Intramuscular Fatty Acid Composition of the Longissimus Muscle of Unweaned Minhota Breed Calves at Different Slaughter Age

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Received on: 15 May 2019
Revised on: 27 Aug 2019
Accepted on: 31 Aug 2019
Online Published on: Mar 2020

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Online version is available on: www.ijas.ir

ABSTRACT

Meat productions from sixteen local Portuguese cattle breeds represent high economic and cultural value for local populations. Among these, Minhota is one of the most important on meat aptitude located on the northwest of the country. This breed is used for high-quality meat. This study describes the influence of slaughter age, corresponding veal (6 months) and beef (9 months) and sex, reared in a traditional production system, on intramuscular fatty acid composition of the longissimus muscle and nutritional quality. Samples of the longissimus thoracis (LT) muscle were analysed for fatty acid composition of intramuscular fat from 41 Minhota breed animals. Unweaned veal (22) and beef (19), corresponding to males (n=25) and females (n=16) were analysed. Both groups of animals were reared in a traditional production system. The meat from the Minhota breed is a valuable source of polyunsaturated fatty acid (PUFA), particularly the C-20 and C-22 n-3 fatty acids in the human diet. A low n-6:n-3 ratio show that fat in this breed has a good nutritional value. The differences in fat composition from veal and beef could be attributed to the fact that maternal suckling is more important in the youngest animals.

KEY WORDS beef, fatty acids, healthy nutrition, meat quality, veal.

INTRODUCTION

Consumers are increasingly aware of the relationships between diet, health and well-being resulting in choices of foods that are healthier and more nutritious (Hocquette et al. 2012). Recent research in food fat composition has been one of the most studied topics in human diet (Billingsley et al. 2017). Long chain omega-3 polyunsaturated fatty acids, such as eicosapentaenioc acid (EPA) (20:5, n-3) and docosahexaenoic acid (DHA) (22:6, n-3), have beneficial effects over other fatty acids present in diets for the maintenance of long term health (McAfee et al. 2010). Beef and other ruminant products are important dietary sources of conjugated linoleic acid (CLA) of which the most prominent is cis-9, trans-11 isomer, which has been identified to contain a range of health promoting beneficial properties (Salter, 2013; Royan and Navidshad, 2015). Beef lipids also contain trans fatty acids (TFA) of which the
most dominant is trans vaccenic acid (TVA; trans-11 18:1). There is much interest in TFA produced by ruminants (RTFA) with an emphasis on potential protective effect against the development of coronary heart diseases, as distinct to industrial trans fatty acids (ITFA) (Salter, 2013). Hence considerable effort has been devoted to improving the fatty acid composition of beef. The fatty acid (FA) composition and content can be highly influenced by breed, gender, age at slaughter and feeding management of ruminants. Many studies have been done to determine the influence of feeding systems on the nutritional value of intramuscular fat content (Moreno et al. 2007; Alfaia et al. 2009; Muchenje et al. 2009a; Cafferky et al. 2019). These authors also pointed out that a positive feature of grass feeding increase the nutritionally important long chain PUFA i.e. EPA and DHA. Veal is widely prized by consumers for its excellent nutritional value. Calf meat is generally obtained from young (immature) bovine animals up to eight months of age or in some cases from older animals, but no more than twelve months. There are several calf production systems, but in terms of consumer health the most beneficial meat is obtained from animals reared on pasture with natural suckling Domaradzki et al. (2017).

Minhota is a local cattle breed, which is also called ‘Galega’ by some authors, and is found in the region of Minho and Douro in Norwest Portugal, an important agriculture and forestry region in Portugal. This breed resulted from the Gelbvieh and Galician Blond cross breeding between 1970 and 2000. As a result, Minhota cattle presents a short history and was recognized as Portuguese local breed in 1996, when the Associação Portuguesa dos Criadores de Raça Minhota (APACRA), i.e. “Portuguese Association for the Minhota Breed Farmers” was founded. In 1997, this breed entered at the Genealogical Book (Araújo, 2011).

Minhota is an indigenous breed that is reared using traditional feeding and management system, whose main use is to produce meat. The livestock production system of this breed mainly involves small family farms, using indoor systems or traditional grazing. While in former times traction force, milk and meat production were the main reasons for farmers keeping Minhota cattle. Most studies on improving meat quality have been conducted in high input large-scale production systems and more studies needed to be evaluated in low input systems (Muchenje et al. 2009b).

In this work this breed reared in a traditional low input system was studied. The sale of cattle for slaughtering is carried out between the age of 5-7 months (150 kg of carcass weight) and 8-10 months (195 kg of carcass weight) (Araújo, 2006), which is the product most sought by industry, butchers and supermarket retailers (Araújo et al. 2016).

