

## **-LACTOGLOBULIN GENETIC VARIANTS IN BROWN-SWISS DAIRY CATTLE AND THEIR ASSOCIATION WITH MILK YIELD AND QUALITY TRAITS**

Unsal Dogru

Department of Animal Sciences, College of Agriculture, Ataturk University, 25240, Yakutiye, Erzurum, Turkey

Corresponding Author's email: udogru@atauni.edu.tr

### **ABSTRACT**

Milk protein polymorphism such as  $\beta$ -Lactoglobulin ( $\beta$ -Lg) of Brown-Swiss dairy cows were investigated in this study. The relationships between milk protein genotypes and some milk production traits were determined. Genetic variants of milk protein were identified by horizontal starch-gel electrophoresis containing mercaptoethanol and urea. The allelic frequencies of A and B were found to be 0.375 and 0.625 respectively. Milk production traits chosen in relation to  $\beta$ -Lg variants were: actual milk yield, 305 d milk yield, actual fat yield, 305 d fat yield, fat percentage in milk, lactation length. Genotype frequencies were in accordance with the Hardy-Weinberg equilibrium. The effect of  $\beta$ -Lg genotypes on milk production traits were analyzed using a general linear model (GLM). There was no significant association between different genotypes of  $\beta$ -Lg and milk production traits of the analyzed cows.

**Keywords:**  $\beta$ -Lactoglobulin, protein polymorphism, dairy cow, milk production traits.

### **INTRODUCTION**

Since the discovery of alleles A and B of  $\beta$ -lactoglobulin in cattle, genetic polymorphism in milk proteins has raised great interest in animal breeding and dairy industry due to the relationship between milk proteins and milk production traits, composition, and quality (Aschaffenburg and Drewry, 1957; Ng-Kwai-Hang *et al.*, 1990; Aleandri *et al.*, 1990; Caroli *et al.*, 2004; De Marchi *et al.*, 2008). Selection and breeding of animals with fascinating genotypes are of crucial importance for the genetic improvement of dairy cattle. The genetic causes of this relationship between milk protein polymorphism and production traits were thought to be due to pleiotropy and linkage (Soysal, 1983).

Polymorphism in bovine milk proteins has been investigated recently as being of great importance in breeding and hybridization strategies. Studies on population structure and preservation of the indigenous genetic resources were also important. A number of studies indicate that milk protein polymorphism has a strong influence on milk qualitative and quantitative traits and technological properties (Di Stasio and Mariani, 2000; Martin *et al.*, 2002). Determining association between and among the genes controlling protein polymorphic polygenic traits related to the productive traits in livestock species is of great economic importance for animal breeding and may increase overall productivity (Nemeš *et al.*, 2012; Luka *et al.*, 2013). Some investigators described that this relationship was not important for milk production traits (Wegner *et al.*, 1974; Janicki, 1980; Eidrigevich *et al.*, 1982; Nakayama *et al.*, 1996; De-Lange *et al.*, 1990; Eenennaam and Medrano, 1991). However, contrarily Mclean *et al.*

(1985), Khaertdinov (1990), Ng-Kwai-Hang *et al.* (1991), Chung *et al.* (1993), Matejcek *et al.* (2007) and Strzalkowska *et al.* (2009) remarked that genetic variant of milk protein could be a criterion for selection for the improvement of dairy cattle production. Furthermore, Ulutas and Yildirim (2009) did not report any relationship between milk yield, fat content and genotypes of  $\beta$ -Lg, but they found that rennet clotting time of milk was significantly related with genotypes of  $\beta$ -Lg. The aim of the present study was to determine the genetic structure of cows in terms of  $\beta$ -Lg genotype and also to investigate some relationship between milk protein genotypes and some production traits.

### **MATERIALS AND METHODS**

Milk samples were obtained from 129 multiparous Brown-Swiss cows reared at Research and Application Farm of College of Agriculture, Ataturk University maintained during 1995 to 2012 to study  $\beta$ -Lg protein polymorphism. A total of 615 production records were utilized for the present investigation. Year was divided into four calving seasons (December-February, winter; March-May, spring; June-August, summer and September-November, autumn). Parities more than five were merged with 5<sup>th</sup>. Lactation milk yields records were adjusted according to Anonymous (1976).

