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Effect of the *αs1*-, *αs2*-, *β*- and *κ*-casein genotypes on the milk production parameters in Czech goat dairy breeds

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Abstract The aims of this study were to type genetic variants in the casein genes in Czech dairy goat population and to assess an association within genotypes group at the casein loci on the following milk production parameters: mean daily milk yield, protein yield, and protein production during 240-days milking in 389 animals of Czech mixed dairy goat breeds in first till third lactation. Different molecular techniques: AS-PCR, PCR-RFLP and PEA (SNaP Shot) were used to determine genetic variants. Molecular analysis confirmed that the *F* (0.622) allele at *CSN1S1* locus was predominant compared to the *A* allele and prevalent genotype was *FA* (0.524). At CSN2 locus allele *C* was predominant (0.669) compared to A allele (0.331) and genotype *AC* (0.486) and *CC* (0.427) had similar appearance. Frequency of allele *F* and *N* was similar (0.478, 0.522, respectively) at *CSN1S2* locus. Frequencies of allele *B* (0.765) and genotype *BB* (0.619) at the *CSN3* locus were dominant in goat population. Associations between milk production parameters and casein gene polymorphism were investigated, and significant effects were found on protein content, at loci *CSN1S1*, *CSN1S2*, *CSN3* ($P < 0.001$), and *CSN2* ($P < 0.05$). Also, significant effect was found on protein production at 240d of lactation at locus *CSN3*. These results indicate that casein genetic polymorphism loci should be used as a molecular marker for marker-assisted selection (MAS) for improving of milk composition and protein production.

Klíčová slova milk protein genes, genetic polymorphism, goat, lactation

1. INTRODUCTION

Ruminant milk composition is influenced by several factors e.g. breed, age, stage of lactation, nutrition, and environmental and genetic factors (1). Interest in the use of goat milk has increasing tendency. In order to manufacture new products, or to optimize the manufacturing procedure of existing products, more information is required concerning the chemical composition of goat milk and its technological properties.

In goat milk, the most abundant proteins are caseins. The four caseins expressed in goat milk, *αs1*, *β*-, *αs2*-, and *κ*- casein, are coded by the *CSN1S1*, *CSN2*, *CSN1S2* and *CSN3* genes, respectively. They are located within a 250-kb segment of caprine chromosome 6. In goats and other ruminants, casein represents about 80% of the total proteins in milk. (2)

A number of genetic variants of the casein genes that affect milk production traits have been identified and characterized in different species. Caroli et al (3) have reported a comparison among casein genetic variants in cattle, goat and sheep. Analysis of caseins in goats is complex due to extensive polymorphism in the four casein loci (4). So far, more than 17 alleles (*A*, B_1, B_2, B_3, B_4, *C*, *D*, *E*, *F*, *G*, *H*, *I*, *L*, *M*, *N*, *01* and *02*) have been detected and grouped into four classes based on different expression levels of *αs1*-casein in the milk. Each allele has unique characteristic, from single nucleotide substitutions/deletions to large insertion/deletions or even interallelic recombination (6, 7, 8, 9). The *CSN2* gene is a major casein fraction in goat milk (10). Genetic variants: *A*, *B* and *C* are associated with normal *β* – casein content in milk and two null alleles (0 and 0´) results in absence or a reduced level of *β* – casein (10, 11). Caroli et al. (4) and Marleta et al. (10) have reviewed the genetic variants of CSN1S2; seven variants have been identified among which five (*A*, *B*, *C*, *E* and *F*) are associated with a normal *αs2*- casein level, one with a low level (*D*) and one resulting in no *αs2*-casein (0) (10, 13). At present, 16 genetic variants have been characterized, resulting in 13 protein variants of the *CSN3* gene (4, 5, 10). All these variants are divided into two groups on the basis of their isoelectric point (IP): A^{IEF} (A, *B*, *B'*, *B''*, *C*, *C''*, *F*, *G*, *H*, *I*, *J*, *L* – IP = 5.29) B^{IEF} (*D*, *E*, *K*, *M* - IP 5.66) (14).

