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Original Paper

CSN1S1 Genotype and the Effect of the F Allele on Milk Characteristics in Slovak White Shorthaired Goats

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Various genetic and environmental factors influence the composition of milk. For the cheese manufacturing industry, one of the most important genes is the CSN1S1 gene, which influences the composition of several milk components as well as technological properties. Much attention has been paid to the F allele, which has been identified as undesirable for the manufacturing cheese. The aim of this study was to determine the genotypic structure of the Slovak population of the Slovak White Shorthaired goat (*Capra hircus*) for the CSN1S1 gene and to evaluate the influence of the CSN1S1 F allele on milk composition characteristics. Hair roots of 86 goats were collected and used as genetic material. Genotyping was performed using PCR and PIRA PCR. Phenotypic data from 101 lactations (first and/or second lactation only) with performance control were used for association studies. The occurrence of the F allele was 0.291. An association study was performed between genotypes consisting of F and non-F alleles. Significantly higher (p >.05) milk, protein, lactose and dry matter production was observed in non-F/non-F individuals than in F/non-F individuals. Genotyping of the CSN1S1 gene could be the basis for the use of marker-assisted selection in a breeding programme for the Slovak White Shorthorn goat. Further analyses involving the effect of the CSN1S1 genotype on cheese production could be performed to quantify the impact of composition on farm economics.

Keywords: goat, milk, CSN1S1

1 Introduction

Goat breeding is out of major interest in Slovakia. Majority of breeding goats are kept in small-scale farming system with self-sufficiency impact on animal breeder or are kept on private farms with on farm cheese production (Makovický et al., 2020).

The economy of a farm focusing on cheese production stands and falls on the quantity and quality of the cheese produced. It was the effort to understand this process that led to the discovery of the casein fraction of milk protein. Further research focused on finding key elements that could improve cheese manufacturing, which led to the discovery of the α_{s1}-casein polymorphism (Clark & García 2017; Rahmatalla et al., 2022).

α_{s1}-casein is a milk protein fraction encoded by the CSN1S1 gene. It represents up to 25% of milk protein. The gene is highly polymorphic with a direct impact on casein synthesis. Alleles are divided into groups according to production potential into strong (A, B, C, H, L and M) producing 3.6 g.l^{-1}, medium (E and I) with 1.1–1.6 g.l^{-1}, weak (D, F and G) representing production of 0.45–0.6 g.l^{-1} and null (01, 02 and N) without production of α_{s1}-caseine (Rahmatalla et al., 2022).

Saanen is a breed characterized by a higher occurrence of the E allele (>0.50) and F allele (>0.30). The F allele has one nucleotide deletion (exon 9) that causes a frameshift, and two additional insertions (11 and 3 bp) in intron 9 that may be responsible for the omission of exons 9, 10, and 11, resulting in the absence of amino acids from position 59 to 95 in the mature protein (Rahmatalla et al., 2022).

Milking performance was affected by the weak allele both positively and negatively depending on breed. Protein and fat content were found to be higher in goats

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carrying the strong allele. Fat and protein contents were found to be higher in AA individuals (Tumino et al., 2023). Nutrition alone has not been shown to cause a change in the concentrations of individual caseins, however, insufficient energy intake decreases casein production in goats carrying strong alleles (Tumino et al., 2023). Dincel et al. (2020) observed a demonstrable effect of the CSN1S1 genotype on free fatty acids content in milk in the Saanen goat.

A massive decrease in the abundance of the F allele was observed in highly selected, progeny-tested males of the French Saanen population due to selection targeting on α_{s1}-casein (Carillier-Jacques et al., 2016). The F allele is considered to unwilling for cheese manufacturing due to its weak curd firmness and rennet properties. This allelic effect is linked to a larger mean diameter of casein micelles in weak alleles E and F. It causes that the quality and quantity of cheese produced during coagulation is reduced. Larger distance between micelles caused by the bigger diameter is not allowing to coagulate properly (Widodo et al., 2022).

The aim of this study was to determine the genotypic structure of the study population for the F and non-F alleles of the CSN1S1 gene and to investigate their effect on milk production traits.

