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Alpha S1-casein genetic variations in Bulgarian sheep breeds and significance on milk casein fractions

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Abstract

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Single nucleotide polymorphism (SNP) of the exon III at CSN1S1 gene encoding alpha S1-casein (αS1-CN) was investigated by means of the PCR-RFLP analysis in two sheep breeds – Bulgarian Dairy Synthetic Population (BDSP, n = 89) and Pleven Blackhead sheep (PLBH, n = 38) with an aim to establish the possible effect of a particular genotype on the casein content and distribution of milk fractions. The homozygous CC genotype was observed in 63.2% of the studied ewes, while the homozygous AA genotype was established in 4.5 % of the individuals. The calculated mean values of observed (Ho = 0.323) and expected (He = 0.321) heterozygosity at CSN1S1 locus indicated a moderate degree of genetic variability in the examined sheep populations. The estimated negative values of the coefficient (Fis = –0.001 ÷ –0.006) showed a low level of inbreeding. The results of the associative analysis indicated that CSN1S1 genotypes were significantly associated with the milk αS1-CN in the BDSP 2 population ($P < 0.05$). The highest casein percentage in this population (35.24 ± 3.96) was associated with ewes carrying the heterozygous AC genotype. No significant differences ($P > 0.05$) were established for CSN1S1 genotypes in terms of casein content in the studied PLBH sheep population.

*Keywords***:** sheep milk; caseins; CSN1S1gene; SNP; PCR-RFLP

Introduction

Casein in ovine milk consists of 4 fractions: αs1-casein, αs2-casein, β-casein and κ-casein (Balthazar et al. 2017). The main casein components contain a variable number of phosphoserine residues. Alpha S-caseins contain 8-10 seryl phosphate groups, while β-casein contains about 5 phosphoserine residues, which makes it more hydrophobic than αs- and κ-caseins (Whitney, 1988). The most strongly phosphorylated by calcium-sensitive caseins is αs2-casein (αs2-CN). It is found in sheep's milk in several forms and differs in the level of phosphorylation (Othman et al. 2013). β-casein accounts for approximately 60% of all casein in sheep's milk and is represented by the multi-phosphorylated forms of β1- and β2-casein, which have a similar amino acid composition to bovine β-casein (Balthazar et al. 2017). The pres-

ence of multi-phosphorylated forms of β-casein can affect the stability of casein micelles and the degree of phosphorylation of each form affects their binding to calcium (Amigo et al. 2000). Kappa casein (κ-CN) is highly heterogeneous, soluble in the presence of calcium, and differs significantly in structure from calcium-sensitive caseins (Othman et al. 2013). Studies show that the relative proportions of the four casein fractions affect the physicochemical, nutritional, and technological properties of ruminant milk (Ramunno et al. 2005). The proportions of the milk casein fractions varied in different ruminant species (Raynal-Ljutovac et al. 2007). Alpha S1-casein is genetically polymorphic due to amino acid substitutions or deletions in the triplet codon (Chessa et al. 2010).

The genetic variation of caseins in sheep's milk makes it possible to apply effective approaches to identify important economic characteristics for the improvement of sheep breeds aimed at the production of specific milk proteins (Giambia et al. 2010). The polymorphism in casein genes is well known and of great importance due to its influence on the quantitative characteristics and technological properties of milk used in the production of dairy products of sheep origin (Ceriotti et al., 2004; Georgescu et al., 2016). Alpha S1 casein gene (CSN1S1) encodes the milk protein αS1-CN is closely linked to the genes CSN1S2, CSN2, and CSN3, which are responsible for the expression of proteins from other casein fractions, and together form the casein locus. The genetic structure of this locus is well described in humans, mice, cattle, and goats (Yahyaoui et al. 2001). In sheep, based on non-isotopic hybridization, the casein locus is mapped on chromosome 6 of the genome of the species *Ovis aries* and covers an area of about 250 kb in the length of genomic DNA (Amigo et al. 2000). With the development of molecular biology, various techniques have been applied to identify polymorphic variants in the CSN1S1 gene in sheep at the DNA level. Based on the PCR-RFLP method, 3 allelic variants were found in exon III of this gene: A, C and D, subsequently found in different breeds of sheep (Pilla et al. 1998). At the DNA level, the replacement of the amino acid serine with proline (Ser / Pro) at position 13 of the αS1-CN polypeptide chain results in the expression of allele C instead of allele A. Based on PCR-SSCP, a nucleotide substitution of type T has been identified / C transition to αS1-CN, water to replace isoleucine (Ile) with tryptophan (Thr), in which variant C is transformed into C' (Amigo et al. 2000). At present, based on various electrophoretic techniques (alkaline, capillary), HPLC analysis, mass spectrophotometry, immunological and other methods identified a total of 9 codominant alleles of the αS1-CN gene: A, B, C, D, E, F, H, G, and I (Giambra et al. 2010). In comparison, a total of 16 alleles of this gene have been identified in goats, identified at both protein and DNA levels (Ramunno et al. 2005). The remaining genes from the casein locus in sheep (αS2-CN, β-CN, and κ-CN) are characterized by a lower level of genetic polymorphism.