About 10 ml of milk was collected from each animal and 20 mg potassium dichromate was added to each sample as a preservative. Fat-free milk samples were stored in a refrigerator at 4°C until they were analyzed. Two or three drops of 2-mercapto ethanol were added to samples before electrophoresis. Milk protein genotyping was carried out by using horizontal starch-

urea gel electrophoresis (Aschaffenburg and Michalak 1968; Dogru, 1994). Direct counting was used to estimate gene and genotypic frequencies of the  $\beta$ -Lg proteins. The chi-squared test was used to check whether the population was in Hardy-Weinberg equilibrium (Soysal, 1998). The data on the milk production traits of the different  $\beta$ -Lg genotypes were subjected to Analysis of variance (ANOVA) using the General Linear Model (GLM) from the Statistical Analysis Software (SPSS Statistics 17.0). The following statistical model used was:

$$Y_{ijkl} = \mu + G_i + A_j + S_k + e_{ijkl}$$

Where:

$Y_{ijkl}$  = is the observation on each trait of the  $ijkl$ th animal

$\mu$  = is the general mean of each trait

$G_i$  = is the fixed effect of  $i$ th  $\beta$ -Lg genotype ( $i=1,2,3$ )

$A_j$  = is the fixed effect of  $j$ th parity number ( $j=1, 2, \dots, 5$ ; parity number  $>5$  were pooled with parity of 5)

$S_k$  = is the fixed effect of the  $k$ th season of calving ( $k=1, 2, \dots, 4$ )

$e_{ijkl}$  = is the random error effect associated to the  $ijkl$ th observation.

## RESULTS AND DISCUSSION

The aim of this study was to identify  $\beta$ -Lg A and  $\beta$ -Lg B alleles and  $\beta$ -Lg AA,  $\beta$ -Lg AB and  $\beta$ -Lg BB genotypes of  $\beta$ -Lg in a population of Brown-Swiss cows. Out of 129 studied cows, genotypic frequencies of  $\beta$ -Lg genotypes were: 22 cows of the  $\beta$ -Lg AA genotype, 53 of genotype AB, and 54 of BB genotype. Chi-squared test for deviations from the Hardy-Weinberg equilibrium were carried out to determine statistical significance. Deviations from the Hardy-Weinberg equilibrium was not significant ( $\chi^2=2.047$ ). Similar findings have been well documented by a number of other investigators (Eenennaam and Medrano, 1991; Chung *et al.* 1995; Eser, 2011). The frequencies reported by Rachagani *et al.* (2006) in Sahiwal and Tharparkar breeds are similar to those reported for Gyr, Nelore, and Sindi breeds by Del Lama and Zago, (1996). These findings indicate that the frequency of A allele in the *Bos indicus* breeds of cattle is lower than the European breeds.

**Table 1. Least square means and standard errors of milk production traits of  $\beta$ -Lg genotype, parity and season of calving.**

