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Short Communication

EFFECTS OF *FASN* AND *SCD* GENE POLYMORPHISM ON THE COMPOSITION OF SHEEP'S MILK

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ABSTRACT

In the study, genotypes and allele frequencies for SNP of *FASN* and *SCD1* genes in the herd of Zošľachtená Valaška sheep in the second and third lactation were determined. In the obtained samples, the basic composition of milk, urea content and proportion of protein fractions were estimated. Blood samples were tested to determine *FASN* and *SCD1* genes frequencies. After genotyping conducted in the sheep herd, three possible genotypes in *SCD1*/*Cfr*I polymorphism and only two out of three possible genotypes in *SCD1*/*Pvu*II and *FASN*/*Aci*I polymorphisms (*AA*, *AG* and *CC*, *CT*, respectively) were found. Analyses have shown changes in milk composition and proportions of protein fractions in *FASN* and *SCD1* genes depending on SNP genotypes. The analysis of the obtained results for *FASN* polymorphism allowed to establish that the milk of animals with CT genotype had the highest content of protein, fat and dry matter. The most desired profile of protein fractions was found in *SCD*/*Cfr*I genotype among the homozygous *CC* genotype carriers: it allows to determine that the milk of these sheep has good technological traits.

Key words: sheep, milk composition, *FASN* , *SCD1*

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INTRODUCTION

Fatty acid synthase (FASN or FAS) is an enzyme catalyzing *de novo* synthesis of fatty acids (Jensen-Urstad and Semenkovich2012). During lactation, FASN from the mammary gland participates in the synthesis of lipids secreted with milk. This enzyme is involved in the conversion of carbohydrates into lipids in lipogenic organs (the liver and adipose tissue) (Anderson *et al.,* 2007). In mammals, FASN is a homodimeric and multifunctional protein complex found in the cytoplasm of cells, with the average mass of 250 kDa. Thanks to two catalytic centres, this protein performs seven different enzymatic functions. In a number of studies, it was suggested that *FASN* may be a candidate gene for several *QTL*s coding such characteristic traits as fat content and composition of fatty acids in milk of cattle and sheep (Garcia-Fernandez *et al.*, 2009 b).

Stearoyl-CoA desaturase isan endoplasmic reticulum enzyme that catalyzes monounsaturated fatty acids (MUFA) from saturated fatty acids (SFA) supplied through diet or de novo synthesis. This protein, with the mass of 37 kDa, is composed of 359 amino acids(Dobrzyn and Ntambi 2005). It performs a key function in many metabolic processes, including the functioning of adipose tissue through the regulation of fatty acids conversion and maintenance of energy homeostasis (Ntambi and Miyazaki 2004; Flowers and Ntambi 2008). In mammals, SCD has a similar gene structure, i.e. it is 15-24 kbp and is composed of 6 exons and introns (Bernard *et al.*, 2001). In sheep, stearoyl-CoA desaturase coding gene has been mapped to chromosome 22 (Kuchel*et al*., 2004). The expression of SCD gene in sheep has been found in adipose tissue, mammary glands, muscles, lungs, kidneys, the liver, heart, pancreas and spleen (Ward *et al*., 1998).

The research was undertaken to estimate the frequency of SNP alleles and genotypes in in *FASN* and *SCD1* genes in the herd of Zošľachtená Valaška sheep, as well as correspondence between genotypes and milk composition (basic milk composition, urea level and proportion of protein fractions).

MATERIALS AND METHODS

Animals: The study was conducted in a herd of 50 Zošľachtená Valaška sheep. Animals during lambing and lactation were kept in special buildings that meet the

requirements of the European Union Directive (Journal of Laws 2010 No. 116, item 778) and were fed: hay *ad libitum*, wheat middlings 250g/pc., hay and silage 3kg/pc. The sheep were in 2nd and 3rd lactation and at 25-30 day of milking. Milk samples were collected into sterile containers and transported to the laboratory at a temperature of 4°C. In addition, samples of peripheral blood from the internal jugular vein were collected into tubes containing anticoagulant (K_3EDTA) to isolate DNA.

