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Production system and slaughter age effects on intramuscular fatty acids from young Tudanca bulls.

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Abstract

Thirty-three young bulls from Tudanca local breed were used to investigate the effect of two production systems (semi-extensive vs. intensive) and two slaughter ages (12 vs. 14 months) on meat fatty acid (FA) composition. *Longissimus thoracis* from semi-extensive animals had a lower percentage of intramuscular fat ($p \leq 0.001$), saturated FAs ($p \leq 0.05$), *trans*-18:1 ($p \leq 0.001$), n-6/n-3 ratio ($p \leq 0.001$) and a higher percentage of branched chain FAs ($p \leq 0.001$), polyunsaturated FAs ($p \leq 0.001$) and conjugated linoleic acid (CLA) ($p \leq 0.001$). Few differences were observed in FA composition between slaughter ages. Hence, meat from semi-extensive animals slaughtered at 12 or 14 months displayed a healthier FA profile from a consumer´s point of view.

Keywords: beef, local breed, feeding system, lipid profile, conjugated linoleic acid.

1. Introduction

Tudanca cattle are an endangered local breed from Cantabria, northern Spain, used for meat production. Although the Tudanca breed is included in a Protected Geographical Indication (P.G.I.) “Carne de Cantabria” (Commission Regulation (EC) Nº 1483/2004), there are few animals commercialized using this brand due to their low carcass yield, which does not fit commercially into the Spanish beef market where current emphasis is placed on carcass quality over meat quality.

Tudanca breeding females are utilized in suckler herds using semi-extensive production systems. Calves are weaned at approximately 5 months of age and sold for finishing elsewhere. Strategies to finish these animals include semi-extensive (pasture based) or intensive production systems (concentrate based). Feeding a high energy concentrate diet has the potential for faster weight gains and increased levels of intramuscular fat (IMF) which can convey improved consumer satisfaction (Moloney, 2002). Finishing on high concentrate diets, however, has been found to increase levels of saturated and *trans* fatty acids associated with coronary heart disease (CHD) in humans (Hodgson, Wahlqvist, Boxall, & Balazs, 1996; Aldai, Nájera, Dugan, Celaya, & Osoro, 2007), while finishing on pasture has been noted to yield healthier beef fatty acid (FA) compositions (i.e. increased levels of FAs beneficial to consumer health including omega-3, *t*11-18:1 and *c*9,*t*11-18:2 FAs) (Enser, Hallett, Hewett, Fursey, Wood, & Harrington, 1998; Steen, Lavery, Kilpatrick, & Porter, 2003; Realini, Duckett, Brito, Dalla Rizza, & De Mattos, 2004).

In Spain, beef obtained from bulls at about 12 months of age represents 35% of beef consumption (M.A.R.M., 2010) and correspond to the age of their peak economic value. Increasing age, however, provides better carcass finish and increased levels of IMF (important factors when grass-finishing animals). Extending the finishing period from 12 to 14 months for Tudanca cattle might, therefore, be a strategy for production where increases in IMF content, fatness and intramuscular FA composition could add

to their credence attributes and help provide a basis for value addition and niche marketing.

The objectives of the present study were, therefore, to examine the effect of semi-extensive (pasture plus concentrate in a limited quantity) and intensive production strategies (indoors feeding *ad libitum* concentrate plus cereal straw) and two slaughter ages (12 vs. 14 months) on IMF content and intramuscular FA composition of Tudanca bulls.

2. Materials and methods

2.1. Location of experiment

This study was conducted between September 2008 and October 2009 at the experimental farm "Finca Aranda", Cóbreces, northern Spain (latitude: 43º23'15''N, longitude: 04º11'32''W) with an elevation of about 84 m above sea level. The climate of this area is Atlantic, with about 1,112 mm of annual rainfall, a 22.9ºC mean maximum temperature and a 4.6ºC mean minimum temperature.

2.2. Animals management, diet compositions and sample collection

Thirty-three purebred Tudanca bull calves were raised on pasture with their mothers until weaning at 5 months of age. After a post-weaning adjustment period of 15 days, they were finished semi-extensively (SE) or intensively (IN) until slaughtered at 12 or 14 months of age with treatment abbreviations as follows: 12SE (n=9), 14SE (n=7), 12IN (n=8) and 14IN (n=9). No significant differences ($p > 0.05$) were found between the four treatments for average weaning weight (121.5 ± 25.94 kg). Throughout the experiment, the animals were handled according to the principles from European Union for the care of animals (Council Directive 98/58/EC).

The experimental protocol for the four treatments is presented in Figure 1. During pasture periods, 12SE and 14SE groups were located into two paddocks (1.67 and 2.05 ha, respectively) and grazed an improved pasture with *Lolium perenne, Holcus*

lanatus and *Trifolium repens* as dominant species. These animals were also group fed a concentrate supplement at 1% of mean body weight. The concentrate included 0.5 kg crush barley plus a commercial finishing concentrate. During the wintering period, animals assigned to SE treatments were group fed *ad líbitum* grass silage, 0.5 kg crush barley plus 1.5 kg commercial finishing concentrate per animal. For intensively managed treatments, the 12IN and 14IN groups were transferred to two feedlot pens after weaning and fed *ad líbitum* on a commercial finishing concentrate and barley straw until slaughter. The ingredient composition of the commercial finishing concentrate is detailed as a footnote in Table 1.

Samples from all feedstuffs (concentrate, straw, crush barley and pasture) were collected every two weeks and analysed for chemical composition. Furthermore four feedstuff samples, equally spaced over the feeding period, were also collected and stored at -20ºC for subsequent FA analysis. Feedstuff fatty acid methyl esters (FAMEs) were prepared and analysed according to Sukhija & Palmquist (1988).

Animals were weighed monthly and the day before slaughter. Average daily gain was calculated by linear regression. Animals were transported 35 km and slaughtered at either 12 or 14 months of age in a commercial abattoir according to standard commercial practices. Carcasses were graded by trained slaughterhouse staff following the European Normative (Council Regulation (EEC) Nº 1208/81). However, in order to be more precise, carcass conformation scores were evaluated using an 18 instead of a 6-point scale with 18 as the best conformation and 1 the worst. In addition, carcass fatness scores were based on a 15 instead of a 5-point scale with 15 being very high fat and 1 very low fat. Post-grading, carcases were cooled for 24h at 2ºC. The day after slaughter, carcasses and perirenal fat were weighed and the *Longissimus thoracis* muscle from the 6th to the 7th rib was excised, ground and frozen at -80ºC until FA analysis.

2.3. Intramuscular fatty acid analysis

2.3.1. Lipid extraction

Intramuscular fat was extracted according to Bligh & Dyer (1959). Briefly, 25 ml of chloroform: methanol 1:2 (v/v) was added to 25 g of muscle, homogenized in a blender (Ultra-Turrax® T25 Basic), then centrifuged and filtered (Whatman nº1). Afterwards, 25 ml chloroform was added to residual tissue, homogenised, centrifuged and filtered. Both filtrates were combined and 25 ml of KCl 0.88% (w/v) was added and then shaken vigorously. After centrifugation at 2,600 x g for 20 minutes, the chloroform phase was evaporated using a rotavapor (Büchi® Heating Bath B-490 and Rotavapor R-200) at 40ºC. The extracted fat was stored at -20ºC under nitrogen gas.

2.3.2. Fatty acid methyl ester preparation, identification and quantification

Fatty acid methyl esters were prepared at room temperature following the cold methylation procedure outlined in section 5 of IUPAC Nº 2301 (1987). The internal standard was initially added to tubes (100 µl of 30 mg 19:0/ml chloroform) and then chloroform was removed by evaporation under nitrogen. Afterwards lipid (0.03 ± 0.005 g) was added to tubes and dissolved in 2 ml of hexane, shaken for 30 s and 1 ml of methanolic KOH (2N) was added. Tubes were then shaken vigorously for 30 s and left until phase separation occurred. The upper (hexane) phase was then removed for subsequent analysis.

For the measurement of FAMEs a Perkin Elmer Autosystem XL-FID GC was used with a flame ionization detector (FID) and a Varian CP-Sil 88 (100 m x 0.25 mm x 0.2 µm) column. The oven temperature was initially set at 70ºC and held for 4 minutes. The temperature was then ramped to 110ºC at 8ºC/min for 9 min, and then to 170ºC at 5ºC/min and held for 10 min and then ramped to 240ºC at 4ºC/min and held for 14.5 min. The injector and detector were set at 250ºC and 260ºC respectively. The carrier gas was helium with a flow rate of 1 ml/min, the injection volume was 0.5 µl and the split ratio 20:1. With this temperature program, major *trans*-18:1 (*t*-18:1) and conjugated linoleic acid (CLA) isomers were not resolved and additional analyses were

conducted. For *t*-18:1 isomer analysis, a temperature program with an extended 150ºC plateau was used as reported by Kramer, Hernandez, Cruz-Hernandez, Kraft, & Dugan (2008). For these analyses, a Varian CP-3800 GC was used equipped with a CP-8400 autosampler, FID, and a Varian CP-Sil 88 (100 m x 0.25 mm x 0.2 µm) column. Injector and detector temperatures were 250ºC and hydrogen was used as carrier gas (1 ml/min). For CLA isomer analysis, Ag^{+}-HPLC (Varian Prostar 230 HPLC equipped with a Varian Prostar 410 autosampler and a Varian Prostar 335 diode array detector set at 233 nm) was conducted using the conditions outlined by Cruz-Hernández, Deng, Zhou, Hill, Yurawecz, Delmonte, Mossoba, Dugan, & Kramer (2004).

