Milk production in sheep – genetic basis and phenotype characteristics

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Abstract

Milk is a product with highly dynamic characteristics. Its composition varies according to the breed, lactation period, nutrition, health status of the animal, as well as the conditions of milking and storage. Investigation of genetic potential and productive traits of farm animals is essential for every producer. The phenotypic characteristics of sheep are the result of the complex interaction of a wide range of genetic and non-genetic factors. They are slightly influenced by traditional selection. Therefore, it is essential to study the genetic pattern of inheritance of these traits. In the last decades of the 20th century, several researchers have studied genetic variation in populations of different breeds of farm animals, using genetic markers of class I, such as blood types and polymorphisms of proteins in blood and milk. Modern methods of molecular biology make it possible to use class II genetic markers in research to identify genetic polymorphisms in the loci of molecular markers and associate them to certain phenotype characteristics, study the laws of organization and functioning of the genome, decipher the mechanisms for the realization of genetic information. The obtained data from these analyzes are successfully applied in the development of breeding programs in animal husbandry practice, to speed up the realization of genetic progress. Some of the main major candidate genes that have been studied related to milk productivity in sheep are: prolactin, α s1, α s2, β and κ -casein, α - and β-lactoglobulin, PIT-1.

Key words: milk production, sheep breeds, candidate genes, PCR-RFLP analysis, phenotype traits

Introduction

Sheep breeding is the second most important branch in agriculture, which meets the need for milk and dairy products, hence the consumer's interest is constantly growing. In many countries, this sector has significant potential for development and opening of job vacancies in small rural and suburban areas. The local breeds raised on such farms and preserved as a genetic information are a source of ecological raw materials for the production of traditional dairy products. Their distribution area and the successful marketing of the products can have a significant social impact on sustainable rural development. Indigenous breeds are raised in smaller herds, where maintenance selection is implemented. They often reduce the genetic diversity in breeds and increase the value of the inbreeding rate, which is associated with a decline in the health status and productivity of animals (Peter et al., 2007; Santos-Silva et al., 2008; Li et al., 2008; Ligda et al., 2009; Kusza et al., 2009).

Molecular markers and the use of DNA methods are an opportunity to determine the allelic variants of genes associated with the quantity and quality of milk productivity of animals. By identifying the polymorphic variations and their phenotypic expression, a database for managing the genetic realization of economically important traits can be accumulated (Arranź and Gutiérrez-Gil, 2012; Gras et al., 2016; Hofmannová et al., 2018).

Milk productivity in sheep is an indicator that can be assessed for each lactation period. Depending on the breed and individual qualities of the animals, this interval varies from 150 days to 270 days (Nenov et al., 1991). Modern milk breeds are characterized by high milk yield, while local (autochthonous) breeds have lower milk yield. A number of factors affect milk productivity: breed and individual characteristics of animals, age, lactation, nutrition and breeding, seasonality, health status (Nenov et al., 1991).

This review aims to trace and summarize scientific research in the field of sheep milk productivity. The review includes research related to the genetic basis of milk productivity and phenotypic characteristics of milk quality and quantity as well.

Genetic markers of for milk production and milk composition

The genes encoding the main hormones associated with lactation, as well as those responsible for the secretion of proteins in milk, are the main molecular markers that are being studied as candidate genes for milk productivity in sheep. The development of molecular DNA techniques has led to their use as routine research methods in countries with a high culture of animal breeding. PCR-RFLP is one of the most widely used methods for genotyping and establishing polymorphic variants of different genes. It was first described in 1974 (Maheswaran, 2004). The main advantage of this method is the easy identification of different genotypes due to cleavage with specific restriction enzymes. Arranz, J. J., and Gutiérrez-Gil, B. (2012) pointed out that thanks to the work of scientists on the Sheep HapMap project developed by the International Sheep Genomics Consortium (ISGC http://www.sheephapmap.org/), many polymorphic alleles have been identified in the genome of sheep. As part of this project, complete genome sequencing has been done and a database has been developed (http://www.livestock genomics. csiro.au/sheep/oar3.0.php), which is an important resource for sheep associations.

Worldwide, a large number of authors have studied various candidate genes associated with milk productivity in sheep prolactin, α s1-, α s2-, β - and κ -casein, α - and β - lactoglobulin, PIT-1 (Barillet et al., 2005; Cohen et al., 1996; Fischberg et al., 1994; Hristova, 2011; Gras et al., 2016).

When it is talked about the quality of milk, it refers to its ability to be processed into various dairy products, as well as obtaining a larger amount of product per liter of raw milk. Worldwide, the bulk of sheep's milk is used to make cheese. The high content of proteins and fats, as well as the total dry matter content of milk guarantees high quality dairy products. The application of genetic marker selection (MAS and Gene assisted selection – GAS) based on the detection of polymorphisms in genes related to milk productivity will help to develop adequate breeding strategies to increase milk yield during lactation and will improve the quality of milk, resp. of dairy products obtained during its processing.

Gras et al. (2016) studied the genetic polymorphism of three genes: prolactin (PRL), kcasein (CNS3) and β -lactoglobulin (LGB) and their influence on milk yield and composition in *the Teleorman Black Head sheep breed* (Romania). A positive relationship was found between polymorphism in the LGB and PRL loci and the observed parameters. In conclusion, it is stated that these candidate genes can be implemented as DNA markers in breeding programs.

Similar results were confirmed by Staiger et al. (2010), who also investigated the influence of these three genes on milk yield in *East Friesian dairy sheep* population from the Old Chatham Sheepherding Company, New York. Genotypes were determined by PCR amplification followed by digestion with HaeIII and RsaI for PRL and β -LG, respectively, and by PCR amplification for CSN3.

