**HINFI POLYMORPHISM OF K-CASEIN AND PIT1 GENES IN ROMANIAN SIMMENTAL CATTLE**

**POLYMORFISMUL HINFI DIN INTERIORUL GENELOR K-CAZEINEI ȘI PIT1 LA RASA BĂLȚATĂ ROMÂNEASCĂ**

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Using molecular markers, number and position of valuable alleles for some quantitative loci can be identified. An ideal genetic marker must be: polymorphic, multiallelic, codominant and environmentally not sensitive. These conditions are accomplished by RFLP markers. Pit 1 and k-casein gene have been shown to be involved in milk yield/quality (protein, fat content) in cattle. The alleles favourable for selection are B for k-casein and A for Pit1, both being associated with higher protein content and superior milk yield for Pit-1. The present study was carried out to establish the genotype and allele frequencies for k-casein and Pit-1 genes using PCR-RFLP/HinfI technique in 76 Romanian Simmental cattle.

**Key words:** k-casein, Pit-1, PCR-RFLP, HinfI, genetic marker, MAS

**Introduction**

Up to few years ago, classic selective improvement was the only way to increase the genetic potential of domestic animals. The molecular genetic provided to scientists a strong tool for genetic improvement – molecular markers. In last decades, due to reasons independent of environment and their objective character lot of genetic markers types: biochemical and molecular (DNA) were successful developed in plants and animals. The marker assisted selection (MAS) could be put into practice for both sexes, for sex limited traits.

The pituitary transcription factor (Pit-1) is the cellular specific transcription factor for activating expression of the prolactin, thyrotropin, and GH genes in the anterior pituitary gland (Tuggle & Trenkle, 1996). Bovine Pit-1 is a 291 amino acid protein with DNA-binding POU domain. This gene is another candidate for milk production marker because of its role in regulating expression of bGH and the prolactin genes. The bovine Pit1 gene was located in centromeric region of chromosome 1 in bovine, between TGLA57 and RM95 loci. This location creates a chain transmission of the following group of genes: TGLA49-RM95-PIT1-TGLA57 (Moody et al., 1995). The primers used for localization of gene on bovine chromosome 1 were made up according to structure of human Pit1 gene, located on chromosome 4. The region between exones 5 and 6 of Pit1 gene was used for design of primers flanking an intron of about 1.1 kb.
Milk protein polymorphism has been studied intensively because of their effect on the yield and processing properties of milk and its products. k-casein constitutes about 25% of the casein fraction of milk. The B variant of k-casein is associated with an increase in milk protein and fat contents as well as cheese production (Van Eenennaam and Medrano, 1991).

The polymorphism within Pit-1 and k-casein genes was studied in several cattle breeds with different techniques (Angus, Holstein, Brown Schwitz, Hereford, Red Holstein x Romanian Simmental hybrids, Pinzgauer) and association among different genotypes and milk yield/quality, and also with different conformation traits, were performed (Vlaic, et al., 2001, 2003, 2005; Gaboreanu Ioana et al., 2005; Zhao et al., 2004; Zwierzckowski et. al, 2002; Renaville et al., 1997). The alleles favorable for selection are B for k-casein and A for Pit1 (genotypes favorable/unfavorable, respectively, AAPit1BBk-casein vs. BB Pit1AAk-casein) having a superior milk production with a higher protein content. This effect represents about 30% of genetic gain of North American cattle population. Only allele A of Pit1 gene was associated to a superior milk quantity and conformational traits which advantage higher protein content in milk, while allele B of k-casein was unilaterally associated with increased cheese production. Milk with a superior protein, caseins, and k-caseins content has a superior coagula percent and a higher processing yield.

Materials and Methods

Genomic DNA was obtained from unrelated Romanian Simmental cattle (N=76) from two local farms from Transylvania (SCDP Jucu & Basto srI). The DNA was extracted from blood using Wizard®DNA Purification kit (Promega).

The polymorphism at Pit-1 locus was studied according to Moody & al., 1995, using PCR/RFLP for amplification of a 1355 pb fragment, corresponding to an intron of 1.1 kb flanked by exons 5 and 6. The 18 base (forward and reverse) primers were used, and PCR products were submitted to digestion using Hinf1 enzyme.

Primer sequences (Microsynth) were: 5’ primer 5’-CAA TGA GAA AGT TGG TGC-3’; and 3’ primer – 5’-TCT GCA TTC GAG ATG CTC-3’. The PCR was performed in 25 µl final volume using 50 ng genomic DNA, 200µM each dNTP, 25 mM MgCl2; 10 pmol of each primer (forward and reverse), 5x Green Go Taq Reaction buffer, 0.5 U of GoTaq DNA Polymerase (Promega). Thermal cycling began with an initial cycle of 95°C for 2 minutes, 55°C for 1 min and 72°C for 2 minutes followed by 29 cycles of 1 minute at 94.55 and 72°C, and concluded with a final extension for 12 minutes. The amplification reaction resulted in a single product of 1.35 kb. Polymerase chain reaction products were digested with Hinf1 (37°C for 2 hours) and separated by electrophoresis in 3% agarose gels in TBE buffer, stained with etidium bromide. The molecular weight marker used was 100 pb DNA ladder (Promega).
The digestion products of Pit-1 gene correspond to the following genotypes: AA (660, 425 and 270 pb), AB (660, 425, 385 and 270 pb) and BB (660, 385 and 270 pb) (Fig. 1).

