

Dace Smiltiņa, Andris Bāliņš, Ziedonis Grīslis
Latvia University of Agriculture, Latvia

POLYMORPHISM OF α_{s1} – CASEIN (*CSN1S1*) IN THE LATVIAN BROWN (LB) DAIRY CATTLE BREED

Abstract

The aim of this study in Laboratory of Molecular Genetic Research of Faculty of Agriculture in LLU was oriented to the identification of α_{s1} –casein (*CSN1S1*) gene polymorphism and to analysis of genotype structure in population of the Latvian Brown dairy cattle. The PCR products were digested with the restriction enzyme *MaeIII*. Blood samples of 100 living individuals of LB breed and representing different ages were genotyped, resulting in a 0,985 frequency of B allele and in 0,015 frequency of C allele. The discovery of the α_{s1} –casein allele C in such a small endangered population is critical in the content of genetic resources preservation.

Key words: *dairy cattle, α_{s1} – casein, alleles B and C, PCR-RFLP*

Introduction

Since the discovery of the milk protein genetic variants, attempts have been made to correlate milk characteristics and milk production with the genotype. Studies on milk protein genetic variability started almost fifty years ago by detecting bovine beta-lactoglobulin main variants (Aschaffenburg et al. 1957:376), and were intensively developed during the following years, discovering high polymorphism, with important differences among cattle species and breeds (Formaggioni et al. 1999). A recent revision of milk protein nomenclature (Farrell Jr et al. 2004: 1645) indicates the 8 α_{s1} -casein, 4 α_{s2} -casein, 12 β -casein, 11 κ -casein, 11 β -lactoglobulin, and 3 α -lactoalbumin variants within the cattle species. More than 95% of the proteins contained in ruminants' milk are coded by 6 structural genes, now well characterised (Martin et al. 2002: 434). Strongest effects on first lactation milk and protein yields seem to be associated with the *CSN1S1* locus (Lin et al. 1986:704).

From the first detected polymorphism in the cattle α_{s1} -casein (*CSN1S1*) locus (Thompson et al. 1962: 1001) through 2004, nine genetic variants were identified at this locus: A, B, C, D, Eyak, Ebali, F, G, and H (Falconer et al. 1996). The *CSN1S1* B variant is the most common in European breeds, with the highest frequency (90-100%) in Holstein (Formaggioni et al. 1999). In Yak and Zebu, C allele is predominant (Mahé et al. 1999: 239). The *CSN1S1* B variant has its phylogenetic origin from C, which was likely, the ancestral allele; all the other known variants in *Bos Taurus* were derived from the *CSN1S1* allele B (Kawamoto et al. 1992:563).

Eenennaam and Medrano (1991) found high milk yield as well as protein content associated with *CSN1S1* genotype CC as compared to genotypes BB and BC (Eenennaam et al. 1991:1730).

Comparisons of two *CSN1S1* promoter alleles discriminated by a *Mae* III RFLP (Koczan et al. 1993: 74) and the two alleles B and C of the protein coding region revealed up to four haplotypes depending on the breed investigated (Jann et al. 2002: 13).

The aim of our present study were to identify allelic variation in *CSN1S1* of Latvian Brown cattle breed population as it had not done never before in Latvia. Also Latvian Brown cattle breed is one of the Latvia genetic resources' populations.

Materials and Methods

Animals were chosen at random from each heard. The blood was taken from the jugular vein, and was collected in K3-EDTA coated sterile vaccutainers and stored at - 20 °C until used for DNA extraction. Research has been done in the Laboratory of the Molecular Genetic Researches of the Faculty of Agriculture of LLU (Latvia University of Agriculture).

DNA was extracted using the Fermentas Genomic DNA Purification Kit # KO512 and DN Easy Blood@Tissue Kit which had extracted by QIAcube (QIAGEN, USA). The *CSN1S1* alleles were identified using the PCR - RFLP (Polymerase Chain Reaction and Restriction Fragment Length Polymorphism) method in the accordance with methodology provided by Koczan (1993). We have used primers: *aS1 forward* 5'-TGC ATG TTC TCA TAA TAA CC-3' and *aS1revers* 5'- GAA GAA GCA GCA AGC TGG-3' from methodology of Koczan (Koczan et al. 1993).

