.58ab	3.56ab	3.82a	3.53b	3.30b	3.87a	0.18	**	***	NS
17:0	1.39b	1.15c	1.03c	1.69a	1.37	1.36	1.22	0.11	***	NS	NS
17:1	0.96b	0.87b	0.86b	1.63a	1.04	1.06	1.12	0.06	***	NS	NS
18:0	13.10a	12.70ab	12.09b	10.82c	12.42a	12.61a	11.50b	0.44	***	***	NS
18:1 <i>trans</i>	3.22b	2.73bc	2.25c	4.35a	3.16ab	3.46a	2.79b	0.24	***	***	*
18:1	31.18c	33.52ab	35.09a	32.84bc	30.95c	33.31b	35.20a	0.90	***	***	NS
18:2 <i>n-6</i>	3.41b	3.61b	3.93b	6.19a	4.07	4.38	4.41	0.34	***	NS	NS
18:3 <i>n-3</i>	1.30a	0.89b	0.74b	0.28c	0.85a	0.84a	0.73b	0.07	***	*	NS
CLA	0.72a	0.61b	0.58b	0.31c	0.51b	0.57a	0.58a	0.03	***	***	***
20:3 <i>n-3</i>	0.40	0.41	0.43	0.50	0.46	0.46	0.39	0.06	NS	NS	NS
20:4 <i>n-6</i>	1.12	1.13	1.34	1.55	1.15	1.20	1.50	0.21	*	*	NS
20:5 <i>n-3</i>	0.52a	0.30b	0.26b	0.12c	0.33	0.27	0.32	0.07	***	NS	NS
22:4 <i>n-6</i>	0.07c	0.11b	0.15b	0.21a	0.13	0.13	0.15	0.03	***	NS	NS
22:5 <i>n-3</i>	0.70a	0.51b	0.49b	0.30c	0.55	0.45	0.55	0.09	***	NS	NS
22:6 <i>n-3</i>	0.43a	0.18b	0.14b	0.16b	0.33a	0.27a	0.10b	0.05	***	***	***

abc: Mean values in rows having different letters are significantly different. SE, standard error; NS, not significant;  $p > 0.05$ ; \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

The *n-6* PUFA 18:2 was affected by the diet (6.19% in T4 vs. 3.41, 3.61 and 3.93 for T1, T2 and T3, respectively). Small or no differences were found in other *n-6* PUFA metabolites, 20:3, 20:4 or 22:4.

The *n-3* PUFA decreased linearly as the amounts of grain in the diet increased. Linolenic acid (18:3 *n-3*) 1.30, 0.89, 0.74 and 0.28%, eicosapentanoic acid (20:5 *n-3*) 0.52, 0.30, 0.26 and 0.12% and docosapentanoic (22:5 *n-3*) 0.70, 0.51, 0.49 and 0.30% for T1, T2, T3 and T4, respectively. The hyperlipidemic effects of 20:4 *n-6* are counteracted by EPA (20:5 *n-3*) (Scientific Review Committee, 1990). The concentrations of DHA (22:6 *n-3*), widely recognized as the essential nutrient in the brain for normal functioning of neural tissue were 0.43, 0.18, 0.14 and 0.16% for T1, T2, T3 and T4, respectively. The biosynthesis of long-chain PUFAs from 18:3 *n-3* appears to be a minor pathway in several species (Pawlosky, Hibbeln, Novotay, & Salem, 2001). In contrast, 20:5 *n-3* may be well utilized for synthesis of other long-chain PUFAs such as 22:6 *n-3*. A feedback control mechanism responsive to the plasma concentrations of 22:6 *n-3* may affect processes that regulate its own synthesis, thereby maintaining 22:6 *n-3* homeostasis during dietary changes (Pawlosky et al., 2003). DPA (22:5 *n-3*) is an intermediate in the production of DHA from EPA and it is the predominant of long *n-3* PUFA in meat HPUFA. Intake recommendations, and health claims often omit DHA. Burbge, Jones, and Wootton (2002) found that the conversion of 18:3 *n-3* was 7.9% to EPA and 8.1% to DPA in men, with hardly any further conversion through to DHA, although there was a considerably greater conversion of DPA to DHA in women.

