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POLYMORPHISMS IN *CSN3*, *CSN2* AND *LGB* GENES AND THEIR RELATION TO MILK PRODUCTION IN DAIRY CATTLE IN THE CZECH REPUBLIC

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Abstract

The principal objective of this study was to perform genotyping of the *CSN3*, *CSN2* and *LGB* genes in selected dairy cattle breeds in the Czech Republic, to determine genotype and allele frequencies and to perform an associative analysis on milk yield in kg, the percentage of fat and protein content, and fat and protein production in kg. The *AA* was the most frequent genotype for *CSN3* with a frequency of 0.474 and the *A* allele (0.694). The *BB* genotype (0.814) and the *B* allele (0.895) predominated in *CSN2* gene. Other genotyping was performed for the *A1* and *A2* alleles, the most frequent were the *A2A2* genotype (0.475) and the *A2* allele (0.680). The heterozygous *AB* genotype with a frequency of 0.900 was the most frequent in the *LGB* gene. A statistically significant higher protein content was found in the *AB* genotype in comparison to the *BB* in gene for *CSN2*. In the remaining cases, the effect of *CSN3*, *CSN2* and *LGB* polymorphic variations on the milk production indicators was not statistically significant.

Keywords: dairy cow, milk, milk proteins, heritability

INTRODUCTION

Milk from ruminants is an important component in the human diet. Europe has the highest cow milk production. Milk is the source of a number of valuable nutrients, especially proteins. Their content in cow's milk ranges between 3.0 to 3.5 %. The Holstein Association USA states for cows in performance test 3.11 % (Holstein Association USA, 2018), Kvapilík *et al.* (2018) 3.36 % for Holstein Friesian cows in the Czech Republic, and 3.55 % for Czech Simmental cows.

Milk proteins of dairy milk consist of two groups – casein and whey proteins. The casein fraction represents approximately 80 % of milk proteins and includes a total of 4 types of caseins: αs1-casein (*CSN1S1*), α_{s2}-casein (*CSN1S2*), β-casein (*CSN2*) a κ-casein (*CSN3*). Whey proteins comprise about 20 %. They are represented by α-lactalbumin (*LALBA*) and β-lactoglobulin (*LGB*) as well as by other proteins (Hoffman and Falvo, 2004; Galila and Darwish, 2008).

The study of genetic polymorphisms associated with dairy yields has been in the spotlight of cattle breeders all over the world for a long time. Their attention polymorphisms is primarily focused on milk protein polymorphism. A number of studies have suggested potential correlations to against milk yield, milk composition, or milk technological traits affecting cheese making industry (Ng-Kwai-Hang, 1998; Gurses *et al.*, 2018). *CSN3*, *CSN2* a *LGB* loci have been the most examined in this context. There is a large amount of research demonstrating the influence of the above-mentioned genes on quantitative and qualitative components of milk production and on selected milk quality indicators (Ozdemir *et al.*, 2018; Neamt *et al.*, 2017). On the other hand, however, there are also studies that have not confirmed the aforesaid correlations (Duifhuis-Rivera *et al.*, 2014; Dogru, 2015). For these reasons, it is necessary to continue to work on further detailed studies to obtain more polymorphisms precise answers to questions related to gene polymorphism and dairy production.

The study involved an analysis of genotype and allele frequencies in selected milk protein genes of dairy cattle in the Czech Republic and an association analysis between polymorphisms and milk yield indicators.

MATERIAL AND METHODS

The study was carried out using five dairy breeds. This cow population consisted predominantly of first lactation cows. From the breeders' perspective, the analysed sample was represented by dairy cows classified as Czech Simmental cattle (35 %), Holstein Friesian cattle (44 %) and a crossbreed group (21 %) with a prevailing portion of Czech Simmental cattle.

The genotypes in the three genes contributing to protein synthesis of cow's milk were determined in the study: *CSN3*, *CSN2* and *LGB*. The *A*, *B* alleles and the *A1*, *A2* alleles were determined in the *CSN2* gene, *A*, *B* and *E* alleles in the *CSN3* gene, and *A*, *B* alleles in the *LGB* gene. The *CSN3* gene was analysed in 728 cows, the *CSN2* gene of the *A, B* allele was the subject of an analysis in 721 cows, the *A1, A2* alleles in 668 cows and the *LGB* gene in 719 cows.

