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ASSOCIATION OF FABP3 GENE POLYMORPHISM WITH MILK PRODUCTION IN EWES FROM THE BULGARIAN DAIRY SYNTHETIC POPULATION

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Abstract

The purpose of the research is to explore the relationship of the polymorphism of the FABP3 gene with milk yield per standard 120-day milking period (TMM120) in ewes from the Bulgarian Dairy Synthetic population from the herd of the Agricultural Institute-Shumen. In the experiment were involved 111 ewes of different lactations. Ewes were selected by birth type (single, twins, triplets) and had 343 milk yield records for a standard 120 days milking period. Two alleles and two genotypes were identified in the studied animals in exon 2 of the FABP3 gene (SNP3) by the PCR-RFLP technique with BseDI endonuclease. The association of the FABP3 gene polymorphism with the milk productivity of sheep was investigated by the one-way analysis of variance ANOVA model. In this study of BDSP ewes, the presence of a homozygous GG genotype at SNP3 of FABP3 resulted in increased milk yield in 2nd lactation ewes and in co-twin ewes.

Key words*: birth type, FABP3 gene, milk yield, parity, sheep.*

INTRODUCTION

Biodiversity in animal species playing a role in the food security of the population is related to the variation of genes, traits and breeds, and is the result of millennial adaptation to the environment and domestication. Genetic diversity provides greater adaptability and ability to survive environmental changes. One of the first domesticated animals was sheep. They are grown in extensive or intensive production systems under diverse climatic conditions, facing challenges such as different diseases and environmental conditions (Kijas et al., 2012). Adaptation to highly differentiated geographical and climatic conditions and specialization for different productivity have led to the creation of a huge number of breeds with significant phenotypic diversity (Gutiérrez-Gil et al., 2014) and it is of great interest to study the diversity of genes related to productive traits in breeds from different directions and geographical-climatic regions.

More than 30 breeds of sheep are bred in Bulgaria, and regardless of whether they are for specific objectives or local breeds, almost all of them are milked because the production of sheep's milk is of particular importance due to its use for the preparation of traditional products such as yogurt, white brine cheese, yellow cheese and others. Sheep milk is often produced from breeds that have low to medium yields and only a few sheep breeds have been created specifically for milk production among which is the Bulgarian Dairy Synthetic population (BDSP) (Figure 1) (Stancheva et al., 2022).

The components of sheep milk are synthesized in mammary epithelial cells and originate from blood plasma (Kulig et al., 2013). The lipids in milk are produced by fatty acids that bind to specific proteins called fatty acid-binding proteins (FABPs). Intracellular fatty acid-binding proteins (FABPs) are cytoplasmic proteins 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). They are thought to affect various cellular processes, in particular lipid metabolism. Heart-type fatty acid-binding protein (H-FABP, FABP3), whose molecular weight is 15 kD, is present in many tissues, especially those with a high demand for fatty acids, such as cardiac muscle, skeletal muscle, and the mammary gland during lactation (Calvo et al., 2002; Lanier and Corl, 2015).

The FABP protein family members are intracellular lipid-binding proteins with low molecular weight and high capacity for binding long-chain fatty acids. Currently 12 tissue-specific cytoplasmic FABPs (FABP1-FABP12) have been established in vertebrates so far that are differentially expressed in tissues, but not all FABP gene members occur in the same species (Chmurzynska, 2006; Wang et al., 2019). They are small in size - they include from 126 to 134 amino acids (Lang et al., 2017) and are found in all animal species (Cho et al., 2011; Wang et al., 2015; Wang et al., 2016; El-Mansy et al., 2019; Al-Janabi, 2019; Ye et al., 2022). These proteins regulate the fatty acid content of cells and thus influence various cellular processes such as lipid metabolism, cell growth and proliferation (Kulig et al., 2013).

The sheep FABP3 gene has been mapped to chromosome 2 (Calvo et al. 2002).

Figure 1. Ewe from BDSP with the offspring (Own source)

The influence of the gene has been studied by various authors, who demonstrate the influence of the different genotypes of the gene on the metabolism of fatty acids in both muscle and milk and, as a final result, affects the milk fat content and the marbling of the meat (Calvo et al., 2004; Aurora et al., 2014; Öner et al. 2014; Kowalewska-Łuczak et al., 2017).

