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GENETIC VARIANTS OF BETA-CASEIN IN HOLSTEIN DAIRY CATTLE IN SLOVAKIA

E. HANUSOVÁ[1]*, J. HUBA[1], M. ORAVCOVÁ[1], P. POLÁK[1], I. VRTKOVÁ[2]

[1]Animal Production Research Centre Nitra, Slovak Republic;
[2]Mendel University Brno, Czech Republic

ABSTRACT

The aim of the study was to detect allelic and genotypic frequencies of A variants of beta- casein (CSN2) in Holstein cows and bulls in Slovakia and to analyze milk production traits of tested cows in dependence on their CSN2 genotypes. Totally, 92 cows at the first parity and 5 bulls were tested. The samples were taken from cow's vaginal smear and bull's semen. DNA analyses were performed using ACRS-PCR (artificially created restriction site – polymerase chain reaction) method. Frequencies of A1 and A2 allele of CSN2 in cows were 0.54 and 0.46. Frequencies of A1 and A2 allele of CSN2 in bulls were 0.60 and 0.40. CSN2 genotypes and their frequencies in cows were A1A1 (0.13), A1A2 (0.83), A2A2 (0.04). Only A1A1 and A1A2 genotypes with frequencies 0.20 and 0.80 were found in bulls. High proportion of heterozygote individuals and genetic disequilibrium were recorded. In the tested cows, least square means for milk yield, protein and fat content (%) were - 8430.8±560.71; 3.17±0.0517 and 3.77±0.167 for the A1A1 genotype, 8434.9±222.80; 3.20±0.020 and 3.90±0.066 for the A1A2 genotype, and 7902.3±971.17; 3.28±0.089 and 3.90±0.288 for the A2A2 genotype. No significant differences in milk traits among CSN2 genotypes of Holstein cows were found.

Key words: Holstein cattle; beta-casein; A1 allele; A2 allele; milk traits

INTRODUCTION

Milk from a variety of animal species has been included in the diet not only for infants, but also for human adults. Milk and other milk products mainly from dairy cows provide a high quality source of energy, proteins and selected micronutrients such as calcium, magnesium and phosphorus to most human population. Cow's milk contains six major proteins: four caseins (alpha-s_1, alpha-s_2, beta and kappa) and two whey proteins. Casein proteins make up about 80 % of proteins in milk of cows.

Beta-casein (CSN2) is one of the major proteins which can vary depending on genetic make-up of cows. Current knowledge assumes existence of 13 variants of CSN2 polymorphism in cattle depending on breed. Variants A1 and A2 of CSN2 are the most common and can be found in many dairy breeds. Their level varies considerably in milk depending on the breed. The beta-casein molecule consists of 209 amino acids. The difference between A1 and A2 types is only in one amino acid at the position 67 of the peptide chain. A1 variant contains histidin and A2 variant contains prolin. Allele of triplets A2- DNA is C**A**T, A1- DNA is C**C**T (Swaisgood, 1992). CSN2-A2 is the original form (Formaggioni et al., 1999) and can be found in old breeds (Zebu, Guernsey). CSN-A1 has evolved more recently and is common in modern cattle breeds (Ng-Kwai-Hang et al., 1990, Ceriotti et al., 2004).

CSN2-A2 reduces the serum cholesterol and decreases concentration of LDL lipids which play an important role in prevention of a wide range of human vascular diseases. Metabolism of CSN2-A1 is partially

***Correspondence:** E-mail: hanusova@cvzv.sk
Emília Hanusová, Animal Production Research Centre Nitra,
951 41 Lužianky, Slovak Republic,
Tel.: +421 37 6546 360 Fax: +421 37 6546 361

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different and induces the occurrence of the beta-casomorphin -7 substance (Kaminski et al., 2007). Some epidemiological studies indicated that consumption of CSN2-A1 may be associated with a higher occurrence of cardiovascular heart disease (CVD) and type I diabetes of in humans. McLachlan (1995) found a correlation between the appearance of ischemic heart diseases and CSN2-A1 (r=0.86), not only among countries but also within the individual country. The same hypothesis was affirmed by Laugesen and Elliot (2003). The data from 20 countries showed that consumption of CSN2-A1 milk increased heart disease rates.

Relationships between heart diseases and CSN2-A1 in animals were reported in several studies (Kritchevsky et al., 1988, Kim et al., 1978, Barth et al., 1984). Tailford et al. (2003) found that the rabbits fed more CSN2-A2 than CSN2-A1 had fewer signs of cardiovascular damage. A2 fed animals showed no damage to arteries and reduced damage even after cholesterol intake. Rabbits fed CSN2-A1 had significantly higher proportion of surface area of the aorta covered by fatty streaks and had a greater thickness in the fatty streak lesions in the aortic arch than those fed CSN2-A2 (P≤0.005).

