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POLYMORPHISM OF *FABP3* GENE AND ITS EFFECT ON LITTER SIZE AND MILK PRODUCTION OF SYNTHETIC POPULATION BULGARIAN MILK EWES

Aim. The purpose of this work was to study polymorphism of *FABP3* (heart-type fatty acid binding protein) gene and its effect on litter size and milk production. The experiment included 30 ewes of Synthetic Population Bulgarian Milk breed from Institute of Animal Science – Kostinbrod.

Methods. By PCR-RFLP method with endonuclease *BseDI* in SNP3 of *FABP3* gene were detected two genotypes – GG and AG. **Results.** In SNP3 of *FABP3* gene the frequency of allele G was 0.85 and the frequency of allele A was 0.15. The genotype GG was with frequency 0.70 and AG – with 0.30.

Conclusions. In this study of ewes from SPBM breed, the presence of heterozygous genotype AG in SNP3 of the *FABP3* was associated with increased litter size, while the presence of the homozygous genotype GG led to increased milk productivity during lactation.

Keywords: sheep, *FABP3* gene, litter size, milk production.

Sheep farming is traditional for Bulgaria because of the suitable environmental and climate conditions. This sector provides livelihood to a large part of the rural population both in the plains and in the mountain areas.

Over the last decade, there has been an intensive search for candidate genes associated with milk and meat production and their quality in order to improve available sheep breeds [1–3]. The allelic diversity of candidate genes for productive traits is a mandatory basis of this process and contributes to their differentiation and study. Knowledge of the different polymorphic variants of these genes would help choose the best breeding strategy depending on the production area [4, 5].

FABP3 or H-*FABP* is a small cytoplasmic protein associated with cardiac activity that is released by cardiac myocytes after an ischemic event.

It plays a role in the active metabolism of fatty acids by transporting fatty acids from the cell membrane to the mitochondria for oxidation [6]. The *FABP3* gene is located on chromosome 2 of the sheep genome [7, 8]. It is found in many tissues such as the heart muscle, skeletal muscle and mammary gland during lactation [9, 10]. Studies have shown that the expression levels of the H-*FABP* gene affect the final stage of cell differentiation after mitosis, which is mainly influenced by fatty acid metabolism [11]. This gene is involved in muscle development and fatty acid metabolism, and it is associated with milk fat content and marbling of meat.

The purpose of this study was to investigate polymorphism of *FABP3* gene and its potential effect on litter size and milk production of ewes of Synthetic Population Bulgarian Milk (SPBM) breed.

Material and methods

The investigation was carried out in the Laboratory of Genetics of Agronomy Faculty, University of Forestry – Sofia, Bulgaria.

Animals and blood collection. In present study were tested 30 ewes of Synthetic Population Bulgarian Milk breed from Institute of Animal Science – Kostinbrod. Blood samples were collected from *v. jugularis* in vacuum tubes containing EDTA.

DNA extraction. Genomic DNA was isolated from whole blood with manual commercial kit for DNA purification according to the manufacturer's instruction (Illustra Blood GenomicPrep DNA Purification Kit, *GE Healthcare*). DNA quality and quantity were determined using spectrophotometer Biodrop and agarose electrophoresis on 1 % agarose gel (Bioline) and 1x TAE buffer (Jena Bioscience).

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PCR amplifications were carried out in total volumes of 10 µl, containing 40 ng DNA template, 0.2 µl dd-H₂O, 20 pM of each primer and 5 µl of 2×(1.5 mM MgCl₂) MyTaq™ HS Red Mix 2x (Bioline). The primer set (F: 5'-GGTTTTGCTAC-CAGGCAGGT-3' and R: 5'-TTCCCTATTCCCC-TTCAGGG-3') used for the amplification is according to Calvo et al. [9] and produced 222 bp size of PCR product. PCR reactions were accomplished by thermocycler QB-96 (Quanta Biotech) under the following conditions: primary denaturation at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 30 s, elongation at 72°C for 1 min. The reaction was completed by final extension at 72°C for 10 min and storage at 10°C forever.

Restriction Fragment Length Polymorphism

The genotypes of all investigated animals for *FABP3* gene were established using RFLP (restriction fragment length polymorphism) analysis. PCR products were digested separately in 10 µl final volume, containing 6 µl PCR product, 2.5 µl ddH₂O, 10 U/µl restriction enzyme (Thermo). It was used endonuclease *BseDI* and digestion reactions were carried out at 60°C for 3 h in thermo-block. The fragment sizes were identified using Ready-to-Use DNA Ladder, 50 bp (Thermo) on 2.5 % agarose (Bioline) gel and stained by RedGel Nucleic Acid Stain (Biotium). The obtained PCR products and restriction fragments were visualized under UV light.

Obtained data was analyzed by statistical software Statgraphics Centurion XVIII.

