International Journal of
Biological Engineering and Agriculture

ISSN: 2833-5376
Volume 2 | No 6 | jun -2023

Genetic Testing of Sheep for Prolactin (PRL) Gene

M. Suleymanova [1], D. Rizayeva [2], M. Khuseinova [3]

[1] doctoral student of Samarkand State University of Veterinary Medicine, Livestock and Biotechnologies

[2] Doctor of Philosophy in Agricultural Sciences (PhD), assistant of Samarkand State University of Veterinary Medicine, Livestock and Biotechnologies

[3] Doctor of Philosophy in Agricultural Sciences (PhD), assistant of Samarkand State University of Veterinary Medicine, Livestock and Biotechnologies

Annotation: Modern breeding methods are aimed at searching for molecular genetic markers that interact with economically useful traits. One of the main characteristics of markers is polymorphism, which is a change in the nucleotide sequence in the DNA molecule caused by various mutations. Its manifestation is the allelic spectrum. The PCR-RFLP method is considered the standard point mutation analysis for the diagnosis of allelic polymorphism of candidate genes. One of the promising genes that are considered markers of sheep productivity is the prolactin (PRL) gene.

Keywords: DNA marker, milk, gene, protein, polymorphism, genotype, sheep, allele, prolactin, casein, texel, prekos, karakul sheep, test.

Introduction

An important place in animal husbandry is occupied by DNA technologies in a number of promising methods used in the study of productivity.

At present, a number of DNA markers have been identified for the development of quantitative and qualitative traits in farm animals that affect meat and dairy productivity. The prospect of selection with the use of markers (Marker Assisted Selection, MAS) is that they are stable in ontogeny, are determined at an early age, which is very important, independent of environmental conditions, their determination does not require large expenditures, but at the same time the quality of breeding is significantly improved. process and improve its efficiency. An important characteristic of markers is polymorphism, which is a change in the nucleotide sequence of the DNA marker due to various types of mutations, its manifestations are the allelic spectrum.

The protein composition of sheep's milk has not been studied enough or is presented even to a lesser extent than milkiness. There are only reports on the content of complex proteins in sheep milk such as total protein, casein and whey protein, as well as on the genetic polymorphism of some protein fractions.

Recently, however, up to 20 different protein components have been found in milk (Tepel A., 1979; Gorbatova K.K., 2001), but they were found mainly in cow's milk (Kharetdinov R.A., Gataullin AM, 2000).

Currently, the physicochemical and technological properties of milk are being studied depending on its protein composition and genetic polymorphism of proteins. These studies will be applied when using sheep's milk as a raw material for the dairy industry. In countries where, due to natural and climatic conditions, the breeding of dairy cattle is associated with great difficulties, the only source

of milk production is dairy sheep and goat breeding (Mills O., 1989). These livestock industries are intensively developing in Iraq, Iran, Pakistan and some Mediterranean countries (Italy, Spain, France, Greece, Portugal), are important in the agricultural economy of the Caucasian countries of the CIS (Azerbaijan, Armenia, Georgia), as well as the North Caucasian republics of Russia (Dagestan, North Ossetia, Alania).

Research in sheep breeding is aimed on the use of the genetic potential of sheep in milk production.

Selection based on genetic markers of productivity is aimed at working with animals with a high genetic potential for live weight gain and milk yield.

PRL and β-LG genes are promising marker genes associated with milk production traits.

Improvement of the breed, based on a more complete assessment of the genotype of animals using marker technologies, using hereditary protein polymorphism. A change in the frequency of a certain allele, type of protein or blood group during selection in the herd may indicate a relationship between immunogenetic properties and productivity [1]. This made it possible to use marker genes in practical breeding [2]. For example, in the Karakul breed, it was found that the AA genotype for β-Lg affects milk productivity: individuals with this genotype produce more milk compared to other genotypes [3]. According to other studies, milk from sheep with a heterozygous AB genotype is better suited for cheese production [4]. For the production of cheese, the kCn protein, type B, is a priority [5].