A number of studies have been devoted to studying the level of genetic variation in the exon three of gene encoding alpha S1-casein (Gencheva & Georgieva, 2019; Giambra et al., 2010; Ivankovic & Dovc, 2004; Pirisi et al., 1999; Ramos et al. 2009). On the other hand, in the Romanian sheep breeds Botosani Karakul, Carabasa and Milk Line no polymorphism was found in this locus. Therefore, Kevorkian et al. (2009) have concluded this exon was monomorphic.

In Bulgaria, sheep breeding is a thousand-year livelihood of the population. The diverse ecological and economic conditions in the country and the different needs and interests of the local people have made possible the development of a large number of sheep breeds. The private farmers tend to raise sheep for milk mainly. The most widespread are the sheep of the breeds Pleven Blackhead and Bulgarian Dairy Synthetic Population. They have a significant contribution to ovine milk production in Bulgaria. The Pleven Blackhead sheep breed is one of the autochthonous sheep breeds in the country, a transitional form of a crossing between Tsigai and Tsakel breeds. Bulgarian Dairy Synthetic Population was established by crossing ewes of fine-wool, semi-finewool and local dairy breeds: Pleven Blackhead sheep, East-Friesian sheep, Awassi sheep, and Local Stara Zagora sheep. Partial crossing with sheep of the Chios breed and Lacaune breed has been applied in the last years. Mainly internal breeding is carried out in the pure-breed flocks (EASRAB, 2017).

The aim of the present research was to identify the genetic polymorphism of the CSN1S1 locus and establish the possible effect of a particular genotype on milk casein fractions in Bulgarian in Dairy Synthetic Population and Pleven Blackhead sheep breed.

Materials and Methods

Animals and sample collection: The present study was conducted with sheep from the two most common milk sheep breeds in Bulgaria – Pleven Blackhead and Bulgarian Dairy Synthetic Population. The investigation was performed with a total of 127 unrelated ewes, representing two sheep breeds grown in private farms located in different regions of Bulgaria: Bulgarian Dairy Synthetic Population from sheep-farm in Agricultural institute – Shumen (BDSP1, n = 35) and two sheep populations – Bulgarian Dairy Synthetic Population (BDSP2, n = 54) and Pleven Blackhead (PLBH, n = 38) from the sheep-farm in village General Kantardzhievo, municipal-

ity Aksakovo, district Varna. The animals were grown under the control of the Executive Agency for Selection and Reproduction in Animal Breeding (EASRAB). Blood samples were obtained in a total volume of 3 ml. from *vena jugularis* into vacuum tubes containing K2EDTA (Becton Dickinson, UK), in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

DNA extraction and PCR amplification

The genomic DNA was extracted from the whole blood samples using a commercial DNA Purification kit (Illustra Blood GenomicPrep GE Healthcare, UK) according to the manufacturer's instructions and stored at – 20° C until the amplifications were performed. The concentration and quality of the obtained genomic DNA were determined using NanoVue Plus Spectrophotometer (GE Healthcare). PCR reactions were carried out in a total volume of 20 µL, containing 80 ng DNA template, 10 pM of each primer and 2×Red Tag DNA Polymerase Master mix (VWR, Belgium). The sample amplifications were performed with primers suggested by Pilla et al. (1998), and accomplished on Doppio (2 × 48 well) Gradient thermal cycler (VWR®, Germany) under the following PCR conditions: an initial denaturation step at 95 °C / 5 min, followed by 35 cycles of 95 °C / 30 s, primer annealing at 53 °C/55 s, extension 72 °C / 1 min, final extension at 72 °C / 10 min, and storage at 4° C / ∞.

