EXCELLENT PUBLISHERS

International Journal of Current Research in Biosciences and Plant Biology

Volume **8** • Number **10** (October-2021) • ISSN: 2349-8080 (Online)

Journal homepage: www.ijcrbp.com

Original Research Article **doi:** https://doi.org/10.20546/ijcrbp.2021.810.005

Relationships of αs_1-casein (CSN_1S_1) and β-casein (CSN_2) genotypes and their association with milk quality and coagulation properties in Bulgarian Brown cattle

Teodora Angelova*, Jivko Krastanov, Daniela Yordanova

Agricultural Institute – Stara Zagora, Agricultural Academy, Stara Zagora 6000, Bulgaria

*Corresponding author; e-mail: teslacow@abv.bg

Article Info

Keywords:
Brown cattle
Coagulation ability
Milk quality
αs_1-casein genotype
β-casein genotype

Abstract

The aim of the present investigation was to establish the relationships of αs_1-casein (CSN_1S_1) and β-casein (CSN_2) genotypes in Bulgarian Brown cattle and to evaluate their association with milk quality and milk coagulation properties. Milk proteins' polymorphism was found out in 155 tissue samples from cows reared at 4 farms. Innovative protocols and techniques were used for determination of genetic polymorphism of αs_1-casein (CSN_1S_1) and β-casein (CSN_2) in Brown cattle. After collection, tissue samples were shipped to the University in Padova, Italy for PCR-RFLP analysis. The analysis of milk composition was done in the lab of the Agriculture Institute – Stara Zagora on Lactoscan ultrasound milk analyzer, whereas coagulation properties of individual milk samples were evaluated on a Computerized Renneting Metter – Polo Trade, Italy. During the study, the following parameters were studied: milk fat and protein contents (%), rennet coagulation time (RCT, min), curd firmness (a_{30}, mm) and curd firming time (k_{20}, min). Heterozygous cows for both studied milk proteins: CSN_1S_1 and CSN_2, had higher milk fat and milk protein percentages, 4.71% fat and 3.45% protein in genotype BC of CSN_1S_1 and 5.00% fat and 5.33% protein in genotype AB of CSN_2. The milk of cows from different CSN_1S_1 and CSN_2 genotypes was outlined with moderate curd firmness and good coagulation time. Homozygous cows for both studied milk proteins had higher curd firmness (32.31 mm) for the BB genotype of CSN_1S_1 and 31.29 mm for the AA genotype of CSN_2.

• *Received: 01 August 2021* • *Revised: 20 September 2021* • *Accepted: 25 September 2021* • *Published Online: 6 October 2021*

Introduction

The appearance of genome-wide analysis and the development of proteomics tools, the determination of milk proteins' polymorphism evidences a considerable progress with regard to the understanding of relationships between nucleotide polymorphisms and protein structure and expressions, and their effect on milk protein composition (Martin et al., 2013). Monitoring of variations of milk protein in the different

cattle breeds allows avoiding the increase in alleles with adverse effects on milk quality during cheese making (Caroli et al., 2000; Comin et al., 2008; Pärna et al., 2012).

Cipolat-Gotet et al., (2018) evaluated the effect of protein fractions of αS1-, αS2-, β- and κ-casein (CN), α-lactalbumin and β-lactoglobulin on the efficiency of cheese making in Swiss Brown cattle. A number of research teams (Rachagani and Gupta, 2008; Caroli et al., 2009; Mota et al., 2020) confirmed that genetic variants of milk proteins had a positive effect on milk production traits, milk cheese making properties and milk nutritional value. Some studies reported considerable differences between CSN_2 genotypes in different cattle breeds with regard to daily milk yield (Heck et al., 2009; Gurcan, 2011; Molee et al., 2015), milk protein content (Cardak, 2005; Micinski et al., 2006), milk fat content (Dogru, 1994; Cardak, 2005) by Ozdemir et al. (2018). Ikonen et al. (1999) outlined that the BB genotype of CSN_2 could be used as a tool in breeding schedules aimed at improvement of milk yield and milk fat content.

