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GENE FREQUENCIES OF CAPRINE ALPHA S_1 CASEIN POLYMORPHISM IN MONTENEGRIAN BALKAN GOAT BREED

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The alleles and genotypes at the locus αS_1 - casein were determined for 98 goats of domestic Balkan goat breed from Montenegro distributed in two flocks.

The occurrence of two alleles (A and F) and three genotype forms (AA, AF and FF) were discovered by using the method of sequencing of cDNA and part of genomic DNA of αS_1 casein. High domination of the strong A allele (0.629) in comparison with the "weak" F allele with a frequency of 0.371 was obtained in this study. The knowledge of distribution on allelic variants at the locus αS_1 – casein in the population of Balkan goats will contribute to a better valorization and improving of this breed and the preservation of its genetic variability.

Key words: allele, alpha S_1 – casein, Balkan goat, frequency, polymorphism

INTRODUCTION

In many dairy goats, milk protein content ranges between 2.5 and 4.5%. The four types of caseins (αS_1-Cn, αS_2-Cn, β-Cn and κ-Cn) are the main components accounting in ruminants with 76 – 86% of the total protein (Jordana *et al.*, 1996; Ricordeau *et al.*, 1999; Caroli *et al.* 2006).

The casein at the locus αS_1 is highly polymorphic, with seven alleles recognized so far using different biochemical and molecular techniques (Feligini *et al.* 2005). Each allele is associated with a different level of casein synthesis. "Strong" alleles, such as αS_1-Cn A, B and C, are associated with high content (3.6 g/L) of αS_1 casein in milk; "medium" allele αS_1-Cn E is associated with 1.6 g/L of casein; "weak" alleles αS_1-Cn F and D are associated with 0.6 g/L.

The presence of the "null" allele αS_1-Cn O in the homozygote state has been associated with the total absence of α_{S1}- casein in milk (Boulanger *et al.*, 1984; Grosclaude *et al.,* 1994; Enne *et al.*, 1997; Grosclaude and Martin, 1997). Different alleles of αS_1-casein in goat milk has been recognized by biochemical and genetic methods, and several differences in composition, relative proportions and

physical-chemical properties have been elucidated (Martin, 1993; Greppi and Roncada, 2005).

Due to the important economic contribution of goat milk to dairy production in Montenegro the aim of this work was to determine the genetic frequencies at the locus αS_1-casein in the goat population of Balkan goat breed in Montenegro.

MATERIAL AND METHODS

The genotyping of alleles of áS_1 casein carried out for total 98 heads of Balkan goat breed distributed in two flocks (39 in one and 59 in the other).

Domestic Balkan goat breed is an autochthonous breed of the Balkan peninsula and it makes the majority of the goat population in Montenegro. There are several varieties of Balkan goat breed regarding the color of hair (red, black and multicolored). The system of breeding is usually extensive or semi extensive. All of these varieties are well adapted to the extensive semi arid conditions, with moderate milk yield (150 kg per lactation), and the content of fat and protein in milk of 3.38 and 3.30%, respectively. The milk is used mainly for production of home-made cheese.

DNA extraction, PCR amplification and sequencing. Blood samples (5 mL) were taken from randomly chosen goats from two flocks. Samples were collected in EDTA tubes and frozen at 20 ^{o}C. A standard phenol-chloroform DNA extraction method was used.

The complete coding sequence of αS_1 casein was amplified by PCR. A typical 40 cycles of PCR were carried out with the following PCR-mixture: 10 μL 10xPCR buffer (500 mM KCl, 200 mM Tris-HCl, pH 8.4); 8 μL of 25 mM $MgCl_2$; 8 μL 10 mM of each dNTP; 4 μL of 10 pmol of forward primer (m) designed: 5' - CATTCTTTACTCCTGGGAAAG - 3'; 4 μL of 10 pmol of reverse primer (p) designed: 5' - AGCACTTTTGGGAACAATTTC - 3'; 0.5 μL 2.5 U AmpliTaq DNA polymerase; 50 ng goat genomic DNA and H_2O to a final volume of 100 μL. Denaturation; 1' at 94^{o}C, annealing; 45" at 58^{o}C, extension; 1' and 30" at 72^{o}C. The PCR products were purified by Microcon (Millipore Corporation, MA) and sequencing was carried out by ABI PRISM®BigDye™ Terminator Cycle Sequencing Ready Reaction Kit and the automatic DNA - sequencer Model 377.

