

# ADIPOSE TISSUE-SPECIFIC EXPRESSION OF LIPOGENIC GENES IN DIFFERENT CATTLE BREEDS: RELATIONSHIP TO FATTY ACID COMPOSITION

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**Abstract** – The objective of the present study was to determine the mRNA expression of selected genes associated with *de novo* FA synthesis and desaturation (ACACA, FASN, SCD1) in cod, subcutaneous, and intramuscular adipose tissues (AT) of 38 Aberdeen Angus (AA), Gascon (GS), Holstein (HO), and Fleckvieh (FL) bulls. Furthermore, the relationship between gene expression levels measured by RT PCR and phenotypic fatty acid composition was evaluated. Gene expression levels significantly differed between various AT depots ( $P < 0.01$ ), with the lowest relative amount observed in the intramuscular AT. The genes studied were generally more highly expressed in the AT of AA and HO compared to GS and FL bulls. The breed differences found in gene expression levels corresponded to those in the total FA content in intramuscular AT. In addition, the differences in gene mRNA levels between breeds were partly reflected in the contents of MUFA and *de novo* synthesized SFA in all the AT examined.

**Key Words** – Adipose tissue, lipogenic gene mRNA, fatty acid.

## I. INTRODUCTION

The fatty acid (FA) composition of beef adipose tissue is of particular consumer concern because specific FA can have detrimental, but also beneficial, effects on human health [1]. It has been previously reported that FA composition varies between cattle breeds [2] and adipose tissue types [3]. A better understanding of the underlying mechanisms leading to these differences is needed. Differences in the expression of genes related to adipogenesis and lipogenesis have been studied in different breeds [4] and in cattle of similar genetics differing in adipose tissue accretion in various depots [5]. However, it remains largely unknown whether variations in selected lipogenic gene expression measured at slaughter are related to breed differences in the FA composition in depot-specific adipose tissues. Therefore, the

objective of this study was to measure the mRNA expression of selected genes associated with *de novo* FA synthesis and desaturation in different adipose depots of bulls of four different breeds, and to evaluate its relationship to FA composition.

## II. MATERIALS AND METHODS

A total of 38 bulls of four breeds substantially differing in the maturity age and the propensity to deposit fat (Aberdeen Angus, AA,  $n = 9$ ; Gascon, GS,  $n = 10$ ; Holstein, HO,  $n = 9$ ; Fleckvieh, FL,  $n = 10$ ) were used. The bulls were finished under identical housing and feeding conditions and slaughtered at a similar age and live weight of approximately 17 months and 650 kg, respectively. Immediately after slaughter, cod (from the scrotal area; CAT), subcutaneous (from the brisket; SAT) and intramuscular (from *m. longissimus lumborum*; IAT) adipose tissue samples were collected. The IAT samples were physically separated from the muscle. All the samples were submerged in 200  $\mu$ l of  $2 \times$  Nucleic Acid Purification Lysis Solution (Applied Biosystems, Foster City, CA, USA), homogenized using a rotor-stator homogenizer and stored at  $-20^{\circ}\text{C}$  until analyzed. After defrosting, 200  $\mu$ l of PBS and 5  $\mu$ l of proteinase K were added to the samples and left at room temperature for 1 h. RNA was isolated using 6100 Nucleic Acid PrepStation (Applied Biosystems, Foster City, CA, USA) following the manufacturer's protocol. In addition, a step with Absolute RNA Wash Solution was included. All RNA samples were reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's protocol. Real-time PCR was performed with the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Genes of interest included acetyl-

