с беспривязным содержанием коров на комплексе. Рентабельность производства молока на комплексе составила 51,5 %, что выше, по сравнению с фермой, на 1,2 п. п.

Следовательно, производство молока на комплексе с беспривязным содержанием и доением в доильном зале экономически более выгодно по сравнению с привязным содержанием коров и доением в молокопровод.

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## MOLECULAR GENETIC TESTING OF SHEEP BY GENE (PRL) AND (CSN1S)

**Suleymanova Mukhabbat Kasimovna, Kravsevich Valdemar Petrovich, Rizayeva Dilfuza Takhirovna, Ruzimuradov Rabbimkul Rayimkulovich, Khuseinova Maysara**

Samarkand State University of veterinary medicine, animal husbandry and biotechnology
E-mail:kadi15@mail.ru; cell phone.: (+99890) 504-24-46

Introduction. Polymorphism, which is a change in the nucleotide sequence in a DNA molecule produced by numerous mutations, is one of the most important properties of markers. The allelic spectrum is one of its manifestations. For identifying allelic variation in candidate genes, the PCR-RFLP technique is regarded the gold standard. Prolactin (PRL) and -casein are two interesting genes that have been proposed as sheep productivity markers (CSN1S).

Methods. The perchlorate method, polymerase chain reaction (PCR), DNA restriction analysis (PFLP), and gel electrophoresis methods were used to screen sheep for the (PRL) and (CSN1S) genes.

Results. The polymorphism of the allelic spectrum of PRL genes in Texel, Prekos, and Karakul sheep was identified as a result of the investigation using the PCR-PFPL method. The information gathered can be used as

a genetic feature of this breed's sheep population, as well as in breeding and pedigree work aimed at maintaining genetic variety.

Conclusion. The study discovered that the AA genotype has an effect on sheep milk productivity, with this genotype being more productive than other genotypes.

The information gathered can be used to assess the state of the breed gene pool and forecast productivity.

Key words: DNA marker, milk, gene, protein, polymorphism, genotype, sheep, allele, prolactin, casein, Texel, precoc, karakul sheeptest.

**Introduction.** Among the potential methodologies employed in the research of productivity, DNA technologies play an essential role in animal breeding.

A number of DNA markers for the development of quantitative and qualitative features in farm animals that affect meat and dairy productivity have recently been found. The advantage of marker-assisted selection (MAS) is that it is stable in ontogenesis, can be determined at an early age, which is critical, and is independent of environmental conditions. Its determination does not cost a lot of money, but it improves the quality and efficiency of the breeding process significantly. Polymorphism, which is variations in the nucleotide sequence of the marker DNA due to various types of mutations; its manifestations include the allelic spectrum, is an important feature of markers. A prerequisite for using a locus as a possible genetic marker is the presence of two or more alleles [5].

Sheep's milk protein content hasn't been thoroughly examined, and it's even less well represented than dairy milk. Only the total protein, casein, and whey protein content of sheep milk, as well as the genetic polymorphism of specific protein fractions, have been reported. Recently, however, up to 20 different protein components have been detected in milk (Tepel A., 1979; Gorbatova K.K., 2001), but they have been identified mainly in cow's milk (Kharetdinov R.A., Gataullin A.M., 2000).

Research in sheep breeding is aimed at using the genetic potential of sheep in milk production.

Selection on the basis of genetic markers of productivity is aimed at working with animals with high genetic potential for live weight gain and milk yield. PRL and β-LG genes are promising marker genes associated with milk production traits.

The application of DNA markers allows us to accelerate the process of accumulation of genes that carry desirable traits for productivity, and therefore increase the productivity and economic profitability of sheep breeding. Markers in this case can be DNA-sequences characterizing: different allelic variants of meat productivity genes, SNP (Single Nucleotide Polymorphism)

points, which can be located in exons, introns or regulatory regions of genes, but in any case have a significant impact on the manifestation of economically important features of the animal. Livestock performance indicators are complex, depending on the total action of a significant number of genes, as well as the interaction between genes, with the ability of the animal to show its genetic potential determined also by the impact of environmental factors. Current research is focused on genomic selection (or whole-genome selection) aimed at calculating the summation effect of many genes, as well as the possible epistatic effect (Goddard, Hayes, 2007). This is a promising area, and will be based on it in the future.

