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Effect of genotypic variation in the FABP3 gene on milk productivity and fertility in Awassi ewes

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Abstract

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A small family of low-molecular-weight intracellular fatty acid-binding proteins (FABPs), influence the transport and metabolism of long-chain fatty acids in the cell, as among them is fatty acid-binding protein 3 (FABP3). The aim of the study was to investigate the effect of genotypic variation in SNP3 of the FABP3 gene on milk productivity and fertility in Awassi sheep. The study included 61 ewes of different ages from a private herd in the village of Sredkovets, Shumen district, Bulgaria. Animal genotypes were established using PCR-RFLP analysis with application of restriction enzyme *BseDI* and subsequent gel electrophoresis. The presence of two alleles was found – the wild allele A (with fragments – 36 bp and 186 bp) and the mutant allele G (with fragments of 36 bp, 43 bp and 143 bp) with frequencies of 0.91 and 0.09, respectively, as well as availability of two genotypes – the homozygous wild GG and the heterozygous AG with frequencies of 0.84 and 0.16, respectively. No individuals with the homozygous mutant genotype AA were detected. A proven higher milk productivity was found in the ewes carriers of the homozygous genotype GG (126,989 l) compared to those with the heterozygous genotype AG (122,141 l). No significant differences in litter size were found between carriers of the two genotypes.

Keywords: Awassi sheep; FABP3 gene; genotype variation; milk yield; litter size

Introduction

Climatic changes on a global scale lead to the need to look for breeds that are more resistant to temperature fluctuations in our country as well. The Awassi is the most numerous and widespread sheep breed in southwest Asia, having been raised in the area for at least 5,000 years. Individual herds differ in milk yield, size and wool quality (Epstein, 1985). These fat-tailed sheep adapt to extreme conditions thanks to their physiological ability to regulate their thermal balance during the seasons of the year at different temperatures and humidity. Awassi sheep are disease and parasite resistant, resilient to large grazing transitions, have a strong herd instinct and resistance to fluctuations in husbandry (Galal et al., 2008; Gürsoy, 2011; Jawasreh et al., 2022). Awassi sheep possess huge genetic diversity and fertility within up to 120% (Üstüner & Oğan, 2013; İnal et al., 2021) and high potential for milk production (Epstein, 1985; Meydan et al., 2024). According to studies in different countries, the milk yield of Awassi sheep varies from 73 kg to 506 kg (Galal et al., 2008; Al-Samarai and Al-Anbari, 2009; Gootwine, 2011; Rashaydehet al., 2020; Meydan et al., 2024), which the au-

thors attribute to the influence of various factors – level of selection, duration of lactation, climatic influences, parity, milking frequency, production year and not lastly genetic diversity (Ali et al., 2020; Meydan et al., 2024). Research shows that Awassi milk fat has a higher omega-3 content, a lower n6/n3 ratio and a relatively lower hypocholesterolemia index than other sheep breeds (Ayadi et al., 2024), i.e. the breed has a significant impact on milk and meat quality, especially on essential fatty acids as the Awassi has healthier fatty acid profiles and correspondingly higher quality meat and milk compared to other breeds (Merzah et al., 2023).

The synthesis of lipids is carried out by specific proteins called fatty acid-binding proteins (FABP). Intracellular fatty acid-binding proteins (FABP) are essential for the transport and metabolism of long-chain fatty acids in the cell by accelerating their absorption and delivering them to cellular organelles (Lanier and Corl, 2015). Intracellular lipid chaperones (FABPs) coordinate lipid trafficking and signaling in cells, with some isoforms largely associated with metabolic and inflammatory pathways. This small family of low molecular weight cytoplasmic proteins are important in the transport and exchange of fatty acids from the plasma membrane to sites of oxidation, triacylglycerol and phospholipid synthesis (Chmurzynska, 2006; Jurie et al., 2007). FABPs play a critical role in hormone action and cellular functions in adipocytes and other cells (Furuhashi and Hotamisligil, 2008). There are 9 different types of FABPs, that differ from each other depending on the type of tissue from which they were originally isolated, among them is the one isolated from the heart – H-FABP or FABP3. Fatty acid-binding protein 3 (FABP3) is involved in signal transduction pathways and in the uptake and utilization of long-chain fatty acids (Chmurzynska, 2006; Bai et al., 2013). Heart-type fatty acid binding protein (H-FABP) is present in many tissues, especially those with a high demand for fatty acids, such as heart muscle, skeletal muscle, and the mammary gland during lactation (Calvo et al. 2002; Jurie et al., 2007; Lanier, Corl, 2015). The sheep FABP3 gene is located on chromosome 2 and is composed of five exons separated by introns (Calvo et al. 2002).

