ANALYSIS OF POLYMORPHISM OF BETA CASEIN OF SLOVAK PINZGAU CATTLE BY PCR-RFLP FOR ALLELS A1 AND A2

ANALIZA POLIMORFISMULUI GENEI β-CAZEINEI PRIN METODA PCR-RFLP PENTRU ALELELE A1 ŞI A2 LA TAURINELE DE RASĂ PINZGAU SLOVACĂ

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The work was oriented to identification of β-casein gene polymorphism and analysis of genotype structure in population of Slovak Pinzgau cattle. The material involved 89 cattle. Bovine genomic DNA was isolated by fenol-chlorophorm deprotenization and ethanol precipitation and used in order to estimate β - casein genotypes by means of PCR-RFLP method. The PCR products were digested with DdeI restriction enzyme. In the population included in the study there were homozygote genotype A1A1 (27 animals), heterozygote genotype A1A2 (46 animals) and homozygote genotype A2A2 (16 animals). In the total population of cattle heterozygotes A1A2 – 0.5168 were the most frequent, while homozygotes A2A2 – 0.1798 were the least frequent ones. This suggests a slight *superiority of allele* A1– *0.5618.*

Keywords: cattle, PCR-RFLP, β-casein

Introduction

The beta - casein (CSN2) constitutes up to 45 % of the casein of bovine milk. CSN2 is localised in bovine chromosome 6 (Ferretti, Leone, Sgaramella, 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 β - casein gene has 12 known genetic variants in the coding sequence of the gene: A, B, C (Aschaffenburg, 1961), A1, A2, A3 (Kiddy, 1966), D (Aschaffenburg, 1968), E (Voglino, 1972), F (Visser, 1991), X (Visser et al., 1995), G (Chin,Ng-Kway-Hang, 1997), H (Han et al., 2000), I (Jann et al., 2002).

The most common forms of beta-casein in dairy cattle breeds are A1 and A2, while B is less common, and A3 and C are rare (Farrell et al., 2004; Kamiński, Cieślińska, Kostyra, 2007; Keating et al., 2008). 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).

The β-casein A1 variant was associated with the incidence of diabetes mellitus 1st type, coronary heart disease and autism (Elliot et al., 1988). Fat percentage was estimated with CSN2 genotype A1A1. CSN2 genotype significantly influenced milk yield, fat yield, and protein yield with the highest yields obtained for the genotype A2A2. The A2 variant reduces serum cholesterol. The A3 variant of bovine CSN2 is associated with higher milk yield while higher protein, casein yields and fat are associated with the B variant. (Panicke, Freyer and Erhardt, 1997).

Materials and Methods

The material involved 89 cattle. Bovine genomic DNA was isolated by fenol-chlorophorm deprotenization and ethanol precipitation (Sambrook et al., 1989) and used in order to estimate CSN2 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 PCR reaction elaborated by McLachlan (2006) was modificated.

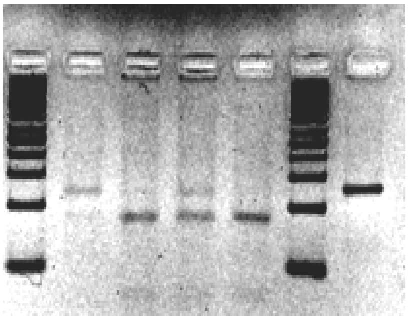
The reaction mixture in the total volume 25 µl containing 50 ng DNA, 1.5 U Taq polymerase (Fermentas), 3 mM $MgCl_2$, 200 µM dNTP, 5 pM of each primer, 0,006 mg BSA. The following amplification parameters were applied: 95oC for 5 minutes followed by 30 cycles: 95 °C for 40 seconds, 58 °C for 60 seconds, 72 °C for 90 seconds. The reaction was completed by the final synthesis: 72 °C for 10 minutes.

The PCR products of 121 bp were digested with 5 units of the *Dde*I restriction enzyme (Promega). Restriction digestion fragments were loaded on 3 % agarose gel (Invitrogen) containing ethidium bromide and the gel were analyzed in the UV rays.

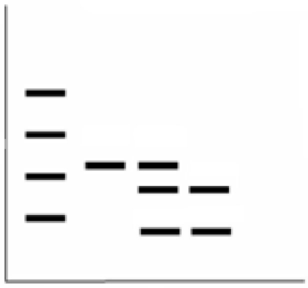
Results and Discussion

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

In the population of Slovak Pinzgau cattle we detected all genotypes: homozygote genotype A1A1 (121 bp) 27 animals, heterozygote genotype A1A2 (121 bp, 86 bp, 35 bp) 46 animals and homozygote genotype A2A2 (86 bp, 35 bp) 16 animals.



Picture 1: Representatively results of analysis PCR-RFLP for *CSN2* gene by *Dde*I on 3 % agarose gel
1, 6 - marker 50 bp DNA Ladder (Fermentas)
2 - genotype A1A1(121bp,)
3,5 - genotype A2A2 (86 bp, 35 bp)
4 - genotype A1A2 (121 bp, 86 bp, 35 bp)
7 - PCR product (121 bp)



Scheme 1: Schematic image of PCR-RFLP product *CSN2* gene
1 - DNA ladder 50 bp,
2 - genotype A1A1 (121 bp)
3 - genotype A1A2 (121 bp, 86 bp, 35 bp)
4 - genotype A2A2 (86 bp, 35 bp)

Table 1.
Freqeuncy of genotypes and alleles of *CSN2* gene in the population of cattle

Frequency	Genotypes (n =89)			Allels		χ^2 d.f. = 2	p
	A1A1	A1A2	A2A2	A1	A2		
Absolute	27	46	16	100	78	0.2243	0.8939
Relative	0.3034	0.5168	0.1798	0.5618	0.4382		

Frequencies of genotypes and alleles determined in the total population (89 animals), are presented in Table 1. In the total population of cattle heterozygotes A1A2– 0.5168 were the most frequent, while homozygotes A2A2 – 0.1798 were the least frequent ones. This suggests a slight superiority of allele A1– 0.5618.

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

Frequencies of A1 allele in our population were similar to those of CSN2 gene as reported by Bech et al. (1990) for Black-and-White breed and Ehrmann (1997) for Red-and-White breed. Ikonen (1997) reported slight superiority of allele A1 for Ayrshire breed.

The higher frequency of the allele A2 in population of Pinzgau cattle was reported by Beja-Pereira et al. (2003).

Manga et al., (2006) present lower frequency of the allele A1 in population of Czech Spotted and Czech Holstein breed.

Conclusions

It may be concluded that CSN2 is a polymorphic gene with a slight superiority of genotype A1A2 and a slight superiority of allele A1. Genetic structure examined in population of Slovak Pinzgau cattle remained within the range quoted in literature for other cattle breeds.

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