

Short Communication

POLYMORPHISM OF β -LACTOGLOBULIN IN PRAMENKA SHEEP BREED IN BOSNIA AND HERZEGOVINA

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ABSTRACT

The aim of this study was to determinate allele frequency β -lactoglobulin in Pramenka breed sheep in Bosnia and Herzegovina. The DNA was extracted from blood of 283 sheep, collected from four flocks around Sarajevo, Bosnia and Herzegovina. The gene β -lactoglobulin was amplified using polymerase chain reaction – restriction fragment length polymorphism (PCR–RFLP) technique. Two genetic variants (A and B) and three genotypes (AA, AB and BB) of β -lactoglobulin have been identified. Genotype frequencies of β -lactoglobulin were with the following values: AA (0.325), AB (0.445), and BB (0.229), while the frequencies of allele A and B were 0.547 and 0.453 respectively. The highest frequency of genotype AA is present in the location Treskavica (38%), genotype AB in the location Vlačić (52%), while the genotype BB is mostly present in location Bjelašnica (29%). The population of Pramenka breed sheep was in Hardy-Weinberg equilibrium. It is expected that in a future the programs in sheep breeding in Bosnia and Herzegovina certainly would take into account the genetic variants of β -lactoglobulin that could contribute to better production and economic benefit.

Keywords: β -lactoglobulin; genetic polymorphism; Pramenka breed.

INTRODUCTION

Beta - (β) lactoglobulin is the main fraction in the milk whey proteins. Its polymorphisms is highly correlated with some productive and technological properties of the sheep milk. Genetic polymorphism of β -lactoglobulin and its influence on the technological properties of milk have been investigated by (Pilla *et al.*, 1995; Giaccone *et al.*, 1997; Kukovics *et al.*, 1998; Nudda *et al.*, 2000; Corral *et al.*, 2010;). Currently, three polymorphic genetic variants (A, B and C alleles) of sheep β -lactoglobulin are described (Recio *et al.*, 1997). The variants of β -lactoglobulin A and β -lactoglobulin B are present in all breeds and differ at amino acid position 20, where genetic variant β -lactoglobulin A has Tyr, and β -lactoglobulin B has His (Kolde and Braunitzer, 1983). Genetic variant β -lactoglobulin C is subtype of β -lactoglobulin A with exchange of Arg to Gln at position 148 (Erhardt, 1989). Molecular mass of β -lactoglobulin is about 19.9 Da and contains 180 amino acids (www.uniprot.org/uniprot/P67976). The locus of β -lactoglobulin gene in sheep is on the third chromosome.

The objective of this research was to investigate genetic polymorphism of the β -lactoglobulin in Pramenka breed sheep in Bosnia and Herzegovina.

MATERIALS AND METHODS

The study included 283 sheep belonging to Pramenka sheep breed. Blood samples collected in flocks located close to Sarajevo (Bjelašnica n=42), (Treskavica n=63), (Crna Rijeka n=106) and (Vlačić n=72). DNA isolation from blood was performed using Wizard genomic DNA Purification Kit Protocol by Promega Corporation, (2001) at the Laboratory of Molecular Genetics at the Faculty of Agriculture and Food Science in Sarajevo. β -lactoglobulin genotyping was performed in the Laboratory of Biochemistry, Molecular Biology and GMO at the Faculty of Agricultural Sciences and Food in Skopje, Macedonia.

Amplification of the polymorphic fragment from β -lactoglobulin gene was performed by PCR (Feligini *et al.*, 1998) using primers (forward 5'-CAACTCAAGGTCCCTCTCCA-3'; and reverse 5'-CTTCAGCTCCTCCACGTACA-3'). The PCR profile included an initial denaturation step 95 °C for 10 min, 35 amplification cycles of 30 s at 93 °C, 30 s at 64 °C, 45 s at 72 °C, and final extension step 10 min at 72 °C. The amplified fragments of 120 bp analyzed using 2.5% agarose gel electrophoresis. Digestion of 10 μ l amplification product was performed using RsaI restriction enzyme (2 U/ μ l) for 2h at 37°C. The digested product was electrophoresed in 3 % agarose gel. Direct counting was used to estimate allele frequencies β -lactoglobulin. Genotypes were determined based on

number/length DNA fragments, AA (66 bp, 37 bp, and 17 bp), AB (103 bp, 66 bp, 37 bp, 17 bp) and BB (103 bp, 17 bp).

