Expression of SCD and LPL genes and fatty acid profile of subcutaneous adipose tissue of two Portuguese cattle breeds

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Abstract – Adipose tissue is a major site of fatty acid desaturation in bovines and is directly involved in endogenous synthesis of *c*9, *t*11- conjugated linoleic acid (CLA) from vaccenic acid. In the present study, the fatty acid composition and expression levels of stearoyl-CoA desaturase (SCD) and lipoprotein lipase (LPL) genes in subcutaneous adipose tissue (SAT) were investigated in two Portuguese cattle breeds (Alentejana and Barrosã) fed high (HS) or low silage (LS) diets. In addition, the relationship between levels of expression of SCD and LPL genes and tissue content in both cis-9, trans-11 CLA and monounsaturated fatty acid (MUFA) was evaluated. Relative SCD and LPL mRNA expression was assessed through real time quantitative PCR, in parallel with the determination of the fatty acid profile in the SAT. The results revealed that Barrosã breed presents higher levels of CLA in adipose tissue than Alentejana. While MUFA content was higher in the SAT of Barrosã bulls ($P<0.001$), Alentejana had the highest levels of saturated fatty acids (SFA) ($P<0.001$). Expression of the SCD gene was highest in Barrosã animals ($P<0.01$) regardless of the diet. In addition, LPL gene expression was also determined by breed, as a higher expression of this gene was observed in Barrosã than in the Alentejana breed ($P<0.05$). LPL, like SCD, is expressed late in adipogenesis. Thus, increased gene expression would be consistent with more active adipocytes in the Barrosã breed. Overall, this study revealed a breed-specific variation in the levels of SCD and LPL gene expression in SAT, which may modulate CLA, MUFA and SFA contents. Furthermore, the SCD gene appears to exert a direct influence on fatty acid composition in cattle, regardless of the diet.

Keywords: Bovine, Subcutaneous adipose tissue, Real Time PCR

I. INTRODUCTION

Adipose tissue is a major site of fatty acid desaturation in bovines [1]. Fat deposition during beef cattle finishing is the result of both hyperplasia and hypertrophy [2]. The fatty acids deposited in the adipocyte originate from diet or *de novo* fatty acid synthesis. In addition, finishing systems can dramatically alter fat deposition, thus indicating that lipogenic enzymes are influenced by the dietary energy level and, possibly, the energy source.

Stearoyl-CoA desaturase (SCD) is the rate-limiting enzyme that converts palmitoyl- and stearoyl-CoA to palmitoleoyl- and oleoyl-CoA, respectively [3]. In ruminants, it is codified by the SCD gene and participates in the formation of conjugated fatty acid (CLA), from trans-11 C18:1 in animal tissues [4]. The expression of SCD in bovine adipose tissue is regulated by numerous factors, namely breed and diet [5], which also influence fatty acid profile in bovines.

Lipoprotein lipase (LPL), a glycoprotein enzyme, is codified by the LPL gene. This enzyme is the rate-limiting enzyme in the hydrolysis of triacylglycerols (TAG) [4], and plays an important role in the differentiation and maturation of adipocytes [6]. Moreover it controls the TAG partitioning between adipose tissue and muscles [7].

It has been shown that breed [1] and diet composition [8] influence the expression patterns of lipogenic genes. Therefore, we hypothesised that varying the silage/concentrate ratio could alter lipogenic gene expression of subcutaneous adipose tissue (SAT). An experimental trial was then conducted to clarify the relationship between gene expression and fatty acid profile in the SAT of bulls from distinct genetic backgrounds.

III. MATERIAL AND METHODS

The experiment was conducted under the guidelines for the care and use of experimental animals of Unidade de Produção Animal, L-INIA, INRB (Fonte Boa, Vale de Santarém, Portugal). Forty young bulls from Alentejana (large-framed) and Barrosã (small-framed) breeds, were assigned to either high silage (HS, 30% concentrate/70% silage) or low silage (LS, 30% silage/70% concentrate) diets. The initial average weight was 266 ± 10.5 kg for Alentejana and 213 ± 3.64 kg for Barrosã bulls. One Alentejana bull from fed the HS diet was removed from the study due to illness. All animals were slaughtered at 18 months old. The SAT samples were collected immediately after

slaughter for fatty acid analyses and gene expression quantification, directly above the *Longissimus dorsi* muscle.

