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Analysis of the Genetic Structure of Slovak White Shorthaired Goat breed using *CSN1S1* gene

Analýza genetickej štruktúry plemena slovenskej bielej kozy krátkosrstej pomocou génu *CSN1S1*

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ABSTRACT

Milk is one of the basic sources of nutrition. With the increasing incidence of allergies and the spread of knowledge about a healthy lifestyle, consumers are changing their eating habits, which partly leads to the reintroduction of goat's milk into the diet. This study aimed to evaluate and describe the genotypic and allelic structure of Slovak White Shorthaired goats for the *CSN1S1* gene. For genotyping, PCR, AS-PCR and PIRA-PCR methods. The He and F_{IS} were used for the description of the diversity of the population. Alleles E and F were the most frequent 0.3710 and 0.2944 respectively followed by B (0.1492) and A (0.1169). The less abundant allele was N with an occurrence of 0.0685. The most abundant genotypes were EF (0.2339), BE (0.1693) AN and EE with an occurrence of 0.1129 both. The White Shorthaired goat breed exhibits high values of heterozygosity (0.7661), polymorphism information content (0.6921), the effective number of alleles (3.7736) and level of possible variability realization (74.1%) for the *CSN1S1* gene. The inbreeding level of the population represents a negative value (-0.0355), which indicates enough heterozygotes in the population.

Keywords: White Shorthaired goat, milk, *CSN1S1*, genetic structure

ABSTRAKT

Mlieko je jedným zo základných zdrojov výživy. S narastajúcim výskytom alergií a rozširovaním poznatkov o zdravom životnom štýle spotrebitelia menia svoje stravovacie návyky, čo vedie k čiastočne opätovnému zavedeniu kozieho mlieka do jedálnička. Cieľom tejto štúdie bolo zhodnotiť a popísať genotypovú a alelickú štruktúru slovenskej bielej kozy krátkosrstej pre *CSN1S1* gén. Na genotypovanie boli použité PCR, AS-PCR a PIRA-PCR metódy. Na popis diverzity populácie boli použité He a F_{IS}. Alely E a F mali najväčšiu frekvenciu 0,3710 a 0,2944, po ktorých nasledovali B (0,1492) a A (0,1169). Najmenej zastúpená bola alela N s výskytom 0,0685. Najviac zastúpené genotypy boli EF (0,2339), BE (0,1693), AN a EE s výskytom 0,1129 u oboch. Biela krátkosrstá koza vykazuje vysoké hodnoty heterozygotnosti (0,7661), informačného obsahu polymorfizmu (0,6921), efektívneho počtu alel (3,7736) a úrovne možnej realizácie variability (74,1 %) pre gén *CSN1S1*. Úroveň inbrídingu populácie predstavuje zápornú hodnotu (-0,0355), čo poukazuje na dostatok heterozygotov v populácii.

Kľúčové slová: biela koza krátkosrstá, mlieko, *CSN1S1*, genetická štruktúra

INTRODUCTION

Goat milk plays an important role in the human diet. Although its composition is much like other ruminants, its unique features turn it mainly into medical and gourmet market articles (Mohsin et al., 2019).

Goat breeding in Slovakia has a tradition on small-scale farms with cheese production under domestic conditions often connected with agrotourism (Lauková et al., 2020, Kováčová et al., 2021). In 2019, 28 485 000 L of goat milk was produced in Slovakia out of which 9 088 183 L were produced by White Shorthaired goats breed (Čopaková, 2022). Almost the same number was produced by goats of unidentified or unspecified breeds, with the majority being crossbreeds of the White Shorthaired goat. (CRHZ 2022; Čopaková, 2022). According to research conducted on Slovak White Shorthaired goats published by Kováčová et al. (2021) the production of goat cheese from unpasteurized milk is safe under Slovak conditions. However, high levels of somatic cell counts may be caused by subclinical mastitis, which may result in a food safety risk. According to the authors, there is not enough information from local studies on the safety of raw goat milk products in Slovakia.

Milk production traits are affected more by environmental factors than genetics, however, focusing on major genes can improve selection effectiveness (Brzáková et al., 2021). The *CSN1S1* gene significantly impacts milk composition and technological properties for cheese production (Massender et al., 2023). The heritability of protein percentage was estimated at a value of 0.441 and for lactose percentage at 0.326 in Jamunapari goats (Verma et al., 2019).