Prolactin is a polypeptide hormone with many functions. It is encoded by a single PRL gene, which was found in all vertebrates (Orford et al., 2010). The PRL gene is located on ovine chromosome 20 (Mateescu et al., 2010). It is secreted mainly by the anterior lobe of the pituitary gland, but also by the uterus, immune system cells, brain, mammary glands, prostate, skin and adipose tissue. Prolactin receptors (PRL-R) are present in almost all tissues. Its main biological function is to stimulate the production of breast milk by the mammary glands after birth.

Casein is a milk protein, and combines several types of proteins found in mammalian milk. It is the only protein in nature with nutritional function, not structural or metabolic. It coagulates under the influence of rennet enzymes but does not coagulate under the influence of high temperatures. It contains four types of proteins: α s1-, α s2-, β - and κ -casein. Casein proteins and their genetic variants have been reported as important factors related to lactation, milk protein content in milk, clotting time and cheese yield efficiency (Bozgo et al., 2020; Gras et al., 2016; Kalaydjiev et al., 2014; Othman et al., 2013).

Othman et al. (2013) determined the genetic polymorphism of α s1- and α s2-Casein genes in three native Egyptian sheep breeds: *Rahmani*, *Barki* and *Ossimi* by PCR-SSCP and PCR-RFLP analysis. They identified allele specificity in both loci, which are registered at GenBank, with the access numbers KF018339 (α s1- casein T allele) and KF018340 (α s1- casein C allele) and JX080380 (α s2- casein gene), respectively.

The importance of studying and preserving local sheep breeds, as the main source of milk, meat and wool for Albanian farmers was highlighted by Bozgo et al. (2020). The aim of their study was to detect the genetic polymorphism of exon 17 in α -casein gene and exon 4, in k-casein gene in three Albanian local sheep breeds: *Bardhoka, Ruda* and *Shkodrane*, by the use of PCR-SSCP and PCR-RFLP respectively. The sequence analysis showed a single nucleotide polymorphism (SNP) at position 137 (C > T) and at 168 (T > C) of exon 17 CSN1S1 and exon 4 CSN3, respectively.

Gencheva and Georgieva (2019) and Gencheva et al. (2020) determined the genetic polymorphism in exon III of the alpha S1-casein (CSN1S1) in sheep breeds reared in Bulgaria: Sofia sheep population (Elin-Pelin, SEPL), Copper- Red Shumen (CRSH), Local Karnobat (LKNB), Pleven Blackhead (PLBH), Stara Zagora (STZG) and Breznik sheep breeds (BRNK). The results of the association analysis indicated that CSN1S1 AC genotype was significantly associated (P < 0.05) with the highest percentages of the fat, protein, casein, solids-nonfat and total solids in SEPL ewes. The genotype CSN1S1 CC was associated with the highestnoncasein protein percentage, while the genotype AA was linked with the highest lactose percentage. The CSN1S1genotype did not show a significant effect (P >0.05) in the SEPL in relation to the renneting time. The authors conclude that, the established singlenucleotide polymorphism in the CSN1S1 locus could be used as a potential genetic marker for ovine milk composition traits, as well as for developing an effective conservation strategy for traditional sheep breeds in Bulgaria.

 β -lactoglobulin (LGB) is a globular protein that is present in the milk of many mammalian species including ruminants, such as cows and sheep. It represents approximately 75% of the albumin fraction encoded by the LGB gene. The main mutation and the best-studied one in the locus of this gene is the replacement of the amino acid Tyr20 with His in the polypeptide chain, which is easily determined by PCR-RFLP analysis (Arora et al., 2010; Elyasi et al., 2010). There are a large number of studies on the influence of genetic variation in the locus of this gene on the characteristics of milk and its coagulation properties in sheep. The conclusions presented by the authors are contradictory (Jyotsana et al., 2014; Kawecka, A. and Radko A., 2011; Selvaggi et al., 2014; Yang et al., 2012; Yousefi et al., 2013). Insome cases the results are not comparable due to differences in the size of the sample, breed, frequency of genotypes considered, and statistical models used for data analysis (Selvaggi et al.,

2015). This fact does not allow LGB to be clearly defined as a genetic marker and requires further research.

Gencheva (2019) investigated the single nucleotide polymorphism in exon II of the β -lactoglobulin gene in four Bulgarian sheep breeds: *Bulgarian Dairy Synthetic Population sheep* (BDSP), *Copper-red Shumen sheep* (CRSH), *Stara Zagora sheep* (STZG) and *Pleven Blackhead sheep* (PLBH). The results obtained from the study confirmed the presence of the SNP polymorphism in exon II of the β -lactoglobulin gene. Therefore, the genetic variability established in this polymorphic locus could be applied in further association studies with milk production traits in sheep.

The gene Pituitary-specific positive transcription factor 1 (PIT-1), also known as POU1F1, is a member of the POU transcription factors, which regulate mammalian growth. It is a specific factor responsible for pituitary gland development and it is associated with the expression of genes encoding growth hormone (GH; MIM 139250) and prolactin (PRL; MIM 176760). The POU family owes its name to the first 3 identified members are PIT1 and OCT1 (MIM 164175) in mammals and Unc-86 in Caenorhabditis elegans (Herr et al., 1989). PIT1 contains 2 protein domains, called POU-specific and POU-homeo, which are required for the binding of high affinity DNA to these genes. The locus of this gene is being studied as a candidate genetic marker for weight development and milk characteristics. Cohen et al. (1996) and Fischberg et al. (1994) state that a mutant Pit-1 may be aberrant in GH and Prl gene activation.

Sumantri et al. (2009) through PCR-RFLP and restriction endonuclease Hinf1 they studied polymorphism in locus Pit-1 and its association with some phenotypic characteristics of milk productivity and weight development in local sheep at the Jonggol Animal Science Teaching and Research Unit (JASTRU), Fact. Anim. Sci. Bogor. Agric. Univ. A total of 161 blood samples were collected from 3 local sheep breeds, namely *Garut* from Wanaraja, Garut from Margawati and lactating ewes from JASTRU farm in Bogor. It can be concluded that Pit-1 genotypes had no positive correlation between body weight and milk production. This result indicateds that the use of single locus Pit-1-Hinf1 in Pit-1 is less effective in studying of body weight and milk production in these three local sheep.

