Chemical composition, fatty acid profile and CLA levels in the *Longissimus* muscle of Caracu and Caracu *vs.* Charolais cattle

Composição química, perfil de ácidos graxos e níveis de CLA no músculo *Longissimus* de bovinos Caracu e Caracu *vs.* Charolês

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Abstract

This work was conducted in order to study the chemical composition, fatty acid profile, as well as the levels of *n*-3 and *n*-6 fatty acids and conjugated linoleic acid (CLA) present in the *Longissimus* muscle of Caracu and Caracu *vs.* Charolais genetic groups of cattle. This study was carried out at the Experimental Farm of the Agronomic Institute of Paraná, in southern Brazil. Twenty animals (10 Caracu – CAR and 10 Caracu *vs.* Charolais – CAC) were used, with an initial average age between 8 and 10 months. The young bulls were slaughtered at 450 kg and 18 months of age. The moisture and crude protein percentages were similar between the two genetic groups. However, the percentage of ash was higher in the CAC group. Conversely, total lipid levels was lower in the CAC group. The fatty acid profiles were similar for CAR and CAC bulls. Percentages of polyunsaturated fatty acids (PUFA) and *n*-6 were higher in the CAC group. The monounsaturated fatty acid (MUFA), saturated fatty acid, and *n*-3 percentages, as well *n*-6/*n*-3 and PUFA/MUFA ratios, were similar between CAR and CAC bulls. The 18:2 *n*-6, 18:2 *c* – 9 *t* – 11 and 20:3 *n*-6 contents (mg/g of total lipids) were higher in the CAR group.
Key words: CLA, fatty acids, *n*-3, *N*-6, young bulls

Resumo

Este trabalho foi realizado para estudar a composição química, perfil de ácidos graxos e a quantificação dos ácidos graxos *n*-3, *n*-6 e ácido linoléico conjugado (CLA) no músculo *Longissimus* de bovinos inteiros dos grupos genéticos Caracu e Caracu *vs.* Charolês. Este estudo foi realizado na Fazenda Experimental do Instituto Agronômico do Paraná. Foram utilizados vinte animais (10 – Caracu – CAR e 10 Caracu *vs.* Charolês – CAC) com idade inicial de 8-10 meses. Os animais foram abatidos com peso médio de 450 kg e 18 meses. A percentagem de umidade e proteína total foram similar entre os dois grupos genéticos. Entretanto, a percentagem de cinzas foi maior no grupo CAC. Ao contrário, a percentagem de lipídeos totais foi menor no grupo CAC. O perfil de ácidos graxos foi similar entre os animais CAR e CAC. A percentagem de ácidos graxos poliinsaturados (AGPI) e *n*-6 foi maior para animais do grupo CAC. As percentagens de ácidos graxos monoinsaturados (AGMI), ácidos graxos saturados (AGS), *n*-3 e as razões de *n*-6/*n*-3 e AGPI/AGS foram similar entre os animais CAR e CAC. A quantificação dos ácidos graxos 18:2 *n*-6, 18:2 cis 9 trans 11 and 20:3 *n*-6 (mg/g of total lipids) foi maior em animais do grupo CAR.
Palavras-chave*:* CLA, ácidos graxos, *n*-3, *N*-6, animais precoces

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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. From this total, about 30% (2.4 milliontons) are exported to several countries around the world (ANUALPEC, 2008).

Beef consumption ranks third worldwide, behind pork and poultry, mainly due to its higher cost as compared to other types of meat, larger space requirements for breeding in comparison with those required for swine and poultry, and the association of beef fat with human health problems.

The Caracu is a tropical criollo beef breed adapted to Brazil. Few studies have investigated the meat characteristics of Caracu, instead focused more on reproduction and performance (PEROTTO et al., 2000). Marbling and fatty acid composition of Caracu cattle, especially when compared to other pure breeds and crossbreds, remain little studied (DUCATTI et al., 2009; PRADO et al., 2009; ROTTA et al. 2009a). Genetic variability consists of differences between species, differences between breeds or lines, differences due to the crossing of breeds, and differences between animals within breeds. The latter source of variation can be estimated by heritability and genetic correlations. Breed effects may be influenced by segregation of the main genes, from which the double-muscled gene in cattle is generated. It is sometimes difficult to assess the real contribution of genetics to differences in meat quality. Breed comparisons are often confused by other effects like fat levels, live weight or age at slaughter and production system (PRADO et al., 2008a; b; c; d).