CLA percentages were affected by diet and the differences ( $p < 0.01$ ) were relevant between T1 and T4 (0.72 and 0.31%), respectively for T1 and T4. The concentration of CLA in fat is higher in ruminants fed on leafy grasses than in those fed on stored forages or concentrates (French et al., 2000). Increased PUFA in the diet may limit ruminal production of CLA and VA and/or may depress stearyl-CoA desaturase expression or activity in lean tissues, which in turn limits CLA formation and accretion in tissues (Griswold et al., 2003). Increased dietary forage tended to increase 18:0 and 18:3 *n-3* suggesting that increased forage may mitigate toxic effects of PUFA on ruminal biohydrogenation thereby increasing the pool of CLA and VA available for CLA formation and accretion in tissues. Rumenic acid distribution is not similar to other PUFA, as it was independent of fat location (Santos-Silva, Bessa, & Mendes, 2003) and according to Banni et al. (2001) in rat livers was incorporated priority in neutral lipids. In ruminant muscles, it is known that CLA is mainly associated to the triacylglycerol fraction (Bauchart, Gladine, Gruffat, Leloutre, & Durand, 2005) which is linked to the fat content of tissues (Ashes, Siebert, Gulati, Cuthbertson, & Scott, 1992).

### 3.2. Changes in fatty acids composition according to breed

Breed affected 14:0, 16:0 and 18:0 concentrations (Table 2). HA and CHA×A beef presented the lower percentages of 14:0 compared to A. HA the lower percentages of 16:0 and 18:0 compared to A and CHA×A. Breed differences in 15:0, 17:0 and 16:0 were also found by Laborde et al. (2001) comparing Simmental and Red Angus breeds.



Monounsaturated fatty acids 16:1, 18:1 *trans* and 18:1 *n*-9 were also affected by breed. The higher values for 16:1 and 18:1 *n*-9 were for HA compared with A and CHA×A and the lower values for 18:1 *trans*. 18:1 *trans* shows a small but significant interaction diet × breed. This breed effects on 18:1 *n*-9 differed with the finding of Siebert, Deland, and Pitchford (1996).

The *n*-6 polyunsaturated fatty acids were not affected significantly by breed. Small differences were found in 20:4 *n*-6. At similar amounts of 18:2 any difference in 20:4 between breeds only could be explained by a genetic difference due to a lesser  $\Delta^5$  desaturase activity responsible for the conversion of 20:3 to 20:4 or lower elongase activity as results of the relative accumulation of 18:3 *n*-6.

The *n*-3 polyunsaturated acids 18:3 and 22:6 were affected by breed being lower in HA compared to A and CHA×A. A significant interaction diet × breed was detected for 22:6.

CLA percentages were affected by breed with the low values for A compared with the other breeds. A significant ( $p < 0.01$ ) interaction diet × breed was detected.

### 3.3. Diet and breed effects on intramuscular fat, H<sub>2</sub>O and cholesterol

Diet affected the muscle lipids percentages (Table 3). The lower values were found in grass beef (T1) compared with the feedlot (T4) and supplemented beef (T3). Grass

beef (T1) presented generally the higher H<sub>2</sub>O% content. These differences could be explained by the lower content of intramuscular fat in T1. These results agree with the literature that shows that even at similar carcass finish grass fed beef is leaner than feedlot or supplemented beef (French et al., 2000). The main difference in carcass characteristics between pasture and grain-finished cattle is that grain-finished carcasses are generally fatter than pasture-finished carcasses regardless of whether animals were slaughtered at the same weight or at the same age. This is because energy intake influences growth rate and carcass fatness: higher energy intake tends to increase the fatness of animal carcasses.

Some differences in total beef cholesterol content according to diet and breed were detected (Table 3). Lower values were found for grass beef compared with T2, T3 and T4. HA presented the lowest values compared with the other breeds. These results were similar to the found in previous studies (García et al., 1996). Small increases in cholesterol content is unlikely to be of nutritional consequence because dietary cholesterol rarely affects plasma cholesterol unless changes are large.

