

## GENETIC POLYMORPHISM OF B-LACTOGLOBULIN IN LITHUANIAN BLACKFACE AND LITHUANIAN NATIVE COARSEWOOLED SHEEP

Jūrate Kučinskienė<sup>1</sup>, Gediminas Vagonis<sup>1</sup>, Jolanta Malevičiūtė<sup>1</sup>, Ilma Tapio<sup>2</sup>

<sup>1</sup>*Department of Animal Breeding and Genetic, Lithuanian Veterinary Academy, Tilzes 18, LT-47181 Kaunas*

<sup>2</sup>*MTT Agrifood Research Finland, Animal Breeding Research FI-31600 Jokioinen, Finland*

**Summary.** We describe the polymorphism of the  $\beta$ -LG milk protein locus in the Lithuanian Blackface sheep breed obtained using isoelectric focusing (IEF) method and in the Lithuanian Native Coarsewooled sheep – using PCR-RFLP method. In Lithuanian Blackface sheep two genetic variants A and B with allele frequencies of A=0.52 and B=0.48 were identified. In Lithuanian Native Coarsewooled sheep allele frequencies were A=0.69 and B=0.31. Mean observed heterozygosity value ( $H_{obs}$ =0.511) was slightly lower than mean expected heterozygosity ( $H_{exp}$ =0.667) in Lithuanian Blackface sheep. In Lithuanian Native Coarsewooled sheep mean expected heterozygosity value ( $H_{obs}$ =0.461) was similar to the mean observed heterozygosity ( $H_{exp}$ =0.434) and deviation from Hardy-Weinberg equilibrium was not detected in any of those breeds.

**Keywords:** Lithuanian sheep,  $\beta$ -lactoglobulin, isoelectric focusing (IEF) method, PCR-RFLP.

## BETA LAKTOGLOBULINO GENO POLIMORFIZMAS LIETUVOS ŠIURKŠČIAVILNIŲ IR LIETUVOS JUODGALVIŲ AVIŲ VEISLĖSE

**Santrauka.** Taikant izoelektrinio fokusavimo (IEF) ir polimerazės grandinės reakcijos-restrikcinių fragmentų ilgio polimorfizmo (PGR-RFIP) metodus, Lietuvos juodgalvių (LJ) ir Lietuvos šurkščiavilnių (LŠ) avių populiacijose rasti du A ir B beta laktoglobulino aleliai. Alelio A dažnis LJ populiacijoje – 0,52, o alelio B dažnis – 0,48. Genotipų AA, AB ir BB dažniai atitinkamai – 19,5%, 66,4%, 14,1%. Juodgalvių avių vidutinis nustatytas heterozigotiškumas ( $H_{obs}$ ) buvo 0,511, o teorinis heterozigotiškumas ( $H_{exp}$ ) – 0,667. Lietuvos šurkščiavilnių avių populiacijoje alelio A dažnis tirtose populiacijose – 0,69, o alelio B dažnis – 0,31. Mažiausias paplitimo dažnis genotipo BB – 7,8%. Genotipai AA ir AB sudarė po 46,1% tirtos avių grupės atitinkamai. Šurkščiavilnių avių vidutinis nustatytas heterozigotiškumas ( $H_{obs}$ ) buvo 0,461. Jis artimas teoriniam heterozigotiškumui ( $H_{exp}$ ) – 0,434. Nukrypimo nuo Hardy-Weinberg pusiausvyros nenustatyta nė vienoje iš tirtų avių veislių.