For the identification of FAMEs by GC, the Supelco 37 Component FAME Mix from Sigma-Aldrich, Inc. was used. In addition, the #463 and #603 standards from Nu-Check Prep, Inc. (Elysian, MN) were used to identify FAMEs not contained in the Supelco 37 FAME mix. Branched-chain FAMEs were identified using GC reference standard BC-Mix1, purchased from Applied Science (State College, PA). For identification of CLA isomers the #UC-59-M standard from Nu-Chek Prep, Inc. was used, which contains the *c*9,*t*11-, *t*8,*c*10-, *c*11,*t*13-, *t*10,*c*12-, *c*8,*c*10-, *c*9,*c*11-, *c*10,*c*12-, *c*11,*c*13-, *t*11,*t*13-, *t*10,*t*12-, *t*9,*t*11- and *t*8,*t*10-CLA isomers.

Fatty acid methyl esters were quantified using peak areas and internal standard (19:0) based calculations. Final FA contents were reported as a percentage of total identified FAMEs (weight %).

2.4. Statistical analysis

Data were analysed using the GLM procedure of SPSS 17.0 (2008). The model included the fixed effects of production system, slaughter age and their interaction applying the equation: $y_{ij} = \mu + A_i + B_j + A_i \times B_j + e_{ij}$ where y_{ij} = the observed value of the i age and j production system, µ = mean value common to all observations, A_i = fixed effect of slaughter age, B_j = fixed effect of production system, $A_i \times B_j$ = interaction between slaughter age and production system and e_{ij} = the error term. Interaction was

eliminated from the model when it was not significant. Duncan's test was used to separate treatment means and they were considered to be significantly different when $p \leq 0.05$.

3. Results and discussion

3.1. Feedstuff composition, animal performance and carcass traits

Chemical and FA composition of feedstuffs are presented in Table 1. Feedstuff ether extracts ranged from 0.6% for straw to 4.6% for concentrate. As expected (Nuernberg, Nuernberg, Ender, Lorenz, Winkler, Rickert, & Steinhart, 2002; Noci, Monahan, French, & Moloney, 2005), pasture provided a greater percentage of 18:3n-3 and polyunsaturated fatty acids (PUFAs) and lower proportions of 16:0, 18:2n-6, saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) than concentrate and crush barley.

Concentrate intake of IN groups was almost four times the concentrate intake of SE groups (Table 2). The average daily gain (ADG) from weaning to slaughter for 14IN animals was higher than 12IN (1,086 vs. 986 g/day). The ADG for 14SE animals were also significantly higher ($p \leq 0.001$) than 12SE during the wintering (876 vs. 368 g/day) and post-wintering pasture periods (1,355 vs. 1,153 g/day). The differences found in ADG during wintering were due to the different conditions of wintering initiation between the SE treatments. While 12SE treatment started wintering after 15 days post-weaning adjustment period, the 14SE treatment started wintering two months later.

Final live weights and cold carcass weights differed significantly ($p \leq 0.001$) with production system and slaughter age. For both variables, 14IN had the greatest weights and 12SE the lowest, with no differences ($p > 0.05$) detected between 14SE and 12IN treatments. Despite the differences found in carcass weights, there were no significant differences ($p > 0.05$) in conformation between treatments. Carcass fatness was only significantly affected ($p \leq 0.001$) by production system (Moloney, Fallon,

Mooney, & Troy, 2004). Animals in the 12SE treatment had the lowest carcass fatness scores ($p \leq 0.05$) compared to the other three treatments. Perirenal fat weight, however, was significantly affected ($p \leq 0.001$) by both production system and slaughter age. Semi-extensive groups had the lowest content of perirenal fat compared to intensively finished animals ($p \leq 0.05$), and between each production system, younger animals had less perirenal fat than older animals ($p \leq 0.05$). These differences are consistent with expectations due to differences in the energy content of the diets and the high precocity of perirenal adipose tissue (Vernon, 1986; Aldai *et al*., 2007; Serrano, Cornu, Kondjoyan, Agabriel, & Micol, 2011).

3.2. Fatty acid profile

The IMF content of SE animals (1.1-1.3%) was lower ($p \leq 0.001$) than IN animals (3.0-2.7%) (Table 3) and this is related to the high energy content of concentrate diets and their availability for fat synthesis (Ponnampalam, Mann, & Sinclair, 2006; Wood, Enser, Fisher, Nute, Sheard, Richardson, Hughes, & Whittington, 2008; Leheska, Thompson, Howe, Hentges, Boyce, Brooks, Shriver, Hoover, & Miller, 2008; Garcia, Pensel, Sancho, Latimori, Kloster, Amigone, & Casal, 2008). However, no significant differences ($p > 0.05$) were observed for IMF between 12 and 14 months for either of the two systems.

Production system affected the percentage of most FAs in IMF (Tables 3, 4, 5, and 6). These results are in accordance with other studies comparing production strategies differing in concentrate intake (Garcia *et al*., 2008; Leheska *et al*., 2008). Only, some minor MUFAs (*c*12-18:1, *c*15-18:1, *t*12-18:1) and PUFAs (20:2n-6, *t*9,*t*11-18:2) and a major PUFA (18:2n-6), were not affected ($p > 0.05$) by production system. On the other hand, slaughter age showed fewer effects on FA composition, and likely relates to the limited difference between slaughter ages (Rule, MacNeil, & Short, 1997).

Palmitic acid (16:0), stearic acid (18:0), oleic acid (*c*9-18:1) and linoleic acid (18:2n-6) were the major FAs in IMF and this concurs with results reported by Raes, Balcaen,

Dirinck, De Winne, Claeys, Demeyer, & De Smet (2003) from *Longissimus lumborum* muscle of 4 different origins (Belgian Blue, Limousin, Irish and Argentine beef) where Belgian Blue and Limousin animals were fattened under high intensive production conditions and Irish and Argentine beef were fattened in their respective countries using semiextensive production systems. These four FAs accounted for over 71% of total FAs in the four treatments.

3.2.1. Saturated fatty acids

The levels of SFAs in beef are of nutritional importance because 16:0 and miristic acid (14:0) increase serum cholesterol, whereas 18:0 has a neutral impact (Hegsted, McGandy, Myers, & Stare, 1965; Leheska *et al*., 2008). In the present experiment, no differences due to slaughter age were found for total or individual SFA ($p > 0.05$), except for arachidic acid (20:0) ($p \leq 0.001$) (Table 3). The percentages of individual and total SFAs were, however, affected by production system ($p \leq 0.05$). Levels of SFA in 12SE animals were significantly lower than 12IN and 14IN ($p \leq 0.05$). Concentrate rich diets have higher energy and bovines accumulate this excess energy in adipocytes as triglycerides (rich in SFA, principally 16:0). Realini *et al*., (2004) found no differences in SFAs when comparing steers finished on pasture (130 d) versus concentrate (100 d).

Branched chain fatty acids (BCFAs) constituted a small proportion of total FAs in IMF. The origin of BCFAs, including *iso* and *anteiso* acids, is controversial as they may be derived from *de novo* synthesis in rumen bacteria and adipose tissue of ruminants (Vlaeminck, Fievez, Cabrita, Fonseca, & Dewhurst, 2006). Effects of BCFA have recently become of interest due their antitumor activity in human breast cancer cells (Wongtangtintharn, Oku, Iwasaki, & Toda 2004), which may relate to effects on cell replication through modulation of FA metabolism. In the current trial, pasture feeding significantly increased the percentage of BCFA (average 1.4% vs. 1.2% for SE and IN groups, respectively). Among BCFA, *iso*-16:0 has been found to have the highest anti-tumor activity (Wongtangtintharn *et al*., 2004). The present study showed that *iso*-16:0

levels were significantly greater ($p \leq 0.001$) in IMF from SE than IN treatments. These results are in agreement with those reported by Manner, Maxwell, & Williams (1984) for forage finished beef.

3.2.2. Monounsaturated fatty acids

The production system had a major impact ($p \leq 0.001$) on the total and *c*-MUFA (Table 4). As observed by others (Realini *et al*., 2004; Descalzo, Insani, Biolatto, Sancho, Garcia, Pensel, & Josifovich, 2005; De la Fuente, Díaz, Álvarez, Oliver, Font i Furnols, Sañudo, Campo, Montossi, Nute, & Cañeque, 2009), IN animals had higher percentages of total MUFA and *c*-MUFA ($p \leq 0.001$) than SE animals. This difference was mainly due to increased oleic acid in IN animals and would be associated with a higher gene expression of stearoyl-CoA desaturase (SCD) in adipose tissues (Daniel, Wynn, Salter, & Buttery, 2004). From the point of view of consumers' health, higher levels of oleic acid in the diet would be considered as positive as it has been found to increase HDL-cholesterol and decrease LDL-cholesterol concentrations in human plasma, both of which protect against CHD (Mattson & Grundy, 1985; Katan, Zock, & Mensink, 1994).

As shown by Malau-Aduli, Siebert, Bottema, & Pitchford (1997) in the adipose tissue in Limousine and Jersey cattle, total MUFA content tended to increase ($p \leq 0.1$) with age. This is consistent with increases in SCD activity known to occur in adipose tissue of growing ruminants (Castillo, Pabon Restrepo, Olivera, & Carulla, 2010).

