The influence of αs1-casein and αs2-casein genes on milk productivity of alpine and nubian goats

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Abstract. An important factor affecting the performance of goats is the presence in their body of various genetic variants of casein, the main protein of milk. Studies show that Alpha-S1-casein and Alpha-S2-casein play an important role in the milk production of goats, determining the quality and characteristics of goat milk. This article presents the data obtained as a result of the study of the polymorphism of CASN1S1 and CASN2S2 genes in the Alpine and Nubian goat breeds. 124 goats of the Alpine breed and 98 goats of the Nubian breed were progenotyped. It was shown that animals with the $CSN1S2^{AA}$ genotype exceeded the carriers of the $CSN1S2^{AB}$ and $CSN1S2^{BB}$ genotypes with high reliability in terms of the content of the mass fraction of protein and casein in milk. According to the highest milk yield and the minimum number of somatic cells in milk, carriers of the $CSN1S2^{AA}$ genotype were distinguished, which were significantly superior to animals of other genotypes. To increase cheese production, it is preferable to select animals of the complex genotype $CSN1S1^{CC}$/$CSN1S2^{AA}$.

1 Introduction

Dairy goat breeding is an important area of agriculture, which is undergoing significant development in Russia [1-7]. With the ever-increasing demand for quality dairy products, research into the genetic traits of livestock plays a critical role in improving the productivity and efficiency of goat production [3].

On the one hand, goat's milk and dairy products are a valuable source of nutrients such as protein, calcium, vitamins and minerals, while being more tolerable than cow's milk. On the other hand, thriving dairy goat farming makes a significant contribution to agriculture, contributing to rural development.

Significant differences in the incidence of certain variants of casein in different goat breeds in different regions can be explained by specific breeding objectives in terms of preferred features of milk production and processing systems, specific properties for nutrition and human health, and adaptation to unique environmental features. Casein is the

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main insoluble protein component of milk, making up about 80% of the total milk protein fraction [1]. Milk caseins have basic nutritional value, since they provide the intake of calcium and phosphorus into the body, which is especially important during the dairy period of feeding offspring [3]. In goats, as in other mammals, there are four evolutionarily conserved casein genes. The genes encoding alpha-S1-, beta-, alpha-S2-, and kappa-casein are CSN1S1, CSN2, CSN1S2, and CSN3, respectively. In our study, we focused on the CSN1S1 and CSN1S2 genes [4, 6, 7].

We chose the Alpine and Nubian goat breeds, as they are one of the most popular and common in Russia. The Alpine breed is known for its strength and endurance, as well as excellent milk qualities. The Nubian breed, in turn, attracts goat breeders with its large size and high fat content in milk.

Therefore, the study of dairy goats – Alpine and Nubian – bred in the Russian Federation, the determination of gene polymorphism affecting their milk quality productivity, is an actual task.

2 Material and methods

The object of the study was 124 goats of the Alpine breed (Farm "Bylinkino", Lukhovitsy, Moscow region) and 97 goats of the Nubian breed (Peasant Farm "Lyashenko S.N.", Pushkinsky, Moscow Region). Genotyping was carried out in the genetic laboratory of the Service Laboratory for Complex Analysis of Chemical Compounds of the Russian State Agrarian University-Moscow Timiryazev Agricultural Academy. DNA was isolated from blood samples using the ExtractDNA Business & Cells kit (Eurogen, Moscow) in accordance with the manufacturer's protocol. For genotyping, the CSN1S1 B1, B2, B3, B4, C, and CSN1S2 A and B alleles were selected, coupled with the formation of αs1-casein and αs2-casein proteins, respectively. Genotypes determined by RT-PCR using the primers given in Table 1.