The purpose of the research is to explore the relationship of the polymorphism of the FABP3 gene with milk yield per standard 120-day milking period (TMM120) in ewes from the Bulgarian Dairy Synthetic population from the herd of the Agricultural Institute - Shumen.

MATERIALS AND METHODS

The object of the research are ewes from the Bulgarian Dairy Synthetic population, bred at the Agricultural Institute - Shumen. Data on the establishment and genealogy of the herd are indicated in our previous studies (Stancheva, 2003; Stancheva et al., 2014; 2016; 2017; 2023). The sheep are reared on barn and pasture under a semi-intensive system. Milking is mechanized and takes place twice, after the lambs are weaned. The suckling period is in the range of 55-60 days, and the duration of the milking period is within 150-180 days.

In the experiment participated one hundred and eleven ewes on the 1st to the 4th lactation. Ewes were selected by birth type (singleton, twins, triplets) and had 343 milk yield records for a standard 120-day milking period. Milk yield data were obtained by measuring the amount of milk in liters milked during the lactation period of the animals according to the AC method specified in the nomenclature of the International Animal Control Committee (ICAR). Milk yield for a standard 120-day milking period (TMM120) is the average daily milk yield for a milking period multiplied by 120 days (TMM120 = ADMYmilking period * 120). TMM120 is calculated only for ewes with a milking period of not less than 120 days.

The main experimental part of the DNA analysis was conducted in the Laboratory of Genetics at the Faculty of Agronomy part of the University of Forestry, Sofia, Bulgaria, according to the methodology described in another our study for BDSP sheep from the same flock (Stancheva et al., 2023).

The association of the FABP3 gene polymerphism with milk productivity depending on the parity and type of birth of the ewes was determined using the one-way analysis of variance ANOVA model.

RESULTS AND DISCUSSIONS

As described in a previous study a 222 bp fragments from exon 2 of the sheep FABP3 gene were amplified using the PCR method. The PCR products that were produced after the amplification were cut with enzyme *BseDI*. After restriction analysis of the samples, two alleles were detected in SNP3 of FABP3. The mutant allele G was presented with three different fragments - 143, 43 and 36 bp fragments. The wild allele A had two fragments -186 and 36 bp. The frequencies for alleles G and A were 0.14 and 0.86, respectively (Table 1). Two genotypes were detected. The homozygous genotype GG was with frequency 0.73 and the heterozygous genotype AG was with frequency 0.27 (Figure 2). The homozygous genotype AA in the animals of this herd of the Agricultural Institute - Shumen was not detected.

Table 1. Allele and genotype frequencies of SNP3 of FABP3 gene

Locus	n	Allele frequency		Genotype frequency			Heterozygosity		Fis	χ^2	p
		G	A	GG	AG	AA	Ho	He			
FABP3	111	0.86	0.14	0.73	0.27	0.00	0.272	0.240	-0.133	3.62	0.05

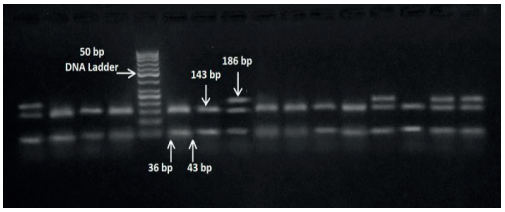

Figure 2. Restriction fragments of FABP3 gene visualized on 2.5% agarose gel under UV light

The established frequency of allele A in the studied breed has a lower value compared to studied foreign breeds where the frequency varies between 0.26-0.46 (Calvo et al., 2002; 2004; Öner et al., 2014), but also compared to the other studied Bulgarian breeds (Dimitrova et al., 2020). Another Bulgarian sheep breed, studied for genetic diversity according to SNP3 of the FABP3 gene and showed the presence of these two genotypes, is the Black-headed Pleven breed, which is close in terms of productivity to the studied Bulgarian Dairy Synthetic population (Bozhilova-Sakova, Dimitrova, unpublished results).

Milk productivity for ewes from the Bulgarian Dairy Synthetic population is determined for a 120-day standard milking period. Table 2 shows the total average milk yield values for a 120-day standard milking period (TMM120) of the studied animals, as well as TMM120 depending on the consecutive lactation and the type of birth of the sheep. Regarding the established milk yield for a 120-day standard milking period (115.205 l), the studied sheep exceeded the minimum selection limit for the Elite class of the population, which is 105 l. In a similar study, Dimitrova et al. (2021) found a lower value for the milk productivity (94.356 l) in sheep of the same breed raised at the Institute of Animal Husbandry - Kostinbrod.

