

ANALYSIS OF POLYMORPHISM OF ALPHA S1 CASEIN OF SLOVAK PINZGAU CATTLE BY PCR-RFLP

ANALIZA POLIMORFISMULUI GENEI CAZEINEI α S1 PRIN METODA PCR – RFLP LA RASA PINZGAU SLOVACĂ

MARTINA MILUCHOVÁ, ANNA TRAKOVICKÁ, M. GÁBOR

Slovak University of Agriculture in Nitra, martina.miluchova@centrum.sk

The work was oriented to identification of α -s1 casein gene polymorphism and analysis of genotype structure in population of Slovak Pinzgau cattle. The material involved 93 cattle. Bovine genomic DNA was isolated by fenol-chlorophorm deprotenization and ethanol precipitation and used in order to estimate α -s1 casein genotypes by means of PCR-RFLP method. The PCR products were digested with MaeIII restriction enzyme. In the population included in the study there were homozygote genotype BB (81 animals) and heterozygote genotype BC (12 animals). Homozygote genotype CC has not been observed. In the total population of cattle homozygotes BB – 0.871 were the most frequent, while BC – 0.129 were the least frequent ones. This suggests a superiority of allele B – 0.9355.

Key words: cattle, PCR-RFLP, α -S1 casein

Introduction

The caseins are the major milks proteins of mammals. Their dual function for the suckling infant is to serve as a major source of amino acid, as well as to transport phosphate and calcium in sufficient amounts to support growth of bones (Koczan, Hobom, Seyfert, 1991). Effects of milk protein polymorphism on milk production traits (milk, fat, and protein yield and also fat and protein percentage) have been investigated during the past decades and, in some cases, results are still conflicting (Aleandai et al, 1990, Bovenhuis et al, 1992, Prinzenberg et al., 2003).

α -S1- casein (CSN1S1) is localised in bovine chromosome 6 (Ferretti, Leone, Sgaramella, 1990; Womack, 1989). CSN1S1 genotype significantly influenced milk yield, fat yield, and protein yield with the highest yields obtained for the genotype BB. Protein percentage was influenced by CSN1S1, with the genotypes BC and BB, respectively, having the highest percentages. Significantly higher lactation cheese yields were estimated with CSN1S1 genotype BB (Aleandai et al, 1990).

Materials and Methods

The material involved 93 cattle. Bovine genomic DNA was isolated by fenol-chlorophorm deprotenization and ethanol precipitation (Sambrook et al.,

1989) and used in order to estimate CSN1S1 genotypes by means of PCR-RFLP method.

DNA primers described by Koczan (1993) were used to PCR amplification: forward primer 5'- TGC ATG TTC TCA TAA TAA CC - 3' and reverse primer 5' - GAA GAA GCA GCA AGC TGG - 3'.

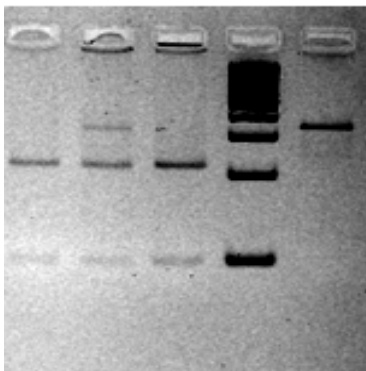
The PCR reaction elaborated by Koczan (1993) was modified.

The reaction mixture in the total volume 25 µl containing 50 ng DNA, 0.5 U Taq polymerase (Fermentas), 1 mM MgCl₂, 200 µM dNTP, 10 pM of each primer. The following amplification parameters were applied: 94°C for 3 minutes followed by 30 cycles: 94 °C for 30 seconds, 59 °C for 30 seconds, 72 °C for 30 seconds. The reaction was completed by the final synthesis: 72 °C for 10 minutes.

The PCR products of 310 bp were digested with 6 units of the *Mae*III restriction enzyme (Roche). Restriction digestion fragments were loaded on 3 % agar gel (Invitrogen) containing ethidium bromide and the gel were analyzed in the UV rays.

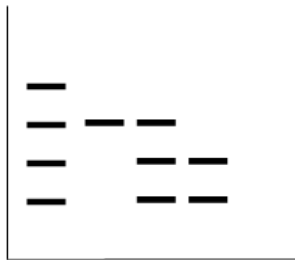
Results and Discussion

*Mae*III digestion of the PCR product, analyzed for 3% agarose-gel electrophoresis. Allele B produced 214 and 96 bp fragments, and allele C produced a 310 bp fragment as the PCR-RFLP.



Picture 1: Representatively results of analysis PCR-RFLP for *CSN1S1* gene by *Mae*III on 3 % agarose gel

- 1 – genotype BB (214 bp, 96 bp)
- 2 – genotype BC (310 bp, 214 bp, 96 bp)
- 3 – genotype BB (214 bp, 96 bp)
- 4 – marker 100 bp DNA Ladder (Fermentas)
- 5 – PCR product (310 bp)



Scheme 1: Schematic image of PCR-RFLP product *CSN1S1* gene.

- 1 – DNA ladder 100 bp,
- 2 – genotype CC (310 bp),
- 3 – genotype BC (310 bp, 214 bp, 96 bp),
- 4 – genotype BB (214bp, 96 bp)

In the population of Slovak Pinzgau cattle we detected two genotypes: homozygote genotype BB (310 bp, 236 bp) 81 animals and heterozygote genotype BC (310 bp, 236 bp, 130bp) 12 animals. Homozygote genotype CC has not been observed.

Table 1. Frequency of genotypes and alleles of *CSN1S1* gene in the population of cattle

Frequency	Genotypes (n = 93)			Allels		χ^2 d.f. = 1	p
	BB	BC	CC	A	B		
Absolute	81	12	-	174	12	0.10	0.75
Relative	0.8710	0.1290	-	0.9355	0.0645		

Frequencies of genotypes and alleles determined in the total population (93 animals), are presented in Table 1. In the total population of cattle homozygotes BB – 0.871 were the most frequent, while BC – 0.129 were the least frequent ones. This suggests a superiority of allele B – 0.9355.

Genetic equilibrium of analysed population was evaluated on the base χ^2 -test. In the population included in the study non-significant differences in frequencies of genotypes were found. Frequencies of alleles in our population were similar.

Frequencies of alleles in our population were similar to those of *CSN1S1* gene as reported by Sadik et al. (1972). Grosclaude et al. (1974) reported slight superiority of allele B for Jersey, Guernsey, Normande, Reggiana. The higher frequency of the allele B found in Pinzgau cattle population reported by Beja-Pereira et al. (2003). Caroli et al., (2004) present lower frequency of the allele C in population of Reggiana breed.

Conclusions

It may be concluded that *CSN1S1* is a polymorphic gene with a slight superiority of genotype BB and a superiority of allele B. Genetic structure examined in population of Slovak Pinzgau cattle remained within the range quoted in literature for other cattle breeds.

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