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KAPPA-CASEIN GENE POLYMORPHISM (CSN3) AND ITS EFFECT ON MILK PRODUCTION TRAITS

POLYMORFIZMUS GÉNU KAPPA-KAZEÍN (CSN3) A JEHO VPLYV NA UKAZOVATELE PRODUKCIE MLIIEKA

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The aim of this study was to evaluate the relationship between the polymorphism in the kappa - casein gene and milk production traits in the crosses of Simmental and Holstein cattle breeds. A total of 80 hair roots samples were used to investigate how CSN3 gene polymorphisms affect milk production traits. The polymorphism of kappa - casein gene on bovine chromosome 6 was genotyped by using the polymerase chain restriction fragment length polymorphism. The digestion of PCR products with restriction enzyme *Hind*III revealed two alleles: the A allele gave one fragment, 443 bp in length and the B allele gave two fragments of 348 and 95 bp. The predominant allele was A with observed frequency 0.76. All three genotypes AA, AB and BB were detected in population. The most frequent genotype was AA with frequency 0.575. The results from the statistical association analysis between CSN3 genotypes and milk production parameters – milk, protein and fat yield (kg) in standard length of lactation were not significant.

Keywords: dairy cattle, kappa - casein gene, milk production, polymorphism

The increase of component contents in milk and improved quality of its technological properties are pressured through the use of main selection factor of the program for cattle milk performance improvement (Ziemiński et al., 2005). Milk components in cattle are quantitative traits, being influenced by environmental and genetic factors (allelic variations at many loci). The bovine milk specific proteins include four casein genes: α_{s1} -, β -, α_{s2} - and κ -casein (CSN1S1, CSN2, CSN1S2 and CNS3, respectively) producing approximately 80 % of the protein content of cow's milk (Farrel et al., 2004). The genes that are correlated with performance parameters explain a part of the genetic variance and can improve the estimation of breeding value. Therefore, they can be used as a suitable supplement to conventional breeding procedures (Příbyl, 1995). Their polymorphism, related to the differences in animal performance can be taken into account in the selection process. Casein genes of cattle are located in the q31 – 33 regions on chromosome 6. They form a cluster of four closely linked genes located in the following order: α_{s1} , β , α_{s2} and κ . The entire cluster is 200 kb in length (Rijnkels et al., 1997). The α_{s1} -, β - and α_{s2} -casein genes are linked most closely and form an evolutionary related family, whereas the κ -casein gene (CSN3) is at least 70 kb away from the others (Ferretti et al., 1990). Casein synthesis occurs at a definitive developmental stage, it is tissue specific and under complex multigene control. Genetic polymorphisms in the casein genes allow the determination of casein haplotypes. The relative concentration of caseins in the mammary gland is also affected by casein genetic variants and has a significant impact on micelle size and technological properties of milk (Juszczak et al., 2001). The α_{s1} - and α_{s2} -casein genes are the least polymorphic. Study of the β -casein polymorphism at the protein level showed that cattle had 11 allelic variants of the gene encoding it, A1, A2, and B alleles being the most frequent (Sulimova et al., 2007). Kappa-casein considerably differs from other caseins in structure and other properties. They are highly homologous to the γ chain of fibrinogen and fulfil a function similar to the function of this protein: they serve a stabilizing factor during the formation of

micelles structure in curdling milk (Grosclaude, 1988). Kappa-casein constitutes about 25% of the casein fraction of milk and several polymorphisms have been found for this protein. The CSN3 gene is located on chromosome 6q31 and consists of 5 exons (Martin et al., 2002). The overall length of the gene is close to 13 kb, but most of the coding sequences for the mature protein are contained in the fourth exon (Azevedo et al., 2008). Eleven genetic haplotypes of CSN3 that differ in the coding region have been identified to date (Farrell et al., 2004), the most frequent being the A and B alleles (Prizenberg et al., 1999). Kappa-casein variants A and B differ by two amino acid substitutions, Thr 136/Ile and Asp148/Ala (Lin et al., 1992). These two point mutations are located relatively close to several glycosylation sites and probably affect the structure of the protein and glycosylation patterns (Fox, 1992). These genetic variants are related to milk performance traits of cows, milk instability for processing, and to cheese production in particular. The B variant of CSN3 gene is associated with an increase in milk protein as well as cheese production because it plays an important role in preserving the other caseins from precipitation, with superior coagulation properties that give the milk higher properties for cheese production (Van, 1991). The B allele only promotes an increase in cheese yield and improves cheese quality, however, it also correlates with other valuable parameters of milk productivity and chemical composition of milk. The effect of the CSN3 allele on the milk performance traits has also been reported in many studies involving Kamiński (2004), Kučerová et al. (2006), Heck et al. (2009) or Bartoňová et al. (2012). The aim of this work was to evaluate the effect of SNP CSN3 genotype on milk production traits in standard length of lactation in crosses of Simmental and Holstein cattle breeds.

