

## ASSOCIATION OF BETA-LACTOGLOBULIN (LGB) GENOTYPES WITH QUALITY COMPOSITION OF MILK AND COAGULATION PROPERTIES IN BULGARIAN BLACK-AND-WHITE CATTLE

**Daniela Yordanova, Teodora Angelova, Jivko Krastanov**

*Agricultural Institute, Stara Zagora, Bulgaria*

*E-mail: dida.sz@abv.bg*

### ABSTRACT

The aim of the present study was to establish the relationship of LGB genotypes with the traits characterizing the qualitative composition of milk and its coagulation ability in cows of the BBW cattle.

Milk proteins' polymorphism was evaluated in 132 tissue samples from cows reared at 4 farms by PCR–RFLP analysis. During the study, the following parameters were studied: daily milk yield (kg), fat and protein contents (%), rennet coagulation time, curd firmness and curd firming time.

The presence of 2 alleles of LGB – A, B, which determine three genotypes – AA, AB, BB.

Homozygous animals carrying the BB genotype of LGB are characterized by the highest average daily milk yield – 31.00 kg.

It was found that animals with genotype AA produce milk with the highest fat content – 3.90%, the fastest rennet coagulation time – 19.82 min and the hardest coagulum – 25.99 mm.

**Key words:** LGB, genotypes, Bulgarian Black–and–White cattle, coagulation, milk.

### Introduction

Beta-lactoglobulin (LGB) is the first milk protein for which polymorphism has been detected (Aschaffenburg and Drewry, 1955). Beta-lactoglobulin accounts for about 50% of the total milk whey protein in ruminants (Walstra et al., 2006). LGB is described by a total of 15 alleles, among which A and B are the most commonly encountered (Matejcek et al., 2007).

Numerous researchers (Zaglool et al., 2016; Botaro et al., 2009; Stipp et al., 2013; Singh et al., 2014) have investigated the relationship between kappa casein (CSN<sub>3</sub>), beta-lactoglobulin (LGB) genotypes and milk yield, milk composition and cheese making properties.

The effect of CSN<sub>3</sub> and LGB on milk yield has been studied in 278 Holstein dairy cows during the first two lactations (Tsiaras et al., 2005). LGB genotypes were associated with milk yield (cows with genotype AB had higher milk yield than those with genotype AA), fat content (BB > AA > AB) and protein content of milk (AB > AA).

These results are in line with other reports: Piątkowska et al. (2011); Heidari et al., (2012); Vidovic et al., (2014) that demonstrated highest milk yields in cows with LGB genotype AA.

Neamt et al. (2017) and Pečiulaitienė et al., (2007) observed highest milk yield and milk fat content in cows with LGB genotype AB, whereas Czerniawska–Piatkowska et al. (2011) affirmed that cows with LGB genotype AA showed the highest milk yield for all lactations, and that cows from the BB genotype produced milk with higher fat content.

The association between polymorphism of  $\kappa$ -casein and beta-lactoglobulin and milk coagulation traits has been subject of a number of studies.

According to Ikonen et al., 1997; 1999; Di Stasio and Mariani, 2000; Wedholm et al., 2006; Hallén et al., 2007, 2008 the presence of allele B of  $\beta$ -CN,  $\kappa$ -CN and  $\beta$ -LG was beneficial for milk coagulation and its further processing into cheese.

Molina et al., (2006b) found out that the milk of cows from LGB genotype AA had substantially firmer curd ( $P \leq 0.05$ ) as compared to the milk of cows from genotype AA.

Jensen et al., (2012) reported that animals with  $\beta$ -LG genotype BB exhibited better milk coagulation properties.

*The purpose of the present study was to establish the association of LGB genotypes with milk composition and coagulation traits in Bulgarian Black-and-White cows.*

### Materials and methods

**Data:** For evaluation of milk coagulation properties and milk composition, 132 individual milk samples were obtained from 132 Bulgarian Black-and-White cows from 4 farms. Milk samples were collected during the morning milking, without adding any preservative.