In recent years, there has been great interest in studies related to fatty acid composition in beef (PRADO et al., 2008a; b). Beef has excellent nutritional properties, with high levels of protein, vitamins and minerals (PADRE et al., 2006).

Different studies have demonstrated the beneficial effects of polyunsaturated fatty acids (PUFA), especially conjugated linoleic acids (CLA), which can be found in beef (ARICETTI et al., 2008). Some isomers in this acid, particularly cis-9,trans-11 and trans-10,cis-12, have been identified as having cancer-inhibiting (NOCI et al., 2005), arthrosclerosis-reducing (ROACH et al., 2002), antioxidant (SECKIN et al., 2005) and anti-diabetic (BAUMAN et al., 2000) properties.

Conjugated linoleic acid refers to a mixture of positional and geometric linoleic isomers, with two double bonds separated by a single bond. Furthermore, each double bond can be arranged in the *cis* or *trans* configuration (McGUIRE; McGUIRE, 1999). The main form present in ruminant products is the *cis 9*, *trans 11* CLA, known as rumenic acid (KRAMER et al., 1998). In terms of their benefits to human health, products containing CLA (considered nutraceutic, or functional foods) have anticarcinogens (especially rumenic acid) and anti-obesity properties (isomer *t*-10, *c*-12) (EVANS; BROWN; McINTOSH, 2002). These products also help prevent atherosclerosis, are antioxidants due to their conjugated double bonds, and contribute to prevent non insulin-dependent diabetes mellitus (SEBÉDIO; GNAEDING; CHARDIGNY, 1999).

The objective of this study was to evaluate the chemical composition of the *Longissimus* muscle (moisture, ash, crude protein and total lipids), fatty acid profile and the levels of *n*-3, *n*-6 and CLA fatty acids of Caracu and Caracu *vs.* Charolais cattle.

Materials and methods

Animal management and sampling

The State University of Maringá animal care and ethics committee approved the use of bulls in this study (COUNCIL FOR INTERNATIONAL ORGANIZATIONS OF MEDICAL SCIENCES-CIOMS, 1985).

This study was carried out at the Experimental Farm of the Agronomic Institute of Paraná, in southern Brazil. From birth to the age of 90 days,

the calves, who were born between June and September, the calves followed their mothers in the annual winter pastures. After weaning (at 90 days of age) until the end of March of the year following birth, the calves were kept in pastures of *Hemarthria altissima* with concentrate supplementation (1.5 kg/animal/day of a mixture made up of 25% soybean meal + 75% cracked corn). Starting in April, the animals were divided into two genetic groups. Twenty (10 Caracu – CAR; 10 Caracu *vs.* Charolais – CAC) with an initial average of 8 – 10 months old were used. The set for young bulls were kept in feed-lot until reaching the slaughter weight of ± 450 kg.

The animals were kept separate in individual pens (8 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 (NATIONAL RESEARCH COUNCIL-NRC, 1996).

The animals were weighed in the beginning of the study and every 28 days, and on the day before slaughter, after 12-hour fasting.

The animals were stunned using a captive bolt stunner. Next, they were bled through exsanguination by cutting the neck vessels, and removal of the head, hide, viscera, tail, legs, diaphragm and excess internal fat. Afterward, the carcass was divided medially from the sternum and spine, resulting in two similar halves, which were weighed to calculate hot carcass weight. Next, the half-carcasses were washed, identified and stored in a cold chamber at 4ºC, where they remained for a 24-hour period. Twenty-four hours later, after chilling, LM samples were taken between the 12th and 13th ribs, according to the method by Hankins e Howe (1946), adapted by Müller (1980). The samples were identified and stored in closed plastic bags, then immediately taken to the Food Analysis Laboratory of the Chemistry Department at the State University of Maringá, where they were frozen at –18ºC for later analysis. At the start of the analyses, the meat samples were unfrozen at room temperature and homogenized using a meat grinder. Next, tests were conducted to analyze the levels of moisture, ash, crude protein, total lipids and fatty acid profile.

