The influence of complex genotypes in the PRL and β-LG genes in Lacon sheep breed on cheese producing

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Abstract. For the first time, an analysis of the distribution of allele variants in the PRL and β-LG genes was conducted on the Russian population of Lakon sheep. The research established the influence of polymorphism in the studied genes on the quantitative and qualitative characteristics of Adyghe cheese. The analysis of data on 248 Lakon sheep showed a positive correlation between β-LG^{BB} and PRL^{BB} – with protein content in milk and better technological qualities for cheese production. The highest fat content in milk was observed in sheep with the β-LG^{BB} genotype (7.14%). The cheese curd from milk of all genotypes was characterized by optimal density and good elasticity. The yield of Adyghe cheese obtained from milk of animals with the genotype PRL^{BB}β-LG^{BB} was higher compared to the same parameter from milk of sheep with the genotypes PRL^{AA}β-LG^{AA}, PRL^{BB}β-LG^{AA}, PRL^{AA}β-LG^{BB} by 5.5, 4.5, and 3.0%, respectively, and it also had a higher fat content by 3.04, 2.90, and 1.10%, respectively. The practical significance of the obtained data lies in the prospect of selecting carriers of desirable alleles of the PRL and β-LG genes for targeted selection of parental pairs and obtaining a larger number of offspring with homozygous genotypes. Targeted selection will provide a higher proportion of sheep in the herd with better quantitative and qualitative indicators of milk productivity for with better parameters for cheese production. The work was carried out by order of the Ministry of Agriculture of the Russian Federation No. 082-03-2023-213 dated March 07, 2023.

1 Introduction

Sheep are one of the oldest, most versatile, and adaptable domestic animals. Thanks to these characteristics, as well as the variety of products obtained from them, sheep as farm animals have become the most widespread. In recent years, there has been a noticeable trend worldwide towards increasing the share of dairy sheep productivity. Interest in dairy

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sheep farming is also growing in Russia, as evidenced by a 7.5-fold increase in sheep milk production from 2012 to 2019, and a 14.5-fold increase compared to 2000 [1].

The growing interest in sheep milk determines the expansion of research directions based on the use of modern molecular genetic methods to identify desirable allelic variants of genes associated with sheep milk productivity. Such an approach will contribute to the effectiveness of breeding work and accelerate its development.

There is sufficient evidence of the association between genotypes of beta-lactoglobulin (β-LG), prolactin (PRL), kappa-casein (CSN3), and other genes with milk productivity and cheese-making qualities of cattle. In sheep breeding, the marker-associated approach based on DNA genotyping and selection of animals with desirable genotypes is promising [2, 3]. Among the candidate genes that affect important economic traits of dairy sheep, the genes PRL, and β-LG have been identified. In several studies, it has been demonstrated that β-LG polymorphism is significantly associated with milk yield, fat, protein, and lactose content, cheese yield, and composition. The influence of PRL on the quantitative and qualitative indicators of sheep milk productivity has been established [4, 5, 6].

However, there is still insufficient research on the influence of polymorphism of different genes on sheep milk productivity.

Therefore, the study of dairy sheep bred in the Russian Federation, the determination of gene polymorphism affecting their cheese productivity, is an important task.

2 Material and methods

The scientific research was conducted during the period of 2019-2021 at the farm "Nikolaev" in the Krasnodar region of the Crimea district. The object of the study was the Lacon sheep breed (n = 248). Animal experiments were conducted in accordance with the principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.

Molecular genetic analysis was carried out in a licensed laboratory of immunogenetics and DNA technologies, the genetics and biotechnology department of the VNIIOK branch of the Federal Scientific Center "North Caucasus Federal Agrarian Scientific Center" (certificate ПИ-77 No. 008326 dated April 18, 2018).

DNA isolated from whole blood of animals served as biological material for the research. Peripheral blood was collected by puncturing the jugular vein of sheep into vacuum tubes of the Vacuette type with the addition of ethylenediaminetetraacetic acid (EDTA-K2) as an anticoagulant at a final concentration of 2.0 mg/ml. With adherence to the temperature regime in a thermos bag with coolants, the blood was delivered to the laboratory within a day.