coenzyme A carboxylase  $\alpha$  (ACACA), fatty acid synthase (FASN) and stearoyl-coenzyme A desaturase 1 (SCD1), whereas actin  $\beta$  (ACTB), ribosomal protein 19 (RPL19) and peptidylprolyl isomerase A (PPIA) were used as reference genes. Primer and probe sequences were designed using Primer Express 3.0 software (Applied Biosystems, Foster City, CA, USA), and they are presented in Table 1. Each PCR reaction was performed in triplicate in a total volume of 10  $\mu$ l with gene specific primers concentration of 500 nM and TaqMan MGB probes concentration of 200 nM, 5  $\mu$ l Fast TaqMan Universal Master Mix (2 $\times$ ) (Applied Biosystems, Foster City, CA, USA), 1  $\mu$ l cDNA, and nuclease-free water up to volume. The PCR program (Fast protocol) was as follows: 95  $^{\circ}$ C for 20 s followed by 40 cycles of 95  $^{\circ}$ C for 2 s and 60  $^{\circ}$ C for 20 s. Three candidate internal reference genes were evaluated using the RefFinder tool

(<http://www.leonxie.com/referencegene.php>) integrating computational programs geNorm, Normfinder, BestKeeper, and the comparative  $\Delta$ Ct method. The PPIA was selected as the most stable and used to normalize gene expression data in this study. Relative transcript levels of target genes were calculated for each sample as  $2^{-\Delta C_t}$ , where  $\Delta C_t$  ( $C_t$  – threshold cycle) was calculated as  $C_{t(\text{target gene})} - C_{t(\text{PPIA})}$ .

The FA composition of CAT, SAT and muscle (*m. longissimus lumborum*) was determined after extraction of total lipids in accordance with [6]. Alkaline trans-methylation of FA was performed in accordance with [7]. Gas chromatography of FA methyl esters was performed using the HP 6890 gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA) with a programmed 60 m DB-23 capillary column (150 to 230 $^{\circ}$ C). FA were identified on the basis of retention times corresponding to standards (PUFA 1, PUFA 2, PUFA 3, 37 Component FAME Mix; Supelco, Bellefonte, PA, USA). The proportions of FA were expressed as percentages of the total area of injected methyl esters. The contents of FA were determined with nonadecanoic acid as an internal standard.

Gene expression data were evaluated using a mixed linear model with repeated measures (MIXED procedure of SAS, [8]). Parameters were estimated by the REML method. The model was

structured to determine the effect of breed and adipose tissue on gene expression (dependent variable) with slaughter date entered as random. Random (co)variances between types of adipose tissue within animal were summarized by residual R matrix (block diagonal with unstructured covariance structure). FA data were analyzed using the GLM procedure of SAS [8] with the model including breed as the fixed effect. Least squares means were calculated, and multiple comparisons were made, with P-values adjusted using Tukey's procedure.

Table 1 Primer and TaqMan MGB probe sequences

| Gene  |                                     |
|-------|-------------------------------------|
| ACACA | Forward: CCTTCTTCCTTGCCAAACC        |
|       | Reverse: CACGGAGCCAATAATGAATCG      |
|       | Probe: FAM TGGAGCTGAACCAGC NFQ      |
| FASN  | Forward: GGACATGGTGACAGATGATGACA    |
|       | Reverse: GCCGAGGCAGGCCATATAG        |
|       | Probe: FAM CGGTGGAAGGCCGG NFQ       |
| SCD1  | Forward: CCGACGTGGCTTTTTCTTCT       |
|       | Reverse: TGGGTGTTTGCGCACAAAG        |
|       | Probe: FAM TCACGTGGGTTGGCT NFQ      |
| ACTB  | Forward: CCTGCGGCATTACAGAA          |
|       | Reverse: GCGGATGTCGACGTCACA         |
|       | Probe: FAM CTACCTTCAATTCCATCATG NFQ |
| RPL19 | Forward: AAGGCAGGCATATGGGTATAGG     |
|       | Reverse: CTCGGGCATTCGAGCATT         |
|       | Probe: FAM AAGCGAAAGGGTACTGC NFQ    |
| PPIA  | Forward: GCTTTGGGTCCAGGAATGG        |
|       | Reverse: TTTGTCCACAGTCAGCAATGG      |
|       | Probe: FAM AGACCAGCAAGAAGAT NFQ     |

### III. RESULTS AND DISCUSSION

The mRNA expression of three genes involved in FA synthesis (ACACA, FASN, SCD1) was measured in different adipose tissue depots of 38 bulls. The ACACA and FASN genes encode key enzymes (ACC and FAS, respectively) regulating *de novo* fatty acid synthesis, whereas the SCD1 gene encodes the enzyme responsible for the conversion of saturated into monounsaturated FA by inserting a double bond in the  $\Delta^9$  position [9].