Genotype analysis: DNA extraction was performer using the MasterPure™ Complete DNA and RNA Purification Kit (Epicentre®an Illumina company) according to the isolation protocol recommended by the producer. The analysis focused on two polymorphisms in *FASN* gene located in intron 31 (G56A) and exon 32 (C257T), and two SNP polymorphisms in *FASN* gene located in the promoter sequence (C31A, referred to as SCD01) and in intron 2 (A1473G, referred to as SCD02). Primer sequence designed on the basis ofAGQ150557 sequence was used for *FASN* gene, where mismatched nucleotide in position 259 was introduced to reverse primer in order to make incision for restrictive enzyme, while for SNPs of *FASN* gene primer sequence based on FJ513370.1 sequence was designed. Table 1 shows the used primer sequences, restriction enzymes, PCR product sizes, and size of fragments after restriction endonuclease digestion.

The analysis of milk: The content of fat, total protein, lactose and solids was determined in milk using an Infrared Milk Analyzer 150 camera from Bentley Instruments Inc. The urea content was determined with the CHEMSPEC.

The shares of protein fractions: serum albumin, *α* + *β*- and *κ*-casein, *α*-lactalbumin, were determined in the collected samples by electrophoresis according to the Laemmli method (1970) on polyacrylamide gel in sodium dodecyl sulfate (SDS-PAGE), in accordance with the methodology of Pecka *et al*., (2012).

Statistical analysis: Test results were statistically analyzed using one-way ANOVA in the program Statistica 10.0.[10] The significance of differences between groups was determined using Duncan's test. Hardy-Weinberg equilibrium and frequency of alleles were calculated using software PopGene version 1.32 (Yeh *et al*., 1997).

RESULTS

Genotyping the herd allowed to determine three possible genotypes in *FASN*/*Cfr*I polymorphism, and two out of three possible genotypes in *FASN*/*Aci*I and *FASN*/*Pvu*II polymorphisms (Figure 1, 2, 3); in the case of *FASN/Mun*I polymorphism monomorphism was discovered. Frequencies of alleles and genotypes are presented in Table 2.

In Table 3, protein, fat, lactose, dry matter and urea contents in sheep's milk in relation to particular *SCD* and *FASN* genotypes are shown.

The results obtained through statistical analysis of *FASN* gene polymorphism showed higher ($P< 0.05$) content of fat and increased ($P< 0.01$) proportion of dry matter in the milk of sheep with CT heterozygous genotype in comparison to animals with CC homozygous genotype: for lactose and dry matter the reverse situation occurred. The analysis of the obtained results for *SCD/Cfr*I polymorphism allowed to establish that the milk of animals with CC homozygous genotype had the highest content of protein, fat and dry matter. For heterozygous animals with the studied polymorphism the highest proportion of lactose and urea was demonstrated.

The next stage of statistical analysis was to determine the connection between genotypes of the studied polymorphisms and the proportion of protein fractions in milk, i.e. serum albumin, α + β – casein, κ – casein, and α –lactalbumin (Table 4).

Analysis of relationship between genotypes of *SCD/Cfr*I polymorphism showed that milk obtained from heterozygous sheep had the lowest level of serum albumin and the highest proportions of κ –casein and α – lactalbumin; at the same time, the milk from homozygous animals with the CC genotype contained the highest level of α + β – casein and the lowest level of κ – casein. The milk of sheep with the AA genotype for *SCD/Cfr*I polymorphism was characterized by the highest proportion of serum albumin and the lowest level of α– lactalbumin. In the case of analysis of *FASN*/*Aci*I polymorphism, it was observed that animals with the CT genotype had higher levels of fractions such as serum albumin, α + β – casein and α – lactalbumin. Only in the milk of the sheep with the CC homozygous genotype the proportion of κ – casein was increased.

Table 1. Conditions of polymorphisms analyzed by PCR-RFLP (mismatched nucleotide is underlined).