Results and discussion

After PCR amplification of exon 2 of *FABP3* locus (SNP3) the obtained PCR products with size 222 bp were cut by *BseDI* restriction enzyme. Two alleles were produced: allele *G* – three fragments

with sizes 143, 43 and 36 bp and allele *A* – two fragments with sizes 186 and 36 bp. For *FABP3* gene were established two alleles *A* and *G* with frequencies 0.15 and 0.85, respectively. Two genotypes were revealed – homozygous genotype *GG* and heterozygous genotype *AG* with frequencies 0.70 and 0.30, respectively. Observed heterozygosity was 0.150 and expected heterozygosity was 0.250 with $P > 0.01$.

In this experiment was studied the relationship between different genotype variants of the tested locus and litter size and milk yield of included in this investigation ewes from Synthetic Population Bulgarian Milk.

A comparative analysis of litter size of ewes of the SPBM breed with genotype variants *AG* and *GG* of the *FABP3* gene was performed using the statistical method ANOVA (Tables 1, 2).

Table 1 showed various statistics for litter size for each of the two genotypes of *FABP3* gene – *AG* and *GG*. Coefficient of variation for total analysis was less than 30 % and analysis was acceptable with P -value = 0.0289. There was a statistically significant relationship between higher litter size and heterozygous genotype *AG* compared to homozygous genotype *GG* in 5 % significance level (Table 2 and Fig. 1).

Table 2 showed the Multiple Range Tests to determine if there was statistically significant difference between mean values of litter size and different genotypes of *FABP3* gene in 95.0 % confidence level.

Figure 1 showed that there was statistically significant difference between intervals.

Results from the comparative analysis of milk production in ewes of the SPBM breed with genotype variants *AG* and *GG* of *FABP3* gene performed using the statistical method ANOVA were presented in Tables 3, 4 and Figure 2.

Table 1. Summary Statistics for Litter size for *FABP3* gene

Locus <i>FABP3</i>	Average	Standard deviation	Coeff. of variation
<i>AG</i>	1,43778	0,359332	24,9922 %
<i>GG</i>	1,186	0,224954	18,9674 %
Total	1,26414	0,292031	23,1012 %

Table 2. Multiple Range Tests for Litter size for *FABP3* gene. Method: 95.0 % LSD

<i>FABP3</i> gene	Mean	Homogeneous Groups	Contrast	Sig.	Difference	+/- Limits
<i>AG</i>	1,43778	x	<i>AG - GG</i>	*	0,251778	0,223838
<i>GG</i>	1,186	x				

Note. * denotes a statistically significant difference.

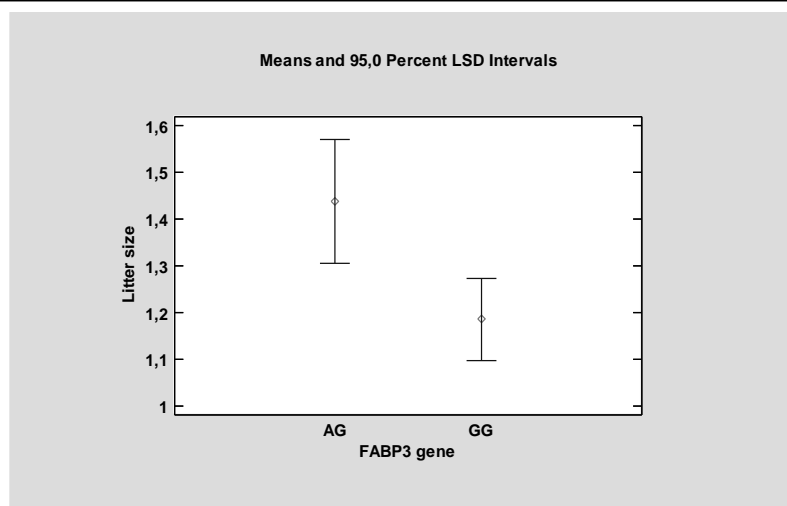


Fig. 1. Means of Litter size at 95 % LSD intervals for *FABP3* gene.

Table 3. Summary Statistics for Milk Production for *FABP3* gene

Locus <i>FABP3</i>	Average	Standard deviation	Coeff. of variation
AG	88,8	6,3	7,09459 %
GG	97,1333	9,58209	9,86488 %
Total	94,3556	9,39531	9,95734 %

Table 4. Multiple Range Tests for Milk production for *FABP3* gene. Method: 95.0 % LSD

<i>FABP3</i> gene	Mean	Homogeneous Groups	Contrast	Sig.	Difference	+/- Limits
AG	88,8	x	AG - GG	*	-8,33333	7,28818
GG	97,1333	x				

Note. *statistically significant difference.

Table 3 showed various statistics for Milk production for each of the two genotypes of *FABP3* gene – AG and GG. Coefficient of variation for total analysis was less than 10 % and the analysis was acceptable. The F-ratio in our case was 5.5. P-value was 0.0267. Since the P-value of the F-test were less than 0.05, there was a statistically significant difference between the mean values of two genotypes of *FABP3* gene in 5 % significance level.

Table 4 showed Multiple Range Tests to determine if there was statistically significant difference between mean values of the two genotypes of *FABP3* gene. The asterisk marked a statistically significant difference between two genotypes in 95.0 % confidence level.

