RELATIONSHIP BETWEEN GENE EXPRESSION OF STEAROYL-COA DESATURASES (*SCD*1 & *SCD5*) AND THE FATTY ACID PROFILE IN ADIPOSE TISSUE OF CATTLE BREEDS IN THE BASQUE REGION

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Abstract – The objectives of the present study were firstly to determine the correlations between *SERBP*1 and *SCD* isoforms and, secondly, to measure associations between the gene expression of *SCD1* and *SCD5* and the fatty acid profile in backfat tissue of Pirenaica, Salers and Friesian cattle breeds. Gene expression of *SREBP1* and *SCD1* in Pirenaica (autochthonous breed) was higher than in the other breeds. Gene expression correlation between *SREBP1* and *SCD*1 was highly significant in Pirenaica breed. However, *cis*-monounsaturated fatty acid content was similar in Pirenaica and Salers, while lower in Friesian cattle. Overall, the effect of *SCD5* on fatty acid profile of backfat tissue showed an opposite trend in comparison to *SCD1*, although other confirmatory studies are necessary.

Key Words – backfat, lipogenic enzyme, monounsaturated fatty acid, *SREBP1*.

I. INTRODUCTION

In recent years, there has been an increasing concern of consumers regarding the amount and type of fat in the diet. The negative implications of saturated FAs (SFA) are well known [1] while some kind of monounsaturated FAs (MUFA) and polyunsaturated FAs have been recognized as beneficial for human health [2]. The stearoyl-CoA 9-desaturase (*SCD*; EC 1.14.19.1 or Δ9-desaturase), is an enzyme of the endoplasmic reticulum that introduces a double bond at the delta 9 position in a large variety of FAs [3]. The *SCD1* genotype has been associated with the FA composition in Japanese Black cattle [4], Canadian Holstein and Jersey breeds [5], Fleckvieh bulls [6] and Korean native cattle [7]. Recently, a novel isoform of SCD was described in cattle brain (*SCD5*; [8]) which was also related with the SFA to unsaturated FA ratio in milk [9]. Therefore, it seems that several isoforms of bovine *SCD* could be associated to the fat composition in cattle. The *SCD* gene and other lipogenic genes involved in the FA biosynthesis are controlled by a couple of key transcription factors such as the sterol regulatory element binding protein-1 (*SREBP1*) [10]. SREBP1 polymorphisms have been previously correlated with fat composition [11] so it is assumed that differences in the expression level of *SREBP1* gene could affect expression and differences in FA composition of adipose tissues in cattle.

The objectives of the present study were 1) to determine the correlations between *SERBP*1 and *SCD* isoforms, and 2) to determine any association between gene expression of *SCD* isoforms (*SCD1* & *SCD5*) and FA profile in backfat tissue of various bovine breeds (Pirenaica, Salers and Friesian) in the Basque Country (Spain).

II. MATERIALS AND METHODS

A total of 160 backfat tissue samples were collected from pure breed cattle slaughtered in a commercial abattoir located in the Basque Country, northern Spain (Urkaiko S. Coop., Zestoa, Gipuzkoa, Spain) during 12 arbitrary days over 5 weeks in June-July of 2014. For the present study, 62 Pirenaica, 12 Salers and 15 Friesian were selected. Even though management details were not controlled, age and gender data were provided by the abattoir. Pirenaica and Salers were bulls of 12 months old while Friesian were cull cows (> 30 months of age).

Backfat samples for FA and RNA analysis were obtained from the left half carcass between the 5th-

6th ribs. Samples for FA analysis were stored in zipp-lock plastic bags while samples for RNA analysis were preserved in RNAlater™ (Ambion, Austin, TX). All were transported to the laboratory in insulated coolers and stored at -80°C.

Fifty mg of fat tissue were weighed, freeze-dried and directly methylated with sodium methoxide [12]. For quantitative purposes, internal standard (23:0ME) was added. Fatty acid methyl esters (FAME) were analyzed by GC/FID using a 100 m SP2560 column with two complementary GC temperature programs [13]. To determine CLA, samples were subjected to a second GC/FID analysis using a 100 m ionic liquid SLB-IL111 column [14]. For identification, several reference standards (NuCheck Prep Inc., Supelco, Matreya and Larodan), FAME fractions by Ag^{+}- solid phase extraction [13], and retention times and elution orders reported in the literature [15] were used. Data were reported in percentages.