Some population studies which indicated relationships between the risk of diabetes I and milk consumption were also done. Birgisdottir et al. (2006) indicated that drinking CSN2-A2 milk at 2 years of age was linked with lower risk of childhood diabetes in Iceland and Scandinavia. Studies by McLachlan (2001), Laugesen and Elliot (2003), Thorsdottir et al. (2000) indicated that drinking CSN2-A1 milk in early childhood was linked with diabetes.

Studies published in medical literature suggested a link between the development of ischemic heart disease and specific milk protein intake (McLachlan, 2001; Laugesen and Elliot, 2003; Tailord et al., 2003). Population study in East Africa showed that people who originally lived in this region had virtually no heart diseases despite consuming a diet rich in animal milk. That milk came from Zebu cattle which is a breed that carries A2 allele exclusively. Western countries which had similarly high bovine milk consumption from predominantly Holstein breed, had a greater incidence of CVD than nations with low milk consumption (McLachlan, 2001).

The aim of the study was to detect allelic and genotypic frequencies of CSN2 in Holstein cattle in Slovakia. Milk production traits of tested cows were analyzed in dependence on their CSN2 genotypes.

MATERIAL AND METHOD

Totally, 92 first parity Holstein cows and five Holstein bulls were tested. The samples were taken from cow's vaginal smear and bull's semen. DNA analyses were performed using ACRS-PCR (artificially created restriction site – polymerase chain reaction) method (McLachlan, 2006). The allelic and genotypic frequencies of A variants of beta-casein (CSN2) were calculated. Hardy-Weinberg equilibrium was tested using Fisher's exact test (FREQ procedure, SAS/STAT 9.2, 2002-2008). The test was preferred to χ^2 goodness-of-fit test as less than five cows of A2A2 genotype were identified.

Milk trait records of cows tested for CSN2 allelic and genotypic frequencies were provided by the Breeding Services of the Slovak Republic. An identical statistical model (general linear model) with class effect of CSN2 genotypes (GLM procedure, SAS/STAT 9.2, 2002-2008) was used to analyze milk production traits (milk yield, protein and fat content):

$$y_{ij} = \mu + C_i + e_{ij}$$

where:

y_{ij} individual observation of milk yield (kg), protein and fat content (%)

μ intercept

C_i class effect of CSN2 genotypes (A1A1, A1A2, A2A2); $\sum_i C_i = 0$

e_{ij} random error, $N(0,\sigma^2)$

The estimated least squares means were compared using Scheffe's multiple range test.

RESULTS

The allelic frequencies of CSN2 in Holstein cows were 0.54 (A1) and 0.46 (A2). The allelic frequencies in Holstein bulls were 0.60 (A1) and 0.40 (A2). CSN2 genotype frequencies are given in Table 1.

Table 1: CSN2 genotypes and their frequencies in Holstein cattle in Slovakia

CSN2 genotypes	Cows		Bulls	
	N	Frequencies	N	Frequencies
A1A1	12	0.13	1	0.2
A1A2	76	0.83	4	0.8
A2A2	4	0.04	0	0

Detected CSN2 genotypes are given in Figure 1. With cows, 12 animals were of A1A1 genotype, 76 animals were of A1A2 genotype and 4 animals were of A2A2 genotypes, i.e. the frequencies in cows were 0.13 (A1A1), 0.83 (A1A2) and 0.04 (A2A2). With bulls, 1 animal was of A1A1 genotype and 4 animals were of A1A2 genotypes, i.e. the frequencies in bulls were 0.20 (A1A1) and 0.80 (A1A2). Bull´s (absence of A2A2

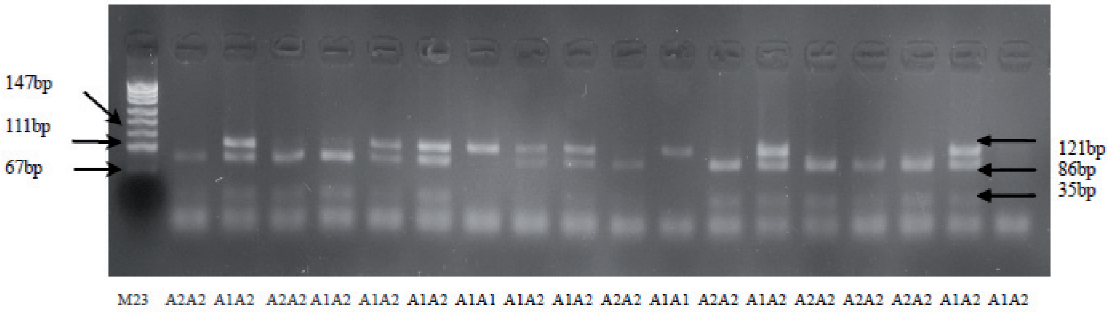

Fig. 1: Electrophoresis results for the ACRS genotyping method

Alela A1 - 121bp
Alela A2 - 86bp, 35bp

Table 2: Least square means for milk production traits in CSN2 genotypes in Holstein cows in Slovakia