### 3.4. Effects of diet and breed in nutritional and health issues related to fatty acid composition

SFA percent (Table 3) was lower ( $p < 0.01$ ) in T4 compared with T1, T2 and T3 (35.33 vs. 38.40, 38.26 and

Table 3  
Breed, diet and interaction effects on IMF%, H<sub>2</sub>O%, cholesterol, fatty acids ratios and desaturase indices of Longissimus dorsi of steers fed different diets (% by weight of total fatty acids)

Item	Diet				Breed			S.E.	Diet	Breed	B × D
	Pasture	P + 0.7%	P + 1%	Feedlot	A	CHA×A	HA				
IMF%	2.86b	3.62ab	4.09a	3.85a	3.64	3.73	3.45	0.45	*	NS	NS
H <sub>2</sub> O%	73.88a	73.53ab	72.15b	72.50ab	72.49	73.00	73.56	0.70	**	NS	NS
Col g/100 g	40.3b	45.1a	42.4ab	45.8a	45.3a	44.2ab	40.8b	2.08	***	***	NS
SFA% <sup>g</sup>	38.40a	38.26a	37.85a	35.33b	38.28a	38.11a	36.00b	0.78	***	***	*
MUFA% <sup>h</sup>	37.74b	39.89a	40.89a	40.77a	37.65c	40.07b	41.87a	0.91	***	***	NS
PUFA% <sup>i</sup>	7.95ab	7.14b	7.50b	9.31a	7.81	7.92	8.20	0.66	***	NS	NS
<i>n</i> -3 <sup>k</sup>	2.95a	1.88b	1.63b	0.86c	2.00	1.82	1.69	0.19	***	NS	*
<i>n</i> -6 <sup>j</sup>	5.00b	5.25b	5.86b	8.15a	5.81	6.10	6.51	0.51	***	NS	NS
<i>n</i> -6/ <i>n</i> -3	1.72c	2.82b	3.77b	10.38a	4.30b	4.12b	5.59a	0.33	***	***	***
18:2/18:3	2.62c	4.09c	5.67b	22.63a	7.77b	8.38b	10.11a	0.81	***	***	*
20:4/20:5	2.14c	3.75bc	5.47b	15.80a	4.40c	7.08b	8.88a	1.04	***	***	***
18:2/CLA	4.99b	6.12b	6.94b	21.11a	9.28	9.85	10.24	1.43	***	NS	NS
18:3/CLA	1.90a	1.51b	1.28bc	0.97c	1.61a	1.43ab	1.20b	0.17	***	***	NS
P/S <sup>l</sup>	0.21b	0.19b	0.20b	0.27a	0.21	0.21	0.23	0.02	***	NS	NS
DS 16:0 <sup>m</sup>	12.15b	13.29b	13.14b	14.65a	13.18b	12.42b	14.70a	0.53	***	***	NS
DS 18:0 <sup>n</sup>	70.31c	72.49b	74.30ab	75.16a	71.33b	72.54b	75.38a	0.94	***	***	NS

abc: Mean values in rows having different letters are significantly different.

SE, standard error; NS, not significant;  $p > 0.05$ ; \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

<sup>g</sup> Total saturated fatty acids (14:0 + 16:0 + 18:0).

<sup>h</sup> Total monounsaturated fatty acids (16:1 + 18:1).

<sup>i</sup> Total polyunsaturated fatty acids (*n*-6 + *n*-3).

<sup>j</sup> *n*-6 (18:2 + 18:3 + 20:3 + 20:4 + 22:4).

<sup>k</sup> *n*-3 (18:3 + 20:5 + 22:5 + 22:6).

<sup>l</sup> P/S (*n*-6 + *n*-3)/(14:0 + 16:0 + 18:0).

<sup>m</sup> Index of  $\Delta^9$  desaturase enzyme activity on the conversion of 16:0 to 16:1 *n*-9 = 100 (16:1 *n*-9/(16:1 *n*-9 + 16:0)).