RFLP genotyping

The obtained PCR products were digested using 1U/µL MboII restriction endonuclease (BioLabs) in a determined specific site at 5'...GAAGA (N8)↓...3'. The digestion reactions were carried out in a total volume of 25 µl, containing 10 µl PCR product and incubated at 37 ºC / 15 h. The stained restriction fragments with a fluorescent dye (GelRed®, Biotium, USA) were separated on 2.5 % agarose gel (TopVision agarose, Fermentas) dissolved in 1 × TBE buffer and visualized using Electrophoresis Gel Imaging Analysis System (Bio-Imaging Systems, Israel) under UV light. The individual CSN1S1genotypes of the analyzed samples were identified based on the number of the restriction fragments and their size identified in the agarose gel and verified through the DNA marker, 50bp (GeneRuler™, Fermentas).

Analysis of milk casein fractions

A total of 87 milk samples (51 from BDSP2 and 36 from PLBH sheep population) were analyzed. The solid, raw casein fraction was washed up twice with 1 mM Ammonium acetate (pH 4.3), centrifuged at 3000g for 10 min. The solid was rinsed with acetone, filtrated and was air-dried in shade at room temperature. The samples were stored in the dark and cool rooms at 16 – 18 °C prior to the analysis. The quantification of casein fraction was carried out by the HPLC method, developed and validated by Bobe et al. (1998) with slight modifications. Analytical HPLC was performed with a C18 column Hypersil Gold (5 µm; 150 mm × 4.6 mm) on a Thermo system composed of a Surveyor LC Pump Plus, Surveyor Autosampler Plus, and Surveyor photodiode array detector PDA Plus. Two mobile phases were prepared: A – Acetonitrile / Water / Trichloroacetic acid (100 + 900 + 1), and B – Acetonitrile / Water / Trichloroacetic acid (900 + 100 + 1), and filtered through a 0.45 µm membrane and degassed before use. The chromatographic conditions were as follows: Total run time – 35 min.; Column temperature – 46°C; Detection wavelength – 220 nm. Injection volume of final sample solution – 20 mL; Eluent flow rate – 1 ml / min in the gradient mode: 1 – 5 min – 29 % B; 5 – 10 min – from 29 up to 37% B; 10 12 min – from 37 up to 41% B; 12-14 min – from 41 up to 42.5% B; 14 –16 min – 42.5% B; 16-17 min – from 42.5 up to 43% B; 17 – 19 min – 43% B; 19 – 21 min – from 43 up to 47% B; 21 – 23 min – 47% B%; 23 – 25 min – from 47 up to 54% B; 25 – 27 min – 54% B; 27 – 28 min – from 54 up to 100% B; 28 – 30 min – 100 % B; 30 – 35 min – from 100 up to 29% B. A sample of 0.15 g (± 0.0001 g) was diluted in a mixture of eluents A and B in ratio 70 : 30, up to 50 ml. A small quantity of each casein extract was transferred into a screw-capped vial and placed in the HPLC system autosampler. The amount of casein fractions were expressed as relative weight percent (% w/w).

Statistical Analyses

The calculation of the main population genetic parameters – allele and genotype frequencies, observed (Ho) and expected (He) heterozygosity, as well as coefficient of inbreeding (Fis) within each sheep group was performed through the package ARLEQUIN software, version 3.5.1.3 (Excoffier & Lischer, 2010). The same program was applied in order to check the Hardy-Weinberg equilibrium deviation (HWE), by the method of Guo & Thommson (1992). The influence of the particular genotype of studied CSN1S1 locus on casein content (%) in two sheep populations – BDSP2 and PLBH were examined via univariate data analysis using the following model:

$$Y = \bar{x} + G + e,$$

where Y are the measurements of the casein content, $\bar{x}$ are the overall mean values, G are the fixed factors (the different genotypes of CSN1S1 locus), and e are the residuals of the model. Significant differences between the different least-square means (LSM) of the genotypes were calculated by the

Post Hoc multiple comparisons with Tamhane test at P-value < 0.05. The IBM SPSS Statistics 17.0 WinWrap Basic, Copyright 1993-2007 statistical package was used to process the data (SPSS Statistics, 2007).

Results and Discussion

PCR-RFLP analysis and genotyping at polymorphic CSN1S1 locus

The obtained results based on PCR-RFLP analysis showed that 63.2% of the studied ewes were homozygous (genotype CC) and their restriction profile revealed two electrophoretic bands (306 and 66 bp). 32.3% of the individuals were heterozygous (AC genotype), presented with four fragments (306, 160, 146 and 66 bp). The homozygous AA genotype was identified by the lowest frequency (4.5%) and was expressed with three bands (160, 146 and 66 bp) on the agarose gel (Figure 1).