**Laboratory analysis:** The analysis of individual coagulation properties of milk was done in the lab of the Agricultural Institute – Stara Zagora, by means of Computerized Renneting Metter – Polo Trade, Italy. The test of milk coagulation properties took 30 min. Milk samples (10 ml) were preheated (35°C) before addition of 200  $\mu$ L chymosin. Naturen Plus 215 /0.8L chymosin was used, with milk coagulation activity of 215 IMCU/ml. During the study, the following parameters were studied: daily milk yield (kg), milk fat and protein contents (%), rennet coagulation time (RCT, min), curd firmness ( $a_{30}$ , mm) and curd firming time ( $k_{20}$ , min).

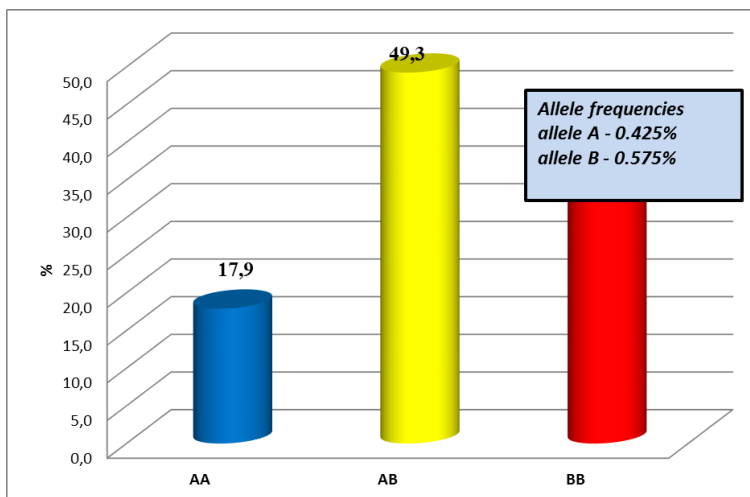
Milk proteins' polymorphism was evaluated in 132 tissue samples from cows reared at 4 farms. To this end, specialized pliers and marks with a vial containing desiccant were used to obtain and seal the tissue specimen at the time of identification of the animal. The genetic polymorphism of milk proteins was determined by PCR-RFLP analysis in the laboratory of the University of Padova, Italy.

**Statistical analysis:** The data were processed with statistical software products Systat 13 and graphs were plotted in MS Excel.

### Results and discussion

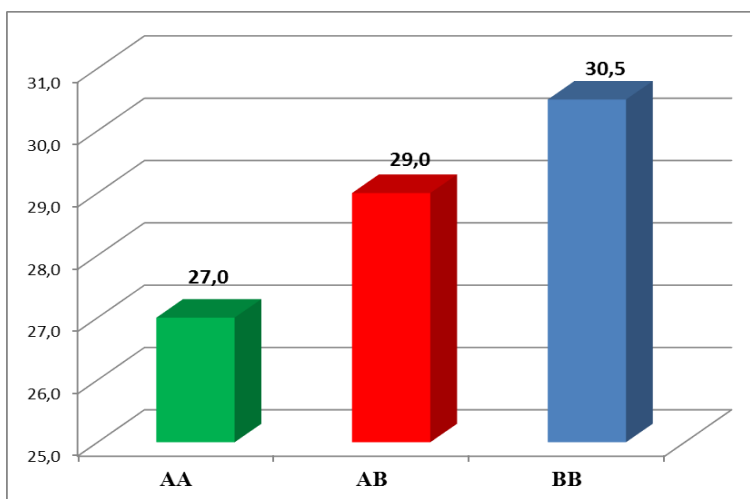
The prevalence of LGB genotypes and allele frequencies in Bulgarian Black-and-White cows are presented on Figure 1. Milk proteins of LBG were characterised by three genotypes – AA, AB and BB. The cows from the AB genotype were the most numerous – 49.25%, followed by cows with genotype BB – 32.84%. Genotype AA was the least frequent – 17.91%. The results reported by Soyudal et al., (2019) in a study with Holstein dairy cows in Turkey were very close to ours – highest proportion of heterozygous cows from the AB genotype – 53.44%, followed by those with genotype BB – 28.04% and AA – 18.52%.

The frequency of allele B was higher than that of allele A – 0.575 and 0.425%. Comparable results for predominance of allele B were also reported by Ivanković et al., (2011) and Soyudal et al., (2019).