Levels of *c*9-16:1, *c*9-17:1 and *c*11-20:1 were significantly affected by production system ($p \leq 0.01$) and slaughter age ($p \leq 0.05$) and these may also relate to the increased expression of SCD. In addition, Christie (2011) reported that *c*9-16:1 strongly stimulates the action of insulin in muscle and is a unique FA that serves as a marker for *de novo* lipogenesis from glucose, as would be typical when concentrate based diets are fed. Effects of age have not, however, been consistent across trials as Cifuni, Napolitano, Pacelli, Riviezzi, & Girolami (1999) and Polak, Rajar, Gasperlin, & Zlender

(2008) found an increase in *c*9-16:1 and a decrease in *c*11-20:1 and *c*9-17:1 with increasing age.

Total *trans*-MUFA and *t*-18:1 isomers were significantly influenced ($p \leq 0.001$) by production system with 14IN animals having higher percentages ($p \leq 0.05$) compared to the other three treatments, mainly due to a higher content ($p \leq 0.001$) of *t*10-18:1 in IN vs. SE treatments. Conversely, SE animals were found to have higher contents of *t*11-18:1 (vaccenic acid) and minor *t*-18:1 isomers including *t*13/14-, *t*15- and *t*16-18:1 ($p \leq 0.001$). These findings are consistent with Purchas, Knight, & Busboom (2005), Dugan, Kramer, Robertson, Meadus, Aldai, & Rolland (2007) and Leheska *et al*., (2008) who compared concentrate versus grass finished beef. It is known that *t*-18:1 isomers are a product of incomplete biohydrogenation of 18:2n-6 and 18:3n-3 in the rumen and these isomers are incorporated into meat lipids (Griinari, Corl, Lacy, Chouinard, Nurmela, & Bauman, 2000; Bessa, Santos-Silva, Ribeiro, & Portugal, 2000). For humans, increasing consumption of *trans* fatty acids has been discouraged due to their undesirable effects on blood cholesterol (Mensink & Katan, 1993). However, different *t*-18:1 isomers have different biological activities and consumption of *t*11-18:1 is not associated with CHD, and may also have other healthful effects (Field, Blewett, Proctor, & Vine, 2009).

3.2.3. Polyunsaturated fatty acids and ratios

The percentage of n-3 FAs in IMF was significantly affected ($p \leq 0.001$) by production system and this effect was consistent with the findings of French, Stanton, Lawless, O'Riordan, Monahan, Caffrey, & Moloney (2000) (Table 5). The lowest values ($p \leq 0.001$) resulted from IN (average 1.2%) versus SE finishing (average 7.9%). This was related to the low 18:3n-3 content in the IN diet (Table 1). Fredriksson Eriksson & Pickova (2007) reported that higher 18:3n-3 in meat from pasture-fed bulls could be enhanced by its association with thylakoid membranes in chloroplasts, which may protect against ruminal biohydrogenation. Furthermore, the presence of secondary

plant metabolites might inhibit microbial biohydrogenation activity within the rumen (Lourenço, Van Ranst, Vlaeminck, De Smet, & Fievez, 2008).

The main long chain FA in the n-3 family included 20:5n-3 (EPA), 22:5n-3 (DPA) and 22:6n-3 (DHA). Percentages of these FAs were influenced by the production system ($p \leq 0.001$) and slaughter age ($p \leq 0.05$). The lowest values ($p \leq 0.001$) for these three FAs were in IN animals. This is consistent with expectations, as pasture contains a high percentage of 18:3n-3 which is the precursor for elongation and desaturation to EPA, DPA and DHA, albeit this process is known to operate with low efficiency. These results are in accordance with those obtained by De la Fuente *et al*., (2009) in Friesian bulls fed concentrate and cereal straw *ad líbitum* until 10-11 months versus Fleckvieh breed and Limousin cross bulls raised at pasture and finished on corn silage *ad líbitum* supplemented with restricted soy and cereal meal until 19-24 moths. The levels of EPA, DPA and DHA were also found to decrease ($p \leq 0.05$) with age which could relate to decreased Δ6-desaturase activity (De Gomez Dumm & Brenner, 1975), or may also be a consequence of dilution due to endogenous fatty acid synthesis. Again, increased levels of these fatty acids in SE animals would be associated with positive health outcomes in humans, as Simopoulos (1991) indicates EPA and DHA can play crucial roles in the prevention of atherosclerosis, heart attack, depression and cancer. In addition, beef can be an important source of these fatty acids in areas where consumption of marine products is limited (Howe, Meyer, Record, & Baghurst, 2006).

The percentage of n-6 fatty acids showed a tendency ($p \leq 0.1$) to be higher in SE (average 17.3%) than IN animals (average 14.1%). The tendency for a higher % n-6 in SE animals may be related with a high content of phospholipids which are rich in PUFA including n-6 FA. On the other hand, IN animals have more triglycerides (rich in SFA and *cis*-MUFA) which would dilute PUFA. In addition, the highest % n-6 ($p \leq 0.05$) was found in 12SE treatment which also had a lower IMF content. In a similar study, Alfaia, Alves, Martins, Costa, Fontes, Lemos, Bessa, & Prates (2009), reported no significant

differences for the % n-6 in muscle between pasture and concentrate fed cattle. This could be explained by the similar IMF content between treatments and lower dilution of PUFA by SFA and MUFA when feeding concentrate.

The ratio of n-6/n-3 FAs is an index commonly used to assess the nutritional value of fats (Santos-Silva, Bessa, & Santos-Silva, 2002). However, Stanley, Elsom, Calder, Griffin, Harris, Jebb, Lovegrove, Moore, Riemersma, & Sanders (2007) question the validity of the n-6/n-3 ratio to predict effects on cardiovascular health and emphasize that absolute intake of long chain n-3 may be more useful. Recommendations for humans are to increase levels of n-3 PUFA in the diet, so that this ratio is below 4:1 (Department of Health., 1994). Feeding cattle diets rich in forage is known to decrease the n-6/n-3 ratio. French *et al*., (2000) showed crossbreed steers fed 12 kg of grazed grass dry matter plus 2.5 kg of concentrate had a lower ratio (2.5) than animals fed 8 kg concentrate plus 1 kg of hay (4.2). Furthermore, Cifuni, Napolitano, Riviezzi, Braghieri, & Girolami, (2004) reported that Podolian cattle finishing using barley-based concentrate and straw *ad líbitum* until 16-18 months produced meat containing a higher ratio of n-6/n-3 (11.4). The n-6/n-3 ratio in SE was low compared to IN animals ($p \leq 0.001$). In the present study, a significant production system by age at slaughter interaction was found for the n-6/n-3 ratio ($p \leq 0.001$). Intensive animals showed an increased n-6/n-3 ratio, however, SE animals displayed a decreased n-6/n-3 ratio as age increased. Again, in terms of human health, the n-6/n-3 ratio in SE beef was within recommended values (2.3-2.1) compared to IN beef (10.6-12.8).

Lower proportions ($p \leq 0.001$) of total PUFA were found in IN animals (average 16.5%) compared to SE animals (average 27%) (Table 6). This can in part be explained by the lower PUFA content in the concentrate diet (Table 1) and a greater rate of complete hydrogenation of PUFA and MUFA to SFA by rumen microorganisms (Choi, Enser, Wood, & Scollan, 2000). Similar results were obtained by Alfaia *et al*., (2009) in

Alentejana breed bulls grazed on pasture (28.9%) until ~23 months compared to concentrate-fed animals (19.1%) until ~15 months.

The PUFA/SFA ratio is another index used to assess the nutritional value of fat, with a recommended value for human consumption of 0.45 (Department of Health, 1994), with a higher ratio considered favorable as it may reduce cholesterolaemia (Alfaia, Ribeiro, Lourenço, Quaresma, Martins, Portugal, Fontes, Bessa, Castro, & Prates, 2006). Intramuscular fat from SE bulls had a significantly higher PUFA/SFA ratio ($p \leq 0.001$) compared to IN bulls, and this difference is in accordance with results reported by French *et al*., (2000).

3.2.4. CLA isomer profile

Conjugated linoleic acid isomers are typically found in products derived from ruminant sources (dairy, beef and lamb) and are intermediates in the process of bacterial biohydrogenation of PUFAs in the rumen (Lourenço, Ramos-Morales, & Wallace, 2010). There are few studies that have reported the effect of pasture vs. concentrate feeding or different slaughter ages on the CLA isomer profile of beef, which is only achieved by Ag^{+}-HPLC (Fritsche, Fritsche, Solomon, Mossoba, Yurawecz, Morehouse, & Ku, 2000; Dannenberger, Nuernberg, Nuernberg, Scollan, Steinhart, & Ender, 2005). Knowing the CLA isomer profile is important, however, as CLA isomers have differing health effects (Pariza, Park, & Cook, 2001).

Production system had a significant effect ($p \leq 0.001$; Table 6) on the proportion of total CLA. In contrast, slaughter age had no significant effect ($p > 0.05$). Animals finished on pasture had significantly greater ($p \leq 0.001$) total CLA (8.1 mg/g FAs) compared to IN animals (4.6 mg/g FAs). Several authors (Realini *et al*., 2004; Lorenzen, Golden, Martz, Grün, Ellersieck, Gerrish, & Moore, 2007) have shown that including pasture in the diet of beef cattle increases CLA in beef. Garcia *et al*., (2008) reported total CLA values in *Longissimus dorsi* muscle of 5.8 vs. 3.1 mg/g FAs in steers fed on pasture

supplemented with cracked corn grain (1% live weight) compared to a corn-based concentrate with alfalfa hay.