Chemical composition

Beef analyses were carried out in laboratory conditions two months later. The samples were unfrozen at room temperature (20°C), ground, homogenized and three replications were used to estimate the traits.

Beef moisture and ash contents were determined according to AOAC (CUNNIF, 1998); crude protein was obtained through the Kjeldahl method (CUNNIF, 1998); total lipids were extracted by the Bligh e Dyer method (BLIGH; DYER, 1959) using a mixture of chloroform/methanol; fatty acid methyl esters (FAME) were prepared by the methylation of triacylglycerol according to the ISO method (INTERNATIONAL ORGANIZATION FOR STANDARTIZATION-ISO, 1978). Cholesterol was evaluated using the modified method of Rowe et al. (1999). A water solution with 60%-potassium hydroxide (w/v) was added into samples in quantities of 2 mL h^{-1} of reflux. The residual component was dissolved again in 2 mL of hexane containing 0.2 mg mL^{-1} 5-α cholestane internal standard (IS) (Sigma, USA).

Fatty acid methyl esters analysis

Fatty acid methyl esters (FAMEs) were analyzed in a gas chromatograph (Varian, USA) equipped with a flame ionization detector and a fused silica capillary column CP-7420 (100 m, 0.25 mm, and 0.39 μm o.d., Varian, USA) Select Fame. Column temperature was programmed at 165 °C for 18 min, 180 ºC (30 °C min^{-1}) for 22 min, and 240 ºC (15 ºC min^{-1}) for 30 min at 45 psi. The injector and detector were kept at 220 ºC and 245 ºC, respectively. The gas flows (White Martins) used were: carrier gas (H_2), 1.4 mL min^{-1}; make-up gas (N_2), 30 ml min^{-1}; H_2 and synthetic flame gas, 30 mL min^{-1} and 300 ml min^{-1}, respectively. Sample injection split mode was 1:80.

Fatty acids were identified by comparing relative FAME peak retention times of samples and fatty acids methyl ester standards from Sigma (USA) by spiking samples with standards. The peak areas were determined by Star software (Varian). The data were expressed as percentages of fatty acid normalized area (ROWE et al., 1999; MILINSK et al., 2005).

Concentrations of n-6, n-3 and CLA

Polyunsaturated fatty acids were measured in mg/g of total lipids, through internal standardization and using fatty acid methyl ester standards. Tricosanoic acid methyl acid (23:0, methyl- tricosonoate) was used as the internal standard.

The calculations were conducted according to the methodology of Joseph e Ackman (1992), as shown in the equation:

$$AGPI\ (mg/g) = [(Ax \times Mp \times Fcx) / (Ap \times M_A \times Fc)]$$

In which:

Ax = PUFA area;

Ap = Internal standard area;

Fcx = PUFA theoretical correction factor;

Mp = Mass of the internal standard added to the sample, in mg;

M_A = Massa of the total lipid sample, in g;

Fc = Conversion factor to express the results in mg of fatty acids per g of total lipids, based on PUFA methyl esters.

Experimental design and statistical analysis

The experiment design consisted of 2 treatments: 10 Caracu – CAR; 10 Caracu *vs.* Charolais – CAC. The data were submitted to an analysis of variance (F-test), using SAS Statistical Software (SAS INSTITUTE INC., 2000), according to the following mathematical model:

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

In which:

Y_{ij} = observation of animal j, subjected to treatment i;

μ = overall constant;

t_i = treatment effect i = 1 and 2;

e_{ij} = random error associated with each observation.