**Figure 1: Prevalence of genotypes and allele frequencies of LGB in Bulgarian Black-and-White cows.**

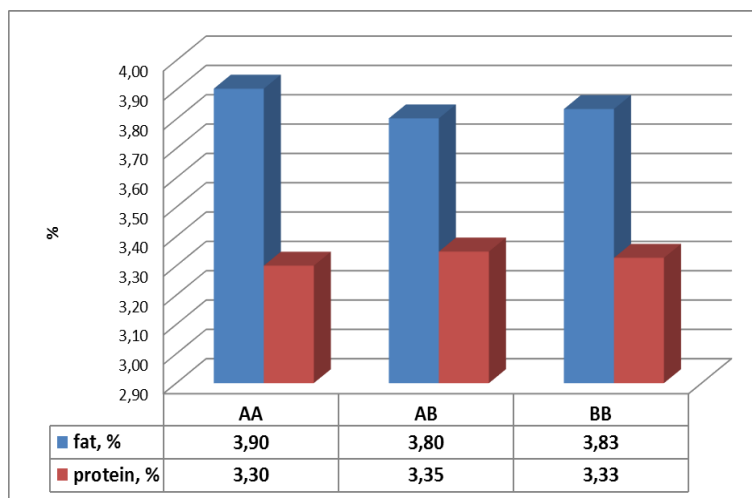
Figure 2 presents the average milk yield of cows from different LGB genotypes. Homozygous cows from genotype BB were outlined with highest average milk yield – 30.5 kg, followed by those from genotype AB – 29.0 kg. The lowest milk yield was demonstrated by cows from genotype AA. These data were in line with the results of Čítek et al., (2021), Tsiaras et al., (2005) and Neamt et al. (2017) about higher milk yield in cows with genotype AB.



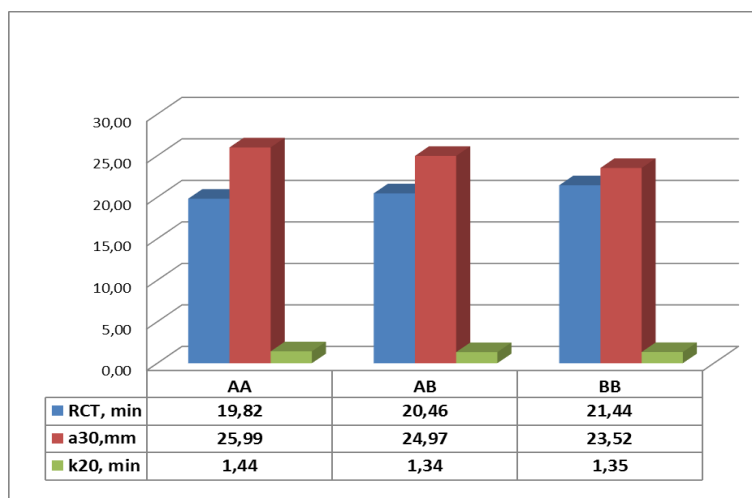
**Figure 2: Average milk yield in cows from different LGB genotypes.**

Fig. 3 depicts average milk fat and milk protein percentages in Bulgarian Black-and-White cows from the different LGB genotypes. Homozygous AA cows produced milk with the highest milk fat content yet lowest milk protein content – 3.90% and 3.30%, respectively (Figure 3). For the other two genotypes (AB and BB), milk fat and protein percentages were similar. Our results corresponded with those of Vidovic et al., (2014) as the latter researchers reported slightly higher milk fat content in cows with genotype AA and BB vs those from genotype AB. Milk protein percentage was higher in cows from genotypes BB and AB. Opposite data were found out by Zagloul et al.,

(2016) in Holstein cows from the genotype AA, which demonstrated higher milk yield and produced milk with higher protein content and milk solids-non-fat.



**Figure 3: Average milk fat and protein percentage in Bulgarian Black-and-White cows from different LGB genotypes.**



**Figure 4: Milk coagulation traits in cows from different LGB genotypes.**

Figure 4 shows that the shortest rennet coagulation time of 19.82 min. was observed in cows from genotype AA along with highest curd firmness – 25.99 mm. The longest RCT was found out in homozygous animals from genotype BB – 20.46 min. Heterozygous AB cows showed the shortest curd firming time – 1.34 min. Similar results – better coagulation properties of milk from cows carrying allele A of kappa casein and beta-lactoglobulin, were reported by Ketto et al., (2019). The authors established shorter rennet coagulation time (RCT) in cows from the genotype AB vs those from genotype BB.

