CHARACTERIZATION AND VARIABILITY OF FATTY ACID COMPOSITION OF LAMB COMMERCIALIZED IN NORTHERN SPAIN: EMPHASIS ON *TRANS*-18:1 AND CLA CONTENT AND PROFILE

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Abstract - Lamb survey was performed in northern Spain (Basque Country and Navarra) in order to characterize its fatty acid composition emphasizing on trans and CLA profiles. Samples were collected in spring (n=24) and winter (n=24) of 2013. Subcutaneous fat was analyzed by GC-FID. In general, very few differences were observed between collection periods. High variability was observed for trans-18:1 content but irrespective of collection periods 2 different groups were clearly identified: 1) when *trans*-18:1 content was $\leq 8\%$ in which 11*t*-18:1 was the predominant isomer; 2) when trans-18:1 content was > 8%, 10t-18:1 was the main isomer. The samples from the 2 groups were clearly separated in the principal component analysis. High variability observed among samples could be associated with differences in genetics and feeding strategies.

Key Words – sheep, survey, vaccenic acid.

I. INTRODUCTION

Mediterranean countries are the major producers of sheep meat in the EU (55%). Spain represents 20% of the total EU production [1] and even though sheep production in northern Spain is not as significant as in other regions, most of the sheep-milk is directed to cheese-making, known as 'Idiazabal' PDO cheese. This product provides a significant added value to the ovine sector in northern Spain.

'Latxa' is the local sheep breed in the Basque Country and Navarra. The production system of 'Latxa' flocks involves primarily part-time grazing in the spring and full-time grazing during the summer. Lambing is concentrated between winter and spring seasons when offspring's suckle their mothers for about a month before they are slaughtered. Then ewe's milk is fully dedicated to cheese-making [2,3]. Local butcher-shops and grocery stores are then provided with ovine meat from very young lambs [5] or ovine meats imported from other Spanish or foreign regions (i.e., UK, New Zealand) that may produce lambs under different management systems in terms of breed, age at slaughter and feeding strategies [2-4]

Based on the availability of the different sources of ovine meat, considerable variation in their fatty acid (FA) composition would be expected. Several reports have shown that *trans* FA (TFA) and conjugate linoleic acid (CLA) pattern in ruminant products can be altered depending mainly on diet fed, but also breed [5-6]. Although several lamb surveys have been published [7-8], the emphasis has not been on TFAs. So, the present survey was undertaken to provide a detailed FA composition of ovine meats commercialized in the northern regions of Spain with special emphasis on TFA and CLA isomers that would affect the quality of these meats.

II. MATERIALS AND METHODS

Lamb chops from the Basque Country and Navarra regions were collected in May (spring, n=24) and December (winter, n=24) of 2013, from 24 different retail stores that included eleven small butcher-shops and thirteen large grocery stores [2]. Precise details of production systems for lamb used in the present survey are unknown. Lamb samples collected during spring would likely have been those that grazed on valley pastures, while animals slaughtered in December would likely be directly slaughtered after weaning. After collections, subcutaneous (SC) fat was sampled from a single lamb chop collected at each location that was then vacuum packed and stored at -80°C for further FA determination.

Fifty mg of SC fat were weighed, freeze-dried and directly methylated with sodium methoxide [9]. For quantitative purposes, equal amounts of two internal standards were added prior to methylation (13:0 & 23:0 FA methyl ester (FAME), Nu-Chek Prep Inc., Elysian, MN, USA). Obtained FAMEs were analyzed using a GC-FID using a 100m SP-2560 column (Supelco, Bellefonte, PA, USA) with two complementary GC temperature programs [6,10] and a 100m SLB-IL111 ionic liquid column (Supelco, Bellefonte, PA, USA) [11] to obtain the separation and identification of several biohydrogenation intermediates and CLA. For peak identification purposes, several reference standards, individual FAMEs and retention times and elution orders reported in the literature were used [10-17]. Identifications were also confirmed using FAME fractions obtained from Ag⁺-SPE cartridges [10,18]. The statistical analysis was conducted using SPSS 20 for Windows (SPSS Inc., IBM Corporation, NY).