Material and methods

Animals and DNA extraction method

The total numbers of hair roots and blood samples were taken from 80 cows (Crosses of Simmental and Holstein). Genomic

DNA for genotyping was extracted from hair roots with isolation kit Extract-N-Amp™ Tissue PCR (Sigma-Aldrich) and from blood samples with standard phenol – chloroform extraction method (Miller et al., 1988). DNA concentrations were estimated by spectrophotometer measuring the optical density at wave length of 260 nm.

Analyses of polymorphisms

A 443 bp fragment containing analyzed polymorphic site of CSN3 gene was amplified by PCR using forward and reverse primers according to Schlieben et al. (1991). In case of blood samples PCR was carried into 25 µl final volume containing: 1 × PCR buffer with (NH₄)₂SO₄, 1.5 mM MgCl₂, 200 µM of dNTPs, 0.5 µM of primers, 1 U Tag DNA polymerase and 50 ng genomic DNA template. The PCR reaction of genomic DNA isolated from hair roots was prepared according to protocol of isolation kit into 20 µl final volume containing: Extract-N-Amp reaction mixture, 0.5 µM of each primers and DNA extract. Thermal cycling conditions included: an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min, 72°C for 1 min and a final extension at 72 °C for 8 min. Genotype analyses were performed using PCR-RFLP method. The PCR products of CSN3 gene were digested with 1 µl of *Hind*III (Promega) restriction enzyme at 37 °C in time 3 hours. The digestion products were separated by horizontal electrophoresis in 2% agarose gels in 0.5 × TBE (130 V for 30 min) stained with GelRed (Biotium) prior to visualization under UV light.

Table 1 Primer sequences of CSN3 locus

Locus (1)	Primer sequence (2)
CSN31	F 5' - GCT GAG CAG GTA TCC TAG TTA T - 3'
	R 5' - CTT CTT TGA TGT CTC CTT AGA G - 3'

F = forward, R = reverse

Source: Schlieben et al., 1991

Tabuľka 1 Sekvencie primerov lokusu CSN3
(1) lokus, (2) sekvencia primeru

Statistical analysis

The allele and genotype frequencies of CSN3 polymorphism were examined for deviation from Hardy – Weinberg equilibrium using χ^2 test. The effect of CSN3 genotype on milk production traits in standard length of lactation – milk, protein and fat yield were analysed using SAS 9.1 software.

Results and discussion

A single nucleotide polymorphisms of the bovine CSN3 gene located on chromosome 6 based on the use of restriction fragment length polymorphism was detected. After digestion of 443 bp PCR products with restriction enzyme *Hind*III three genotypes were determined. The digested AA PCR product exhibited one fragment of 443 bp, whereas the BB genotype gave two fragments of 348 and 95 bp. Figure 1 shows PCR product size and restriction patterns of all the three genotypes AA, AB and BB.

On the basis of the Hardy-Weinberg equilibrium (law), the expected frequencies of A and B alleles were in population of 80 cows 0.76 and 0.24, respectively. The observed frequencies of genotypes were 0.575 ($n = 46$), 0.363 ($n = 29$), 0.062 ($n = 5$) for AA, AB and BB genotype, respectively. The expected

