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Genetic polymorphism at *CSN1S1*, *CSN2* and *CSN3* loci in the Holstein and Czech Fleckvieh breeds kept in the Czech Republic.

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Abstract

The dairy industry continuously strives to improve the quality of its products. It has long been known that manufacturing properties of milk are influenced by the relative composition of its proteins. The bovine casein cluster contains four milk protein genes, which are closely linked, in the order *CSN1S1*, *CSN2*, *CSN1S2* and *CSN3*. Several polymorphisms have been described for each casein gene. The objective of this work was to determine genetic polymorphism in the candidate genes *CSN1S1*, *CSN2* and *CSN3* in Holstein and Czech Fleckvieh breeds kept in the Czech Republic. The casein genes from a 184 Holstein (H) and 502 Czech Fleckvieh (CF) were determined by PCR-RFLP methods and LightCycler analysis. Results showed that at the locus *CSN1S1,* allele *B* was predominant in both breeds (0.965 and 0.852). *A*llele *A2* was predominant in both breeds (0.818 and 0.644) at the *CSN2* locus, with allele *A* predominant (0.788 and 0.640, respectively) at the locus *CSN3*.

Introduction

Casein proteins are the major constituents (80%) of total protein in bovine milk. These proteins have been extensively studied, and it has been reported that specific genetic variants of the caseins affect cheese making properties (Grosclaude et al., 1988; Martin et al., 2002). Significant associations with several milk production traits have also been identified for sites of polymorphism within noncoding regions in the casein complex (Martin et al., 2002). The casein loci have been physically mapped within a 200-kb fragment on the bovine chromosome 6 (*BTA6q31-33*) (Treadgill and Womack, 1990) in the order *CSN1S1-CSN2-CSN1S2-CSN3.* The loci are considered to influence milk production traits (Bovenhuis et al., 1992). Antibacterial activities of derived peptides (Malkoski et al., 2001) may also affect the biological fitness of the offspring.

Casein genes could thus be subjected to natural and artificial selection (Ward et al., 1997). Genetic polymorphisms in the casein genes allow the determination of casein haplotypes. The latter can be used for studies concerning quantitative traits (Lien et al., 1995; Velmala et al., 1995) or phylogeny (Mahe et al., 1999), since they provide more information than individual genes (Lien and Rogne, 1993).

Most of the casein alleles have been identified at the protein level and differ from each other by a few base substitutions that cause one or two amino acid changes in the protein. The methods, which are used to detect genetic variation on DNA level are based on PCR and its modifications e.g. PCR-RFLP, AS-PCR, PCR-SSCP. Further techniques include the hybridization probes (Hoffmann et al., 2007) and microarray technology or ligation detection reaction (LDR) combined with a universal array (UA) (Chessa et al, 2007).

This study aimed at examining the frequency of genotypes and alleles determining individual milk protein fractions (*CSN1S1*, *CSN2* and *CSN3*) in Holstein and Czech Fleckvieh, cattle breeds in the Czech Republic.

Material and Methods

Blood samples were collected from 686 animals (184 Holstein and 502 Czech Fleckvieh) for genomic DNA extraction using ABI PRISM 6100 analysis (Nucleic acid Prep. Station, Applied Biosystem, Co) according to the standard protocol. DNA fragments of the three casein genes were amplified by standard PCR technology.

Genetics variants *B* and *C* at the *alpha* S1 (*CSN1S1*) gene was typed for *Mae*III polymorphism in the promoter region (*CSN1S1prom*) by PCR-RFLP according to Koczan et al. (1993) with some modifications (primer sequence). *Beta* casein (*CSN2*) genetic variants *A1, A2, A3* and *B* were genotyped by PCR and using the LightCycler analysis (unpublished data*)*, whereas *kappa* casein (*CSN3*) gene was genotyped by PCR-RFLP according to the protocol (Schlieben et al., 1991; Lien and Rogne, 1993). In the latter the genetic variants: *A, B* and *E* have been evaluated.

Statistical analysis

Allele and genotype distribution, Hardy-Weinberg equilibrium, polymorphic information contents and heterozygosity were estimated using PowerMarker software V3.25 (Liu and Muse, 2005).

Results

Results of the molecular analysis of the bovine *CSN1S1*, *CSN2* and *CSN3* casein genes are presented in Table 1 and 2. Litter difference in the occurrence and frequencies of different alleles and genotypes at the casein loci were found between in Holstein and Fleckvieh breeds kept in the Czech Republic.

