Casein Polymorphism in Relation to the Milk Production Traits of Slovak Spotted Cattle

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Summary

The aim of this study was to identify polymorphisms in the bovine genes encoding CSN1A1 and CSN3 caseins and analyse the structure of population of Slovak Spotted cattle. Subsequent analysis was carried out to estimate the effect of both polymorphisms on long-life milk production traits. The genomic DNA was extracted from totally 180 blood samples. All of analysed animals were genotyped by using the PCR-RFLP method. In population the prevalence of homozygote animals was found. For CSN1A1 gene the BB genotype was noted as predominant. In terms of CSN3 gene the genotype frequencies was more balanced. The most frequent genotype were AA. The population genetic indices signalised the decrease of genetic variability in population mainly for CSN1A1 gene. Except F_{IS} index, each of parameter reflected the high proportion of BB homozygous individuals in population. The values of observed and expected heterozygosity for CSN3 gene indicated similarly the prevalence of homozygote individuals in population, but the decrease of heterozygosity was not so high. The effect of polymorphisms on production traits was tested based on the GLM procedure. In case of both SNPs we were able to describe the variability of analysed traits on 95%. The study results clearly indicated the positive effects of $CSN1A1^{BB}$ and $CSN3^{AA}$ genotypes in order to improve the milk production traits in Slovak Spotted cattle. In addition, the statistical analysis confirmed the key role of casein in milk production and its composition.

Key words

casein, cattle, genetic polymorphism, milk yield and composition, PCR - RFLP

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Introduction

Main goals of the dairy cattle breeding are the search of an economically efficient way of the improvement of milk production and the qualitative milk traits. Selection and breeding of animals with desirable genotypes is of crucial importance for the genetic improvement of dairy cows. Milk protein genetic polymorphisms of the genus Bos provoke a significant scientific interest, mainly associated with their evolution, population structure, breeding and hybridization. Over the last decades, studies have been concentrated on the influence of the genetic variants of the major milk proteins on the quantitative and qualitative milk traits and their technological properties (Hristov et al., 2012).

Casein is a family of milk proteins that exists in several molecular forms and is the main protein present in the bovine milk. Casein content in bovine milk ranges from 2.4% to 2.6%, which represents about 78% of all milk proteins (Żukiewicz et al., 2012). Bovine milk proteins are classified by two groups; one group is soluble at pH 4.6, on the other hand, the other group is not soluble at pH 4.6. The soluble fraction is called whey protein such as α lactalbumin (LALBA), β lactogloulin (LGB). However, the insoluble part constitutes four casein protein such as α s1 casein (CSN1S1), α s2 casein (CSN1S2), β casein (CSN2) and κ-caseins (CSN3) (Gurcan, 2011; Gras et al., 2016). Caseins are encoded for by single autosomal genes that occupy approximately 200 Kbp on the sixth chromosome (Zwierzchowski et al., 2001). The linked genes are present in the sequence: αS1, β, αS2, κ, which means that their expression during lactation is regulated in a synchronic way. It is possible that a LCR (locus control region) exists for the casein genes (Żukiewicz et al., 2012). Most of the studies have been focused on CSN1S1 and CSN3 of the group of caseins and LGB of the whey proteins. These proteins have a great effect on milk production and milk components. The genetic variants of the CSN3 gene have a dominant role due to the protein influence of this protein on the formation, structure and stabilization of the casein micelles with respect to milk technological properties and cheese production (Hristov et al., 2012; Deb et al., 2014).

Five genetic variants of CSN1S1 have been identified: A, B, C, D, and E. The allele B is of the highest frequency in *Bos taurus*, whereas C is the most common in *B. indicus* and *B. gruniens* (Eigel et al., 1984). The allele B encodes glutamine whereas C encodes glycine, both at position 192 (Żukiewicz et al., 2012). The CSN3 gene comprises a 13 kb sequence divided into 5 exons (Martin et al., 2007). This gene encoded protein that affect the yield, composition as well as technical properties of milk. It is a standout amongst the most vital milk proteins in the mammals that assumes a crucial part in the milk quality and coagulation. Up to now, within the sequence of CSN3 gene eleven genetic variants have been distinguished (Trakovická et al., 2012). The most widely recognized alleles are A and B that differ in two amino acids at position 136 (Thr → Ile) and 148 (Asp → Ala) (Awad et al., 2016).