The polymorphism at k-casein locus was studied on amplification of the 350 pb DNA fragment according to Medrano and Aguilar-Cordova (1990). The PCR reaction was performed in 25 µl final volume using 100 ng genomic DNA, 10 mM each dNTP, 25 mM MgCl₂; 10 pmol of each primer (forward and reverse), 5x Green Go Taq Reaction buffer, 0.6 U of GoTaq DNA Polymerase (Promega). Thermal cycling began with an initial denaturation at 95°C for 3 minutes, followed by 35 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min and concluded with a final extension for 12 minutes. Polymerase chain reaction products were digested with HinfI enzyme. The reaction mixture which included 10 µl of PCR product, 10x Neb bufer, 7.3 µl dd H₂O, 0.2 µl BSA and 6 units of HinfI was incubated at 37°C for 2 hours. Fragments were separated by electrophoresis in 2.5% agarose gels in TBE buffer, stained with etidium bromide (Fig. 2). The molecular weight marker used was 100 pb DNA ladder (Promega).

Results and Discussions

Description of polymorphism

Pit 1 polymorphism: Digestion of PCR products with HinfI of Pit 1 gene revealed two alleles corresponding to the following genotypes: AA (660, 425 and 270 pb), AB (660, 425, 385 and 270 pb) and BB (660, 385 and 270 pb) (Figure 1). The A allele was found to be superior for milk and protein yields, inferior for fat percentage, and superior for body depth, angularity and rear leg set (Renaville et al., 1997; Zwierzckowski et. al, 2002; Vlaic et al, 2003; Zhao, Q. et al, 2004). This result indicated that the cows carrying the A allele had higher yields, deeper bodies, and greater dairness. Fat percentage was lower because of the higher milk yield, but nearly constant fat yield, associated with the A allele.

Allele frequencies: The frequency of allele A was 0.22 and 0.78 for allele B respectively. These alleles generated three patterns, and frequencies were: 0.118, 0.197 and 0.685 for AA, AB and BB, respectively.

Renaville et al. (1997) obtained similar results for allele frequencies in 89 Italian Holstein-Friesian bulls using PCR-RFLP/HinfI method for 451 pb fragment of Pit-1 gene.

k-casein polymorphism

Digestion of PCR products with HinfI of k-casein gene revealed two alleles corresponding to the following genotypes: AA (132/134 and 84 pb), AB (266, 132/134 and 84 pb) and BB (266 and 84 pb) respectively (Figure 2).

Allele frequencies: The frequency of A allele was 0.7 and 0.3 for B allele. Genotype frequencies for k-casein locus were 0.45 for AA, 0.50 for AB and 0.05 for genotype BB respectively. K-casein A allele frequencies of Romanian
Simmental population in this study were similar with allele frequencies reported in Hereford population (Moody et al. 1996) but lower than the frequencies reported in Holstein and Pinzgauer breeds (Medrano, 1990; Vlaic et al. 2005).

Figure 1 - Partial genomic structure and Hinfl polymorphism between exon 5 and 6 of Pit-1 gene. The genotypes of the different animals are shown at the top of each line (AA, AB and BB). The small 40 pb fragment of allele B is not visible in the gel.

Figure 2 - Hinfl PCR/RFLP at the k-casein locus in Romanian Simmental cattle. At left molecular weight marker – 100 pb DNA step ladder (Promega) and the genotypes of different animals are shown at the top of each line (AA, AB and BB).

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POLYMORPISMUL HINF\textsc{I} DIN INTERIORUL GENELOR K-CAZEINEI ŞI PIT1 LA RASA BĂLŢATĂ ROMÂNEASCĂ

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Cu ajutorul markerilor moleculari, pot fi puse în evidență numărul și poziția aleelor valoroase de la loci caracterelor cantitative. Un marker genetic ideal trebuie să fie: polimorfic, multialelic, codominant și insensibil la mediu. Aceste condiții sunt îndeplinite de markerii RFLP. S-a demonstrat că genele k-cazeinei și Pit-1 sunt asociate cu unele insuşiri calitative (conținut de proteiină, procent de grăsimi) și cantitative ale producției de lapte la vaci. Alelele favorabile în procesul de selecție sunt B pentru k-cazeină și A pentru Pit-1. În lucrarea de față se prezintă studiul efectuat pe 76 vaci din rasa Bălțată românească în vederea stabilirii frecvențelor de genă și genotip la loci k-cazeinei și Pit-1 prin tehnica PCR-RFLP/Hinf\textsc{I}. 

Cuvinte cheie: k-cazeină, Pit-1, PCR-RFLP, Hinf\textsc{I}, markeri genetici, MAS