Casein polymorphism is important and well known due to its effect on qualitative and quantitative parameters in milk (3, 4, 5, 6). Technological properties of milk are also affected by protein composition especially in cattle and goats while in sheep the results are controversial (Giambra et al., 6).

The objectives of the current study were first to determine alleles and genotype frequencies at casein loci; second investigate an association between the variants of milk protein genes (*CSN1S1*, *CSN2*, *CSN1S2* and *CSN3*) and production parameters: mean daily record (kg), protein yield (%) and protein production during 240d milking in first till third lactation in Czech dairy goat breeds (White and Brown Shorthaired goats and their crosses).

2. MATERIAL AND METHODOLOGY

In our experiment 389 animals of Czech mixed dairy goat breeds from local bio ecology intensive farm, were used. They were freely lairaged throughout the year on the farm with free-run and year-round fed mixed ration for 3 years of experiment (2004-2006).

Genomic DNA was extracted from blood by using ABI PRISM 6100 analysis (Life Technologies, Co., USA) by standard protocol.

Casein genotyping

Genotyping at the calcium-sensitive casein loci (*CSN1S1*, *CSN2* and *CSN1S2*) and *CSN3* were performed by various methods (Table 1). The amplification products and the restriction patterns were visualized on agarose gel (PCR-agarose, Top-Bio, Ltd., CR) in TBE buffer stained with Ethidium Bromide.

Milk samples and data collection

Phenotype data were obtained from Czech dairy goats during 240d milking in the first till the third lactation in the years 2004-2006 and were taken from the official database of the Goat and sheep Breeders´ Association.

Statistical analysis

Genotype frequencies were determined for each group by direct counting. Analysis of variance was carrying out using the General Linear Model (GLM) procedure of the SAS software package v. 9.1.

Data performance was subsequently tested by model:

$$y_{ijkl} = \mu + O_i + P_j + L_k + G_l + e_{ijklm}$$

where

μ = constant
y = is the parameter monitored production;
O_i = effect of kidding (2003-2008);
P_j = effect of breed (strain B and H);
L_k = effect of lactation number (lactation 1, 2 and 3);
G_l = effect of the gene (alternatively genotypes genes *CSN1S1*, *CSNS1S2*, *CSN2* and *CSN3*);
e_{ijklm} = the residual effect.

3. RESULTS AND DISCUSSION

We typed four genetic variants: *A* (A*=A, B, C, D)*, *E*, *F* and *0* at *CSN1S1* loci. Allele *A* is related to a high content of *αs1-casein* in the milk, allele *E* correspond to an intermediate content, allele *F* is related to a low level, and allele 0 allele is associated with absence of *αs1*-casein in milk. Analysis of *CSN1S1* locus showed a prevalence of *F* allele (0.672) in observed dairy goat breeds. The frequency of allele *A**, *E* and *01* in dairy goats breeds were 0.278, 0.031 and 0.019, respectively. Comparison of our results with those available in the literature (15; 16; 17) result showed a similarity to the Alpine and Saanen breeds characterized by a high frequency of the *F* allele. The most common genotype was *A***F* (0,524) followed by the *FF* (0.360) genotype in population. Genotypes: *EF* (0.061), *F0* (0.039) and *AA* (0.015) occurred with a very low frequency in observed dairy goat breeds.

At *CSN2* locus, 3 alleles: *A*, *C* and null were typed. Even if *C* allele was predominant (0.669) compared to allele *A* (0.331) and the most predominant genotype was *AC* followed genotype CC (Table 3). Similar results were described in Italian goat populations, especially in the Saanen and Camosciata breeds (19; 4, 20). The absence of null allele in experimental population was compatible with the results obtained in other breeds characterized by absence or very low frequencies of null allele (22; 17; 21; 4).