Gene/SNP	Primers	PCR	RE	D
FASN intron 31, G56A	**F:**TGAGATGGGGCAGCAGGCCT	275	*Mun*I	*G*:275 *A*: 222, 53
FASN exon 32, C257T	**R:**GGAACACTGTTCGCTTGCGGG		*Aci*I	*C*: 149, 107, 19 *T*: 168, 107
SCD1	**F:**CAGGGGCAGGGGCAGAGGCA	225	*Cfr*I	*C*: 194, 31

promoter region C31A	**R:** CGCTGGCAGCCGGTGACTGTG				*A*: 225
SCD1 intron 2, A1473G	**F:** CCCTAGGAGCTTCTTTGCTTC **R:**CCTGGTGAGAGTTCCTCTACT	225	*Pvu*II	*A*: 225 *G*: 133, 92

Explanations: PCR - PCR product size (bp); RE - restriction endonuclease; D - digestion product size (bp)

Table 2. Frequencies of genotypes and allele polymorphisms in *FASN* and *SCD1* genes in the herd under study

		Genotype			Allele	
*SCD1/Cfr*I		*AA*	*CA*	*CC*	*A*	*C*
	N	5	27	18		
	Frequency	0.100	0.540	0.360	0.370	0.630
		Genotype			Allele	
*SCD1/Pvu*II		*AA*	*AG*	*GG*	*A*	*G*
	N	49	1	0		
	Frequency	0.980	0.020	0.000	0.990	0.010
		Genotyp				Allele
*FASN/Aci*I		*CC*	*CT*	*TT*	*C*	*T*
	N	43	7	0		
	Frequency	0.560	0.360	0.080	0.740	0.260

Table 3. Milk composition and urea level depending on *SCD1* and *FASN* genotypes.

SNP	Genotype	n	Fat (%)	Protein (%)	Lactose (%)	Dry matter (%)	Urea (mg l^{-1})
SCD/*Cfr*I	*AA*	5	3.00±0.70	5.40±1.10	5.47±0.51	14.56±1.62	84.85±33.32
	AC	27	3.22±1.23	5.61±0.75	5.59±0.47	15.12±1.58	103.31±26.12
	CC	18	3.39±1.05	5.62±0.65	5.52±0.28	15.24±1.50	92.29±27.95
*FASN/Aci*I	*CC*	43	3.16±1.08^{a}	5.51±0.73^{A}	5.57±0.38	14.93±1.53^{A}	99.15±27.30
	CT	7	3.88±1.21^{a}	6.09±0.63^{A}	5.46±0.59	16.20±1.11^{A}	87.32±30.30

Explanations: mean values in rows marked with the same case letters differ significantly at: ^{a} - $P \leq 0.05$; ^{A} - $P \leq 0.01$

Table 4. Proportion of protein fractions in sheep's milk depending on particular *SCD* and *FASN* genotypes.

SNP	Genotype	N	Serum albumin (%)	α + β – casein (%)	κ – casein (%)	α –lactalbumin (%)
SCD/*Cfr*I	*AA*	5	15.38±6.45	40.53±7.35	12.08±2.45	10.48±1.85
	AC	27	13.50±3.60	42.59±6.60	12.57±4.99	12.28±5.12
	CC	18	13.99±2.00	46.00±5.13	10.98±1.82	10.99±2.11
*FASN/Aci*I	*CC*	43	13.68±3.56	42.97±6.73	12.28±4.40	11.28±3.06
	CT	7	14.44±3.43	44.69±5.71	11.43±2.92	12.50±5.83

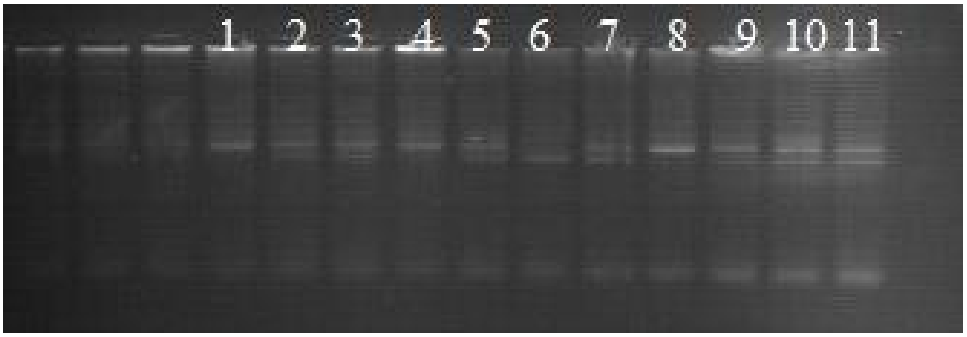

Fig 1. The digested PCR product SCD1/*Cfr*I; line: 1, 4, 8 - CA genotype, line: 2,3,5,7,9,10,11 - AA genotype, line 6 – CC genotype