The *B* and *C* variants of the *CSN1S1* casein gene were observed in both Holstein and Czech Fleckvieh breeds with the *B* variant predominant in both breeds (Table 1). The *CSN1S1 CC* genotype was absent and the heterozygote genotype *BC* showed a low frequency only in the Holstein population. In the Czech Fleckvieh breed genotype *BC* and *CC* presented a low frequencies with the highest frequency found for genotype *BB*. According to Lien and Rogne (1993); Jann et al., (2004), and Chessa et al. (2007) the superiority of the frequency of alleles *CSN1S1 B* and *CSN1S1 C* is characteristic for almost all breeds of European cattle.

In both breeds, the genetic variant *A2* at the *CSN2* casein gene was predominant, followed by *A1*. Allele *A3* and *B* showed a very low frequency in both breeds (Table 1). Genotype frequencies were observed in the order *A2A2 > A1A2 > A1A1 > A2A3 > A1A3 >A2B*, *A3B* in the Czech Fleckvieh breed. In the Holstein breed, the order was *A1A2*, *A2A2 > A1A1 > A2B > A1B > A2A3*. Genotypes *A1A3*, *A3A3*, *A3B* and *BB* in the Czech Fleckvieh, and *A1B*, *A3A3* and *BB* in the Holstein population were absent (Table2).

At the *CSN3* casein gene, variant *A*, was predominant in both breeds, with different trends depending on breed and origin. Allele *B* and *E* showed low frequencies in both breeds (Table 1). In the Czech Fleckvieh population, *AA* and *AB* were found to be the predominant genotype compared to genotypes *AE*, *BB* and *BE*. Genotype *EE* was absent in both cattle populations. In Holsteins, genotypes *AE*, *BB* and *BE* were observed at low frequencies. Genotype *AB* and *AE* had a medium frequency, and genotype *BB* and *BE* showed very low frequencies in the Holstein population.

Large similarities regarding the casein loci, *CSN1S1*, *CSN2* and *CSN3* have been reported among Holstein populations (Poli and Antonini, 1991) and in some European bovine breeds (Jann et al., 2004).

All loci were tested for the Hardy-Weinberg equilibrium and characterised by expected (H_{exp}) heterozygosity and polymorphic information content (PIC) in both cattle populations. The Czech Fleckvieh population followed the Hardy-Weinberg equilibrium at all evaluated loci. However, the Holstein population did not follow the Hardy-Weinberg equilibrium the *CSN2* locus. The smallest heterozygosity was observed at the *CSN1S1* in the Holstein breed and the highest was described at the *CSN3* in the Czech Fleckvieh breed. PIC showed a very low value at the *CSN1S1* locus in Holsteins, with the highest value observed at the *CSN3* locus in the Czech Fleckviehs.

Conclusion

Allele and genotype distribution at all loci in Holstein and the Czech Fleckvieh agree with results previously described in northern, central European and southern European cattle breeds as well as in Holstein populations. Differences in allele distribution depend on origin, intensity of selection and domestication history among cattle breeds.

Although further studied are needed to evaluate the haplotype distribution on the both Holstein and the Czech Fleckvief breeds and effect on milk protein production.

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Table 1 Allele distributions, heterozygosity and PIC of the *CSN1S1*, *CSN2* and *CSN3* loci in Holstein and Flecvieh cattle breed.

		Czech Fleckvieh			Holstein		
Locus	Frequency	Heterozygosity	PIC	*CSN1S1*	Frequency	Heterozygosity	PIC
CSN1S1							
B	0.852			*B*	0.964		
C	0.148	0.245	0.221	*C*	0.036	0.07	0.066
CSN2				*CSN2*			
A1	0.328			*A1*	0.155		
A2	0.644			*A2*	0.818		
A3	0.001			*A3*	0.022		
B	0.027	0.472	0.387	*B*	0.005	0.289	0.274
CSN3				*CSN3*			
A	0.64			*A*	0.788		
B	0.326			*B*	0.106		
E	0.034	0.509	0.394	*E*	0.106	0.375	0.328

Table 2 Genotype distributions of the *CSN1S1*, *CSN2* and *CSN3* loci in Holstein and Czech Fleckvieh cattle breed.

		Czech Fleckvieh		Holstein	
Locus	Genotype	No.	Frequency	No.	Frequency
CSN1S1	*BB*	366	0.729	171	0.929
	BC	123	0.245	13	0.071
	CC	13	0.026	-	-
CSN2	*A1A1*	56	0.112	5	0.027
	A1A2	209	0.416	44	0.24
	A1A3	-	-	3	0.016
	A1B	8	0.016	-	-
	A2A2	209	0.416	126	0.685
	A2A3	1	0.002	4	0.022
	A2B	19	0.038	1	0.005
	A3A3	-	-	-	-
	A3B	-	-	1	0.005
	BB	-	-	-	-
CSN3	*AA*	201	0.400	113	0.614
	AB	222	0.442	30	0.163
	AE	19	0.038	34	0.185
	BB	45	0.090	2	0.011
	BE	15	0.030	5	0.027
	EE	-	-	-	