Results and discussion

Chemical composition

The percentages of moisture were similar (P>0.05) in the *Longissimus* muscle of Caracu (CAR – 74.2%) and Caracu *vs.* Charolais (CAC – 74.7%) genetic groups (Table 1). Padre et al. (2006, 2007) found lower values (72.8%) in Nellore *vs.* Aberdeen Angus crossbred cattle. In general, moisture levels vary little as functions of genetic group (MOREIRA et al., 2003).

Table 1. Chemical composition of the *Longissimus* muscle of animals from the CAR breed and CAC genetic group, finished in feedlot.

Parameters	CAR	CAC
Moisture, %	74.2 ± 0.73	74.7 ± 1.09
Ash, %	0.96 ± 0.04[b]	1.13 ± 0.06[a]
Crude protein, %	21.4 ± 0.59	22.2 ± 0.72
Total lipids, %	2.68 ± 0.15[a]	1.66 ± 0.26[b]

Letters in the same line differ by the F-test (P<0.05).

The percentage of ash was higher (P<0.05) in animals from the CAC genetic group (1.13%) as compared to CAR breed (0.96%) (Table 1).Ash levels show little variation as a function of diet, handling, age, gender and genetic group (MOREIRA et al., 2003; PADRE et al., 2006, 2007; KAZAMA et al., 2008). The lower percentage of ash observed in the *Longissimus* of CAR cattle is related to the higher fat levels in these animals. Because it is hydrophobic, fat features only trace amounts of ash (ENSER et al., 1998).

The percentages of crude protein were similar (P>0.05) for both CAR (21.4%) CAC (22.2%) animals. Crude protein percentages show little variation as a function of diet, handling, age, gender and genetic group (MOREIRA et al., 2003; PADRE et al., 2006, 2007; KAZAMA et al., 2008).

CAR animals featured a higher (P<0.05) percentage of total lipids in the *Longissimus* muscle (2.68%) when compared to animals from the CAC genetic group (1.66%). Total lipid percentage is the parameter that has the greatest variation as a function of diet, handling, age, gender and genetic group (MOREIRA et al., 2003; PADRE et al., 2006, 2007; KAZAMA et al., 2008). The lowest percentage of total lipids observed in animals from the CAC genetic group could be related with the breeds that make up this genetic group (Charolais). Charolais breed animals slaughtered at an early age and light weight (450 kg) feature low levels of total lipids in the *Longissimus* muscle (DUCATTI et al., 2009; PRADO et al., 2009a; PRADO et al., 2009b; ROTTA et al., 2009). Charolais cattle and their crossbreeds should be slaughtered at a weight over 550 kg in order to feature a high level of total lipids in the *Longissimus* muscle, which characterizes marbling in beef (ABRAHÃO et al., 2005).

Nevertheless, total lipid levels observed in both groups are within the maximum level regarded as acceptable for the prevention of diseases related to fat content in beef, according to recommendations from the English Health Department (HMSO, 1994).

Fatty acid profile

The fatty acids with the highest levels were C 16:0 (palmitic acid – 25.4%), C 18:0 (stearic acid – 20.4%) and C 18:1 *n*-9 (oleic acid – 35.0%) (Table 2). In ruminants in general, these are the predominant fatty acids (MOREIRA et al., 2003; PADRE et al., 2006, 2007; KAZAMA et al., 2008). The fatty acid profiles were similar for the *Longissimus* of CAR and CAC animals – with the exception of fatty acid C 23:0 (tricosanoic acid), which has higher (P<0.05) in animals of the CAC genetic group (0.28%) as compared to CAR animals (0.13%). However, the level of this fatty acid has very little influence in human health.

As demonstrated in several different studies, the fatty acid profile of the *Longissimus* muscle is little influenced by breed (WOOD et al., 2003) age (OWENS; DUBESKI; HANSON, 1993), gender (PADRE et al., 2006), diet (KAZAMA et al. 2008), handling (MARQUES et al., 2006), or finishing system (MOREIRA et al., 2003).

Table 2. Fatty acid profile of the *Longissimus* muscle of animals from the CAR breed and CAC genetic group, finished in feedlot.