Gene expression levels significantly differed between various adipose tissue depots (Figure 1). The expression of ACACA was greater in CAT than in SAT and IAT depots ( $P < 0.001$ ). The CAT depot exhibited higher ( $P < 0.01$ ) FASN expression than the IAT depot. The expression of SCD1 was lower ( $P < 0.001$ ) in IAT than in

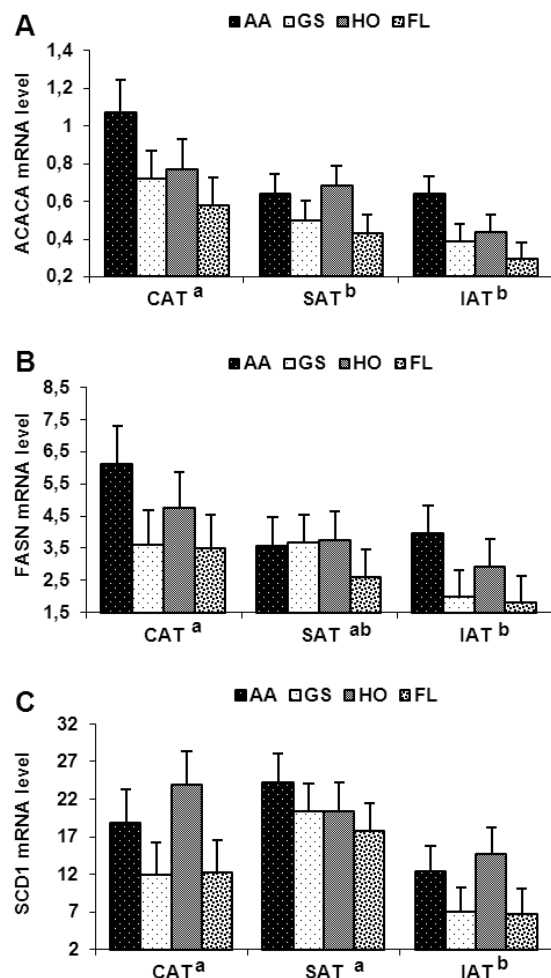


Figure 1: Relative expression of ACACA (A), FASN (B) and SCD1 in cod (CAT), subcutaneous (SAT) and intramuscular (IAT) adipose tissue of Aberdeen Angus (AA), Gascon (GS), Holstein (HO), and Fleckvieh (FL) bulls. Symbols a and b indicate differences ( $P<0.01$ ) between adipose tissue depots across breeds.

both CAT and SAT. Similar differences between IAT and SAT were previously reported for relative transcript levels of FASN [5] and SCD1 [10].

Breed differences between relative lipogenic gene expression levels were quite distinct but, due to a high variation within breeds, they mostly lacked statistical significance. Numerically the highest expression levels were observed in AA and HO breeds, regardless of adipose tissue type. Interestingly, a similar pattern of breed differences in the expression of the three genes studied was observed between