Table 1. Oligonucleotide primers for RT-PCR and positions of nucleotide substitutions that determine alleles of CSN1S1 genes

Alleles CSN1S1	Nucleotide Position	Primers (5′ – 3′)
C	5048–5070 Complementary to: 5222–5241	Forward: AACAGCACTGTTAAATGTATAAT Reverse: TCATCAGTTAAGCTACACAA
B4	16914–16931 Complementary to: 17287–17304	Forward: AGAACAGTGGAAAGACTG; Reverse: CCCACACTGCATTCTAAT
B3	12064–12084 Complementary to: 12274–12294	Forward: TTAGTTTCCCATTCTTTACTC Reverse: GAAGCTCTAACATGATTTGAT
B2	5995–6016 Complementary to: 6284–6304	Forward: TTCAAATGGAAAAACATTCTCC Reverse: GTCAAATGTATAGGTACAGAT
B1	10463–10483 Complementary to: 10752–10773	Forward: GAAAAGAGAACATGTACTTTG Reverse: CATCTTCCTTTTGAATGTACTT

Amplification was carried out on a CFX96 device (BioRad, USA) in a volume of 20 µl, including 10 µl of 10x PCR buffer, 1 µl of MgCl2 (Synthol, Moscow), 0.2 µl of SynTaq DNA polymerase 5 U/µl (Synthol, Moscow), 2 µl of dNTP mixture (2.5 mM), 5.6 µl of

bidistilled water and 1.2 µl of DNA. For genotyping by alleles B1, B2, B3, C, a single amplification protocol was used: initial denaturation 97°C – 2 min, 31 cycles (94°C – 45 sec, annealing 55°C – 45 sec, elongation 72°C – 45 sec), final elongation 72°C – 10 min. To identify the nucleotide substitution G→A at position 139 of exon 17, which distinguishes the B4 allele from B3 (Table 2), a separate amplification protocol was selected: initial denaturation 97°C – 2 min, 29 cycles (94°C – 45 sec, annealing 55.8°C – 45 sec, elongation 72°C – 90 sec + 4 sec to each cycle), final elongation 72°C – 10 min.

Table 2. Position of nucleotides and amino acids in the CSN1S1 gene in goats

Alleles CSN1S1	Position of nucleotides and amino acids					
	nucleotides 16/17 (exon 3)	nucleotide 8 (exon 4)	nucleotide 23 (9 exon)	nucleotide 22 (exon 10)	nucleotide 14 (exon 12)	nucleotide 139 (exon 17)
B1	CA His^{8}	T Leu^{16}	C Ser^{66}	G Glu^{77}	G Arg^{100}	A Thr^{196}
B2		C Pro^{16}				
B3		C Pro^{16}			A Lys^{100}	
B4		C Pro^{16}			A Lys^{100}	G Ala^{196}
C	AT Ile^{8}	C Pro^{16}			A Lys^{100}	G Ala^{196}

The analysis of goat milk components by the following parameters: mass fraction of fat (MFF), protein (MPF, total), lactose, casein, monounsaturated fatty acids (FA), polyunsaturated FA, saturated FA, somatic cell count, differential somatic cell count (lymphocytes and polymorphonuclear neutrophils) was carried out at the L.K. Ernst Federal Research Center of Animal Husbandry based on the ONIS BioTekhZh using a CombiFoss 7 DC multiparametric automatic milk analyzer (FOSS, Denmark). Milk samples were collected individually and preserved using Broad Spectrum Microtabs II tablets (Bentley Instruments, USA) during control milkings for three months. Milk yield for 305 days of lactation was determined based on the results of control milkings. In the studied sample of goats, animals of 1, 2, 3 and older lactations were represented in equal proportions.

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

Of the 15 possible genotypes for the CSN1S1 gene, 14 were identified in alpine goats and 13 in Nubian goats. In the Nubian breed, the CC genotype was the most common and was detected in 41.0% of individuals, while in the Alpine breed it was not detected at all (Table 3).

In the Alpine breed, the B1B3 genotype was found with the highest frequency at the level of 30.0%, which has less frequency in Nubian goats. The B1B2, B2B3, and CB1 genotypes occurred with a similar frequency – from 3.0 to 7.0%, and the B4B4 genotype from 10.0 to 13.0% in the studied breeds.

Table 3. Genotype frequencies for the CSN1S1 and CSN1S2 genes in the studied animals of different breeds

Genotypes	Breed	
	Alpine (n=124)	Nubian (n=97)
Locus *CSN1S1*		
(B1, B1)	0,01	-
(B1, B2)	0,04	0,05

(B1, B3)	**0,30**	0,09
(B1, B4)	**0,12**	0,04
(B1, C)	0,07	0,04
(B2, B2)	0,06	-
(B2, B3)	0,03	0,04
(B2, B4)	0,02	0,05
(B2, C)	0,02	0,03
(B3, B3)	0,01	0,01
(B3, B4)	**0,15**	0,06
(B3, C)	0,04	0,02
(B4, B4)	**0,10**	**0,13**
(B4, C)	0,03	0,02
(C, C)	-	**0,41**
Locus *CSN1S2*		
AA	0,59	0,79
AB	0,14	0,21
BB	0,27	-

Genotype AB was found in the Alpine and Nubian breeds with similar frequencies (0.14 and 0.21, respectively). The greatest differences were noted in the genotype BB, which was found with a frequency of 0.27 in the Alpine breed and was not found in the Nubian breed.