III. RESULTS AND DISCUSSION

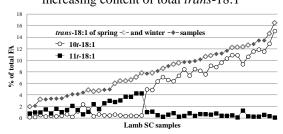
Season effect on the FA composition of the lamb SC fat is summarized in Table 1. There were only few significant differences between collection periods or season. No differences were found for the saturated (SFA) and branched-chain FAs (BCFA). In terms of monounsatured FAs (MUFA), slight differences were found between collection periods. In general, samples collected in spring tend to have a higher content of vaccenic acid (11t-18:1). There were no differences in polyunsatured FAs (PUFA) content while significant differences were observed in the CLA content, mainly related to the second major isomer (7t, 9c-18:2). Total non-conjugated diene content was also higher in the spring collection as individual isomers (not all reported) were in general significantly higher in this period.

Even though no significant differences were found for the total *trans*-18:1 content, the 10*t*-18:1 isomer represented a higher percentage than 11t-18:1 in both collections (4.93% vs 1.72% in spring, and 5.05% vs 1.02% in winter, respectively). It has been well documented that grass/forage fed ruminants have a higher 11t-/10t-18:1 ratio in their tissues [13,19] Furthermore, independently to the collection period, the content of 10t- and 11t-18:1 showed great variation depending on the content of total *trans*-18:1. It was evident that lamb SC samples with less than 8% total *trans*-18:1 of total FAME were clearly associated with higher levels of 11t-18:1. However, when the *trans*-18:1 content was above 8% of total FAME, 10t-18:1 was the predominant isomer (Figure 1).

Table 1. Major individual and groups of fatty acids of lamb subcutaneous fat from both collections

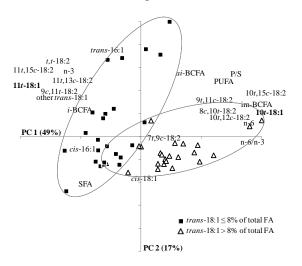
| lamb subcutaneous fat from both collections | | | | |
|---|--------|--------|---------|---------|
| Percentage | Spring | Winter | SEM | P value |
| ∑SFA | 40.9 | 40.7 | 0.670 | ns |
| 16:0 | 22.5 | 21.8 | 0.474 | ns |
| 18:0 | 14.0 | 14.4 | 0.564 | ns |
| ∑BCFA | 3.59 | 3.44 | 0.227 | ns |
| ai-15:0 | 0.260 | 0.220 | 0.0137 | ns |
| <i>i</i> -16:0 | 0.248 | 0.220 | 0.00917 | ns |
| <i>i</i> -17:0 | 0.358 | 0.335 | 0.0123 | ns |
| ai-17:0 | 0.741 | 0.700 | 0.0299 | ns |
| ∑im-BCFA | 1.56 | 1.55 | 0.208 | ns |
| 16:0-4Me | 0.211 | 0.202 | 0.0274 | ns |
| 16:0-12Me | 0.228 | 0.229 | 0.0309 | ns |
| ∑MUFA | 43.0 | 44.1 | 0.700 | ns |
| $\overline{\Sigma}$ cis-MUFA | 34.0 | 36.1 | 0.588 | ns |
| 9 <i>c</i> -16:1 | 1.44 | 1.35 | 0.0501 | ns |
| 9 <i>c</i> -18:1 | 29.1 | 31.3 | 0.573 | ns |
| ∑ <i>trans</i> -MUFA | 9.00 | 7.91 | 0.541 | ns |
| - 10 <i>t</i> -18:1 | 4.93 | 5.05 | 0.672 | ns |
| 11 <i>t</i> -18:1 | 1.72 | 1.02 | 0.190 | + |
| 11 <i>t</i> -/10 <i>t</i> - | 3.91 | 1.94 | 0.663 | ns |
| ∑PUFA | 3.88 | 4.40 | 0.215 | ns |
| $\overline{\Sigma}$ n-6 | 3.12 | 3.70 | 0.231 | ns |
| | 2.87 | 3.44 | 0.220 | ns |
| 20:4n-6 | 0.128 | 0.126 | 0.00665 | ns |
| ∑n-3 | 0.760 | 0.699 | 0.0501 | ns |
| | 0.545 | 0.496 | 0.0396 | ns |
| 22:5n-3 | 0.118 | 0.0939 | 0.00770 | ns |
| n-6/n-3 | 6.02 | 5.91 | 0.535 | ns |
| P/S | 0.144 | 0.152 | 0.00887 | ns |
| ∑CLA | 1.14 | 0.781 | 0.0896 | * |
| - 7 <i>t</i> ,9 <i>c</i> - | 0.0648 | 0.0458 | 0.00403 | * |
| 9 <i>c</i> ,11 <i>t</i> - | 0.786 | 0.509 | 0.0864 | ns |
| ∑nc-dienes | 1.21 | 0.918 | 0.0514 | * |
| | 0.190 | 0.107 | 0.0224 | + |
| 10t,15c-18:2 | 0.136 | 0.135 | 0.0159 | ns |
| ∑Trienes | 0.160 | 0.134 | 0.0107 | + |