Table 2. Overall mean and analysis of variance for a milk yield per standard 120-day milking period (litters)

Variable	Milk yield per standart 120-day milking period (TMM^{120}) (l)			*P-value*
	n	*Average*	*SD*	
TMM^{120}, total	343	115.205	26.55	
TMM^{120} by parity				
1st lactation	102	122.378	27.78	***0.0084**
2nd lactation	105	110.201	26.71	
3rd lactation	90	113.693	24.08	
4th lactation	46	113.678	25.47	
TMM^{120} by type of sheep birth				
Singles	135	114.761	28.21	0.1209
Twins	184	116.850	25.50	
Triplets	24	105.092	23.35	

*P≤0.01

Depending on the succession of lactation, the milk yield for a 120-day standard milking period is the highest, and above the general average, for 1st lactation of ewes (122.378 l) and the lowest for 2nd lactation of ewes (110.201 l). On the 3rd and 4th lactations, the values of TMM120 were equalized. Analysis of variance reported a significant effect of the factor consecutive lactation on milk yield for a 120-day standard milking period (P≤0.01).

The milk yield results for a 120-day standard milking period, according to the type of birth of the ewes, showed a certain superiority of animals born as twins (116.850 l) compared to those born as singletons and triplets (114.761 and 105.092 l), but the differences found were without statistical significance.

The milk productivity for a 120-day standard milking period (total, by consecutive lactation and type of birth) of the determined genotypes was presented in Table 3. In our previous study, we found that carriers of the homozygous genotype GG were 72.72% of the studied sheep and showed a tendency for larger litter size compared to ewes with heterozygous AG genotype (Stancheva et al., 2023).

Table 3. Association of FABP3 gene polymorphism with milk yield for a standard 120-day milking period (litters)

Variable	Genotype AG			Genotype GG			*P-value*
	n	*Average*	*SD*	n	*Average*	*SD*	
TMM^{120}. total	89	113.154	23.28	254	115.924	27.62	0.397907
TMM^{120} by parity							
1st lactation	29	121.143	21.62	73	122.869	29.99	3.936143
2nd lactation	28	100.913*	20.12	77	113.579*	28.09	***0.030993**
3rd lactation	25	115.177	24.31	65	113.123	24.16	3.949321
4th lactation	7	121.793	21.61	39	112.221	26.08	4.061706
TMM^{120} by type of sheep birth							
Singles	23	120.046	26.06	112	113.676	28.62	0.325841
Twins	59	110.367*	22.72	125	119.910*	26.24	***0.017403**
Triplets	7	114.000	14.31	17	101.424	25.64	0.23848

*P≤0.05

In the present study, carriers of the homozygous GG genotype demonstrated the potential for higher milk yield per 120-day standard milking period (115.924 l) compared to animals with the heterozygous genotype AG (113.154 l), and the differences found were not statistically proven. In sheep of the same breed from the herd of the Institute of Animal Husbandry - Kostinbrod, Dimitrova et al., (2021) found that the presence of a homozygous GG genotype of the FABP3 gene led to a proven higher milk productivity.

There was no clearly expressed tendency for higher milk yield for the 120-day standard milking period of the animals with homozygous genotype GG, depending on the consecutive lactation and the type of birth of the sheep. Analysis of variance reported a significant effect of the GG genotype on milk yield over a 120-day standard milking period in ewes of the 2nd lactation and in twin-born animals (P≤0.05).

CONCLUSIONS

The total milk productivity of the studied sheep for a 120-day standard milking period (115.205 l) exceeded the minimum threshold limit for the Elite class of the population. It is the highest in ewes of the 1st lactation and in animals born as twins.

A reliable effect of the factor "consecutive lactation" on milk yield over a 120-day standard milking period was established.

The carriers of the homozygous genotype GG in SNP3 of the FABP3 gene demonstrate the potential for higher milk yield over a 120-day standard milking period compared to animals with the heterozygous genotype AG.

A reliable effect of the GG genotype on milk yield for a 120-day standard milking period was established in ewes on second lactation and in animals born as twins.

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