The allele and genotype frequencies at the polymorphic CSN1S1 locus were estimated for examined sheep breeds and summarized in Table 1.

The mean value of frequency for allele A at CSN1S1 locus was 0.207 across the studied sheep populations and varied from 0.171 in the BDSP1 population to 0.237 in PLBH sheep. According to allele C, the mean value of frequency was 0.793 and ranged from 0.763 in PLBH sheep to 0.829 in BDSP1. The homozygous CC genotype was found with the highest frequency of 0.686 in the BDSP population, whereas for PLBH it was lower (0.579). Considering the whole sample containing a total of 127 individuals, the observed heterozygosity (Ho) ranged from 0.286 in the BDSP1 sheep population to 0.368 in the PLBH sheep population with a mean of 0.323, while expected heterozygosity (He) varied from 0.284 BDSP1 sheep population to 0.362 in PLBH sheep population with a mean 0.321. As a whole, the Ho was higher than the expected one, resulting in a negative coefficient of inbreeding (Fis), varied from –0.001 in the PLBH sheep to –0.006 in the BDSP1 sheep population (Table.1). The chi-square test for Hardy-Weinberg equilibrium pointed values of χ^2 with a level of probability P > 0.9, at a degree of freedom (df = 1) confirming the validity of the Hardy-Weinberg equilibrium for all studied sheep populations (Table 1).

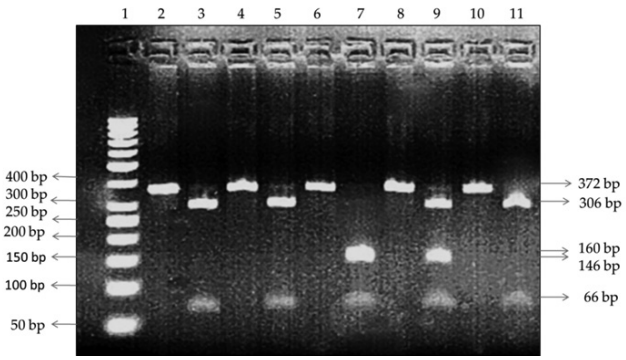

Fig. 1. Electrophoregram with restriction fragments of amplified PCR products at the polymorphic locus (exon III) of the CSN1S1 gene obtained with MboII restriction enzyme in BDSP and PLBH sheep populations in 2% agarose gel

Lane 1 – DNA Ladder, 50 bp; lanes 2, 4, 6, 8 and 10 – the obtained PCR (amplicons) of CSN1S1 polymorphic locus with 372 bp size; lanes 3, 5 and 11 – individuals with CC homozygous genotype (two bands of 306 and 66 bp); lane 9 – individuals with AC heterozygous genotype (four fragments of 306, 160, 146 and 66 bp); lane 7 – individual with AA homozygous genotype (three bands of 160, 146 and 66 bp).

The distribution of allele frequency in all sheep breeds demonstrated a higher prevalence of allele C (mean value 0.793) compared to allele A (mean value 0.207) in the studied sheep populations. Ramos et al. (2009) have been

Table 1. Distribution of the allele and genotype frequencies, observed (Ho) and expected (He) heterozygosity, chi-square (χ2) for HWE and coefficient of inbreeding (Fis) obtained for the polymorphic exon III at ovine CSN1S1 locus in the studied sheep populations

Sheep population	Allele frequencies		Genotype frequencies			Heterozygosity		Fis	χ^2
	A	C	AA	AC	CC	Ho	He		
BDSP 1 n = 35	0.171	0.829	0.028 (1)	0.286 (10)	0.686 (24)	0.286	0.284	–0.006	0.003 (0.958)*
BDSP 2 n = 54	0.213	0.787	0.055 (3)	0.315 (17)	0.630 (34)	0.315	0.316	–0.005	0.001 (0.974)*
PLBH 1 n = 38	0.237	0.763	0.053 (2)	0.368 (14)	0.579 (22)	0.368	0.362	–0.001	0.001 (0.972)*
Mean	0.207	0.793	0.045	0.323	0.632	0.323	0.321	–0.004	

