Lucrări ştiinţifice **Zootehnie şi Biotehnologii**, vol. **41 (1)** (**2008**), Timişoara

GENETIC POLYMORPHISM AT THE B-LACTOGLOBULIN LOCUS IN A DAIRY HERD OF ROMANIAN SPOTTED AND BROWN OF MARAMURES BREEDS

POLIMORFISMUL GENETIC DIN LOCUSUL GENEI B-LACTOGLOBULINEI ÎNTR-UN EFECTIV DE BOVINE DIN RASELE BĂLȚATĂ ROMÂNEASCĂ ŞI BRUNĂ DE MARAMUREŞ

ILIE DANIELA*, SĂLĂJEANU AURELIA*, MAGDIN ANUȚA*, STANCA CLAUDIA**, VINTILĂ I.**

**Research and Development Station for Bovine Raising - Arad*
***Faculty of Animal Science and Biotechnology, Timişoara, România*

The objective of the present study was focused on possibilities to estimate the allele and genotype frequencies of β-lactoglobulin (BLG) gene polymorphisms in dairy cattle belonging to two different genetic groups from the Research and Development Station for Bovine Raising Arad in order to have breeding programs that target an increase in the frequency of the B allele in the dairy cattle population. Genotyping was performed on 20 Romanian Spotted and 18 Brown of Maramures cattle.In order to differentiate the favorable genotype for superior composition and higher cheese yield, we used simple DNA extraction method from fresh blood and techniques based on DNA analysis, which include polymerase chain reaction and restriction fragment length polymorphisms (PCR-RFLP) methods. Employing these techniques we were able to determine the (BLG) genotype of all individuals in a given population under selection, regardless of sex, age or physiological stage. As a result, it is now possible to include information on milk protein genotypes into marker assisted selection programs and consequently improve response to selection.
Key words β-lactoglobulin, PCR-RFLP analyses, DNA genotype

Introduction

Molecular genetic techniques that are currently available allow direct genotyping for milk proteins using DNA from different sample tissue as blood or semen samples and permits selection of bulls and cows at any age.

β-lactoglobulin (BLG) is a major component of whey protein, which consists 162 amino acids and, of its several known genetic variants, A and B are predominant in bovine breed [1, 3]. The chemical differences between these two variants of protein are small. They are consisting in two point mutation occurred in codon 64 for Asp and for Val from 118 position. Association studies shows that

genetic variants (A and B) identified in BLG locus are also associated with lactation performance and have a major influence on the composition of milk and on its processing properties, including cheese yield [5]. The AA genotype is associated with higher milk yield; the BB genotype is associated with higher fat and casein contents and is, therefore, more desirable for cheese making [2, 4, 6].

Increasing the frequency of favorable allelic variant of BLG in the current population and selecting the most desirable β-lactoglobulin genotype are of particular interest to dairy cattle breeders. Hereby, the aim of this study was to work out, the DNA tests for bovine BLG gene genotype detection, which enabled to determine very precisely which are the carrier individuals of the favorable genotypes for superior milk quality and cheese yield in a dairy herd from the Research and Development Station for Bovine Raising Arad. Using just them in the reproduction program, we can increase the frequency of B allele and so we can obtain substantial genetic progress for milk quality and quantity.

Material and Methods

In order to detect the polymorphism of BLG gene, we used PCR (Polymerase Chain Reaction) procedure for gene amplification, and RFLP (Restriction Fragment Length Polymorphism) analyses for marker allele identification.

Animals: 38 Brown of Maramures (N=18) and Romanian Spotted (N=20) cows were used maintained at the Research and Development Station for Bovine Raising Arad. The average production of Brown of Maramures cows was 8532 kg milk/lactation (305 days) with 3.88% fat and 3.2% protein. For Romanian Spotted cows the average production was 9113 kg milk/lactation (305 days) with 3.9% fat and 3.3% protein. During the lactation period animals were milked twice a day and milk analysis was performed as part of the official production control every month.

DNA purification: blood sample for DNA genotyping was collected from jugular vein on EDTA anticoagulant. The DNA isolation from fresh blood sample (300 µl) was performed with a rapid method using Wizard Genomic DNA Purification Kit from Promega. The total amount of isolated DNA was resuspended in sterile distilled water, measured spectophotometrically and diluted to 100 ng for each reaction.