RNA was extracted using RNeasy Lipid Tissue kit (Qiagen Inc., Valencia, CA, USA) and following manufacturer's instructions. Then, DNase digestion step was performed to remove any contaminating genomic DNA. A NanoDrop Spectrophotometer (ND-2000c, Wilmington, DE, USA) was used to analyze RNA samples for quantity (OD260 nm) and purity (OD260 nm/OD280 nm). Aliquots of RNA were stored at -80ºC and dehydrated in RNAstable® 96-Well Plates for international shipping. Total RNAs were reverse-transcribed to synthesize single-strand DNA by using ReverTra Ace (TOYOBO, Osaka, Japan). Quantitative PCR (qPCR) was performed with TaqMan probes (Sigma-genosys, Hokkaido, Japan) in a 7500 Real-Time PCR System (Applied Biosystems). Gene expression of *SREBP1*, *SCD1* and *SCD5* was analyzed using TaqMan Endogenous Control Eukaryotic 18S rRNA gene for the relative quantification of all genes examined. The comparative threshold cycle method (ΔCt) was employed to calculate the relative quantification of gene expression based on the following formula: $\Delta Ct = (Ct_{\text{target gene}} - Ct_{\text{18S rRNA gene}})$.

Statistical analysis was performed using IBM SPSS Statistics 20 for Windows. Non-parametric tests were utilized (age and gender data were not taken into account). Differences in gene expression were examined using the Kruskal Wallis test. Spearman correlations were calculated to assess associations between FAs and mRNA expression for each gene. Differences were considered significant at $P \leq 0.05$.

III. RESULTS AND DISCUSSION

The expression of *SCD1*, *SCD5* and *SREBP1* mRNA was detected in measureable quantities in backfat samples. When genes were expressed in relative amount (-ΔCt), significant differences ($P \leq 0.01$) were observed among genes (Fig. 1). This expression pattern was previously observed by Lengi and Corl [8] when several tissues were studied.

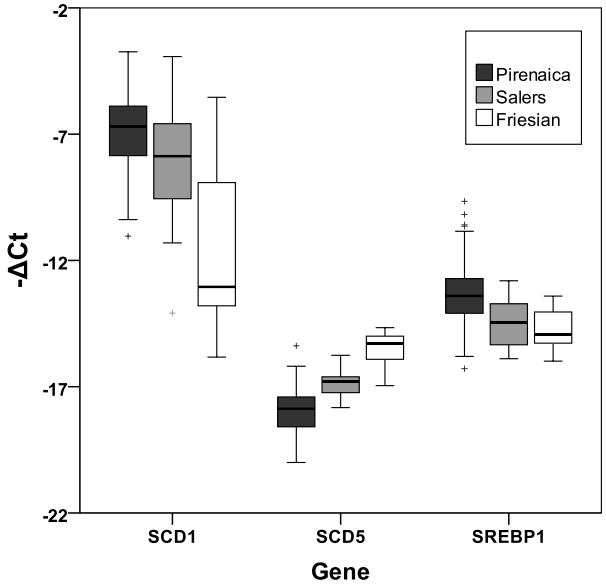

Figure 1. Relative expression levels of *SCD1*, *SCD5* and *SREBP1* mRNA of Pirenaica, Salers and Friesian cattle

SREBP1 is known to regulate the transcriptional activation of *SCD1* [16]. Total sample correlation analysis showed that *SREBP1* and *SCD1* are positively correlated ($P \leq 0.001$). Within each breed, however, correlation between *SREBP1* and *SCD1* was positive and highest for Pirenaica ($P \leq 0.001$), a trend was observed for Salers ($P \leq 0.1$) and it was not significant for Friesian cattle (Table 1). This indicates that some other factors may act as regulators. On the other hand, the correlation between *SREBP1* and *SCD5* was not clear due to differences between breeds.