The genetic markers FABP3, SLC27A3 and ABCG2 were also being studied as major genes for sheep milk composition.

Intracellular lipid chaperones known as fatty acid-binding proteins (FABPs) play an important role in the transport and metabolism of fatty acids in the cell. FABP are a group of molecules that coordinate lipid responses in cells and are also strongly linked to. FABPs are abundantly expressed 14–15 kDa proteins that reversibly bind hydrophobic ligands, such as saturated and unsaturated long-chain fatty acids, eicosanoids and other lipids, with high affinity (Furuhashi & Hotamisligil, 2008). The transport of fatty acids is also supported by a group of FATP proteins (fatty acid transport proteins) which are encoded by the family of SLC27A genes (Kowalewska-Luczak et al., 2017).

Polymorphisms in the Fatty Acid Binding Protein 3 gene (FABP3) have been analyzed by many researchers on the Spanish (*Aragonesa, Awasi, Assaf, Manchega*), Turkish (*Kıvırcık*) and Slovak (*Zoslachtena Valaska*) sheep breeds (Calvo et al., 2002, 2004: Öner et al., 2014; Kowalewska-Łuczak et al., 2017). The author's teams report genetic diversity in the studied breeds, and the allele and genotype frequency was different in the studied breeds.

Kowalewska-Łuczak et al. (2017) declared that genetic differences in genes FABP3 and SLC27A3 might be related to the productivity and composition of sheep's milk. A herd of 50 Slovak sheep breed *Zoslachtena Valaska* (*Zošľachtená Valaška*, in Slovak) was studied. Polymorphism in the loci of both studied genes was found, as well as the influence of some of the alleles on phenotypic traits. The authors stated that because their findings did not show a good trend, more research with different breeds and numbers of animals should be done.

ABCG2 belongs to the family of transporters, which contains the ATP-binding domain and it is responsible for the transport of various cytostatic and xenobiotic drugs across the cell membrane (Hofmannová et al., 2018; Sarkadi et al., 2004). The ABCG2 gene is located on chromosome 6 of the genome of domestic sheep (*Ovis aries*). It has 20 exons separated by 19 introns and it is expressed in some tissues, including the mammary gland (Al-Mamun et al., 2015). This gene has been selected as a candidate gene influencing milk production traits in sheep based on its function by a number of researchers (Árnyasi et al., 2013; Gutiérrez-Gil et al., 2014; Hofmannová et al., 2018).

Hofmannová et al. (2018) investigated the ABCG2 locus on 1747 records from 387 dairy sheep of *the Lacaune breed* (n = 139) and *the East Friesian breed* (n = 248) in the Czech Republic and identified a mutation c.683-80_46del in the intron 5 region. The effect of this mutation on somatic cell count has been established. The mutation c.683-80_46del in intron 5 of the ABCG2 gene was shown to be a candidate gene for somatic cell count, however more research in other sheep breed populations is needed to confirm this potential association. Similar results were reported by Árnyasi et al. (2013).

In Bulgaria, four breeds were genotyped for ABSG2 locus: *Bulgarian Dairy Synthetic population, Askanian Merino, Caucasian Merino* and *Karnobat Merino sheep breeds*. The results showed polymorphism in ABSG2 locus in investigated sheep. The comparative analysis was performed using the statistical method ANOVA, but no positive trend was observed (Dimitrova et al., 2019; Bozhilova-Sakova et al., 2021).

In order to find QTL markers for milk productivity and milk composition in sheep chromosomes, some authors conducted a complete genome scan (Mateescu and Thonney, 2010; Gutierrez-Gil et al., 2009).

A wide study (Mateescu and Thonney, 2010) involved 188 animals backcrossed pedigree using dairy *East Friesian* rams and non-dairy *Dorset* ewes and analyzed polymorphism at the loci of ninety-nine microsatellite markers. Ovine chromosomes 2, 12, 18, 20 and 24 were identified to harbor putative QTL for different measures of milk production. The QTL mapped on *Ovis aries* chromosomes 2 (OAR2) and 20 (OAR20) were similar to QTL that has already been mapped in other studies, whereas QTL on OAR 12, 18 and 24 were unique to the backcross pedigree and have not been reported previously.

By means of genome-wise multi-marker regression analysis, Gutierrez-Gil et al. (2009) accomplished similar research in a commercial population of Spanish Churra sheep to identify chromosomal regions associated with phenotypic variation observed in milk production traits. Eleven half-sib families, including a total of 1213 ewes, were analysed following a daughter design. Significant QTL for milk protein percentage on chromosome 3 has been demonstrated. Eight other regions, localized on chromosomes 1, 2, 20, 23 and 25, showed eventual significant linkage with some of the analyzed traits. In the article, the authors emphasize the importance of these studies, as improving productivity in dairy sheep breeds allows the production of high quality cheese and increases the competitiveness of sheep breeding in the regions of the Mediterranean countries, which are less favorable.

Phenotypic characteristics of milk production in sheep

Milk is a product with highly dynamic characteristics. Its composition varies according to the breed, lactation period, nutrition, health status of the animal, as well as the conditions of milking and storage. Studies on the milk composition of *the Bulgarian Dairy Synthetic population* have existed since the creation of the breed (Petrova and Nedelchev, 2000; Raicheva et al., 2004 a, 2004 b; Boikovski et al., 2005).