Genomic DNA was isolated using the commercial kit "DiatomtmDNAPrep200" according to the protocol provided by the manufacturer, LLC "Laboratory Isogen", Russia. The yield of pure DNA was 3-5 μg from 100 μl of blood with an absorption coefficient of 260/280 in the range of 1.6-2.0.

Commercial kits "GenPak™ PCR Core" (Isogen, Russia) were used for polymerase chain reaction (PCR) to amplify DNA. Master mixes contained all necessary reaction components, including Taq DNA polymerase inhibited for "hot start", a mixture of highly purified 2'-deoxynucleoside-5'-triphosphates (dATP, dTTP, dGTP, dCTP), and a dye for electrophoresis.

The PCR-restriction fragment length polymorphism (PCR-RFLP) method was used to genotype the studied sheep population for the genes PRL, and β-LG on a programmable four-channel thermocycler "Tertsik" from DNA Technology (Russia) in a total reaction volume of 20 μl using specific nucleotide sequences (primers) synthesized in the scientific-production laboratory "Syntol" (Moscow).

The number and length of the restriction fragment in an agarose gel (PRL - 3.0%, β-LG - 1.8%) of different concentrations with the presence of 10.0% ethidium bromide (10.0 μl) were determined by the horizontal gel electrophoresis method under ultraviolet light. The standard set M50 "GenePakDNAMarkers" (Isogen, Russia) was used as a marker of molecular masses.

Methodology for determining milk productivity of sheep and milk suitability for cheese production. Milk productivity was determined by conducting control milkings of ewes every 14 days, as well as using data from the farm's zootechnical and breeding records, such as annual reports and primary zootechnical records. Using the "Lactoscan M" analyzer, according to the research protocol, the qualitative indicators of milk - fat and protein content - were determined. Milk samples for research were selected in accordance with GOST 26809.1-2014.

Cheese of the "Adyghe" type was made from milk obtained from sheep of different genotypes for the prolactin and beta-lactoglobulin genes at the cheese factory "Dolina Lefkadia", and its organoleptic characteristics were evaluated according to GOST 32263-2013 "Soft cheeses. Technical conditions".

Mathematical data processing. Digital research material was processed using the BioStat computer program, the "Microsoft Office" software package, and the method of variation statistics with determination of the significance of differences by the Student's t-test at three levels of probability ($p < 0.05$; $p < 0.01$; $p < 0.001$).

3 Results and discussion

Genetic polymorphism in the PRL gene. As a result of molecular genetic research, a deletion g.460_483del located in the second intron of the prolactin gene was identified in Lacon sheep breed. Two alleles were detected: PRL^{A} and PRL^{B}, determining three genotypes: PRL^{AA}, PRL^{BB}, and PRL^{AB} with different frequencies of occurrence (Table 1).

Table 1. Frequency of occurrence of alleles and genotypes of the prolactin gene

Gene/ genotypes	n	Frequency of occurrence ± sp		H_{obs}	H_{ex}	Heterozygosity test	χ^2
		alleles	genotypes				
PRL^{AA}	186	A – 0.81±0.019 B – 0.19±0.035	0.75±0.032	0.15	0.22	-0.07 $H_{obs} < H_{ex}$	52.95
PRL^{AB}	32		0.13±0.059				
PRL^{BB}	30		0.12±0.059				
Notes: sp - error in genotype/allele frequency; significance level $p < 0.05$.							

A high frequency of occurrence of the PRL^{A} allele and a low allele of PRL^{B} were established, the prevalence of the PRL^{AA} genotype was 75.0%.

The level of observed heterozygosity (Hobs) of the PRL gene is 31.8% lower than expected (Hex). The heterozygosity test for the PRL gene was negative and amounted to 0.07. The Pearson criterion (χ^2) in the PRL gene was 52.95, which indicates that the actual distribution of genotypes does not correspond to the theoretically expected one due to the predominance of homozygous individuals.