The PRL gene is located on chromosome 20; the PRLA and PRLB alleles differ in position at amino acid 38 (His/Tyr). The gene is responsible for the production of protein and lactose in milk, encodes the enzyme prolactin, which plays an important role in the development of the mammary gland and milk secretion [7,8]

The sheep genome was sequenced in 2012 (The International Sheep Genomics Consortium et al., 2010). The combination of the decoded genome with a high-density SNP-chip makes it possible to discover significant genetic polymorphism in meat and dairy productivity.

The foregoing predetermined the purpose of the present research and served as the basis for the study of gene polymorphism, PRL, in the α-casein gene (CSN1S1).

Materials and methods. The studies were carried out during 2020-2021 at the KSUP "Khvinevichi" of the Republic of Belarus in the Republic of Belarus and at the farm "Nurabad Keng Dalasi" of the Republic of Uzbekistan.

The analysis was carried out in the DNA laboratory of the Grodno State Agrarian University. The experiment was carried out on a population of sheep of the Karakul breed of sheep (10), prekos (10), texel (10).

Modern breeding methods are aimed at searching for molecular genetic markers that interact with economically useful traits. One of the main characteristics of markers is polymorphism, which is a change in the nucleotide sequence in the DNA molecule caused by various mutations. Its manifestation is the allelic spectrum [3, 4]. The PCR-RFLP method is considered the standard point mutation analysis for the diagnosis of allelic polymorphism of candidate genes [4]. Some of the promising genes considered markers of sheep productivity are prolactin (PRL), beta-lactoglobulin (β-LG) genes.

Detection of the received results

Restriction fragments are analyzed by gel electrophoresis (on a 2-4% agarose gel stained with ethidium bromide at a voltage of 120-140V for 30-60 min) followed by visualization on the GelDoc XR+, BIORAD gel-documentation system.

Genetic and statistical analysis of the obtained results was carried out using formulas. Calculation of the frequency of occurrence of genotypes was determined by the formula: p =n/N, where p is the frequency of a certain genotype, n is the number of animals with a certain genotype, N is the total number of animals. WITH

The calculation of the frequency of occurrence of alleles was carried out according to the formula: P(A) = $2N1+N2/2n$, where P is the allele frequency; A - allele; N1 is the number of homozygotes for the studied allele; N2 is the number of heterozygotes; n is the sample size of animals. (9.10)

The allele frequency (for two-allelic systems) was determined by formulas (1), (2).

P(A) = (2AA + AB) / 2n, (1)

q(B) = (2BB + AB) / 2n, (2)

where P (A) is the frequency of allele A;

AA, BB are the number of individuals with a homozygous genotype;

AB is the number of individuals with a heterozygous genotype;

n is the number of individuals in groups;

q(B) is the B allele frequency.

The determination of genetic balance was carried out using the $\chi 2$ test, according to the law

Hardy-Weinberg, according to the formula (3):

$\chi 2 = (\Phi - T)2/T$, (3)

Results and its discussion. Prolactinproteinaceoushormoneproduced by specializedcellsanterior lobepituitary glandvertebrates.

The PRL gene is located on chromosome 20; the PRLA and PRLB alleles differ in position at amino acid 38 (His/Tyr). The gene is responsible for the production of protein and lactose in milk and encodes the prolactin enzyme, which plays an important role in the development of the mammary gland and milk secretion [7,8].

Sheep genotyping for the prolactin gene (PRL)

The following primers were used to amplify the prolactin gene fragment:

- for - 5' ACCTCTCTTCGGAAATGTTCA – 3'
- rev – 5' CTGTTGGGCTTGCTCTTTGTC– 3'.

PRL: PCR program "hot start" - 3 min at 95°C; 30 cycles: denaturation - 1 min. at 940C, annealing - 2 min. at 580C, synthesis - 2 min. at 720C; completion - 5 min at 720C.

