Analysis of Beta-Casein Gene (CSN2) Polymorphism in Different Breeds of Cattle

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Abstract

The goal of work was identification of β - casein gene polymorphism in different breeds of cow. The beta - casein constitutes up to 45 % of the casein of bovine milk. The most common forms of beta-casein in dairy cattle breeds are A1 and A2, while B is less common. The β-casein A1 variant was associated with the incidence of diabetes mellitus 1st type, coronary heart disease and autism. The A2 variant reduces serum cholesterol. The material involved 287 cows (Simmental breed – 111 cows, Pinzgau breed – 89 cows, Holstein breed – 87 cows). 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. In the populations included in the study were detected all three genotypes – homozygote genotype A1A1, heterozygote genotype A1A2 and homozygote genotype A2A2 with frequencies 0.1261, 0.3333 and 0.5405 in Simmental breed; 0.1379, 0.4598 and 0.4023 in Holstein breed, 0.3034, 0.5168 and 0.1798 in Pinzgau breed. In population of Simmental breed and Holstein breed was higher frequency of allele A2 (0.7072 and 0.6322). In opposite, in population of Pinzgau breed was present higher frequency of the allele A1 (0.5618).

Keywords: beta-casein, cattle, PCR-RFLP

1. 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 [1-3].

The beta - casein (CSN2) constitutes up to 45 % of the casein of bovine milk. CSN2 is localised in bovine chromosome 6 [4]. The primary sequence of b-casein has been reported by Ribadeau-Dumas *et al*. (1972) [5]. 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 [6-8]. The original beta-casein protein in bovine milk was A2. A1 beta-casein is a consequence of a mutation [9]. The β - casein A1 and B variants differ from the A2 variant at position 67 where a histidine (codon CAT) replaces a proline (codon CCT). 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 [10-12]. Beta-casomorphin (BCM7) have also been suggested to show many immunological activities like chronic inflammatory responses, such as allergy, mucin production, lymphocyte proliferation, skin reactions [13]. Human milk, goat milk, sheep milk and other species are „A2-like“ with proline at the equivalent position [14, 15].

The β-casein A1 variant was associated with the incidence of diabetes mellitus 1st type, coronary

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heart disease and autism [16]. The A2 variant reduces serum cholesterol [17].
The goal of work was identification of β - casein gene polymorphism in different breeds of cow.

2. Materials and methods

The material involved 287 cows (Slovak spotted breed – 111 cows, Pinzgau breed – 89 cows, Holstein breed – 87 cows). 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) [18] 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 (NH4)2SO4, 0.1% Tween 20), 6 mM MgCl2, 200 μM dNTP, 5 pM of each primer. The following amplification parameters were applied: 95°C for 5 minutes followed by 30 cycles: 95 °C for 10 seconds, 58 °C for 30 seconds, 72 °C for 30 seconds. The reaction was completed by the final extension: 72 °C 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 gel (Invitrogen) containing GelRed™ (Biotium) in 1 × SB buffer [19] 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.

3. Results 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.

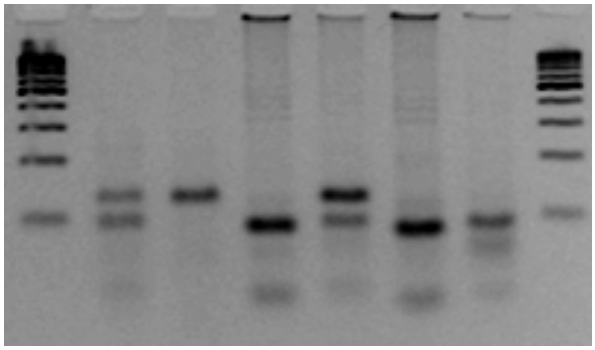

Figure 1. Representatively results of analysis PCR-RFLP for *CSN2* gene by *Dde*I on 3 % agarose gel. 1,8 – marker 100 bp DNA Ladder (Fermentas); 2,5 – genotype A1A2 (121 bp, 86 bp, 35 bp); 3 – genotype A1A1(121bp); 4,6,7 – genotype A2A2 (86 bp, 35 bp)