The *CSN1S1* is a 16,828 bp long gene encoding milk protein α_{S1}-casein. The gene is located on chromosome six between 85.978 and 85.995 Mb. There are at least 18 described variants of α_{S1}-casein, which are divided into four groups, according to their effect on the level of produced α_{S1}-casein, on strong (A, A3, B1, B2, B3, B4, C, H, L and M) producing 3.6 g/L per allele of α_{S1}-casein, intermediate (E and I) 1,1-1,6 g/L per allele, weak (D, F and G) producing 0.45-0.6 g/L per allele and null (N, 01 and 02), which cause absence of α_{S1}-casein in homozygous carriers (Rahmatalla et al., 2022).

Genotypes which are a combination of A and B alleles have the highest protein content, on the other hand, the FF homozygotes have the lowest. It was observed that genotype AB has higher protein content than BF, AF, and FF (Verma et al., 2019). The difference between genotype AA and FF was measured as 4.8 g protein/kg, while AA to EE was 3.8 g protein/kg (Moioli et al., 1998). Likewise, Markovic et al. (2020) described the effect of the *CSN1S1* genotype on fat, total solid not fat and protein content, when AA genotype individuals produced significantly higher amounts than AF and FF individuals in all of these properties.

Besides milk production, goat farmers are, depending on region, focusing on manufacturing cheese (Cabral et al., 2020) and fermented products (Lu and Miller, 2019). Pizarro et al. (2020) suggest that a high fat-to-protein ratio could be a crucial factor affecting technological properties, especially curd formation performance and high cheese yield, which they claim could have *CSN1S1* AE heterozygotes. On the other hand, Mangia et al., (2019) preferred milk from individuals carrying weak D, F or G alleles for processing milk into fermented products.

The low level of binding IgE to the protein was according to Mansor et al. (2023) related to the amount of expressed casein which is related to the genetic variability of proteins. Results also identified α_{S1}-casein as one of the major allergens in goat's milk. The mouse model shows that individuals allergic to cow's milk are less sensitive to goat's one (Zhang et al., 2022).

Major genes and their effect on production traits are in focus of researchers since the beginning of the genomics era. The *CSN1S1* gene was applied in Marker-assisted selection since it has an indirect impact on the production and technological qualities of milk on farm economics (Cisternas and Strahsburger, 2019; Gipson, 2019).

Including the α_{S1}-casein genotype in estimating breeding values can enhance the efficiency of breeding

programs which are focused on the improvement of dairy goats (Pizarro Inostroza et al., 2019). The semen companies propose as the best animals for artificial insemination (AI), bucks homozygous for strong (AA and BB) and intermediate alleles (EE), but also the heterozygous BE and AE (Frattini et al., 2014).

Livestock breeding has a major impact and great influence on agriculture even today (Johnson, 2016). Genetic changes in populations are made by livestock breeders when they mate with individuals that are different from average (Bennett et al., 2014). Genetic evaluation procedures used in selection programs allow the comparison of populations across breeds and countries (Mateescu, 2020). For optimal management of the genetic resources of local breeds, it is necessary to characterize their genetic diversity (Bhati et al., 2020).

The aim of this study was to genotype and evaluate the genetic structure for the *CSN1S1* gene of the local breed population of Slovak White Shorthaired goats. Valid information can lay basement for improving and conservation of this breed. The polymorphism of the *CSN1S1* gene has significant impact on production, technological quality, and milk content. Marker assisted selection focused on desired alleles with positive effect on these production traits can have significant effect on economics.

MATERIALS AND METHODS

Animals

A total of 124 White Shorthaired goats were used for the present study. This breed excels in milk production, good fertility, and fitness. It is suitable for individual and herd-keeping. The morphology of tits makes it suitable for machine milking. It is the most represented goat breed in Slovakia. The breed was created by crossing Saanen bucks imported from Switzerland and Germany with local rustic-type goats in the first half of the 20th century mainly in the area of the Moravian Region, which is nowadays located in the Czech Republic (Sztankóová et al., 2009; Makovický et al., 2022). In 1954, it was recognized as a separate breed with a population size of approximately one and a half million heads (Vostra-Vydrova et al., 2020). The current status of the breed is 7 514 individuals (CRHZ, 2022) out of which are 708 registered purebred females and 50 registered purebred males. The breed is recognized as an endangered breed (Tomka et al., 2022). The samples were collected from two breeding farms located in northern Slovakia. Breeding farms are regulated by national legislation, intended to produce mating bucks or pure-breed goats for the purpose of improving the gene pool of the breed concerned (The Act no. 194/1998, 1998).