Comparative studies between Holstein and Brown cattle breeds were conducted by Ozdemir and Dogru (2004). Ozdemir et al. (2018) demonstrated that αs_1-Cn had a statistically significant effect on milk yield and milk butterfat in Holstein cattle, whereas no positive effect on these traits was detected in Brown cattle. Also, there was not a significant effect of genotypes CSN_1S_1 and CSN_2 on studied milk production traits /milk yield, milk fat and protein percentages.

Bittante et al., (2012) outlined the breed of cattle as one of main factors influencing milk coagulation properties. The milk produced by Swiss Brown cattle has very good coagulation properties compared to Holstein milk. The coagulation properties of Swiss Brown milk comprise shorted rennet coagulation time with higher curd firmness than the milk of Holstein cows (De Marchi et al., 2008; Dal Zotto et al., 2007; Penasa et al., 2014). Milk coagulation properties were also influenced by milk quality composition (Toffanin et al., 2012; Pretto et al., 2013).

The aim of the present investigation was to establish the relationships of αs_1-casein (CSN_1S_1) and β-casein (CSN_2) genotypes in Bulgarian Brown cattle and to evaluate their association with milk quality and milk coagulation properties.

Materials and methods

Animal and experimental design

Milk proteins' polymorphism was found out in 155 tissue samples from cows reared at 4 farms. Innovative protocols and techniques were used for determination of genetic polymorphism of αs_1-casein (CSN_1S_1) and β-casein (CSN_2) in Brown cattle. After collection, tissue samples were sent to the University of Padova for analysis. 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. After collection, tissue samples were shipped to the University in Padova, Italy for PCR-RFLP analysis.

Laboratory analysis of tissue samples

DNA was purified by means of a Maxwell®16 Tissue DNA purification kit (Promega) according to the manufacturer's instructions. DNA is precipitated with three volumes of cold 70% ethanol, 0.1M sodium acetate (pH 5.2) and 2 mL glycogen 2 mg/mL, the pellets are then washed twice in cold 70% ethanol and dissolved in 50 mL of dH_2O. The DNA concentration is determined on a Qubit® fluorimeter (Invitrogen). A 221 bp fragment of exon IV was amplified using the following primers:

Bub - F 5'- TGCCAAGCCCAGCCAACTACC-3'
Bub - R 5'- CGACGGTTGAAGTAACTTGGGCTG-3'

For PCR amplification, 40 ng DNA was used, 0.20 µM of each primer, 1x HF- buffer (Finnzymes), 0.2 mM dNTPs and 0.2 U Phusion-HF DNA polymerase (Finnzymes). The PCR analysis conditions comprised: initial denaturation at 98 °C for 30 sec, followed by 40 cycles at 98 °C for 7 sec, 64 °C for 15 sec and 72 °C for 20 sec, and a final extension at 72 °C for 7 min. PCR products were purified with Agencourt Purification AMPure System (Beckman Coulter) and sequencing was done with GenomeLab ™ DTCS Quick Start Kit for Dye Terminator Cycle Sequencing as per manufacturer's instructions. The Agencourt CleanSEQ purification system (Beckman Coulter) was employed for purification of sequencing products. The screening was performed by a Genetic CEQ8000 assay system (Beckman Coulter), and the single nucleotide polymorphism (SNP) determination with Beckman Coulter genetic analysis software v.9.00. The results from polymorphism analysis are presented in previous

publications of ours (Yordanova et al., 2014; Yordanova et al., 2017).

Laboratory analysis of milk samples

The analysis of milk composition was done in the lab of the Agriculture Institute – Stara Zagora on Lactoscan ultrasound milk analyzer, whereas coagulation properties of individual milk samples were evaluated on a Computerized Renneting Metter – Polo Trade, Italy. Milk samples were obtained by milk meters. The milk was analysed within 3 hours after sample collection. Naturen Plus 215 /0.8 L chymosin was used, with milk coagulation activity of 215 IMCU/mL. During the study, the following parameters were studied: milk fat and protein contents (%), rennet coagulation time (RCT, min), curd firmness (a_{30}, mm) and curd firming time (k_{20}, min).

