BREED EFFECT ON *TRANS*-18:1, CONJUGATED LINOLEIC ACID AND POLYUNSATURATED FATTY ACID PROFILE OF VEAL MEAT PRODUCED IN MOUNTAIN AREAS OF NORTHERN SPAIN

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Abstract – This study was designed to compare the fatty acid (FA) composition of veal meat produced from 'Tudanca x Charolais' cross (n=6) and Limousin (n=6) breeds when allowed to feed freely on mountain pastures and suckled naturally from birth to 7 months of age. After 80 days of age calves also had access to concentrate, while mothers did not. Beef from Tudanca x Charolais genotype provided higher fat content (*P*<0.05) than Limousin. In general, Tudanca x Charolais had a better FA profile than Limousin beef, especially in terms of the content of polyunsaturated (PUFA; *P*<0.05) and highly unsaturated long-chain fatty acids (HUFA; *P*<0.05), as well as vaccenic acid (VA; *P*<0.1) and the overall *trans*-18:1 isomer profile. The improved nutritional quality provided an added value to the veal meat produced by the local farmers.

Key Words – fatty acid, genotype, highly unsaturated fatty acids, rumenic acid, vaccenic acid.

I. INTRODUCTION

Mountain areas of northern Spain are traditionally dedicated to beef production utilizing mostly local breeds and resources [1]. The most common beef production system in these mountain areas is centered on calf production which farmers sell to bigger feedlots for final intensive-fattening and commercialization (8-9 months of age at slaughter). According to recent findings [2] it was concluded that this system did not provide much income to local farmers and therefore an alternative systems was suggested where farmers could produce veal meat locally as opposed to finishing elsewhere. This production system meets a number of expectations in terms of sustainability and multi-functionality of rural areas while at the same time a nutritionally healthier meat could be obtained in terms of their FA composition [3].

In order to test the FA composition of veal meat obtained according to aforementioned local production system two breeds were investigated: a local cross ('Tudanca x Charolais') versus a foreign breed that has been well-adapted to the area (Limousin). Emphasis has been made on *trans*(*t*)-18:1 and conjugated linoleic acid (CLA) isomeric distribution, and their PUFA contents.

II. MATERIALS AND METHODS

Twelve male calves from Tudanca x Charolais cross (n = 6) and Limousin (n = 6) were studied. From birth (March-April 2010), calves were naturally suckled by their mothers in mountain areas of Cantabria (Nansa Valley, northern Spain). The major botanic species in the fields were *Lolium perenne*, *Agrostis capillaris* and *Trifolium repens*. After an average of 80 days of age calves received *ad libitum* access to concentrate up to a maximum of 3 kg/day, while mothers did not. The concentrate meal was composed approximately by 40% corn meal, 45% barley meal, 10% soya meal, 2% fat, 3% minerals, vitamins and oligoelements. On dry matter (DM) basis, chemical composition of the concentrate was as follows: 16.2% crude protein, 6.7% ash, and 3.9% ether extract, while the percentages of major FAs were as follows: 18% 16:0, 2.1% 18:0, 20% 9*c*-18:1, 50% 18:2n-6, and 3.3% 18:3n-3.

Calves were slaughtered at 7 months of age in a commercial abattoir. Live weight at slaughter was 255±10.5Kg and 318±14.5Kg for Tudanca x Charolais and Limousin, respectively. At 11 days *post-mortem*, the rib joint between the 5th to the 9th ribs of the left half carcass was cut and transported to the laboratory. From the 6th rib the *longissimus thoracis* muscle was ground, freeze-dried, and stored at -80 ºC.

Lipids were extracted from 1 g of freeze-dried muscle using a mixture of chloroform - methanol (1:1, v/v) [4] as described in [5]. Lipid aliquots (~10 mg) from each steak were methylated separately using acidic (methanolic HCl) and basic (sodium methoxide) reagents [6]. For quantitative purposes, 1 mL of internal standard (1 mg / mL of 23:0 methyl ester, N-23-M from Nu-Chek Prep Inc., Elysian, MN, USA) was added prior to methylation. FA methyl esters (FAME) were analyzed using a GC equipped with a flame ionization detector (Agilent Technologies, Model 7890A, Wilmington, DE, USA). The FAMEs, including the *trans*-18:1 isomers, were analyzed using a 100 m SP-2560 column (Supelco, Bellefonte, PA, USA) and two complementary GC temperature programs plateauing at 175 ºC and 150 ºC [7]. The CLA isomers were separated and identified using a 100 m SLB-IL111 ionic liquid stationary phase column (Supelco, Bellefonte, PA, USA) as described by [8].

