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 1 x TAE buffer (Jena Bioscience).
**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 TM HS Red Mix 2x (Bioline). The primer set (F: 5’-GGTCTTAC-CAGGCAGGT-3’ and R: 5’-TTTCCTATTCCCC-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 UV light.

Obtained PCR products and restriction fragments were visualized on agarose (Bioline) gel and stained by RedGel Nucleic Acid Stain (Biotium). The obtained PCR products were digested separately in 10 μl final volume, containing 6 μl PCR product, 2.5 μl ddH2O, 0.2 μl ddMgCl2, 2×(1.5 mM MgCl2) MyTaq TM HS Red Mix 2x (Bioline) and 0.2 μl restriction enzyme (Thermo). It was used endonuclease BseDI and digestion reactions were carried out at 60°C for 3 h in thermoblock. 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.
Fig. 1. Means of Litter size at 95% LSD intervals for FABP3 gene.

Table 3. Summary Statistics for Milk Production for FABP3 gene

| Locus FABP3 | 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

| FABP3 gene | Mean  | Homogeneous Groups | Contrast   | Sig. | Difference +/- Limits |
|------------|-------|--------------------|------------|------|-----------------------|
| AG         | 88.8  | x                  | AG - GG    | *    | -8.33333              |
| GG         | 97.1333 | x                | AG - GG    | *    | 7.28818               |

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 was 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 frequency 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 Kivrcek 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.
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.

This research was part of the project KII-06-H 26-9/18.12.2018 (2018–2021) “Investigation of DNA markers associated with production in sheep breeds reared in Bulgaria” financed by NSF – the Ministry of Education and Science, Republic of Bulgaria.

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ПОЛІМОРФІЗМ ГЕНА *FABP3* ТА ЙОГО ВПЛИВ НА РОЗМІР ПОСЛІДУ ТА МОЛОЧНУ ПРОДУКТИВНІСТЬ ШТУЧНОЇ ПОПУЛЯЦІЇ БОЛГАРСЬКОЇ МОЛОЧНОЇ ВІВЦІ

**Мета.** Метою роботи було вивчення поліморфізму гена *FABP3* (який відповідає за біл, 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’язано з 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.