## Conclusion

1. Two LGB alleles have been detected – A, B as well as three genotypes – AA, AB, BB.
2. Homozygous cows from LGB genotype BB were outlined with the highest average milk yield – 31.00 kg.
3. It was found out that cows with genotype AA produced milk with the highest fat content – 3.90%, *shortest rennet coagulation time* – 19.82 min and *firmest curd* – 25.99 mm.

## Acknowledgements

The authors acknowledge the close cooperation with renowned scientists from the University of Padova – Italy within the framework of Project DO–02–249 of the Ministry of Education and Science, with leader Prof. Jivko Krastanov.

## References

1. Aschaffenburg R., Drewry J. (1955). *Occurrence of different beta-lactoglobulins in cow's milk*. Nature, v.176, p. 218–219.
2. Botaro B.G., Y. V. R. Lima, C. S. Cortinhas, L. F. Silva, F. P. Rennó; M. V. Santos. (2009). *Effect of the kappa-casein gene polymorphism, breed and seasonality on physicochemical characteristics, composition and stability of bovine milk*. Revista Brasileira de Zootecnia, v.38, n.12, p. 2447–2454.
3. Čítek J., M. Brzáková, L. Hanusová, O. Hanuš, L. Večerek, E. Samková, Z. Křížová, I. Hoštičková, T. Kávová, K. Straková, L. Hasoňová. (2021). *Gene polymorphisms influencing yield, composition and technological properties of milk from Czech Simmental and Holstein cows*. Anim Biosci Jan 34(1):2–11. doi: 10.5713/ajas.19.0520.
4. Czerniawska-Piatkowska E., M. Szewczuk, A. Olszewska, Chocilowicz, E. (2011). *Association between beta-lactoglobulin (LGB) polymorphism and yield and composition of milk Holstein-Friesian cows imported from Sweden*. Acta Sci Pol Zootechnica 10: 9–18.
5. Di Stasio L., Mariani, P. (2000). *The role of protein polymorphism in the genetic improvement of milk production*. Zoot. Nutr. Anim. 26:69–90.
6. Hallén E., A. Wedholm, A. Andrén, Lundén, A. (2008). *Effect of  $\beta$ -casein,  $\kappa$ -casein and  $\beta$ -lactoglobulin genotypes on concentration of milk protein variants*. J. Anim. Breed. Genet. 125:119–129.
7. Hallén E., T. Allmere, J. Naslund, A. Andren, Lunden, A. (2007). *Effect of genetic polymorphism of milk proteins on rheology of chymosin-induced milk gels*. Int. Dairy J. 17:791–799.
8. Heidari M., M. A. Azari, S. Hasani, A. Khanahmadi, Zerehdaran, S. (2012). *Effect of polymorphic variants of GH, Pit-1, and beta-LG genes on milk production of Holstein cows*. Genetika 48(4):503–7. DOI: 10.1134/S1022795412040060.
9. Ikonen T., K. Ahlfors, R. Kempe, M. Ojala, Ruottinen, O. (1999). *Genetic parameters for the milk coagulation properties and prevalence of noncoagulating milk in Finnish dairy cows*. J. Dairy Sci. 82:205–214.
10. Ikonen T., M. Ojala, Syvaaja, E. L. (1997). *Effects of composite casein and  $\beta$ -lactoglobulin genotypes on renneting properties and composition of bovine milk by assuming an animal model*. Agric. Food Sci. Finland 6:283–294.
11. Ivanković A., J. Ramljak, A. Dokso, N. Kelava, M. Konjačić, Paprika, S. (2011). *Genetski polimorfizam  $\beta$ -laktoglobulina i  $\kappa$ -kazeina pasmina goveda u Hrvatskoj*. Mljekarstvo 61 (4), 301–308.