Traits	CSN2 genotypes		
	A1A1 ($\mu \pm s_\mu$)	A1A2 ($\mu \pm s_\mu$)	A2A2 ($\mu \pm s_\mu$)
Milk yield (kg)	8430.8±560.71	8434.9±222.80	7902.3±971.17
Protein content (%)	3.17±0.052	3.20±0.020	3.28±0.089
Fat content (%)	3.77±0.167	3.90±0.066	3.90±0.288

genotype) and cow´s genotypes (P=0.0053) were found to deviate from Hardy-Weinberg equilibrium according to Fisher's exact test.

No effect of CSN2 genotypes on milk yield, protein and fat content was found for tested cows. Least square means and standard error estimates are given in Table 2. Tendencies in milk trait pattern may only be outlined, as the differences were found to be statistically insignificant, according to Scheffe's multiple range test (P>0.05). Milk yield was similar in Holstein cows of A1A1 and A1A2 genotypes (8430.8 and 8434.9 kg). Lower milk yield (7902.3 kg) and higher protein (3.28 % vs. 3.17 % and 3.20 %) content were found in cows of A2A2 genotype. Fat content was slightly higher in cows of A1A2 and A2A2 genotypes in comparison with cows of A1A1 genotype (3.90 % vs. 3.77 %).

DISCUSSION

The findings on allelic frequencies in cows (A1=0.54) partially agree with those of Kaminski et al. (2007), who reported high frequency of A1 allele (0.66) in Holstein. On the contrary, Manga et al. (2006) reported considerably lower frequency of A1 allele (0.286), as well as low proportion of A1A1 homozygote animals (0.07) in Czech Holstein. A deviation from Hardy-Weinberg equilibrium, indicated according to distribution of CSN2 genotypes, in our study contrasted with findings of Ng-Kwai-Hang et al. (1990), who reported a balance of A1 and A2 alleles (6,460 Holstein cows from Quebec, Canada). A quarter of tested cows had exclusively A1 genes, a quarter had exclusively A2 genes and a half had both A1 and A2 genes. Lower frequencies of A1A2 heterozygote animals in Slovak Pinzgau breed (0.40 and 0.5168) were reported in studies of Hanusová et al. (2009) and Miľuchová et al. (2009). Higher frequencies of A2 allele were reported by Beja-Pereira et al. (2003) and Hanusová et al. (2009) for Pinzgau breed. Cardak (2005) reported high frequencies of A2 allele in Holstein-Friesian and Simmentaler cows from South-West Germany (0.667 and 0.463, resp.).

Similar to our analyses of milk traits in Holstein cows, Manga et al. (2006) reported no significant differences in Czech Holstein cows according to CSN2 genotypes. The only exception was in SCC, which was found significantly higher in A1A1 Czech Holstein cows than in A1A2 and A2A2 Czech Holstein cows. No significant differences in milk production traits (milk yield, protein and fat content) according to CSN2 genotypes in Holstein-Friesian cows were reported by Cardak (2005). On the other hand, the author reported significantly higher milk yield in A2A2 Simmentaler cows in comparison to A1A1 and A1A2 Simmentaler cows and significantly higher protein and fat content in A1A1 Simmentaler cows in comparison with A2A2 and A1A2 Simmentaler cows. Hanusová et al. (2009), who focused on analyses of Slovak Pinzgau cattle, reported significantly higher fat content in A1A1 cows in comparison to A2A2 cows (P=0.014).

CONCLUSIONS

The allelic frequencies of CSN2 in Holstein cows were 0.54 (A1) and 0.46 (A2). The allelic frequencies of CSN2 in Holstein bulls were 0.60 (A1) and A2 (0.40). High proportion of heterozygote individuals was detected in tested subpopulation. No effect of CSN2 genotypes on milk production traits and the disproportions in distribution of cows over genotypes were found. Further research and a larger collection of tested animals are needed to indicate whether genetic disequilibrium was due to sample effect or selection practice.

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