At *CSN1S2* locus, we typed three alleles: *F*, *D*/*0* and *N**, (*N**= *A*, *B*, *C*, *E* alleles). Alleles *F* and *N* had similar frequency (0. 478, 0.522) respectively. The most predominant genotype was heterozygote *FN*, compared to homozygous *FF* and *N* N** that had low frequency of occurence in our experimental population (Table 3). Ramunno et al. (23) identified *F* allele as being associated with a normal – "high" level of *αs2*-casein content. Typing of the allele *F* allows detection only *N** allele, which described group of alleles associated with a high level of *αs2*-casein. In observed population the genetic analysis did not identify the intermediate *D* and "null" alleles. Results obtained in experimental population, in this respect, are similar to those described in Italian breeds (23; 17; 21; 4).

We typed four alleles: *A*, *B*, *C* and *D* alleles at *κ*- casein locus, and allele B was observed in highest proportion compared to alleles *A*, *C* and *D*. We observed that the most predominant genotype was *BB* (0.619), followed genotype *AB* (0.275). Genotypes, *AA* (0.07) followed *AD* (0.02), *BC* (0.01) and *BD* (0.01) occurred with very low frequency in population. Caroli et al. (4) and Sacchi et al. (21) have reported similar results in Italian, Spanish or German goat populations and described that allele *B* and *A* are the most popular. Sacchi et al. (21) have found that *C* allele had prevalent frequency in Italian population compared to alleles *A* and *B*, which have intermediate or low frequency of occurrence. This difference could be caused by breed, migration of animals as well as selection.

The relationships between polymorphism at the individual casein loci and production parameters are described in Table 2 and 3.

Mean daily milk yield: Production parameter was not being significant different ($P< 0.05$). On average, the mean value of daily milk yield did not differ markedly within genotype groups at the individual casein loci (Table 2 and 3). At *CSN1S1* loci, *AA* genotype, associated with a high level of αs_1 - casein in milk, had the lower mean value (2.20kg) compared to mean value (2.58kg) of *FE* genotype that is associated with a medium and low level of αs_1 - casein in milk (Table 2). Mean value of the parameter was almost equal within genotype groups at *CSN2* loci (Table 2). To the contrary situation has been occurred at *CSN1S2* and *CSN3*, where mean value of monitored feature has different values within genotype group. The mean values of parameter has been moved from 2.16 (*FF* genotype) to 2.68kg (genotype *NN*) at *CSN1S2* locus (Table 3). And the highest value (2.78kg) occurred at *BD* genotype, and decreased up to value 2.27 at genotype *BC* at *CSN3* locus (Table 3).

Protein yield: Significant statistical differences were found between protein content ($P<0.001$) and polymorphism at the individual casein loci: *CSN1S1*, *CSN2S1* and *CSN3*, and ($P< 0.05$) at *CSN2* locus in experimental samples. Identical results have been demonstrated by Grosclaude et al. (15), Pirisi A. (24), Dagnachew et al. (12) and Marletta et al. (10) that genetic variability affect protein content, in particular protein synthesis at individual casein loci. But also, they also refer that there is a tight linkage among casein genes and milk production traits should be improved by considering the entire casein haplotype instead of single gene typing.

Protein production during 240d milking in first till third lactation: Significant effect on protein production during 240d milking in first till third lactation was also found within groups at *CSN3* locus ($P < 0.05$). We assume, that this association could be caused by that there has been a direct effect of *CSN3* locus on milk quality and technological properties in milk. Similar knowledge have been reviewed by Marletta et al. (10) that B^{IEF} variants being associated with higher casein content in milk than A^{IEF} and confirmed by Caravaca et al. (31). Likewise, this association could be due to the direct effect of the different alleles as well as to the haplotype combinations, in which B^{IEF} variants are associated with strong alleles at *CSN1S1* and *CSN1S2* loci.

We assume that differences in the mean value of milk production parameters can be caused by differences in casein gene sequences e.g. single nucleotide polymorphism (SNP), insertion/deletion and differential splicing patterns, heterogeneity of genetic variants and their haplotype formation. Lastly, as Marletta et al. (10) point out, casein heterogeneity in milk can also be caused by post-translational modifications, such as different levels of phosphorylation and glycosylation.