The frequency of alleles and genotypes were calculated using Hardy and Weinberg's method for equilibration in a population.

RESULTS AND DISCUSSION

Sequencing of complementary DNA (cDNA) and part of genomic DNA (gDNA) where the main coding region of alpha S_1 casein, exon 12 (fragment between 3' end of intron 11 and 5' end of exon 13, with length of approximately 750 base pears), showed numerous single base substitutions, deletions and insertions.

Comparison with standard sequence of αS_1 casein (Figure 1), the occurrence of two alleles (A and F) was found. The A allele should include alleles

A, B, C, D, E, and any other forms of 0 and F that were not tested for. In the Balkan goats, no case of the 0 - allele was found. This gives three possible genotype forms (AA, AF and FF).

cattctttactcctgggaaagggatgccatgatagatggtatccatgaaattgacaatatattttctcttgaatagGAACA
GCTTCTCAGACTGAAAAAATACAAAGTACCCCAGCTGgtaatgttttattataataatat
aaaattaagtctacagaattaaaataattaagtgaaatttactttgactaaattctacatcaaatcatgctagagcctcccaaat
gattcgttatactctggaatttattctgtatttattttcccaaattcagttctgagtggtgattccgtatatatgcctccttgtgaaa
gcacagatcattgccaaataaacattccatttaagaaaacagtgtagactctgtagaggaatcagggatcccatacattcaa
attgcttttgtcaaaattttctaagaaagaatcggcaagttgattgatctcatttgctctagacccgtttcctttatagttactttc
atttcctgacattgttttgaatattgaatgagcaatctcctattcagttatccctgaggctagcttgtggcaagtccaataaatgt
gttcataaagtctctccttcccctaatccctaagctctataaaatttgctatgttcatgagacctttgacaatattatgaagatttt
gttttgtttaattacagataagctatgatgtgtctggttaattagcatttttattttgaatgtaaatcaatgtcataaaactaataata
cattgttttttatttttttaaggaaattgttcccaatagtgctgag

Figure 1. Standard sequence of 750 bp with coding region of exon 12 (underlined caps lock)

Occurrence of allele A and its homozygote form AA was characterized by two single base substitutions in the coding region of exon 12. That occured on position 80, where A was substituted by C and amino acid lysine substituted by asparagine, as well as on position 83 where A was substituted with G and amino acid valine was substituted with methionine (Figure 2).

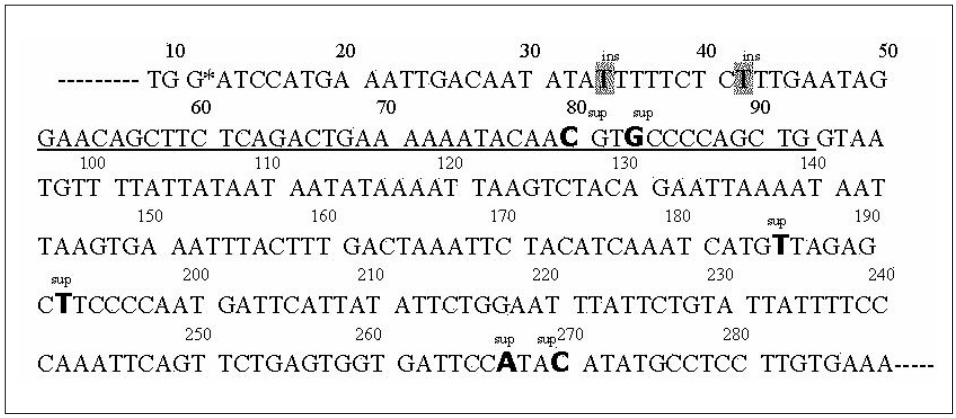

Figure 2. Part of identified sequence of AA genotype, with underlined coding region