The distribution pattern of *t*,*t*-CLA isomers was consistent with other experiments (Nuernberg *et al*., 2002; Dannenberger *et al*., 2005), in which meat from animals fed on pasture presented higher ($p \leq 0.001$) values than animals fed on concentrate. In fact, the contribution of total of *t*,*t*-CLA isomers was about 16% and 12% of total CLA in SE and IN animals, respectively. Semi-extensive groups had significantly higher percentages ($p \leq 0.001$) of *t*11,*t*13-CLA and *t*12,*t*14-CLA. These isomers are considered sensitive grass intake indicators (Alfaia *et al*., 2009).

Regarding total *c,t/t,c*-CLA isomers, these provided 81.5% and 85.9% of total CLA isomers in SE and IN bulls, respectively. Among *c,t/t,c*–CLA isomers, the most abundant was *c*9,*t*11-CLA (rumenic acid). The majority of this isomer originates from PUFA biohydrogenation to *t*11-18:1 in the rumen and then endogenous resynthesis by Δ9-desaturation (Griinari *et al*., 2000). Semi-extensive animals had a higher percentage ($p \leq 0.001$) of *c*9,*t*11-CLA than IN animals (4.9 vs. 2.3 mg/g FAs, respectively). These results are in accordance with French *et al*., (2000), who reported that diets containing proportionally higher levels of 18:3n-3, such as fresh grass, grass silage and concentrates containing linseed, result in an increased deposition of *c*9,*t*11-CLA in muscle.

Another CLA isomer that has been shown to exhibit biological activity is *t*10,*c*12-CLA (Pariza, Park, & Cook, 2000). Both *c*9,*t*11-CLA and *t*10,*c*12-CLA have been shown to have equal anticancer activity (Ip, Yan, Ip, Banni, Carta, Angioni, Murru, Spada, Melis, & Saebo, 2002). Beyond anticancer effects, the *t*10,*c*12-CLA is responsible for body fat reduction and inhibition of SCD activity (Pariza *et al*., 2000; Park, Storkson, Ntambi, Cook, Sih, & Pariza, 2000; Storkson, Park, Cook, & Pariza, 2005). Because mammals do not have Δ12-desaturase, *t*10,*c*12-CLA cannot be endogenously produced from *t*10-18:1 and hence comes directly from intestinal absorption (Pariza *et al*., 2000; Kraft,

Collomb, Möckel, Sieber, & Jahreis, 2003). Production system ($p \leq 0.001$) and slaughter age ($p \leq 0.05$) had a significant effect on *t*10,*c*12-CLA. Higher percentages ($p \leq 0.001$) were found in IN compared to SE bulls as shown by Alfaia *et al*., (2009). Moreover older animals had the highest ($p \leq 0.05$) percentage of *t*10,*c*12-CLA, probably due to their higher intake and longer duration of concentrate consumption which provided a rich supply of rapidly fermented carbohydrate, which has been associated with increased levels of *t*10,*c*12-CLA (Bauman & Griinari, 2003; Shingfield, Reynolds, Lupoli, Toivonen, Yurawecz, Delmonte, Griinari, Grandison, & Beever, 2005).

The second most abundant CLA isomer in IN bulls was *t*7,*c*9-CLA and in SE bulls it was *t*11,*c*13-CLA. This is consistent with differences seen when feeding high (Yurawecz, Roach, Sehat, Mossoba, Kramer, Fritsche, Steinhart, & Ku, 1998; Fritsche at al., 2000) and low (Nuernberg *et al*., 2002; Dannenberg *et al*., 2005) forage to concentrate ratios. A significant ($p \leq 0.01$) increase in *t*7,*c*9-CLA content was also observed in 14 versus 12 month old animals. Corl, Baumgard, Griinari, Delmonte, Morehouse, Scollan, & Bauman (2002) studied the metabolic pathway for the formation of *t*7,*c*9-CLA in the rumen of dairy cows and showed that this CLA isomer is synthesized endogenously almost exclusively by Δ9-desaturase using rumen derived *t*7-18:1 as the substrate. Biological effects of both *t*7,*c*9-CLA and *t*11,*c*13-CLA are, however, not known and worthy of further investigation.

4. Conclusions

Pasture fed animals presented lower levels of intramuscular fat and increasing slaughter age from 12 to 14 months did not increase intramuscular fat content. Pasture fed animals also presented lower percentage of SFA, a higher percentage of BCFA, PUFA and CLA, a lower n-6/n-3 ratio, and a *t*-18:1 profile with a higher proportion of *t*11- versus *t*10-18:1. As a consequence, meat from semiextensive production systems would have a FA profile more favourable to health conscious consumers. This could

provide niche marketing advantages versus conventionally (high concentrate) finished beef. Moreover, the large difference in concentrate intake between the semiextensive an intensive systems (~900 kg/animal) only resulted in a difference in carcass weight of around 30 kg/animal. Nevertheless, bearing in mind the important role of intramuscular fat content on consumer satisfaction, complementary information about meat sensory traits needs to be considered to globally to assess these production systems.

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References

Aldai, N., Nájera, A. I., Dugan, M. E. R., Celaya, R., & Osoro, K. (2007). Characterisation of intramuscular, intermuscular and subcutaneous adipose tissues in yearling bulls of different genetic groups. *Meat Science, 76*, 682-691.

Alfaia, C. M. M., Ribeiro, V. S. S., Lourenço, M. R. A., Quaresma, M. A. G., Martins, S. I. V., Portugal, A. P. V., Fontes, C. M. G. A., Bessa, R. J. B., Castro, M. L. F., & Prates, J. A. M. (2006). Fatty acid composition, conjugated linoleic acid isomers and cholesterol in beef from crossbred bullocks intensively produced and from Alentejana purebred bullocks reared according to Carnalentejana-PDO specifications. *Meat Science, 72*, 425-436.

Alfaia, C. P. M., Alves, S. P., Martins, S. I. V., Costa, A. S. H., Fontes, C. M. G. A., Lemos, J. P. C., Bessa, R. J. B., & Prates, J. A. M. (2009). Effect of the feeding system on intramuscular fatty acids and conjugated linoleic acid isomers of beef cattle, with emphasis on their nutritional value and discriminatory ability. *Food Chemistry, 114*, 939-946.

Bauman, D. E., & Griinari, J. M. (2003). Nutritional Regulation of milk fat synthesis. *Annual Review of Nutrition, 23*, 203-227.

Bessa, R. J. B., Santos-Silva, J., Ribeiro, J. M. R., & Portugal, A. V. (2000). Reticulo-rumen biohydrogenation and the enrichment of ruminant edible products with linoleic acid conjugated isomers. *Livestock Production Science, 63*, 201-211.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry Physiology, 37*, 1911–1912.

Castillo, J. A., Pabon Restrepo, M. L., Olivera, M., & Carulla, J. E. (2010). Role of stearoyl CoA desaturase on conjugated linoleic acid concentration in bovine milk: A review. *Revista Colombiana de Ciencias Pecuarias, 23*, 493-500.

Choi, N. J., Enser, M., Wood, J. D., & Scollan, N. D. (2000). Effect of breed on the deposition in beef muscle and adipose tissue of dietary n-3 polyunsaturated fatty acids. *Animal Science, 71*, 509-519.

Christie, W. W. (2011). Fatty acids: straight-chain monoenoic – structures, occurrence and biochemistry. http://lipidlibrary.aocs.org/lipids/fa_mono/index.htm

Cifuni, G. F., Napolitano, F., Pacelli, C., Riviezzi, A. M., & Girolami, A. (1999). Effect of age at slaughter on carcass traits, fatty acid composition and lipid oxidation of Apulian lambs. *Small Ruminant Research, 35*, 65-70.

Cifuni, G. F., Napolitano, F., Riviezzi, A. M., Braghieri, A., & Girolami, A. (2004). Fatty acid profile, cholesterol content and tenderness of meat from Podolian young bulls. *Meat Science, 67*, 289-297.

Commission Regulation (EC) N^{o} 1483/2004 supplementing the Annex to Regulation (EC) N^{o} 2400/96 on the entry of certain names in the Register of protected designations of origin and protected geographical indications ('Carne de la Sierra de Guadarrama', 'Ternera de Navarra' or 'Nafarroako Aratxea', 'Carne de Vacuno del País Vasco' or 'Euskal Okela', 'Ternera Asturiana' and 'Carne de Cantabria'.

Corl, B. A., Baumgard, L. H., Griinari, J. M., Delmonte, P., Morehouse, K., Yurawecz, M. P., & Bauman, D. E. (2002). *Trans*-7,*cis*-9 CLA is synthesized endogenously by ∆9-desaturase in dairy cows. *Lipids, 37*, 681-688.

Council Directive 98/58/EC of 20 July (1998) concerning the protection of animals kept for farming purposes. *Official Journal of the European Union, L221*, 23-27.

Council Regulation (EEC) Nº 1208/81 of 28 April 1981 determining the Community scale for the classification of carcasses of adult bovine animals. *Official Journal of the European Union*, *L123*, 3.

