STEAROYL-COA DESATURASE GENE AND HIS ASSOCIATION WITH FATTY ACIDS IN BEEF

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Abstract: The quality of meat in cattle is influenced by many genes; one of them is *SCD1* gene (stearoyl-CoA desaturase). This gene is associated with composition of fatty acids in meat and milk. In this study, the total of 260 bulls of Czech Fleckvieh breed were genotyped using the PCR-RFLP method. The frequencies of alleles and genotypes were determined in this population and the association analysis between fatty acids in fat extracted from *musculus longissimus dorsi* and genotypes was performed. Statistically significant ($p<0.0001$) association between genotypes and myristoleic acid (C14:1) was found. *CC* genotype had higher median value. No other associations were found.

Key Words: Czech Fleckvieh breed, fatty acids, *SCD1*

INTRODUCTION

Stearoyl – CoA desaturase (SCD) is the rate limiting enzyme catalysing the synthesis of monounsaturated fatty acids (MUFA) from saturated fatty acids (SFA) (Ntambi, Miyzaki 2004). The *SCD* gene is highly expressed in white adipose tissue, brown adipose tissue, meibomian gland, Harderian and preputial gland under normal dietary conditions (Dobrzyn, Dobrzyn 2006). In ruminants, fatty acids in the feed are chemically reduced by microorganisms in the rumen and absorbed as saturated fatty acids. The composition in fatty acids stored in the fat depots reflects the previous action of SCD on substrates such as stearic acid and palmitic acid (Kim, Ntambi 1999). Some SFA, commonly found in meat, especially myristic and palmitic acids are one of the risk factors of heart diseases (Erkkila et al. 2008). Diet high in SFA tends to increase blood cholesterol levels while diet high in MUFA tent to lower blood cholesterol levels. Cholesterol is carried in the bloodstream as lipoproteins. Low – density lipoprotein (LDL) cholesterol is the "bad" cholesterol because elevated LDL levels are associated with an increase risk heart disease. In contrast, high – density lipoprotein (HDL) cholesterol is the "good" cholesterol since high HDL level is associated with less heart disease (Jiang et al. 2008).

There were characterised two different isoforms of *SCD* gene. Isoform *SCD5* is localised on the 6th chromosome and isoform *SCD1* on the 26th chromosome (Lengi, Corl 2007). Previously it was described that the structure of bovine *SCD1* gene has 6 exons and 5 introns and is 17 kb long. But according to new findings, *SCD1* consists of 4 exons and 3 introns (Ensemble 2015). Eight single nucleotide polymorphisms (SNP) were found in Japanese Black cattle in exon 5 (recently exon 3) (Taniguchi et al. 2004) and three of them were also found in Canadian Holstein cattle and Jersey cattle (Kgwatalala et al. 2007). Also those three SNPs were found in 11 Italian breeds (Milanesi et al. 2008). However only SNP on 878 position in the sequence causes alanine/valine substitution in SCD1 protein (Barton et al. 2010). The *C* allele is coding amino acid alanine and *T* allele is coding amino acid valine. Alanine is associated with higher MUFA content in intramuscular fat (Taniguchi et al. 2004).

MATERIAL AND METHODS

Animals

In this study we analysed samples of DNA from *musculus longissimus dorsi* of 260 bulls of dual-purpose Fleckvieh breed obtained at the Department of Animal Morphology, Physiology and Genetics of Mendel University in Brno.

Chemical analysis

The content of fatty acids was analysed using the gas chromatograph HP4890 with capilar column DB–23 (60m x 0.25mm x 0.25 μm). Extracted fat from meat samples of *musculus longissimus dorsi* was used. For the measurements was chosen a thermal programme from 100˚C * 3 min * 10˚C/min * 170˚C * 0 min * 4˚C/min * 230˚C * 8 min * 5˚C/min * 250˚C * 15 min, injector temperature 270˚C, temperature of detector 280˚C. Final chromatograms were processed by the CSW station program (v1.7, Data Apex). The following FA were determined:

- Saturated FA: C12:0, C14:0, C16:0, C18:0, C20:0
- Monounsaturated FA: C14:1, C16:1, C18:1 n-9, C20:1
- Diunsaturated FA: C18:2 n-6t, C18:2 n-6c, C18:2 n-9
- Polyunsaturated FA: C18:3 n-6, C18:3 n-3, C20:4 n-6, C20:5 n-3, C22:4 n-6, C22:5 n-6, C22:5 n-3, C22:6 n-3

PCR - RFLP

For genotyping the PCR – RFLP method was used. DNA samples were mixed with PPP Master Mix and specific primers. Primers were designed according to Barton et al. (2010) (Table 1). The length of amplified fragment is 144 base pair (bp). The PCR consisted of the following temperature profile: 95˚C for 5 min followed by 35 cycles (95˚C/30 s, 60˚C/30 s and 72˚C/45 s) and final elongation at 72˚C for 7 min. The cycler PTC-200 was used for PCR (Bio - Rad, Hercules, USA).