2 Material and methods

2.1 Animals

In this study, genotypic and phenotypic data of 86 purebred Slovak White Shorthaired goats from one dairy farm were analysed. In the present study were used only goats on their first and/or second lactation. The breed originated in the early 1920s, when the indigenous goat breeds from Moravia were crossed with Saanen bucks imported from Germany and Switzerland (Makovický et al., 2020). Later in 1954, it was recognized as a separate breed, which today is divided into Slovak and Czech White Short-haired goat breeds (Vostrá-Vydrová et al., 2020).

The farm is located in central northern Slovakia and is operated in an intensive farming system using quality housing, feeding a high balanced granulated total mix ratio, with milking twice a day. Groups of housed animals are formed based on milk productivity. The farm processes milk in its own dairy, which produces goat milk powder and colostrum.

2.2 Sampling of genetic material

Hair root samples were collected on farm in April 2022. Samples were collected according to guidelines for taking samples for DNA testing provided by Breeding Service of Slovak Republic (PSSR, 2022).

Hair roots samples were used as a source of genomic DNA for genotypic analyses. The DNA was extracted using the commercial reagent DNAzol® Direct (Molecular Research Center, Cincinnati OH, USA). Approximately 10 hair roots were incubated with the 100 µl of DNAzol for 20 minutes at 90 °C. Afterwards the mixture was centrifuged at 6,000 × g for 1 minute at room temperature. Obtained lysate was stored at 4 °C.

2.3 Genotyping

Polymerase chain reaction (PCR), and primer-introduced restriction polymerase chain reaction (PIRA-PCR) were used to detect F allele. FIREPol HS polymerase (Solis BioDyne) was used to amplify the target regions of the CSN1S1 gene. The 1 bp deletion was detected using (PIRA-PCR) the specific restriction enzyme FastDigest TaqI (Thermo Scientific BioScience). The PCR reaction for both mutations was performed using a gradient thermocycler C1000 TouchTM (Biorad). Reaction mixture has total volume of 25 µl and consisted of 2 µl template DNA, 1 X $(NH_4)2SO_4$ buffer, 1 U FIREPol HS polymerase (Solis BioDyne), 0.2 mM dNTP mix, 0.2 pM of each primer and 4 mM MgCl2 in both PCR reactions. The PCR cycling regime was the same for both reactions as follows: 95 °C for 15 min, followed by 35 cycles (30 cycles for 1 bp deletion detection) of 95 °C for 5 seconds, 59 °C for 20 seconds and 72 °C for 20 seconds. The reaction was completed by a final elongation: 72 °C for 5 min. In the case of PIRA-PCR methods the PCR products were subsequently digested with a restriction enzyme.

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 R: GGGTTGATAGCCTTGTATGTT	Allele I+: 170 bp (ins) Allele I-: 159 bp (wild)	Wang et al., 2018
PIRA PCR*	1 bp deletion	F: TTCTAAAAGTCTCAGAGGCAG R: ATAAAAATGGTATACCTCACTTGTC	Allele D+: 92 bp (del) Allele D-: 67 bp + 26 bp (wild)	Ramuno et al., 2000 Bevilacqua et al., 2002

* 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)

Table 2 Allele determination combination of the occurrence of the CSN1S1 gene mutation

Allele	9888-90 Ct deletion	9981-2 CCGTAATGTTT insertion	Conventional allele
non-F	no	no	A
non-F	no	yes	B*
non-F	yes	no	N
F	yes	yes	F

Source: Pizarro et al., 2019
* including allele E

To separate amplified PCR products and restriction fragments, an agarose electrophoresis with GelRed(TM) intercalating dye (Biotium) was used. It was used a 3% agarose gel, separated fragments were visualized using an UV transilluminator and Olympus C-7070 documentary system. Detection method summary is provided in Table 1.

A combination of occurrence of C deletion in position 9888 and 11 bp insertion at position 9981 are commonly used for distinguishing alleles A, B (including E allele), F and N. For the purpose of this study, we used this method to identify alleles F and non-F. In the Table 2 there is a combination key for determining the allele by these mutations.