Cruz-Hernández, C., Deng, Z., Zhou, J., Hill, A. R., Yurawecz, M. P., Delmonte, P., Mossoba, M. M., Dugan, M. E. R., & Kramer, J. K. G. (2004). Methods for analysis of conjugated linoleic acids and trans-18:1 isomers in dairy fats by using a combination of gas chromatography, silver-ion thin-layer chromatography/gas chromatography, and silver-ion liquid chromatography. *Journal of AOAC International, 87*, 545–562.

Daniel, Z. C. T. R., Wynn, R. J., Salter, A. M., & Buttery, P. J. (2004). Differing effects of forage and concentrate diets on the oleic acid and conjugated linoleic acid content of sheep tissues: The role of stearoyl-CoA desaturase. *Journal of Animal Science, 82*, 747-758.

Dannenberger, D., Nuernberg, K., Nuernberg, G., Scollan, N., Steinhart, H., & Ender, K. (2005). Effect of pasture vs. concentrate diet on CLA isomer distribution in different tissue lipids of beef cattle. *Lipids, 40*, 589-598.

De Gomez Dumm, I. N. T., & Brenner, R. R. (1975). Oxidative desaturation of α-linolenic, linoleic, and stearic acids by human liver microsomes. *Lipids, 10*, 315-317.

De la Fuente, J., Díaz, M. T., Álvarez, I., Oliver, M. A., Font i Furnols, M., Sañudo, C., Campo, M. M., Montossi, F., Nute, G. R., & Cañeque, V. (2009). Fatty acid and vitamin E composition of intramuscular fat in cattle reared in different production systems. *Meat Science, 82*, 331-337.

Department of Health. (1994). Nutritional Aspects of Cardiovascular Disease. Report on Health and Social Subjects. London: HMSO*, 46*.

Descalzo, A. M., Insani, E. M., Biolatto, A., Sancho, A. M., Garcia, P. T., Pensel, N. A., & Josifovich, J. A. (2005). Influence of pasture or grain-based diets supplemented with vitamin E on antioxidant/oxidative balance of Argentine beef. *Meat Science, 70*, 35-44.

Dugan, M., Kramer, J., Robertson, W., Meadus, W., Aldai, N., & Rolland, D. (2007). Comparing subcutaneous adipose tissue in beef and muskox with emphasis on *trans* 18:1 and conjugated linoleic acids. *Lipids, 42*, 509-518.

Enser, M., Hallett, K. G., Hewett, B., Fursey, G. A. J., Wood, J. D., & Harrington, G. (1998). Fatty acid content and composition of UK beef and lamb muscle in relation to production system and implications for human nutrition. *Meat Science, 49*, 329-341.

Field, C. J., Blewett, H. H., Proctor, S., & Vine, D. (2009). Human health benefits of vaccenic acid. *Applied Physiology, Nutrition, and Metabolism, 34*, 979–991.

Fredriksson Eriksson, S., & Pickova, J. (2007). Fatty acids and tocopherol levels in M. *Longissimus dorsi* of beef cattle in Sweden - A comparison between seasonal diets. *Meat Science, 76*, 746-754.

French, P., Stanton, C., Lawless, F., O'Riordan, E. G., Monahan, F. J., Caffrey, P. J., & Moloney, A. P. (2000). Fatty acid composition, including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage, or concentrate-based diets. *Journal of Animal Science, 78*, 2849-2855.

Fritsche, J., Fritsche, S., Solomon, M. B., Mossoba, M. M., Yurawecz, M. P., Morehouse, K., & Ku, Y. (2000). Quantitative determination of conjugated linoleic acid isomers in beef fat. *European Journal of Lipid Science and Technology, 102*, 667-672.

Garcia, P. T., Pensel, N. A., Sancho, A. M., Latimori, N. J., Kloster, A. M., Amigone, M. A., & Casal, J. J. (2008). Beef lipids in relation to animal breed and nutrition in Argentina. *Meat Science, 79*, 500-508.

Griinari, J. M., Corl, B. A., Lacy, S. H., Chouinard, P. Y., Nurmela, K. V. V., & Bauman, D. E. (2000). Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ9-desaturase. *Journal of Nutrition, 130*, 2285-2291.

Hegsted, D. M., McGandy, R. B., Myers, M. L., & Stare, F. J. (1965). Quantitative effects of dietary fat on serum cholesterol in man. *The American Journal of Clinical Nutrition, 17*, 281-295.

Hodgson, J. M., Wahlqvist, M. L., Boxall, J. A., & Balazs, N. D. (1996). Platelet *trans* fatty acids in relation to angiographically assessed coronary artery disease. *Atherosclerosis, 120*, 147-154.

Howe, P., Meyer, B., Record, S., & Baghurst, K. (2006). Dietary intake of long-chain ω-3 polyunsaturated fatty acids: contribution of meat sources. *Nutrition, 22*, 47-53.

Ip, C., Yan, D., Ip, M. M., Banni, S., Carta, G., Angioni, E., Murru, E., Spada, S., Melis, M. P., & Saebo, A. (2002). Conjugated linoleic acid isomers and mammary cancer prevention. *Nutrition and Cancer, 43*, 52-58.

IUPAC N^{o} 2301. (1987). Standard methods for the analysis of oils, fats and derivatives. Preparation of the fatty acid methyl esters. Oxford, UK: International Union of Pure and Applied Chemistry, Blackwell.

Katan, M. B., Zock, P. L., & Mensink, R. P. (1994). Effects of fats and fatty acids on blood lipids in humans: an overview. *The American Journal of Clinical Nutrition, 60*, 1017S-1022S.

Kraft, J., Collomb, M., Möckel, P., Sieber, R., & Jahreis, G. (2003). Differences in CLA isomer distribution of cow's milk lipids. *Lipids, 38*, 657-664.

Kramer, J. K. G., Hernandez, M., Cruz-Hernandez, C., Kraft, J., & Dugan, M. E. R. (2008). Combining results of two GC separations partly achieves determination of all *cis* and *trans* 16:1, 18:1, 18:2 and 18:3 except CLA isomers of milk fat as demonstrated using Ag-ion SPE fractionation. *Lipids, 43*, 259–273.

Leheska, J. M., Thompson, L. D., Howe, J. C., Hentges, E., Boyce, J., Brooks, J. C., Shriver, B., Hoover, L., & Miller, M. F. (2008). Effects of conventional and grass-feeding systems on the nutrient composition of beef. *Journal of Animal Science, 86*, 3575-3585.

Lorenzen, C. L., Golden, J. W., Martz, F. A., Grün, I. U., Ellersieck, M. R., Gerrish, J. R., & Moore, K. C. (2007). Conjugated linoleic acid content of beef differs by feeding regime and muscle. *Meat Science, 75*, 159-167.

Lourenço, M., Van Ranst, G., Vlaeminck, B., De Smet, S., & Fievez, V. (2008). Influence of different dietary forages on the fatty acid composition of rumen

digesta as well as ruminant meat and milk. *Animal Feed Science and Technology, 145*, 418-437.

Lourenço, M., Ramos-Morales, E., & Wallace, R. J. (2010). The role of microbes in rumen lipolysis and biohydrogenation and their manipulation. *Animal, 4*, 1008-1023.

M.A.R.M. (2010). El medio ambiente y el medio rural y marino en España 2010. *Ministerio de Medio Ambietne y Medio Rural y Marino*, 1092.

Malau-Aduli, A. E. O., Siebert, B. D., Bottema, C. D. K., & Pitchford, W. S. (1997). A comparison of the fatty acid composition of triacylglycerols in adipose tissue from Limousin and Jersey cattle. *Australian Journal of Agricultural Research, 48*, 715–722.

Manner, W., Maxwell, R. J., & Williams, J. E. (1984). Effects of dietary regimen and tissue site on bovine fatty acid profiles. *Journal of Animal Science, 59*, 109-121.

Mattson, F. H., & Grundy, S. M. (1985). Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *Journal of Lipid Research, 26*, 194-202.

Mensink, R. P., & Katan, M. B. (1993). *Trans* monounsaturated fatty acids in nutrition and their impact on serum lipoprotein levels in man. *Progress in Lipid Research, 32*, 111-122.

Moloney, A. P. (2002). The fat content of meat and meat products. In J. Kerry, J.Ledward, & D. Kerry (Eds.), *Meat Processing – Improving Quality* (pp.137-153). Cambridge: Wood head Publishing Ltd.

Moloney, A. P., Fallon, R. J., Mooney, M. T., & Troy, D. J. (2004). The quality of meat and fatness of bulls offered ad libitum concentrates, indoors or at pasture. *Livestock Production Science*, *87*, 271-276.

Noci, F., Monahan, F. J., French, P., & Moloney, A. P. (2005). The fatty acid composition of muscle fat and subcutaneous adipose tissue of pasture-fed beef heifers: Influence of the duration of grazing. *Journal of Animal Science, 83*, 1167-1178.

Nuernberg, K., Nuernberg, G., Ender, K., Lorenz, S., Winkler, K., Rickert, R., & Steinhart, H. (2002). N-3 fatty acids and conjugated linoleic acids of *longissimus* muscle in beef cattle. *European Journal of Lipid Science and Technology, 104*, 463-471.

Pariza, M. W., Park, Y., & Cook, M. E. (2000). Mechanisms of action of conjugated linoleic acid: evidence and speculation. *Proceedings of the Society for Experimental Biology and Medicine, 223*, 8-13.