BDSP – Bulgarian Dairy Synthetic Population sheep; PLBH – Pleven Blackhead sheep; n – number of the individuals in studied sheep populations; Ho and He – observed and expected heterozygosity; * Degree of probability (P); The number of individuals corresponding to different genotype is given in parenthesis.

reported similar results in Serra da Esttrela, White Merino and Black Merino sheep breeds, with allele A frequencies of 0.023, 0.022 and 0.036, and for allele C – 0.923, 0.916 and 0.902, respectively, as well as by Giambra et al. (2010) in a total of 57 individuals of Black Faced Mutton sheep. The results corresponded with these reported by Rustempasic et al. 2013 for the Pramenka flocks located in Bosnia and Herzegovina, while the frequencies are 0.020 for allele A and 0.0980 for allele C. Based on PCR-RFLP analysis, Hristova (2011) has obtained frequencies similar to these from the present study – 0.020 for allele A and 0.980 for allele C, in a total of 90 individuals belonging to local sheep breeds – Karakachan, Local Karnobat and Copper-red Shumen reared in Bulgaria. The opposite, the lower values of frequencies for allele C, varied from 0.54 to 0.72 have been observed by Amigo et al. (2000) in different and sheep breeds in Italy and Spain, but in general, they were significantly higher than the frequencies of allele A (0.006 – 0.060).

A number of researchers point out the C allele as favorable in terms of better technological performance of sheep's milk. This variant occurs with the highest frequency in a number of sheep breeds originating in Italy and Spanish – Manchega (0.600), Merino (0.547), Segurenã (0.890) and their crosses – Langhe × Comisana (0.720) Amigo et al. (2000). Amigo et al. (2000) associate allele A with lower casein content in sheep's milk and therefore considered unfavorable in terms of technological parameters of sheep's milk. In addition, variant D (also colled "Welsh" variant) occurs an extremely low frequency, and is most common in the sheep breeds Welsh, Hampshir and Sarda.

It should be noted that the trend found in the genotyping of animals from the population of Synthetic BDSP by the casein locus and respectively its higher frequencies by the C allele (0.829 for BDSP1 and 0.787 for BDSP 2) must be chosen specifically, selected by phenotype. In support of this assertion, we could find the fact that this population has the highest milk yield, compared to other breeds reared in Bulgaria in accordance with data in Livestock breeds in Republic of Bulgaria (EASRAB, 2017). Other reasons for these results could be the influence of various factors, such as breed affiliation, population size, including sample size, and BDSP2 breeding strategies. On the other hand, a moderate level of heterozygosity was observed in BDSP2. We believe that the probable reason for the average level of genetic diversity is the participation of several breeds in the process of BDSP creation.

Association analysis based on single-gene approach at polymorphic CSN1S1 locus

The obtained results for population genetic structure and a moderate level of genetic diversity established in the BDSP2 and PLBH sheep populations indicate the important potential for breeding selection. Therefore, this gave us a reason to perform an association analysis based on the single-gene approach for CSN1S1 locus. However, the ewes with homozygous AA genotype of both sheep populations were not included in the association analysis due to their minor individuals and low genotype frequencies (0.055 in BDSP2 and 0.053 in PLBH sheep). Levene's test of equality of variances showed that the CSN1S1 genotypes samples are none homogeneous ($P < 0.05$) which determines the usage of the Tamhane test of significant differences between the genotypes.

In Table 2, the obtained coefficients of determination (R^2) for the observed casein content in the examined sheep populations are shown.

The higher influence ($R^2 = 2.4\%$) at the particular genotype was established compared to casein content was established for the BDSP2 population. In the other sheep group – PLBH, the lower insignificant influence ($R^2 = 1.8\%$) was estimated.

The calculated mean values of milk casein content presented in Table 2 and Figure 2 revealed that polymorphism in the CSN1S1 gene had a significant influence on this trait in the BDSP sheep population.