2.4 Milk performance

The performance record data was provided by the Breeding Service of the Slovak Republic (PSSR, š.p.) and is used with the written permission of the farm owners. Milk samples were taken in between years 2018 and 2021 only from goats in the first and/or second lactation. For the statistical model, there was used 101 lactations from 86 goat. There were used 15 goats with both, first and second lactations. Partial milk samples were taken monthly during milking once a day in a volume of 300 ml for at least 5 months during one lactation with a maximum interval of 34 days between samplings. A record was kept of the quantity of milk milked from each individual. The milking period was at least 150 days with a lactation interval of at least 190 days. Milk samples from individuals were analysed in the certified lab of PSSR, š. p. for lactose, protein, fat, and dry matter content. Overall milk was calculated out from partial milking samples using following formulae (PSSR, 2012):

$$P_o = P_1 \cdot D_1 + 0\sum_{i=1}^{k-1}(P_i + P_{i+1})\cdot D_i / 2 + P_k \cdot 15$$

where: P_o – overall milk production for the observed milking period (kg); P_1 – total milked volume during the first sampling; D_1 – number of days between kidding and the first sampling (D_1 = 40 days); P_i – total milked volume during i-th sampling; D_i – number of days between *i*-th and *i* + 1-th sampling, where *i* = 1... *k* - 1; P_k – total milked volume during last sampling; 15 – estimated number of days between last sampling and dry-off

The production of lactose, protein, fat, and dry matter as well as their content was calculated based on partial milk samples analysis by the PSSR, š. p.

To analyse effect of CSN1S1*F allele on the production traits was used N-Way ANOVA model described by following equation:

$$y_{ijkl} = m + G_i + Y_j + L_k + e_{ijkl}$$

where: y_{ijkl} – observed parameter; m – overall mean; G_i – genotype of CSN1S1 gene ($n = 3$); Y_j – year in which the lactation was measured; L_k – lactation number; e_{ijkl} – random residual effect

Scheffe's multiple comparison test was used to evaluate the differences between the means of the CSN1S1 genotype groups for all parameters.

All analyses were conducted using SAS version 9.4 (SAS Institute Inc).

3 Results and discussion

3.1 Gene and genotype frequencies

Since the basic biological equation says that the phenotypic manifestation is formed by the sum of genetics and the environment, it is necessary to determine to what extent these variables are involved in the result. Knowledge of the genotypic structure of the population is important for the breeder only if the gene under study has a direct influence on the health status, production, and reproductive indicators of the population, but especially on the farm economy. This knowledge can be used to focus on increasing the prevalence of genotypes with the highest impact on the desired traits. On the other hand, they can also be used to reduce the incidence of genotypes with an undesirable influence on the selected parameter. The result of genotyping is presented in Table 3 and

