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ASSOCIATION OF *FABP3* GENE POLYMORPHISM WITH LITTER SIZE IN EWES FROM THE BULGARIAN DAIRY SYNTHETIC POPULATION

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Abstract

The Bulgarian Dairy Synthetic Population (BDSP) is the most numerous breed in Bulgaria. FABP3 gene plays a crucial role in hormone action and cellular functions. The aim of the study is to investigate the relationship of FABP3 gene polymorphism with litter size in ewes of BDSP. This experiment involved 110 ewes from the herd of the Agricultural Institute-Shumen. Ewes were selected by birth type (singles, twins, triplets) and with records of the number of lambs born from a minimum of two consecutive lambing. The average number lambing of ewe is 3.94. 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 FABP3 gene polymorphism with total litter size and litter size depending on the parity and type of birth of ewes was studied by the one-way analysis model of variance ANOVA. A certain superiority was observed in the examined traits of the animals born as twins and triplets, but no significant differences were found between the individual groups.

Key words*: birth type, FABP3 gene, litter size, sheep.*

INTRODUCTION

FABPs are members of a family of conserved intracellular lipid-binding proteins with low molecular weight and high binding capacity for long-chain fatty acids, and 9 tissue-specific cytoplasmic FABPs (FABP1-FABP9) have been identified so far, and they are differentially expressed in different tissues (Chmurzynska, 2006). These small intracellular proteins having a molecular size of 14 to 16 kDa with 126 to 134 amino acids (Lang et al., 2017) and they 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). FABPs regulate intracellular levels of fatty acids and thereby control various cellular processes and lipid metabolism, cell growth and proliferation (Kulig et al., 2013).

The FABP3 protein is involved in the transport and exchange of fatty acids from the cell membrane to intracellular sites for fatty acid utilization (Veerkamp et al., 1995) and is predominantly expressed in tissues with a high demand for fatty acids such as cardiac and skeletal muscles, mammary gland during of lactation, liver or adipose tissues (Calvo et al, 2004; Lanier and Corl, 2015). Among the FABPs, FABP3, also known as Heart FABP (H-FABP) because it is mainly expressed in cardiac muscle and was originally isolated there (Gerbens et al., 1999).

The fatty acid binding protein 3 (*FABP3*) gene is an important candidate-gene for both the quality of the meat and the properties of dairy products such as the production of cheese due to its possible effects on the content of dairy fat (Calvo et al, 2004). The genetic variants of *FABP3* have been reported to affect the content of intramuscular fat in both sheep and pigs, and in pigs is considered to be a candidate-gene for fat characteristics (Gerbens et al., 1999; Uemoto et al., 2007). In the sheep, the *FABP3* gene is mapped in the distal part of Chromosome 2 of *Ovis aries* genome. Sheep

gene FABP3 and its chromosomal location were established in 2002 (Calvo et al., 2002) and as a result 13 SNPs, one CTC insertion/deletion and a variable polyA tract have been found. Two of the established SNPS, located in exon 2 and intron 13, respectively, have been studied for associations and found that heterozygous genotypes for the two SNPS are related to the milk fat content (Calvo et al., 2002; Oner et al., 2014).

Studies in pigs have shown that the *FABP3* gene is associated with carcass fat content, intramuscular fat and meat quality, and its main functions are to regulate fatty acid uptake and intracellular transport (Chmurzyńska, 2006; Hong et al., 2015). The *FABP3* gene can be used as a genetic marker for increased intramuscular fat content (Chen et al., 2014; Gondim et al., 2019). Adipose tissue, in turn, often affects reproductive traits, as it plays an endocrine role influencing the metabolism of sex hormones (Pinto, 2014).

The reproductive performance is an indicator of reproductive efficiency and degree of genetic progress in both crossbreeding and selection programs (Nuraddis et al., 2011). Selection using a gene marker complements traditional methods, allowing selection of animals with higher accuracy and at an earlier age. Identifying candidate genes responsible for phenotypic variation is challenging because they are usually controlled by many genes and influenced by environmental factors (Andersson, 2001; Al-Janabi, 2019).

Prolificacy is the main economically significant trait for sheep of all breeds, and according to some authors, the number of lambs born per lambing was more important than the profit generated from the realized lambs (Petrović, 2012). Notter et al. (2000) indicated that fertility also determined the biological performance of sheep.

Sheep from the Bulgarian Dairy Synthetic Population are the most numerous, commercial and adaptable breed in Bulgaria. The animals have the potential for high milk yield - from 150 to 200 l (for the milking period) and good fecundity - 150 lambs from 100 ewes. Regarding fertility, the goal of selection is to increase the number of ewes having mainly 2 lambs per birth (Stancheva et al., 2014). It is known that the improvement of economically important traits by traditionally known selection methods will take a long period of time. Knowledge of the genetic background of sheep under selection control is of paramount importance to increase animal productivity and production efficiency. Therefore, it is necessary to accumulate sufficient information about their genetic diversity and the relationship of established polymorphic variants with economically useful traits. This is extremely important for commercial breeds that are grown in very different production systems and have difficulty expressing their genetic potential for high productivity. In Bulgaria, studies to identify a genetic polymorphism of the FABP3 gene in sheep of different breeds are limited, and an association of the established polymorphism of FABP3 with the fertility of sheep was performed in only one of them (Dimitrova et al., 2021).

