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The effect of breed on the chemical composition and fatty acid profile of the *Longissimus dorsi* muscle of Brazilian beef cattle

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ABSTRACT

The study was conducted to evaluate the chemical composition and fatty acid profile of the *Longissimus dorsi* muscle of first (PUR1) and second (PUR2) generations of Purunã-breed cattle, and compare them to their ancestral generations (PUR). Thirty-nine young bulls with an average initial weight of 236 kg and initial age of 10 months were used. The animals were slaughtered at the age of 16 months with an average final weight of 464 kg. The content of water, ash, crude protein and total cholesterol in the muscles were similar (P>0.05) among the different genetic groups. The levels of total intramuscular fat were higher (P<0.05) for PUR in comparison to PUR1 and PUR 2. The percentage shares of 14:0, 14:1 *n*-7 and 16:0 fatty acids were higher (P<0.05) as a percentage of total fatty acids in PUR2 and PUR in comparison to PUR1. Thus, the 22:6 *n*-6 ratios were greatest (P<0.05) for the PUR1 genetic group. The lowest (P<0.05) percentage was observed in PUR. There were no difference (P>0.05) in SFA, MUFA, PUFA, *n*-3, or in the PUFA:SFA and *n*-6:*n*-3 ratios, when comparing the first and second Purunã generations to their ancestors.

KEY WORDS: beef, generation, cholesterol, CLA, fatty acids, meat

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INTRODUCTION

Brazil has the largest commercial cattle herd in the world, with approximately 159 million animals and a production of approximately 8.2 million tons of carcass each year (Anualpec, 2007). From this total, about 30% (2.4 million tons) is exported to several countries around the world.

The consumer market for beef has become increasingly demanding as a result of negative factors associated with meat production and quality. Among these factors are the undesirable relationships between beef consumption and heart disease, atherosclerosis, intestinal cancer, and obesity, among other diseases (Kwiterovich, 1997).

The fatty acid composition of meat (muscle and adipose tissue) is important for two main reasons: it determines nutritional value, and it affects various aspects of meat quality, including shelf-life and flavour. Nutritional value is determined in part by the ratio between saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) in meat, as well as the balance between fatty acids of the *n*-6 and *n*-3 series. In general, a ratio of PUFA to SFA (termed P:S) above 0.45 and a ratio of *n*-6:*n*-3 below 4.0 are required in the diet to combat various "lifestyle diseases", such as coronary heart disease and cancers (Hu, 2001).

Several factors in beef production affect fatty acid composition, including breed and diet. Breed affects the fat content in meat, and fat content itself is a determining factor for fatty acid profile (Prado et al., 2008b). Ruminants naturally consume a diet low in fat but high in PUFA, whether as fresh grass, conserved grass or the concentrate portion of the diet.

The State of Paraná, located in southern Brazil, features a milder climate as compared to other regions of the country. Consequently, researchers have been conducting studies since the 1980s on the crossbreeding between Zebu and European breeds, with the objective of increasing production (Perotto et al., 2000) and meat quality of bulls (Aricetti et al., 2008; Macedo et al., 2008; Prado et al., 2008a,b,c,d; Ducatti et al., 2009; Maggioni et al., 2009; Prado et al., 2009). After several stages of crossbreeding, an ideal crossbreeding ratio was found as the best adapted for the region. Initially, Nellore specimens were crossbred with Charolais, Angus, Caracu and Canchim cattle (Perotto et al., 200), giving rise to a breed Purunã. Purunã bulls are very well adapted to subtropical and tropical regions, and show good weight gain potential. Their carcass has better conformation and greater fat thickness than Zebu bulls. Genetics is the most important factor for fat deposition and composition. The detailed mechanisms of variation, however, is not fully understood, nor whether and how they can be manipulated. British breeds are well-known for their highly marbled meat, while the Zebu breeds contain less fat and more connective tissue (Rotta et al., 2009). In the warmer regions of Brazil, adapted breeds of cattle are primarily limited to *Bos indicus* cattle bred with Brazilian Nellore.