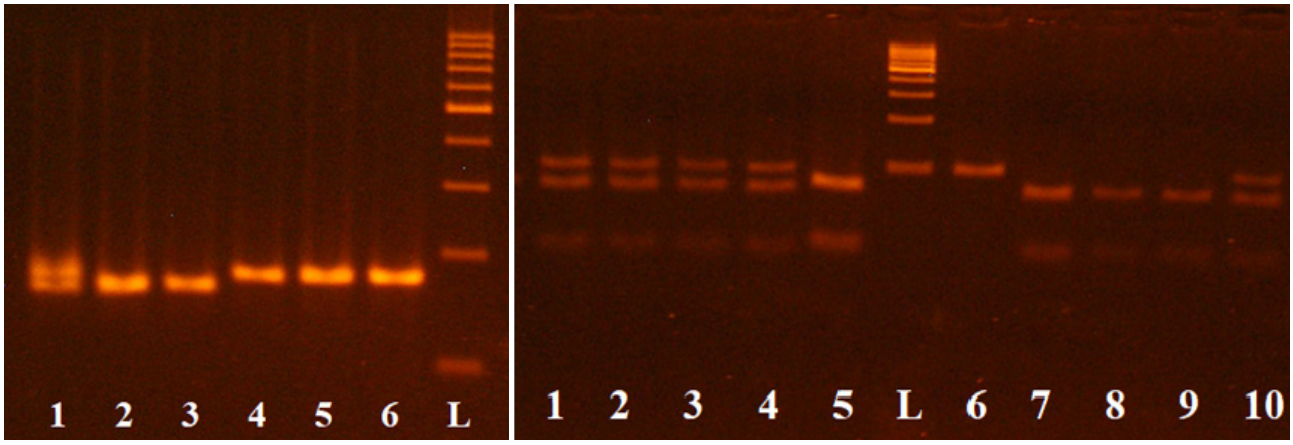

Figure 1 Images of PCR and PIRA-PCR electrophoresis gels for the determination of selected mutations
a) 11 bp insertion: 2 and 3 – homozygous I- (159 bp); 4, 5 and 6 homosygous I+ (170 bp); 1 – heterozygous I+I-(159 bp and 170 bp); L – 100 bp ladder (Thermo Scientific BioScience); b) 1 bp deletion: 5, 7, 8 and 9 – homozygous D- (26 bp and 63 bp); 6 – homozygous D+ (92 bp); 1, 2, 3, 4 and 10 heterosygous D-D+ (26, 67 and 92 bp); L – 100 bp ladder (Thermo Scientific BioScience)

gel images from PCR and PIRA-PCR electrophoresis are shown in Figure 1.

Table 3 The result of genotyping with allelic and haplotype frequencies and genetic structure

Alleles	Number	Frequencies
F	50	0.2907
Non-F	122	0.7093
Genotype		
F/F	7	0.0814
F/non-F	36	0.4186
non-F/non-F	43	0.5000

According to literature, it was thought that the most common CSN1S1 genotypes for French breeds are A, B, E, F and N (Vázquez-Flores et al., 2012). Many studies point to the prevalence of E and F alleles in Saanen goats, regardless of the country, such as France (E: 0.41 and F: 0.43) (Grosclaude et al., 1997), USA (E: 0.71; F: 0.30) (Maga et al., 2009) or Indonesia (F: 0.39) (Widodo et al., 2023). Saanen population from northern Italy shows significantly different frequency of allele F (0.15) (Frattini et al., 2014) as well as Czech White Shorthaired goat (F: 0.66) (Sztankóová et al., 2007). Nevertheless, populations with an increased occurrence of the A allele have recently appeared in the European region, due to selective crossing to fix the occurrence of alleles with high protein production (Widodo et al., 2023) and lower the frequencies of alleles with low protein content, especially allele F (Carillier-Jacquin et al., 2016). This can be seen in the results of the genotyping of French breeds Saanen and Alpine between 1985 and 2000, where the frequency of the F allele decreased from the original 0.43 and 0.41 in 1985 (Grosclaude & Martin, 1997) to 0.1940 and 0.1650 in 1990 and 0.0766 and 0.0545 in 2000 (Ouafi et al., 2002).

If we compare occurrence of allele F among breeds with different production indicators, i.e. Alpine (F: 0.41) and Saanen (F: 0.46) as highly productive breeds, Murciano-

Table 4 Summary statistics of all observed parameters of performance control

Parameter	No.	Mean	Standard Deviation	Minimum	Maximum
Milk (l)	101	890.38	286.28	150.13	1,334.18
Fat (kg)	101	28.64	8.68	5.33	45.90
Protein (kg)	101	26.08	8.61	3.96	39.76
Lactose (kg)	101	39.97	12.77	6.73	59.37
Dry matter (kg)	101	54.73	16.94	9.29	85.66
Fat (%)	101	3.29	0.49	2.37	4.47
Protein (%)	101	2.92	0.17	2.61	3.47
Lactose (%)	101	4.49	0.09	4.16	4.67

No. – number of observations

Granadina (F: 0.05) with excellent cheese yield and Rove (F: 0.10), originally a meat breed, which has very creamy and thick milk which is use to production of exclusive cheese, we can observe differences that could be related to some traits that are associated with different production uses (Martin & Lerox, 2000; Duclos, 2009; Irvine, 2018; Magro et al., 2022). Wang et al. (2018) observed a new 11 bp insertion in intron 8 of CSN1S1 gene, which was significantly associated with litter size in Shaanbei White Cashmere goat. Further researcher of this insertion on three goat breeds discovers positively association with several milk traits and negatively association with body measurement traits compared to individuals without the insertion (Zhang et al., 2019).

3.2 Milk production traits

Based on performance control provided by the Breeding Services of the Slovak Republic (PSSR), genotypic data were combined with phenotypic data for each lactation: milk production, fat production, protein production, lactose production, dry matter production as well as fat content, protein content, and lactose content. Individuals for which all the criteria for evaluating the milk yield of goats were not met were not included in further calculations (PSSR, 2012). Summary statistics of all observed parameters of performance control according are shown in table 4.