Stancheva (2003) reported the mean values of the milk composition in sheep of the same breed, respectively for the first and second lactation as follows: fat 7.324% and 7.457%; protein 5.428% and 5.568%; dry matter 17.914% and 18.026%. The authors (Stancheva et al., 2011) determined the physicochemical composition of sheep at a different age, the traits and technological characteristics of pooled milk samples for the lactation period and obtain mean values for the milk composition that describe it as responding to the standard for raw sheep milk (fat 7.32%, protein 5.35%, solid non fat 10.63% and dry matter 17.94%).

Raicheva et al. (2004 a) determined the values of the milk composition for the day during the milking period in *Bulgarian Dairy Synthetic* population sheep at second lactation at the stage of developing the breed (dry matter 18.09–19.43%, solid non-fat 11.04–11.38%, fat 6.84–7.81% and protein 5.24–6.17%). While researching the milk yield parameters of early fertilized sheep, Raycheva and Ivanova (2011) discovered the following mean milk composition values: milk – 7.31%, proteins – 5.69%, dry matter – 18.58%, and solid non fat – 11.27%.

In examining pooled samples of milk in *Kara-kachanska breed* Ivanova (2011) found the following milk composition: dry matter of 19. 22%, protein 6.74%, and fat 7.28%.

Thomas et al. (2000) in crosses between *East Friesian and Lacaune* at first lactation with a 30-day sucking period, determined milk composition as follows: fat 5.46%–5.65% and protein 4.46%–4.68%, as the authors found an advantage of the *East Friesian* crosses in milk yield. There are many similar studies on the milk composition of *East Friesian* crosses (McKusick et al., 1999 a; McKusick et al., 1999 b; Thomas et al., 1999).

Gonzalo et al. (1994) studied the effect of various factors on the milk composition in *Churra* sheep, milked automatically. The authors found variation within the range of 5.99–8.20% for fat and 5.87–6.85% for proteins.

Cappio-Borlino et al. (1997) studied the milk composition in the dairy breed *Vale del Belice* at first, second and more lactations and reported a mean value of the fat at first lactation as 6.84% and for the second lactation as 6.90%.

Kalaydzhiev et al. (2021) determined a correlation between phenotypic characteristics of chemical composition, somatic cell count and rennet coagulation of sheep milk during different stages of lactation in three Bulgarian autochthonous sheep breed – *Local Stara Zagora sheep, newly developed breed* – *Bulgarian dairy synthetic population* (BDSP), and *Lacaune sheep breed*. From the data obtained about the chemical composition of sheep milk, it was established that *Bulgarian dairy synthetic population sheep breed* had the highest fat value – 9.50%, whereas for the other breeds the fat value was approximately 8.30%. Strong negative correlation between somatic cell count value and parameters of milk coagulation ability was also established – SSC:RCT (-0.170); SCC:K20 (-0.142) and SCC:A30 (-0.254).

The phenotypic characteristics of sheep are a result of the complex interaction of a wide range of genetic and non-genetic factors. In the literature available, there are a large number of studies on the parameters of selection traits in specialized sheep breeds and dairy sheep *Valle del Beliche*. Cappio-Borlino et al. (1997) reported an average milk yield at first and second lactation - 1.681 1 and 1.938 1, respectively in the dairy breed for 225-d standard lactation.

According to Fernandaz et al. (1997), the milking milk yield for the 120-d milking period of *Churra* dairy breed is 146.98 l. In sheep of the same breed, Othmane et al. (2002) obtained similar values for the 120-d milking milk yield depending on the sequence of the lactation (93 l for the first and 101 l for the second lactation).

When studying crossbreeds – *Eastfrisean* and *Lacaune* born in the same year, Thomas et al. (2000) reported milk yield of 88.3–104.0 l.

In *Slovak Lacaune* the standard milking period is 150 days. Oravcova (2007) evaluated the data for 7 years and registered variation of the milking milk yield within the range of 156–189,2 l.

Panayotov et al. (2018) obtained results for the milk yield of *Lacaune sheep* with an average value for 150-d milking period of 213.29 l. Such milk yield, according to the authors, the sheep of *Bulgarian dairy synthetic population* and *Pleven Blackface sheep* might achieve for approximately 200 days of milking. At the first control from the studies sheep the milk obtained was 2.279 l, while for some animals, the milk was above 3.000 l (max. 3.310 l).

The average daily milk yield of the dairy *Sar-da breed* for the milking period in sheep from the second to the fourth lactation was 0.623 kg at double milking (Nudda et al., 2002).

Legarra and Ugarte (2001) reported an average milk production of 127.12 l of the sheep from *Latksa breed*, milked twice and controlled according to the AC method.

In Bulgaria, in purebred local Stara Zagora dairy sheep controlled in 2009, Dzhorbineva et al. (2011) reported a variation of the 120-day milking milk yield from 111.3 l to 129.4 l.

When studying the lactation milk yield of the sheep from *Bulgarian Dairy Synthetic Population*, a specialized Bulgarian dairy breed at the stage of its creation, Stancheva (2003) reported 194.5 l for the sheep at first lactation and 199.9 l at second lactation, as the average value of the milking milk yield at first lactation was 99.178 l, whereas at the second it was 101.957 l.

Boikovski et al. (2006) reported a 105.67 l milk yield of the *Bulgarian dairy synthetic population* sheep from the herd at the Agricultural Institute – Shumen, Bulgaria at first lactation. The authors found a tendency for higher milk yield in the second lactation compared to the first. In a study, with the same herd, Stancheva (2013) reported the highest average milk yield at first lactation in those born in 2008 (107.01 l) followed by those born in 2007 (96.97 l) and 2004 (95.62 l) years, and the lowest milk yields were in animals born in 2006 and 2005 (90.62 l and 91.00 l).