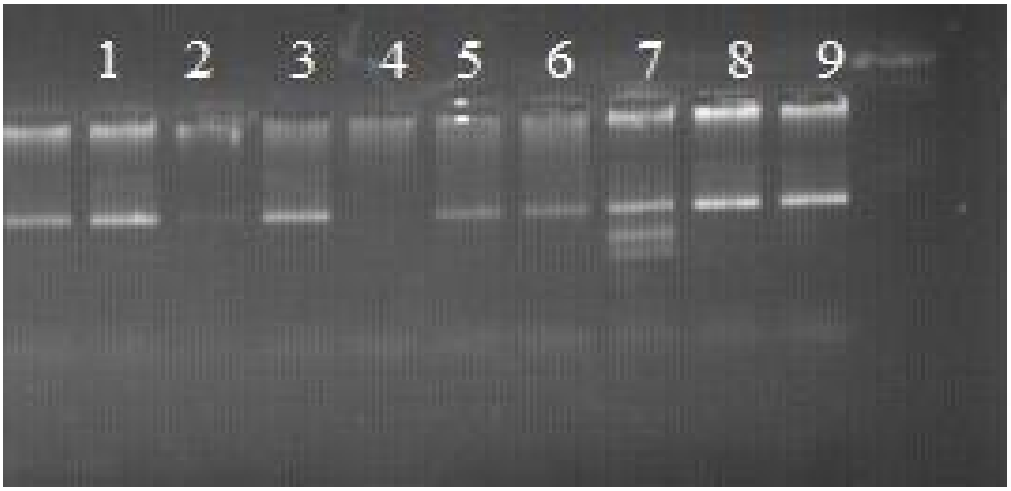

Fig 2. The digested PCR product SCD1/*Pvu*II; line: 1-6, 8, 9 - GA genotype, line: 7 - AA genotype

Fig 3. The digested PCR product FASN/*Aci*I; line: 1, 4 - TT genotype, line: 2,3,5,6,7 - CT genotype

DISCUSSION

Economically vital traits are mostly quantitative ones: they are controlled by many genes, each of which influences a given trait. The major gene model suggests that there may be a gene pool which is responsible for most of genetic diversity. Such major genes, which are usually involved in the biology of a given trait, may also be candidate genes for the trait marker, or may be considered qualitative trait *loci* (QTL) for a given trait. Due to key functions they perform in the synthesis of lipids secreted with milk, the regulation of transduction of fatty acids and energy homeostasis *FASN* and *SCD1* genes are promising candidate genes.

Apart from our research, few other authors have analyzed *FASN* gene polymorphism in sheep. In Crisa *et al.* (2010), the study of three sheep breeds (Altamurana, Gentile and di Puglia Sarda) demonstrated monomorphism at the intron 31 (G56A). In the C257T polymorphism, the frequency of allele *C* was 0.93. In this particular study positive substitution effect of allele T on medium-chain fatty acids was demonstrated. In the work of Garcia-Fernandez *et al*. (2009 b), in the herd of Spanish Churra sheep, 11 genetic markers localized in OAR11 – including two SNPs in *FASN* gene – were analyzed to detect QTL, which are responsible for the composition of fatty acids in milk; no association between the analyzed markers and the milk traits was found.

The polymorphism in *SCD1* gene examined in our research was also analyzed by Garcia-Fernandez *et al*. (2009 a, 2010) in eight sheep breeds from Spain (Churra, Ojalada, Castellana), France (Lacaune, Berrichon du Cher), Egypt (Ossimi, Rahmani) and Israel (Assaf), where the minor allele frequency for C31A polymorphism was within 0-0.45 range, and 0-0.25 for A1473G polymorphism.