Pariza, M. W., Park, Y., & Cook, M. E. (2001). The biologically active isomers of conjugated linoleic acid. *Progress in Lipid Research, 40*, 283-298.

Park, Y., Storkson, J. M., Ntambi, J. M., Cook, M. E., Sih, C. J., & Pariza, M. W. (2000). Inhibition of hepatic stearoyl-CoA desaturase activity by *trans*-10,*cis*-12 conjugated linoleic acid and its derivatives. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, 1486*, 285-292.

Polak, T., Rajar, A., Gasperlin, L., & Zlender, B. (2008). Cholesterol concentration and fatty acid profile of red deer (*Cervus elaphus*) meat. *Meat Science, 80*, 864-869.

Ponnampalam, E. N., Mann, N. J., & Sinclair, A. J. (2006). Effect of feeding systems on omega-3 fatty acids, conjugated linoleic acid and *trans* fatty acids in Australian beef cuts: potential impact on human health. *Asia Pacific Journal of Clinical Nutrition, 15*, 21-29.

Purchas, R. W., Knight, T. W., & Busboom, J. R. (2005). The effect of production system and age on concentrations of fatty acids in intramuscular fat of the *longissimus* and *triceps brachii* muscles of Angus-cross heifers. *Meat Science, 70*, 597-603.

Raes, K., Balcaen, A., Dirinck, P., De Winne, A., Claeys, E., Demeyer, D., & De Smet, S. (2003). Meat quality, fatty acid composition and flavour analysis in Belgian retail beef. *Meat Science, 65*, 1237-1246.

Realini, C. E., Duckett, S. K., Brito, G. W., Dalla Rizza, M., & De Mattos, D. (2004). Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. *Meat Science, 66*, 567-577.

Rule, D. C., MacNeil, M. D., & Short, R. E. (1997). Influence of sire growth potential, time on feed, and growing-finishing strategy on cholesterol and fatty acids of the ground carcass and *longissimus* muscle of beef steers. *Journal of Animal Science, 75*, 1525-1533.

Santos-Silva, J., Bessa, R. J. B., & Santos-Silva, F. (2002). Effect of genotype, feeding system and slaughter weight on the quality of light lambs: II. Fatty acid composition of meat. *Livestock Production Science, 77*, 187-194.

Serrano, E., Cornu, A., Kondjoyan, N., Agabriel, J., & Micol, D. (2011). Traceability of grass feeding in beef: terpenes, 2,3-octanedione and skatole accumulation in adipose tissue of young bulls. *Animal*, *5*, 641-649.

Shingfield, K. J., Reynolds, C. K., Lupoli, B., Toivonen, V., Yurawecz, M. P., Delmonte, P., Griinari, J. M., Grandison, A. S., & Beever, D. E. (2005). Effect of forage type and proportion of concentrate in the diet on milk fatty acid composition in cows given sunflower oil and fish oil. *Animal Science, 80*, 225-238.

Simopoulos, A. P. (1991). Omega-3 fatty acids in health and disease and in growth and development. *The American Journal of Clinical Nutrition, 54*, 438-463.

SPSS 17.0 (2008). *User´s guide. SPSS Inc.*

Stanley, J. C., Elsom, R. L., Calder, P. C., Griffin, B. A., Harris, W. S., Jebb, S. A., Lovegrove, J. A., Moore, C. S., Riemersma, R. A., & Sanders, T. A. B. (2007). UK Food Standards Agency Workshop Report: the effects of the dietary n-6:n-3 fatty acid ratio on cardiovascular health. *British Journal of Nutrition*, *98*, 1305-1310.

Steen, R. W. J., Lavery, N. P., Kilpatrick, D. J., & Porter, M. G. (2003). Effects of pasture and high-concentrate diets on the performance of beef cattle, carcass composition at equal growth rates, and the fatty acid composition of beef. *New Zealand Journal of Agricultural Research, 46*, 69-81.

Storkson, J. M., Park, Y., Cook, M. E., & Pariza, M. W. (2005). Effects of *trans*-10,*cis*-12 conjugated linoleic acid and cognates on apolipoprotein B secretion in HepG2 cells. *Nutrition Research, 25*, 387-399.

Sukhija, P. S., & Palmquist, D. L. (1988). Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. *Journal of Agricultural and Food Chemistry, 36*, 1202-1206.

Vernon, R. G. (1986). The growth and metabolism of adipocytes. In: Buttery, P.J., Haynes, N.B., Lindsay, D.B. (Eds.), *Control and Manipulation of Animal Growth* (pp.67-83). Butterworth, London.

Vlaeminck, B., Fievez, V., Cabrita, A. R. J., Fonseca, A. J. M., & Dewhurst, R. J. (2006). Factors affecting odd- and branched-chain fatty acids in milk: A review. *Animal Feed Science and Technology*, *131*, 389-417.

Wongtangtintharn, S., Oku, H., Iwasaki, H., & Toda, T. (2004). Effect of branched-chain fatty acids on fatty acid biosynthesis of human breast cancer cells. *Journal of Nutritional Science and Vitaminology, 50*, 137-143.

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., Hughes, S. I., & Whittington, F. M. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science*, *78*, 343-358.

Yurawecz, M., Roach, J., Sehat, N., Mossoba, M., Kramer, J., Fritsche, J., Steinhart, H., & Ku, Y. (1998). A new conjugated linoleic acid isomer, 7 *trans*, 9 *cis*-octadecadienoic acid, in cow milk, cheese, beef and human milk and adipose tissue. *Lipids*, *33*, 803-809.

Figure 1

Experimental protocol for the four treatments: 12SE (12 months slaughter age, semi-extensive), 14SE (14 months slaughter age, semi-extensive), 12IN (12 months slaughter age, intensive) and 14IN (14 months slaughter age, intensive). The format date used is: dd/mm/yy

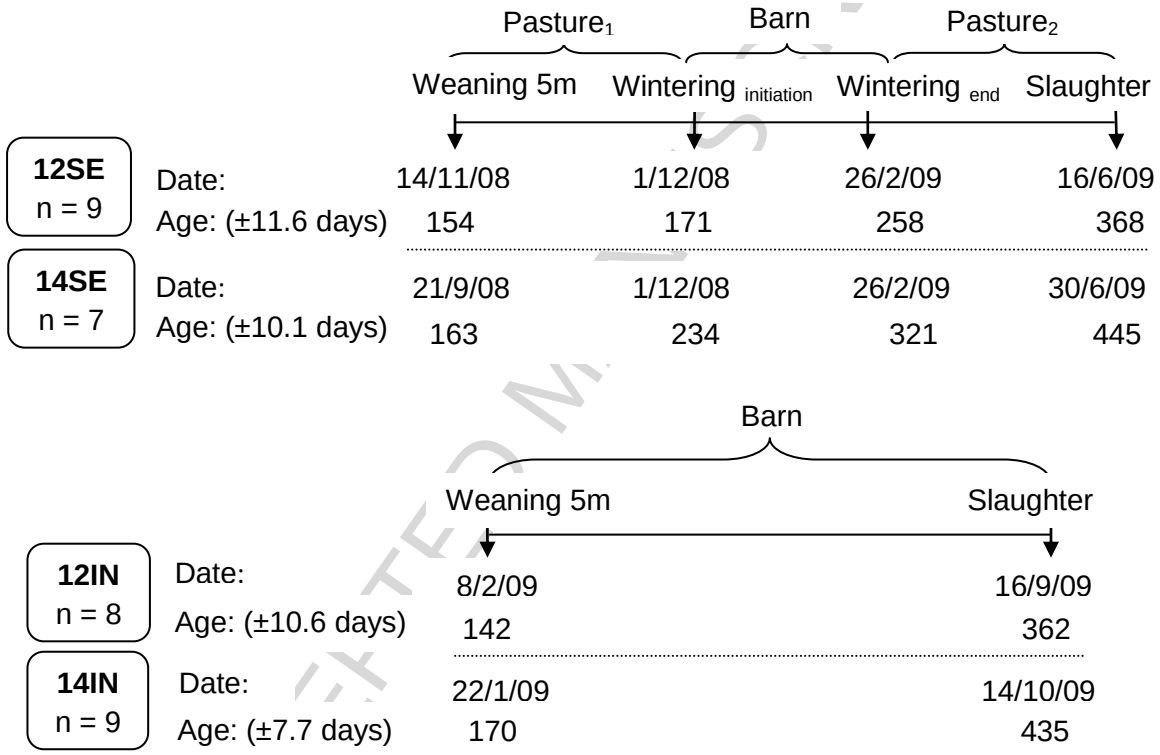

Table 1

Chemical and fatty acid composition of feedstuffs.