The use of N-way ANOVA showed that there was a statistically significant difference in the mean of the CSN1S1 genotypes between at least two groups for the five performance control parameters which can be seen in table 5.

The low value of the coefficient of determination for lactose content (0.29) means that the observed results are not well reproduced by the model, so we do not consider this model to provide statistically significant evidence for the effect of genotype on milk lactose content.

According to literature, French goat breeds have a protein, casein and fat content significantly associated (p <0.05) with the CSN1S1 gene genotype. AA homozygotes have a higher proportion of casein, protein, and fat than FF homozygotes (Grosclaude & Martin, 1997). On the other hand, Manfredi et al. (1995) observed that AF genotype produces lower percentages of fat and protein content than FF. The Jumanapari population shows that protein content in milk decreases according to genotype as follows: AA > BB > BF > AF > FF (Verma et al., 2020). This was supported by Moioli et al. (1998), who observed the same effect in the Alpine goats for protein content: AA > AE > AF > EE > EF > FF, and that AA, AE and AF genotypes has significantly higher percentage of milk fat than EE and FF. Yue et al. (2011) observed that homozygous NN genotype has significantly lower amounts of protein than individuals with FF genotype, which is placing it

Table 5 N-Way ANOVA results for CSN1S1 genotype

Parameter	Type III Sum of Squares	Mean Square	F-value	P-value
Milk (l)	121,540.30	60,770.15	4.66	0.0118*
Fat (kg)	101.68	50.84	2.01	0.1394
Protein (kg)	148.58	74.29	5.59	0.0051**
Lactose (kg)	318.68	159.34	5.95	0.0037*
Dry matter (kg)	494.13	247.06	3.56	0.0325*
Fat (%)	0.01	0.00	0.03	0.9703
Protein (%)	0.04	0.02	1.54	0.2205
Lactose (%)	0.04	0.02	3.16	0.0470*

* – p <0.05; ** – p <0.01

Table 6 Least square means (±SE) of CSN1S1 genotypes groups for parameters of performance control on which genotype CSN1S1 has a significant effect

Parameter	F/F	F/non-F	non-F/non-F	R2
No.	11	42	48	–
Milk (l)	749.99 ±42.29	698.19a ±29.56	787.70a ±40.71	0.86
Protein (kg)	21.22 ±1.35	22.83b ±0.94	22.84b ±1.30	0.84
Lactose (kg)	33.65 ±1.91	31.10c ±1.34	35.69c ±1.84	0.85
Dry matter (kg)	45.32 ±3.09	42.94d ±2.16	48.69d ±2.97	0.78
Lactose (%)	4.48 ±0.03	4.46e ±0.02	4.51e ±0.03	0.29

No. – number of observations; smal letter shows significant differences between groups within confidence interval of 95%

at the bottom of the line. Several studies have observed that a significantly lower percentage of protein and fat in FF individuals is accompanied by a higher amount of milk yield compared to genotypes AA and AB (Yue et al., 2011; Vacca et al., 2020; Verma et al., 2020).

The influence of the CSN1S1 genotype on milk yield in French breeds (Alpine and Saanen) is thought to be not entirely clear, with the AF and FF genotypes showing opposite effects between individuals and breeds (Caravaca et al., 2009). Tumino et al. (2023) concluded that milk yield was lower in Rossa Mediterranea with weak alleles than was found in Girgentana, which was inconsistent with Alpine breeds. Also lactose was lower in individuals with AA. For fat and protein content, no significant difference was observed between FF and AF individuals. Caravaca et al. (2011) also described the existence of a breed-specific allelic effect within genotypes of casein genes on milk characteristics. Thus, the setting of selection objects for the CSN1S1 gene needs to be performed after evaluating the allelic effect in the target population.

4 Conclusions

CSN1S1 genotypes may play an important role in milk composition. To improve milk performance in a desired way, either to increase milk yield or to increase a selected milk component, the CSN1S1 gene could be used in a breeding strategy. In order to identify an undesirable allele for elimination, its effect for the target breed needs to be evaluated for the possibility of a different effect in other breeds.

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