Parameter	N	Actual milk yield (kg)	305d milk yield (kg)	Actual fat yield (kg)	305d fat yield (kg)	Fat in milk (%)	Lactation length (d)
$\beta$ -Lg genotype							
AA	101	3325.2 $\pm$ 92.8 <sup>NS</sup>	3099.9 $\pm$ 72.1 <sup>NS</sup>	132.7 $\pm$ 5.2 <sup>NS</sup>	124.3 $\pm$ 4.6 <sup>NS</sup>	3.94 $\pm$ 4.9 <sup>NS</sup>	313.5 $\pm$ 7.2 <sup>NS</sup>
AB	259	3383.6 $\pm$ 60.9	3152.0 $\pm$ 47.3	131.8 $\pm$ 3.4	122.1 $\pm$ 2.9	3.93 $\pm$ 3.2	320.9 $\pm$ 4.7
BB	255	3406.9 $\pm$ 60.1	3140.0 $\pm$ 46.7	135.1 $\pm$ 3.4	125.7 $\pm$ 2.9	3.88 $\pm$ 3.1	320.5 $\pm$ 4.7
Parity							
1	126	3043.8 $\pm$ 84.2b	2714.6 $\pm$ 65.4c	119.6 $\pm$ 4.7c	107.4 $\pm$ 4.1c	4.01 $\pm$ 4.5a	338.4 $\pm$ 6.5a
2	119	3273.6 $\pm$ 86.1b	3058.5 $\pm$ 66.9b	128.3 $\pm$ 4.8bc	120.3 $\pm$ 4.2b	3.93 $\pm$ 4.6ab	319.7 $\pm$ 7.6b
3	91	3567.3 $\pm$ 99.2a	3331.1 $\pm$ 76.9a	139.6 $\pm$ 5.5ab	130.7 $\pm$ 4.9ab	3.87 $\pm$ 5.3ab	315.7 $\pm$ 7.7b
4	78	3740.5 $\pm$ 99.8a	3492.1 $\pm$ 83.7a	147.1 $\pm$ 6.0a	137.5 $\pm$ 5.3a	3.95 $\pm$ 5.7a	324.5 $\pm$ 8.3ab
5	201	3234.4 $\pm$ 71.3b	3056.9 $\pm$ 55.4b	131.4 $\pm$ 3.9bc	124.4 $\pm$ 3.5b	3.81 $\pm$ 3.8b	293.1 $\pm$ 5.5c
Season of calving							
Spring	179	3189.7 $\pm$ 72.8b	2989.7 $\pm$ 56.5b	128.4 $\pm$ 4.1 <sup>NS</sup>	119.9 $\pm$ 3.6 <sup>NS</sup>	3.93 $\pm$ 3.8 <sup>NS</sup>	311.8 $\pm$ 5.6b
Summer	124	3378.5 $\pm$ 86.6ab	3091.2 $\pm$ 67.2ab	133.1 $\pm$ 4.8	122.1 $\pm$ 4.2	3.84 $\pm$ 4.6	316.1 $\pm$ 6.7b
Autumn	101	3573.9 $\pm$ 96.3a	3264.9 $\pm$ 74.8a	140.8 $\pm$ 5.4	130.2 $\pm$ 4.7	3.92 $\pm$ 5.1	327.9 $\pm$ 7.5a
Winter	211	3345.6 $\pm$ 67.0ab	3176.8 $\pm$ 52.0ab	130.5 $\pm$ 3.7	124.0 $\pm$ 3.3	3.97 $\pm$ 3.5	317.2 $\pm$ 5.1b
Overall Mean	615	3371.9 $\pm$ 43.8	3130.7 $\pm$ 34.0	132.2 $\pm$ 2.4	124.0 $\pm$ 2.1	3.92 $\pm$ 2.3	318.3 $\pm$ 3.4

NS: Non-Significant

a, b, c: Means with same superscripts are not significantly different ( $P < 0.05$ ) from one another

Table 1 shows the effect of  $\beta$ -Lg genotypes, parity and season of calving on milk production traits in Brown-Swiss cattle. The results indicate that  $\beta$ -Lg genotypes do not have any statistically significant effect on all examined milk production traits. Similar results coincide with those reported by various workers (Wegner *et al.*, 1974; Janicki, 1980; Eidrigevich *et al.*, 1982; DeLange *et al.*, 1990; Ng-Kwai-Hang *et al.*, 1990; Eenennaam and Medrano, 1991; Nakayama *et al.*, 1996; Lunden *et al.*, 1997; Ojala, *et al.*, 1997; Eser, 2011). On

the other hand, our result disagree with the literature data demonstrated by Mayer *et al.* (1990) and Tsiaras *et al.* (2005) who claimed that there were significant relationship between  $\beta$ -Lg genotypes with higher milk yield and fat yield traits and this relationship could be used in indirect selection.  $\beta$ -Lg BB is associated with higher casein and fat contents, which are favorable for cheese making (McLean *et al.*, 1984; Aleandri *et al.*, 1990; Lodes *et al.*, 1997). Cornberg *et al.* (1964) and Samarineanu *et al.* (1984) found an association of  $\beta$ -Lg

AA phenotype with milk yield in Black and White cattle and Brown-Swiss cattle breeds, respectively. The AA genotype of -Lg has also been shown to have a favorable effect on protein yield (Bovenhuis *et al.*, 1992)

Among genotypic groups concerning protein polymorphism, relative or statistically difference for yield, composition, and quality characteristics varies with breeds or herds within same breed. It may be due to genotype-environment interaction and polymorphism in traits. Therefore, more studies are needed to be conducted in different herds to confirm the evidence found in present study.