**Raktažodžiai:** Lietuvos avys, beta laktoglobulinas, IEF, PGR-RFIP.

**Introduction.** Among specific genes that may affect economically important traits in sheep, the  $\beta$ -lactoglobulin ( $\beta$ -LG) locus has been extensively studied. Three genetic variants of this protein: A, B (Kolde et al., 1983; Shlee et al., 1993) and C (Erhardt, 1989) have been identified. The genetic variants A and B differ at amino acid position 20, where variant A has a His and variant B has a Thr (Kolde et al., 1983). The variant C is a subtype of variant A with a single amino acid exchange of Arg to Glu at position 148 (Erhardt, 1989). Polymorphism of  $\beta$ -LG has been detected in several breeds, and studies of the effect of  $\beta$ -LG alleles on sheep production traits have given different results. Genotype BB is linked with higher milk yield, while AA and AB genotypes seem to be superior in protein and casein content and curd yield (Bolla et al., 1989; Garzon et al., 1992). However, other studies failed to detect any effect of the gene on milk production traits (Barillet et al., 1993; Recio et al., 1997). Nevertheless, Bocharov (1998) found associations between  $\beta$ -LG variant AB with higher body weight, while genotype AA could be linked with sheep wool density.

Lithuanian Native Coarsewooled is a native, low productive meat-wool type sheep breed that was mainly bred in Lithuania at the end of the 19<sup>th</sup> beginning of the 20<sup>th</sup> century. The breed was created by crossing Pomeranian, Polish long-tailed, thin-tailed sheep, mainly bred in southern part of Lithuania with Northern short-tailed sheep that were bred in the eastern districts of the country. Lithuanian Native Coarsewooled breed as watched internationally and included into the FAO World

Watch List for domestic animal diversity (Scherf, 2000). Lithuanian Blackface is a modern meat-wool type sheep breed, created in the middle of the 20<sup>th</sup> century by crossing Lithuanian Native Coarsewooled ewes with wool type Shropshire and meat type German Blackface rams. The productivity traits of the animals have been continuously studied (Zapasnikienė, 2002). Also the genetic diversity within these breeds using microsatellite markers and mitochondrial DNA sequencing has been evaluated (Grigaliūnaite, 2003), the polymorphism of  $\beta$ -LG in Lithuanian Native Coarsewooled and Lithuanian Blackface sheep so far has not been studied.

The aim of the present study was to describe the genetic polymorphism of the  $\beta$ -LG milk protein locus in the Lithuanian Blackface sheep breed obtained using isoelectric focusing (IEF) method and in the Lithuanian Native Coarsewooled sheep – using PCR-RFLP method.

### Materials and methods.

#### IEF.

Phenotyping of skim milk was carried out by IEF in 0.3 mm thin polyacrylamide gel using carrier ampholytes according to the method developed by Erhardt (7). The gel solution was made of 8.4 ml of gel stock solution (5.78 % (wt/vol) acrylamide, 0.15 % (wt/vol) NN'-methylene-bisacrylamide, 51 % (wt/vol) urea) and 0.527 ml of the following mixture of carrier ampholytes: 40 % (vol/vol) Servalyte pH 2.5-5; 34.9 % (vol/vol) Pharmalyte pH 4.5-5.4 and 25 % Ampholyte pH 4-6.5. As catalysts 1 ml ammoniumpersulfate (0.7 % wt/vol) and 15  $\mu$ l TEMED were added. After prefocusing at 3000 V

limit, 20 mA constant current for 150 Vh, 10 µl of each sample preparation containing 10 % (vol/vol) whole milk, 2.7 % (wt/vol) 2-β-mercaptoethanol in H<sub>2</sub>O were applied 3 mm in front of the anode. Final focusing was for 3000 Vh constant and 40 mA limit. Identification of the genetic variants was carried out after staining the gels with Coomassie Brilliant Blue R-250 according to Erhardt (1989).

#### PCR-RFLP

DNA from the hair roots was extracted using lysis buffer (10 mM Tris/HCl pH 8.3 (Sigma, USA), 50 mM KCl (Sigma, USA), 0.5 % Tween (Amresco, USA) and 20mg/ml Proteinase K (Merck, Germany). Mixture was placed to 56 °C overnight. PCR was carried out in GeneAmp®PCRSystem 2700 (Applied Biosystem, USA) thermocycler with end volume of 30 µl containing 100ng DNA, standard 10×PCR buffer, 25 mM MgCl<sub>2</sub>, 2 mM of each dNTP, BSA (20 mg/ml), primers – the forward 5'-AAA AGC CCT GGG TGG GCA GC-3' and reverse 5'-TTG GGT TCA GTG TGA GTC TGG -3' and 2U *Taq*-polymerase. All PCR reagents are produced by MBI Fermentas (Lithuania). DNA was amplified 33 cycles (95 °C 5 min; 95 °C 50 s, 65 °C 40 s, 72 °C 40 s) with final elongation for five minutes at 72 °C. 10 µl of the PCR product (452 bp) was digested with restriction endonuclease *RsaI* (5U) (MBI Fermentas, Lithuania) followed by incubation for one hour at 37 °C. After digestion products were separated on the 2 % agarose gel (Top Vision™GQ Agarose, MBI Fermentas, Lithuania)

