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ORIGINAL PAPER

INVESTIGATION ON RELATIONSHIPS OF THE FABP3 AND SLC27A3 GENES WITH MILK PRODUCTION TRAITS IN SHEEP*

Inga Kowalewska-Łuczak[1], Ewa Czerniawska-Piątkowska[2], Ewa Pecka-Kiełb [3]

[1]Department of Genetics and Animal Breeding
[2]Department of Ruminant Science, Laboratory of Molecular Cytogenetics
West Pomeranian University of Technology, Szczecin, Poland
[3]Department of Biostructure and Animal Physiology
Wroclaw University of Environmental and Life Sciences, Poland

Abstract

The aim of this study was to determine whether genetic differences in genes FABP3 and SLC27A3 may be related to the productivity and composition of sheep's milk. A herd of 50 Slovak sheep breed Zoslachtena Valaska (Zošľachtená Valaška, in Slovak) was studied. The frequencies of the most common alleles were as follows: FABP3 SNP13 0.82, SLC27A3 C/T 0.64, SCL27A3 A/G 0.74. The results of the statistical analysis for polymorphism in the FABP3 (SNP13) gene showed that animals with the homozygous AA genotype had the highest content of fat, protein and solids in the milk of tested sheep. The analysis of the results for the SLC27A3 C/T polymorphism allowed us to conclude that the milk of animals with the homozygous TT genotype was characterized by the lowest content of fat, protein and solids and the highest content of lactose. In the case of SLC27A3 A/G polymorphism, sheep with the heterozygous genotype were characterized by the highest fat content in milk and the lowest content of protein and lactose. The milk of sheep with the heterozygous genotype FABP3 was characterized by the highest share of serum albumin, $\alpha+\beta$ – caseins and α-lactalbumin. In contrast, animals with the homozygous AA genotype were characterized by the highest content of κ-casein. The relationships between genotypes of the SLC27A3 C/T polymorphism showed that sheep with the heterozygous genotype were characterized by the lowest content of serum albumin, and the highest content of $\alpha+\beta$ – caseins in milk. The analysis conducted for the SLC27A3 A/G polymorphism demonstrated that animals with the AA genotype were characterized by the lowest content of serum albumin in milk. Because most of the results were not confirmed statistically, we should continue research using different breeds of sheep and herds with a larger number of animals.

Keywords: livestock, gene polymorphism, FABP3, SLC27A3, PCR-RFLP, milk components.

dr hab. inż. Ewa Czerniawska-Piątkowska, Department of Ruminant Science, Laboratory of Molecular Cytogenetics, West Pomeranian University of Technology, Szczecin, Poland, e-mail: ewa.czerniawska-piatkowska@zut.edu.pl

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INTRODUCTION

Intracellular fatty acid binding proteins (FABP) are cytoplasmic proteins that are essential for the transport and metabolism of fatty acids in the cell, by accelerating the absorption of long-chain fatty acids and delivering fatty acids to intracellular organelles (Lanier, Corl 2015). Furthermore, FABP can modulate intracellular levels of fatty acid and thereby regulate a variety of cellular processes and lipid metabolism. Heart-type fatty acid binding protein (H-FABP, FABP3), whose molecular weight is 15 kD, is present in many tissues, particularly the ones with high fatty acids demand, such as the heart muscle, skeletal muscle and mammary gland during lactation (Calvo et al. 2002, Lanier, Corl 2015). The transport of fatty acids is also supported by a group of FATP proteins (fatty acid transport proteins) which are encoded by the family of SLC27A genes. Fatty acid transport proteins have a molecular weight ranging between 63-80 kDa and are integral membrane proteins with at least one transmembrane domain. All members of the FATP family have a highly conserved sequence, which is composed of 311 amino acids, known as FATP, as well as an AMP-binding enzyme located at the C-terminal domain. This region is responsible for binding and absorption of LCFA (Anderson, Stahl 2013). The FABP3 gene in sheep was mapped on chromosome 2and is composed of four exons separated by introns, while the SLC27A3 gene was mapped on chromosome 1 and is composed of 10 exons separated by introns (Calvo et al. 2002, 2006*a*).

The study aimed to determine the prevalence of alleles and genotypes in relation to the SNP polymorphisms in SLC27A3 and FABP3 genes in a herd of sheep (Zošľachtená Valaška), and to determine possible relationships between genotypes and qualitative characteristics of sheep's milk.