The production of unweaned young calves, involves indoor management in which calves are fed with maternal suckling, grass hay and complementary concentrate-based diet (Araújo, 2006). Several studies have been published on the effect of sex and slaughter age on the carcass and muscle organoleptic parameters of this breed (Araújo, 2006; Araújo, 2011; Araujo et al. 2016). Nevertheless, there is a lack of information about FA composition of the muscle. Likewise, there is scarcity of studies on the FA composition in suckled calves slaughtered at early age for most of the cattle breeds (Moreno et al. 2006; Moreno et al. 2007; Bispo et al. 2010), although abundant information on the FA profile is available for steers slaughtered at larger ages.

The aim of this study was to provide information concerning the meat fatty acid composition and nutritional quality in veal and beef of male and female animals, in Minhota cattle reared in a traditional production system.

MATERIALS AND METHODS

Animal management

Forty-one unweaned pure Minhota calves aged 12 months or less, 25 males and 16 females were used from the Northwest region of Portugal. These animals were classified as veal, V (14 males and 8 females) and beef, Z (11 males and 8 females), according EC Regulation (2007). This regulates the categories of animals being V, animals aged 8 months or less and Z, animals aged more than 8 months but not more than 12 months. The animals in this study were born between August and May and were slaughtered between April and November on accredited abattoirs in the north-west of Portugal. Hot carcass weight after 45 min post mortem and cold carcass weight after the refrigeration period (24 h at 4°C) was obtained.

Calves came from 22 private farms and were reared using a traditional intensive system with indoor management. All the calves were in pens separated from their mothers. They suckled twice a day until the slaughter. From the first few weeks, animals were supplemented with farm products like grass hay ad libitum with commercial concentrate (see composition in Table 1), until slaughter. Although, all animals have a similar feeding system, it is relevant to emphasize that the maternal suckling is less important in older animals because mothers have less milk production. Mothers have also an indoor management and were fed with fresh grass, corn silage and commercial concentrate, depending of the farm management.

Samples and measurements

The animals were weighted before being slaughtered conventionally at a commercial abattoir. Carcasses were weighted and classified with respect to fat and conformation scores according to the European Union grading system and chilled (0-4°C) for 24 h before sampling.
Samples of the longissimus thoracis (LT) muscle from the left half of the carcass were collected from the 10th rib at 24 hours post mortem. Cores from the central part of the muscle with no visible adhering subcutaneous adipose tissue was analysed for fat and fatty acid composition. Contents of intramuscular fat were determined with near infrared spectrophotometer (Foss Tecator NRS 6500, Denmark), in duplicate as reported by Moreno et al. (2007).

**Preparation of fatty acid methyl esters**

Muscle samples were trimmed of residual adipose tissue. After blending in a commercial grinder, samples were individually packed under vacuum (97%) and stored frozen at -20°C. LT samples were thawed and homogenized using Heidolph Diax 900 processor (Heidolph-Instruments GMBH and COKG, Schwabach, Germany). Intramuscular lipids were extracted from 50 g of homogenised muscle, following the Bligh and Dyer (1959) method as described by Moreno et al. (2007).

Intramuscular lipids of LT were frozen at –20°C and their total fatty acids (TFA) were extracted and converted into fatty acids methyl esters by transesterification with sodium methanolate (1 M) and BF3-methanol (14% vol/vol) at 20°C.

Fatty acid analyses were performed according to ISO 5508 (1990) and the following methyl esters of butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, tridecanoic acid, myristic acid, myristoleic acid, pentadecanoic acid, pentadecanoic acid, palmitic acid, palmitoleic acid, heptadecanoic or margaric acid, heptadecenoic or margaroleic acid, stearic acid, elaidic acid, oleic acid, transvaccenec acid, linolelaidic acid, linoleic acid, arachidonic acid, a-linolenic acid, eicosapentanoic acid, a-linolenic acid, conjugated linoleic acid, eicosadienoic acid, behenic acid,dihomo-α-linolenic acid, eicosatrienoic acid, arachidonic acid, lignoceric acid, eicosapentaenoic acid, nervonic acid, docosahexaenoic acid were quantified.