For FAME identification reference standards #463 and #603, individual 21:0 and 23:0 ME, and CLA mixture #UC-59M were used (Nu-Chek Prep Inc., Elysian, MN, USA). Many of the *trans*-18:1 and CLA isomers, not included in the standard mixtures, were identified according to the literature [6,7,8,9].

The statistical analysis was conducted using SPSS 19 for Windows (SPSS Inc., IBM Corporation, NY, USA). One-way ANOVA analysis was applied to test differences between breeds (Tudanca x Charolais cross, Limousin) for all variables studied. Significance was declared at $P<0.05$ and trend was declared at $P<0.1$.

III. RESULTS AND DISCUSSION

Beef obtained from Tudanca x Charolais calves had significantly greater amount of total FAMEs per 100 g of fresh meat (2.04%; $P<0.01$) than in Limousin (1.20%). In addition, the absolute amount of most of the FAs (individual and groups) were significantly higher in Tudanca x Charolais beef with few exceptions (data not shown).

Trans isomer 10*t*-18:1 tended to be greater in Limousin (11.3 mg / 100 g meat) than in Tudanca x Charolais beef (7.2 mg / 100 g meat, $P<0.1$), and 11*t*-18:1 isomer (VA) tended to be greater in Tudanca x Charolais (57 mg / 100 g meat) than in Limousin beef (28 mg / 100 g meat; $P < 0.1$). The relative abundance of all the *trans*-18:1 isomers to total *trans*-18:1 are shown in Figure 1. Interestingly, 10*t*-18:1 was significantly lower ($P<0.01$) in Tudanca x Charolais compared to the Limousin breed. Moreover, 6*t*/7*t*/8*t*-18:1 also tended to be lower in Tudanca x Charolais, while other isomers like 12*t*- ($P<0.05$), 13*t*/14*t*- ($P<0.01$), 15*t*- and 16*t*- 18:1 were significantly or numerically higher in Tudanca x Charolais.

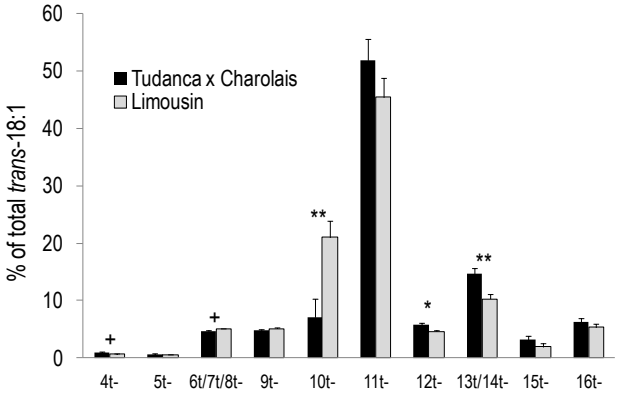

Figure 1. Breed effect on the relative isomeric distribution of individual *trans*-18:1 isomers

It was interesting to note that neither the absolute nor the relative content of rumenic acid (RA, 9*c*11*t*-18:2) or total CLA were significantly different between these two breeds (data not shown). Most of the total CLA consisted of RA which represented over 76 % of the total CLA. When representing the individual isomers relative to total CLA (Figure 2) only one isomer (11*t*,13c-) was significantly different between breeds.