MATERIAL AND METHODS

The animals

The study material included samples of peripheral blood and milk taken from 50 sheep of the Slovak breed Zošľachtená Valaška, which were in 2nd and 3rd lactation and at 25-30 day of milking. During lambing and lactation, the animals were kept in special buildings that meet the essential requirements mentioned in the Directive of the European Union (OJ 2010 No. 116, item 778) and fed with: hay *ad libitum* wheat middling 250 g/pc., hay silage 3 kg/pc. Lambs prior to the milking were left to stand for four hours. The samples of peripheral blood were collected from each individual animal from the jugular vein into tubes containing K_3EDTA as an anticoagulant factor. In addition, milk samples were collected into sterile containers and transported to the laboratory at 4°C.

The analysis of genotype

DNA extraction was carried out using a DNA isolation kit MasterPure™ Complete DNA and RNA Purification Kit (Epicentre® an Illumina company), according to the insulation protocol included in the package. The analysis focused on four polymorphisms of SNP type, two in each of the genes. The FABP3 gene was tested for SNPs located in exon 2 and intron 3, called, respectively, SNP3 (G/A) and SNP13 (G/A) (AY157617). The analyses involved the use of primer sequences, PCR thermal profile and restriction enzymes developed by Calvo et al. (2004). The SLC27A3 gene was analyzed for polymorphisms located in intron 4 (SLC27A3 C/T) and exon 9 (SLC27A3 A/G) (AY996127). The primer sequences were developed based on the work of Calvo et al. (2006*a*) and the AY996127 sequence (Table 1). Thermal conditions of the SLC27A3 gene fragments were adjusted to the annealing temperature indicated in Table 1.

Table 1

Conditions for PCR-RFLP of the analyzed *SLC27A3* polymorphisms

Primers	Source	Annealing temp. (C°)	PCR product size/ Digestion product size
GACAGTGTTCCAGTACATCG TACGTCTCCAGCACCTGCA	Calvo et al. (2006*a*) own project	50	444 pz / C: 240, 204 pz; T: 444 pz
CTTGGAGGCCCTGGACTTTC TGGGCAGGCACTGCTTTTTCTC	Calvo et al. (2006*a*) own project	54	440 pz / A: 205, 80, 71, 43,41 pz; G: 246, 80, 71, 43 pz

Amplification reactions were conducted in a final volume of 20 µl, 0.2 mm of each dNTP, 10 pmol of each primer (forward and reverse), 50-100 ng ovine genomic DNA, containing 1 unit of Taq DNA polymerase in a standard PCR buffer and sterile water. The products obtained from amplification of the FABP3 gene fragment (exon 2 – 222 bp and intron 3 – 355 bp) were digested with restriction enzymes BsaJI and BanII (respectively). Restriction enzymes proposed by Calvo et al. (2006*a*) were used for digestion of the SLC27A3 gene fragments: intron 4 – BsaHI and exon 9 – TseI. The digestion of amplicons was carried out under specific temperature conditions for specific enzymes and the resulting restriction fragments were separated in agarose gels, in the presence of pUC19/MspI pattern.

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

Statistical analysis

Test results were statistically analyzed using one-way analysis of variance (ANOVA) in Statistica 10.0 (StatSoft®). The significance of differences between groups was determined using the Duncan's test.

RESULTS AND DISCUSSION

The presence of all possible genotypes between SNPs was revealed in the case of three analyzed polymorphisms. The presence of heterozygotes was revealed in a fragment of the FABP3 gene, digested with the BsaJI enzyme. The turnout of alleles and genotypes was presented in Table 2.

Table 2

Frequencies of genotypes and alleles for the studied polymorphisms in FABP3 and SLC27A3 gene

SNP	Genotype frequencies		Allele frequencies	
SNP13	AA AG GG	0.66 0.32 0.02	A G	0.82 0.18
SLC27A3 C/T	CC CT TT	0.34 0.60 0.06	C T	0.64 0.36
SLC27A3 A/G	AA AG GG	0.56 0.36 0.08	A G	0.74 0.26

Table 3 summarizes the basic composition (the content of fat, protein, lactose and solids) and urea content in sheep's milk for each genotype of the tested polymorphisms of FABP3 and SLC27A3 genes. When considering the results of the statistical analysis for polymorphism in the FABP3 (SNP13) gene, it was observed that animals with the homozygous AA genotype had the highest content of fat, protein and solids in the milk of tested sheep. The sheep with the heterozygous genotype demonstrated the highest content of solids and urea in milk. The analysis of the results for the SLC27A3 C/T polymorphism allowed us to conclude that the milk of animals with the homozygous TT genotype was characterized by the lowest content of fat, protein and solids and the highest content of lactose. This difference was statistically confirmed for the lactose content ($P \leq 0.05$). The lowest level of urea in sheep's milk was detected for animals with the heterozygous genotype in respect to the relevant polymorphism.