The aim of this study was to identify polymorphisms in CSN1S1 and CSN3 genes and to analyse their effect on long-life milk production traits in population of Slovak Spotted cattle.

Material and methods

In the study, overall 180 Slovak Spotted cow have been included to determine the impact of genes located in bovine casein locus on long-life milk production traits. The Slovak spotted cattle, belonging to the dual-purpose production type, is genetically related with Simmental cattle and has a long tradition in Slovakia.

The genomic DNA was extracted from blood samples according to protocol of Miller et al. (1988). Subsequently, the concentration and purity of genomic DNA were tested based on the spectrophotometry measurements by the optical density at wave length of 260 nm. The polymorphisms in CSN1S1 and CSN3 genes were analysed according to Miluchová et al. (2014) using RFLP methods. The products of PCR reaction and restriction fragments have been separated and visualised using horizontal electrophoresis in 2% agarose gels (130 V for 50 min) and stained with day GelRed.

The genotypic structure of population and allele frequencies have been determined using Genalex version 6.1 (Peakall and Smouse, 2012). The Hardy-Weinberg equilibrium in population was tested based on the significance between observed and expected genotype frequencies using Chi-square test. The diversity indices including observed (Ho) and expected heterozygosity (H_e), effective allele numbers (N_e), polymorphic information content (PIC), and F_{IS} index was calculated using Genalex version 6.1 (Peakall and Smouse, 2012). Associations of the animal genotypes with long-life milk production traits were determined by analyses of quantitative traits. Statistical analysis was performed using SAS Enterprise Guide 4.2 software (SAS Institute Inc., 2009) and significance of differences based on genotypes effect of production traits were tested by following general linear models:

$$Y_{ijklm}^{1,2,3} = G_i + BT_j + S_k + L_l + BY_m + e_{ijklm}$$

where: $Y_{ijklm}^{1,2,3}$ – milk, protein and fat yield, G_i – effect of genotype, BT_j – effect of breed type, S_k – effect of sire, , L_l – effect of number of lactations, BY_m – date of birth, e_{ijklm} – random error.

Results and discussion

In population of Slovak Spotted cows both analysed SNPs was successfully genotyped. For the CSN3 gene have been identified all three genotype (AA, AB, and BB). In case of CSN1A1 only two genotype was detected (BB and BC), the CC genotype was not observed. The allele and genotype frequencies are listed in Table 1. For CSN1A1 gene the BB genotype was predominant in population, which also indicated considerably higher frequency of B allele in comparison to C allele. In terms of CSN3 gene the genotype frequencies was more balanced. The most frequent genotypes were AA and AB genotypes. The lowest frequency was found for homozygous BB genotype. In case of both SNPs the differences between observed and expected genotype frequencies were not significant that indicate the Hardy-Weinberg equilibrium in population and low impact of effect such as selection, inbreeding or migration. However, the population genetic indices signalised the decrease of genetic variability in population mainly for CSN1A1 gene (Table 1). Except F_{IS} index, each of parameter reflected the high proportion of BB homozygous

Table 1. Summary of genotype structure and population indices evaluated in population

Locus	Genotypes frequency			Alleles frequency		χ2 test	H_o	H_e	N_e	PIC	F_{IS}
CSN1S1	BB 0.96	BC 0.04	CC -	B 0.98±0.01	C 0.02±0.01	ns	0.04	0.04	1.04	0.04	-0.019
CSN3	AA 0.51	AB 0.36	BB 0.13	A 0.69±0.02	B 0.31±0.02	ns	0.35	0.42	1.74	0.34	0.17

H_o – observed heterozygosity, H_e – observed heterozygosity, N_e – effective allele number, PIC – polymorphic information content, F_{IS} – fixation index, ns – not significant

Table 2. Basic statistical variation measurements of long-life milk production in analysed population

Trait	N	Mean	SD	Min	Max
MY (kg)	180	19395.31	15372.86	733.00	55102.00
FY (kg)	180	702.06	544.50	23.00	2007.00
PY (kg)	180	619.17	495.91	22.00	1763.00

n – number of individuals, mean – average value, SD – standard deviation, Min – minimum, Max – maximum, MY – milk yield, FY – fat yield, PY – protein yield

Table 3. Impact of fixed effects included in GLM model on milk production traits in analysed population