## REFERENCES

- Aleandri, R., L. G. Buttazzoni, J. C. Schneider, A. Caroli, and R. Davoli (1990) The effect of milk protein polymorphism on milk components and cheese-producing ability. *J. of Dairy Sci.* 73:241–255.
- Anonymous, (1976). Internationales komitee zur ermittlung der wirtschaftlichkeit von milchtieren. Das Teirzuchtrecht in der Bundesrepublik Deusschland Ntb, 310.
- Aschaffenburg, R. and J. Drewry (1957). Genetics of the beta-lactoglobulin of cow's milk. *Nature*, 180: 376-378.
- Aschaffenburg, R. and W. Michalak (1968). Simultaneous phenotyping procedure for milk proteins improved resolution of the Beta Lactoglobulin. *J. Dairy Sci.* 51: 1319-1320.
- Bovenhuis, H., J.A.M. Van Arenduk, and S. Korver (1992). Associations between milk protein polymorphism and milk production traits. *J. of Dairy Sci.* 75: 2459-2559.
- Caroli, A., S. Chessa, P. Bolla, E. Budelli, and G.C. Gandini (2004) Genetic structure of milk protein polymorphisms and effects on milk production traits in a local dairy cattle. *J. Anim. Breed Genet.* 121: 119-127.
- Chung E. R., C. H. Yu, H. Y. Chung, J. E. Kim, K. J. Chun, S. K. Han, and Y. C. Shin (1993). Studies on polymorphism of milk proteins as genetic markers in beef cattle. *Koren J. of Ani. Sci.* 35(3):181-190.
- Chung, E. R., S. K. Han, and T. J. Rhim (1995). Milk protein polymorphism as genetic marker in Korean native cattle. *Asian Australian J. of Ani. Sci* 8(2): 187-194.
- Cornberg, G., H. Meyer, and M. Growing (1964). Die beziehung der Beta-Laktoglobulin typen beim rind zu erstkol healter, milcmange und fettgehalt. *Zuchtungskunde*, 36: 248-255.
- De Marchi, M., G. Bittante, R. Dal Zotto, C. Dalvit, and M. Cassandro (2008). Effect of Holstein Friesian and Brown Swiss breeds on quality of milk and cheese. *J. Dairy Sci.* 91: 4092–4102.
- Del Lama, S. N. and M. A. Zago (1996). Identification for the kappa-caesinand beta-actoglobulin genotypes in Brazilian Bos indicus and Bubalis bubalis population. *Brazilian J. Genet.* 19: 73-77.
- De-Lange, D.J., E. H. H. Meyer, L. Van-Rensburg, and A. Swanepoel (1990). The polymorphism protein system in cow's milk: I. The association with the productifon of milk protein and fat during lactation. *Suid Afrikaanse Tydskrif vir Suiwelkunde*, 22(4): 67-71.
- Di Stasio, L., and P. Mariani (2000). The role of protein polymorphism in the genetic improvement of milk production. *Zootecnica e Nutrizione Animale.* 26(3): 69-90.
- Dogru, U, (1994). Genetic structure of several polymorphic blood and milk proteins of Brown-Swiss, Holstein, Simmental and Eastern Anatolian Red cattle breeds and their differences according to several production traits. (In Turkish). Ataturk University PhD. Thesis Erzurum.
- Eenennaam, A. A. V., and J. F. Medrano (1991). Milk protein polymorphism in California dairy cattle. *J. Dairy Sci.* 74: 1730-1742
- Eidrigevich, E., V. Petrenko, and V. Karnozhitskii (1982). Polymorphic protein systems in the selection of animals. *Anim. Breed. Abst.* 50: p. 165.
- Eser, K. G. (2011). Association between milk protein polymorphism and milk production traits in Black and White dairy cattle in Turkey. *Afr. J. of Biotech.* 10(6):1044-1048
- Janicki, C. (1980). Polymorphism of -Lactoglobulin in Polish Red and White Lowland cattle. *Anim. Breed. Abst.* 48: p. 504.
- Khaertdinov, R. A. (1990). The effect of genotype on milk protein in Kholmogor and Ayrshire cows. *Anim. Breed. Abst.* 58: p. 7175.
- Lodes, A., I. Krause, J. Burcberger, J. Aumann, and H. Klostermeyer (1997). The influence of genetic variants of milk proteins on the compositional and technological properties of milk. 3. Content of protein, casein, whey protein and casein number. *Milchwissenschaft* 52:3–8.
- Luka, D.V. Vidovic, Z. Nemes, M. Stupar, and A. Popovic-Vranjes (2013). Genotypic frequencies of the -lactoglobulin, -casein and transferrin in Serbian Holstein-Friesian dairy cattle. *Mljekarstvo.* 63(4): 203-210.
- Lunden, A., M. Nilsson, and L. Janson (1997). Marked effect of beta-Lactoglobulin polymorphism on the ratio of casein to total protein in milk. *J. Dairy Sci.* 80: 2996-3005.
- Martin, P., M. Szymanowska, L. Zwierzchowski, and C. Leroux (2002). The impact of genetic