The chi-square test (χ^2) was used to check whether the population in Hardy-Weinberg equilibrium. All calculations and the χ^2 analyses were carried out using statistical program SAS (Statistical Analysis System Version 9.3. 2011. USA).

RESULTS AND DISCUSSION

Genetic polymorphism analysis of the β -lactoglobulin revealed the presence three genotypes (AA, AB and BB) and two allelic variants (A and B) in Pramenka breed.

After digestion, fragments of 103, 66, 37, 17 bp were obtained, but the last one (17 bp) wasn't visible on the gel. Depending on the fragments presented in the gels allelic variants were determined in the studied animals. The digested PCR product is shown in Figure 1, while distributions of the β -lactoglobulin genotypes Figure 2.

In this research was found the most genotype AB (44.60%), followed by AA (32.50%) and BB (22.90%). High prevalence of heterozygous variant AB β -LG compared to AA and BB β -LG was found in studies in (Čubrić-Čurik *et al.*, 2002; Ivanković, 2004; Mroczkowski *et al.*, 2004; Kučinskiene *et al.*, 2005;

Mohammad *et al.*, 2006; Mele *et al.*, 2007; Nassiry *et al.*, 2007; Kawecka and Radko, 2011).

Allele frequencies are shown in Table 1, while the distribution of genotypes among regions is given in Table 2 and Figure 3.

Allele A β -LG was found as dominant compared to allele B of β -LG. Comparing with the results of other studies dealing with the allele frequency of allele A and B of β -LG, our finding (0.547:0.453) was similar with the results obtained by Erhardt, (1989) in Pleven breed (0.53:0.47), Recio *et al.*, (1997) in Merino (0.58:0.42), Kučinskiene *et al.*, (2005) in Lithuanian Blackface breed (0.52:0.48), and Çelik and Özdemir (2006) in Morkaraman breed (0.56:0.44), Kusza *et al.*, (2015) in Rack sheep (0.64: 0.36). Some authors as dominant allele found B β -LG as follow: Giaccone *et al.*, (1997) in Valle del Belize (0.35:0.65), Čubrić-Čurik *et al.*, (2002) in Pag breed (0.48:0.52), Mohamadi *et al.*, (2006) in Afshari (0.34:0.66).

The critical value of chi-square test for a second degree of freedom and level of significance is 0.05 to 5.991 and 0.01 to 9.210. The value of chi-square test was 2.90 lower than the critical range, between these genotypes the statistically significant deviation from HW equilibrium has not been found. Deviations from HWE law are not found in the research of (Ivanković, 2004; Dario *et al.*, 2005; Elmaci *et al.*, 2006).

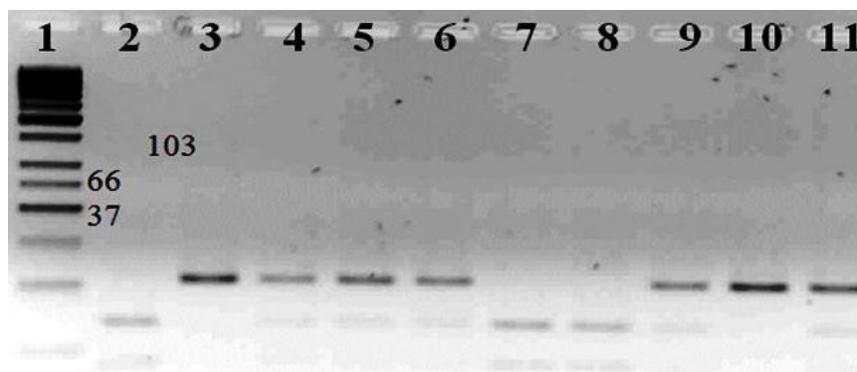


Fig 1. The digested PCR product β -LG . #1-DNA Lader, #2 AA genotype, #3 BB genotype, #4-6 AB genotype, #7-8 AA genotype, #9 AB genotype, #10 BB genotype, #11 AB genotype .