<sup>n</sup> Index of  $\Delta^9$  desaturase enzyme activity on the conversion of 18:0 to 18:1 *n*-9 = 100 (18:1 *n*-9/(18:1 *n*-9 + 18:0)).

37.85%, respectively) and in HA breed (36.00 vs. 38.28 and 38.11 for A and CHA×A, respectively).

MUFA% (Table 3) was higher in HA beef (41.87 vs. 40.07 and 37.65% for CHA×A and A, respectively) and lower in T1 than in T2, T3 and T4.

PUFA percent were affected only by diet (7.95, 7.14, 7.50 and 9.31 for T1, T2, T3 and T4, respectively). The value of PUFA is the sum of the different contribution of  $n-6$  and  $n-3$  fatty acids from diet.  $n-6$  PUFA (5.00, 5.25, 5.86 and 8.15%) and  $n-3$  PUFA (2.95, 1.88, 1.63 and 0.86% for T1, T2, T3 and T4, respectively). The  $n-6$  PUFA were apparently not affected by breed but  $n-3$  PUFA presented significant ( $p < 0.05$ ) interactions between breed and diet. The PUFA content remains relatively constant across different beef types. This is mainly due to the relatively constant proportion of phospholipids in the cell membranes, and increasing deposition of triglycerides in the adipocytes with increasing intramuscular fat content (Vernon & Flint, 1988).

The recommended intake for humans of  $n-6$  fatty acids is approximately 4% of dietary energy with a minimum of 1.5% and the intake of  $n-3$  fatty acids should be about 0.75% of dietary energy to avoid essential fatty acid deficiency (Innis, 1996). There is great evidence that increased consumption of  $n-3$  fatty acids protect from CHD and the excessive consumption of  $n-6$  fatty acids at the expense of  $n-3$  promote CHD and other diseases. Literature comparisons (Cordain et al., 2002) showed tissue lipids of wild ruminants were similar to pasture-fed cattle, but dissimilar to grain-fed cattle. The lipid composition of wild ruminant tissues was suggested as a model for dietary lipid recommendations. Overall relative content of  $n-3$  PUFA was 59% lower in T4 than in T1 ( $p < 0.05$ ).

The 18:2  $n-6$ /18:3  $n-3$  and  $n-6/n-3$  ratios were linearly and significantly ( $p < 0.05$ ) affected for the contribution of grain to the diet (Table 3). HA breed presented the higher ratio values compared with the other breeds. The less  $n-3$  PUFA percentages in HA explain this difference. The  $n-6/n-3$  PUFA ratio is an important index to evaluate the nutritional value of fat. According to the Department of Health (1994) it should be lower than four in human diet. It is interesting to notice that EPA and DHA only are formed in significant amounts if the ratio  $n-6/n-3$  is less than 10:1. The  $n-6/n-3$  is influenced mainly by the diet. A low ratio is not difficult in a grass feeding system, as the supply of  $n-6$  is low.

The 18:2  $n-6$ /18:3  $n-3$  and 20:4  $n-6$ /20:5  $n-3$  ratios followed a similar pattern and could be used as very good indicators of the ruminant diets. They are easy to determine from chromatogram data (García, Pensel, Margaría, Rosso, & Casal, 2003). Some minor breed differences between A and the other breeds were also detected.

P/S ratio was affected significantly by diet being significantly higher in T4 compared with the other treatments (0.21, 0.18, 0.20 and 0.26 for T1, T2, T3 and T4, respectively). These results show that to increase P/S to the appropriate level, 0.45 or above (Williams, 2000), in terms

of nutritional recommendations would be difficult when beef are fed either pasture or concentrate. According to Raes, De Semet, and Demeyer (2004) P/S dietary source does not affect the P/S ratio, but is mainly influenced by genetics, in particular, the overall fat level of the animal and less for nutrition.