Statistical analysis

To determine the effect on αs_1-casein (CSN_1S_1) and β-casein (CSN_2) genotypes on traits, characterization of chemical composition and coagulation ability of milk, the following was applied after the last mixed linear model:

$Y_{ijklmnop} = HYM_i + Farm_j + PL_k + Testdim_l + Sire_m + CSN_1S_{1n} + CSN_{2o}$ + eijкlmnop where:
Yijklmnop – p-th corresponding observation of a trait;
HYM_i – fixed effect on the i $^{-th}$ herd - year - month of milk sampling;
$Farm_j$ – fixed effect of the j -th farm;
PL_k – fixed effect of the k -th parity;
$Testdim_l$ – random regression effect of the lactation days to the respective control day of the respective lactation of the animal;
$Sire_m$ – random effect of the sire;
CSN_1S_{1n} - fixed effect of the n-th genotype of CSN_1S_1;
CSN_{2o} – fixed effect of the o-th genotype of CSN_2;
eijklmnop – random effect of unobserved factors;
The data were processed with statistical software products PEST (Groeneveld et al., 1990), SYSTAT 13 and graphs were plotted in MS Excel.

Results and discussion

Fig. 1 depicts the average milk fat and protein percentages in cows with different CSN_1S_1 genotypes. Milk fat percentage was higher that milk protein for both genotypes as followed: for genotype BB – 4.43% fat and 3.21% protein vs 4.71% fat and 3.45 protein for genotype BC. The significantly higher milk fat and protein contents of cows from genotype BC of CSN_1S_1 should be emphasized. According to Ozdemir and Dogru (2004; 2018) αs_1-Cn has a substantial effect on milk yield and milk butterfat percentage in Holstein cows, whereas such a positive effect in Swiss Brown cows was absent.

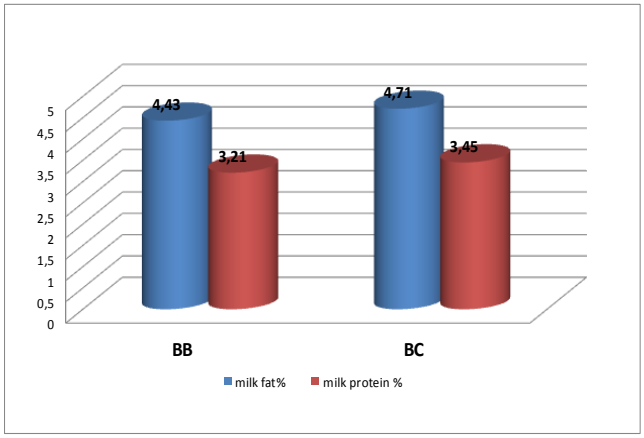

Fig. 1: Average milk fat and milk protein percentages in cows from different CSN_1S_1 genotypes.

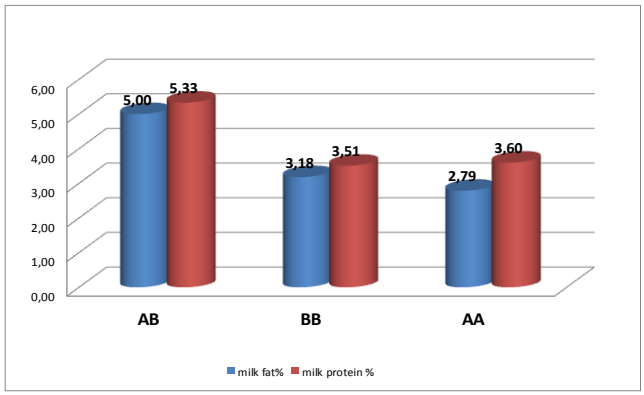

Fig. 2: Average milk fat and milk protein percentages in cows from different CSN_2 genotypes.