The SAT samples were preserved in RNAlater and subsequently stored at –80ºC until RNA extraction. RNA was isolated using RNeasy Lipid Tissue Mini Kit according to the manufacturer's protocol. RNA samples were reverse transcribed to cDNA and the Real-Time PCR was performed using the 7300 Real-Time PCR System. The Ct data were analysed and transformed using the relative standard curve method [9]. Gene expression levels of the target genes (SCD and LPL) were normalized against acidic ribosomal protein P0 (RPLP0) as the internal control. Relative transcription levels of the target genes were calculated as described by Larionov *et al*. [9].

Total lipids from SAT samples were extracted by the method of Folch *et al*. [10], modified by Carlson [11], and converted to methyl esters as described by Raes *et al*. [12], using sodium methoxide in anhydrous methanol, followed by hydrochloric acid in methanol. Fatty acid methyl esters (FAME) were extracted with *n*-hexane. The resulting FAME were then analysed as described by Bessa *et al*. [13].

Relative gene expression data was analysed using the GLM procedure of SAS [14] with a model which included breed, diet and their respective interaction as independent variables. Results were expressed as mean ± standard error of the mean (SEM). Differences between groups were examined for statistical significance using PDIFF option. The SAS CORR procedure was used to examine correlations between the relative gene expression and the fatty acid profile.

III. RESULTS AND DISCUSSION

The fatty acid composition of SAT from the four experimental groups is shown in Table 1. While Alentejana bulls presented higher proportions of SFA but lower MUFA, the inverse pattern was observed for the Barrosã bulls ($P<0.001$). High MUFA proportion in the SAT has been previously reported [1] and may be due to elevated SCD activity. A breed×diet interaction was found for the trans (TFA) and polyunsaturated fatty acids (PUFA), being higher in Alentejana bulls fed the LS diet ($P<0.05$). The branched chain fatty acids (BCFA), which are closely related to the rumen activity, were higher in the HS than the LS fed animals ($P<0.001$). However, a breed-related variation in the BCFA proportions was also observed, favouring Barrosã bulls ($P<0.05$). Total CLA was higher in Barrosã than in Alentejana bulls, regardless of the diet ($P<0.001$).

Both SCD and LPL (Fig. 1) showed higher relative expression levels in the Barrosã breed in comparison to Alentejana ($P=0.003$ and $P=0.021$, respectively), corresponding to a fold-change of 1.74 and 1.56, respectively. Greater relative LPL mRNA expression could indicate that Barrosã bulls have more adipocytes undergoing differentiation compared to Alentejana bulls, as LPL was suggested to be a good indicator of adipocyte differentiation [6]. In opposition, and although diet influenced the percentages of some classes of fatty acids, it failed to produce a significant variations in the expression of both genes.

As shown in Table 2, the correlation analysis between oleic acid (18:1*c*9) proportions and SCD expression levels showed a positive and significant correlation ($P<0.01$). This result is in agreement with previous reports [8] that increased SCD activity is, at least partially, responsible for elevated oleic acid, the main MUFA, content in ruminant. Similarly, a significant correlation was also found between CLA percentage and SCD expression levels.

Concerning the relationships between the fatty acids and LPL expression, the correlation analysis revealed close relationships between LPL and SCD, oleic acid, CLA and CLA/PUFA ratio (at least $P<0.05$). These results reinforce the role of LPL in the control of TGA uptake and, consequently, the fatty acid profile. In addition, correlation analysis showed a close relationship ($r=0.449$, $P=0.0046$) between SCD expression and Δ^9 desaturase index, suggesting that the later can be used to infer SCD activity (Fig. 2).

Both SCD and LPL are expressed late in adipogenesis, thus increased gene expression would be consistent with more active adipocytes. Taken together, these results point out that Barrosã bulls may have more differentiated adipocytes capable of storing fat in the subcutaneous depot than Alentejana bulls.

IV. CONCLUSION

Overall, this study showed a breed-specific variation in the expression levels of SCD and LPL genes, which may account, at least in part, for the distinct SAT fatty acid profile of Alentejana and Barrosã breeds.

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Table 1. Fatty acid partial sums (g/100 g total fatty acids) of subcutaneous adipose tissue from Alentejana and Barrosã breeds fed high (HS) or low (LS) silage diets.