12. Jensen H. B., J.W. Holland, N. A. Poulsen, Larsen, L. B. (2012). *Milk protein genetic variants and isoforms identified in bovine milk representing extremes in coagulation properties*. Journal of Dairy Science. Volume 95, Issue 6, June 2012, pp. 2891–2903.
13. Ketto I. A., A. Abdelghania, A. G. Johansen, J. Øyaas, Skeie, S. B. (2019). *Effect of milk protein genetic polymorphisms on rennet and acid coagulation properties after standardisation of protein content*. International Dairy Journal Volume 88, January 2019, pp. 18–24.
14. Matějčec A., J. Matějčková, E. Němcová, O. M. Jandurová, M. Štípková, J. Bouška, Frelich, J. (2007). *Joint effect of CSN3 and LGB genotypes and their relation to breeding values of milk production parameters in Czech Fleckvieh*. Czech Journal of Animal Science 52, 83–87.
15. Molina L.H., T. Benavides, C. Brito, B. Carrillo, Molina, I. (2006b). *Relationship between A and B variants of  $\kappa$ -casein and  $\beta$ -lactoglobulin and coagulation properties of milk (Part II.)*. International Journal of Dairy Technology 59, 188–191.
16. Neamt R.I., G. Saplacan, S. Acatincai, L.T. Ciszter, D. Gavojdian, Ilie, D.E. (2017). *The influence of CSN3 and LGB polymorphisms on milk production and chemical composition in Romanian Simmental cattle*. Acta Biochim Pol 64: 493–497.
17. Pečiulaitienė N., I. Miceikienė, R. Mišeikienė, Kriauziene, J. (2007). *Genetic factors influencing milk production traits in Lithuanian dairy cattle breeds*. Zemėsukio Mokslai 32–38.
18. Piątkowska C.E., M. Szewczuk, A. Olszewska, Chociłowicz, E. (2011). *Association between beta-lactoglobulin (lgb) polymorphism and yield and composition of milk of Holstein Friesian cows imported from Sweden*. Acta Scientiarum Polonorum Zootechnica, 10 (1):9–18.
19. Singh U, R. Deb, S. Kumar, R. Singh, G. Sengar, Sharma, A. (2014). *Association of prolactin and beta-lactoglobulin genes with milk production traits and somatic cellcount among Indian Frieswal (HF × Sahiwal) cows*. Biomarkers and Genomic Medicine, 7 (1):38–42.
20. Soyudal B., S. Ardicli, H. Samli, D. Dincel, Balci, F. (2019). *Association of polymorphisms in the CSN2, CSN3, LGB and LALBA genes with milk production traits in Holstein cows raised in Turkey*. Journal of the Hellenic Veterinary Medical Society, 69(4), 1271–1282. doi:<https://doi.org/10.12681/jhvms.19617>.
21. Stipp A.T., P.R. Bignardi, R.C. Polifrederico, K. Sivieri, Costa, M. K. (2013). *Polimorfismos genéticos da kappa-caseína e da betalactoglobulina produção de leite em bovinos*. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, v.65, n.1, pp. 275–280.
22. Tsiaras, A. M., G. Bargouli, G. Banos, Boscós, C.M. (2005). *Effect of Kappa-Casein and Beta-Lactoglobulin loci on milk production traits and reproductive performance of Holstein cows*. J. Dairy Sci. 88:327–334.
23. Vidovic V., D. Lukac, Z. Nemes, Trivunovic, S. (2014). *B-lactoglobulin genetic variants in Serbian Holstein-Friesian dairy cattle and their association with yield and quality of milk*. Animal Science Papers and Reports 32(2):179–182.
24. Walstra P., J. T. M. Wouters, Geurts, T. J. (2006). *Dairy science and technology*. 2nd ed. Boca Raton: Taylor & Francis, 782p.
25. Wedholm A., L. B. Larsen, H. Lindmark-Mansson, A. H. Karlsson, Andren, A. (2006). *Effect of protein composition on the cheese-making properties of milk from individual dairy cows*. J. Dairy Sci. 89:3296–3305.
26. Zagloul, A.W., A. Awad, S. El, A. El, El-Bayom, K. M. (2016). *Association of  $\beta$ -Lactoglobulin Gene Polymorphism with Milk Yield, Fat and Protein in Holstein-Friesian Cattle*. World. v.6, n.3, pp. 117–122.