Fig. 2 shows an interesting fact – slight variation in average milk fat and protein percentages in the two homozygous CSN_2 genotypes (AA and BB): for genotype AA – 2.79% milk fat and 3.60% milk protein and for genotype BB – 3.18 fat and 3.51 protein content. The results demonstrated that animals from the AB genotype produced milk with the highest milk fat and protein percentage, respectively. The three CSN_2

genotypes were characterized with higher protein content in milk than milk fat percentage. Kevin Forde /Editor of THAT'S FARMING/ affirmed that the Swiss Brown cattle was the breed producing milk of excellent quality, used mainly for production of cheese. It contained high fat (4.17%) and protein (3.5%) concentrations. The milk of this breed was unique, differing from the milk of other breeds by several parameters: higher content of long-chain fatty acids; the closest fat/protein ratio; lower size of fat globules. Mistry et al. (2002) reported that the milk produced by recently calved Swiss Brown and Holstein cows was Pasteurized and used for production of Cheddar. The milk of Holsteins has an average fat content of 4.06%, protein 3.01% and dry matter of 12.23%. The respective values for Brown cattle are 4.15%, 3.36% and 13.11%.

Fig. 3 presents the average values of milk coagulation properties, namely RCT – rennet coagulation time, A_{30} – curd firmness and K_{20} – curd firming time in Brown cattle. Shorter coagulation time of 15.46 min was observed in cows from the BC genotype. Curd firmness and curd firming time for this genotype were respectively 29.81 mm and 1.61 min. The other genotype (BB) was characterized with longer RCT of 17.81 min, higher curd firmness (32.31 mm) and longer curd firming time (2.69 min).

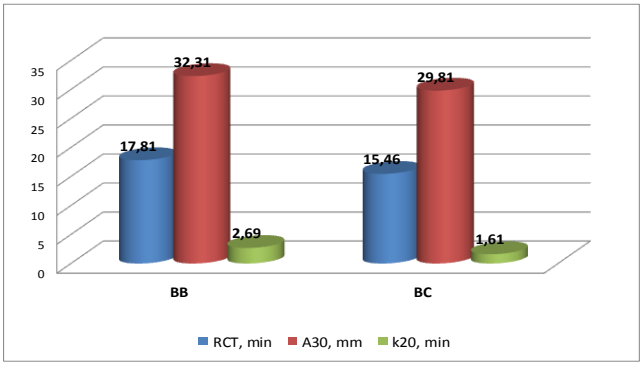

Fig. 3: Rennet coagulation time, curd firmness and curd firming time in the milk of cows from different CSN_1S_1 genotypes.

The milk of Swiss Brown cattle forms a curd 15 minutes earlier than Holstein milk, and the coagulum is firmer (Mistry et al., 2002). The authors reported that addition of $CaCl_2$ is necessary in order to decrease rennet coagulation time and to obtain a firmer coagulum from Holstein milk. One of primary factors influencing coagulation properties of milk, is breed (Bittante et al., 2012). The milk of Swiss Brown cattle has very good coagulation properties than that of Holstein breed. The milk of the former breed is outlined with shorter coagulation time and increased curd firmness (Dal Zotto et al., 2007; De Marchi et al., 2008; Penasa et al., 2014).

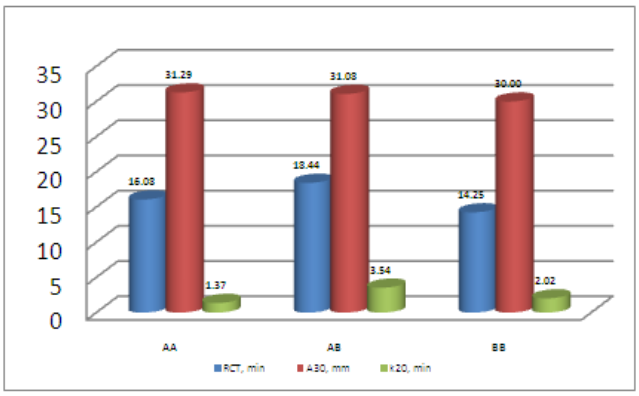

Fig. 4: Rennet coagulation time, curd firmness and curd firming time in the milk of cows from different CSN2 genotypes.

The milk coagulation traits in the three CSN_2 genotypes varied within moderate ranges. Obtained average values were as followed: for genotype AA RCT – 16.08 min, A30 – 31.29 mm, K_{20} – 1.37 min, for genotype AB RCT – 18.44 min, A_{30} – 31.08 mm, K_{20} – 3.54 min. The genotype BB was characterized with shortest coagulation time (RCT 14.25 min), A_{30} – 30.00 mm, K_{20} – 2.02 min. In general, the milk of cows from different CSN_2 genotypes formed a firm curd.