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 of a protein type or blood group during selection in a herd can indicate a link between immunogenetic traits and productivity [1]. This made it possible to use marker genes in practical breeding [2]. For example, in the Karakul breed, it has been found that the AA genotype of 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 cheese production, the priority protein is kCn - type B [5].

At the protein level, six genotypes AA, AB, BB, AE, BE, and EE were identified in goats. The correlation between genotype variant and milk production parameters (milk yield, fat and protein content) was also established (Bartowska J. etc., 2007; Ricordeau etc., 2000]. In 2004, Ramunnoetc., 2004 identified 17 αS1 genetic variants in goats - A, B1, B2, B3, B4, C, D, E, F, G, H, I, L, M, N, 01, 02. The αS2-casein gene is the longest of the four casein-coding genes and includes 18 exons. The total length of the gene is about 18483 bp [Groenen M etc., 1993]. αS2-casein is a family of proteins - αS2, αS3, αS4, αS6. Their difference lies in the different number of phosphate groups (3, 12, 11, and 10, respectively).The αS2 protein consists of 207 amino acids and has a molecular weight of 25,150 kDa. Its sensitivity to calcium ions due to its high degree of phosphorylation is higher than that of other caseins. Two segments (50-123 and 132-207) in the peptide chain of αS2-casein are homologous. This suggests that αS2 may have arisen from a duplication of primitive genes [Ribadeau-Dumas B, 1979].

Modern breeding methods are aimed at finding molecular genetic markers that interact with economically useful traits. One of the main characteristics of markers is polymorphism, which represents a change in the nucleotide sequence in a DNA molecule caused by various mutations. Its manifestation is the allelic spectrum [3, 4]. The PCR-PFPL method is con-

sidered the standard point mutation analysis for diagnosing allelic polymorphism of candidate genes [4]. Some of the promising genes considered as markers of sheep productivity are the genes, prolactin (PRL), α-casein (CSN1S).

The PRL gene is located on chromosome 20; the PRLA and PRLB alleles differ in their position in amino acid 38 (His/Tyr). The gene is responsible for protein and lactose production in milk and encodes the enzyme prolactin, which plays a major role in mammary gland development and milk secretion [5, 6]

The sheep genome was sequenced in 2012. (The International Sheep Genomics Consortium etc., 2010). The combination of the decoded genome with the high-density SNP-chip enable the discovery of significant genetic polymorphism, for meat and dairy productivity.

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

**Materials and methods.** The research was conducted during 2020-2021 at CMSP «Hvinevichi» RB.

The analysis was performed in the DNA laboratory of Grodno State Agrarian University. The experiments were performed on a population of Karakul sheep (10), Prekos (10), and Texel (10).

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

Nuclear DNA extraction from FABRIC by perchlorate method

1. 0.1 g of tissue is washed with distilled water, shredded with scissors, and transferred to a

1.5 ml microcentrifuge tube.

2. 200 µl of 1xSTE buffer is added (this mixture can be kept in the refrigerator for 24 hours).

3. 75 µl of 10% SDS is added to the test tube.

4. Add 10 µl of proteinase K (freshly prepared), 12 µl after 2 days storage of the solution. Stirwellon a vortex.

5. The sample is placed in a thermostat overnight at 37 ºC.

6. 50 µl of 5M sodium perchlorate solution is added to the tube with the lysate.

7. Add 300 µl of CIA (chloroform) to the test tube.

8. Stir vortex intensively.

9. Centrifuge for 10 min at 13000 rpm.

10. The upper DNA-containing phase is carefully transferred into a clean 1.5 ml tube without touching the intermediate layer.