Sampling

Genomic DNA was extracted from hair root samples using the commercial reagent DNAzol® Direct (Molecular Research Center, Cincinnati OH, USA). The 100 µl of DNAzol was incubated with 10 hair roots at 90 °C for 20 minutes and then centrifuged at 6,000 x g for 1 min at room temperature. The lysate was stored at 4 °C.

Genotyping

For evaluating the studied population of Slovak White Shorthaired goats there were determine five alleles of the caprine *CSN1S1* gene: A, B, E, F, and N, which are reported to affect technological, nutritional and production properties of the goat's milk and are also the most represented among Saanen breed and crossbreeds breeds.

Genotyping was performed using Polymerase Chain Reaction (PCR), Primer-Introduced Restriction Analysis Polymerase Chain Reaction (PIRA-PCR) and Allele Specific Polymerase Chain Reaction (AS-PCR). Amplification of specific regions of the *CSN1S1* gene was performed by FIREPol HS polymerase (Solis BioDyne) and the identification of the presence of 1 bp deletion (PIRA-PCR) was performed by specific restriction enzyme FastDigest *TaqI* (Thermo Scientific BioScience). The PCR reaction was realized with gradient thermocycler C1000 TouchTM (Biorad). The reaction mixture in the total volume of 25 µl contained 2 µl template DNA, 1 U FIREPol HS polymerase (Solis BioDyne), 1X $(NH_4)_2SO_4$ buffer, 4 mM (2.5 mM for 475 bp LINE element) $MgCl_2$, 0.2 mM dNTP mix and 0.2

pM of each primer. PCR cycling condition included 95 °C for 15 minutes followed by 35 cycles (30 cycles for 1 bp deletion) of 95 °C for 5 seconds, 59 °C for 20 seconds (56 °C for 25 seconds for 475 bp LINE element) and 72 °C for 20 seconds. The reaction was completed by the final elongation step of 72 °C for 5 minutes. In the case of PIRA-PCR methods, which were used for the detection of 1 bp deletion, the PCR products were subsequently digested with the restriction enzyme.

Amplified PCR products and restriction products were separated by agarose electrophoresis with the GelRed(TM) intercalating dye (Biotium). A 3% agarose gel was used for the 11 bp insertion and 1 bp deletion, and a 2% agarose gel was used for the 475 bp LINE element. Electrophoresis was performed in 1 x SB solution (Brody and Kern, 2004) at 180 V for 25 minutes for the 11 bp insertion, 20 minutes for the 1 bp deletion and 15 minutes for the 475 bp LINE element. After electrophoresis, the resulting fragments were visualized using a UV transilluminator and an Olympus C-7070 documentary system. A summary of molecular genetic methods used to detect selected mutations of the *CSN1S1* gene is summarized in Table 1.

A, B, F and N alleles are determined using a combination of C deletion in position 9888 and an 11 bp insertion at position 9981. For determining allele E is decisive 457 bp LINE element (Pizarro et al., 2019) (Table 2).

Table 2. Combination of *CSN1S1* gene mutations for allele determination (Pizarro et al., 2019)

Allele	9888-90 C deletion	9981-2 CCGTAATGTTT insertion	457 bp LINE insertion
A	No	No	No
B	No	Yes	No
E	No	Yes	Yes
F	Yes	Yes	No
N	Yes	No	No

Genetic Structure

The genotypic structure and allelic frequencies for the tested population were established for the polymorphism of the *CSN1S1* gene based on molecular genetic analysis. The chi-square statistic was used to verify the statistical significance of the difference between expected and observed genotype frequencies. Assessment of allele efficacy was performed using the following parameters: expected heterozygosity (He_{exp}), observed heterozygosity (He_{obs}), polymorphism information content (PIC), expected homozygosity (E), effective number of alleles (ENA), level of possible variability realization (V%) and population inbreeding level (F_{IS}).