This study was conducted to evaluate the effects of generation in the frame of the same breed on the *Longissiums dorsi* muscle chemical composition and fatty acid profile of its intramuscular fat.

MATERIAL AND METHODS

Animals and management

The Committee of Animal Production at the State University of Maringá approved this experiment, which was carried out at the Experimental Farm of the Agronomic Institute of Paraná (Brazil) and followed the guiding principles of biomedical research on animals .

Thirty-nine bulls (13-PUR1, 13-PUR2 and 13-PUR) were selected, all resulting from industrial crossbreeding and belonging to the experimental herd of the Agronomic Institute of Paraná (IAPAR).

Diets

The animals were set for young finishing bulls in feedlot with an average age of 10 months, with an initial liveweight of 236 kg.

From birth to the age of 90 days, the calves followed their mothers in the annual winter pastures. After earty weaning (at 90 days of age), the calves were kept in pastures of *Hemarthria altissima* with concentrate supplementation (1.5 kg/animal/day of a mixture made up of 25% soyabean meal, 73% cracked maize, 2% limestone and urea).

The animals were kept separate in individual pens (5 m^2 for each animal), and fed twice a day. They were given access to a diet formulated to meet requirements for fattening beef cattle (NRC, 1996). The animals were fed maize silage *ad libitum* along with a concentrate made up of 25% soyabean meal, 73% cracked maize, and 2% salt, calculated as 1.2% of animal liveweight/day. The young bulls were weighed at the beginning of the experiment. Thereafter, they were weighed every 28 days, observing a 16-h fast of solids, accomplished by removing all feed at 4 p.m. on the day prior to weighing. Silage was provided *ad libitum*, with adjustments made according to the previous day's intake. Around 5 to 10% extra was left in the trough, in order not to limit intake. The experimental period lasted 180 days, during which the animals reached an average final liveweight of 464 kg. The development of the thickness fat cover was monitored every 28 days after a period of adaptation for the animals, using an ultrasound device (Aloka 500 with a Ust-5049-3.5 transducer). After reaching 4 mm cover fat thickness and an average age of 16 months, the animals were slaughtered.

Slaughter protocol, sampling and analysis

The animals were slaughtered at a commercial slaughterhouse 100 km away from the Lapa Research Farm, following the usual practices of the Brazilian beef industry. Thereafter, the carcasses were identified and weighed before chilling at 4°C for 24 h. After chilling, the right part of the carcass was used to determine the quantitative characteristics. Twenty-four h later, samples from *Longissimus dorsi* muscle were taken by a complete cross-section between the 12th and 13th ribs. The fat cover was discarded and the muscle sample was frozen at -20^{o}C for further analyses. Laboratory analyses of beef were carried out about four months after sampling. The samples were thawed at 0°C, ground, homogenized, and analysed in triplicate.

Beef moisture and ash contents were determined according to Cunniff (1998), crude protein according to the Kjeldahl method (Cunniff, 1998). Forage and beef intramuscular fat were extracted by the Bligh and Dyer method (1959) with a chloroform/methanol mixture. Fatty acids methyl esters (FAME) were prepared by triacylglycerol methylation according to ISO method 5509 (1978). All reagents and solvents used in the analysis were of analytical reagent quality and were purchased from Merck (Darmstadt, Germany).

Cholesterol analysis was carried out through direct saponification according to Al-Hasani et al. (1993). Cholesterol content was analysed in a 14-A gas chromatograph (Shimadzu, Japan), equipped with a flame ionization detector and a fused silica capillary column (25 m long, 0.25-mm internal diameter, and 0.20 μm Ohio Valley-30). Injector, column, and detector temperatures were 260, 280 and 280^{o}C, respectively. Ultra-pure gas fluxes (White Martins) of 1.5 ml min^{-1} H_2 as carrier gas, 30 ml min^{-1} N_2 as make-up gas, 300 ml min^{-1} synthetic gas, and 30 ml min^{-1} N_2 for flame were used. The ratio of the areas of cholesterol and 5-α cholestane was plotted against the cholesterol concentration for injected volumes of 0.0, 2.0, 3.0, 4.0 and 5.0 μl. The curve obtained was used for cholesterol analysis in mg 100 g^{-1}.