In the case of SLC27A3 A/G polymorphism, it was observed that sheep with the heterozygous genotype were characterized by the highest fat and

Table 3

The composition of sheep's milk and the urea content according to the genotypes FABP3 and SLC27A3

SNP	Genotype	*n*	Fat (%)	Protein (%)	Lactose (%)	Dry matter (%)	Urea (mg l^{-1})
SNP13	AA	33	3.51±1.23	5.66±0.83	5.45±0.40	15.34±1.76	96.79±8.52
	GA	16	2.81±0.63	5.43±0.54	5.71±0.33	14.64±0.87	100.39±27.01
SLC27A3 C/T	CC	17	3.20±0.93	5.52±0.60	5.64±0.35	15.08±1.28	99.24±29.68
	CT	30	3.39±1.17	5.68±0.81	5.46^{a}±0.39	15.24±1.66	96.40±28.00
	TT	3	2.22±1.36	5.10±0.79	6.03^{a}±0.63	14.00±1.58	98.60±19.08
SLC27A3 A/G	AA	28	3.17±1.17	5.64±0.80	5.59±0.44	15.12±1.60	91.20±5.66
	AG	18	3.40±1.14	5.48±0.73	5.47±0.39	15.05±1.62	110.30±28.37
	GG	4	3.21±0.74	5.73±0.28	5.69±0.26	15.34±0.73	83.93±21.54

Explanation: mean values in rows marked with the superscript differ significantly at: $^{a, b} - P \leq 0.05$.

urea content in milk and the lowest content of protein and lactose. The analysis of the tested polymorphism demonstrated that the milk of animals with the homozygous GG genotype was characterized by the highest content of solids.

The next stage of the statistical analysis was to estimate the relationships between genotypes of the tested polymorphisms and the shares of individual protein fractions in milk (Table 4). When considering two of the three possible genotypes for the FABP3 polymorphism (the homozygous GG genotype was identified in only 1 individual), it was observed that the milk of sheep with the heterozygous genotype was characterized by the highest share of serum albumin, $\alpha+\beta$ – caseins and α-lactalbumin. In contrast, animals with the homozygous AA genotype were characterized by the highest content of κ-casein.

The relationships between genotypes of the SLC27A3 C/T polymorphism showed that sheep with the heterozygous genotype were characterized by the lowest content of serum albumin, and the highest content of $\alpha+\beta$ – caseins in milk. On the other hand, the milk obtained from animals with the homozygous GG genotype was characterized by the highest content of κ-casein α-lactalbumin.

The analysis conducted for the SLC27A3 A/G polymorphism demonstrated that animals with the AA genotype were characterized by the lowest

Table 4

Fraction of protein in the milk of sheep depending on the particular FABP3 and SLC27A3 genotype

SNP	Genotype	*n*	Serum albumin (%)	$\alpha + \beta$-casein (%)	κ-casein (%)	α-lactalbumin (%)
SNP13	AA	33	13.68±3.56	42.97±6.73	12.28±4.40	11.28±3.06
	GA	16	14.44±3.43	44.69±5.71	11.43±2.92	12.50±5.83
SLC27A3 C/T	CC	17	15.03±4.77	42.80±8.09	12.23±5.61	11.91±5.56
	CT	30	12.99±2.32	44.11±5.40	11.69±2.59	11.21±2.61
	TT	3	15.79±2.45	42.67±5.45	13.25±5.11	14.34±6.19
SLC27A3 A/G	AA	28	13.66±3.46	43.39±6.93	11.82±4.48	10.71±2.52
	AG	18	14.01±3.82	43.49±5.95	12.32±3.41	13.25±5.65
	GG	4	14.90±1.91	45.62±3.87	11.22±1.61	10.79±1.103

content of serum albumin in milk. In contrast, sheep with the homozygous GG genotype were characterized by the highest content of $\alpha+\beta$ – caseins in milk. The milk of sheep with heterozygous genotypes was characterized by the highest content of κ-casein α-lactalbumin.

There have been several reports of analyses concerning loci affecting performance traits of dairy cattle. In recent times, the researchers in Europe have observed an increase in consumer demand for good quality products made from sheep's milk. In comparison to cattle, the performance of dairy sheep depends on several important factors: in sheep, the use of artificial insemination is more or less limited, while natural mating plays an important role in the reproduction and improvement of performance traits is more expensive in sheep than it is in cattle. Therefore, the use of marker-assisted selection (MAS) and gene-assisted selection (GAS), which may accelerate the selection or reduce the costs of improving performance traits, appears to be more attractive in the case of dairy sheep (Barillet et al. 2005).