In the populations included in the study were detected all three genotypes. In the population of Slovak Spotted breed we detected homozygote genotype A1A1 (14 cows), heterozygote genotype A1A2 (37 cows) and homozygote genotype A2A2 (60 cows). 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).
In Holstein breed was observed homozygote genotype A1A1 (12 cows), heterozygote genotype A1A2 (40 cows) and homozygote genotype A2A2 (35 cows) with frequencies 0.1379, 0.4598 and 0.4023, subsequently. In Pinzgau breed was detected homozygote genotype A1A1 (27 cows), heterozygote genotype A1A2 (46 cows) and homozygote genotype A2A2 (16 cows) with frequency 0.3034, 0.5168 and 0.1798, subsequently. . In population of Slovak spotted breed and Holstein breed was higher frequency of allele A2 (0.7072 and 0.6322). In opposite, in population of Pinzgau breed was present higher frequency of the allele A1 (0.5618). Genetic equilibrium of analysed populations was evaluated on the base χ2-test. In the populations included in the study non-significant differences in frequencies of genotypes were found.
Detailed genotype and gene frequencies per breed are presented in Table 1.

Table 1. Genotype and allele frequencies of CSN2 gene in different breeds of cow

BREED	*COWS*	*GENOTYPE FREQUENCIES*			*ALLELE FREQUENCIES*		X^2 *d.f.=2*	*P*
		A1A1	**A1A2**	**A2A2**	**A1**	**A2**		
Slovak Spotted breed	111	0.1261	0.3333	0.5406	0.2928	0.7072	4.232	0.1205
Pinzgau breed	89	0.3034	0.5168	0.1798	0.5618	0.4382	0.22	0.896
Holstein breed	87	0.1379	0.4598	0.4023	0.3678	0.6322	0.011	0.9945
TOTAL	**287**							

Table 2. Effectiveness of alleles for CSN2 gene in different breeds of cow

Breed	Locus	Alelles	H_{obs}	H_e	PIC	E	ENA	V%
Slovak Spotted breed	**CSN2**	*A1;A2*	0.3333	0.4142	0.3285	0.5858	1.7071	41.80
Pinzgau breed			0.5168	0.4924	0.3712	0.5076	1.9701	49.78
Holstein breed			0.4598	0.4650	0.3568	0.535	1.8692	47.04

The expected homozygosity for gene CSN2 is in all populations stated a slight increase in homozygosity (Slovak Spotted breed – 0.5858, Pinzgau breed – 0.5076, Holstein breed – 0.535). This caused a slight decrease in the level of possible variability realization (Slovak Spotted breed – 41.80%, Pinzgau breed – 49.78%, Holstein breed – 47.04%), which corresponds to the effective number of alleles (Slovak Spotted breed – 1.7071, Pinzgau breed – 1.9701, Holstein breed – 1.8692).

Frequencies of A2 allele in population Slovak Spotted breed and Holstein breed were similar to those of CSN2 gene as reported by Beja-Pereira *et al*. (2003) [20] for Pinzgau cattle. The predominance of CSN2 A2 allele (0.764) detected Caroli *et al*. (2008) [21] in population of Carora cattle. Manga *et al*. (2006) [22] presented lower frequency of the allele A1 in population of Czech Spotted and Czech Holstein breed. In population of Pinzgau breed we detected slight superiority of allele A1. The higher frequency of the allele A1 was reported by Bech *et al*. (1990) [23] for Black-and-White breed and Ehrmann *et al*. (1997) [24] for Red-and-White breed. Ikonen *et al*. (1997) [25] reported slight superiority of allele A1 for Ayrshire breed and Hanusová *et al*. (2010) [26] for Holstein bulls.

4. Conclusions

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

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