Milk samples were taken from dairy cows included in the study and DNA was isolated by using the DNA/RNA extractor MagCore HF16 Plus (RBC Bioscience). Isolation was performed according to the manufacturer's instructions using MagCore DNA Whole Blood Kit and MagCore Genomic DNA Tissue Kit (RBC Bioscience). Quality and quantity of the isolated DNA were verified by electrophoresis and spectrophotometry.

Genotyping of all loci was done by the PCR/RFLP method. Methodologies of individual genotyping were based on the following studies. The *CSN3* gene was analysed according to the methodology of Mitra *et al.* (1998), the *CSN2* gene (*A, B* mutation) according to Medrano and Sharrow (1991), the *CSN2* gene (*A1, A2* mutation) according to McLachlan (2006) and as for the *LGB* gene, the methodology was based on the study of Strzalkowska *et al.* (2002). The resulting genotypes were electrophoretically determined, genotype and allelic frequencies were calculated.

The differences between observed and expected frequencies of genotypes were tested using a χ^2 test in order to verify Hardy-Weinberg Equilibrium (HWE) on the significance level $P < 0.05$ and $P < 0.01$.

The milk performance data was obtained from the breeding documentation. Statistical analysis was done using the Statistica CZ 12 (Statsoft CR). ANOVA and Student t-test were used to evaluate differences in milk production traits among the different genotypes. Indicators of milk productivity as follows were evaluated: milk yield in kg, fat and protein percentage, and fat and protein yield in kg. The differences among genotypes were tested on the significance level $P < 0.05$ and $P < 0.01$.

RESULTS AND DISCUSSION

For the *CSN3* gene, six different genotypes were discovered. The highest frequency had the *AA* genotype discovered in 345 dairy cows with a relative frequency of 0.474. The second most frequent genotype was the *AB* genotype, with a frequency of 0.419. The next sequence of genotypes was as follows: *BB* (frequency of 0.050), *BE* (0.029) and *AE* (0.022). The least frequent was the *EE* genotype, occurring only in 5 dairy cows out of a total of 728 (Tab. I). In a number of studies, the *AA* genotype is the most common (Strzalkowska *et al.*, 2002; Neamt *et al.*, 2017). On the other hand, Bartoňová *et al.* (2012) found out that the most frequent genotype in Czech Simmental cattle was the *AB* genotype (0.487), followed by the *AA* (0.296) and *BB* (0.156) genotypes. The authors found out the lowest frequencies for the *BE* (0.033) and *AE* (0.026) genotypes. The *EE* genotype was not determined. Our analysis revealed that the *A* allele predominated whereas the *E* allele was the least frequent. In fact, identical results for Czech Simmental cattle were reported by Kučerová *et al.* (2006) and Bartoňová *et al.* 2012). Neamt *et al.* (2017) discovered the marked predominance of the *A* allele (0.785) above the *B* allele (0.215). Research of Trakovická *et al.*, 2012) reported similar results.

The study showed that the *BB* genotype and the B allele were predominant in the *CSN2* gene. In genotyping of the *A1* and *A2*, the *A2A2* (0.475) genotype, the *A2* (0.680) allele and then *A1A2* (0.404) genotype were the most frequent in the given cattle population. The same order of genotypes based on the established frequencies was described in Slovak Simmental cattle by Miluchová *et al.* (2013). The frequency of the most common *A2A2* genotype in their study was rather higher (0.541) than in our population as well as the frequency of the least numerous *A1A1* genotype (0.126). Masella *et al.* (2017) determined that the most common genotype in the cow population in northern Italy was the *A1A2* genotype (0.403),

I: *Frequencies of Genotypes and Alleles in bovine CSN2, CSN3 and LGB genes.*

CSN2 **alleles** ***A, B***

Genotype	n	Frequency	χ2	Allele	Frequency
AA	18	0.025	2.191^{ns}	A	0.105
AB	116	0.161		B	0.895
BB	587	0.814			