Polymorphisms in the FABP3 gene considered in the present study have been also analyzed by other researchers – the tests were conducted on the Spanish (Aragonesa, Awasi, Assaf, Manchega) and Turkish (Kıvırcık) sheep breeds (Calvo et al. 2002, 2004, Öner et al. 2014). The turnout of A allele for the FABP3/BanII (SNP13) polymorphism was higher than that indicated by Calvo et al. (2002, 2004), where it ranged between 0.26-0.46 depending on the breed of sheep. On the other hand, the turnout of A allele for the FABP3/BsaJI (SNP3) polymorphism ranged between 0.26-0.46 (Calvo et al. 2002, 2004, Öner et al. 2014). There are few publications concerning polymorphisms in the SLC27A3 gene in sheep (Calvo et al. 2006*a*,*b*) and only one of them provides turnout for the polymorphism located in intron 4, which amounts to 0.55 for C allele, and is slightly lower than that obtained in the aforementioned study (Calvo et al. 2006*a*).

Calvo et al. (2004) conducted an analysis of the effect of genotypes for the analyzed polymorphisms in the FABP3 gene on performance traits of dairy sheep, where the within-family analysis showed a statistically significant effect of genotypes on estimated breeding value for milk-fat content. Another study analyzed two other polymorphisms in the FABP3 gene of Indian sheep with regard to meat quality traits (Arora et al. 2014), but there was no relationship between genotypes and performance traits. As in the case of studies concerning the analysis of allele turnout for polymorphisms located in the SLC27A3 gene, there are very few publications dealing with the assessment of relationships between individual genotypes and performance traits of dairy sheep. Calvo et al. (2006*a*) conducted a within-family analysis of the polymorphism located in intron 4 and found no direct relationship between genotypes and the tested performance traits of dairy sheep.

Studies concerning polymorphisms in the FABP3 gene were also conducted for performance traits of cattle. They revealed a relationship of polymorphisms with fat and protein content in the milk of Jersey cows (Kulig et al. 2010), while the regression analysis showed a link between polymorphism and the estimated breeding value for the performance of fat in the milk of Polish Holstein-Friesian cows (Kulig et al. 2013).

The level of lactose in sheep's milk depends on the age of animals, stage of lactation and health status (Othmane et al. 2002, Nudda et al. 2003, Kuchtík et al. 2008, Sabahelkhier et al. 2012). On the other hand, the level of urea in milk is affected by animal welfare, climatic conditions, quality of pastures, stage of lactation, bacterial infections, etc. (Kuchtík et al. 2008, Bendelja et al. 2009, Matutinović et al. 2014, Pecka-Kiełb et al. 2016). The concentration of urea in milk is also an indicator that provides information about a proper balance of feed rations in terms of energy and protein in feed (Bendelja et al. 2009). Our study revealed quite equal levels of urea and lactose with respect to all of the analyzed polymorphisms and individual genotypes, which indicates good condition and nutrition of animals.

The main source of calcium and phosphorus, for newborn animals during feeding with colostrum and milk, is the presence of this element in casein micelles, which are a major component of protein in milk. Caseins constitute approximately 70% of total protein (Summer et al. 2010, Hamed et al. 2012, Pecka et al. 2012*b*). The level of casein in milk is related to the phase of lactation, age and time of year (Othmane et al. 2002, Nudda et al. 2003, Hamed et al. 2012, Matutinović et al. 2014). Bacterial infections of the mammary gland also affect the level of α- and κ-casein in milk (Pecka-Kiełb et al. 2016). The effect of reducing the level of casein is a lower content of minerals such as calcium and phosphorus (Summer et al. 2010). However, from a technological point of view, it is desirable to obtain milk with an increased share of caseins, in particular κ-casein (Brophy et al. 2003).

CONCLUSIONS

Despite the fact that sheep milk is much richer in nutrients compared to the milk of goats and cows, it is rarely consumed directly by drinking, and is used primarily for the production of a wide variety of different cheeses, and in some countries for the production of yoghurt or whey. As a result of scientific and technical progress, it was possible to deepen our knowledge about the properties of sheep's milk and the role of each of its components during the production of cheese, which is crucial for the development of the cheese industry. Performance traits of milk are controlled by many genes and the polymorphism of milk protein is strongly associated with quantitative and qualitative parameters of milk. The inclusion of molecular genetic markers for any selection in the direction of improving milk production and its composition slowly becomes another goal in sheep farming (Selvaggi et al. 2014). Therefore, this study may be a prelude to the development of species and can help in the selection that would ensure appropriate quality of milk. Obviously, all information concerning the tested polymorphisms in FABP3 and SLC27A3 genes should be confirmed during tests using different breeds of sheep and herds with a larger number of animals.

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