Statistically significant differences ($P < 0.05$) were observed between the individuals with the heterozygous AC

Table 2. Effect of CSN1S1 genotypes on the alphaS1-CN content (%) in BDSP2 and PLBH sheep populations carrying different CSN1S1 genotype

Sheep population	$\bar{x} \pm SD$ CSN1S1 genotype s				R^2 (%)
	n	AC	n	CC	
BDSP 2	17	35.24 ± 3.96^{a}	34	33.90 ± 5.52^{b}	2.4*
PLBH	14	35.61 ± 5.74^{ns}	22	35.42 ± 2.71^{ns}	1.8*

^{a,b} Defferent superscripts within the same row represent significant differences at the level of significance $P < 0.05$; ^{ns} – insignificant differences ($P > 0.05$); R^2 – coefficients of determination based on observed means through Tamhane test; $\bar{x}$±SD – mean values ± standard deviation; n – number of the individuals; * Degree of probability (P)

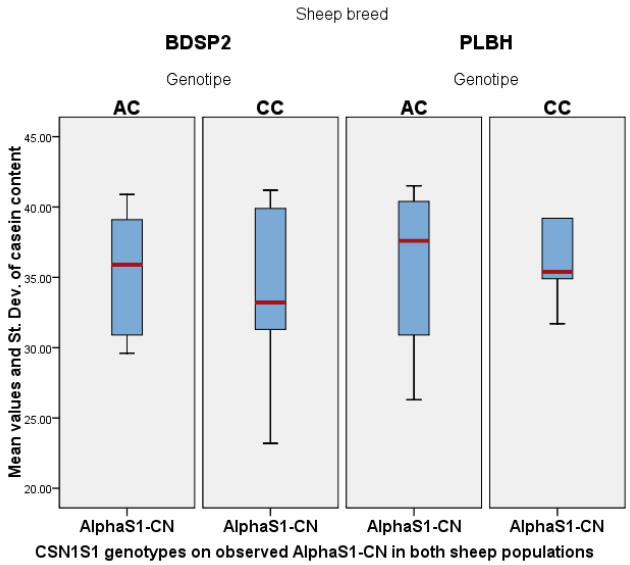

Fig. 2. Influence of different CSN1S1 genotypes on observed AlphaS1-CN in Bulgarian Dairy Synthetic Population (BDSP2) and Pleven Blackhead (PLBH) sheep populations

Influence of different CSN1S1 genotypes on observed AlphaS1-CN in Bulgarian Synthetic Population (BDSP2) and Pleven Blackhead (PLBH) sheep populations

genotype and homozygous CC genotype. Thus, the highest casein percentage in the Synthetic BDSP population (35.24 ± 3.96) was associated with ewes carrying the heterozygous AC genotype. No significant differences (P > 0.05) between the individuals with the heterozygous AC genotype and homozygous CC genotype for CSN1S1 genotypes in terms of casein content in the studied PLBH sheep population (Table 2 and Figure 2) were established. In addition, the highest casein percentage in the PLBH population (35.61 ± 5.74) in AC genotype was established.

Concerning the ovine CSN1S1 polymorphism, Amigo et al. (2000) reported that milk samples containing genotype CC showed the best technological behavior, due to the higher amount of casein. Mroczkowski et al. (2004) investigated Merino sheep from central Poland within the αS1-CN and concluded that the CC genotype exhibited a statistically significant advantage in terms of fat and solids percentage compared to the sheep carrying the AC αS1-CN genotype. Martini et al. (2006; 2008) pointed out that the allele C of alpha S1-casein is most favorable for cheese making. Although the available results in the literature till now, they still do not allow associating specific variants of the ovine milk with the compositions traits.

A number of researchers have associated the CC genotype CSN1S1 with higher milk yield in sheep (Ramos et al. 2009). Other authors have found a positive correlation between this genotype and parameters that are important from the point of view of technology in the production of dairy products – higher casein content, small micelle diameter and high coagulability of sheep's milk (Pirisi et al. 1999).

Comparative analysis of milk casein fractions (αS1-, αS2-, β- and κ-CN)

The study of milk samples by the RH-HPLC method showed that in the milk of the studied populations of sheep, the highest percentage is that of β- and αS1-casein fractions, while αS2- and k-CN, are relatively less represented (Table 3).

The observed trend of the predominance of β-casein over total% casein is in line with the results reported by other authors. Upon separation of milk proteins by SDS-PAGE electrophoresis in 15% polyacrylamide gel, Marin et al. (2012) found that β-casein had the highest expression, followed by k-, αS1-, αS2-casein, respectively. RP-HPLC analysis of casein fractions from some Greek sheep breeds (Karogouniko, Boutsiko, Chios and Frisarta) also showed that β-CN is the best-represented casein fraction (37 ÷ 42.3 %) compared to total casein, followed by alpha S1-casein (Moatsou et al. 2004).