Genetic polymorphism in the β-LG gene. The rs430610497 mutation was studied in the β-LG gene, that leads to the replacement of the CAC codon by TAC and, accordingly, the amino acid histidine by tyrosine (p.Tyr36His). Two alleles $β\text{-}LG^{A}$, $β\text{-}LG^{B}$ and three genotypes $β\text{-}LG^{AA}$, $β\text{-}LG^{BB}$, $β\text{-}LG^{AB}$ with different frequencies of occurrence were identified, while the allele $β\text{-}LG^{B}$ was almost twice as dominant over the $β\text{-}LG^{A}$ allele (Table 2).

Table 2. The frequency of occurrence of alleles and genotypes of the beta-lactoglobulin gene

Gene/ genotypes	n	Frequency of occurrence ± sp		H_{obs}	H_{ex}	Heterozygosity test	χ^2
		alleles	genotypes				
β-LG^{AA}	27	A – 0.34±0.028 B – 0.66±0.023	0.11±0.060	0.86	0.81	+0.05 Hobs > Hex	0.26
β-LG^{AB}	115		0.46±0.046				
β-LG^{BB}	106		0.43±0.048				
Notes: sp - error in genotype/allele frequency; significance level p <0.05.							

The test of heterozygosity, indicating the level of genetic diversity of the population, in the β-LG gene had a positive value and amounted to 0.05. According to the Hardy-Weinberg equation and the χ2 criterion, it was proved that the genetic balance in the β-LG gene is observed, the Pearson criterion did not exceed the critical value (p <0.05).

The degree of homozygosity (Ca) indicating the consolidation of genes in the PRL gene was 69.22%, in the β-LG gene - 55.12%. The level of polymorphism, an indicator of the number of active effective alleles (Na), was 1.45 and 1.82 for the PRL and β-LG genes, respectively. The degree of genetic variability (V) was relatively uniform in the PRL genes - 30.6 and β-LG - 44.6.

The value of information polymorphism (PIC) is determined by the ability of the marker to detect population polymorphism depending on the number of detected alleles and the distribution of their frequencies. For genetic markers, such as PRL, β-LG, the calculation of the PIC value showed their approximately equal selection value - 0.31 and 0.45, respectively.

Milk productivity of sheep of the Lacone breed of complex genotypes for the PRL and β-LG genes. Due to the polygenic nature of the formation of lactation traits, the analysis and forecast of milk productivity indicators should be carried out taking into account the genotype for several genes.

Of the nine theoretical possible complex genotypes, all nine genocomplexes were identified in the study of livestock. Animals with the complex genotype PRL^{AA}β-LG^{AB} (33.5%) and PRL^{AA}β-LG^{BB} (32.2%) were the most common, four genocomplexes - PRL^{AA}β-LG^{AA}, PRL^{BB}β-LG^{AB}, PRL^{AB}β-LG^{BB}, PRL^{AB}β-LG^{AB}, had a frequency of occurrence from 5.2 to 9.3% (Figure 1).

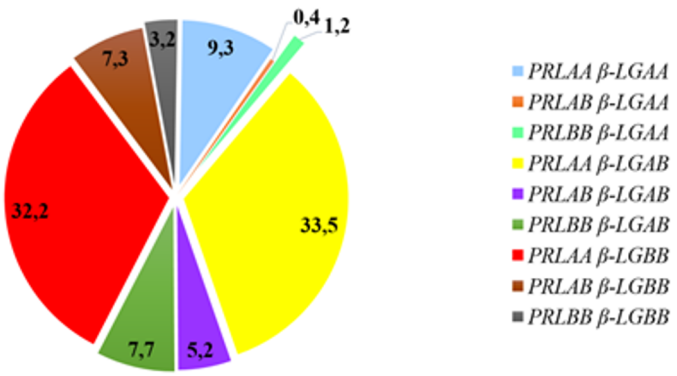

Fig. 1. Frequency of occurrence (%) of complex PRL and β-LG genotypes in Lacon sheep.