HaeIII endonuclease was used to restrict the amplified region of the PRLR gene. 2 µl. buffer for restriction enzymes, 1 µl. endonuclease HaeIII, 2 µl. H2O. The reaction was carried out at a temperature of 37°C.

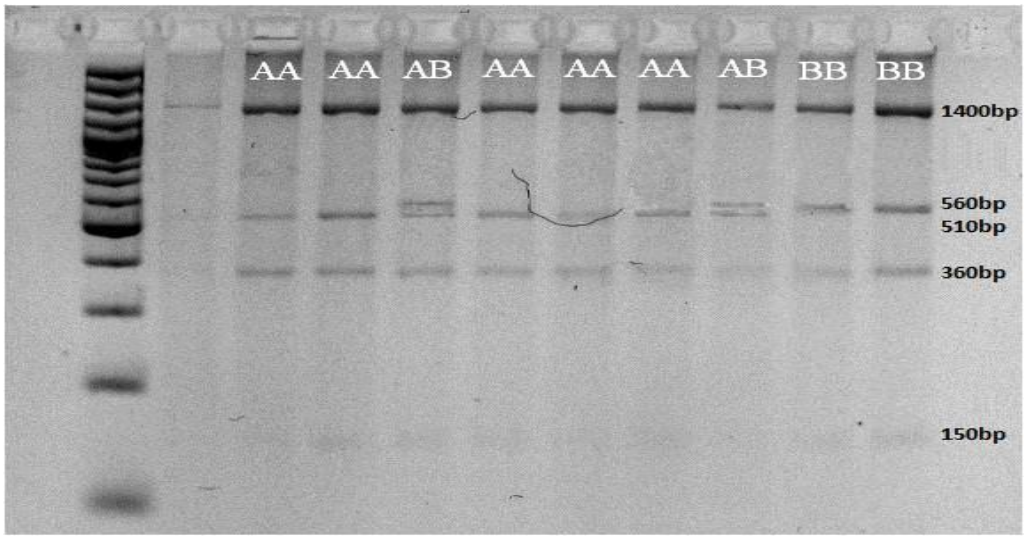
Restriction gene products were separated electrophoretically in a 2% agarose gel (at 130 V) in TBE buffer under UV light using ethidium bromide on a GelDocRX+ (BIORAD) gel-documentation system.

Digestion of amplification products with HaeIII restriction enzyme at 370C identified the following genotypes.

- PRL AA - 1400 b.p., 510 b.p. 360 bp 150 bp
- PRLAB - 1400 b.p., 530 b.p., 510 b.p. 360 bp 150 bp;
- PRLBB– 1400 b.p., 530 b.p., 360 b.p. 150 bp.

Analysis of polymorphism of prolactin genes revealed the presence of two alleles - PRLA and PRLB and three genotypes, homozygous (PRLAA, PRLBB;) and heterozygous (PRLAB) (Table 1)

Electrophoregram of PCR-RFLP prolactin (PRL) result



Picture 1. Electrophoretic analysis of restriction products, when determining the prolactin genotype

Table 1 Occurrence of alleles and genotypes of PRL- gene in sheep

Index	n	Genotype frequency						Allele frequency		$\chi 2$
		AA		*AB*		*BB*				
		n	%	n	%	n	%	*A*	*IN*	
Texel										
O	10	7	70.0	1	10.0	2	20.0	0.75	0.25	1.8
E		5	50	2.5	25	2.5	25			
Prekos										
O	10	4	40.0	3	30.0	3	30.0	0.55	0.45	1.81
E		4.5	45	1.5	15	4	40			
Karakulskaya										
O	10	4	40.0	6	60.0	-	-	0.70	0.30	2.75
E		6.5	60	3.5	20	-	-			

O- actually observed indicator, E - theoretically expected indicator

Prolactin polymorphism is characterized by a high (0.75) concentration of the A allele in the Texel and Karakul breeds (0.70), an average concentration in the Prekos (0.55) PRLA and a low (0.25) PRLB allele in the Texels and Karakul breeds, which found reflected in the presence of homo- and heterozygous genotypes: PRLAA - 7 (70.0%) in texels, 6 (60.0%) karakul, 4 (40%) prekos. PRLBB - 2 (20%) in texels and 3 (30.0%) preskews, absent in Karakul.; PRLAB - texels 1 (10.0%), precuts 3 (30.0%) and karakul 6 (60.0%).