stained with ethidium bromide and examined by ultraviolet light. Digestion of the AA genotype resulted in to four fragments of the size 175 bp, 170 bp, 66bp and 41bp, respectively. AB genotype was digested in to five fragments of the size 236 bp, 175 bp, 170 bp, 66 bp and 41bp respectively. BB genotype gave three fragments: 236 bp, 175 bp and 41 bp.

#### Statistical analysis

The frequencies of A and B alleles and β-LG genotype frequencies were calculated by direct counting. The mean expected unbiased and mean observed heterozygosities were calculated using Pop100gene program:

(<http://www.ensam.inra.fr/URLB/pop100gene/pop100gene.html>)

**Results and discussion.** In Lithuanian Blackface sheep two genetic variants A and B with allele frequencies of A=0.52 and B=0.48 (Table 1) were identified. The most frequent genotype in Lithuanian Blackface breed, detected in 66.7 % of studied individuals, was heterozygous genotype AB. Homozygous genotypes AA and BB were observed at frequencies of 19.0 % and 14.3 %, respectively. In Lithuanian Native Coarsewooled sheep two genetic variants A and B with allele frequencies of A=0.69 and B=0.31 were identified. The BB genotype was not frequent (7.8 %) in Lithuanian Native Coarsewooled breed. The genotypes AA and AB were observed at frequency of 46.1 %.

Table1. Distribution of β-lactoglobulin genotype and allele frequencies in Lithuanian Blackface and Lithuanian Native Coarsewooled sheep breeds

		Lithuanian Blackface	Lithuanian Native Coarsewooled
Genotype	AA	4	12
	AB	14	12
	BB	3	2
Genotype frequency	AA	0.19	0.46
	AB	0.67	0.46
	BB	0.14	0.08
Allele frequency	A	0.52	0.69
	B	0.48	0.31
P-value		0.20	0.06

Mean observed heterozygosity value ( $H_{obs}=0.511$ ) in Lithuanian Blackface sheep was slightly lower than mean expected heterozygosity ( $H_{exp}=0.667$ ). In Lithuanian Native Coarsewooled sheep mean expected heterozygosity value ( $H_{obs}=0.461$ ) was similar to the mean observed heterozygosity ( $H_{exp}=0.434$ ). The deviation from Hardy-Weinberg equilibrium was not detected in any of those breeds.

The polymorphism of the β-LG locus in Lithuanian Blackface and Lithuanian Native Coarsewooled sheep breed is comparable to the results for some dairy and wool type sheep breeds described in literature. Relatively similar amount of heterozygous individuals (> 50 %) was observed also in Pag (Cubric-Curik et. Al., 2002) and Karakul (Nassiry et. al., 2002) breeds (Figure 1). However, the frequency of AA genotype in Lithuanian Blackface was smaller in comparison to the group of

sheep, represented by Romney-Marsh (Bocharev, 1998), British Milk sheep, Hungarian Merino (Anton et. Al., 1997) and Finnish Landrace (15), in which the AA genotype was detected in 79 % of studied individuals. Results obtained for BB genotype in Lithuanian Blackface and Lithuanian Native Coarsewooled sheep were similar with observations in British Milk (Anton et. Al., 1997), Hungarian Merino (Anton et. Al., 1997) and Ghezel sheep (Elyasi et. Al., 2004) breeds. Nassiry et al (2002) didn't find BB genotype in Russian Karakul sheep breeds. Highest frequency of this genotype was reported in Lacaune sheep (Anton et. Al., 1997). The most frequent allele in Lithuanian Blackface and Lithuanian Native Coarsewooled sheep was allele A (Table1). This allele was reported in breeds Spanish Manchega and Segurena and in Italian Barbaresca-siciliana and Massene

breeds (Elyasi et. Al., 2004) with frequencies 0.68, 0.67, 0.62, 0.62 respectively.