Table 1. Summary of molecular genetic methods used for selected mutations of the *CSN1S1* gene

Method	Mutation	Primer sequences 5´- 3´	Allele identification	References
PCR	11 bp insertion	F: GCTGGAAGCAGTTCGTCA	Allele I+ : 170 bp (ins)	Wang et al., 2018
		R: GGGTTGATAGCCTTGTATGTT	Allele I- : 159 bp (wild)	
PIRA PCR*	1 bp deletion	F: TTCTAAAAGTCTCAGAGGCAG	Allele D+ : 92 bp (del)	Ramuno et al., 2000
		R: ATAAAAATGGTATACCTCACTTGTC	Allele D- : 67 bp + 26 bp (wild)	Bevilacqua et al., 2002
AS-PCR	475 bp LINE element	F EF: CTATCATGTCAAACCATTCTATCC	Allele L+ : 225 bp (LINE)	Pizarro et al., 2019
		F E-EF: TCCCATTCTCCCAAATCATC	Allele L- : 133 bp (wild)	
		R: CAATTTCACTTAAGGATGTTACAC		

* PIRA-PCR was constructed by combining primer forward (Ramunno et al., 2000) and primer reverse with modification to create a cleavage site for the restriction enzyme *TaqI* (Bevilacqua et al., 2002)

Expected heterozygosity (He_{exp}) (Nei, 1973)

$$He_{exp} = 1 - \sum (p^2 + q^2)$$

Polymorphism information content (PIC) (Botstein et al., 1980)

$$PIC = 1 - \sum (p^2 + q^2) - \left(\sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 \, 2p_i^2 \right)$$

Expected homozygosity (E) (Crow and Kimura, 1970)

$$E = \sum p_i^2$$

Effective number of alleles (ENA) (Crow and Kimura, 1970)

$$ENA = \frac{1}{p^2 + q^2}$$

Level of possible variability realization (V%) (Crow and Kimura, 1970)

$$V = \frac{1 - C_a}{1 - \frac{1}{N}} \times 100$$

Population inbreeding level (F_{IS}) (Wright, 1969)

$$F_{IS} = 1 - \frac{He_{obs}}{He_{exp}}$$

RESULTS AND DISSCUSION

The *CSN1S1* polymorphism of Slovak White Shorthaired goat were detected using PCR, PIRA-PCR and AS-PCR. Obtained fragments of selected mutations in *CSN1S1* gene were visualized, depending on mutation, in 2% or 3% agarose gel (Invitrogen, Waltham, MA, USA) which contained GelRed™ dye (Biotium) in 1 x SB buffer (Brody and Kern, 2004) at 180 V for 15 to 25 minutes, depending on the mutation. Fragments are shown in Figure 1.

Gene and genotype frequencies

The genetic structure of the population is very useful information for establishing and correcting breeding strategies with the aim of improving production traits while conserving the livestock genetic resources. The result of the genotyping is shown in Table 3.

Of the 124 goats of the White Shorthaired breed analysed 10 genotypes were observed: the most abundant genotype was EF observed in 29 goats followed by genotype BE in 21 goats. Genotypes FF were observed in 15 goats, genotypes EE and AN were observed in 14