Fatty acids	CAR	CAC
C 14:0	2.53 ± 0.25	2.21 ± 0.97
C 14:1 *n*-7	0.35 ± 0.10	0.31 ± 0.14
C 15:0	0.41 ± 0.07	0.36 ± 0.08
C 15:1 *n*-7	0.26 ± 0.04	0.23 ± 0.06
C 16:0	25.6 ± 1.46	25.3 ± 2.52
C 16:1 *n*-7	2.45 ± 0.44	2.55 ± 0.34
C 16:1 *n*-9	0.55 ± 0.09	0.51 ± 0.11
C 17:0	0.89 ± 0.13	0.78 ± 0.14
C 17:1 *n*-7	0.612 ± 0.11	0.66 ± 0.07
C 18:0	21.4 ± 2.10	19.5 ± 2.40
C 18:1 *t*-9	0.29 ± 0.05	0.95 ± 0.70
C 18:1 *n*-9	34.7 ± 1.51	35.3 ± 4.09
C 18:1 *n*-7	0.94 ± 0.15	0.89 ± 0.12
C 18:2 *t*-6	0.25 ± 0.11	0.31 ± 0.10
C 18:2 *n*-6	3.84 ± 1.26	5.07 ± 1.62
C 18:3 *n*-6	0.07 ± 0.01	0.10 ± 0.07
C 18:3 *n*-3	0.19 ± 0.02	0.18 ± 0.05
C 20:0	0.39 ± 0.14	0.38 ± 0.08
C 18:2 *c*-9 *t*-11	0.33 ± 0.03	0.37 ± 0.04
C 18:2 *t*-10 *c*-12	0.09 ± 0.01	0.09 ± 0.02
C 20:1 *n*-9	0.14 ± 0.005	0.16 ± 0.04
C 20:3 *n*-6	0.14 ± 0.04	0.21 ± 0.07
C 22:0	0.98 ± 0.30	1.31 ± 0.31
C 20:5 *n*-3	0.09 ± 0.06	0.08 ± 0.03
C 23:0	0.13 ± 0.07[a]	0.28 ± 0.13[b]
C 22:2 *n*-6	nd	nd
C 24:0	nd	nd
C 24:1 *n*-9	0.09 ± 0.04	0.15 ± 0.09
C 22:6 *n*-3	0.06 ± 0.01	0.09 ± 0.04

Letters in the same line differ by the F-test ($p < 0.05$).

The percentages of polyunsaturated fatty acids (PUFA) and *n*-6 were higher ($P<0.05$) in animals from the CAC genetic group (6.78 and 5.77%, respectively) in comparison to CAR breed animals (5.20 and 4.39%, respectively) (Table 3). The average PUFA level observed was similar to literature (PADRE et al., 2006, 2007; KAZAMA et al., 2008). Similarly, the percentage of *n*-6 is close to results found in cattle, regardless of diet and genetic group (MOREIRA et al., 2003; MARQUES et al., 2006; KAZAMA et al., 2008).

The levels of monounsaturated fatty acids (MUFA), saturated fatty acids (SFA), *n*-3 and the *n*-6/*n*-3 and PUFA/SFA ratios were similar ($P>0.05$) between CAR and CAC animals (Table 3).

As ruminant diets contain low fat concentrations, the majority of the adipose tissue is synthesized from lipogenesis. Fatty acids are elongated up to C

18:0 and then converted into C 18:1 by desaturation (RULE; MACNEIL; SHORT, 1997). As the adipose tissue increases, the deposition of C 18:1 content also increases and that of C 18:2 is reduced.

Oleic acid raises human HDL-Cholesterol (High Density Lipoprotein) and lowers LDL-cholesterol (Low Density Lipoprotein) concentrations in the blood (KATAN; ZOOCK; MENSINK, 1994). Studies demonstrated a strong relationship between LDL-cholesterol levels and human cardiovascular diseases, and an inverse relation of HDL-cholesterol with the risk of cardiovascular diseases (KWITEROVICH, 1997).

The average PUFA/SFA ratio was 0.12; this value is below 0.40, the limit recommended by the English Health Department (HMSO, 1994). This PUFA/SFA ratio has been significant for health care, because it reduces the risk of coronary diseases, although the optimal ratio has been a matter of debate (HU, 2001).