The ratio 18:3  $n-3$ /CLA was significantly ( $p < 0.05$ ) affected by diet (1.90, 1.51, 1.28 and 0.97 for T1, T2, T3 and T4, respectively) and also for breed (1.61, 1.43, 1.20 for A, CHA×A and HA, respectively).

The ratio 18:2  $n-6$ /CLA was affected significantly only by diet. T1, T2 and T3 differed ( $p < 0.01$ ) from T4 (4.99, 6.12, 6.94 vs. 21.11, respectively). This could be important because some of the effects attributed to CLA are also due to CLA-induced changes in 18:2  $n-6$  metabolism. It is likely, that metabolites such as 20:3 and 20:4 of the CLA isomers are involved in some aspects whereby CLA induces its physiological effects (Pariza et al., 2001).

### 3.5. Indices of desaturase enzyme activity

The index of  $\Delta^9$  desaturase enzyme activity on the conversion of 16:0–16:1 and 18:0–18:1 increased as the grain in the diet increased and was higher in HA than in the other breeds. The lower values of the desaturase and higher values for CLA in T1 could be explained according to Daniel et al. (2004) that the increased concentrations of CLA in forage rich diets is associated with an increase in substrate (18:1 *trans* 11) availability and not with an increase in SCD gene expression. The HA presented the higher values for both  $\Delta^9$  desaturase which may explain the genetic basis for breed differences ( $p < 0.05$ ) in 16:1 and C18:1 between HA and A (Siebert, Pitchford, Kruk, Kuchel, & Deland, 2003).

### 3.6. Dietary beef lipid contribution

Considering that the mg of fatty acids per 100 g muscle are nutritionally more useful than the percentages we show in Table 4, the estimated contribution (mg/100 g muscle) of all fatty acids, SFA, MUFA,  $n-6$  and  $n-3$  PUFA.

Diet affected the contribution of all fatty acids and the values for the different ratios (Table 4). Comparing grass and feedlot beef (Table 4) is possible to say that 100 g of grass beef has more 18:3  $n-3$  (44 vs. 11 mg), CLA (20 vs. 12 mg), 20:5  $n-3$  (20 vs. 11 mg), 22:5  $n-5$  (20 vs. 11 mg), 22:6  $n-3$  (12 vs. 6 mg) and  $n-3$  PUFA (84 vs. 32 mg). Feedlot beef has more SFA (1372 vs. 1081 mg), MUFA (1574 vs. 1078 mg), PUFA (350 vs. 227 mg) and  $n-6$  PUFA (318 vs. 143 mg).

Breed affected significantly ( $p < 0.01$ ) the contribution of 22:6  $n-3$  and the total amounts ( $p < 0.05$ ) of  $n-3$  fatty acid but have no significant effects on total intake of SFA, MUFA, PUFA and  $n-6$  fatty acids (Table 3). The manipulation of fatty acid profiles in cattle should be selected for individuals or breeds types capable of transmitting to descendants the ability to accumulate lipids with less

Table 4  
Fatty acid contribution of beef according to diet and breed (mg/100 g)