Hinkovski et al. (2008) observed a tendency for a higher 120-d milking milk yield of the herd from the Bulgarian dairy synthetic population in the Institute of Animal Sciences – Kostinbrod, Bulgaria at second lactation (116.5 l) when compared to the first (110.69 l). For the same herd, Ivanova and Raicheva (2008) determined the average value of the standard 120 l milking milk yield in the sheep at second lactation (115.512 l), which was significantly higher (P < 0.01) than this trait at first lactation (68.139 l). When examining the milk yield for a 120-day milking period in early-bred sheep at first lactation, an average value of 90.75 l was reported (Ivanova and Raycheva, 2010). In Bulgarian dairy synthetic population sheep at different ages and lactations from the herd of the Institute of Animal Sciences - Kostinbrod, Bulgaria.

Ivanova et al. (2015), registered a milk yield during the sucking period of 57.54 l, milk yield of 106.28 l and lactation milk yield of 162.29 l. The variation of the indicator was high (up to 38%). When assessing the genealogical lines of the sheep from the same flock, the highest milk yield found for a standard 120-day milking period was 241 l (Raycheva and Ivanova, 2015).

Ivanova et al. (2010) determined the level of 120-d milking milk yield of the sheep of *Bulgarian diary synthetic population* from the herd of the Agricultural Institute – Stara Zagora, Bulgaria at first lactation of 104.39 l. When studying the trends of change of the milk yield in the same sheep, Slavova et al. (2015) found the highest milking milk yield of the animals at second lactation – 122.022 l for a period of 131.330 days. The average daily milking milk yield increased with the sequences of the lactations as follows: at I lactation – 0.843 l; at II lactation – 0.929 l; at III – 0.974 l and at IV – 0.997 l.

No significant differences in the average values of the milk yield during the various periods of lactation in the purebred *Bulgarian dairy synthetic population* and their crosses with *Awassi* in their comparative study were observed. The maximum milk yield of the purebred sheep was 105.83 l, whereas for the crossbreeds it was 86.85 l (Ivanova et al., 2015 a).

Conclusion

Traditional selection methods have little influence on milk productivity. Therefore, further and in-depth studies are needed on the polymorphism of candidate milk genes and their association with phenotype traits of milk production.

The studies included in the review find a statistically significant effect of a large part of the genetic variation in genetic markers on the expression of phenotype traits for milk productivity and milk composition in sheep. This allows us to conclude that genes related to milk productivity can be used for so-called assisted selection in animal husbandry.

The knoweledge of the genetic basis of the required qualities offers the breeding associations a method based on modern molecular techniques, the advantage of which is early assessment and maximum accuracy of expected phenotypic variation. The identification of genetic markers allows clear selection and excluding of genes which are not favorable for the productive direction.

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References:

Al-Mamun, H. A., Kwan, P., Clark, S. A., Ferdosi, M. H., Tellam, R., & Gondro, C. (2015). Genome-wide association study of body weight in Australian Merino sheep reveals an orthologous region on OAR6 to human and bovine genomic regions affecting height and weight. *Genetics Selection Evolution*, 47(1), 1-11.

Arranz, J. J., & Gutiérrez-Gil, B. (2012). Detection of QTL underlying milk traits in sheep: An update. *Milk production—advanced genetic traits—cellular mechanism—animal management health*, 97-126. https://www. intechopen.com/chapters/39318

Árnyasi, M., Komlósi, I., Kent, M. P., Czeglédi, L., Gulyás, G., & Jávor, A. (2013). Investigation of polymorphisms and association of the ABCG2 gene with milk production traits in sheep. *Livestock Science*, *154*(1-3), 64-68.

Arora, R., Bhatia, S., Mishra, B. P., Sharma, R., Pandey, A. K., Prakash, B., & Jain, A. (2010). Genetic polymorphism of the β -lactoglobulin gene in native sheep from India. *Biochemical Genetics*, 48(3), 304-311.

Barillet, F., Arranz, J. J., & Carta, A. (2005). Mapping quantitative trait loci for milk production and genetic polymorphisms of milk proteins in dairy sheep. *Genetics selection evolution*, *37*(Suppl. 1), S109-S123.

Boikovski, S., Stancheva, N., Dimitrov, D., & Stefanova, G. (2005). Milk composition of the sheep from the newly created milk breed. *Bulgarian Journal of Agricultural Science (Bulgaria)*, 11(5), 619-632.

Boikovski, S., Stefanova, G., & Stancheva, N. (2006). Milk Yield for milking period in the Sheep from the Newly Created Milk Breed in Bulgaria. *Bulgarian Journal of Agricultural Science, 12*(1), 145-152. **Bozgo, V., Hoda, A., Hysi, L., & Papa, L.** (2020). Genetic polymorphism detection of Casein genes in Albanian local sheep breeds. *Livestock Research for Rural Development, 32*(5). 71. http://www.lrrd.org/lrrd32/5/ ahoda32071.html

Bozhilova-Sakova, M., Dimitrova, I., Ivanova, T., Koutev, V., & Ignatova, M. (2021). Polymorphism of ABCG2 gene and its effects on litter size and milk production of synthetic population bulgarian milk ewes. *Tradition And Modernity In Veterinary Medicine* https:// scij-tmvm.com

Calvo, J. H., Vaiman, D., Saidi-Mehtar, N., Beattie, A., Jurado, J. J., & Serrano, M. (2002). Characterization, genetic variation and chromosomal assignment to sheep chromosome 2 of the ovine heart fatty acid-binding protein gene (FABP3). *Cytogenetic and genome research*, *98*(4), 270-273.

Calvo, J. H., Marcos, S., Jurado, J. J., & Serrano, M. (2004). Association of the heart fatty acid-binding protein (FABP3) gene with milk traits in Manchega breed sheep. *Animal Genetics*, *35*(4), 347-349.

Cappio-Borlino, A., Portolano, B., Todaro, M., Macciotta, N. P. P., Giaccone, P., & Pulina, G. (1997). Lactation curves of Valle del Belice dairy ewes for yields of milk, fat, and protein estimated with test day models. *Journal of Dairy Science*, 80(11), 3023-3029.