High intra-breed and inter-breed polymorphism in the CSN1S1 and CSN1S2 genes was also identified (Table 4).

When analyzing the frequencies of the CSN1S1 gene alleles, the most pronounced interbreed difference was noted in the frequency of the C allele. Thus, in Nubian goats, its frequency reached 0.451, which is 5.3 times higher than among the Alpine breed animals. Smaller differences were found in the distribution of the B3, B2 and B1 alleles. Thus, the frequency of the $CSN1S1^{B3}$ allele was the highest - 0.264 in Alpine goats and two times lower in Nubian goats - 0.122. The $CSN1S1^{B2}$ allele was detected with similar frequencies in animals of the Alpine and Nubian breeds - 0.123 and 0.093. In relation to the $CSN1S1^{B1}$ allele, a low frequency was found in the Nubian (0.110), while in the Alpine breed this allele was detected with a frequency of 0.264.

No differences were found in the frequency of the $CSN1S1^{B4}$ allele, which was found in animals of all the studied breeds (from 0.22 to 0.26).

Polymorphism was also found in the CSN1S2 gene. In both breeds, the frequency of the $CSN1S2^{A}$ allele was higher than the $CSN1S2^{B}$ allele.

Table 4. Allele frequencies of the CSN1S1 and CSN1S2 gene in goats of different breeds

Allele	Breed	
	Alpine goats (n=124)	Nubian goats (n=97)
CSN1S1		
B1	0.27	0.11
B2	0.12	0.09
B3	0.27	0.12
B4	0.26	0.22
C	0.08	0.46
CSN1S2		
A	0,66	0,88
B	0,34	0,12

Analysis of milk productivity of goats of different genotypes for the highly polymorphic gene CSN1S1 did not reveal a reliable superiority of animals of one genotype over others.

This coincides with the results obtained by G. Cosenza et al. [2], who found that animals carrying alleles B1, B2, B3, B4, C of the CSN1S1 gene are characterized by approximately equal values of the main traits of milk productivity.

The minimum average level of milk productivity in the Alpine breed was 621 kg, the maximum - 795 kg, the average for all animals studied - 676 kg. The protein content in milk ranged from 2.05% to 3.75%, and the fat content - from 2.97% to 4.49%. The limits of the casein content in milk were from 2.09% to 2.53%. In the Nubian breed, the minimum average was 548 kg, the maximum was 596 kg, with an average of 567 kg. The protein content in the milk ranged from 2.44% to 3.96%, the fat content from 3.90% to 4.61%, and the casein content in the milk was more uniform, from 3.54% to 5.04%.

Comparison of milk productivity indices of goats of different breeds and different genotypes for the CSN1S2 gene allowed us to establish that, according to the parameters characterizing quantitative characteristics, goats of the heterozygous genotype $CSN1S2^{AB}$ stood out in the Alpine breed, which reliably ($p<0.001$) surpassed animals with homozygous genotypes in milk yield (Table 5).

Table 5. Milk productivity of goats of different genotypes according to the CSN1S2 gene