**Gas-liquid chromatography analysis of fatty acid methyl esters**

TFA were achieved by GC using a Varian 3900 (Varian, USA) gas chromatograph equipped with a flame ionization detector (FID) and a silica capillary column (Supelco SP2560, 100 m×0.25 mm, 0.25 μm). Helium was used as the carrier gas (1 mL/min), in conditions of split injection (1:20). Injector and detector temperatures were 250 °C and 260 °C, respectively. The oven temperature, to analyze total fatty acids and cis isomers, was programmed at 70 °C for 4 min, then increased from 70 to 110 °C at 8 °C/min and increased from 110 to 170 °C at 5 °C/min, held at 170 °C for 10 min, increased from 170 to 240 °C at 3 °C/min and finally held at 240 °C for 15 min. Identification of individual FA was based on comparison of their retention time with commercial FA standardmixtures (FAME Mix C4-C24, Supelco; methyl oleate 99%, Sigma). Fatty acids were quantified using the internal standard after adjusting for response determined using the Sigma–Aldrich standard mixtures as reported by Moreno et al. (2006).

**Statistical analysis**

For the assessment of fatty acid composition in Minhota bovine breed, ANOVA using the general linear model using XLSTAT 2014.4.10 add-in (Addinsoft, Paris, France) for Microsoft Excel 2010 (Microsoft, Redmond, United States of America) was performed for all variables considered in the study.

Effects of sex and slaughter age were included in the model; however, the season of birth was not included because no significant differences were found in relation to any of the variables studied:

\[
Y_{ij} = \mu + B_i + F_j + (B \times F)_{ij} + e_{ij}
\]

Where:
- \(Y_{ij}\): observation of the dependent variable.
- \(\mu\): overall mean.
- \(B_i\): effect of sex (i=1, 2).
- \(F_j\): effect of the slaughter age (j=1, 2).
- \((B \times F)_{ij}\): interaction term of sex and age of slaughter.
- \(e_{ij}\): residual random error associated with the observation.

**RESULTS AND DISCUSSION**

**Carcass parameters and beef chemical composition**

Significant differences (P<0.001) were obtained in slaughter weight between bovines V, 6 months and Z, 9 months of age (Table 2). These results are in agreement with Moreno et al. (2008) study that showed higher weight values as the slaughter age of cattle increased.
In addition, Araújo et al. (2016) also observed higher carcass weight in Minhota breed, slaughtered at 9 months compared to 6 months. In general, dairy and local breeds produced lighter weight carcases than specialized beef breeds (Alberti et al. 2008).

Significant differences (P<0.01) were achieved in dressing percentage between males and females, 55.77% vs. 52.34%, respectively. Monserrat et al. (1998) and Carballo (2003) also obtained differences in this indicator between sexes on Galega breed, but no significant differences (P>0.05) were observed between males and females on Avileña-Negra Ibérica breed (Daza et al. 2014). On this study yields obtained for male, 55.8% (Table 2), were similar to those observed by Arthur et al. (1995) who found dressing percentages of 55.7%, 56.6% and 55.6% for Angus, Hereford and Charolais breeds, respectively. However, Piedrafita et al. (2003) observed higher percentages, above 60%, in Asturiana de los Valles, Bruna dels Pirineus, Pireneus and Gasconne breeds. Alberti et al. (2008) found that dairy and local breeds had lower percentages; the lowest for Jersey, 50.1%, whereas the specialized breeds, Charolais, Pirenaica, Asturiana de los Valles, Piemontese and Limousin, had values over 60%. Sharifi et al. (2015), noticed dressing percentages between 51.3% and 55.1% on Holstein. Evidently, comparisons should be made with carefullness because the use of genetically selected animals, different rearing systems with or without intensive finishing period or differences in carcass measurements could also influence yields.

Fat content of longissimus thoracis muscle

The results of the fatty acid composition of muscle are presented as the percentage by weight of total fatty acids in Table 3. Two effects were studied, animal age and sex and it was not observed interaction between these in the thirty fatty acids analysed, P > 0.05 (Table 3).

There were significant differences in the percentages of all n-3 fatty acids and almost all n-6 fatty acids when comparing the animals with 6 and 9 months age (Table 3), with higher values found in the younger animals. From Table 4 we can be observed a P < 0.01 for the total polyunsaturated n-3 fatty acids and P= 0.09 for the n-6 fatty acids with significant differences in C18:2n-6 and C20:4n-6.

Concerning the conjugated linoleic acids, the predominant CLA in ruminant fats is the cis-9, trans-11 isomer, that accounts for more than 80% of total CLAs (Chin et al. 1992). Trans-unsaturated fatty acid CLA isomer cis-9, trans-11 content has no significant differences with age (Table 3).
The only source of CLA in ruminant intramuscular fat is the plant linoleic and α-linolenic acid which can be transformed to CLA by bacteria in the rumen (Kepler et al. 1966), probably because all groups of animals from this study had the same feeding system, no differences were observed. The trans vaccenic acid (trans C18:1 t11, TVA), another trans-unsaturated fatty acid, was found in Minhota breed with significant differences between the two age groups, also with higher values for the younger animals (Table 3).
TVA is a trans fatty acid that increases bad cholesterol in serum (Judd et al. 2002), however, the conversion to CLA is a benefit for human health.