The *n*-6 and *n*-3 fatty acids have a significant role in reducing the risk of coronary heart disease, but it is still a matter of debate (HU, 2001).

Table 3. Percentage of fatty acids in the *Longissimus* muscle of animals from the Caracu (CAR) breed and from Caracu *vs.* Charolais (CAC) genetic group.

Parameters	CAR	CAC
Polyunsaturated fatty acid	5.20 ± 1.27[b]	6.78 ± 1.67[a]
Monounsaturated fatty acid	40.4 ± 1.59	41.7 ± 4.17
Saturated fatty acid	52.14 ± 2.61	49.8 ± 3.64
n-6	4.39 ± 1.27[b]	5.77 ± 1.66[a]
n-3	0.39 ± 0.07	0.55 ± 0.06
n-6/*n*-3[6]	0.09 ± 0.04	0.10 ± 0.03
AGPI/AGS[7]	0.10 ± 0.03	0.14 ± 0.04

Letters in the same line differ by the F-test ($p < 0.05$).

Concentration of n-3, n-6 and CLA fatty acids

The concentrations of C 18:2 *n*-6 and C 20:3 *n*-6 fatty acids in the *Longissimus* were higher ($P<0.05$) in animals of the CAR breed in relation to animals from the CAC genetic group (Table 4). However, the concentration of C 18:3 *n*-6 and C 20:5 *n*-3 were similar ($P>0.05$) between both genetic groups.

Animals from the CAC genetic group (1.72 mg/g total lipids) featured higher ($P<0.05$) concentrations of C 18:2 *c*-9 *t*-11 (conjugated linoleic acid – CLA) as compared to CAR breed animals (1.31 mg/g total lipids) (Table 4). PADRE et al. (2007) reported 9.20 mg/g total lipids in steers finished in pasture systems. The genetic group can affect the concentration of CLA (MIR et al., 2004). Nevertheless, the most important factor in altering CLA concentration is diet. Animals finished in pasture systems show higher concentrations of CLA in the *Longissimus* muscle when compared to animals finished in feedlot (PADRE et al., 2007). The diet of animals finished in pasture is richer in CLA *c*-9, *t*-11 if compared to that of animals finished in feedlot (MENDONZA et al., 2005). The animals in the present experiment were finished in feedlot; this could therefore be an explanation for the low concentration of CLA in the *Longissimus* muscle of these animals.

CLA *c*-9, *t*-11 is the predominant CLA isomer. Padre et al. (2006), while evaluating the LM of steers finished in pasture systems, found 58.89% of total CLA as CLA *c*-9, *t*-11. Mendonza et al. (2005) evaluated the LM of different Zebu crossbreeds with European breeds fed *Brachiaria* spp., and observed a CLA content of 74.4% for the *c*-9, *t*-11 CLA isomer.

Table 4. Concentration of polyunsaturated fatty acids in the *Longissimus* muscle of animals from the Caracu (CAR) breed and from Caracu *vs.* Charolais (CAC) genetic group.

Fatty acids	CAR (mg/g of TL)	CAC (mg/g of TL)
C 18:2 *n*-6	59.5 ± 7.54[a]	51.8 ± 12.2[b]
C 18:3 *n*-6	1.17 ± 0.32	1.09 ± 0.08
C 18:2 *c*-9 *t*-11	1.31 ± 0.73[b]	1.72 ± 0.20[a]
C 20:3 *n*-6	3.13 ± 0.81[a]	2.57 ± 0.50[b]
C 20:5 *n*-3	2.81 ± 0.84	2.81 ± 0.51

Letters in the same line differ by the F-test ($p < 0.05$).

Conclusion

Crossbreeding has been used as a tool for raising performance and improving feed efficiency in animals. On the other hand, meat quality also depends on genetics. However, the crossbreeding between two Continental breeds (Charolais *vs.* Caracu) had little influence on the chemical composition, fatty acid profile CLA levels in the *Longissimus* of young bulls.

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