Fatty acids methyl esters (FAMEs) were analysed in a gas chromatograph (Varian, USA) equipped with flame ionization detector and fused silica capillary column CP-7420 Select FAME (100 m, 0.25 mm, and 0.25 μm film, Varian, USA). Fatty acids were identified by comparing sample relative retention times of FAME peaks with those of FAME standard-spiked samples (Sigma Chemical Co., St. Louis, MO, USA). The peak areas were determined by Star software (Varian).

Experimental design and statistical analysis

The experimental design with 3 treatments (PUR1, PUR2 and PUR) and 13 replications (animals) within each treatment was completely randomized. The

data were submitted to a one-way analysis of variance using SAS statistical software (2000), according to the following mathematical model:

$$Y_{ij} = \mu + t_i + e_{ij;}$$

were: Y_{ij} - observation of animal j, subjected to treatment i; μ - overall constant; t_i - treatment effect i - 1, 2, 3; e_{ij} - random error associated with each observation.

RESULTS AND DISCUSSION

Chemical composition. Moisture, ash, crude protein contents and total cholesterol were similar (P>0.05) among the three groups (Table 1). Average

Table 1. Chemical composition of the *Longissimus dorsi* muscle from young bulls of different genetic groups

Composition	Genetic groups			SE[4]	P < F
	PUR1[1]	PUR2[2]	PUR[3]		
Water, %	73.2	74.3	74.5	0.52	NS
Ash, %	1.01	0.97	0.96	0.02	NS
Crude protein, %	23.1	22.4	21.8	0.57	NS
Intramuscular fat, %	2.68[b]	3.20[b]	5.60[a]	0.64	0.05
Total cholesterol[5]	41.7	40.7	43.9	2.34	NS

[1]first-generation Purunã; [2] second-generation Purunã; [3]pure breed; [4]standart error; [5]mg/100g of muscle; NS - non-significant

moisture content was 74.0%, which is similar to other studies (Moreira et al., 2003; Prado et al., 2009; Rotta et al., 2009). Padre et al. (2006) found 73.7% of moisture in ½ Nellore vs ½ Aberdeen Angus. Variations in moisture percentages occur when there is a variation in lipid percentages in the *Longissimus dorsi* muscle. The higher percentage of intramuscular fat does the water get lower (Prado et al., 2008a,b).

The average ash percentage was 0.98%. Padre et al. (2006, 2007) observed ash percentages similar to those in this study. Thus, ash percentage is little influenced by diet or genetic group (Moreira et al., 2003; Padre et al., 2007). Ash is important to the supply of zinc and iron, which are of great nutritional importance to humans.

The average protein percentage in the *Longissimus dorsi* muscle was 23.7%. In the literature (Marques et al., 2006; Padre et al., 2007), there are reports of average crude protein percentages in *Longissimus dorsi* muscle varying between 21 and 24%. Thus, it can be concluded that the genetic group would not alter protein percentage in the muscles of bovines. Protein is important in meat, as it features a peptide known as carnitine, which plays an essential role in metabolism

by facilitating energy production from fat reserves. When ready for human consumption, 100 g of beef supply about 50% of the protein needs for a 60-kg adult (HSMO, 1994).

Intramuscular fat was greater (P<0.05) in the *Longissimus dorsi* muscle of animals from the PUR genetic group, in comparison to animals from the PUR1 and PUR2 genetics groups. However, there was no observed difference (P>0.05) in the *Longissimus dorsi* muscle of animals from the PUR1 and PUR2 genetic groups. In general, intramuscular fat in the *Longissimus dorsi* muscle of bulls finished in feedlot is close to 3% (Padre et al., 2006, 2007; Kazama et al., 2008). As such, the animals from the PUR genetic group featured levels over those recommended by the English Health Department (HMSO, 1994). However, the beef from these PUR specimens is considered very tender exactly because of their high lipid levels, as the two characteristics are proportionally similar.