The presence of allele F and homozygote form FF was characterized with two more substitutions in the region of exon 12 comparing to the allele A. The first

substitution of G with A occurred on position 64, followed by substitution of amino acid arginine with lysine, and the second was on position 71, but in the frame of amino acid lysine (Figure 3).

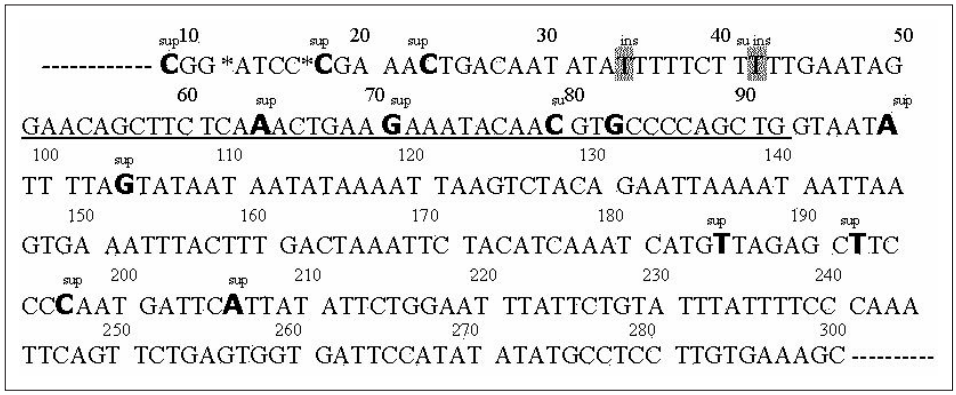

Figure 3. Part of identified sequence of FF genotype, with underlined coding region

Occurrence of alleles A and F together and heterozygote form AF was characterized by the presence of non identified nucleotides labelled as N on the chromatogram. The occurrence of non identified nucleotides was due to the overlapping of two nucleotides as happened on positions 64 and 71 of exon 12 (G - A and A - G) (Figure 4).

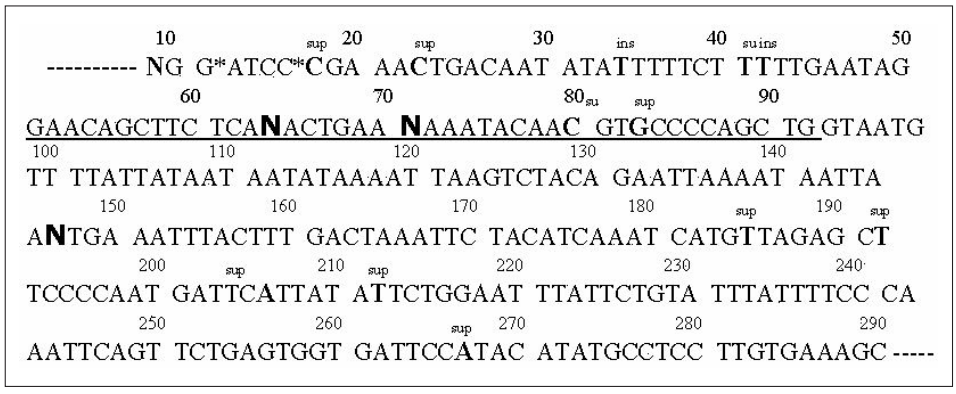

Figure 4. Part of identified sequence of AF genotype, with underlined coding region
Legend: * - deletion, sup – substitution, ins – insertion, N – non identified (overleaping)

Beside the mentioned substitutions in the region of exon 12 all identified genotypes had many other substitutions, deletions and insertions in the areas of intron 11 and intron 12.