Cohen, L. E., Wondisford, F. E., & Radovick, S. (1996). Role of Pit-1 in the gene expression of growth hormone, prolactin, and thyrotropin. *Endocrinology and metabolism clinics of North America*, *25*(3), 523-540.

Dimitrova, I., Bozhilova-Sakova, M., Ignatova, M., Koutev, V. & Genova, K. (2019). Investigation of ABCG2 gene in three fine fleece sheep breeds in Bulgaria, in: *Proceedings of International Congress on Domestic Animal Breeding, Genetics and Husbandry* 2019 (ICABGEH-19), Prague, Czechia, 11 – 13 September 2019, 214-216.

Dzhorbineva, M., Kalaydjiev, G., & Dimitrov, I. (2011). Current state and future perspectives for the local Stara Zagora sheep. *Agrarni Nauki*, *3*(6), 47-51.

Elyasi, G., Shodja, J., Nassiri, M., Tahmasebi, A., Pirahary, O., & Javanmard, A. (2010). Polymorphism of β -lactoglobulin gene in Iranian sheep breeds using PCR-RFLP. *Journal of molecular Genetics*, 2. (1), 6-9.

Fernández, G., Baro, J. A., De la Fuente, L. F., & San Primitivo, F. (1997). Genetic parameters for linear udder traits of dairy ewes. *Journal of dairy science*, *80*(3), 601-605.

Fischberg, D. J., Chen, X. H., & Bancroft, C. (1994). A Pit-1 phosphorylation mutant can mediate both basal and induced prolactin and growth hormone promoter activity. *Molecular Endocrinology*, *8*(11), 1566-1573.

Furuhashi, M., & Hotamisligil, G. S. (2008). Fatty acid-binding proteins: role in metabolic diseases and poten-

tial as drug targets. *Nature reviews Drug discovery*, 7(6), 489-503. https://doi.org/10.1038/nrd2589

Gencheva, D., & Georgieva, S. (2019). Genetic diversity and population structure of Bulgarian autochthonous sheep breeds based on nucleotide variation in Alpha S1-casein gene. *Bulgarian Journal of Agricultural Science*, 25, 3, 95-102.

Gencheva, D. (2020). Single nucleotide polymorphism of the β -lactoglobulin gene in sheep breeds reared in Bulgaria. *Bulgarian Journal of Veterinary Medicine*, 23(3), 295-303.

Gencheva, D., Veleva, P., Naidenova, N., & Pamukova, D. (2020). Genetic polymorphism of alpha S1casein in Bulgarian sheep breeds and its effect on milk composition. *Turkish Journal of Veterinary and Animal Sciences*, 44(4), 860-870.

Gonzalo, C., Carriedo, J. A., Baro, J. A., & San Primitivo, F. (1994). Factors influencing variation of test day milk yield, somatic cell count, fat, and protein in dairy sheep. *Journal of dairy science*, *77*(6), 1537-1542.

Gras, M. A., Pistol, G. C., Pelmus, R. S., Lazar, C., Grosu, H., & Ghita, E. (2016). Relationship between gene polymorphism and milk production traits in Teleorman Black Head sheep breed. *Revista MVZ Córdoba*, 21(1), 5124-5136. http://www.scielo.org.co/scielo. php?script=sci_arttext&pid=S0122-02682016000100004-&lng=en&tlng=en.

Gutiérrez-Gil, B., El-Zarei, M. F., Alvarez, L., Bayón, Y., De La Fuente, L. F., San Primitivo, F., & Arranz, J. J. (2009). Quantitative trait loci underlying milk production traits in sheep. *Animal Genetics*, 40(4), 423-434.

Gutierrez-Gil, B., Arranz, J. J., Pong-Wong, R., Garcia-Gamez, E., Kijas, J., & Wiener, P. (2014). Application of selection mapping to identify genomic regions associated with dairy production in sheep. *PloS one*, *9*(5), e94623.

Herr, W., Sturm, R. A., Clerc, R. G., Corcoran, L. M., Baltimore, D., Sharp, P. A., Ingraham, H. A., Rosenfeld, M. G., Finney, M., & Ruvkun, G. (1988). The POU domain: a large conserved region in the mammalian pit-1, oct-1, oct-2, and Caenorhabditis elegans unc-86 gene products. *Genes Dev*, 2(12A), 1513-1516.

Hinkovski, T., Raicheva, E., & Metodiev, N. (2008). Evaluation of the productivity of sheep from the Synthetic population of Bulgarian dairy. *Livestock sciences*, 3, 35-42. (BG)

Hofmannova, M., Rychtářová, J. A. N. A., Sztankoova, Z., Milerski, M., Vostrý, L. U. B. O. Š., & Svitakova, A. (2018). Association between polymorphism of ABCG2 gene and somatic cell count in Czech dairy sheep breeds. *Medycyna Weterynaryjna*, 74(8), 489-492. Khristova, D. (2011). Genetic polymorphism of alpha S1-casein gene in Bulgarian sheep breeds. *Agricultural Science and technology (Bulgaria). 3*, (1), 8-12. https://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0094623&type=printable

Ivanova, I., Dimova, N., Peeva, Z., Dzhorbineva, M., & Mihaylova, M. (2010). Relationship between some external dimensions and the assessment of the physical condition of sheep from the Synthetic population of Bulgarian dairy. *Livestock sciences*, 4, 14-19. (BG)

Ivanova, S. (2011). Technological regimes of lyophilization of sheep's milk and main indicators of the products. Dissertation, S. 12. Ivanova, S., 2011. Technological regimes of lyophilization of sheep's milk and main indicators of the products. *Dissertation, Sofia*. (BG)

Ivanova, S., Ivanova, T., Angelov, L., & Raicheva, E. (2015). Milk productivity, physicochemical and fatty acid composition of sheep milk from Synthetic Bulgarian dairy population. *Livestock sciences*, LII, 2, 15-24. (BG)