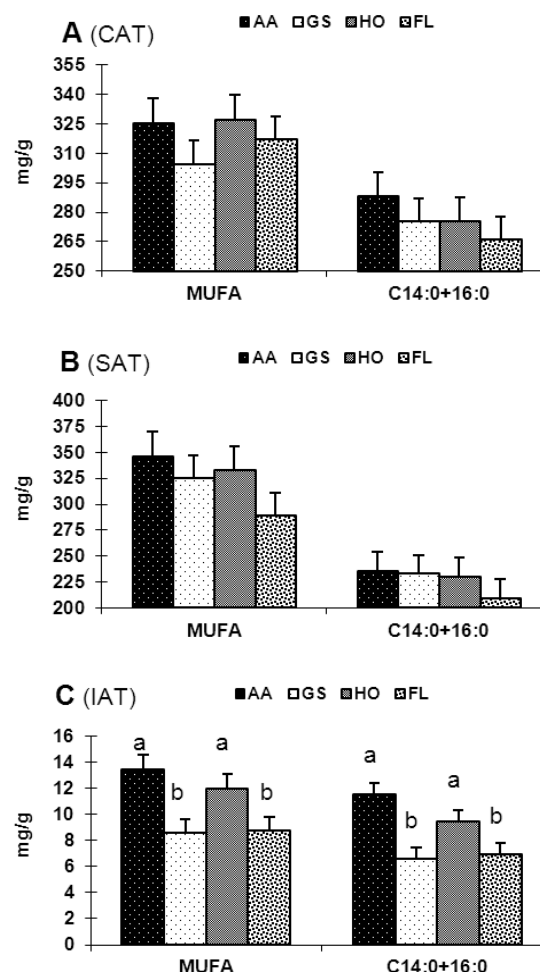


Figure 2: MUFA and C14:0 + C16:0 contents in cod (A), subcutaneous (B) and intramuscular (C) adipose tissue of Aberdeen Angus (AA), Gascon (GS), Holstein (HO), and Fleckvieh (FL) bulls. Symbols a and b indicate differences ( $P<0.05$ ) between breeds within adipose tissue depots.

CAT and IAT depots, whereas it was different for SAT (Figure 1). It seems that the expression of these genes is under depot-specific control, as previously reported in [11].

The amount of total FA in CAT and SAT was similar between breeds, whereas it was higher in AA and HO than in GS ( $P<0.01$ ) in IAT (Table 2). The pattern of breed differences in ACACA, FASN and SCD1 expression levels in IAT reflected the breed differences in IAT total FA contents. Partly in agreement with the present study, intramuscular fat content was positively related to the expression of ACACA and FAS, but not to SCD, enzymes in Limousin and

Aberdeen Angus cattle [12]. The authors suggest that the rate of SFA synthesis might be a major factor in intramuscular fat deposition in ruminants.

Table 2 Total FA content in cod, subcutaneous and intramuscular adipose tissue (mg/g)

|     | AA              | GS              | HO               | FL               | SE   | P     |
|-----|-----------------|-----------------|------------------|------------------|------|-------|
| CAT | 825             | 823             | 841              | 821              | 28.0 | 0.958 |
| SAT | 757             | 735             | 751              | 670              | 47.0 | 0.533 |
| IAT | 36 <sup>a</sup> | 23 <sup>b</sup> | 31 <sup>ac</sup> | 24 <sup>bc</sup> | 2.6  | 0.003 |

<sup>a,b,c</sup> Values in the same row with different superscripts differ at P<0.05.

The contents of MUFA (a product of SCD1 action) and the sum of C14:0 and C16:0 (*de novo* synthesized SFA, a product of ACACA and FASN action) in different adipose tissue depots are shown in Figure 2. The IAT of AA and HO contained greater amounts of MUFA and C14:0+C16:0 (P<0.05), whereas the differences between breeds in the other depots were not significant. From the comparison of Figure 1 and Figure 2 it seems evident that the breed and adipose tissue type differences in MUFA and *de novo* synthesized SFA contents can be at least partly explained by the differences in the relative expression levels of ACACA, FASN and SCD1.

#### IV. CONCLUSION

The mRNA levels of lipogenic genes ACACA, FASN and SCD1 were mostly lower in IAT compared to CAT and SAT. In addition, these genes were more expressed in adipose tissues of AA and HO bulls with a higher propensity to deposit fat compared to leaner animals of the GS and FL breeds. The expression levels partly corresponded to the breed differences in the FA composition of adipose tissue depots.

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