The PCR reaction elaborated by Koczan (Koczan et al. 1993: 74) was modified. The amplification was carried out in Applied Biosystems 2720 Thermal Cycler with the following amplification conditions: 94 °C for 3 min (initial denaturation), then followed 30 cycles with denaturation at 94 °C for 30 sec, annealing at 59 °C for 30 sec, and extension at 72 °C for 30 sec with a final extension step of 72 °C for 10 min. Samples of PCR products (25 µl) were digested with *Mae*III endonucleases according to the manufacturer's recommendations (Roche DiagnosticsGmbH). Restrictive fragments that were obtained this manner were separated in a 3% agarose gel with Ethidium bromide (10 µl EtBr 100 ml⁻¹ of 3% agarose gel). Electrophoresis on 3% agarose gel was used for visualisation of the restricted DNA bands (60V, 150 min) in 0.5X TBE buffer.

The alleles' frequencies were calculated by using the appropriate diallele locus expressions, where the allele's B relative frequency was designated as p, and the relative frequency of C allele - as q. We obtained the p and q expressions:

$$p = \frac{2D + H}{2N} \quad (1) \quad \text{and} \quad q = \frac{2R + H}{2N} \quad (2),$$

where

D, H, R - the number of individuals with genotypes BB, BC, and CC;

N - total number of animals in the analysis;

2N - total number of alleles in the analysis.

Calculations were made by the Microsoft Office Excel 2007 standard package assistance, but the computer program package TFPGA was used as a population genetic basis of the accuracy of testing (Miller 1997). The allele frequencies were estimated by simple allele counting according to the Hardy-Weinberg equilibrium (Falconer et al. 1996).

Results and discussion

For expression of the results of the restricted DNA bands for *CSN1S1* genotypes had used a Figure 1.

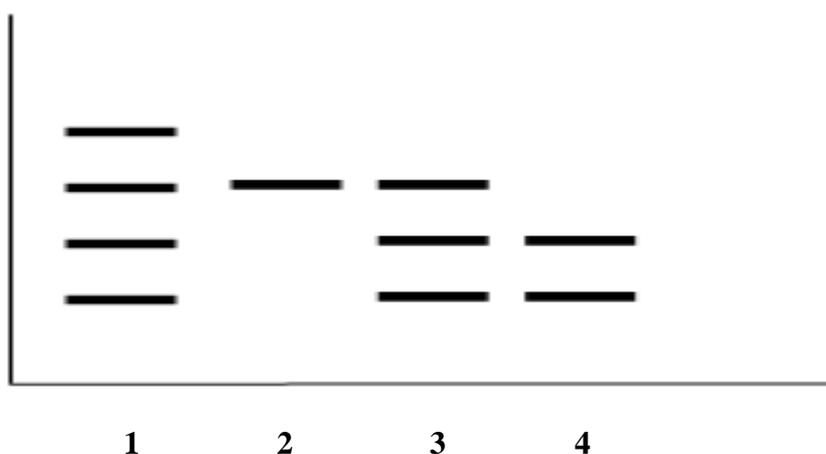


Figure1. Schematic image of the PCR - RFLP product *CSN1S1* gene by *Maelll* (Koczan et al. 1993)

In Figure 1 can see that:

1 – DNA ladder 100 bp,

2 – Genotype CC and PCR product(310 bp,),

3 – Genotype BC (310 bp, 214 bp, 96 bp),

4 – Genotype BB (214bp, 96 bp).

Results of genotypes and alleles of *CSN1S1* in the Latvian Brown breed's population have presented in Table 1. In our research we detected only two genotypes in the population of all Latvian cattle breed: homozygote genotype BB - 97 animals, and heterozygote genotype BC - only 3 animals. Homozygote genotype CC has not been observed at all.

The most common is *CSN1S1* allele B in the Latvian brown breed. Unfortunately, the adverse of frequency of *CSN1S1* allele B in Latvian Brown breed's population have a high level (Table 1); in the preliminary estimation we found frequency of *CSN1S1* allele B 0.985.