Figure 2 showed in the Multiple Range Tests intervals used to determine if there was a statistically significant difference between mean values of the two genotypes of *FABP3* gene. In this study, the intervals were significantly different.

In SNP3 of *FABP3* gene allele G was with frequency 0.85 and allele A with 0.15. The fre-

quency of genotype GG was 0.70 and genotype AG – 0.30. In a previous study, this region of the gene *FABP3* was studied in sheep of three merino (Ascanian, Caucasian and Karnobat) and two local (Cooper-Red Shumen and Karakachan) breeds [12]. The frequencies were as followed: allele D ranging from 0.77 to 0.88 and allele A – from 0.13 to 0.23. In all these five breeds, all three possible genotypes were identified – genotype AA – with a frequency in the range of 0.03 – 0.07, genotype AG from 0.13 to 0.40 and genotype GG from 0.57 to 0.80. The tested animals from SPBM have a relatively lower frequency of the allele A and lack of genotype AA, which were associated with the mouflon, considered as the ancestor of European sheep [9]. The frequency of allele A in domestic sheep breeds varies between 0.26 in Raza Aragonesa to 0.33 in Awasi, 0.38 in Assaf, 0.42 in Kivircik and 0.46 in Manchega breeds [7, 9, 13]. The frequency of allele A and absence of genotype AA showed that this newly created SPBM sheep breed is also genetically more distant from the mouflon compared to the studied Spanish and Turkish breeds.

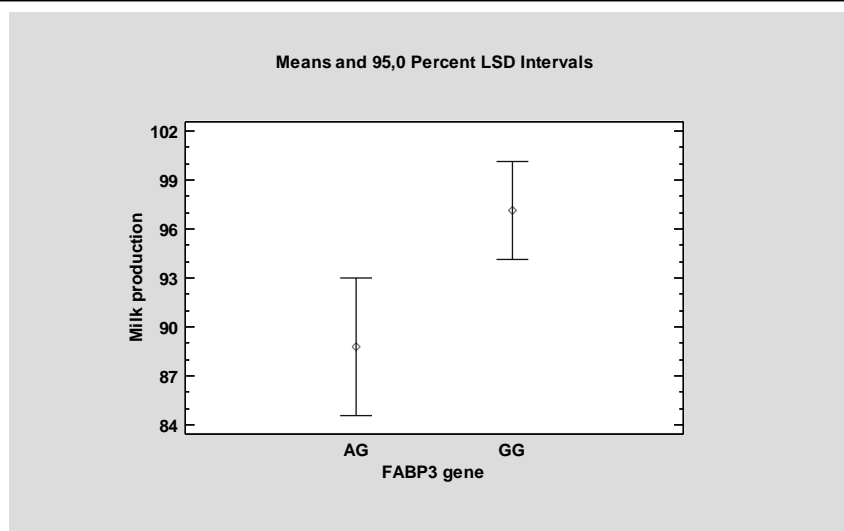


Fig. 2. Means of Milk Production at 95 % LSD intervals for *FABP3* gene.

In this study of sheep from SPBM breed, the presence of heterozygous genotype AG in SNP3 of the *FABP3* gene was associated with increased litter size, while the presence of homozygous genotype GG led to increased milk productivity per lactation.

Conclusions

Two alleles and two genotypes were identified for SNP3 of *FABP3* gene. In this study of sheep from SPBM breed, the presence in SNP3 of

the *FABP3* gene of the AG genotype was associated with increased litter size, while the presence of the GG genotype led to increased milk productivity per lactation. Further investigations are required to validate these associations in a larger population.

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ПОЛІМОРФІЗМ ГЕНА *FABP3* ТА ЙОГО ВПЛИВ НА РОЗМІР ПОСЛІДУ ТА МОЛОЧНУ ПРОДУКТИВНІСТЬ ШТУЧНИХ ПОПУЛЯЦІЙ БОЛГАРСЬКОЇ МОЛОЧНОЇ ВІВЦІ

Мета. Метою роботи було вивчення поліморфізму гена *FABP3* (який відповідає за білок що зв'язує жирні кислоти серцевого типу) і його впливу на розмір посліду та продуктивність молока. В експерименті брали участь 30 овець Synthetic Population Bulgarian Milk породи з Інституту тваринництва м. Костінброд. **Методи.** Методом ПЛР-ПДРФ з ендонуклеазою *BseDI* в SNP3 гені *FABP3* були виявлені два генотипи – ГГ і АГ. **Результати.** У SNP3 гена *FABP3* частота алелі Г становила 0,85, а частота алелі А – 0,15. Генотип ГГ траплявся з частотою 0,70, а АГ – з 0,30. **Висновки.** У цьому дослідженні вівцематок породи SPBM присутність гетерозиготного генотипу АГ у SNP3 *FABP3* було пов'язана зі збільшенням розміру посліду, тоді як наявність гомозиготного генотипу ГГ спричинила збільшення молочної продуктивності під час лактації.

Ключові слова: вівці, ген *FABP3*, розмір посліду, виробництво молока.