Garcia-Fernandez *et al*. (2010) carried out the analysis of 22 traits associated with the composition of fatty acids and three usability traits in sheep's milk and demonstrated that SCD01 polymorphism had a significant effect on fat percentage, the proportion of

linoleic acid (C18:2 cis-9,cis-12), and the n-6/n-3 ratio, while SCD02 polymorphism showed a significant effect on butyric acid (C4:0). Research into polymorphism in *FASN* and *SCD1* genes was conducted in connection with productivity traits in cattle. Numerous authors demonstrate correlation between *FASN* gene polymorphisms and the fat content in milk (Roy *et al*. 2006), its influence on the composition of fatty acids in milk (Morris *et al*., 2007; Schennik *et al*., 2009; Li *et al*., 2011), as well as cows' meat (Zhang *et al*., 2008; Hayakawa *et al*., 2015). Also for *SCD1* gene, the association with the composition of fatty acids in cows' milk was demonstrated.

In the existing literature of the subject, one may find research into the influence of polymorphism in the β-lactoglobulin coding gene on sheep's milk composition (Selvaggi *et al*., 2015; Triantaphyllopoulos *et al*., 2017). *CPT1B* polymorphism in sheep (Dervishi*et al*., 2012), and κ-casein, *DGAT1, SBD1*, *SBD2* polymorphism (Clément *et al*., 2006; Nanekarani *et al*., 2016) have been extensively studied.

Previous research effects of SNPs in the *SLC27A3* and *FABP3* gene in sheep have concentrated on milk composition and milk technological quality. They were the authors' own research (Kowalewska-Łuczak *et al*., 2017, Pecka-Kiełb *et al*. 2018 and 2020). In another research, the aim of the study was to investigate expression changes of *SCD* and *FASN* genes that resulted from crossbreeding the different breeds of sheep (Izadi *et al*. 2016), and gene analysis associated with the profile of fatty acids from milk of sheep (Esteves *et al*. 2019).

However, no research into the influence of genotype on SNPs in *FASN* and *SCD1* genes on the composition and proportion of protein fractions in sheep's milk and the level of protein, lactose or urea has been found.

According to Ciecierska *et al.* (2013), in cows *FASN*/*Aci*I polymorphism affects productivity of fat and protein in milk. Cows with homozygous *AA* genotype had higher fat and protein productivity in comparison to the cows with heterozygous *AG* genotype. In our research a reverse correlation was found: the milk of sheep with *CT* heterozygous genotype had higher levels of protein and fat than that of homozygous sheep.

Protein and fat content in the milk of Tibetan sheep is 4.84% and 6.94%, respectively, while the proportion of caseins to protein in general is 51% (Wang *et al*., 2011); Sabahelkhier*et al.* (2012) determined protein content in sheep milk to be 6.35%, fat - 6.90%, lactose - 5.00%, and dry matter - 19.3%.

In our research, the level of protein, caseins and lactose in milk was within the range of values found in the literature, whereas fat and dry matter contents were much lower, which may be connected with the difference in maintenance and nutrition of animals. Milk composition and urea level were determined by, among others, lactation phase, age, nutrition and health condition (Fuertes *et al*., 1998; Thomas *et al*., 2001; Kuchtík*et al*., 2008; Pecka *et al*., 2013).

In milk, because of cheese yield increased proportion of κ- casein and low level of whey protein is highly desired (Pecka*et al*., 2013). In our research, the most desired profile of protein fractions, protein, fat and dry matter content was found in animals with the *CC* homozygous genotype for *SCD*/*Cfr*I polymorphism.

Conclusions: Conducted research showed changes in the composition of milk and proportion of protein fractions depending on SNPs in *FASN* and *SCD1* genes. The protein, fat, carbohydrates content and proportions of protein fractions - including caseins - determines the technological properties of milk; our research proves that the milk of sheep with *CC* genotype in *SCD/Cfr*I polymorphism has good technological parameters.

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