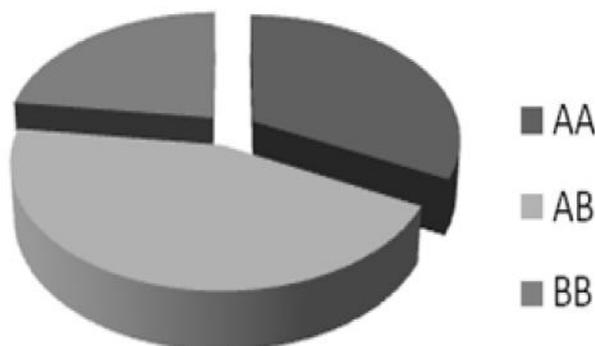


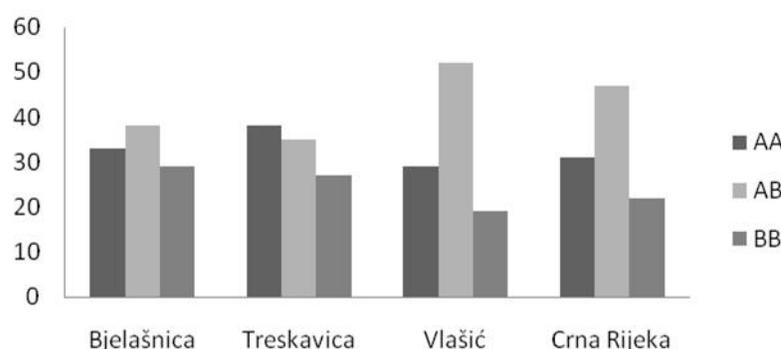
Fig. 2. Frequency β -LG of genotypes

Table1. Allele frequencies β -lactoglobulin in Pramenka breed, and Hardy-Weinberg equilibrium

	Genotype frequencies			Allele frequencies	
	AA	AB	BB	A	B
Observed	92	126	65	0.547	0.453
Expected	84.7	140.25	58.1		
$\chi^2=$	2,90	df=2	<i>p value = 5.991</i>		

Table2. Genotype frequencies β -lactoglobulin by localities

	N	Genotypes		
		AA	AB	BB
Bjelašnica	42	14 0.33	16 0.38	12 0.29
Treskavica	63	24 0.38	22 0.35	17 0.27
Vlašić	72	21 0.29	38 0.52	13 0.19
Crna Rijeka	106	33 0.31	50 0.47	23 0.22

**Fig.3. Distribution of β -lactoglobulin genotypes among the localities**

The highest frequency of genotype AA β -LG is present in the location Treskavica (38%), of genotype AB β -LG in the location Vlašić (52%), while the genotype BB β -LG is mostly present in location Bjelašnica (29%). In location Treskavica, Crna Rijeka and Bjelašnica are present small population of Bosnia and Herzegovina Pramenka represented variant AA and BB β -LG. These results indicate a low variability and inbreeding in small and closed populations. In the area of Vlašić have large population Pramenka and there is no planned selection. There is a major presence heterozygous AB β -LG and a high degree of genetic variability that may contribute to the selection of the best sheep for breeding and selection. The superiority of genotype AB β -LG in relation to the AA and BB can be continuously carried out selection by farmers in the production of sheep's milk and cheese at Mediterranean region is a possible explanation for this phenomenon.

Conclusions: In this study was observed dominance of A allelic variant of β -lactoglobulin gene. Allele B of β -

lactoglobulin gene is associated with better productive and technological properties of sheep milk. It is expected that in a future the programs in sheep breeding certainly would take into account the genetic variants of this gene in Pramenka. Knowledge about genetic profile of breed due to studied polymorphic variants of milk proteins is useful in further breeding development and economic reaffirmation of Pramenka breed in Bosnia and Herzegovina.

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