Results established by PCR-RFLP in the Iranian breeds Lori-Bakhtiari and Zel revealed in g.939A > G SNP (single nucleotide polymorphism) in exon 2 of FABP3 gene exhibiting three genotypes: AA, AG and GG. This polymorphism significantly affects blood triglyceride and cholesterol levels, as well as weaning weight (Javadi-Novashnagh et al., 2015).

Variation in FABP3 was studied in 50 Slovak Zošľachtená Valaška sheep in relation to its influence on milk composition (Kowalewska-Łuczak et al., 2017). SNP3 (G/A) and SNP13 (G/A) located in exon 2 and intron 3, respectively, were studied by PCR-RFLP method. The results of the statistical analysis of the polymorphism in SNP13 of the FABP3 gene showed that animals with the homozygous AA genotype had the highest milk fat, protein and solids content.

In Bulgaria, the Awassi breed was imported for the first time in 1977, and later imports were carried out periodically from Israel and Iraq (Boykovski et al., 2006, 2019). The animals adapted very well, and the obtained average milk yield of the first lactation was 343.3 L for a lactation and 191 L for a 200-day milking period. The breed was used in the creation of the Bulgarian Dairy Synthetic Population, since the quality of the milk of the Awassi breed does not differ from the milk obtained from other breeds of dairy sheep in our country. Currently, Awassi sheep are raised in private flocks.

SNP3 diversity of FABP3 has been studied in several breeds of sheep in Bulgaria – Bulgarian Dairy Synthetic Population, Ascanian, Caucasian, Karnobat, Cooper-Red Shumen and Karakachan (Dimitrova et al., 2020, 2021a, 2021b, 2022), but studies of polymorphism in genes such as FABP3 of animals of the Awassi breed not performed in our country.

The aim of the study was to investigate the effect of genotypic variation in SNP3 of the FABP3 gene on milk productivity and litter size in Awassi sheep.

Materials and Methods

Animals

The study included 61 Awassi ewes of different ages from a private herd owned by Osman Ismail Sali in the village of Sredkovets, Shumen district, Bulgaria (Fig. 1). Blood samples from each animal were collected from the jugular vein into tubes containing EDTA as an anticoagulant and stored at -20°C until DNA extraction.

Fig. 1. Awassi ewes from Osman Ismail Sali's private flock

One hundred and one records of milk productivity of ewes and one hundred and twenty four records of the number of lambs born per ewe were analysed. The data on the milk productivity and fertility of the sheep were provided by the „Dairy Sheep Breeding Association" (RAMO), Sofia, Bulgaria. Milk yield control was performed only for 1st and 2nd lactation ewes using the AC method specified in the nomenclature of the International Committee for Animal Control (ICAR) for a standard 120-day milking period (TMM120).

DNA extraction

In the Laboratory of Genetics at University of Forestry – Sofia was extracted genomic DNA from whole blood using a manual commercial kit for DNA purification (Illustra Blood GenomicPrep DNA Purification Kit, GE Healthcare, US). The DNA concentration was determined by spectrophotometer Biodrop. The quality of the harvested DNA was tested also on 1% agarose (Bioline, UK) gel prepared with TAE buffer (Jena Bioscience, Germany).

PCR amplification

The polymerase chain reaction amplifications were carried out 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 (by Calvo et al., 2004) was as follows:

F: 5′-GGTTTTGCTACCAGGCAGGT-3′ and

R: 5′-TTCCCTATTCCCCTTCAGGG-3'.

Amplification reactions were performed by thermal cycler VerityPro 96-Well (Applied Biosystems by Thermo Fisher Scientific) 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.

RFLP analysis

The genotypes of all tested animals from Awassi breed were determined using restriction fragment length polymorphism analysis (RFLP). All amplification products of the FABP3 gene fragment (from exon 2 – 222 bp) were digested 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 carried out 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 GelRed® Nucleic Acid Gel Stain (Biotium). The obtained PCR products and restriction fragments were visualized under UV light.

Statistical Analysis

The effect of the FABP3 gene polymorphism on milk yield and fertility in sheep was determined using a one-way analysis of variance ANOVA model.

Results and Discussion

When examining the selected group of animals from the Awassi breed, the presence of two alleles was found – the wild allele A (with fragments – 36 bp and 186 bp) and the mutant allele G (with fragments of 36 bp, 43 bp and 143 bp) with frequencies of 0.91 and 0.09, respectively, as well as two genotypes – the homozygous GG and the heterozygous AG with frequencies of 0.84 and 0.16, respectively. No individuals with homozygous genotype AA were found (Table 1; Figure 2), as in other dairy breed – Synthetic Bulgarian Dairy Population (Dimitrova et al., 2022).

The values of expected (He) and observed (Ho) heterozygosity almost coincide, which means that the studied herd has enough genetic diversity and based on the value of Fis (Table 1), which is close to zero, it can be argued that it is almost absent of inbreeding.