CSN2 alleles A1, B2

Genotype	n	Frequency	χ2	Allele	Frequency
A1A1	79	0.118	0.527^{ns}	*A1*	0.320
A1A2	270	0.404		*A2*	0.680
A2A2	319	0.478			

CSN3

Genotype	n	Frequency	χ^2	Allele	Frequency
AA	345	0.474	6.884^{ns}	A	0.694
AB	305	0.419		B	0.273
BB	36	0.049		E	0.032
AE	16	0.022			
BE	21	0.029			
EE	5	0.007			

LGB

Genotype	n	Frequency	χ^2	Allele	Frequency
AA	16	0.022	65.049^{**}	A	0.472
AB	647	0.900		B	0.528
BB	56	0.078			

^{ns}non-significant; **significant ($P < 0.01$)

II: *Milk production traits ($\bar{x} \pm s_x$) of different genotypes of CSN2, CSN3 and LGB in cows of Czech Simmental, Holstein and crosses.*

	CSN2			*CSN2*			*CSN3*					*LGB*		
Genotype	***AA***	***AB***	***BB***	***A1A1***	***A1A2***	***A2A2***	***AA***	***AB***	***BE***	***BB***	***AE***	***AA***	***AB***	***BB***
n	6	77	352	80	269	351	26	69	4	2	6	13	389	32
DIM[1]	268 ±43	286 ±41	289 ±34	294 ±34	294 ±25	293 ±31	290 ±28	287 ±38	303 ±5	303 ±4	305 ±1	267 ±72	289 ±33	288 ±45
Milk (kg)	5728 ±1603	6928 ±1803	7118 ±1929	7750 ±2033	8080 ±2147	7895 ±2238	6691 ±1081	6593 ±1410	7809 192	6214 ±727	6751 ±784	6107 ±2186	7121 ±1899	6821 ±1854
Fat (kg)	248.8 ±69.3	284.4 ±70.4	291.9 ±78.0	322.1 ±83.1	331.1 ±86.7	322.7 ±89.0	273.4 ±49.6	277.1 ±59.8	306.8 ±30.9	241.0 ±25.5	279.7 ±24.2	249.6 ±91.2	292.4 ±76.1	278.3 ±75.5
Fat (%)	4.36 ±0.40	4.12 ±0.35	4.11 ±0.35	4.15 ±0.37	4.12 ±0.33	4.11 ±0.33	4.09 ±0.32	4.22 ±0.36	3.93 ±0.39	3.88 ±0.04	4.16 ±0.17	3.99 ±0.44	4.12 ±0.34	4.06 ±0.42
Protein (kg)	207.5 ±54.4	248.2 ±61.4	249.7 ±60.8	267.1 ±62.8	275.8 ±64.6	270.3 ±66.6	236.5 ±42.7	236.3 ±50.7	269.0 ±16.5	213.0 ±28.3	243.0 ±25.5	218.8 ±82.5	250.9 ±60.2	237.0 ±57.7
Protein (%)	3.65 ±0.22	3.60^{a} ±0.19	3.53^{b} ±0.21	3.47 ±0.23	3.45 ±0.23	3.46 ±0.23	3.53 ±0.21	3.59 ±0.17	3.45 ±0.24	3.43 ±0.05	3.60 ±0.08	3.55 ±0.16	3.55 ±0.21	3.51 ±0.21

[1] DIM – days in milk; a,b means with different superscripts in row differ within genotypes of each gene at *$P < 0.05$*;

followed by the *A2A2* (0.301) and *A1A1* (0.139) genotypes. The minor *F* and *I* alleles were also determined in their study. However, the frequency of a total of seven genotypes with these alleles was only 0.065.

The last gene included in this study was the *LGB* gene. The *AB* heterozygous genotype was determined to be most frequent. Its frequency of 0.900 was largely predominant in the cow population. The same genotype order was also described in Holstein crossbred cows in the study of Molee *et al.* (2015), *AB* 0.535, *BB* 0.289 and *AA* 0.175. The *AB* genotype was discovered to be the most frequent also in the study of Neamt *et al.* (2017). The *LGB* gene was the only gene included in our analysis not to be in HWE.