11. Repeat points 7, 8, 9, 10.

12. 300 µl of 96% ethanol is added to the test tube containing the DNA phase and shaken until a «jellyfish» of DNA appears.

13. DNA is transferred to a 0.6 mL tube with 150 µl of 70 % ethanol using a pipette.

14. After 5 - 10 min the alcohol is removed and the DNA is dried until transparent.

15. Dissolve the DNA in 100 µl of DNA storage buffer.

16. Place the tube with DNA in the freezer for storage.

Composing the reaction mixture

Polymerase chain reaction (PCR) is used for DNA amplification. An optimally chosen composition of the reaction mixture, as well as temperature and time regimes of the PCR are essential for the success of the reaction. Table 1 shows the composition of the reaction mixture and the concentrations of the reagents used for a total volume of 25 µl.

Table 1 – Composition of the reaction mixture and concentrations of the reagents used

| Component | Concentrationpersam |
|---|---|
| 1 x | 1 |
| 2 or 50 mM 2 | 2-5 |
| DSTF | 2-4 |
| Primer | 1-2 p |
| Primer | 1-2 p |
| Taq | 0,5-1,5 |
| DN | 0,5-1 µ |
| $H_2O$ | up to 25 µ |

Performing Restriction

After the amplificant is obtained, a restriction analysis of DNA (PFPL - restriction fragment length polymorphism) is performed by selecting appropriate restriction enzymes, taking into account the temperature modes of restriction.

Detection of the obtained results.

Restriction fragments are analyzed by gel electrophoresis (on a 2-4 % agarose gel stained with ethidium bromide at 120-140V for 30-60 min) fol-

lowed by visualization on a GelDoc XR+ gel-documentation system, BIO-RAD.

Genetic and statistical analysis of the results was performed using formulas. The frequency of genotypes was calculated using the formula: p = $n / N$, wherep – frequency of a certain genotype, n - number of animals with a certain genotype, N – total animals. C

The frequency of alleles was calculated using the formula: P(A) = $2N1+N2 /2n$ ,where P - frequency of occurrence of the allele; A - allele; N1 - number of homozygotes for the studied allele; N2 - number of heterozygotes; n - sample volume of animals.

The allele frequencies (for two-allele systems) were determined using the formulas (1), (2).

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

q (BB + AB) /2n. (2)

whreP(A)) – A allelefrequency;

AA, BB – number of individuals with heterozygous genotype;

AB – the number of individuals with the heterozygous genotype;

n – number of individuals in groups; q(B) – B allele frequency.

Genetic equilibrium was determined using the $\chi 2$ test, according to the Gardi-Weinberg, by the formula (3): $\chi 2 = (\Phi F – T)2 / T$, (3)

where F is the actual number of individuals in the population with

**Results and discussion.** Prolactin is a protein hormone produced by specialized cells of the anterior lobe of the vertebrate pituitary gland.

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

Genetic structure of the studied sheep groups by prolactin gene (PRL)

Genotyping of sheep by prolactin gene (PRL) The following primers were used to amplify a fragment of the prolactin gene:

1 for - 5’ ACCTCTCTTCGGAAATGTTCA – 3’

2 rev – 5’ CTGTTGGGCTTGCTCTTTGTC– 3’.

PRL: Hot-start PCR program - 3 min at 95 $^0$C; 30 cycles: denaturation - 1 min at 94 $^0$C, annealing - 2 min at 58 $^0$C, synthesis - 2 min at 72 $^0$C; completion - 5 min at 72 $^0$C.

HaeIII endonuclease was used for restriction of the amplified region of the PRLR gene. 2 μl of restrictase buffer, 1 μl of HaeIII endonuclease, 2 μl. H2O.The reaction was performed at 37 °C.

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

The following genotypes were identified by cleavage of the amplification products with HaeIIIrestrictase at 37 $^0$C.