The *CSN1S1* genotypes' and alleles' analysis showed that all the analyzed varieties of *CSN1S1* genetic variability have fixated or close to the fixation state of allele B, and allele C was observed in the Latvian Brown breed, moreover, with a very low frequency - $q = 0.015$. In the analysis of the global experience in the data, we found, for example, that in Colombia from 102 dairy cows of the investigated *CSN1S1* genotypes were: BB genotype found in 61 individuals, BC genotype – in 39, and CC genotype – in 2. This indicates that the CC genotype has remained in some breeds there (Convenio 2010: 9). In the USA *CSN1S1* genotype CC is most common for Brown Swiss and Guernsey breeds, but *CSN1S1* genotype BB- for Holstein Friesian breed (Formaggioni et al. 1999). In Poland researchers have investigated that for the cattle with 50-75% HF, the homozygous BB genotype individuals clearly prevailed within the *CSN1S1* polymorphism, reaching 94.74% (Czerniawska-Piatkowska et al. 2004:158).

Table 1

Frequency analysis of α_{s1} – casein (*CSN1S1*) genotypes and alleles estimated in the Latvian Brown (LB) dairy cow breed population

Genotypes and alleles	Latvian Brown breed (n=100)			
	Number of genotype and allele	Frequency of genotype and allele	HW	\pm
BB	97	0.970	0,970	0
BC	3	0.030	0,030	0
CC	0	0.000	0.000	0
Total	100	1.000	1.000	
B (p)	197	0.985	-	-
C (q)	3	0.015	-	-
Total	200	1.000	-	-

HW – Hardy- Weinberg equilibrium

p - allele's B relative frequency

q - allele's C relative frequency

When analyze *CSN1S1* phylogenesis, can see in Figure 2 that for the cattle first were forming *CSN1S1* allele C, from which were forming E and B alleles, but from B allele - A, D, F and H alleles (Formaggioni at al. 1999).

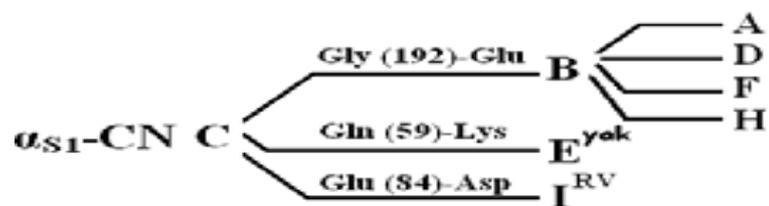


Figure 2. Phylogenetic origin of the α_{s1} – casein (Formaggioni, P. and at al.; 1999)

Bovine *CSN1S1* allele B causes the overwhelming dominance of LB breed population has not known yet, because up to now, such studies were not conducted. Our previous studies with other forms of caseins (κ -and β -CN) showed the existence of genetic variability, although the case can only appear on a possible genetic drift, since targeted gene assists breeding has so far not been done in Latvia.

The *CSN1S1* frequency increases it is necessary to detect the CC and BC genotypes bulls and the maximum use of breeding work. For increasing of the frequency of *CSN1S1* need to discover CC and BC genotypes' bulls and the maximum use of breeding work.

Conclusions

1. In DNA samples (n = 100) of Latvian Brown cattle breed the examination found that the frequencies of *CSN1S1* alleles B and C are 0.985 and 0.015.
2. Frequency of *CSN1S1* allele B more than nine times higher than the frequency of *CSN1S1* allele C and the reason which caused the prevalence of *CSN1S1* allele B is not known yet.
3. Frequencies of *CSN1S1* genotypes BB, BC, and CC in the Latvian Brown breed (n = 100, 0.97, 0.03, 0.00) do correspond to Hardy-Weinberg equilibrium proportions:

$$(0.985 + 0.015)^2 = 0.970 + 0.030 + 0.000,$$

and populations are in genetic equilibrium, but with tendency to fixation state of *CSN1S1* allele B.

4. Critically important is to detect the CC and BC genotypes' breeding bulls and use artificial insemination.

Acknowledgments

The study and publication were financed by the project „Support for doctoral studies in LLU” No.2009/0180/1DP/1.1.2.1.2/09/IPIA/VIAA/017/ agreement No. 04.4-08/EF2.D1.13’.

The study was supported also by financing of the Research Project No. 09.1461 of the Latvian Science Council.