Ivanova, T., & Raicheva, E., (2008). Evaluation of the effect of some factors on milk yield. *Collection of reports* "80 years of agricultural science in the Rhodopes", 67-71. (BG)

Ivanova, T., & Raicheva, E., (2010). Study on the dimensions of the exterior and udder in sheep from the Synthetic Bulgarian Dairy Population. *Livestock sciences,* 5, 3-9. (BG)

Ivanova, T., Raicheva, E., & Tsvetkova, V., (2015 a). Comparative study of productivity in sheep from the Synthetic population of Bulgarian dairy and their crosses with the Awassi breed. *Livestock sciences*, LII, 3, 13-19. (BG)

Jyotsana, B., Kumar, R., Kumari, R., Meena, A. S., Prince, L. L. L., Prakash, V., & Kumar, S. (2014). β-Lactoglobulin gene polymorphism in Indian sheep breeds of different agro-climatic regions. *Indian Journal of Animal Sciences*, *84*(10), 1133-1136.

Kalaydjiev, G., Angelova, T., Yordanova, D., Karabashev, V., Laleva, S., Cassandro, M., Krastanov, J., Oblakov, N., Dimov, D. & Popova, Y. (2014). "D-allele" frequencies in milk α-s1 casein from Bulgarian local sheep breeds. *LXVIII Convegno Sisvet, XI Convegno Aipvet e XII Convegno Sira*, 16-18 June, Pisa, Italy, 142-145.

Kalaydzhiev, G. I., Balabanova, T. B., Ivanova, M. G., & Ivanov, G. Y. (2021). Correlation between phenotypic characteristics of chemical composition and rennet coagulation of sheep milk. In *IOP Conference Series: Materials Science and Engineering* (Vol. 1031, No. 1, p. 012099). IOP Publishing.

Kawecka, A., & Radko, A. (2011). Genetic polymorphism of β -lactoglobulin in sheep raised for milk production. *Journal of Applied Animal Research*, *39*(1), 68-71.

Kowalewska-Łuczak, I., Czerniawska-Piątkowska, E., & Pecka-Kielb, E. (2017). Investigation on relationships of the FABP3 AND SLC27A3 genes with milk production traits in sheep. *J. Elementol*, *22*, 1485-1493.

Kusza, S., Gyarmathy, E., Dubravska, J., Nagy, I., Jávor, A., & Kukovics, S. (2009). Study of genetic differences among Slovak Tsigai populations using microsatellite markers. *Czech J. Anim. Sci*, *54*(10), 468-474.

Legarra, A., & Ugarte, E. (2001). Genetic parameters of milk traits in Latxa dairy sheep. *Animal Science*, *73*(3), 407-412.

Li, J. Y., Chen, H., Lan, X. Y., Kong, X. J., & Min, L. J. (2008). Genetic diversity of five Chinese goat breeds assessed by microsatellite markers. *Czech J Anim Sci*, 53(8), 315-319.

Ligda, C. H., Altarayrah, J., Georgoudis, A., & Econogene Consortium. (2009). Genetic analysis of Greek sheep breeds using microsatellite markers for setting conservation priorities. *Small Ruminant Research*, *83*(1-3), 42-48.

Maheswaran, M. (2004). Molecular markers: history features and applications. *Advanced Biotech*, *51*, 373-378.

Mateescu, R. G., & Thonney, M. L. (2010). Genetic mapping of quantitative trait loci for milk production in sheep. *Animal genetics*, *41*(5), 460-466.

McKusick, B. C., Berger, Y. M., & Thomas, D. L. (1999, November). Effects of three weaning and rearing systems on commercial milk production and lamb growth. In *Proceedings of the5th Great Lakes Dairy Sheep Symposium*. http://www.ansci.wisc.edu/extension-new%20copy/ sheep/Publications_and_Proceedings/symposium%20 PDF/symposium_1999.pdf

McKusick, B. C., Berger, Y. M., & Thomas, D. L. (1999). Preliminary results: effects of udder morphology on commercial milk production of East Friesian crossbred ewes. In *Proc. 5th Great lakes Dairy Sheep Symp. University of Wisconsin-Madison, Dept. Anim. Sci., and University of Vermont, Center for Sustainable Agriculture* (pp. 49-60). http://www.ansci.wisc.edu/extensionnew%20copy/sheep/Publications_and_Proceedings/ symposium%20PDF/symposium_1999.pdf

Nenov, T., Ivanova, S., Atanasova, T., & Peteva, D. (1991). Technologies in animal husbandry, Zemizdat, Sofia. (BG)

Nudda, A., Bencini, R., Mijatovic, S., & Pulina, G. (2002). The yield and composition of milk in Sarda, Awassi, and Merino sheep milked unilaterally at different frequencies. *Journal of Dairy Science*, *85*(11), 2879-2884.

Öner, Y., Orman, A., Üstüner, H., & Yilmaz, A. (2014). Investigation of polymorphisms on ABCG2, AA-NAT and FABP3 genes in the Kıvırcık sheep reared in three different provinces of Turkey. *Kafkas Univ. Vet. Fak. Derg*, *20*, 649-654.

Oravcová, M. (2007). Genetic evaluation for milk production traits in Slovakian Lacaune sheep. *Slovak Journal of Animal Science*, 40(4), 172-179.

Orford, M., Tzamaloukas, O., Papachristoforou, C., & Miltiadou, D. (2010). A simplified PCR-based assay for the characterization of two prolactin variants that affect milk traits in sheep breeds. *Journal of dairy science*, *93*(12), 5996-5999.

Othman, O. E., El-Fiky, S. A., Hassan, N. A., Mahfouz, E. R., & Balabel, E. A. (2013). Genetic polymorphism detection of two α -Casein genes in three Egyptian sheep breeds. *Journal of Genetic Engineering and Biotechnology*, *11*(2), 129-134.

