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RESEARCH CONCERNING THE GENETIC STRUCTURE OF ROMANIAN SIMENTAL AND MARAMURES BROWN BREEEDS AT THE PITUITARY TRANSCRIPTION FACTOR LOCUS

CERCETARI PRIVIND STRUCTURA GENETICA A POPULATIILOR DE BALTATA ROMANEASCA SI BRUNA DE MARAMURES LA LOCUSUL FACTORULUI DE TRANSCRIPTIE PITUITAR

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Abstract: Pituitary transcription factor Pit-1, which belongs to a large POU domain family is a positive regulatory factor of growth hormone, prolactin and thyrotropin β-subunit in the mammalian pituitary. Therefore, the gene encoding Pit-1 was chosen as a candidate gene to investigate its association with lactation performance in cattle. The present study was carried out to establish the genetic structure at this locus in two Romanian cattle breeds: Romanian Simmental and Maramures Brown, to establish the possible association between genotype and milk yield and conformation traits. A strategy employing polymerase chain reaction was used to amplify a 1355- pb fragment from blood DNA and digestion with HinfI enzyme and the genetic structure was estimated for both breeds.

Key words: polymorphism, gene, transcription factor, genetic structure, PCR, *Hinf*I

Introduction

Selection of a particular trait in a mammal is presently very expensive and very slow. Usually the selection process involves a genealogical evaluation of the mammals history over a long period of time. Molecular techniques pursue the detection of existing variations and polymorphisms within the individuals of a population for a specific region of the DNA. One such method is the study of candidate genes to determine whether specific genes are associated with milk production traits in mammals and therefore these genes can be used as molecular markers. This method first requires identification of candidate genes or anonymous genetic markers associated with the traits of interest. Molecular markers are DNA fragments, usually non-informational (hyper-variable DNA regions or introns), sometimes even informational (functional genes) involved in the detection of

chromosomal segments (or genes), which determine the manifestation of a character. The marker assisted selection (MAS) could be put into practice for both sexes and for sex limited traits. The polymorphism within Pit-1 was studied in several cattle breeds (Angus, Holstein, Brown Schwitz, Hereford etc) (Zhao et al., 2004; Zwierzckowski et. al, 2002; Renaville et al., 1997), and association among different genotypes and milk yield/quality, and also with different conformation traits, were performed. The allele A of Pit-1 gene was associated to a higher milk production and a higher content of protein in milk, genotypes favourable for selection being AA and AB. The somatotropin system is quite complicated and involves at a hypothalamic level, somatostatin and somatostatin, at a pituitary level, pituitary-specific transcription factor (Pıt-1) which is responsible for growth hormone expression in mammals, at a hepatic level, growth hormone receptor and growth hormone plasmatic transport protein, and at a cellular level, growth hormone receptor, insulin-growth factor- and insulin growth factor transport protein. 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 a candidate for milk production marker because of its role in regulating expression of bGH and the prolactin genes.

Materials and Methods

Genomic DNA of 76 registered Romanian Spotted cattle from Transylvania local farms (SCDP Jucu & Basto srl) and from 50 Maramures Brown cattle (SC Agrozootehnica SA, Petreşti and Livada SA farms). was extracted from blood using Wizard®DNA Purification kit (Promega) and MagNa Pure LC DNA Isolation kit I (Roche). The DNA quality and quantity was determined on spectrophotometer Nanodrop ND1000. 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 restriction reaction using *Hinf*I enzyme. The sites for restriction enzyme are shown in Figure 1.

The PCR was performed in 25 µl final volume using 50 ng genomic DNA, 200µM each dNTP, 25 mM $MgCl_2$; 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^{o}C for 2 minutes, 55^{o}C for 1 min and 72^{o}C for 2 minutes followed by 29 cycles of 1 minute at 94,55 and 72^{o}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 *Hinf*I (37^{o}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)

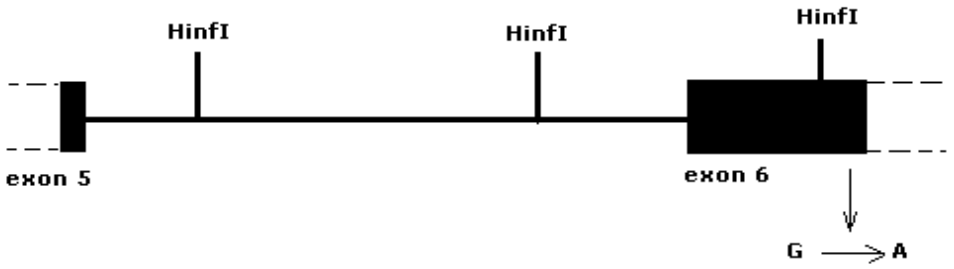

Figure 1- The *Hinf*I restriction sites in Pit1 gene

Results and Discussions

Digestion of PCR products for both breeds with *Hinf*I nuclease 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 2).

Allele frequencies for Romanian Simmental breed (N = 76) -: 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.

For Maramures Brown breed (N=50) the frequency of A allele was 0,16 and 0,84 for B allele. The genotype frequency was AA=0, 06; AB=0, 2 and BB=0, 74

Chi-square test was used to asses the homogeneity of two populations (P< 0,01 for Romanian Simmental breed and P>.0,05 for Maramures Brown breed).

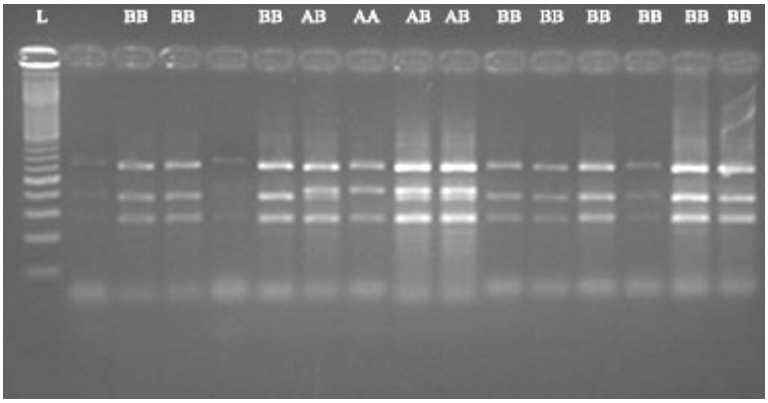

Figure 2. *Hinf*I polymorphism between exons 5 and 6 of Pit-1 gene in Romanian spotted cattle. The genotypes of the different animals are shown at the top of each lane (BB, AB and BB). Sizes are indicated in pb on the right site. The small 40 pb fragment of allele B is not visible in the gel. At left molecular weight marker – 100 pb DNA step ladder (Promega)

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. These preliminary researches were performed to initiate the association between genotype, milk yield and conformational traits in two analyzed breeds. For the Maramures Brown breed we intend to perform further DNA test at Pit-1 locus to assign the accurate results regarded to association between genotype and the quantitative traits.

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