Analysing each FA individually, for male vs. female calves, there are significant differences in fourteen fatty acids. Observing the n-3 and n-6 FAs values (Table 3), there are higher values for male than female. This last remark also occurs with the essential fatty acid, α-linolenic acid (18:3 n-3), which is 53% higher in the muscle of male compared to female.

This important α-linolenic acid PUFA is present in many concentrate feed ingredients but at lower levels than 18:2 n-6 (Wood et al. 2008). In Minhota breed, male reveals the highest balance in the ratio of linoleic:linolenic fatty acids (18:2 n-6: 18:3 n-3), which is 5.7 for male and 7.6 for female.

**Fatty acid ratios and meat quality**

The ratio of n-6:n-3 polyunsaturated fatty acids (PUFA) in primitive man was (1:1) compared with current diets (10:1). This imbalance is thought to be involved in several of so-called “diseases of Western Civilization” as it is a risk factor in cancers and coronary heart disease, especially the formation of blood clots leading to a heart attack (Enser et al. 1996; Enser, 2001), and the recommendation is a ratio less than 4 in n-6:n-3 PUFA (Wood et al. 2003). The contribution of meat to the supply of dietary polyunsaturated C20 and C22 PUFA is an important dietary source since for many people meat is the only source of C20 and C22 n-3 PUFA (Enser et al. 1996). In this study a favourable n-6:n-3 PUFA ratio from a dietetic and health point of view was present in all Minhota bovine breed groups, when considering the two essential fatty acids together with C20 and C22 PUFA (Table 4).

The contribution of C20 and C22 PUFA to balance the ratio n-6:n-3 PUFA is patent in meat from this breed, where n-6:n-3 PUFA ratios fluctuate from 1.59-2.25 in all groups, which is well below the recommended ratio. Comparing this ratio with the Arouquesa, a local breed from the north region of Portugal, the values are similar, 1.95-2.28 (Pestana et al. 2012).

Other portuguese breeds, have very distinct values, between 1.58 and 14.9 for the longissimus muscle (Alfiaia et al. 2006a; Alfiaia et al. 2006b; Alfiaia et al. 2007a; Alfiaia et al. 2007b). In this study all groups of animals are unweaned, reared with indoor management and their mothers reared in an extensive system, which also could influence the good result for the n-6:n-3 PUFA ratio.

Other possible reasons for the different ratio values between these breeds could be the dimension of the herd, where breeds like Minhota, Arouquesa, Barrosã and Mirandes, are small herds, where more care can be given to the unweaned calves receiving the mothers milk, the other two breeds, Alentejana and Merolenga, which have the highest ratio values, belong to large herds, and consequently, the calves are not receiving the same attention. Also, the animals from these two last breeds are slaughtered with at age above sixteen months.

From Table 3, there are significant differences in cis n-3 PUFA between the animals from the two age groups. It comes to evidence that the differences in C20:3 n-3, C20:5 n-3 and C22:6 n-3 indicate that veal animals present higher values. These differences are more relevant in n-3 PUFA than in n-6 PUFA, where only the arachidonic acid (C20:4 n-6) is significantly different between the two age groups.

This observation could be attributed to the fact that maternal suckling is more important in veal animals than in beef ones.

This investigation has determined that the ratio of PUFA to saturated fatty acids (PUFA:SFA) in Minhota breed was between 0.1-0.2 (Table 4) which is below to nutritional advice (Scollan et al. 2006) which recommends a ratio > 0.4. These PUFA:SFA ratio values are in agreement with the obtainable in cattle, e.g. 0.11 (Enser et al. 1996), 0.19-0.25 in Barrosa (Alfiaia et al. 2007a), 0.17-0.21 in Arouquesa (Alfiaia et al. 2007b) and 0.17-0.23 in Mirandes (Pestana et al. 2012).

As feeding management is a major factor to influence the meat quality, more studies have to be carried out on this breed to determine beneficial effects on young animals meat quality derived from alternative systems, as including a grazing period for the mothers and the young bovines.

**CONCLUSION**

The meat from the Minhota breed is a valuable source of PUFA, particularly the C-20 and C-22 n-3 fatty acids in the human diet. A very low n-6:n-3 ratio show that this meat has a good nutritional value compared with other breeds. The differences in fat composition from the two age groups could be attributed to the fact that maternal suckling is more important in younger animals than in older ones. Further researchers on this breed had to be carried out in order to investigate the use of extensive feeding with these unweaned animals, especially for animals above eight months could help to explain the differences established between older and younger groups.

**ACKNOWLEDGEMENT**

This work was supported by the Foundation for Science and Technology (FCT).