The frequency of occurrence of other complex genotypes was insignificant. Thus, the frequency of occurrence of genotypes PRL^{BB}β-LG^{BB}, PRL^{BB}β-LG^{AA} did not exceed 5.0%

and amounted to 3.2 and 1.2%, respectively. The lowest occurrence was noted in the PRL^{AB}β-LG^{AA} genocomplex (0.4%).

The impact on milk productivity and milk quality of complex genotypes, the frequency of which is higher than 5.0%, was studied. The results are presented in Figure 2.

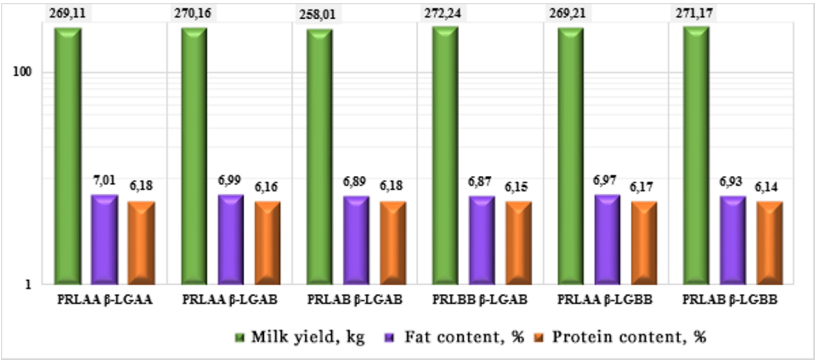

Fig. 2. Indicators of milk productivity in sheep of the Lacon breed of complex genotypes for the PRL and β-LG genes.

Sheep with PRL^{BB}β-LG^{AB} (272.24 kg) and PRL^{AB}β-LG^{BB} (271.17 kg) genotypes differed in the highest rates of milk yield per lactation. Sheep of the other two genotypes, PRL^{AA}β-LG^{AA} and PRL^{AA}β-LG^{BB}, had similar milk yields (269 kg of milk). A high fat content in milk was noted in sheep with the PRL^{AA}β-LG^{AA} (7.01%) and PRL^{AA}β-LG^{BB} (6.97%) genotypes. The protein content in sheep milk was in the range from 6.14 to 6.18%, had a fairly similar value in all presented complex genotypes.

The effect of complex genotypes in the PRL and β-LG genes on the producing cheeses of the Adyghe type. Milk for cheese production was selected from genotyped animals for the PRL and β-LG genes. Ewes were subdivided into four groups with genotypes PRL^{AA}β-LG^{AA}, PRL^{BB}β-LG^{BB}, PRL^{BB}β-LG^{AA} and PRL^{AA}β-LG^{BB}, since the greatest differences were found between these groups in terms of milk production.

The fat content in milk in sheep with the PRL^{AA}β-LG^{AA} genotype was 7.0%, which is higher than in the milk of sheep with the PRL^{BB}β-LG^{AA}, PRL^{BB}β-LG^{BB} and PRL^{AA}β-LG^{BB} genotypes by 0.01, 0.02 and 0.04 percent. respectively. The protein content in sheep milk had a similar value in all presented gene complexes and ranged from 6.17 to 6.19% (Table 3).

Table 3. Characteristics of the suitability of sheep milk of complex genotypes for the PRL and β-LG genes for cheese producing

Genotypes	Parameters				
	Fat content, %	Protein content, %	Acidity, °T	Density, g/cm^3	Coagulation time, min
PRL^{AA}β-LG^{AA}	7,01	6,18	20	1031	60
PRL^{BB}β-LG^{BB}	6,99	6,19	24	1028	57
PRL^{BB}β-LG^{AA}	7,00	6,19	19	1032	62
PRL^{AA}β-LG^{BB}	6,97	6,17	20	1030	61

The density and titratable acidity of milk in all groups were within the parameters established for sheep's milk. An important indicator of the suitability of milk for making cheese is the rate of rennet coagulation. The milk of sheep with the PRL^{BB}β-LG^{BB} genotype coagulated faster than the milk of sheep with the PRL^{AA}β-LG^{AA}, PRL^{BB}β-LG^{AA}, and PRL^{AA}β-LG^{BB} genotypes, respectively, by 3, 5, and 4 minutes. The clot from the milk of all groups was characterized by low density and good elasticity.