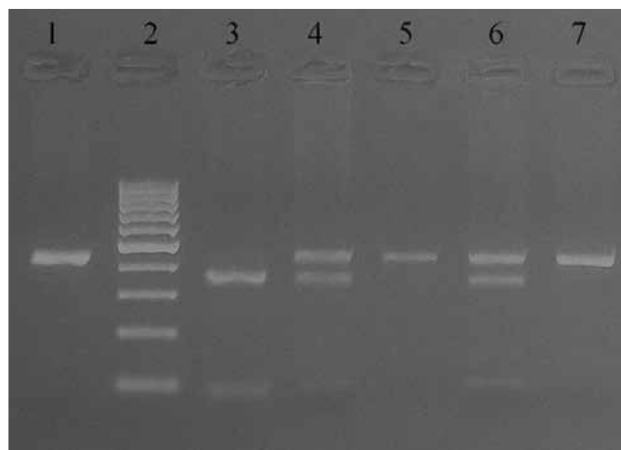


Figure 1 Representative results of PCR – RFLP analysis CSN3 on 2% agarose gel

1 – PCR product (443 bp), 2 – DNA ladder (100 bp), 3 – genotype BB (348 bp, 95 bp), 4 – genotype AB (443 bp, 348 bp, 95 bp), 5 – genotype AA (443 bp), 6 – genotype AB (443 bp, 348 bp, 95 bp)

Obrázok 1 Repräsentatívne výsledky PCR – RFLP analýzy CSN3 na 2 % agarózovom géle

1 – PCR produkt (443 bp), 2 – DNA ladder (100 bp), 3 – genotyp BB (348 bp, 95 bp), 4 – genotyp AB (443 bp, 348 bp, 95 bp), 5 – genotyp AA (443 bp), 6 – genotyp AB (443 bp, 348 bp, 95 bp)

frequencies of the three genotypes were 0.572 (AA), 0.369 (AB) and 0.059 (BB). The most frequent genotype in population of crossbred cows was AA. Table 2 shows frequencies of the three detected genotypes. The calculated χ^2 test values indicate Hardy-Weinberg genetic equilibrium in populations.

Table 2 Frequency of alleles and genotypes of CSN3 locus

Cows (n = 80) (1)	Genotype (2)			Allele (3)		χ^2
	AA	AB	BB	A	B	
Number (4)	46	29	5	121	39	0.0226-
Frequency (5)	0.575	0.3625	0.0625	0.7563	0.2437	

$P > 0,05$

Tabuľka 2 Frekvencie alel a genotypov lokusu CSN3

(1) kravy, (2) genotyp, (3) alely, (4) počet, (5) frekvencia

Our findings were similar to results reported in other studies. Miluchová et al. (2009) identified high frequency of the A allele by using multiplex PCR – RFLP method in population of Pinzgau cattle. In evaluation of Czech Simmental cattle, Kučerová et al. (2006) reported frequency of the A allele 0.60 and the B allele 0.38, but in this study the E allele (0.02) was also detected. Similar results of allele and genotype frequencies in Czech Fleckvieh cattle reported Bartoňová et al. (2012). By using 9 different primer pairs, Keating et al. (2007) identified high dominance of the A allele (0.80) in various dairy cattle population. The PCR – RFLP method and restriction enzyme *Hind*III was also used in study of Simmental and Aberdeen Angus cattle (Curi et al., 2005), when the A allele was dominant. In contrary, Jann et al. (2004) reported in population of British Jersey cattle predominance of the B allele (0.71). The prevalence of the A allele in dairy cattle breeds is very frequent, because the A allele is associated with higher milk yield.

Table 3 shows the average values of milk, protein and fat yield in standard length of lactation. Differences between the average values of analyzed traits in comparison with cows with

Table 3 Basic statistical measurements of milk production in standard length of lactation

Trait (1)	\bar{x}	s	x_{\min}	x_{\max}	n
Milk yield in kg (2)	5042.45	1386.49	1840	7604	80
Fat yield in kg (3)	221.025	67.4516	78	374	80
Protein yield in kg (4)	161.1	42.7346	56	235	80

Tabuľka 3 Základné štatistické ukazovatele produkcie mlieka za obdobie normovanej laktácie (1) ukazovateľ, (2) produkcia mlieka v kg, (3) produkcia tuku v kg, (4) produkcia bielkovín v kg**Table 4** Means and standard deviations of milk production traits in cows of different CSN3 genotypes

Genotype (1)	n	Traits (means \pm SD) (2)			
		Milk yield in kg (3)	Fat yield in kg (4)	Protein yield in kg (5)	N
CSN3					
AA	46	5 114.2 \pm 1 308.3	222.8 \pm 67.3090	162.6 \pm 40.5443	46
AB	29	4 972.9 \pm 1 464.4	218.8 \pm 65.7365	159.4 \pm 44.5064	29
BB	5	4 786.0 \pm 1 877.1	217.8 \pm 92.5051	157.0 \pm 60.3448	5