Sequencing results regarding the number, percentage and frequencies of detected alleles for 59 goats from the first and 39 goats from the second flock, as well as for all examined goats are presented in Table 1.

Table 1. Number, percentage and frequency of alleles and genotypes of αS_1 casein

	Genotypes				Alleles		
	AA	AF	FF	Total	A	F	Total
Flock – I							
Number	28	23	8	59	79	39	118
Percentage	46.55	39.65	13.79	100.00	66.38	33.62	100.00
Frequency	0.441	0.446	0.113	1.00	0.664	0.336	1.00
Flock – II							
Number	11	23	5	39	45	33	78
Percentage	28.20	58.97	12.82	100.00	57.69	42.31	100.00
Frequency	0.333	0.488	0.179	1.00	0.577	0.423	1.00
Total							
Number	39	46	13	98	124	72	196
Percentage	39.17	47.42	13.40	100.00	62.89	37.11	100.00
Frequency	0.396	0.467	0.138	1.00	0.629	0.371	1.00

The strong A allele of αS_1 casein was the highly dominant allele in the population of Balkan goats with frequencies of 0.664 in the first, 0.577 in the second flock and 0.629 for all investigated goats.

As for the weak F allele, its frequency was 0.336 in the first, 0.432 in the second flock and 0.371 for all goats. Frequencies of the AA, AF and FF genotypes were 0.441; 0.446 and 0.113 in the first, 0.333; 0.448 and 0.179 in the second flock and 0.396; 0.476 and 0.138 for all goats respectively.

The percentage of A allele was 63 and of F allele 37, while the percentages of genotypes AA, AF and FF were 39, 47 and 14, respectively.

The high domination of strong A allele (0.629) obtained for Balkan goats breed from Montenegro is in accordance to the results for Spanish Canaria breed, 0.60 (Jordana *et al*., 1996); Italian Gargancia breed, 0.61 (Martin, 1993); as well as to the results for Balkan goat breed from Albania (0.47 – A and 0.46 B allele) and Damascus breed of goats from Greece (0.87), given by Grosclaude and Martin (1997). It is important to note that in the same time the frequency of F allele in the mentioned breeds was very low, even less than in the Balkan goat breed.

Strong alleles (A, B and C) of alpha S_1 casein represent the ancestral sequences of these genes in goats that are highly dominant in old - autochthonous unselected breeds. The frequency of these alleles is usually low in highly selected breeds for milk production, like Alpine and Saanen (Grosclaude *et al.*, 1994).

CONCLUSION

The distribution of A and F alleles and related genotypes of αS_1 casein in Balkan goat breed from Montenegro, as well as high predominance of A allele, is very similar to their distributions in other old unselected breeds, such as Canaria, Gargancia, Maltese, Damascus and Balkan goat breed from Albania. The distribution on allelic variants at the locus αS_1 – casein in Balkan goat breed can be exploited in the future for the development of a breeding program.

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POLIMORFIZAM I FREKVENCIJA ALELA ALFA S1 KAZEINA DOMAĆE BALKANSKE RASE KOZA U CRNOJ GORI

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SADRŽAJ

Frekvencija alela i genotipova na lokusu αS_1 – kazeina utvrđena je za 98 grla domaće balkanske koze gajene u Crnoj Gori.

Pojava dva alela (A i F) i tri genotipa (AA, AF and FF) otkrivena je primjenom metode sekvenciranja cDNK i dijela genomske DNK αS_1 kazeina. U ovim istraživanjima je utvrđena visoka dominantnost "snažnog" A alela (0,629) u poređenju sa "slabim" F alelom čija je frekvencija 0,371. Poznavanje distribucije alelnih varijanti, odnosno genotipova, na lokusu αS_1 kazeina u populaciji domaće balkanske koze u Crnoj Gori doprinijeće boljoj selekciji i genetskom unapređenju populacije ove rase i očuvanju njene genetičke varijabilnosti.