Mix for RFLP consisted of PCR product, buffer G and restriction enzyme *Sat*I (Thermo Fisher Scientific Inc., Waltham, USA). Incubation was performed overnight at 37˚C. The *Sat*I restriction site is 5´…..TG↓YGG…..3´, where Y= *C, T*. After digestion, *C* allele is characterised by the presence of the restriction fragments 29, 47 and 68 bp and the *T* allele by 29 and 115 bp.

Table 1 Primers used for amplification of SCD1 gene

Primer	sequence	Length (bp)	G+C (%)	Tm (˚C)
SCD1-1A	ATG TAT GGA TAC CGC CCT TAT GAC	24	46	60.92
SCD1-2B	TTC TGG CAC GTA ACC TAA TAC CCT	24	46	60.86

Agarose gel electrophoresis

For verifying the presence of amplicons in PCR and identification of fragments after restriction, agarose gel electrophoresis was used with the concentration of 3%. Gel consisted of Agarose (SERVA, DE), TBE buffer (Sigma Aldrich, USA) and ethidium bromide (Top Bio) as visualising colour. The 50 bp and 100 bp DNA Ladders (Thermo Fisher Scientific Inc., Waltham, USA) were used for sizing of the fragments.

Data analysis

Frequencies of alleles and genotypes were calculated as well as the Hardy – Weinberg equilibrium. The phenotypic data for all measurements and the genotypes were analysed using linear mixed model REML using the SAS v8.2. Fixed effects included genotype, farm and regression on the age of slaughter. Effect of the father was used as a random effect.

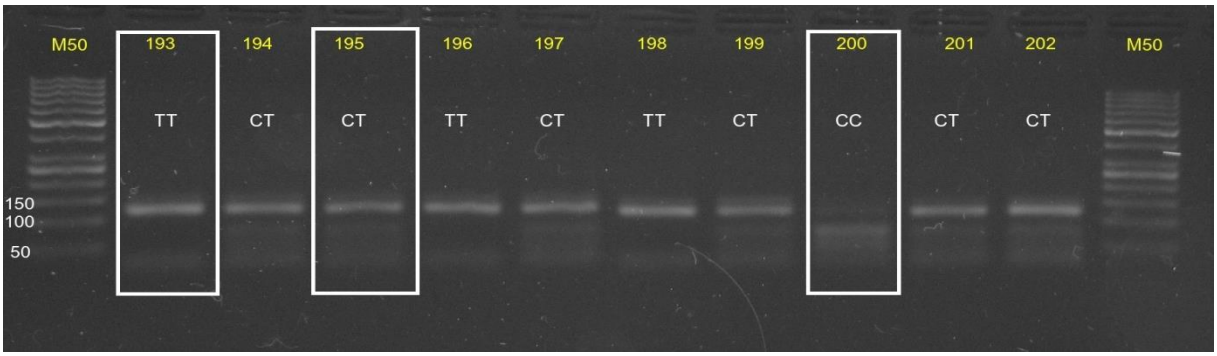
$$f_{ijkl} = \mu + SCD1_i + farm_j + age_k + father_k + e_{ijkl}$$

RESULTS AND DISCUSSION

PCR - RFLP

In this analysed sequence the enzyme *Sat*I had two restriction sites, one nonpolymorphic and one polymorphic. Therefore in every sample the 29 bp long fragment was presented. Polymorphism was located at position 71 in the sequence. After restriction by the *Sat*I enzyme, different lengths of fragment occurred on gel electrophoresis (see Figure 1).

Figure 1 Visualisation of CC, CT and TT genotypes on agarose gel electrophoresis



Data analysis

In nine samples, the PCR amplification was not successful and three samples were taken out because of the high age of the individuals which correlates with higher accumulation of fat so the results were counted from total of 248 samples. Relative frequencies of alleles were almost the same (*C* = 49.8%; *T* = 50.2%) which agrees with relative frequencies of genotypes where the number of heterozygotes was *CT* = 0.60%, while relative frequencies of homozygotes were *CC*= 0.19% and *TT* = 0.20%. Barton et al. (2010) described the frequencies of genotypes of dual – purpose breed of *CT*, *CC* and *TT* as 46.76%, 32.16% and 21.08 %, respectively. Their findings differed in frequency of alleles where allele *C* was 55.54% and allele *T* 44.46%. Similar findings obtained Mele et al. (2007) in Italian Holstein breed with frequency of alleles *C* = 57% and *T*= 43%. Genotype *CT* was the most frequent with 60% (*AA* = 27%; *TT* = 13%). On the other hand frequency of alleles in Piedmontese was 58% (*T*) and 42% (*C*) (Moioli et al. 2007). Difference in allele frequency might be given by differences in breeds or by the fact that T allele causes inhibition of activity in some fatty acids and is not preferred by breeders. According to Hardy Weinberg law, studied population was not in equilibrium which can show negative selection pressure on meat quality traits.