Tabuľka 4 Priemerná hodnota a smerodajná odchýlka ukazovateľov produkcie mlieka v závislosti od CSN3 genotypu (1) genotyp, (2) ukazovateľ (priemerná hodnota \pm smerodajná odchýlka), (3) produkcia mlieka v kg, (4) produkcia tuku v kg, (5) obsah bielkovín v kg

AA, AB and BB genotype were lower and non-significant (Table 4). Significance of observed differences was also affected by number of data available for particular trait. However, based on the analysis it can be concluded that the A allele had in observed population potential positive effect on milk production traits. Milk, protein and fat yield were not significantly higher in cows with AA genotype. Similar results, but statistically significant were reported by Sitkowska et al. (2008) in population of Holstein cows. Higher milk production in cows with AA genotype was also detected in other studies (Tsiaras et al., 2005, Curi et al., 2005, Neubauerová et al., 2001). Heck et al. (2009) reported positive statistically significant effect of the B allele on protein content in milk of Holstein cows. Similar associations with the B allele have been reported in studies by Kučerová et al. (2006), Tsiaras et al. (2005) and Žitný et al. (2001); they suggest the positive effect of this allele on protein and fat contents in milk. The main influence of CSN3 gene on protein content was reported by Matejičková et al. (2010). Generally, it can be said that the A allele has positive effect on milk yield and the B allele on protein and fat yield. Testing the A and B alleles is of practical importance, because the milk of cows that carry the B allele of CSN3 has a better thermal resistance and shorter coagulation time, better curdles, and contains micelles of different size, which is preferable for cheese - making (Grosclaude et al., 1988). The cheese yield from the milk of cows with genotype BB is higher compared to the milk of AA cows (Sulimova et al., 2007). Selection for the B allele of CSN3 can be used as a part of cattle breeding programs in farms with tradition of cheese production. In the future, this association between CSN3 gene polymorphism and production traits in larger cattle population can be validated. That would increase the significance of the statistical analysis and the reliability of the obtained results.

Conclusions

By using PCR-RFLP method, single nucleotide polymorphism in the CSN3 gene was detected. In the studied population of 80 cows (crosses of Simmental and Holstein) all three genotypes AA, AB and BB were detected. The majority of cows (57.5 %) were dominant homozygous with genotype

AA. The Allele A was more frequent than the B allele (0.7563 vs. 0.2437). The observed population was in Hardy-Weinberg genetic equilibrium. Based on the statistical analysis CSN3 polymorphism had no significant effect on evaluated milk production parameters, but the results indicate positive effect of genotype AA on milk, protein and fat yield. This finding was contrary to other studies which reported that the A allele had a significant positive effect on milk production but a negative one for the yield of fat and protein. Genotyping the alleles A and B of CSN3 gene is of practical importance, because the allele B is correlated with commercially valuable parameters of milk productivity and improves the cheese yielding capacity.

Súhrn

Cieľom práce bolo zistiť vzťah medzi polymorfizmom génu kódujúceho kappa - kazeín a ukazovateľmi mliekovej úžitkovosti v skupine kríženiek simentálskeho a holštajnského plemena. Na hodnotenie vplyvu polymorfizmu génu CSN3 na ukazovatele produkcie mlieka bolo použitých celkovo 80 vzoriek pozostávajúcich z chlповých cibúl. Genotypovanie na základe polymorfizmu tohto génu lokalizovaného na bovinom chromozóme 6 bolo uskutočnené s využitím polymorfizmu dĺžky reštrikčných fragmentov. Vplyv polymorfizmu bol analyzovaný pomocou softvéru SAS 9.1. Štiepením produktu PCR reakcie reštrikčným enzýmom *HindIII* sme zistili prítomnosť dvoch alel: alely A na základe štiepneho fragmentu s veľkosťou 443 bp a alely B s dvoma fragmentmi veľkými 348 a 95 bp. Prevládajúcou bola alela A s frekvenciou výskytu 0,76. V populácii sme zistili prítomnosť troch genotypov AA, AB a BB. Najčastejšie sa vyskytoval genotyp AA s frekvenciou 0,575. Výsledky štatistickej analýzy vzťahov medzi genotypmi CSN3 a ukazovateľmi produkcie mlieka za obdobie normovanej laktácie boli nepreukazné.

Kľúčové slová: mliekový dobytok, gén kappa - kazeín, produkcia mlieka, polymorfizmus

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