Conclusions

1. Heterozygous cows for both studied milk proteins: CSN_1S_1 and CSN_2, had higher milk fat and milk protein percentages – 4.71% fat and 3.45% protein in genotype BC of CSN_1S_1 and 5.00% fat and 5.33 protein in genotype AB of CSN_2.
2. The milk of cows from different CSN_1S_1 and CSN_2 genotypes was outlined with moderate curd firmness and good coagulation time.
3. Homozygous cows for both studied milk proteins had higher curd firmness (32.31 mm) for the BB genotype of CSN_1S_1 and 31.29 mm for the AA genotype of CSN_2.

Conflict of interest statement

Authors declare that they have no conflict of interest.

Acknowledgement

The study was performed with close co-operation of renowned researchers from the University of Padova, Italy, and funded by scientific project of the Ministry of Education and Science DO-02-249 with leader Prof. Jivko Krastanov.

References

Bittante, G., Penasa, M., Cecchinato, A., 2012. Invited review: Genetics and modeling of milk coagulation properties. J. Dairy Sci., 95: 6843–6870.

Cardak, A. D., 2005. Effects of genetic variants in milk protein on yield and composition of milk from Holstein-Friesian and Simmentale cows. S. Afr. J. Anim. Sci., 35: 41–47.

Caroli, A., Bolla, P., Budelli, E., Barbieri, G., Leone, P., 2000. Effect of k-casein E allele on clotting aptitude of Italian Friesian milk. Zootec. Nutr. Anim. 26: 127-130.

Caroli, A.M., Chessa, S., Erhardt, G. J., 2009. Invited review: Milk protein polymorphisms in cattle: Effect on animal breeding and human nutrition. J. Dairy Sci., 92: 5335-5352.

Cipolat-Gotet, C., Cecchinato, A., Malacarne, M., Bittante, G., Summer, A., 2018. Variations in milk protein fractions affect the efficiency of the cheese-making process. J. Dairy Sci., 101(10): 8788-8804.

Comin, A., Cassandro, M., Chessa, S., Ojala, M., Dal Zotto, R., De Marchi, M., Carnier, P., Gallo, L., Pagnacco, G., Bittante, G., 2008. Effects of composite β- and κ-casein genotypes on milk coagulation, quality, and yield traits in Italian Holstein cows. J. Dairy Sci., 91: 4022-4027.

Dal Zotto, R., De Marchi, M., Dalvit, C., Cassandro, M., Gallo, L., Carnier, P., Bittante, G., 2007. Heritabilities and genetic correlations of body conformation score and calving interval with yield, somatic cell score, and linear type traits in Brown Swiss cattle. J. Dairy Sci., 90: 5737-5743.

De Marchi, M., Bittante, G., Dal Zotto, R., Dalvit, C., Cassandro, M., 2008. Effect of Holstein Friesian and Brown Swiss breeds on quality of milk and cheese. J. Dairy Sci., 91: 4092-4102.

Dogru, U., 1994. Genetic structure of several polymorphic blood and milk proteins of Brown Swiss, Holstein, Simmental and Eastern Anotolian Red cattle breeds and their differences according to several production traits. PhD Thesis, Ataturk University, Erzurum, Turkey.

Gurcan, E. K., 2011. Association between milk protein polymorphism and milk production traits in black and white dairy cattle in Turkey. Afr. J. Biotech., 10: 1044–1048.

Heck, J. M. L., Schennink, A., Valenberg, H. J. F., Bovenhuis, H., Visker, M. H., Arendonk, J. A. M., Hooijdonk, A. C. M., 2009. Effects of milk protein variants on the protein composition of bovine milk. J. Dairy Sci., 92: 1192–1202.

Ikonen, T., Ojala, M., Ruottinen, O., 1999. Associations between milk protein polymorphism and first lactation milk production traits in Finnish Ayrshire cows. J. Dairy Sci., 82: 1026–1033.