Fatty acid	Diet				Breed			S.E.	Diet	Breed	Diet × Breed
	Pasture	Pasture + 0.7%	P + 1%	Feedlot	Angus	CHA×A	HA				
14:0	63b	78ab	94a	96a	92	84	73	3.50	**	NS	NS
14:1	14b	18ab	24a	26a	21	20	21	3.50	***	NS	NS
15:0	16	17	15	15	19	18	14	3.12	NS	NS	NS
16:0	630b	858ab	966a	859ab	832	876	777	115	**	NS	NS
16:1	96b	131ab	148a	144a	130	124	135	18.39	***	NS	**
17:0	40b	41b	43b	68a	51	51	41	8.59	***	NS	NS
17:1	28b	31b	35b	59a	39	40	36	5.47	***	NS	NS
18:0	376b	455ab	496a	417ab	447	464	397	54.58	**	NS	NS
18:1 <i>trans</i>	91b	100b	92b	175a	119	129	95	20.88	***	NS	NS
18:1	891b	1116a	1225a	1452a	1135	1256	1227	165.44	***	NS	NS
18:2 <i>n</i> -6	98c	123bc	157b	234a	148	161	150	18.26	***	NS	NS
18:3 <i>n</i> -3	44a	31a	30a	11b	29	34	23	3.60	***	NS	NS
CLA	20a	22a	24a	12b	18	21	19	2.66	***	NS	*
20:3 <i>n</i> -6	12b	13ab	17ab	20a	17	14	15	3.88	**	NS	NS
20:4 <i>n</i> -6	32b	37b	53a	56a	40	43	51	7.27	***	NS	NS
20:5 <i>n</i> -3	15a	10b	10b	4c	11	9	10	1.30	***	NS	NS
22:4 <i>n</i> -6	2a	4a	5b	8b	4	5	5	0.62	***	NS	NS
22:5 <i>n</i> -3	20a	17a	19a	11b	17	16	18	2.55	***	NS	NS
22:6 <i>n</i> -3	12a	6b	5b	6b	10a	9a	3b	1.58	***	***	***
<i>n</i> -3 <sup>a</sup>	84a	64b	64b	32c	66a	63ab	54b	7.07	***	*	NS
<i>n</i> -6 <sup>b</sup>	143c	178c	234b	318a	211	223	221	24.02	***	NS	NS
SFA <sup>c</sup>	1081b	1391ab	1556a	1372ab	1380	1424	1247	180.26	**	NS	NS
MUFA <sup>d</sup>	1078b	1457ab	1692a	1574a	1384	1509	1458	198.96	**	NS	NS
PUFA <sup>e</sup>	227c	242bc	298ab	350a	276	286	275	29.07	***	NS	NS

abc: Mean values in rows having different letters are significantly different.

NS: not significant  $p > 0.05$ ; \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

S.E. standard error.

<sup>a</sup> *n*-3 (18:3 + 20:5 + 22:5 + 22:6).

<sup>b</sup> *n*-6 (18:2 + 18:3 + 20:3 + 20:4 + 22:4).

<sup>c</sup> Saturated fatty acids (14:0 + 16:0 + 18:0).

<sup>d</sup> Monounsaturated fatty acids (16:1 + 18:1).

<sup>e</sup> Polyunsaturated fatty acids (*n*-3 + *n*-6).

palmitic acid and/or more oleic and stearic acids because the latter have desirable effects on humans (Bonanome & Grundy, 1988). The contribution of beef to dietary intake of SFA, MUFA and PUFA is an important issue to consumers and consequently the meat industry but the effect of breed type on the contribution of beef 22:6 *n*-3 deserves more research.

Generally grass beef (T1) presented (Table 4) the lowest contribution to the intake of SFA, MUFA and PUFA and *n*-6 fatty acids but the highest *n*-3 fatty acids and CLA than beef produced in feedlot (T4). T2 and T3 were in an intermediate position. Increases in dietary levels of saturated fat particularly 12:0, 14:0 and 16:0 have been identified as the major dietary factor responsible for raising total and LDL serum cholesterol concentrations.

There is evidence that increased consumption of *n*-3 fatty acids protect from CHD and that excessive consumption of *n*-6 fatty acids at the expense of *n*-3 may promote CHD and other chronic diseases. If the recommended intake of *n*-3 PUFA is 100–200 mg/day (Department of Health, 1994) the grass beef contribution (84 mg/100 g beef) could be important. The modest amount of PUFA in muscle tissue phospholipids of lean meat need to be

taken into account when determining dietary PUFA intakes, whereas previous estimates of *n*-3 HPUFA have often been based on consumption of *n*-3 HPUFA of sea-foods only (Lopez-Garcia et al., 2004).

Meat and meat products contribute about 25–30% of the total human CLA intake in Western populations (Schmid, Collomb, Sieber, & Bee, 2006). This intake could be increased through specific feeding strategies as grass feeding.