4. CONCLUSION

In summary, in this study we confirmed effect of casein gene polymorphism on production parameters of milk, especially on protein content as well as effect of polymorphism at *κ*- casein on protein production during 240d of lactation. Such information about variability at milk protein loci (*αs-1*, *β-*, *αs2-*, and *κ-* casein) should be utilised for genetic improvement of Czech dairy goat breeds for increasing both the quality and quantity of milk production and technological properties as well as for conservation.

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Table 1 Analyses applied to the DNA samples for genotyping

Locus	Method	Typed alleles	Reference
CSN1S1	AS-PCR[1]	*E, non E*	Jansà-Pérez et al., (25); Rando et al., (26); Kumar et al., (18)
		01, non 01	Sztankoova et al., (27)
	PCR-RFLP	*A**[3]*, F*	Ramunno et al., (28)
CSN2	AS-PCR[1]	*0´*	Ramunno et al., (22)
	Light Cycler Analysis	*A, C*	Sztankoova et al., (29)
CSN1S2	PCR-RFLP	*D, F, N**[4]*, 0*	Ramunno et al., (13, 23)
CSN3	PEA[2]	*A, B, C, D, E, F, G*	Yahyaoui et al., (30)

[1]AS-PCR = allele specific – PCR
[2]PEA=Primer extension analysis
[3]*A*=A, B, C, D*
[4]*N*=A, B, C, E*

Table 2 Genotype frequency and standard errors for the mean value of production parameters from the first till the third lactation at the α_{s1} – casein and β – casein loci

			Genotype *CSN1S1*					Genotype *CSN2*		
Production parameters	*A*A** (n=6)	*FA* (n=204)	*FE* (n=24)	*FF* (n=140)	*F0* (n=15)	F-test	*AA* (n=34)	*AC* (n=189)	*CC* (n=166)	F-test
Mean daily milk yield (kg)	2.20±0.18	2.47±0.04	2.58±0.09	2.46±0.04	2.52±0.12	0.99	2.54±0.08	2.45±0.04	2.45±0.04	0.64
Protein content (%)	2.93±0.07	2.89±0.02	1.91±0.03	2.88±0.02	2.59±0.05	11.51^{c}	2.94±0.03	2.89±0.02	2.85±0.02	3.98^{a}
Protein production (g)[1]	14633±1476	16319±319	17567±731	16306±353	14739±955	1.71	17539±620	16329±305	15941±334	2.82

[1] protein production during 240d milking in the first till the third lactation; a, b, c (*P*≤0.05; *P*≤0.01; *P*≤ 0.001); * *A*=A, B, C, D*

Table 3 Genotype frequency and standard errors for the mean value of production parameters from the first till the third lactation at the α_{s2} – casein and κ – casein loci

		Genotype *CSN1S2*					Genotype *CSN3*				
Production parameters	*FF* (n=49)	*FN** (n=246)	*N*N** (n=65)	F-test	*AA* (n=26)	*AB* (n=106)	*AD* (n=9)	*BB* (n=241)	*BC* (n=1)	*BD* (n=6)	*F-test*
Mean daily milk yield (kg)	2.16±0.05	2.58±0.05	2.68±0.06	1.87	2.32±0.09	2.52±0.05	2.53±0.15	2.45±0.04	2.27±0.44	2.78±0.18	1.67
Protein content (%)	2.90±0.02	2.88±0.02	2.80±0.02	8.37^{c}	2.81±0.03	2.86±0.02	3.09±0.06	2.89±0.02	3.67±0.17	3.04±0.07	9.57^{c}
Protein production (g)[1]	14548±428	17141±433	17194±507	0.06	15006±715	16384±368	18071±1227	16330±314	17587±3541	20357±1494	2.65^{a}

[1] protein production during 240d milking in the first till the third lactation; a, b, c (*P*≤0.05; *P*≤0.01; *P*≤ 0.001); *N*=A, B, C, E*