The average cholesterol levels found were 42.1 mg/100 g of muscle. Higher values were obtained by Wheeler et al. (1987) while evaluating the cholesterol concentration in the *Longissimus dorsi* muscle of Chianina or Hereford vs Aberdeen Angus crossbreeds, for which they found a concentration of 63.3 mg/100 g of muscle. In that study, it was discovered that the cholesterol levels were highest in animals slaughtered at 455 kg (82.4 mg/100g of muscle). However, young cattle

Table 2. Fatty acid profile of the *Longissimus dorsi* muscle of young bulls of different genetic groups

Fatty acids	Genetic groups			SE[4]	P<F
	PUR1[1]	PUR2[2]	PUR[3]		
14:0	1.64[b]	2.43[a]	2.13[a]	0.19	0.05
14:1 *n*-7	0.15[b]	0.17[a]	0.19[a]	0.03	0.05
16:0	26.7[b]	29.4[a]	27.4[ab]	0.85	0.05
16:1 *n*-7	2.15	2.51	2.53	0.28	NS
17:0	0.59	0.61	0.55	0.05	NS
18:1 *t*-11	0.88	0.76	0.78	0.30	NS
17:1 *n*-9	0.49	0.48	0.51	0.05	NS
18:0	19.6	17.5	18.3	1.35	NS
18:1 *n*-9	40.9	40.6	42.9	1.15	NS
18:2 *n*-6	4.42	3.52	3.2	0.62	NS
18:3 *n*-6	0.13	0.14	0.17	0.05	NS
18:3 *n*-3	0.19	0.11	0.16	0.04	NS
CLA	0.17	0.18	0.25	0.04	NS
20:4 *n*-6	1.21	0.84	0.71	0.23	NS
22:0	0.32	0.22	0.27	0.09	NS
20:5 *n*-3	0.13	0.15	0.15	0.05	NS
22:5 *n*-3	0.14	0.13	0.12	0.01	NS
22:6 *n*-3	0.23[a]	0.17[b]	0.04[c]	0.05	0.05

[1] first-generation Purunã; [2]second-generation Purunã; [3]pure breed; [4]standart error; NS - non-significant

feature low total cholesterol levels in the *Longissimus dorsi* muscle (Padre et al., 2006, 2007; Kazama et al., 2008).

Fatty acid profile. Table 2 shows the fatty acid profile of the *Longissimus dorsi* muscle. Animals from the PUR2 and PUR genetic groups featured higher ($P<0.05$) percentages of 14:0 (myristic acid) and 14:1 *n*-7 as compared to specimens from the PUR1 genetic group. Furthermore, no difference was observed for these two fatty acids between PUR2 and PUR animals. The percentage of 16:0 (palmitic acid) was lower ($P<0.05$) in PUR1 and higher ($P<0.05$) in PUR2 animals. The percentage of 18:2 cis-9 trans-11 (conjugated linoleic acid - CLA) was similar ($P>0.05$) among genetic groups. The term CLA refers to a mixture of linoleic acid isomers, of which the 18:2 cis-9 trans-11 form is the one most commonly found in the meat of ruminants, and is important because of its many beneficial properties for human health, being anticarcinogen, antiatherogenic, antidiabetic (type II) and an immunomodulator (Bauman and Griinari, 2001).

Animals from the PUR1 genetic group featured a higher ($P<0.05$) percentage of 22:6 *n*-3 (cervonic acid - DHA). DHA is considered essential in the formation of nerve and eye tissues, in which it plays an especially important role in the early stages of development before and after birth, as well as on the mother's needs during gestation and lactation.

Among all fat components, the ones that offer the greatest health risks to humans are saturated fatty acids. However, not all fatty acids act in the same manner 14:0 (myristic acid), 16:0 (palmitic acid) and 17:0 (margaric acid) raise the levels of low density lipoprotein-cholesterol in the bloodstream.

As ruminant diets contain low fat concentrations, the majority of the adipose tissue is synthesized from lipogenesis. Fatty acids are elongated up to 18:0 and converted into 18:1 by desaturation. As the adipose tissue increases, the deposition of 18:1 content also increases, and that of 18:2 is reduced.