The aim of the study is to investigate the relationship of the FABP3 gene polymorphism with the litter size in ewes from the Bulgarian Dairy Synthetic Population.

MATERIALS AND METHODS

Subject of the study were ewes from the Bulgarian Dairy Synthetic Population, raised at the Agricultural Institute - Shumen (Figure 1). The flock was created according to a kind of modified scheme, as a genealogical structure was formed and built already at the stage of the applied crossing schemes (Stancheva, 2003; Stancheva et al., 2014; 2016; 2017). For more than 30 years, “interlinear breeding” has been carried out with rams of our own production, applying homogeneous selection combined with moderate inbreeding. The sheep were divided into 3 flocks and were raised in a semi-intensive system. Young animals were kept separately until they reached 18 months and entered the main flock. Their feed was our own production. The breeding process took place as standard - once a year, in the months of June - July. Sheep were artificially inseminated according to an individual plan at random at 18 months after the formation of the flocks. Lambing was usually performed from the second half of November and ends by the middle of January.

A total of 110 ewes of different ages were included in the study. Animals were selected by birth type (singles, twins, triplets), with records of the number of lambs born from a minimum of two consecutive litters. A total of 433 records of the number of lambs born from a mother ewe were analysed, and the average number of lambs from the studied sample was 3.94.

Figure 1. Ewes from the herd of Bulgarian Dairy Synthetic Population in the Agricultural Institute - Shumen

The experimental work on DNA analysis was carried out in the Laboratory of Genetics at the Faculty of Agronomy of the Forestry University, Sofia, Bulgaria. Peripheral blood samples were stored in tubes containing EDTA at -20°C until the DNA extraction process. DNA was extracted by manual commercial kit for DNA purification according to the instruction (QIAamp DNA Blood Mini Kit Qiagen).

The polymerase chain reaction amplifications were performed in total volumes of 10 µl, containing 4 µl DNA template, 0.2 µl sterile H_2O, 0.4 µl of each primer and 5 µl of 2 × (1.5 mM $MgCl_2$) MyTaq TM HS Red Mix 2x (Bioline, UK). The primer set used was suggested by Calvo et al. (2004):

F: 5′-GGTTTTGCTACCAGGCAGGT-3′ and

R: 5′-TTCCCTATTCCCCTTCAGGG-3’.

PCR amplifications were effectuated by thermal cycler QB-96 (Quanta Biotech) under the next conditions: primary denaturation at 94ºC for 2 min, followed by 30 cycles of denaturation at 94ºC for 1 min, annealing at 56ºC for 30 s, elongation at 72ºC for 1 min, and final extension at 72ºC for 10 min.

All animals were genotyped using RFLP analysis. The amplification products of the *FABP3* gene fragment (exon 2 - 222 bp) were processed separately in 10 µl final volume, containing 6 µl PCR product, 2.5 µl dd H_2O, 10 U/µl restriction enzyme *BseDI* (Thermo, US) and 1µl enzyme buffer. The digestion reactions were fulfilled at 60°C for 3 h in thermal block. The fragment sizes were identified using Ready-to-Use DNA Ladder, 50 bp (Thermo, US) on 2,5 % agarose (Bioline) gel and stained by RedGel Nucleic Acid Stain (Bioline, UK). The PCR products and restriction fragments were visualized under UV light.

The allelic and the genotypic frequencies of *FABP3* gene were estimated using simple gene counting method (Falconer and Mackay, 1996). The association of *FABP3* gene polymorphism with total litter size and litter size depending on the parity and type of birth of ewes was established using the one-way analysis model of variance ANOVA.

RESULTS AND DISCUSSIONS

A 222 bp fragment from exon 2 of *FABP3* locus of sheep was amplified using the PCR technique from each of 110 ewes. The obtained PCR products were cut with restriction enzyme *BseDI*. Two alleles - mutant *G* (with 143, 43 and 36 bp fragments) and wild *A* (with two fragments -186 and 36 bp) were detected (Table 1). In the studied Bulgarian Dairy Synthetic Population ewes from flock of the Agricultural Institute - Shumen were identified both possible alleles mutant *G* and wild *A* with frequency 0.86 and 0.14, respectively. Two different genotypes were identified in SNP3 of fatty acid-binding protein 3 gene - homozygous genotype *GG* with frequency 0.73 and heterozygous genotype *AG* with frequency 0.27. Observed heterozygosity (H_o) was 0.272 and expected heterozygosity (H_e) was 0.240. This flock was found to be in Hardy-Weinberg equilibrium.