The *CSN2* association analysis (Tab. II) revealed a statistically significant difference between the *AB* and *BB* genotypes in protein content in milk ($P < 0.05$). The tendency clearly showed a positive effect of the *A* allele on protein content in milk but the differences between the *AA* and *AB* genotypes, and between the *AA* and *BB* genotypes, were not significant, probably also due to the very low number of *AA* homozygotes. Trends in milk production (in kg) were opposite, non-significant, hence the differences in protein production per lactation between the *AB* and *BB* genotypes were minimal. Ozdemir *et al.* (2018) did not detect any significant influence of the gene on milk yield indicators in their extensive meta-analysis.

Similarly, the differences in milk performance indicators between the *A1* and *A2* versions of the *CSN2* gene were not significant. Nguyen *et al.* (2018) made a similar conclusion for the fat, protein, lactose, and total dry matter content. However, they detected subtle differences in the physical properties of milk with the *A1A1* or *A2A2* phenotype and recommend further research in this area.

The most closely observed gene from the casein cluster is *CSN3* due to the repeatedly substantiated significant effect on protein content in milk, renneting, yield and quality of curd. However, the results in our study were influenced by a very low frequency of the *BB*, *BE* and *AE* genotypes and therefore the differences were apparently not statistically significant. Some authors discovered that higher protein content is associated with the presence of the *B* allele in the genotype. Ozdemir *et al.* (2018) specify in their comparison of the *AA* with *BB* genotypes not only a significant effect on the protein content, but also an effect on the fat content in milk. They also discovered a remarkable difference in protein content by comparing the *AA* with *AB* genotypes. In our analysis, however, the differences between the *AA* and *AB* genotypes, which were sufficiently numerous, did not achieve statistical significance.

Gurses *et al.* (2018) also found that the *CSN3* polymorphism significantly affects the protein and non-fat dry matter content of the Holstein Friesian and Brown Swiss cattle. They discovered a significant effect on the fat content in the Jersey cattle. According to the findings of Neamt *et al.* (2017), the *AA* genotype was associated with higher milk production than *BB* and a higher fat content. On the other hand, dairy cows with the *BB* genotype had a higher protein content in milk compared to both the *AA* genotype cows and the *AB* genotype cows.

Also, polymorphic variants of the *LGB* gene were not significantly associated with differences in milk yield in our study. However, the *AB* heterozygous genotype showed the highest milk yield in kg, fat production in kg, fat content in %, and protein production in kg. Differences in fat and protein production in kg were getting closer to the statistical significance limit. Similar results were also discovered by Neamt *et al.* (2017), the *AB* genotype had significantly higher milk and protein production than both homozygous genotypes, a non-significantly higher protein content and fat production. In the association study of these authors, the *BB* genotype had a significantly higher percentage of fat in milk than both homozygous genotypes (*BB* 4.23, *AA* 4.17, *AB* 4.20). Ozdemir *et al.* (2018) describe the genotype succession such as *AB* > *BB* > *AA* for daily milk production, however, homozygous genotypes were better in milk yield over the lactation period. The authors also state that the *B* allele is associated with a higher milk fat content. Nevertheless, they reflect that not all the authors confirm this dependence, which is also true for the effect on protein content.

CONCLUSION

The present study is part of a larger study specialized in determining the impact of selected polymorphisms in milk protein genes on the qualitative and technological milk traits. An analysis of genotype and allele frequencies was performed in the dairy cattle population in the Czech Republic and the association analysis between polymorphisms and milk yield indicators. A relatively low frequency of the *B* variant in the *CSN3* gene has been detected which can be considered an adverse phenomenon with regard to a positive impact on renneting established by a number of authors. It is encouraging that a number of cattle breeding companies declare that their bulls come with genotypes at *CSN3* locus. However, changes in the cattle population frequencies represents a long-term issue. With the exception of one case, the associations were insignificant.

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