	Concentrate [a]	Straw	Crush Barley	Pasture
Chemical composition (% DM)				
Crude protein	15.8	3.46	9.22	14.2
Ether extract	4.57	0.62	2.16	2.70
Crude fiber	10.2	41.9	4.66	23.6
Acid detergent fiber	-	51.4	7.02	29.7
Neutral detergent fiber	24.8	79.3	21.8	52.3
Ash	6.61	7.28	2.46	11.2
Fatty acid composition (%)[b]				
14:0	0.24	5.26	0.27	0.57
16:0	23.5	34.7	23.0	15.6
18:0	3.30	4.46	1.83	2.16
20:0	0.48	4.12	0.32	0.73
22:0	0.36	5.54	0.28	1.16
24:0	0.41	4.13	0.21	1.06
*c*9-18:1	21.8	7.85	13.5	2.89
*c*11-18:1	2.28	0.51	0.63	0.82
18:2n-6	42.5	14.7	53.5	14.9
18:3n-3	3.47	3.92	5.08	46.8
ΣSFA[c]	**28.3**	**58.2**	**25.9**	**21.3**
ΣMUFA[d]	**24.1**	**8.36**	**14.2**	**3.71**
ΣPUFA[e]	**45.9**	**18.6**	**58.6**	**61.7**

[a] The commercial finishing concentrate consisted of 40.4% corn, 12.9% barley, 11% corn dried distillers grains plus solubles, 10.67% soybean, 7.3% soybean husk, 6.5% canola meal, 6% barley straw (treated with sodium hydroxide), 2.45% calcium carbonate, 1.17% palm oil, 1% molasses, 0.5% sodium bicarbonate and 0.43% sodium chloride on an as-fed basis.

[b] Percentage of total fatty acids quantified (%wt./wt.).

[c] Sum of 14:0, 16:0, 18:0, 20:0, 22:0 and 24:0.

[d] Sum of *c*9-18:1 and *c*11-18:1.

[e] Sum of 18:2n-6 and 18:3n-3.

Table 2

Mean dry matter concentrate intake (DMCI), effect of two slaughter ages (12 vs. 14 months) on average daily gain during weaning-slaughter period (ADG $_{weaning\text{-}slaughter}$), wintering period (ADG $_{wintering}$) and post-wintering pasture period (ADG $_{pasture\ 2}$), and effect of two production systems (semi-extensive vs. intensive) and two slaughter ages on final live weight, cold carcass weight, carcass fatness, carcass conformation and perirenal fat weight from youthful Tudanca bulls.

	Production system							
	Semi-extensive		Intensive			*p* values		
	12 mo.	14 mo.	12 mo.	14 mo.	SEM	PS	SA	PSxSA
DMCI (kg MS/animal)	368	494	1198	1493	-	-	-	-
ADG $_{weaning\text{-}slaughter}$ (g/d)	-	-	986^{b}	1086^{a}	0.01	-	***	-
ADG $_{wintering}$ (g/d)	368^{b}	876^{a}	-	-	0.03	-	***	-
ADG $_{pasture\ 2}$ (g/d)	1153^{b}	1355^{a}	-	-	0.02	-	***	-
Final live wt. (kg)	279^{c}	350^{b}	329^{b}	385^{a}	8.94	***	***	ns
Cold carcass wt. (kg)	141^{c}	187^{b}	178^{b}	213^{a}	5.97	***	***	ns
Carcass fatness (1-15)	2.3^{b}	4.4^{a}	5.4^{a}	5.0^{a}	0.30	***	ns	*
Conformation (1-18)	4.0	4.5	3.9	5.0	0.22	ns	ns	ns
Perirenal fat wt. (kg)	0.53^{d}	1.14^{c}	2.17^{b}	2.74^{a}	0.17	***	***	ns

^{a,b,c} Means within a row with different superscripts differ significantly ($p \leq 0.05$), ns = $p > 0.1$, t = $p \leq 0.1$, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$. SEM: standard error of mean. PS: production system and SA: slaughter age. mo.: months.

Table 3

Effect of two production systems (semi-extensive vs. intensive) and two slaughter ages (12 vs. 14 months) on intramuscular fat content (IMF) and on percentage (% wt./wt. of total FAs quantified) of saturated fatty acids (SFAs) and branched chain fatty acids (BCFAs) from *Longissimus thoracis* muscle of youthful Tudanca bulls.

	Production system							
	Semi-extensive		Intensive			*p* values		
	12 mo.	14 mo.	12 mo.	14 mo.	SEM	PS	SA	PSxSA
IMF (%)	1.14 ^{b}	1.31 ^{b}	3.03 ^{a}	2.73 ^{a}	0.195	***	ns	ns
14:0	1.10 ^{b}	1.37 ^{b}	1.77 ^{a}	1.82 ^{a}	0.084	***	ns	ns
15:0	0.37 ^{a}	0.35 ^{a}	0.26 ^{b}	0.27 ^{b}	0.011	***	ns	ns
16:0	17.6 ^{b}	19.1 ^{b}	22.6 ^{a}	21.3 ^{a}	0.465	***	ns	*
17:0	0.84 ^{b}	0.83 ^{b}	0.96 ^{a}	1.03 ^{a}	0.022	***	ns	ns
18:0	16.9	17.6	16.2	16.3	0.275	*	ns	ns
20:0	0.12 ^{b}	0.13 ^{a}	0.10 ^{c}	0.11 ^{b}	0.003	***	***	ns
21:0	0.03 ^{a}	0.03 ^{a}	0.02 ^{b}	0.02 ^{b}	0.001	***	ns	ns
22:0	0.30 ^{a}	0.27 ^{a}	0.07 ^{b}	0.06 ^{b}	0.021	***	ns	ns
23:0	0.03 ^{a}	0.03 ^{ab}	0.02 ^{b}	0.02 ^{b}	0.002	**	ns	ns
24:0	0.03 ^{a}	0.04 ^{a}	0.00 ^{b}	0.01 ^{b}	0.003	***	ns	ns
ΣSFA	**37.4** ^{b}	**39.8** ^{ab}	**41.9** ^{a}	**40.9** ^{a}	**0.588**	*****	**ns**	**ns**
iso-15:0	0.12 ^{b}	0.15 ^{a}	0.07 ^{c}	0.07 ^{c}	0.007	***	ns	ns
anteiso-15:0	0.18 ^{a}	0.18 ^{a}	0.11 ^{b}	0.13 ^{b}	0.007	***	ns	ns
iso-16:0	0.14 ^{a}	0.15 ^{a}	0.09 ^{c}	0.11 ^{b}	0.005	***	*	ns
iso-17:0	0.47 ^{a}	0.47 ^{a}	0.33 ^{b}	0.36 ^{b}	0.014	***	ns	ns
anteiso-17:0	0.38 ^{b}	0.40 ^{b}	0.44 ^{ab}	0.47 ^{a}	0.013	**	ns	ns
iso-18:0	0.08 ^{b}	0.09 ^{b}	0.09 ^{b}	0.12 ^{a}	0.004	**	**	t
ΣBCFA	**1.37** ^{ab}	**1.43** ^{a}	**1.13** ^{c}	**1.26** ^{bc}	**0.032**	*****	**t**	**ns**

^{a,b,c} Means within a row with different superscripts differ significantly ($p \leq 0.05$), ns = $p > 0.1$, t = $p \leq 0.1$, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$. SEM: standard error of mean. PS: production system and SA: slaughter age. mo.: months.

Table 4

Effect of two production systems (semi-extensive vs. intensive) and two slaughter ages (12 vs. 14 months) on percentage (% wt./wt. of total FAs quantified) of monounsaturated fatty acids (MUFAs) from *Longissimus thoracis* muscle of youthful Tudanca bulls.

	Production system							
	Semi-extensive		Intensive			*p* values		
	12 mo.	14 mo.	12 mo.	14 mo.	SEM	PS	SA	PSxSA
*c*9-14:1	0.09 ^{c}	0.13 ^{bc}	0.23 ^{a}	0.18 ^{ab}	0.014	***	ns	*
*c*7-16:1	0.22 ^{a}	0.22 ^{a}	0.14 ^{b}	0.13 ^{b}	0.008	***	ns	ns
*c*9-16:1	1.15 ^{c}	1.59 ^{b}	2.00 ^{a}	2.09 ^{a}	0.094	***	*	ns
*c*9-17:1	0.40 ^{c}	0.43 ^{bc}	0.45 ^{ab}	0.49 ^{a}	0.013	***	*	ns
*c*9-18:1	22.5 ^{c}	26.5 ^{b}	30.4 ^{a}	29.2 ^{ab}	0.711	***	ns	*
*c*11-18:1	1.21 ^{b}	1.11 ^{b}	1.50 ^{a}	1.48 ^{a}	0.047	***	ns	ns
*c*12-18:1	0.32	0.31	0.32	0.30	0.008	ns	ns	ns
*c*13-18:1	0.11 ^{b}	0.13 ^{b}	0.18 ^{a}	0.16 ^{a}	0.006	***	ns	*
*c*14-18:1	0.07 ^{a}	0.07 ^{a}	0.06 ^{b}	0.06 ^{b}	0.002	***	ns	ns
*c*15-18:1	0.13	0.12	0.12	0.12	0.004	ns	ns	ns
*c*11-20:1	0.13 ^{b}	0.14 ^{b}	0.14 ^{b}	0.17 ^{a}	0.004	**	*	ns
Σ*c*-MUFA	**26.4** ^{c}	**30.8** ^{b}	**35.5** ^{a}	**34.3** ^{a}	**0.813**	***	**ns**	*
*t*9-16:1	0.28 ^{a}	0.23 ^{b}	0.10 ^{c}	0.07 ^{c}	0.018	***	*	ns
*t*6-t8-18:1	0.12 ^{b}	0.14 ^{b}	0.25 ^{a}	0.30 ^{a}	0.016	***	t	ns
*t*9-18:1	0.15 ^{b}	0.18 ^{b}	0.29 ^{a}	0.30 ^{a}	0.014	***	ns	ns
*t*10-18:1	0.29 ^{c}	0.29 ^{c}	2.31 ^{b}	3.88 ^{a}	0.324	***	t	t
*t*11-18:1	1.98 ^{a}	1.99 ^{a}	1.16 ^{b}	1.00 ^{b}	0.119	***	ns	ns
*t*12-18:1	0.29	0.28	0.27	0.27	0.007	ns	ns	ns
*t*13/*t*14-18:1	0.64 ^{a}	0.61 ^{a}	0.40 ^{b}	0.41 ^{b}	0.024	***	ns	ns
*t*15-18:1	0.37 ^{b}	0.46 ^{a}	0.31 ^{b}	0.31 ^{b}	0.016	***	ns	ns
*t*16-18:1	0.24 ^{a}	0.25 ^{a}	0.11 ^{b}	0.10 ^{b}	0.014	***	ns	ns
Σ*t*-18:1	4.08 ^{b}	4.19 ^{b}	5.09 ^{b}	6.58 ^{a}	0.261	***	ns	ns
Σ*t*-MUFA	**4.36** ^{b}	**4.42** ^{b}	**5.19** ^{b}	**6.65** ^{a}	**0.253**	***	**t**	**t**
ΣMUFA	**30.7** ^{c}	**35.2** ^{b}	**40.7** ^{a}	**41.0** ^{a}	**0.945**	***	**t**	**t**