From the milk of ewes of selected groups, cheese of the Adygei type was produced. Physical and chemical parameters of cheese from sheep milk of complex genotypes of prolactin and beta-lactoglobulin are shown in Table 4.

Table 4. Physical and chemical parameters of cheese from sheep milk of complex genotypes for the PRL and β-LG genes

Parameters	Complex genotype			
	$PRL^{AA}β\text{-}LG^{AA}$	$PRL^{BB}β\text{-}LG^{BB}$	$PRL^{BB}β\text{-}LG^{AA}$	$PRL^{AA}β\text{-}LG^{BB}$
Milk yield, l	3,0	3,0	3,0	3,0
Yield of cheese, %	26,8	32,3	27,8	29,3
Mass fraction of fat in dry matter, %	55,26	58,30	55,40	57,20
Moisture, %	60,0	60,7	66,3	65,7
Cheese fat content, %	20,33	23,22	22,36	21,65
Dry matter, %	40,0	39,3	33,7	34,3
Cheese pH	4,45	4,39	4,41	4,43

The yield of cheese from ewes carrying the $PRL^{BB}β\text{-}LG^{BB}$ genotype was higher compared with the $PRL^{AA}β\text{-}LG^{AA}$ genotype by 5.5%, with the $PRL^{BB}β\text{-}LG^{AA}$ genotype by 4.5%, and with the $PRL^{AA}β\text{-}LG^{BB}$ genotype by 3.0%.

The mass fraction of fat in cheese produced from milk from sheep with the $PRL^{BB}β\text{-}LG^{BB}$ genotype, after 18 hours of exposure in terms of dry matter, was 58.30%, which is higher compared to cheese from milk $PRL^{AA}β\text{-}LG^{AA}$, $PRL^{BB}β\text{-}LG^{AA}$ and $PRL^{AA}β\text{-}LG^{BB}$ at 3.04; 2.90; and 1.10%, respectively. Cheese from the milk of sheep with the $PRL^{BB}β\text{-}LG^{BB}$ genotype was also characterized by a lower moisture content, which amounted to 50.0%, while in cheese from the milk of sheep with the $PRL^{AA}β\text{-}LG^{AA}$ genotypes, this indicator was at the level of 56.3%.

All the resulting cheeses had a compacted, elastic outer layer, without a pattern, of a delicate, homogeneous texture. Cheese produced from milk from sheep carrying the $PRL^{AA}β\text{-}LG^{AA}$ genotype had a more sour taste; from milk from sheep of other genotypes, it had a pure, sour-milk taste. The color of the dough of cheese from the milk of sheep with the $PRL^{AA}β\text{-}LG^{AA}$ genotype was white with a creamy tint, while for cheese from the milk of other genotypes, it was white with a slightly yellowish tint.

Thus, the milk of sheep with the complex genotype $PRL^{BB}β\text{-}LG^{BB}$ has the best qualities in the cheese producing.

4 Conclusion

The conducted research has provided data of theoretical and practical significance, which complements and expands the knowledge about the polymorphism of PRL and β-LG genes controlling the quantitative and qualitative characteristics of milk in the Lakon sheep.

When producing Adyghe cheese, it is necessary to take into account that the least amount of milk per unit of product is spent when using milk from sheep with a complex genotype of $PRL^{BB}β\text{-}LG^{BB}$. Compared to using milk from sheep with the genotype $PRL^{AA}β\text{-}LG^{AA}$, this reduced costs in monetary terms by 16.9%.

To improve the milk productivity traits of the Lakon sheep, it is recommended to include genotyping of animals for PRL and β-LG genes in the selection and breeding program. It is also necessary to select carriers of desirable genotypes, taking into account that animals with the PRL^{AA} and $β\text{-}LG^{AA}$ genotypes are the most valuable for selection for increased milk production, while $PRL^{BB}β\text{-}LG^{BB}$ genotypes are preferred for selection for improved milk suitability for cheese.

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