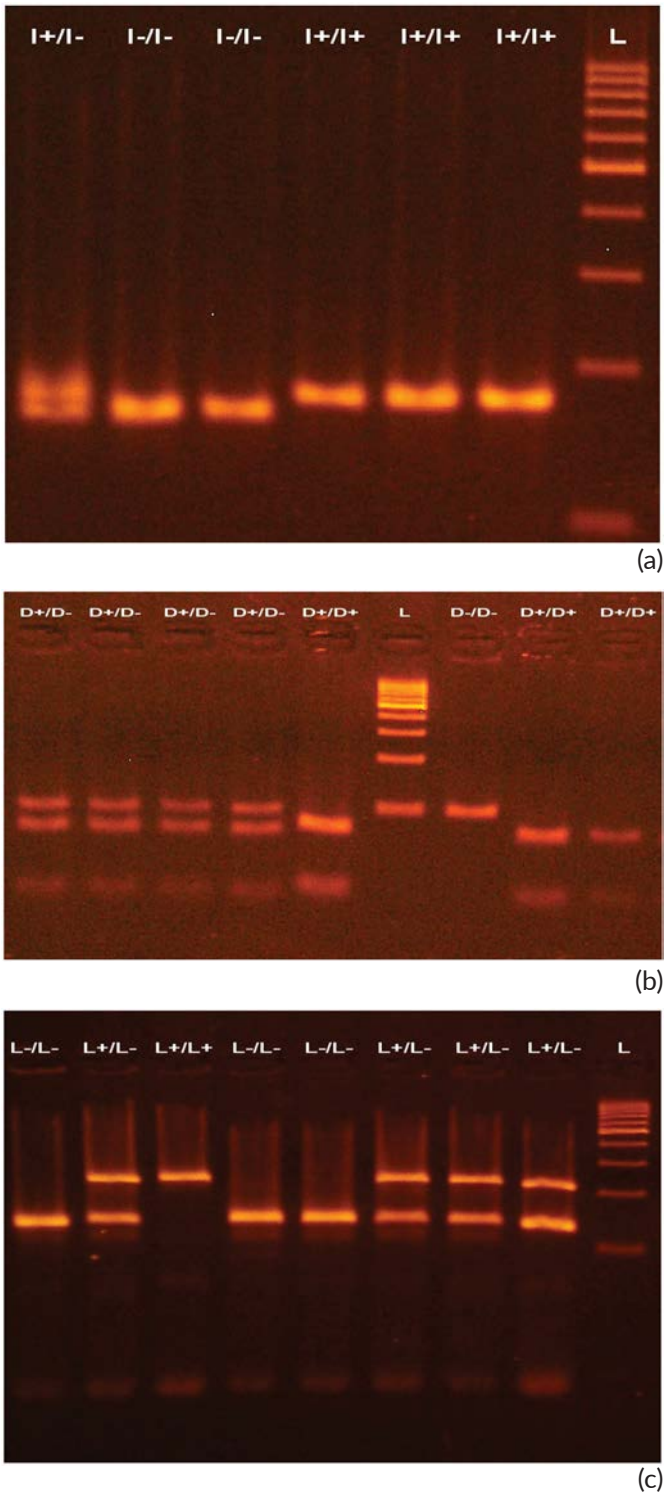

Figure 1. Illustration of *CSN1S1* 9981-2 CCGTAATGTTT insertion, *CSN1S1* 9888-90 C deletion, *CSN1S1* 457 bp line element genotypes on agarose gels. (a) Marker *CSN1S1* 9981-2 CCGTAATGTTT insertion: genotype I+/I- (170 bp, 159 bp), I-/I- (159 bp), I+/I+ (170 bp); (b) Marker *CSN1S1* 9888-90 C deletion: genotype D+/D- (92 bp, 67 bp, 26 bp), genotype D-/D- (67 bp, 26 bp), genotype D+/D+ (92 bp); (c) Marker *CSN1S1* 457 bp line element: genotype L+/L- (225 bp, 133 bp), L-/L- (133 bp), L+/L+ (225 bp). L – ladder 100 – 1000 bp (Thermo Scientific BioScience).

goats both. Genotypes BF (13 goats) and AE (12 goats) were observed likewise. The less abundant genotypes were AB (three goats), EN (two goats) and FN with one observation.

For the α_{S1}-casein was the most abundant allele E with an incidence 37.1%. This allele is categorized by its effect on casein production as an intermediate allele, which is producing 1.1 g/l of α_{S1}-casein. The second most abundant allele was the F allele (29.44%), which is categorized as a weak allele, producing 0.45 g/l of α_{S1}-casein.

Table 3. The result of allelic and haplotype genotyping and genetic structure

Alleles	Number	Frequencies	Chi-square test×
A	29	0.1169	
B	37	0.1492	
E	92	0.3710	
F	73	0.2944	
N	17	0.0685	
Genotype			
AA	-	-	
AB	3	0.0242	
AE	12	0.0968	
AF	-	-	
AN	14	0.1129	102.188***
BB	-	-	
BE	21	0.1693	
BF	13	0.1048	
BN	-	-	
EE	14	0.1129	
EF	29	0.2339	
EN	2	0.0161	
FF	15	0.1210	
FN	1	0.0081	
NN	-	-	

× - X^2 (14, N = 124) = 102.188, P < .0001; *** - P < 0.001

The abundance of strong alleles A and B were 11.69% and 14.92% respectively. These alleles produce on average 3.5 g/l of α_{S1}-casein. Allele N was a less abundant allele with frequencies of 6.85%. This allele is categorized as a null allele which produces no α_{S1}-casein (Caroli et al., 2007; Park et al., 2007).