Othmane, M. H., De La Fuente, L. F., Carriedo, J. A., & San Primitivo, F. (2002). Heritability and genetic correlations of test day milk yield and composition, individual laboratory cheese yield, and somatic cell count for dairy ewes. *Journal of dairy science*, *85*(10), 2692-2698.

Panayotov, D., Sevov, S., & Georgiev, D. (2018). Milk yield and morphological characteristics of the udder of sheep from the breed Lacaune in Bulgaria. *Bulgarian Journal of Agricultural Science*, 24(1), 95-100.

Peter, C., Bruford, M., Perez, T., Dalamitra, S., Hewitt, G., Erhardt, G., & Econogene Consortium. (2007). Genetic diversity and subdivision of 57 European and Middle-Eastern sheep breeds. *Animal genetics*, *38*(1), 37-44.

Petrova, N., & Nedelchev, D. (2000). Milk production, composition and properties of sheep milk during the lactation period. *Bulgarian Journal of Agricultural Science*, 6(5), 583-588.

Raicheva, E., Nedelchev, D., & Ivanova, T. (2004). Variation of composition and properties of the milk for control day. *Bulgarian Journal of Agricultural Science (Bulgaria).* 10, 371-376.

Raicheva, E., Nedelchev, D., & Petrova, N. (2004). Milk yield, composition and properties of milk from dairy sheep breeds. *Bulgarian Journal of Agricultural Science (Bulgaria).* 10, 269-274.

Raicheva, E., & Ivanova, T. (2015). Estimation of the genealogical lines of sheep from the Synthetic population of Bulgarian dairy. *Livestock sciences*, LII, 3, 3-7. (BG)

Raycheva, E., & Ivanova, T. (2011). Preliminary study on some parameters related to milk production in early bred sheep from the Synthetic population of Bulgarian dairy. *Journal of Mountain Agriculture on the Balkans*, 4, 656-666. (BG)

Santos-Silva, F., Ivo, R. S., Sousa, M. C. O., Carolino, M. I., Ginja, C., & Gama, L. T. (2008). Assessing genetic diversity and differentiation in Portuguese coarsewool sheep breeds with microsatellite markers. *Small Ruminant Research*, 78(1-3), 32-40. Sarkadi, B., Özvegy-Laczka, C., Német, K., & Váradi, A. (2004). ABCG2–a transporter for all seasons. *FEBS letters*, *567*(1), 116-120.

Selvaggi, M., Laudadio, V., Dario, C., & Tufarelli, V. (2014). Investigating the genetic polymorphism of sheep milk proteins: a useful tool for dairy production. *Journal of the Science of Food and Agriculture*, *94*(15), 3090-3099. http://dx.doi.org/10.1002/jsfa.6750

Selvaggi, M., Laudadio, V., Dario, C., & Tufarelli, V. (2015). β-Lactoglobulin gene polymorphisms in sheep and effects on milk production traits: A review. *Adv. Anim. Vet. Sci*, *3*(9), 478-484.

Slavova, P., Laleva, S., & Popova, Y. (2015). Study of the change of the productive traits of milk and fertility in sheep from the Synthetic population of Bulgarian dairy as a result of the conducted selection. *Livestock sciences*, LII, 3, 20-25. (BG)

Staiger, E. A., Thonney, M. L., Buchanan, J. W., Rogers, E. R., Oltenacu, P. A., & Mateescu, R. G. (2010). Effect of prolactin, β -lactoglobulin, and κ -casein genotype on milk yield in East Friesian sheep. *Journal of dairy science*, *93*(4), 1736-1742.

Stancheva, N. (2003). Phenotypic and genotypic parameters of the selection traits in the newly created high-milk sheep population in the country. *Doctoral dissertation*, Sofia. (BG)

Stancheva, N. (2013). Productivity and heredity of some traits of sheep from the Synthetic Bulgarian Dairy Population. *Livestock sciences*, *L*, (6), 29-35. (BG)

Stancheva, N., Naydenova, N., & Staikova, G. (2011). Physicochemical composition, properties, and technological characteristics of sheep milk from the Bulgarian dairy synthetic population. *Macedonian J Anim Sci, 1*, 73-76.

Sumantri, C., Herdiana, D., Farajallah, A., & Rahmat, D. (2009). Polymorphism of Pituitary-Specific Transcription Factor-1 (Pit-1) Gene at Locus (Pit-1-Hinf1) and its effects on dam body weight and milk production of local sheeps. *Jurnal Ilmu Ternak dan Veteriner*, *14*(3), 212-229.

Thomas, D. L., Berger, Y. M., & McKusick, B. C. (1999). East Friesian germplasm: Effects on milk production, lamb growth, and lamb survival. In *Proc. Am. Soc. Anim. Sci.*

Thomas, D. L., Berger, Y. M., McKusick, B. C., & Gottfredson, R. G. (2000, November). Comparison of East Friesian-crossbred and Lacaune-crossbred ewe lambs for dairy sheep production first-year results from a multi-year trial. In *Dairy Sheep Symposium*. http://babcock.wisc.edu/sites/default/files/sheepgoat/sg_Comparison.pdf

Yang, F., Li, L., Liu, H., Cai, Y., & Wang, G. (2012). Polymorphism in the exon 4 of β -lactoglobulin variant B precursor gene and its association with milk traits and protein structure in Chinese Holstein. *Molecular biology reports*, *39*(4), 3957-3964. http://dx.doi.org/10.1007/ s11033-011-1175-6

Yousefi, S., Azari, M. A., Zerehdaran, S., Samiee, R., & Khataminejhad, R. (2013). Effect of β-lactoglobulin and κ-casein genes polymorphism on milk composition in indigenous Zel sheep. *Archives Animal Breeding*, *56*(1), 216-224.