The detected high frequency of genotype AB in meat-wool type Lithuanian Blackface and Lithuanian Native Coarsewooled sheep might be in agreement with the observations made by Bocharov (1998). Since the

priority in the development of this breed is given to the improvement of the meat traits, the associations between  $\beta$ -LG variant AB and higher body weight are possible. However, in order to prove this observation, further analyses are needed.

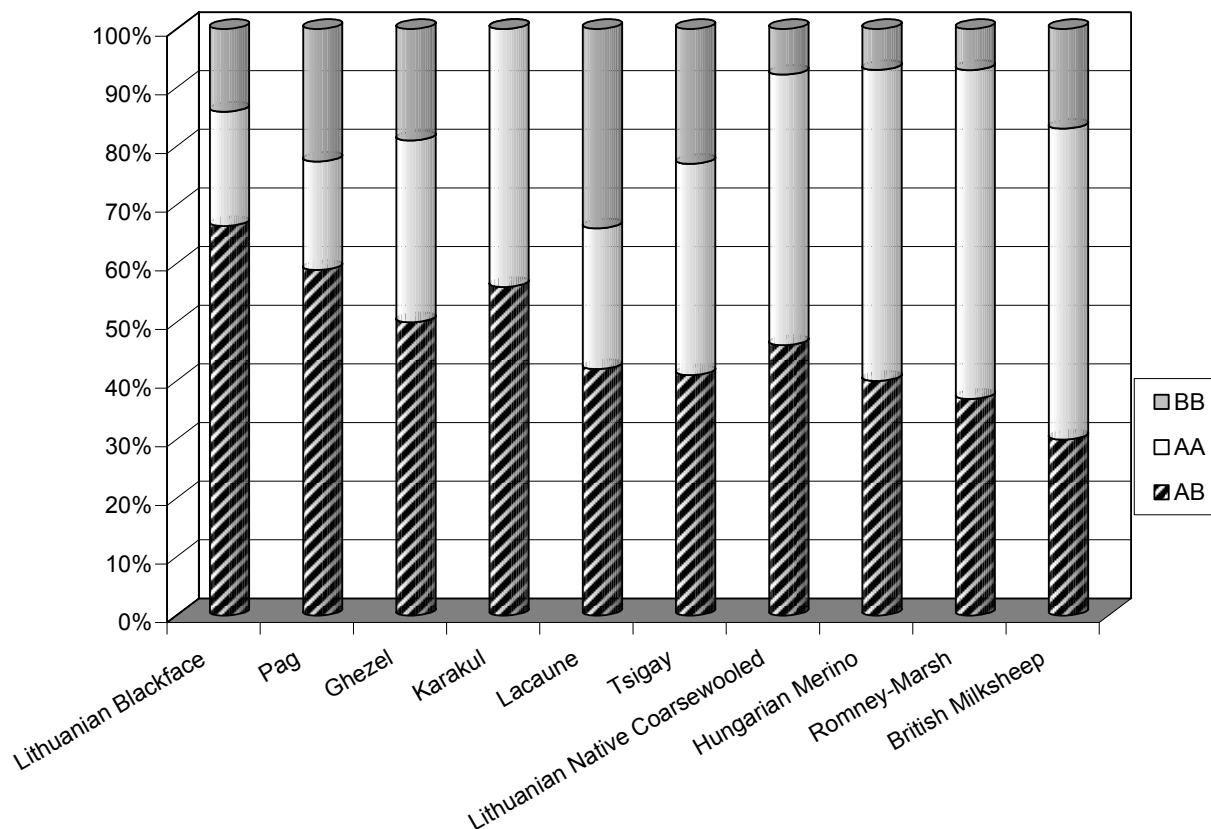


Figure 1. The distribution of  $\beta$ -LG genotypes in Lithuanian Blackface and Lithuanian Native Coarsewooled sheep breed and other sheep breeds, represented in literature

**Acknowledgements.** The authors wish to thank Prof. dr. G. Erhardt from the Institute of Animal Breeding and Genetics at Justus-Liebig University (Giessen) for possibility to use isoelectric focusing method.