Genotypes	N	Traits (in average)			Factors
		MY (kg)	FY (kg)	PY (kg)	
CSN1S1					
BB	169	19719.67***	713.0814***	629.5349***	Breed type*** Sire*** No. of lactations** Birth date**
BC	8	12421.38***	465.125***	396.25***	
CSN3					
AA	91	22357.25**	812.02	716.02**	
AB	62	15481.63**	555.66	489.66**	
BB	24	18477.67**	670.96	593.25**	

MY – milk yield, FY – Fat yield, PY – protein yield, ** P<0.01, *** P<0.0001

individuals in population as well as the absence of CC genotype. The values of observed and expected heterozygosity for CSN3 gene indicated similarly the prevalence of homozygote individuals in population, but the decrease of heterozygosity is not so high. The F_{IS} index on level 0.17 reflects slightly increase of homozygosity in population. According to the classification of PIC (low polymorphism if PIC value < 0.25, median polymorphism if 0.25 < PIC value < 0.5, and high polymorphism if PIC value > 0.5) (Botstein et al., 1980), the analysis show low (CSN1A1) or median (CSN3) levels of polymorphic information content of SNPs across population. The effectiveness of loci allele impact in populations has been expressed by effective allele numbers. Comparison of N_e showed higher effective allele numbers across populations for CSN3 gene and indicated good level of genetic variability in analysed population at the considered locus.

The obtained allele and genotype frequencies of CSN1A1 and CSN3 genes are in agreement with previously published studies. Similarly to our results Kučerová et al. (2006) found in population of Czech Simmental cattle the predominance of $CSN3^A$ allele. In Czech Fleckvieh Bartoňová et al. (2012) confirmed the higher proportion of $CSN3^A$ across analysed individuals. Keating et al. (2007) showed that this proportion of CSN3 allele frequencies is valid in various dairy cattle breeds. In the Slovakia, the CSN3 polymorphism was analysed in Simmental and Holstein cattle. Trakovická et al. (2012) reported in both breeds the predominance of A allele over the B allele.

Table 2 shows the observed average values of analysed production traits in population. The average values for each trait in relation to the specific CSN1A1 and CSN3 genotypes are listed in table 3. The statistical significance of observed differences in association analyses was affected by the number of data available for particular trait. Therefore, only animals with relevant data of production traits were used in subsequent analysis.

In case of both SNPs we were able to describe the variability of analysed traits on 95%. Except genotypes effect, four other fixed effects were included in the GLM model: sire, breed type, total number of lactation in long-life production, and date of birth. As expected due to the biology of milk production each of them showed high statistical significant impact on milk, protein as well as fat yield (Table 3).

The statistical analysis confirmed the key role of casein in milk production and its composition. In both SNPs higher milk production was found in the most frequent homozygous genotypes in population. Cows of homozygous CSN1A1^{BB} genotype were characterized by highest milk, protein and fat yield ($P < 0.0001$). The opposite relationships between milk production and CSN1A1 genotypes were found in the group of cows with CSN1A1^{BC} genotype. The statistical analysis of CSN3 genotype effect on long-life milk production traits shows significant differences ($P < 0.001$) only for milk and protein yield. The genotype CSN3^{AA} was favourable for each of analysed production traits.

The significant impact of caseins on milk production has been reported in many studies. In comparison to our results Micinski et al. (2007) reported the superiority of the BC genotype in population, but they confirm the positive impact of B allele on milk yield. Similarly Hristov et al. (2013) confirmed the predominance of CSN1A1^{B} allele over the CSN1A1^{C} allele and its significant impact mainly in regard to milk yield. For CSN3 genotypes Curi et al. (2005) reported the association between genotype AA and higher milk production. However, Strzalkowska et al. (2002) found that the BB genotype is favourable for milk fat percentage.

Conclusion

The statistical analysis showed significant effect of both analysed SNPs in genes encoding CSN1A1 and CSN3 caseins on long-life milk production of Slovak Spotted cattle. The study results indicated the positive effects mainly in relation to the CSN1A1^{BB} and CSN3^{AA} genotypes in order to improve the economically important traits for farmers. It is generally accepted that both of the presented genes have significant impact on milk production and milk components and are considered as candidate genes. Therefore the analyses of increase genetic gain using marker assisted selection including casein genes can put more exactly genetic evaluation and decrease time, which is needed to realization in cattle breeding.

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