Traits	Genotype		
	CSN1S2^{AA}	*CSN1S2^{AB}*	*CSN1S2^{BB}*
Alpine goats (n=124)			
N	73	17	34
Yield, kg	689***[2]±2	708***[1,3]±4	632 ±3
Fat, %	3.90±0,09	3,76±0,18	3,92±0,14
Monounsaturated FA, %	1,12±0,03	1,05±0,05	1,12±0,04
Polyunsaturated FA, %	0,15±0,01	0,14±0,01	0,15±0,01
Saturated FA, %	2,56±0,07	2,43±0,15	2,60±0,13
Protein (total), %	3,09***[1]±0,05	2,75 ±0,22	2,92±0,11
Casein, %	2,34*[1]±0,04	2,23*[3]±0,04	2,10±0,06
Nubian goats (n=97)			
N	73	24	-
Yield, kg	567±3	568,02±4	-
Fat, %	4,13±0,07	4,23±0,11	-
Monounsaturated FA, %	1,92±0,04	1,81±0,07	-
Polyunsaturated FA, %	0,24±0,01	0,23±0,01	-
Saturated FA, %	4,35±0,03	4,44±0,05	-
Protein (total), %	3,49±0,07	3,40±0,12	-
Casein, %	4,51*[1]±0,08	4,43±0,07	-
Notes: *p<0,05; **p<0,01; ***p<0,001 when comparing genotypes *[1] CSN1S2^{AA} c CSN1S2^{AB}; [2] CSN1S2^{AA} c CSN1S2^{BB}; [3] CSN1S2^{AB} c CSN1S2^{BB}*			

When comparing homozygous genotypes with each other, a highly reliable superiority of $CSN1S2^{AA}$ over $CSN1S2^{BB}$ ($p<0.001$) was found. If we consider the qualitative composition of milk, then animals with the $CSN1S2^{AA}$ genotype had higher indices, reliably surpassing $CSN1S2^{BB}$ goats both in the mass fraction of total protein in milk ($p<0.001$) and in the casein level ($p<0.05$). Animals with the $CSN1S2^{AB}$ genotype also prevailed over $CSN1S2^{BB}$ in terms of casein levels. No reliable difference was found between Alpine breed animals of different genotypes in terms of saturated and unsaturated fatty acids in milk. When examining the productivity of Nubian breed animals, a reliable difference (at $p<0.05$) was found only when comparing casein levels in $CSN1S2^{AA}$ and $CSN1S2^{AB}$ animals.

A new stage of the present research consisted in the analysis of the parameters of milk productivity of goats with different combinations of alleles for the CSN1S1 and CSN1S2

genes to identify those animals that are carriers of such alleles in both genes that are associated with greater milk production or such alleles that determine a higher content of individual components of milk. Such a comprehensive approach to comparing animals of different genotypes made it possible to reveal that goats of the Nubian breed of the $CSN1S1^{CC}/CSN1S2^{AA}$ genotype had a reliably higher level of indicators characterizing the qualitative composition of milk (content of protein, casein and saturated fatty acids), whereas in the Alpine breed no genotypes were found whose owners reliably surpassed goats with other genotypes. To confirm this pattern, revealed both when comparing genotypes in each of the studied genes and in two genes together, additional studies are required on a larger number of animals, as well as with the involvement of other goat breeds.

4 Conclusion

The conducted studies allowed us to obtain information on the frequencies of genotypes and alleles, which has theoretical and practical significance and complements the knowledge of the polymorphism of the CSN1S1 and CSN1S2 genes, which control the quality characteristics of milk in dairy goats [5].

In the Russian population of goats of the Alpine breed, animals with the genotype B1B3 (locus CSN1S1) and genotype AA (locus CSN1S2) are most common. Animals with the genotype CC for the locus CSN1S1 were not found in the population, which necessitates the search for producers who are carriers of this allele. Their use will help to obtain goats both heterozygous and homozygous for the allele C, which is very important, since it is this allele in the homozygous state that determines the hypoallergenic qualities of milk.

In the group of goats of the Nubian breed, on the contrary, the genotypes CC and AA were characterized by the highest frequency. This means that among the goats of this breed, 41% of animals have the desired genotype for the CSN1S1 locus.

In the group of goats of the Alpine breed, animals with the $CSN1S2^{AA}$ genotype reliably exceeded carriers of the $CSN1S2^{AB}$ and $CSN1S2^{BB}$ genotypes in the content of the mass fraction of protein and casein in milk, and among the goats of the Nubian breed - only in the content of casein.

Considering that Nubian and Alpine goats have different characteristics of milk productivity according to production requirements, it is still worth emphasizing that Nubian goats, with a reliably lower milk yield, have a higher content of fat in milk (including different types of fatty acids) and protein in milk (including casein).

Also, to increase cheese production, it is preferable to select goats of both Nubian and Alpine breeds with a complex genotype $CSN1S1^{CC}/CSN1S2^{AA}$.

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