PRL$^{AA}$ – 1400 b.p, 510 b.p 360 b.p. 150 b.p

PRL$^{AB}$ – 1400 b.p, 530 b.p, 510 b.p. 360 b.p. 150 b.p;

PRL$^{BB}$ – 1400b.p, 530 b.p, 360 b.p. 150 b.p.

Analysis of prolactin gene polymorphisms revealed the presence of two alleles, PRLA and PRLB, and three genotypes, homozygous (PRLAA, PRLBB;) and heterozygous (PRLAB) (Table 2)

Table 2 – Frequency of PRL gene alleles and genotypes in sheep

| Indicator | n | Genotypefrequency | | | | | | Allelefrequency | | $\chi^2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | AA | | AB | | BB | | | | |
| | | n | % | n | % | n | % | A | B | |
| Texel | | | | | | | | | | |
| O | 10 | 7 | 70,0 | 1 | 10,0 | 2 | 20,0 | 0,75 | 0,25 | 1,579 |
| 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 | 6 | 60,0 | 4 | 40,0 | - | - | 0,80 | 0,20 | 2,5 |
| E | | 8 | 80 | 2 | 20 | - | - | | | |

*O – actual value, E – theoretically expected rate*

Prolactin polymorphism is characterized by a high (0.75) A allele concentration in Texel and Karakul breeds (0.80), medium PRL$^{A}$ in Precos (0.55) and low (0.25) PRL$^{B}$ allele in Texels and Karakul, as reflected by the presence of homo- and heterozygous genotypes: PRL$^{AA}$ – 7 (70,0 %)in texels, 6 (60,0 %) in karakulas, 4 (40%) in prakos. PRL$^{BB}$ - 2 (20 %) in texels and 3 (30.0 %) precocels, absent in karakul.; PRL$^{AB}$ - texels 1 (10.0 %), precocels 3 (30.0 %0 and karakul 4 (40.0 %).

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

In a group of Karakul sheep, the B-allele was found only in heterozygotes.This distribution of frequencies is probably a consequence of homozygotization predominantly for one allele and can lead to the loss of the gene variant.

In our studies, two alleles A and B with frequencies of 0.80 and 0.020, respectively, were found in Karakul sheep, and the homozygous genotype AA (60 %) is more common. The x values (1.579 - 2.5) indicate genetic equilibrium in the studied animal population. As a result of the study by

PCR-PFPL method, the polymorphism of the allelic spectrum of PRL genes of Texel, Prekos and Karakul sheep was established. The data obtained can be used as a genetic characteristic of the sheep population of this breed and can also be used in breeding and pedigree work aimed at preserving genetic diversity.

Genetic structure of the studied groups of sheep according to the α-casein gene(CSN1S1)

Casein is the main protein in all mammalian milk. It belongs to a group of proteins called phosphoproteins). The alpha-casein group is 43-55 %, beta-casein 24-35 %, kappacasein 8-15 %, gamma-casein group 3-7 %.

Alpha-S1-casein (αS1Cn) is the major fraction of casein, which consists of a mixture of two proteins, a major and a minor component, which have the same primary structure but differ in the degree of phosphorylation.

Genotyping of sheep for the α-casein gene (CSN1S1) The following primers were used to amplify a fragment of the prolactin gene:

CSN1S1-F 5′- GGTGTCAAATTTAGCTGTTAA-3′

CSN1S1-R 5′- GCCCTCTTCTCTAAAAAGGTT-3′.

CSN1S1: PCR-program "hot start" - 5 min at 94 $^0$C; 35 cycles: denaturation - 30 sec at 95 $^0$C, annealing - 30 sec at 62 $^0$C, synthesis - 45 sec at 72 $^0$C; completion - 5 min at 72 $^0$C.

MboII endonuclease was used to restrict the amplified portion of the GH gene. 2 μl of restrictase buffer, 1 μl of MboII endonuclease, 2 μl. H2O.The reaction was performed at 37 °C.

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

CSN1S1 $^{AA}$ – 160 b.p., 146 b.p. 66 b.p

CSN1S1$^{AC}$ – 306 b.p., 160b.p., 146 b.p. 66 b.p;

CSN1S1$^{CC}$ – 306 b.p., 66 b.p.