The study of milk samples, by the method of RH-HPLC, showed greater intrabreed variation with respect to the 4 casein fractions (αs1-CN, αs2-CN, β-CN and κ-CN) in the milk of BDSP2 and PLBH breeds. Despite the differences in the individual fractions within the total casein, no significant differences (P < 0.05) were found between the two studied populations in the mean values for % content of the milk fractions (Figure 3).

Table 3. Effect of CSN1S1 genotypes on the alphaS1-CN content (%) in BDSP2 and PLBH sheep populations carrying different CSN1S1 genotype

Sheep population	n	$\bar{x}$ ± SD			
		αs1-CN, %	αs2-CN, %	β-CN, %	κ-CN, %
BDSP2	51	34.42 ± 4.95	12.87 ± 3.46	40.82 ± 6.38	11.53 ± 3.58
PLBH	36	35.48 ± 3.92	12.03 ± 3.99	41.41 ± 5.17	11.04 ± 3.00

Level of significance P < 0.05; ±SD – Mean values ± Standard Deviation; n – number of the individuals

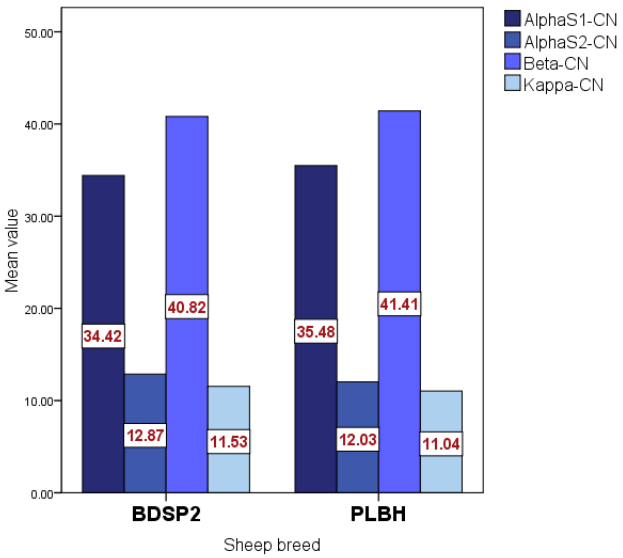

Fig. 3. Variation of milk casein fractions in two sheep breeds – Bulgarian Dairy Synthetic Population (BDSP2) and Pleven Blackhead (PLBH)

Variation of milk casein fractions in two sheep breeds – Bulgarian Synthetic Population (BDSP2) and Pleven Blackhead (PLBH)

This result could be explained by the high milk yield of both breeds, compared to other local breeds bred in Bulgaria. In support of this claim is the similar distribution of allelic and genotypic frequencies in the two populations found in the present study with respect to the CSN1S1 locus.

Conclusions

The results obtained regarding the nucleotide polymorphism in the CSN1S1 gene, as well as those from the analysis of casein fractions of sheep's milk is essential in the production of typical dairy products from sheep, which are traditional resources for Bulgaria.

Based on the PCR-RFLP assay, the homozygous CC genotype was observed in 63.2% of the studied ewes, while the homozygous AA genotype was established in 4.5% of the individuals. The mean values of the expected heterozygosity (He = 0.321) and the coefficient of inbreeding (Fis = –0.004) obtained at CSN1S1 locus indicated a moderate degree of genetic variability and a low level of inbreeding in the Bulgarian Dairy Synthetic Population and Pleven Blackhead sheep population.

Established genotypes were significantly associated with the milk αS1-CN in Bulgarian Dairy Synthetic Population ($P < 0.05$) and the highest casein percentage in this population (35.24 ± 3.96) was related with ewes carrying the heterozygous AC genotype. No significant differences ($P > 0.05$) were established for CSN1S1 genotypes in terms of casein content in the Pleven Blackhead sheep population.

The quality composition and technological properties of milk are directly dependent on the genetic diversity within the individual sheep populations. It is important to note that currently, based on genotype data of individuals in the respective population, genomic selection is applied to accelerate genetic progress. In this sense, the application of DNA marker-assisted breeding strategies (MAS) in sheep farming would significantly accelerate the rate of genetic improvement in the desired productivity characteristics, which are difficult to measure and require significant financial and human resources, as well as time, in case that selection is based only on the phenotype of the animals. The use of DNA polymorphisms as genetic markers in certain loci responsible for productive traits is essential for the conservation and sustainable development of genetic resources in animal husbandry, including sheep farming, as well as for their rational use and enhancement of the selection effect, respectively the quality of the animal products.

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