The prevalence of alleles E (0.3710) and F (0.2944) were expected based on previous studies in Saanen populations in France (E: 0.41 and F: 0.43) (Grosclaude et al., 1987), Italy (E: 0.49 and F: 0.46) (Martin and Leroux, 2000), Mexico (E: 0.629 and F: 0.210) (Vázquez-Flores et al., 2012), USA (E: 0.71 and F: 0.30) (Maga, et al., 2009) and Malaysia (E: 0.31) (Widodo et al., 2022) which was demonstrated as typical for Saanen breed except that Mohammed et al. (2021) reported no occurrence of allele E in their studied population of Saanen.

Accumulation of E and F alleles in European breeds can be explained by breeding strategies focusing on increasing milk yield. The FF homozygotes have the highest milk yield (Verma et al., 2019). This statement is supported by the fact that breeds originating in the Alps are showing the highest daily milk yield and daily fat and protein yield whereas Mediterranean-originated breeds are showing the highest percentage of protein, fat, and lactose (Vacca et al., 2018). Regional differences in preferred dairy products could change the genetic structure of the population in a direction, that is most suitable for a selected product. The effect of α_{S1}-casein genotype on preferred technological quality for fermented dairy production is less suitable for processing the milk into cheese preferred on the Balkan peninsula.

Slovak White Shorthaired shows the presence of the A (0.1169) and B (0.1492) alleles, which are not common for goat breeds origin in the Alps. Results correspond with the Saanen breed and its local crossbreeds described in the literature (Sztankóová et al., 2007).

Indigenous goat breeds on the other hand exhibit high frequencies of A and B alleles with rare occurrences of alleles E and F (Singh et al., 2018; Verma et al., 2019; Anggraeni et al., 2021). Italian breed Garganica has the

most frequent allele A, followed by F and B (Santillo et al., 2022). Montenegro population of Balkan breed shows, that genotype group A* consists of A, B and C alleles and has frequencies of 0.626 and allele F: 0.374 (Marković et al., 2018). In the Carpathian breed was observed the prevalence of alleles A (0.35) and B (0.40), whereas the occurrence of allele E was only 0.07 and F: 0.18 (Anghel et al., 2019). In Indian Jamunapari goat breed alleles A, B and F were detected with frequencies 0.456, 0.503 and 0.041, respectively (Verma et al., 2019), but in another research, it was detected in Indian Jamunapari A: 0.36, B: 0.303, C 0.081, D: 0.057, E: 0.081 and F: 0.14 (Singh et al., 2018).

The most common genotypes in Carpatian were AB and BF, followed by homozygotes AA and BB. The EE, FF, and AE, genotypes were observed in only 0.03 each (Anghel et al., 2019). The most common genotype in the Balkan goat breed was A*F: 0.456 (A* = A, B and C) and the less frequent was genotype FF:0.146 (Marković et al., 2018). The most common genotypes in Jamunapari were AA: 0.274 followed by BE and BC with frequencies of 0.163 each. The less frequent genotype was BF: 0.081 (Singh et al., 2018).

Effectiveness of alleles

The loss of genetic variation is a concern, especially in populations of limited size. In such populations, heterozygosity at a specific locus is commonly used to describe the genetic variation (Gautschi et al., 2003). The effectiveness of alleles in the test population is shown in Table 4. Observed heterozygosity (0.7661) was slightly higher than the expected one (0.735) and much higher than it was described in the literature (0.5) (Anggraeni et al., 2021). The reason may be the mating of mostly unrelated animals, which is supported by the negative value of population inbreeding level F_{IS} (-0.0355). According to the literature, our observed F_{IS} value for goat *CSN1S1* is much lower than in other indigenous breeds like Skopelos (0.0376) (Michailidou et al., 2019) or Jamunapri (0.525) (Vema et al., 2019). The studied population shows a low value of the coefficient of homozygosity (0.265). The effective number of alleles (ENA) was 3.7736 which is higher than the 3.28 (Roy et al., 2020) described in the literature. The PIC value (0.6921) is higher than 0.5, which according to Botstein et al (1980) means a high degree of polymorphism in the observed population. The value is higher than in highly selected breeds as observed Wang et al. (2018) in Shaanbei White Cashmere (0.373). A high value of PIC can be explained by relatively low inbreeding and more balance allelic frequencies. The high level of polymorphism caused the level of possible variability realization to be 74.1%.