Table 3 – Incidence of alleles and genotypes of the CSN1S1 gene in sheep

| Indicator | n | Genotypefrequency | | | | | | Allelefrequency | | $\chi 2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | AA | | AC | | CC | | A | B | |
| | | n | % | n | % | n | % | | | |
| Texel | | | | | | | | | | |
| O | 10 | 7 | 70,0 | 2 | 20,0 | 1 | 10,0 | 0,75 | 0,25 | 1,60 |
| E | | 5 | 50 | 2,5 | 25 | 2,5 | 25 | | | |
| Prekos | | | | | | | | | | |
| O | 10 | 5 | 50,0 | 3 | 30,0 | 2 | 20,0 | 0,70 | 0,30 | 2,53 |
| E | | 4, | 40 | 2 | 20 | 4 | 40 | | | |
| Karakulskaya | | | | | | | | | | |
| O | 10 | 6 | 60,0 | 4 | 40,0 | - | - | 0,80 | 0,20 | 0,4 |
| E | | 5 | 50 | 5 | 50 | - | - | | | |

*O – actual value, E – theoretically expected rate*

*A-allele frequency in our study group.*

The CSN1S1 gene genotypes are characterized by a high (0.75) concentration of the A allele in Texel and Karakul breeds (0.80), an average of the CSN1S1A allele (0.70) in Prekos and a low (0.25) CSN1S1B allele in Texels and Karakul, as reflected in the presence of homo- and heterozygous genotypes: $CSN1S1^{AA}$ – 7 (70,0 %) in texels, 6 (60.0 %) in karakulas, 5 (50 %) in precocci. $CSN1S1^{SS}$, 1 (10 %) in texels and 2 (10.0 %) precocels, absent in karakul.; $CSN1S1^{AC}$, 2 (20.0 %) in texels, 4 (40.0 %) in precocels and 4 (40.0 %) in karakul.

The frequency of the A-allele of the prolactin gene in all experimental groups significantly exceeds the frequency of the B-allele. In Karakul sheep, the C-allele was detected only in the heterozygotes, the frequency of which is -40 %. The maximum frequency of A-allele is recorded in the group of Texel (70 %) and Karakul 60 %.

The x values (0.4 - 2.53) indicate genetic equilibrium in the studied animal population.

Table 4 – Characteristics of Karakul sheep by milk productivity

| | $PRL^{AA}$ | $PRL^{AB}$ |
|---|---|---|
| Productivity, l | 10,5 ± 0,5 | 9,4 ± 0,5 |
| Fat, % | 7,06 ± 0,05 | 7,04 ± 0,05 |
| Drymatter, % | 18,1 ± 0,08 | 17,94 ± 0,08 |
| Protein, % | 5,02 ± 0,04 | 4,95 ± 0,03 |
| Casein, % | 4,06 ± 0,03 | 4,01 ± 0,02 |

Sheep with genotype AA had a high milk productivity of 10.5 liters compared to genotype AB 9.4 in their higher content. Sheep with genotype AA produce more milk compared to genotype AB. Accordingly, sheep with genotype AA had a high fat content of 10.5 %, dry matter 18.1 %, protein

5.02 and casein 4.06 compared to genotype AB fat 7.04 %, dry matter 17.94 %, protein 4.95 % and casein 4.01 %.

**Conclusion**

1. As a result of the study by PCR-PFPL method, the polymorphism of the allelic spectrum of genes, PRL, CSN1S1 of Texel, Prekos, and Karakul sheep were established. The data obtained can be used as a genetic characteristic of the sheep population of these breeds and can also be used in breeding and pedigree work aimed at preserving genetic diversity.

2. As a result of the study, it was found that milk productivity is influenced by genotype $PRL^{AA}$ sheep with this genotype are more productive than those with other genotypes.

The data obtained can be used as a test of the state of the breed gene pool and prediction of productivity.

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