Martin, P., Bianchi, L., Cebo, C., Miranda, G., 2013. Genetic polymorphism of milk proteins. In: Advanced Dairy Chemistry, Vol. 1A: Proteins: Basic Aspects, pp.463-514.

Micinski, J., Klupczynski, J., 2006. Correlations between polymorphic variants of milk proteins, and milk yield and chemical composition in black-and-white and Jersey cows. Pol. J. Food. Nutr. Sci., 15: 137–143.

Mistry, V.V., Brouk, M.J., Kasperson, K.M., Martin, E., 2002. Cheddar cheese from milk of Holstein and Brown Swiss cows. Milchwissenschaft, 57: 19-23.

Molee, A., Mernkrathoke, P., Poompramun, C., 2015. Effect of casein genes-beta-LGB, DGAT1. GH, and LHR-on milk production and milk composition traits in crossbred Holsteins. Genet. Mol. Res., 14: 2561–2571.

Mota, M., L. F., Pegolo, S., Bisutti, V., Bittante, G., Cecchinato, A., 2020. Genomic analysis of milk protein fractions in Brown Swiss cattle. Animals (Basel), 10(2): 336.

Ozdemir, M., Dogru, U., 2004. Relationships between alfa-casein polymorphism and production traits in Brown Swiss and Holstein. J. Appl. Anim. Res., 25: 5–8.

Ozdemir, M., Kopuzlu, S., Topal, M., Bilgin, O. C., 2018. Relationships between milk protein polymorphisms and production traits in cattle: a systematic review and meta-analysis, Arch. Anim. Breed., 61: 197–206.

Pärna, E., Kaart, T., Kiiman, H., Bulitko, T., Viinalass, H., 2012. Milk Protein Genotype Associations with Milk Coagulation and Quality Traits. Open access peer-reviewed chapter In Book: Milk Production - Advanced Genetic Traits, Cellular Mechanism, Animal Management and Health. DOI: 10.5772/29123.

Penasa, M., Tiezzi, F., Sturaro, A., Cassandro, M., De

Marchi, M., 2014. A comparison of the predicted coagulation characteristics and composition of milk from multi-breed herds of Holstein-Friesian, Brown Swiss and Simmental cows. Int. Dairy J., 35: 6–10.

Pretto, D., De Marchi, M., Penasa, M., Cassandro, M., 2013. Effect of milk composition and coagulation traits on Grana Padano cheese yield under field conditions. J. Dairy Res., 80: 1-5.

Rachagani, S., Ishwar, D., 2008. Bovine kappa-casein gene polymorphism and its association with milk production traits. Genet. Mol. Biol., 31: 893-897

Toffanin, V., Marchi, M., Penasa, M., Pretto, D., Cassandro, M., 2012. Characterization of Milk Coagulation Ability in Bulk Milk Samples. 20th Int. Symp. "Animal Science Days", Kranjska Gora, Slovenia, Sept. 19th−21st, 2012.

Yordanova, D., Angelova, T., Karabashev, V., Kalaydhziev, G., Laleva, S., Cassandro, M., Maretto, F., Krastanov, J., Popova, Y., Oblakov, N., 2014. Polymorphism and allelic frequency of CSN_1S_1 in different cattle breeds in Bulgaria. Acta Agraria Kaposváriensis, 18(Supl. 1): 35-39.

Yordanova, D., Angelova, T., Krastanov, Zh., Kalaydzhiev, G., Karabashev, V., Laleva, S., Oblakov, N., Cassandro, M., 2017. Polymorphism and allele frequencies of beta-casein (CSN_2) in different Bulgarian cattle breeds. Anim. Sci. (Sofia), LIV: 29-33.

How to cite this article:
Angelova, T., Krastanov, J., Yordanova, D., 2021. Relationships of αs1-casein (CSN_1S_1) and β-casein (CSN_2) genotypes and their association with milk quality and coagulation properties in Bulgarian Brown cattle. Int. J. Curr. Res. Biosci. Plant Biol., 8(10): 28-33. **doi:** https://doi.org/10.20546/ijcrbp.2021.810.005