Slovak and Czech population comparison

Original White Shorthaired goat breed is a local breed derived from the Saanen in the first half of the 20th century in Czechoslovakia. The dissolution of Czechoslovakia in 1993 led to the division of the breed between two populations of Czech White Shorthaired and Slovak White Shorthaired goat breeds. These breeds are currently recognized as two separate breeds (Sztankóová et al., 2007). Vostry et al., (2022) observed a narrow genetic distance between Slovak and Czech White Shorthaired populations in their genome-wide diversification analysis performed using a 50,000 SNP array chip. Unlike their observations results in the present paper show big differences in the occurrence of allele E, which is visible in Table 4. Vostry et al. (2022) estimated for Slovak White Shorthaired two times lower value of inbreeding than in Czech population.

The sum of alleles A and B is equal to A* and in the Slovak population, it has approximately the same frequencies (0.2661) as the Czech sum of alleles A, B and C (0.269) in the year 2007, which decrease in 2009 to 0.203. In the Slovak population, there is a massive prevalence of allele E (0.3710) which is very rare in Czech populations 0.054 in 2007 compared with 0.004 in 2009. Allele F (0.658 in 2007 and 0.789 in 2009) is present in Czech population with double as high frequencies than in Slovak population (0.2944). It is visible loss in frequencies of A* and E alleles, which are preferable in Saanen, in favour of allele F. Both Czech populations were genotyped with the same methodic within two years (Sztankóová et al., 2007; Sztankóová et al., 2009).

Table 4. Effectiveness of *CSN1S1* alleles in White Shorthaired goat population

Population		Slovak WSH (Present study)	Czech WSH 2007 (Sztankóová et al., 2007)	Czech WSH 2009 (Sztankóová et al., 2009)
Alleles	A*	0.2661*	0.269	0.203
	E	0.3710	0.054	0.004
	F	0.2944	0.658	0.789
	N	0.0685	-	-
He_{obs}		0.7661	0.636	0.423
He_{exp}		0.735	0.491	0.337
PIC		0.6921	0.426	0.285
E		0.265	-	-
ENA		3.7736	-	-
V %		74.1	-	-
F_{IS}		-0.0355	-	-

WSH – White Shorthaired goat breed, A* = A + B+C, * – A (0.1169) + B (0.1492), He_{obs} – observed heterozygosity, He_{exp} – expected heterozygosity, PIC – polymorphism information content, E – expected homozygosity, ENA – effective number of alleles, V % - level of possible variability realization, F_{IS} – population inbreeding level

The most abundant genotype in the Czech population was A*F (0.502), followed by FF (0.345) and EF (0.096). Genotypes A*A* (0.018), 01E (0.012) and 01F (0.002) were rare (Sztankóová et al., 2007). Our studied population dominated genotype A*E (0.2661) and EF (0.2339), followed by FF (0.121), A*N (0.1129), EE (0.1129) and A*F (0.1048). Genotypes A*A* (0.0242), EN (0.0161) and FN (0.0081) were rare. Big difference in genotypic structure, although they originated from one population shows that these two populations had different breeding programs. The Slovak population shows higher diversity than the Czech population which is supported by He_{obs} value of 0.7661 in Slovak compared with 0.636 in the Czech population.

The high value of heterozygosity (0.7661) in the Slovak population can be caused by the introduction of bucks from a population with a different genotypic structure which is commonly done in Slovakia by buying Czech White Shorthaired mating bucks.

CONCLUSIONS

The *CSN1S1* is a major gene with a significant impact on milk production traits. It has been proven by many researchers that alleles A and B are the most suitable for dairy goats due to their positive influence on milk protein content and technological properties in cheese production. The E allele was found to have a positive effect on milk production in the AE and BE genotypes. Alleles F and N are not suitable for cheese production because they have a negative effect on the firmness of the curd. There is evidence of almost no allergenicity in the NN genotype, which can be used in breeding individuals producing less allergenic milk. Breeding to target specific genotypes could lead to a reduction in genetic diversity in the goat population. In this study, no reduction in genetic diversity was observed in the White Shorthaired goat population, which was confirmed by the negative F_{IS} value. The diversity found in the Slovak population could be used to reduce inbreeding. Further studies may

help to improve the breeding strategy of this breed. In order to accurately quantify the influence of genotypes in the studied breed, further correlation analysis should be performed.

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