	Alentejana		Barrosã			Significance level		
	HS	LS	HS	LS	SEM	Breed	Diet	Breed×Diet
Σ SFA	46.9	44.8	39.6	39.2	1.19	***	ns	ns
Σ MUFA	45.1	46.0	50.6	51.5	1.23	***	ns	ns
Σ TFA	2.33^{b}	3.23^{a}	2.95^{ab}	2.98^{ab}	0.211	ns	*	*
Σ PUFA	1.62^{c}	2.58^{a}	2.07^{b}	2.11^{b}	0.123	ns	***	***
Σ CLA	5.16	4.79	9.85	9.92	0.446	***	ns	ns
Σ BCFA	2.69	2.06	2.77	2.25	0.068	*	***	ns

Significance level: not significant (ns), $P>0.05$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

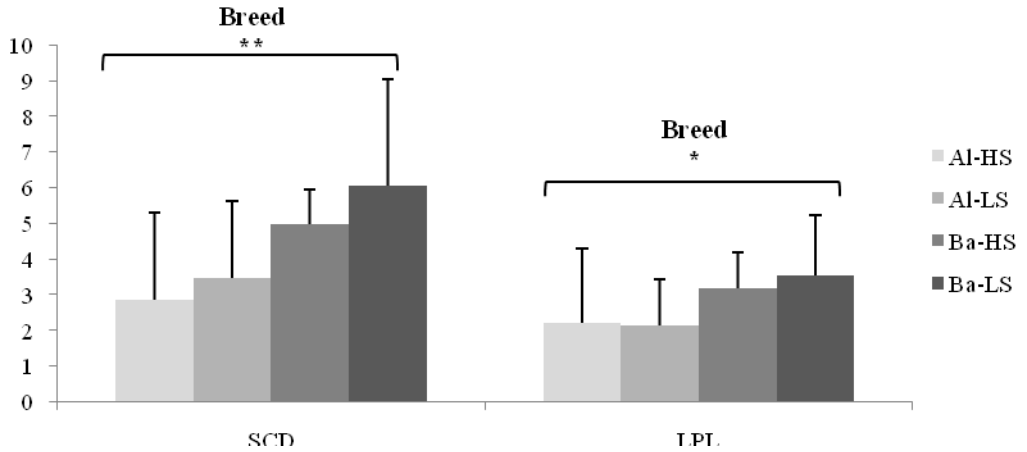

Figure 1. Relative normalized expression of SCD and LPL mRNA in the subcutaneous adipose tissue of Alentejana (Al) and Barrosã (Ba) fed high (HS) or low (LS) silage diets. Error bars represent standard error. Significance level: not significant (ns), $P>0.05$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

Table 2. Pearson correlation coefficients between fatty acid profile and relative gene expression in the subcutaneous adipose tissue.

	18:0	18:1*c*9	CLA	*n*-6/*n*-3	CLA/PUFA	PUFA/SFA	SCD	LPL
18:0	1.00	-0.582***	-0.640***	-0.107	-0.498**	-0.422**	-0.252	-0.158
18:1*c*9		1.00	0.501**	-0.107	0.478**	0.322*	0.484**	0.500**
CLA			1.00	-0.260	0.727***	0.290	0.451**	0.351*
n-6/*n*-3				1.00	-0.0273	0.557***	-0.0055	-0.159
CLA/PUFA					1.00	0.061	0.274	0.358*
PUFA/SFA						1.00	0.144	0.023
SCD							1.00	0.756***
LPL								1.00

Significance level: not significant (ns), $P>0.05$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

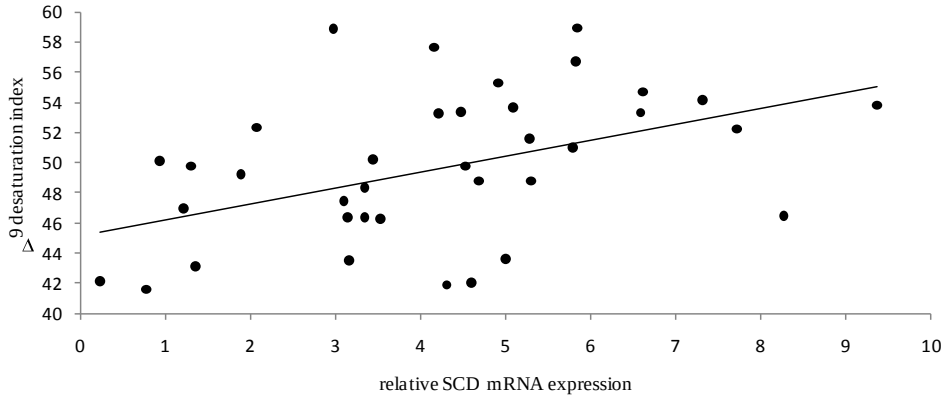

Figure 2. Relationship between relative expression of SCD mRNA in the subcutaneous adipose tissue and the Δ^9 desaturase index.