Total fat content, the ratio of P/S, the contribution of specific fatty acids and the ratio *n*-6/*n*-3 have health importance. In ruminant muscle and adipose tissue PUFA are restricted almost exclusively to the phospholipid fraction (Wood et al., 2003). Consumption of fat trimmed lean beef (2–5% fat) also was associated with reduced LDL cholesterol (Davidson, Hunninghake, Kevin, Kwiterovich, & Kafonek, 1999).

### 3.7. Linear discriminant analysis

The classification functions obtained using the fatty acid composition (%) are presented in Table 5 and Fig. 1. The significant variables selected by the stepwise procedure

Table 5  
Classification functions obtained using the fatty acid composition (%)

Variable	T1	T2	T3	T4
14:1	1.825	-0.735	-0.226	-0.864
17:1	-4.131	-3.635	-3.465	11.232
18:1 <i>trans</i>	-0.171	-2.122	-2.968	5.261
18:2 <i>n-6</i>	-8.361	-3.049	-0.499	11.909
18:3 <i>n-3</i>	10.243	3.612	0.892	-14.747
CLA	4.412	1.657	1.683	-7.751
20:5 <i>n-3</i>	7.619	0.258	-0.994	-6.883
Constant	-17.318	-4.473	-3.963	-39.121
Correctly classified cases (%)	94.4	77.8	77.8	100

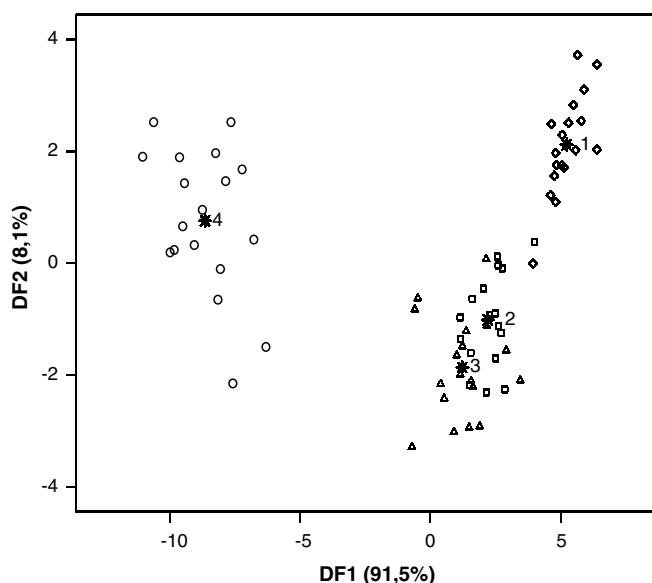


Fig. 1. Linear discriminant analysis applied to the fatty acid composition of Longissimus dorsi intramuscular fat from T1, T2, T3 and T4.

were the percentages of 14:1, 17:1, 18:1 *trans*, 18:2 *n-6*, 18:3 *n-3*, CLA and 20:5 *n-3*. Using these parameters, two discriminant functions were defined that explained 91.5 and 8.1% of the total variance, respectively. This kind of analysis allowed a success rate of correct classification of each dietary treatment into their respective group for both, original cases and after cross validation.

#### 4. Conclusion

The diet was shown to be more important than breed in determining FA composition. Pasture beef had higher percentages of SFA, *n-3* PUFA and CLA and lower percentages of IMF, MUFA, *n-6* PUFA and *n-6/n-3* ratios than feedlot beef. HA beef presented less percentage of SFA and more MUFA with a higher *n-6/n-3* ratio than A and CHA×A. Cattle raised on pasture have a positive impact on fatty acid tissue profile, mainly due to an increase in the proportion of *n-3* fatty acids and of conjugated isomers of linoleic acid (CLA). Those changes in fatty acid composition affect the nutritional value of fat because the

*n-3* PUFA have beneficial effects on human physiology and health preventing the occurrence of coronary heart diseases, hypertension, inflammatory and immune disorders and neurological dysfunctions (Williams, 2000). These differences reflect the lipid composition of the diet, as grass contain a high concentration of 18:3 precursor of the *n-3* series, while concentrates contain high levels of 18:2 precursor of the *n-6* fatty acid family.

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