^{a,b,c} Means within a row with different superscripts differ significantly ($p \leq 0.05$), ns = $p > 0.1$, t = $p \leq 0.1$, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$. SEM: standard error of mean. PS: production system and SA: slaughter age. mo.: months.

Table 5

Effect of two production systems (semi-extensive vs. intensive) and two slaughter ages (12 vs. 14 months) on percentage (% wt./wt. of total FAs quantified) of polyunsaturated fatty acids n-6 and n-3 and the n-6/n-3 ratio from *Longissimus thoracis* muscle of youthful Tudanca bulls.

	Production system							
	Semi-extensive		Intensive			*p* values		
	12 mo.	14 mo.	12 mo.	14 mo.	SEM	PS	SA	PSxSA
18:2n-6	14.2 ^{a}	10.5 ^{b}	9.95 ^{b}	10.7 ^{b}	0.623	ns	ns	t
18:3n-6	0.10 ^{a}	0.08 ^{ab}	0.06 ^{bc}	0.06 ^{c}	0.005	***	ns	ns
20:2n-6	0.14 ^{a}	0.11 ^{ab}	0.10 ^{b}	0.10 ^{b}	0.006	t	ns	ns
20:3n-6	0.85 ^{a}	0.63 ^{b}	0.53 ^{b}	0.48 ^{b}	0.040	**	t	ns
20:4n-6	4.29 ^{a}	2.97 ^{b}	2.55 ^{b}	2.62 ^{b}	0.215	**	ns	t
22:2n-6	0.02 ^{a}	0.02 ^{a}	0.01 ^{b}	0.01 ^{b}	0.001	***	ns	ns
22:4n-6	0.43 ^{ab}	0.34 ^{b}	0.50 ^{ab}	0.56 ^{a}	0.031	*	ns	ns
Σn-6	**19.9 ^{a}**	**14.6 ^{b}**	**13.7 ^{b}**	**14.5 ^{b}**	**0.889**	**t**	**ns**	**t**
18:3n-3	3.54 ^{a}	3.24 ^{a}	0.33 ^{b}	0.37 ^{b}	0.293	***	ns	ns
22:3n-3	0.04 ^{a}	0.04 ^{a}	0.01 ^{b}	0.01 ^{b}	0.003	***	ns	ns
20:5n-3	1.65 ^{a}	1.23 ^{b}	0.18 ^{c}	0.12 ^{c}	0.128	***	*	t
22:5n-3	3.10 ^{a}	2.36 ^{b}	0.69 ^{c}	0.59 ^{c}	0.216	***	*	t
22:6n-3	0.32 ^{a}	0.21 ^{b}	0.09 ^{c}	0.07 ^{c}	0.021	***	**	*
Σn-3	**8.64 ^{a}**	**7.08 ^{b}**	**1.30 ^{c}**	**1.15 ^{c}**	**0.651**	*******	**t**	**ns**
Σn-6/Σn-3	**2.30 ^{c}**	**2.09 ^{c}**	**10.6 ^{b}**	**12.8 ^{a}**	**0.859**	*******	*****	*******

^{a,b,c} Means within a row with different superscripts differ significantly ($p \leq 0.05$), ns = $p > 0.1$, t = $p \leq 0.1$, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$. SEM: standard error of mean. PS: production system and SA: slaughter age. mo.: months.

Table 6

Effect of two production systems (semi-extensive vs. intensive) and two slaughter ages (12 vs. 14 months) on percentage (% wt./wt. of total FAs quantified) of conjugated linoleic acid (CLA), other biohydrogenated FAs (Other biohydrogenated), polyunsaturated fatty acids (PUFA) and PUFA/SFA ratio from *Longissimus thoracis* muscle of youthful Tudanca bulls.

	Production system					*p* values		
	Semi-extensive		Intensive					
	12	14	12	14	SEM	PS	SA	PSxSA
*t*12,*t*14-18:2	0.02 ^{a}	0.02 ^{a}	0.00 ^{b}	0.00 ^{b}	0.002	***	ns	ns
*t*11,*t*13-18:2	0.06 ^{a}	0.06 ^{a}	0.01 ^{b}	0.01 ^{b}	0.005	***	ns	ns
*t*9,*t*11-18:2	0.02	0.03	0.03	0.01	0.002	ns	ns	**
Σ *t*,*t*-CLA	**0.12 ^{a}**	**0.14 ^{a}**	**0.07 ^{b}**	**0.04 ^{b}**	**0.009**	*******	**ns**	*****
*t*11,*c*13-18:2	0.05 ^{a}	0.05 ^{a}	0.01 ^{b}	0.00 ^{b}	0.004	***	ns	ns
*t*10,*c*12-18:2	0.01 ^{c}	0.01 ^{b}	0.02 ^{a}	0.02 ^{a}	0.001	***	*	ns
*c*9,*t*11-18:2	0.49 ^{a}	0.49 ^{a}	0.25 ^{b}	0.21 ^{b}	0.028	***	ns	ns
*t*7,*c*9-18:2	0.03 ^{c}	0.04 ^{c}	0.07 ^{b}	0.08 ^{a}	0.004	***	**	ns
Σ *c*,*t*/*t*,*c*-CLA	**0.65 ^{a}**	**0.67 ^{a}**	**0.40 ^{b}**	**0.39 ^{b}**	**0.028**	*******	**ns**	**ns**
Σ CLA	**0.79 ^{a}**	**0.83 ^{a}**	**0.48 ^{b}**	**0.44 ^{b}**	**0.035**	*******	**ns**	**ns**
*c*9,*t*11,*c*15-18:3	0.09 ^{a}	0.08 ^{a}	0.02 ^{b}	0.01 ^{b}	0.006	***	ns	ns
*c*9,*t*13/*t*8,*c*12-18:2	0.20 ^{a}	0.21 ^{a}	0.13 ^{b}	0.12 ^{b}	0.008	***	ns	ns
other *c*,*c*/*c*,*t*/*t*,*t*-18:2	0.56 ^{a}	0.52 ^{ab}	0.44 ^{b}	0.47 ^{b}	0.015	**	ns	ns
*t*11,*c*15-18:2	0.26 ^{a}	0.23 ^{a}	0.09 ^{b}	0.12 ^{b}	0.015	***	ns	ns
Σ Other	**1.11 ^{a}**	**1.04 ^{a}**	**0.68 ^{b}**	**0.72 ^{b}**	**0.038**	*******	**ns**	**ns**
Σ PUFA	**30.5 ^{a}**	**23.6 ^{b}**	**16.2 ^{c}**	**16.8 ^{c}**	**1.407**	*******	**ns**	**t**
PUFA/SFA	**0.84 ^{a}**	**0.60 ^{b}**	**0.40 ^{b}**	**0.41 ^{b}**	**0.045**	*******	**ns**	**t**

^{a,b,c} Means within a row with different superscripts differ significantly ($p \leq 0.05$), ns = $p > 0.1$, t = $p \leq 0.1$, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$. SEM: standard error of mean. **Σ*t*,*t*-CLA (%)**= *t*12,*t*14-18:2 + *t*11,*t*13-18:2 + *t*10,*t*12-18:2 + *t*9,*t*11-18:2 + *t*8,*t*10-18:2 + *t*7,*t*9-18:2 + *t*6,*t*8-18:2, **Σ*c*,*t*/*t*,*c*-CLA (%)**= *t*12,*c*14-18:2 + *c*12,*t*14-18:2 + *t*11,c13-18:2 + *c*11,*t*13-18:2 + *t*10,*c*12-18:2 + *t*9,*c*11-18:2 + *c*9,*t*11-18:2 + *t*8,*c*10-18:2 + *t*7,*c*9-18:2. **ΣCLA (%)**= Σ*t*,*t*-CLA + Σ*c*,*t*/*t*,*c*-CLA + *c*9,*c*11-18:2. **ΣPUFA** = Σn-6 + Σn-3 + ΣCLA + ΣOther Biohydrogenated FAs. PS: production system and SA: slaughter age. mo.: months.