Table 1. Estimated linear equations between expression level of *SREBP1* and *SCD1* and *SCD5*

	Linear Equation	R^2	*P* value
Pirenaica	ΔCt SCD1=0.92 ΔCt SREBP1 -5.49	0.487	0.000
Salers	ΔCt SCD1=1.50 ΔCt SREBP1 -13.5	0.298	0.055
Friesian	ΔCt SCD1=2.32 ΔCt SREBP1 -22.6	0.343	0.182
Total	ΔCt SCD1=1.49 ΔCt SREBP1 -11.7	0.435	0.000
Pirenaica	ΔCt SCD5=0.12 ΔCt SREBP1 +16.4	0.029	0.284
Salers	ΔCt SCD5=0.15 ΔCt SREBP1 +14.6	0.065	0.430
Friesian	ΔCt SCD5=0.53 ΔCt SREBP1 -7.69	0.371	0.008
Total	ΔCt SCD5=-0.21 ΔCt SREBP1 +20.3	0.047	0.024

In order to determine overall correlations between the gene expression of *SCD1* and *SCD5* with the FA profile of backfat several FAs were selected. From the 98 individual FAs quantified initially, only potential substrates and/or products were selected for correlation purposes. Moreover, 10*t*,12*c*-18:2 was also included as it has been previously described as a potent inhibitor of *SCD1* [17,18]. Correlations between gene expression of *SCD1* and *SCD5* with selected 28 individual FAs of backfat are represented in Fig. 2. In general, it was observed that when *SCD1* mRNA expression was positively correlated with an individual FA, *SCD5* mRNA expression was negatively correlated and vice versa, except for few FAs. This pattern was not that evident when correlations were analyzed within each breed. Salers group, even though the number of animals was the lowest, provided the most similar correlation pattern to the overall population (Fig. 2). Friesians, however, did not show any significant correlations (data not shown) that could be related to the higher age of these animals (average of 70 months) compared to the other breeds.

Even though the expression of *SCD1* was significantly higher in Pirenaica breed, similar *cis*-MUFA contents were found in Pirenaica and Salers breeds. This could be associated to uncontrolled factors like feeding or potential higher expression of *SCD5* in Salers breed as it was observed in Fig. 1.

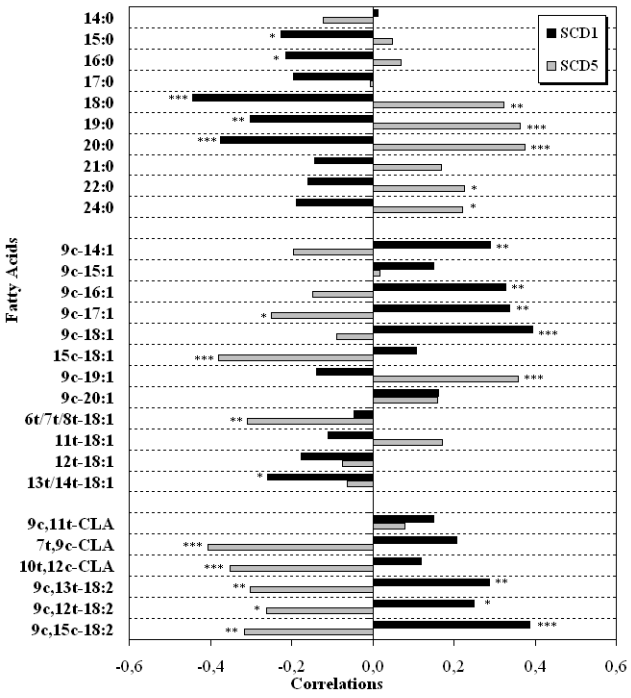

Figure 2. Correlations between expression level of *SCD1* and *SCD5* with selected fatty acids

IV. CONCLUSION

Gene expression of *SREBP1* and *SCD1* in Pirenaica was higher than those in other breeds. Gene expression correlation between *SREBP1* and *SCD*1 was highly significant in Pirenaica breed. Moreover, *cis*-MUFA content in Pirenaica was significantly higher than in Friesian breed. Interestingly, the effect of *SCD5* on FA profile of backfat tissue was in contrast to that of *SCD1*.

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