The effect of the polymorphism of *SCD1* on FAs is shown in Table 2. Significant association of the *SCD1* genotypes were observed for myristoleic acid (C14:1), when the p-value between genotypes *CC* and *CT* is 0.0002, the *CC* and *TT* is <0.0001 and between *CT* and *TT* is 0.003. But the *CC* genotype has the higher content of C14:1 (0.66±0.07) than *CT* (0.53±0.06) and *TT* (0.43±0.07) genotypes. This indicates that the *C* allele has an effect on higher content of C14:1, which is in agreement with findings of Moioli et al. (2007) who also found higher content of C14:1 caused by the *C* allele. Also Mele et al. (2007) and Barton et al. (2010) found an influence of the *CC* genotype on the higher content of C14:1 in dairy and dual-purpose cattle.

No other significant effects of the polymorphism of *SCD1* were found in this study.

Table 2 Effects of the polymorphism of SCD1 (SNP C878T) gene on FA

FA	*SCD1(SNP 878C>T)*			Significant effect		
	CC n = 43	*CT* n = 151	*TT* n = 54	*CC - CT*	*CC - TT*	*CT - TT*
	LSM±SE (g · 100 g^{-1})	LSM±SE (g · 100 g^{-1})	LSM±SE (g · 100 g^{-1})			
C12:0	0.07±0.01	0.07±0.01	0.08±0.01	0.91	0.51	0.48
C14:0	2.78±0.18	2.75±0.17	2.74±0.19	0.73	0.68	0.87
C14:1	0.66±0.07	0.53±0.06	0.43±0.07	0.0002	<0.0001	0.003
C16:0	29.67±0.75	29.17±0.71	29.24±0.78	0.18	0.34	0.86
C16:1	3.03±0.28	3.01±0.26	3.07±0.29	0.86	0.85	0.68
C18:0	17.14±1.13	17.96±1.06	18.24±1.17	0.15	0.11	0.62

C18:1	42.15±1.28	41.91±1.20	41.74±1.33	0.71	0.61	0.79
C18:2, n-6	2.88±0.67	2.94±0.62	2.90±0.70	0.85	0.97	0.88
C18:3, n-6	0.12±0.01	0.13±0.01	0.14±0.02	0.34	0.13	0.35
C18:3, n-3	0.39±0.05	0.40±0.04	0.39±0.05	0.83	0.93	0.91
C18:2, n-9	0.24±0.03	0.22±0.02	0.22±0.03	0.10	0.18	0.98
C20:0	0.13±0.02	0.14±0.02	0.15±0.02	0.48	0.31	0.59
C20:1	0.18±0.02	0.18±0.02	0.18±0.02	0.55	0.98	0.57
C20:4, n-6	0.35±0.24	0.39±0.22	0.37±0.25	0.74	0.89	0.87
C20:5, n-3	0.03±0.04	0.03±0.03	0.04±0.04	0.77	0.49	0.57
C22:4, n-6	0.06±0.05	0.08±0.04	0.08±0.05	0.37	0.65	0.73
C22:5, n-6	0.04±0.04	0.05±0.04	0.06±0.04	0.59	0.35	0.53
C22:5, n-3	0.12±0.07	0.13±0.06	0.13±0.07	0.70	0.75	0.99
C22:6, n-3	0.02±0.03	0.03±0.03	0.04±0.03	0.47	0.27	0.53

CONCLUSION

As the result of this study was to genotype given population of dual – purpose cattle breed for the polymorphism of *SCD1* gene and to determine whether the polymorphism has any influence on the content of FA in fat extracted from meat. It was shown that the *SCD1* polymorphism is significantly associated with content of myristoleic acid, which is preferred by customers as the "good" fatty acid. The positive additive effect of the *C* allele on its level is shown. This might serve as a guide for the breeders which genotype to prefer in selection of cattle but further study is needed because no other associations were proven.

ACKNOWLEDGEMENT

This work was supported by project NAZV QI91A055 and realised in CEITEC - Central European Institute of Technology with research infrastructure supported by the project CZ.1.05/1.1.00/02.0068 financed from European Regional Development Fund. We would like to thank Ing. Eliska Drackova, PhD. for providing the phenotypic data.

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