EXPRESSION OF LIPOGENIC GENES AND TRANSCRIPTION FACTORS MEASURED IN SUBCUTANEOUS FAT OF HEIFERS AND BULLS AT DIFFERENT TIME POINTS

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Abstract – The mRNA of lipogenic genes and transcription factors (ACACA, FASN, SCD1, CEBPA, PPARG, and SREBF1) was measured in subcutaneous fat of heifers and bulls at 15 months of age (Sampling 1) and at slaughter at 19 months of age (Sampling 2). Earlier maturing heifers exhibited higher (P<0.05) mRNA levels of ACACA, FASN, SCD1, and SREBF1 than later maturing bulls at Sampling 1, whereas no significant differences between genders except for ACACA were observed at Sampling 2. Higher expression levels were generally determined in older age of animals but the differences were more pronounced in bulls. Generally, the gene expression data from the present study indicated a clear gender- and age-specific pattern.

Key Words – cattle, gene mRNA, lipid metabolism.

I. INTRODUCTION

Different factors induce strong variability in the site-specific development of various adipose tissue types and their fatty acid (FA) composition in cattle. Heifers generally exhibit greater carcass fatness and intramuscular fat content compared to bulls. Furthermore, fat amounts increase and the proportions of FA change as animals grow older [1]. However, little is known regarding the effect of gender on the expression of genes related to lipid metabolism. Therefore, the objective of this study was to measure the mRNA expression of selected genes associated with fatty acid biosynthesis and desaturation as well as transcriptional regulation at two time-points during the growth period in subcutaneous fat of heifers and bulls.

II. MATERIALS AND METHODS

A total of 24 Fleckvieh cattle (12 heifers and 12 bulls) were loose housed in two pens at the age of approximately 8 months and fed ad libitum an identical diet. The average age and live weight at slaughter of heifers and bulls (mean ± standard deviation) were 561 ± 6 and 556 ± 5 days, respectively, and 611 ± 53 and 670 ± 50 kg, respectively. At the average age of 442 ± 12 days (Sampling 1), subcutaneous fat samples were obtained from live animals from the area above m. longissimus lumborum through a 5-cm skin incision after local anesthesia. Immediately after slaughter (Sampling 2), subcutaneous fat samples from the same location were collected.

Total RNA (tRNA) was isolated using vacuum operated ABI PRISM 6100 Nucleic Acid PrepStation Instrument (Applied Biosystem Co., Foster City, CA, USA) according to the manufacturer´s recommended protocol. Relative levels of target and reference gene mRNA were determined using 2-step RT quantitative PCR (qPCR) with specific TaqMan Gene Bovine Expression Assays (ThermoFisher Scientific, MA, USA). Genes of interest included acetyl-coenzyme A carboxylase α (ACACA), fatty acid synthase (FASN), stearoyl-coenzyme A desaturase 1 (SCD1), CCAAT/enhancer-binding protein α (CEBPA), peroxisome proliferator-activated receptor γ (PPARG), and sterol regulatory element binding transcription factor 1 (SREBF1), whereas eukaryotic translation initiation factor 3K (EIF3K), ceroid-lipofuscinosis, neuronal 3 (CLN3), and ubiquitously expressed transcript (UXT) were used as reference genes. The efficiency of qPCR was determined using standard curves generated for each gene from a 10-fold serial dilution of pooled cDNA. Normalized relative quantities (CNRQs) of target genes were calculated using qBase Plus software (Biogazelle, Belgium) [2].

Gene expression data (lognormal distribution) were analyzed using mixed linear model with repeated measures (unstructured covariance) using the GLIMMIXED procedure of SAS. The model was structured to determine the effects of sex, sampling date, and their interaction. Parameters were estimated by the REML method. The LSM of gene expression data were retransformed to original scale.

III. RESULTS AND DISCUSSION

The mRNA expression level was measured in ACACA and FASN involved in de novo FA synthesis, and in SCD1 responsible for the conversion of saturated into monounsaturated FA [3]. In addition, the expression was determined in CEBPA, PPARG and SREBF1, the key transcriptional regulators of adipogenesis through an induction of the expression of many downstream genes involved in lipid metabolism [4]

The gene expression data (Table 1) from the present study indicate a clear gender- and age-specific pattern. At Sampling 1 (approx. 15 months of age), earlier maturing heifers exhibited higher ($P<0.05$) mRNA levels of ACACA, FASN and SCD1 than later maturing bulls, whereas these differences were less distinct in CEBPA, PPARG and SREBF1. At Sampling 2, no significant differences between genders were observed except for ACACA. In agreement with this study, higher lipogenic enzyme activities were observed in subcutaneous fat of heifer compared to bulls slaughtered at 12 to 14 months of age [5]. In the present study, higher expression levels were generally determined in 19 compared to 15 months of age but the differences were more pronounced in bulls. A similar pattern was demonstrated for SCD1 in our previous study comparing heifers and bulls slaughtered in 14 and 18 months [6]. It is suggested that the genes under study reach their peaks at different age-points in heifers and bulls.

Table 1 Relative expressions of ACACA, FASN, SCD1, CEBPA, PPARG, and SREBF1 in subcutaneous fat of heifers and bulls at the age of 15 months (Sampling 1) and 19 months (Sampling 2). G = effect of gender, S = effect of sampling, G × S = interaction. Different symbols a, b and c indicate differences ($P<0.05$) in case of significant ($P<0.05$) interaction

Gene	Sampling 1		Sampling 2		P – value		
	Heifer	Bull	Heifer	Bull	G	S	G × S
ACACA	4.93^{b}	0.98^{a}	12.05^{c}	7.17^{b}	0.000	0.000	0.012
FASN	7.34^{b}	1.31^{a}	10.61^{b}	10.29^{b}	0.002	0.000	0.001
SCD	9.87^{b}	1.82^{a}	16.82^{c}	13.70^{bc}	0.002	0.000	0.015
CEBPA	5.81^{ab}	2.78^{a}	8.22^{b}	11.23^{b}	0.299	0.000	0.010
PPARG	4.60^{ab}	2.19^{a}	6.81^{b}	7.57^{b}	0.085	0.000	0.012
SREBF1	1.07	0.72	2.06	1.48	0.044	0.002	0.858

IV. CONCLUSION

It was demonstrated that mRNA levels of lipogenic genes ACACA, FASN and SCD1 as well as transcription factor CEBPA, PPARG and SREBF1 were highly gender- and age-dependent. The difference between heifers and bulls were more pronounced in the earlier stage of growth.

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