Oleic acid raises human HDL-cholesterol (high density lipoprotein) and lowers LDL-cholesterol (low density lipoprotein) concentration in the blood. Studies have demonstrated a strong positive relationship between LDL-cholesterol levels and human cardiovascular diseases, while HDL-cholesterol has an inverse relationship with the risk of cardiovascular diseases (Kwiterovich, 1997).

Table 3 shows that the percentages of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), *n*-6 and *n*-3 fatty acids, as well as PUFA:SFA and *n*-6:*n*-3 ratios for lipid in the *Longissimus dorsi* muscle were similar ($P>0.05$) among animals from the different genetic groups. Although the animal diet contained high levels of PUFA, the meat featured high values of SFA due to biohydrogenation in the rumen. The ratio of PUFA:SFA fatty acids plays an important role in reducing the risk of coronary heart disease; however, the optimal balance between these two classes of fatty acids is still a matter of debate (Hu, 2001).

Table 3. Proportion (%) of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), *n*-6 fatty acids, *n*-3 fatty acids, PUFA:SFA and *n*-6:*n*-3 ratios of the *Longissimus dorsi* muscle of young bulls

Fatty acids	Genetic groups			SE[4]	P<F
	PUR1[1]	PUR2[2]	PUR[3]		
SFA	48.3	48.8	46.7	1.13	NS
MUFA	44.6	46.9	44.5	0.84	NS
PUFA	6.50	6.17	7.40	0.47	NS
n-6	4.51[b]	4.08[b]	5.76[a]	0.63	0.05
n-3	0.57	0.47	0.68	0.09	NS
PUFA:SFA	0.13	0.13	0.15	0.01	NS
n-6:*n*-3	9.34	10.7	8.63	1.24	NS

[1]first-generation Purunã, [2] second-generation Purunã, [3] pure breed, [4]standard error, NS - non-significant

Laborde et al. (2001) recommend the intake of at most 11 g of saturated fatty acids, a minimum of 3.300 mg PUFA *n*-6 and at least 500 mg of PUFA *n*-3 per 1,000 kcal of energy.

The *n*-6:*n*-3 ratio recommended by HSMO (1994) is 4-10:1. The ratio obtained in this experiment was around 9:1, which meets the recommendations set by Laborde et al. (2001). Similar or higher values are generally observed in the meat of confined animals, while the opposite is found in animals raised in pasture systems (Padre et al., 2007).

The percentages of MUFA and PUFA at 45.3 and 6.69%, respectively, represent an important contribution to lower levels of bad cholesterol (LDL) and raising good cholesterol (HDL) (Kwiterovich, 1997).

The *n*-3 and *n*-6 fatty acids are precursors of eicosanoids (protaglandin, tromboxanes and leukotrienes), which act in controlling a variety of body functions, including blood pressure, heart rate, vascular dilation, blood coagulation, lipolysis and immunologic responses.

According to De Smet et al. (2003), the PUFA:SFA ratio is influenced mainly by genetics, particularly in the different adipose levels of the animal, with little effect caused by nutrition. The secondary effect of nutrition in the PUFA:SFA ratio is particularly important for ruminants, given that a part of the unsaturated fatty acids in their diet is hydrogenated in the rumen, in contrast to what happens in monogastric species.

However, the *n*-6:*n*-3 ratio may be influenced by the fatty acid composition in animal diets, with the inclusion of *n*-3 sources in the diet of animals increasing total *n*-3 content, along with a decrease in the deposition of intramuscular *n*-6 fatty acids; as the supply of *n*-6 is lowered, the *n*-6:*n*-3 ratio decreases. Ruminants finished on pasture systems have an *n*-6:*n*-3 ratio below two (Padre et al., 2003,

2007), while for ruminants finished in feedlots on a high grain diet, this ratio stays around 6 to 10 (Kazama et al., 2008).

CONCLUSIONS

Animals from the Purunã PUR1 and PUR2 genetic groups produced beef of high quality when compared to other genetic groups, in terms of chemical composition and fatty acid profile. Animals from the PUR genetic group featured higher lipid levels as compared to the other genetic groups.

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