Bibliography

1. Aschaffenburg, R. and Drewry, J. (1957) Genetics of the b-lactoglobulins of cows' milk. *Nature*, 180: 376-378.
2. *Convenio 36-57 de 2006 suscrito entre la Universidad de Nariño y el Ministerio de Agricultura y Desarrollo Rural(2010) INFORME TÉCNICO A 30 DE JUNIO DE 2010. Programa integral de mejoramiento de la calidad composicional de la leche de Bos taurus de material genético selecto utilizando multiovulación, transferencia de embriones y marcadores moleculares.* (Technical Report June 30, 2010. Comprehensive program to improve the compositional quality of milk from *Bos Taurus* selected genetic material using multiovulation, embryo transfer and molecular markers). <http://promegalac.udenar.edu.co/wp-content/uploads/2010/08/Informe-de-Avance-Jun-30-10.pdf> [30 July 2010]: 1-20.
3. Czerniawska-Piatkowska, E., Kamieniecki, H., Pilarczyk, R., Rzewucka, E. (2004) A comparison of protein polymorphisms in milk produced by two dairy farms in West Pomerania. *Archiv Tierzucht, Dummerstorf*, 47-2: 155 – 163.
4. Eenennaam, A., Medrano, J.F. (1991) Milk protein polymorphisms in California dairy cattle. *Journal of Dairy Sciences*, 74: 1730 – 1742.
5. Falconer, D.S., Mackay, T. F. C. (1996). *Introduction to Quantitative genetics*. Edition 4th, Longmans Green, Harlow. Essex, UK.
6. Farrell, H.M., Jimenez-Flores, Jr.R., Bleck, G.T., Brown, E.M., Butler, J.E., Creamer, L.K. (2004) Nomenclature of the Proteins of Cows' Milk-Sixth Revision, *Journal of Dairy Sciences*, 87: 1641-1674.
7. Farrell, Jr, H.M., Jimenez-Flores, R., Bleck, G.T., Brown, E.M., Butler, J.E., Creamer, L. K., Hicks, C. L., Hollar, C.M., Ng-Kwai-Hang, F., and Swaisgood, H. E. (2004) Nomenclature of the proteins of cows' milk-sixth edition. *Journal of Dairy Sciences*, 87: 1641-1674.
8. Formaggioni, P., Summer, A., Malacarne, M., Mariani, P. (1999) Milk protein polymorphism: detection and diffusion of the genetic variants in *Bos* genus. In:*Annali della Facolta di Medicina Veterinaria Universita di Parma*, Volume XIX, Pp. 127 – 165; <http://www.unipr.it/arpa/facvet/annali/1999/formaggioni/formaggioni.htm> [16 June 2009]
9. Jann, O., Prinzenberg, E.-M. , Brandt H., Williams, J. L., Ajmone-Marsan, P., Zaragoza, P., Özbeyaz, C. and Erhardt, G. (2002) Intragenic haplotypes at the bovine *CSN1S1* locus. *Archiv Tierzucht, Dummerstorf*, 45, 1: 13-21.
10. Kawamoto, Y., Namikawa, T.A., Adachi, T., Amano, T., Shotake, T., Nishida, T. (1992) A population genetic study on yaks, cattle and their hybrids in Nepal using milk protein variations, *Animal Science and Technology*, 63: 563-575.
11. Koczan, D., Hobom, G., and Seyfert, H.-M. (1993), Characterization of the bovine α_{s1} -casein gene C-allele, based on a *Mae* III polymorphism. *Animal Genetics*, 24: 74.
12. Lin, C. Y., McAllister, A. J., Ng-Kwai-Hang, K. F., and Hayes, J. F. (1986) Effects of milk protein loci on first lactation production in dairy cattle. *Journal of Dairy Sciences*, 69-3: 704–712.
13. Mahé, M.F., Miranda, G., Queval, R., Bado, A., Zafindrajaona, P.S., Grosclaude, F. (1999) Genetic polymorphism of milk proteins in African *Bos taurus* and *Bos indicus* populations. Characterization of variants aS1-Cn H and k-Cn, *Journal in Genetics Selection Evolution*, 31: 239 – 253.
14. Martin, P., Szymanowska, M., Zwierzchowski, L., and Leroux, C. (2002) The impact of genetic polymorphisms on the protein composition of ruminants milks. *Reproduction Nutrition Development*, 42: 433 - 459.
15. Miller, M.P. (1997) *Tools for population genetic analyses (TFPGA) version 3.1: a Windows program for the analysis of allozyme and molecular population genetic data.* <http://www.marksgeneticssoftware.net/tfpga.htm> [1 February 2010].

16. Thompson, M.P., Kiddy, C.A., Pepper, L., Zittle, C.A. (1962) Variations in the α S1-casein fraction of individual cow's milk, *Nature*, 195: 1001– 1002.