SEM, standard error of the mean; ns, not significant; *, $P \le 0.05$; +, P < 0.1; SFA, saturated fatty acids; BCFA, branched-chain fatty acids; im-BCFA, internal methyl BCFAs; MUFA, monounsatured fatty acids; PUFA, polyunsaturated fatty acids; CLA, conjugated linoleic acids; nc-dienes, non conjugated dienes; P/S, PUFA/SFA. Figure 1. Relative abundance of 10t- and 11t-18:1
(%) in all lamb SC samples collected and sorted by increasing content of total *trans*-18:1



According to the PC analysis depicted in Figure 2, samples with a low total trans-18:1 content $(\leq 8\%)$ had positive relationships with 11*t*-18:1, n-3, rumenic acid (RA, 9c,11t-18:2) and SFA, but also with i-BCFAs, cis- and trans-16:1 isomers, trans-18:1 isomers other than 10t- and 11t-, 11t,15c-18:2, t,t-18:2 and 11t,13c-18:2. All these FAs are characteristically found in ruminants fed high grass/forage proportion in the diet, and their presence was reported in studies where appropriate methodologies were used to resolve TFA and CLA isomers [6,19]. On the other hand, samples with a high total trans-18:1 content (>8%) had positive relationships with 10t-18:1, PUFA, n-6, ai-BCFA, im-BCFA, 10t,15c-18:2 and the CLA isomers 7*t*,9*c*-, 10*t*,12*c*-, 9*t*,11*c*-, and 8*c*,10*t*-18:2. These FAs are characteristically found in concentrate-fed ruminants as previously reported [13,19-20].

Figure 2. Plot of variables and lamb SC fat subcutaneous fat samples distributed in a PCA



IV. CONCLUSION

Only few differences were observed between collection periods (spring, winter) mainly due to the high variation among lamb SC samples that could be related to differences of breed, feeding systems (and diet components) of ovine-meat produced locally or imported from other locations (national or international). 10t-18:1 was the predominant trans-18:1 isomer in over half the samples irrespective of collection periods. Lamb SC samples high in total trans-18:1 were also associated with a high 10t-18:1 content and high levels of im-BCFA, ai-BCFA, n-6 and non RA CLAs in backfat, while samples low in total trans-18:1 were associated with higher levels of 11t-18:1 than 10t-18:1 and higher levels of *i*-BCFA, n-3 and RA. It will require further investigations to identify factors to lower the content of 10t-18:1 and other undesirable FAs in lamb meat.

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