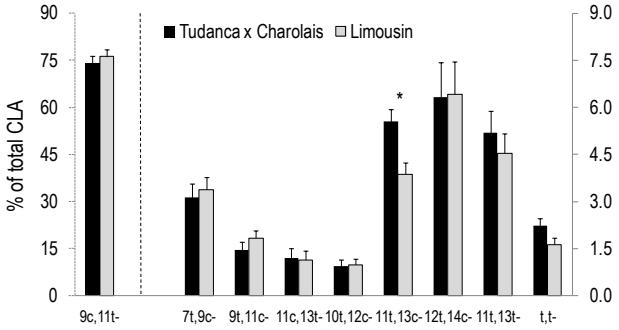

Figure 2. Breed effect on the relative isomeric distribution of individual CLA isomers.
(Rumenic acid has been represented in the left axis while other CLA isomers have been represented in the right axis).

In general, all PUFA and HUFA percentages were significantly higher (P<0.05) in Limousin compared to Tudanca x Charolais beef (data not shown). In Figure 3, absolute amounts of selected PUFAs and HUFAs are compared. In general, all n-6 and n-3 PUFA and HUFAs were either numerically or statistically higher in Tudanca x Charolais compared to Limousin breed.

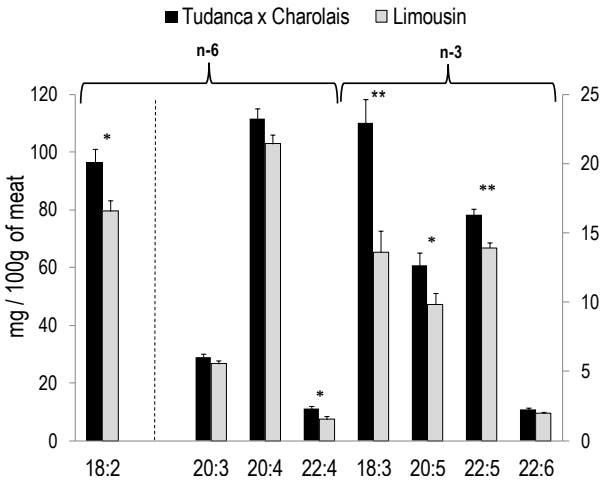

Figure 3. Breed effect on selected n-6 and n-3 PUFA and HUFA (mg / 100 g of meat).
(Linoleic acid has been represented in the left axis while other PUFA and HUFAs have been represented in the right axis).

During the early stage of development, and exclusive suckling period, calves probably derived their *trans* FAs mainly from cow's milk as the reticular groove normally closes, delivering the milk directly into the abomasum bypassing the rumen [10,11]. In the absence of a fully functioning rumen of the calves, the FA composition of cow's milk could not be extensively altered by rumen bacteria. VA (11*t*-18:1) was likely the predominant *trans*-18:1 isomer in the milk because the dams of both breeds were exclusively pasture-fed. After 80 days of age, all calves had free access to concentrate that provided them with residual 18:2n-6 and 18:3n-3 from the barley and soybean meal in the diet. These PUFAs could be converted to either 11*t*- and/or 10*t*-18:1 in the developing rumen of the calves, and the higher level of 10*t*-18:1 was consistent with the greater consumption of concentrate by Limousin calves (346Kg vs 253Kg) [1] which would have been a consequence of the lower milk production of their dams compared to Tudanca dams (farmers communication).

IV. CONCLUSION

From the present study it was concluded that veal meat obtained from Tudanca x Charolais calves had significantly higher contents of recognized metabolites of 18:3n-3 that provided a better FA profile especially in terms of the content of n-3 HUFA, in addition to VA and the overall *trans*-18:1 isomer profile. In general, veal meat obtained under the local mountain production system, without a commercial final intensive fattening period, proved to provide veal product showing a nutritionally desirable lipid profile.

ACKNOWLEDGEMENTS

This work was undertaken within the agreement between Fundación Botín and CSIC (2010) to study and develop a beef production and commercialization system in Nansa - Peñarrubia valley. Help provided by farmers, IGM staff and Rubén Plano from Semark AC Group S.A. is very much appreciated. N. Aldai thanks the European Commission for the contract through the 'Marie Curie (SPACANBEEF, PIOF-GA-2008-220730)' international program and the Spanish Ministry of Science and Innovation for the contract through the 'Ramón y Cajal (RYC-2011-08593)' program.

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