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Analysis of Slovak Spotted breed for bovine beta casein A1 variant as risk factor for human health*

Martina Miluchová✉, Michal Gábor and Anna Trakovická

Department of Genetics and Breeding Biology, Slovak University of Agriculture in Nitra, Nitra, Slovakia

The goal of work was identification A1 variant of bovine beta casein which involves ischemic heart disease and diabetes mellitus in human. The digestion of A1beta casein can result in the production of bioactive beta casomorphin-7 (BCM-7); this is not the case with A2. This bioactive peptide has been linked to physiological traits that may elicit effects on components of the vascular and immune systems. The material involved 111 Slovak Spotted breed. Bovine genomic DNA was extracted from whole blood by using commercial kit, and used in order to estimate beta-casein genotypes by means of PCR-RFLP method. The PCR products were digested with *Dde*I restriction enzyme. In the population included in the study were detected all three genotypes, homozygote genotype A1A1 (14 animals), heterozygote genotype A1A2 (37 animals) and homozygote genotype A2A2 (60 animals). In the total population of cattle homozygotes A2A2–0.5405 were the most frequent, while homozygotes A1A1–0.1261 were the least frequent ones. This suggests a superiority of allele A2 (0.7072) which does not produce BCM-7, and thus is safe for human consumption. The expected homozygosity for gene CSN2 is in the population stated a slight increase in homozygosity (0.5858). This caused a slight decrease in the level of possible variability realization (41.80%), which corresponds to the effective number of alleles (1.7071).

Key words: Slovak Spotted breed, PCR-RFLP, beta-casein

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INTRODUCTION

One of the primary functions of milk is to protect the health of a newborn mammal. Milk contains many peptides and proteins. Milk proteins were classified in two groups: caseins constitute about 80% of the protein content of milk; the remaining about 20% of milk protein content consist of whey proteins (Bagnicka *et al*., 2010; Gálik *et al*., 2011; Szwajkowska *et al*., 2011).

The beta-casein (CSN2) constitutes up to 45% of the casein of bovine milk. CSN2 is localised in bovine chromosome 6 (Ferretti *et al*., 1990). The primary sequence of β-casein gene reported Ribadeau-Dumas *et al.* (1972). Single-polypeptide chain of this protein containing 209 residues with molecular weight of 23983.

The most common forms of beta-casein in dairy cattle breeds are A1 and A2, while B is less common (Farrell *et al*., 2004; Kamiński *et al*., 2007; Keating *et al*., 2008). The original beta-casein protein in bovine milk was A2. A1 beta-casein is a consequence of a mutation (Ng-Kwai-Hang & Grosclaude, 2002). The β-casein A1 and B variants differ from the A2 variant at position 67, where a histidine replaces a proline. In addition, the B variant differs from the A1 variant in a substitution of argine for serine at position 122. Importantly, it is the change to histidine at position 67 that has the potential to result in cleavage occurring upon digestion and a bioactive peptide, beta-casomorphin potentially being liberated (Stewart *et al*., 1987; Damiani *et al*., 1992; Lien *et al*., 1992). Human milk, goat milk, sheep milk and other species are "A2-like" with proline at the equivalent position (Lonnerdal *et al*., 1990; Provot *et al*., 1989).

The β-casein A1 variant was associated with the incidence of diabetes mellitus 1st type, coronary heart disease and autism (Elliot *et al*., 1988). The A2 variant reduces serum cholesterol (Panicke *et al*., 1997).

The goal of work was identification A1 variant of bovine beta casein which involves ischemic heart disease and diabetes mellitus in human.

MATERIAL AND METHODS

The material involved 111 cows of Slovak Spotted breed. Bovine genomic DNA was extracted from whole blood by using commercial kit and used in order to estimate β-casein genotypes by means of PCR-RFLP method.

DNA primers described by McLachlan (2006) were used to PCR amplification: forward primer 5'-CCT TCT TTC CAG GAT GAA CTC CAG G-3' and reverse primer 5'-GAG TAA GAG GAG GGA TGT TTT GTG GGA GGC TCT-3'.

The reaction mixture in the total volume 10 µl containing 50 ng DNA, 1.5 U Taq polymerase (Fermentas), 1X PCR buffer (750 mM Tris/HCl, pH 8.8, 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20), 6 mM $MgCl_2$, 200 µM dNTP, 5 pM of each primer. The following amplification parameters were applied: 95ºC for 5 minutes followed by 30 cycles: 95ºC for 10 seconds, 58ºC for 30 seconds, 72ºC for 30 seconds. The reaction was completed by the final synthesis: 72ºC for 5 minutes.