In our previous study with 30 ewes from the same herd, the allelic frequency was similar as two alleles and two genotypes were found with frequencies of 0.67 for *GG* and 0.33 for *AG* (Dimitrova et al., 2022). Earlier, Dimitrova et al. (2021) also found the presence of two alleles (*A* and *G*) and two genotypes (*AG* with a

frequency of 0.30 and *GG* with a frequency of 0.70) in BDSP sheep reared in Institute of Animal Science - Kostinbrod. In another study, this region of the *FABP3* gene was examined in Bulgarian sheep from three merino and two local breeds (Dimitrova et al., 2020). Two alleles were found with the frequency of allele *G* ranging from 0.77 to 0.87, and allele *A* - from 0.13 to 0.23. All three possible genotypes were identified in all five breeds with the frequency of *GG* genotype - from 0.57 to 0.80, of AG genotype - from 0.13 to 0.40 and of AA genotype - from 0.03 to 0.07. Oner et al. (2014) also identified all three possible genotypes of the FABP3 gene in Kıvırcık sheep bred in three different provinces of Turkey. The authors found genetic diversity in the same region of the *FABP3* gene - SNP3, with the frequency of allele *A* being 0.42 and allele *G* - 0.58. In contrast to the present study, homozygous genotype AA was observed in this Turkish breed with a frequency of 0.30, while the other two genotypes - *GG* (0.46) and *AG* (0.24) had a lower frequency than found in our study.

Our obtained mean litter size value for the Bulgarian Dairy Synthetic Population ewes from the studied flock was 1.59 with trait-specific phenotypic variation (37.73%) (Table 2). In a similar study, Dimitrova et al. (2021) found a lower value for litter size (1.26) for ewes from the same population bred at Institute of Animal Science - Kostinbrod. On another scale, the size of the litter varies from 1.46 pcs. lambs born in the 2nd lambing to 1.66 number of lambs born in the 5th lambing, and according to the type of birth of the sheep, there is a certain superiority of the animals born as twins and triplets. In the analysis of variance, no significant differences were found between the individual groups.

The results for the litter size of the established genotypes are presented in Table 3. Carriers of the homozygous genotype *GG* were 72.72% of the animals studied. Their litter size was 0.45 higher than that of ewes with the heterozygous *AG* genotype, but the differences were not statistically significant. In contrast to us, Dimitrova et al. (2021) found a proven higher litter size in sheep of the same population with a heterozygous genotype of the *FABP3* gene, in the flock of Institute of Animal Science - Kostinbrod. The results for the litter size by successive weaning and the type of birth of the ewes for the two genotypes did not outline a clear trend of superiority of the animals with the homozygous genotype *GG*. The established differences between the individual groups again have no statistical significance.

CONCLUSIONS

The results obtained in this study indicate that fatty acid binding protein 3 in sheep is a polymorphic gene. Genetic diversity was found in SNP3 (the exon 2) of the *FABP3* gene in the 110 animals studied of Bulgarian Dairy Synthetic Population - two alleles (wild *A* and mutant *G*) and two genotypes (homozygous *GG* and heterozygous *AG*). The *G* allele and the homozygous *GG* genotype show a higher frequency. The mean litter size for ewes from the study flock was 1.59, which ranged from 1.46 at 2nd lambing to 1.66 at 5th lambing. According to the type of birth of the sheep, there is a tendency for superiority of those born as twins and triplets. Carriers of the homozygous genotype *GG* show a tendency for a higher litter size compared to ewes with the heterozygous genotype *AG*.

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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	110	0.86	0.14	0.73	0.27	0.00	0.272	0.240	-0.133	3.62	0.05

Table 2. Overall mean for litter size

Variable	n	Average	C.V. %	P-value
Total litter size	433	1.59	37.73	
Liter size by parity				
1-st lambing	110	1.61	38.71	0.268
2-nd lambing	110	1.46	28.77	
3-rd lambing	95	1.65	42.06	
4-th lambing	70	1.63	49.77	
5-th lambing	32	1.66	29.74	
6-th lambing	16	1.56	26.25	
Liter size by type of sheep birth				
Singles	171	1.51	33.36	0.162
Twins	215	1.63	41.23	
Triplets	47	1.64	36.63	

Note. *denotes a statistically significant difference.

Table 3. Association of FABP3 gene polymorphism with litter size

Variable	Genotype *AG*			Genotype *GG*			P-value
	n	Average	C.V. %	n	Average	C.V. %	
Total liter size	119	1.55	33.39	314	1.60	39.44	0.505
Liter size by parity							
1-st lambing	30	1.60	31.72	80	1.61	41.76	0.926
2-nd lambing	30	1.37	24.02	80	1.50	30.38	0.247
3-rd lambing	26	1.62	48.61	69	1.67	40.20	0.733
4-th lambing	18	1.61	36.93	52	1.63	55.02	0.904
5-th lambing	10	1.60	26.67	22	1.68	32.25	0.701
6-th lambing	5	1.80	20.00	11	1.45	27.27	0.223
Liter size by type of sheep birth							
Singles	54	1.52	32.98	117	1.51	33.82	0.952
Twins	44	1.61	38.21	171	1.63	42.23	0.869
Triplets	21	1.52	26.19	26	1.77	42.46	0.166

Note. *denotes a statistically significant difference.

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