- polymorphisms on the protein composition of ruminants milks. *Reproduction Nutrition Development*. 42(5): 433-459
- Matejcek, A., J. Matejckova, E. Nemcova, and J. Frelich (2007). Joint effect of CSN3 and LGB genotypes and their relation to breeding values of milk production parameters in Czech Fleckvieh. *Czech J. of Ani. Sci.* 52: 83-87.
- Mayer, F., G. Erhardt, K. Failing, and B. Senft (1990). Investigation on the relationship between milk yield udder health, milk protein and blood protein polymorphism in cattle. *Anim. Breed. Abst.* 58: p. 2593.
- McLean, D. M., E. R. B. Graham, R.W. Ponzoni, and H. A. McKenzie (1984) Effects of milk protein genetic variants on milk yield and composition. *J. of Dairy Res.* 51:531-546.
- Nakayama, O., M. Onedera, K. Nakayashi, K. Nakamura, T. Yamamoto, K. Arai, A. Kikkawa, and M. Kosugiyama (1996). Effect of  $\beta$ -Casein genotype on milk in yield in Japanese Shorthorn Cows. *Anim. Breed. Abst.* 64: p. 150.
- Nemeš, Z., V. Vidović, L. J. Strbac, D. Lukac, and M. Stupar (2012). Milk quality and processing in relation to polymorphism of  $\kappa$  casein at black and white Holstein cattle. 6th Central European Congress on Food, Novi Sad, 23-26 May, 1666-1669.
- Ng-Kwai-Hang, K.F., H. G. Monardes, and J. F. Hayes (1991). Association between genetic polymorphism of milk protein and production traits during three lactations. *Anim. Breed. Abst.* 59: p. 4614.
- Ng-Kwai-Hang, K.F., H.G. Monardes, and J.F. Hayes (1990). Association between genetic polymorphism of milk proteins and production traits during three lactations. *J. Dairy Sci.* 73: 3414-3420.
- Ojala, M., T.R. Famula, and J.F. Medrano (1997). Effects of milk protein genotypes on the variation for milk production traits of Holstein and Jersey cows in California. *J. Dairy Sci.* 80: 1776-1785.
- Rachagani, S., I. D. Gupta, N. Gupta, and S. C. Gupta (2006). Genotyping of  $\beta$ -lactoglobulin gene by PCR-RFLP in Sahiwal and Tharparkar cattle breeds. *BMC Genet.* 7: 31-34.
- Samorineanu, M., F. Stametescu, I. Granciu, M. Spulber, and N. Sotu (1984). The result of electrophoretic studies of some proteins in the blood and milk of Romanian Brown cows in Moldavia. *Anim. Breed. Abst.* 52: 1-3.
- Soysal, M. I. (1983). The relationship between blood protein polymorphism with several yield traits in sheep population of Ataturk University. (In Turkish). Ataturk University PhD. Thesis Erzurum.
- Soysal, M. I. (1998). *Statistical Methods*. (In Turkish). Agricultural Faculty Public No: 95, Notice No: 64. Namik Kemal University Tekirdag
- SPSS, Statistics. (2008). 17.0 Release 17.0.0 (Aug 23, 2008)
- Strzalkowska, N., A. Jó wik, E. Bagnicka, J. Krzy ewski, J. O. Horba czuk (2009). Studies upon genetic and environmental factors affecting the cholesterol content of cow milk. I. Relationship between the polymorphic form of  $\beta$ -lactoglobulin, somatic cell count, cow age and stage of lactation and cholesterol content of milk. *Animal Science Papers and Reports* 27 (2): 95-103.
- Tsiaras, A.M., G.G. Bargouli, G. Banos, and C. M. Boscos (2005). Effect of  $\kappa$ -casein and  $\beta$ -lactoglobulin loci on milk production traits and reproductive performance of Holstein cows. *J. of Dairy Sci.* 88(1): 327-334.
- Ulutas, Z, and M. Yildirim (2009). Genetic variants of  $\beta$ -Lactoglobulin,  $\kappa$ -Casein and  $\beta$ -Casein of milk in East Anatolian Red Cattle breed and it's association with milk yield, fat content and rennet clotting time on milk. *Asian J. Chem.* 21(1): 795-801.
- Wegner, W., I. Reetz, H. Feder, H. Sponer, G. Ascherman, and R. Plischke (1974). Some qualitative and quantitative characters and their relationship with each other in Germany Black Pied cattle populations, II polymorphic systems and performance. *Anim. Breed. Abst.* 42: p. 968.