The PCR products of 121 bp were digested with 5 units of the *Dde*I restriction enzyme (Fermentas). Restriction digestion fragments were loaded on 3% agarose

✉e-mail: martina.miluchova@centrum.sk, martina.miluchova@uniag.sk

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Abbreviations: BCM-7, beta casomorphin-7; CSN2, beta-casein; d.f., degrees of freedom; He_{obs}, experimental heterozygosity; He_{exp}, theoretical heterozygosity; PIC, polymorphism information content; E, expected homozygosity; ENA, effective number of alleles; V%, level of possible variability realization

Table 1. Genotype and allele frequencies of Slovak Spotted cattle for CSN2

Frequencies	Genotype frequencies			Allelic frequencies		χ^2 d.f. = 2	P
	A1A1	A1A2	A2A2	A1	A2		
Absolute	14	37	60	65	157	4.232	0.1205
Relative	0.1261	0.3333	0.5406	0.2982	0.7072		

Table 2. Effectiveness of alleles for CSN2 gene in population Slovak Spotted cattle

Locus	Alelles	H_{obs}	H_e	PIC	E	ENA	V%
CSN2	A1;A2	0.3333	0.4142	0.3285	0.5858	1.7071	41.80

gel (Invitrogen) containing GelRed™ (Biotium) in 1 × SB buffer (Brody & Kern, 2004) at 180 V for 15 minutes and the gel were analyzed in the UV rays and the documentary system Olympus C-7070 were used to record the results.

RESULT AND DISCUSSION

*Dde*I digestion of the PCR product was analyzed by 3% agarose-gel electrophoresis. Allele A1 produced 121 bp fragment, and allele A2 produced a 86 bp and 35 bp fragments as the PCR-RFLP.

In the population included in the study were detected all three genotypes, homozygote genotype A1A1 (14 animals), heterozygote genotype A1A2 (37 animals), and homozygote genotype A2A2 (60 animals). In the total population of cattle homozygotes A2A2 – 0.5405 were the most frequent, while homozygotes A1A1 – 0.1261 were the least frequent ones. This suggests a superiority of allele A2 (0.7072).

Genetic equilibrium of analysed population was evaluated on the base χ2-test. In the population included in the study non-significant differences in frequencies of genotypes were found.

The expected homozygosity for gene CSN2 is in the population stated a slight increase in homozygosity (0.5858). This caused a slight decrease in the level of possible variability realization (41.80%), which corresponds to the effective number of alleles (1.7071).

Frequencies of A2 allele in our population were similar to those of CSN2 gene as reported by Beja-Pereira *et al.* (2003) for Pinzgau cattle. The predominance of CSN2 A2 allele (0.764) detected Caroli *et al.* (2008) in population of Carora cattle. Manga *et al.* (2006) presented lower frequency of allele A1 in population of Czech Spotted and Czech Holstein breed. The higher frequency of allele A1 was reported by Bech *et al.* (1990) for Black-and-White breed and Ehrmann *et al.* (1997) for Red-and-White breed. Ikonen *et al.* (1997) reported slight superiority of allele A1 for Ayrshire breed and Hanusová *et al.* (2010) for Holstein bulls.

It may be concluded that Slovak spotted breed exhibit a superiority of allele A2 (0.7072) which does not produce BCM-7, and thus is safe for human consumption.

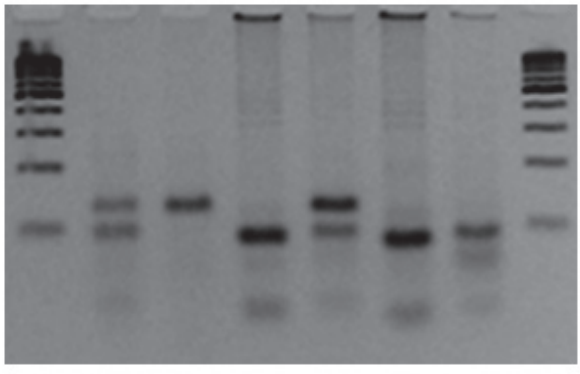

Figure 1. Representatively results of analysis PCR-RFLP for *CSN2* gene by *Dde*I on 3% agarose gel.
1, 8 — marker 100 bp DNA Ladder (Fermentas); 2, 5 — genotype A1A2 (121 bp, 86 bp, 35 bp); 3 — genotype A1A1 (121bp); 4, 6, 7 — genotype A2A2 (86 bp, 35 bp)

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