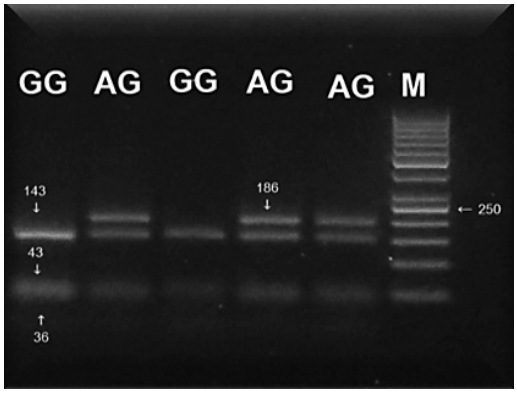

Fig. 2. Gel electrophoresis of the obtained restriction fragments. Genotype GG was presented with three bands; genotype AG was presented with four bands; M – DNA Ladder 50 bp.

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	61	0.91	0.09	0.84	0.16	0.00	0.163	0.164	0.06	1.02	0.31

A polymorphism in SNP3 of the FABP3 gene has also been found in sheep breeds of Spanish (Calvo et al. 2002, 2004), Turkish (Öner et al., 2014) and Bulgarian origin (Dimitrova et al., 2020; Dimitrova et al., 2021a; Dimitrova et al., 2022), in all of which a predominance of the G allele was found. The frequency of the A allele in the studied breeds in our country is lower than in the studied foreign breeds, where the frequency varies between 0.26–0.46 (Calvo et al., 2002, 2004; Öner et al., 2014).

The average values for milk productivity and litter size of the studied sheep are presented in Table 2. Due to the lack of modern scientific studies on the adaptation of the sheep of the Awassi breed to the climatic conditions and the farming systems applied in our country, as well as on the genetic variability of the main selection traits (milk productivity and fertility), we cannot make a comparison for the level of this two traits (126.317 l milk and 1.30 number of lambs born from ewe). Given the genetic potential of the Awassi breed for high milk yield, we can note that despite good care for the animals, the milk yield reached is below the breed's capabilities. The lack of data on the milk productivity of the ewes for the third and subsequent lactations does not give us a reason to draw definite conclusions about the realized level of productivity, bearing in mind the changes in milk yield caused by the age and parity (Bencini and Pulina, 1997). The average fertility achieved in the herd generally corresponds to the potential of the breed.

The results for the total milk productivity and litter size of the established genotypes show a reliable effect of the GG genotype on the milk yield for a 120-day milking period in the sheep of the studied herd ($P \leq 0.01$) (Table 3).

Two other studies in sheep from the Synthetic Bulgarian Dairy Population, in two different flocks, Dimitrova et al. (2021b) and Stancheva et al. (2024) also found that the presence of a homozygous GG genotype in SNP3 of the FABP3 gene leads to proven higher milk productivity.

Regarding fertility, Dimitrova et al. (2021b) reported that the presence of a heterozygous AG genotype in the same region of the FABP3 gene was associated with increased litter size in sheep from the Synthetic Bulgarian Dairy Population from the herd of the Institute of Animal Science – Kostinbrod, but Stancheva et al., (2023) in other flock of the same breed (of the Agricultural Institute – Shumen) did not establish statistically significant differences in the litter size of ewes carriers of the GG and AG genotypes.

In the future, it is necessary to expand the sample of this breed with animals from this and other herds and collect data on third and subsequent lactations to be used in the improvement process of this specialized milk breed.

Conclusion

In the present study, for the first time, the influence of FABP3 gene polymorphism in SNP3 on milk productivity and litter size in Awassi breed sheep in our country was investigated. A higher milk yield has been proven in the carriers of the homozygous genotype GG compared to those with the heterozygous genotype AG. No individuals of the breed

Table 2. Overall mean and analysis of variance for a milk yield per standart 120-day milking period and litter size

Traits	n	*Average*	*C.V.%*	*P-value*
TMM^{120} (l), total	101	126.317	42.77	
TMM^{120} (l) by parity				
1st lactation	61	126.230	33.06	0.87027
2nd lactation	40	126.449	58.78	
Litter size (lambs per ewes), total	124	1.30	21.21	*P-value*
Litter size by parity				
1st lambing	57	1.33	22.62	0.71161
2nd lambing	39	1.28	20.78	
3rd lambing	28	1.25	19.44	

Note. * denotes a statistically significant difference.

Table 3. Effect of the genotype of FABP3 gene on the milk yield for a standart 120-day milking period and litter size

Traits	Genotype AG			Genotype GG			*P-value*
	n	*Average*	*C.V.%*	n	*Average*	*C.V.%*	
TMM^{120}, total	14	122.141	25.44	87	126.989	42.59	0.00935*
Litter size, total	16	1.31	22.92	108	1.30	21.05	0.89588

*$P \leq 0.01$

with homozygous mutant genotype AA were found, and no significant differences in litter size between the carriers of the two genotypes.

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Conflict of interest

The authors declare that there is no conflict of interest.

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