In vitro DNA amplification: sequence surrounding the nucleotide mutation of BLG region was amplified by using following sense and antisense primers [5]:

BLG 1 5'-TGT GCT GGA CAC CGA CTA CAA AAA G-3'

BLG 2 5'-GCT CCC GGT ATA TGA CCA CCC TCT-3'

Genomic DNA was amplified in 25 µl reaction containing PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 25 pmole of BLG sense and antisens primers, and 1.25U of GoTaq Flexi Polymerase. DNA was amplified in five steps: 1) initial denaturation of the double strand at 95°C for 5 min., 2) denaturation at 95°C for 1 min., 3) annealing at 61°C for 1 min.,4) extension at 72°C for 1 min., and 5) final extension at 72°C for 10 min. Steps 2, 3 and 4, corresponding to one cycle, were repeated 30 times.

Enzymatic digestion of PCR product: After PCR reaction the obtained amplicons were digested in a reaction mixture containing 15µl PCR product and 5U of the restriction enzyme *HaeIII* in 1:10 volume of 10 X enzymes buffer with BSA. The digestion mixtures were incubated for 3 h in a thermocycler at 37 °C. After digestion, the PCR-RFLP products were analyzed by electrophoresis in 3.5% agarose gel stained with ethidium bromide, in a horizontal electrophoresis system. For each gel was applied a molecular weight standard to enable calculation of PCR-RFLP fragments. All gels were photographed for analysis of data using gel doc equipment. The genotypes were determined for each polymorphism by analyzing the size of the fragments reported as base pairs (bp).

Results and Discussions

For the determination of the A and B alleles of the LGB gene (chromosome 11), a 247-bp fragment located between nucleotide 23 of exon IV and nucleotide 158 of intron IV was amplified, which contains a polymorphism at the *Hae*III restriction site [5].

Two different alleles (A and B) were found for the BLG/*Hae*III polymorphism. Genotype AA was characterized by the presence of two fragments of 148 and 99 bp, respectively. Genotype BB was identified based on the presence of two bands of 99 and 74 bp, respectively, and three restriction fragments of 99, 74 and 74 bp, respectively. Heterozygous individuals (AB) showed three bands of 148, 99 and 74 bp, respectively.

In the studied population we calculated the genotype and allelic frequencies. All this data studied for BLG in the breeds of cattle studied are shows in table 1.

Table 1

Genotypic and allele frequencies at the BLG locus in Romanian Spotted and Brown of Maramures bovine breeds.

Cattle breed	Genotype	Number of animals (frequency)	Allele frequency
Romanian Spotted	AA	2 (0.100)	A-0.450
	AB	14 (0.700)	B-0.550
	BB	4 (0.200)	
Brown of Maramures	AA	2 (0.111)	A-0.333
	AB	8 (0.444)	B-0.666
	BB	8 (0.444)	

The highest frequencies for the BLG B allele were observed in Brown of Maramures cattle (0.666). In the Romanian Spotted breed only four cattle were BB and the frequencies of the A and B alleles were 0.45 and 0.55, respectively.

Conclusions

Considering that presented results are partial, genotyping different breeds and cross breeds of cattle by PCR-RFLP analysis will continue in our laboratory.

The polymorphism of bovine BLG was determined by PCR-RFLP. Using these methods was possible to identify A and B alleles for studied cattle. In comparison with other bovine breeds, high frequency of BLG B allele (0.666) was observed in the Brown of Maramures cattle.

The calculated BLG A and B allele frequencies for Romanian Spotted and Brown of Maramures cattle were pA=0.450, pB=0.550 and pA=0.333, pB=0.666, respectively. The genotypic frequencies calculated for Romanian Spotted cattle were AA=0.100, AB=0.700, BB=0.200 and for Brown of Maramures cattle were AA=0.111, AB=0.444, BB=0.444.

Selection of BB genotype animals for both K-cn and β-lgb gene should be advantageous to increase this allele in the cattle population and will provide a high demand of superior milk content for dairy industry.

Acknowledgements: This work was supported by the projects: Molecular markers used in animal improvement, grant no. 4490/2005.

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