#### References

- Anton I., Zsolnai A., Kulkovics S., Molnar A., Fesüs L. Genetic polymorphisms of milk proteins in Hungarian dairy sheep breeds and crosses. *Sheep and Goat Production in Central and Eastern European countries. Proceedings of the workshop held in Budapest, Hungary. 1997. P. 224-226.*
- Barillet F., Sanna S., Boichard D., Astruc J.M., Carta M., Casu S. Genetic evaluation of the Lacaune, Manech and Sarda dairy sheep with animal model. *Proceed. 5th Int. Symp. On Mashine Milking of Small Ruminants, Budapest. May 14-20. Hungarian J. Anim. Prod. 1993. 1 (Suppl). P. 580-607.*
- Bocharov V.V. The molecular analysis of  $\beta$ -lactoglobulin locus in the different sheep breeds. *Dubrovicy. Doctoral thesis. 1998. P 1-93. (In Russian).*
- Bolla P., Caroli A., Mezzelani A., Rizzi R., Pagnacco G., Fraghi A. and Casu S. Milk protein markers and production in sheep. *Anim. Gen. 1989. Vol. 20. P 78.*
- Cubic-Curik V., Feligini M., Lukac-Havranek J., Curik I., Enne G. Genetic Polymorphism of  $\beta$ -Lactoglobulin in Native Sheep from the Island of Pag. *Food Technol. Biotechnol. 2002. Vol. 40(1). P. 75-78.*
- Elyasi G., Shodja J., Nassiry M.R., Tahmasebi A., Pirahary O. and Javanmard A. Polymorphism of  $\beta$ -lactoglobulin gene in Iranian sheep breeds using PCR-RFLP. *The Joint Agriculture and Natural Resources Symposium, Tabriz-Ganja. 2004.*
- Erhardt G. Evidence for a third allele at the  $\beta$ -LG locus of sheep and its occurrence in different breeds. *Animal Genetic. 1989. Vol. 20. P 197-204.*
- Garzon A.I., Martinez J.  $\beta$ -LG in Manchega sheep breed. Relationship with milk technological indexes in handcraft manufacture of Manchego cheese. *XXIII Int. Conf. Anim. Genet., Interlaken. 1992.*
- Grigaliūnaite I. Genetic Diversity in Baltic Sheep Breeds. *Doctoral thesis. Lithuanian Veterinary Academy, Kaunas, Lithuania. 2003. P 126.*
- Kolde H.J., Braunitzer G. The primary structure of ovine  $\beta$ -LG. *Milchwissenschaft. 1983. Vol. 38. P 70-72.*
- Recio I., Perz-Rodriguez, M.L., Amigo, L. and Romos, M. Study of the polymorphism of caprine milk casein by capillary electrophoresis. *Journal of Dairy Research. 1997. 64. P. 515-523.*
- Shlee P., Krause I., Rottmann O. Genotyping of ovine  $\beta$ -LG A and B using the PCR. *Arch. Tierz. 1993. Vol. 36. P 519-523.*
- Scherf B.D. (ed.). *World Watch List for domestic animal diversity. 3rd edition. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy. 2000.*
- Zapasnikiene B. The Effect of Age of Ewes and Lambing Season on Litter Size and Weight of Lambs. *Veterinarija ir zootechnika. 2002. Vol. 19(41). P 112-115.*
- Nassiry M.R., J. Shoja and G.Elyasi. The determination of  $\beta$ -lactoglobulin gene polymorphism in some Iranian and Russian sheep breeds. *The 3rd International Iran and Russia Conference, September 18-20, Moscow-Russia. 2002. P.50.*