Homozygous individuals with the BB genotype were not found, which is due to the genotypes of the ancestors of Karakul sheep.

In the group of Karakul sheep, the B-allele was found only in heterozygotes. This frequency distribution is likely due to homozygotization predominantly for one allele and may lead to the loss of a gene variant.

In our studies, two alleles A and B were found in sheep of the Karakul breed with frequencies of 0.80 and 0.020, respectively, and the homozygous AA genotype is more common (60%).

Indicators x (1.8 - 2.75) indicate the genetic balance in the studied animal population.

As a result of the PCR-RFLP study, the breed characteristics of the polymorphism of the allelic spectrum of the PRL genes of sheep of the Texel, Prekos and Karakul breeds were established.

The obtained data can be used as a genetic characteristic of the sheep population of this breed, as well as find application in selection and breeding work aimed at preserving genetic diversity.

Conclusion

As a result of the study, the PCR-RFLP method established the breed characteristics of the polymorphism of the allelic spectrum of genes, PRL sheep breeds texel, prekos, karakul. The data obtained can be used as a genetic characteristic of the population of sheep of this breed, as well as find application in selection and breeding work aimed at preserving genetic diversity.

LITERATURE

1. Погосян, Г.А. Стимуляция молочности у овец имплантацией йодсодержащих препаратов / Г.А. Погосян // Овцы, козы, шерстяное дело. – 2014. – № 1. – С. 37-38.
2. Jawasreh K. Effect and Interaction of β-Lactoglobulin, Kappa Casein, and Prolactin Genes on Milk Production and Composition of Awassi Sheep / K. Jawasreh, A.A.Amareen P.Aad // Animals 2019. 9(6). P. 382.
3. Padilla P. Polymorphisms of α-lactoalbumin, β-lactoglobulin and prolactin genes are highly associated with milk composition traits in Spanish Merino sheep / P. Padilla, M. Izquierdo, M. Martínez-Trancón, J. Carlos Parejo, et al. // Livest. Sci. 2018. 217. P. 26–29.
4. Селионова М. И. Породные особенности аллельного профиля генов, контролирующих молочную продуктивность крупного рогатого скота / М. И. Селионова, Л. Н. Чижова, Е. С. Суржикова и др. // АгроЗооТехника. 2019. № 1. Т. 2. С.
5. Селионова М.И. Геномные технологии в селекции сельскохозяйственных животных / М.И. Селионова, А.-М.М. Айбазов // Сельскохозяйственный журнал. 2014. №7 (1).
6. Молекулярно-генетические исследования сельскохозяйственных животных методом ПЦР-ПДРФ: учебное пособие / Л.В. Гетманцева [и др.]; Донской ГАУ. – Персиановский: Донской ГАУ. 2018. С. 119.
7. Молекулярно-генетические исследования сельскохозяйственных животных методом ПЦР-ПДРФ: учебное пособие / Л.В. Гетманцева [и др.]; Донской ГАУ. – Персиановский: Донской ГАУ. 2018. С. 119.
8. Bobokulovich, B. A., & Kasimovna, S. M. (2021). Dairy Productivity Of Sheep Of Different Genotypes. *European Journal of Agricultural and Rural Education*, *2*(6), 17-19.
9. Сулейманова, М. К., & Ризаева, Д. Т. (2022). Молочная продуктивность овец разных генотипов. In *Инновационное развитие продуктивного и непродуктивного животноводства* (pp. 195-199).
10. Suleymanova, M. K., Kravsevich, V. P., Rizayeva, D. T., Ruzimuradov, R. R., & Khuseinova, M. (2022). MOLECULAR GENETIC TESTING OF SHEEP BY GENE (PRL) AND (CSN1S).