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Allele frequencies and genotypes of kappa casein (CSN_3) and their association with chemical composition and coagulation properties of milk in Brown cattle

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Abstract. *The aim of the present study was to evaluate allele frequencies and genotypes of kappa casein (CSN_3) and their association with milk quality and coagulation properties in Brown cattle. Milk proteins' polymorphism was found out in 155 tissue samples from cows reared at 4 farms. 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 215/0.8L 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). Kappa casein (CSN_3) is characterised by five genotypes – AA, AB, BB, AH and BH, the frequency of which varied within various ranges. The milk of cows with genotype AB was characterized by the highest content of fat and protein: 4.85% and 5.00%, respectively. The milk of heterozygous cows from genotype AB demonstrated the longest rennet coagulation time – 18.04 min. The animals carrying the H allele produced milk with the highest curd firmness – 37.00 mm.*

Keywords: allele frequencies, Brown cattle, coagulation ability, CSN_3, genotypes

Introduction

The Brown cattle, the second most high-producing after the Holstein at a global scale, is also characterized by excellent quality of milk. Along with other benefits of the breed, superior cheesemaking properties of milk is among its most valuable characteristics (Zanon et al., 2020). Sulimova et al. (2007) outlined that alleles A and B of CSN_3 were of practical significance, as allele B was associated with commercially valuable milk parameters (milk protein content and milk yield), as well as with improved profitability from cheese production. Rachagani and Gupta (2008), Caroli et al. (2009) and Mota et al. (2020) also confirmed that genetic variants of milk proteins had a beneficial effect on milk production traits, cheesemaking properties and milk nutritional value. Franzoi et al. (2019) indicated significant differences of protein fractions in milk of the studied dairy and dual-purpose cattle breeds.

Kappa casein is of particular interest for milk protein polymorphism due to its proven relationship with milk quality and composition (Özdemir and Doğru, 2005). CSN_3 is approximately 12% of total milk casein content (Azevedo et al., 2008) and is associated with higher milk protein content and solids non-fat in Holstein and Brown cattle milk (Gurses et al., 2016).

The frequency of the B allele of CSN_3 in the different cattle breeds varies from 0.06 to 0.57 (Ron et al., 1994; Golijow et al., 1999; Sulimova et al., 2007). Another study reported the highest frequency of this allele in Brown Swiss and Jersey cattle breeds – 0.67 and 0.86, respectively (Lein et al., 1999). Ilie et al. (2009) also affirmed that in Brown cattle, the B allele of CSN_3 was the most prevalent, whereas among Simmental cattle, the frequency of genotype AA exceeded that of BB, while the latter genotype of CSN_3 correlated positively with milk protein content in both breeds (Petrovska et al., 2017).

The milk produced by animals offers a perfect balance between yield and quality. The most recent data of ICAR from 2019 about most European countries demonstrate milk yield over 7000 kg, with fat content about 4% and high protein content – about 3.5 to 3.8% (ICAR, 2019). The milk of this breed is appropriate for production of high-quality dairy products, especially cheeses. The fat-to-protein ratio of 1.22 places milk of Brown Swiss cattle in the leading three cattle breeds in France, suitable for production of durable dairy products after Normande (1.23) and Jersey (1.45) cattle breeds (The European Brown Swiss Journal, 2020).

The aim of the present study was to evaluate allele frequencies and genotypes of kappa casein (CSN3) and their association with chemical composition and coagulation properties of milk in Brown cattle.

Material and metods

Animals and experimental design

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The analysis comprised 155 animals (Brown cattle) reared in 4 herds from different regions of the country, regardless of lactation number and days in lactation. Records of animals' origin were obtained from pedigree books. Farm 1 – Stara Zagora region: Cows were housed in free stalls, with individual resting boxes. Feeding was based on corn silage and compound feed, milking was performed twice daily in a herringbone milking parlour. After milking, cows were let to walk in fenced pastures.

Farms 2, 3 and 4 – Sliven region, Sliven municipality: Cows were reared in tie-stalls. Feeding was based on corn silage, hay and compound feed, and milking was performed twice, through a central milking pipeline. After milking, cows were let to walk in fenced pastures.

Milk protein polymorphism was found out in 155 tissue samples from cows reared at the above mentioned 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.

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 dH2O. 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 seconds, followed by 40 cycles at 98°C for 7 seconds, 64°C for 15 s and 72°C for 20 seconds, and a final extension at 72°C for 7 minutes. 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.

Laboratory analysis of milk samples

The analysis of milk composition was done in the lab of the Agricultural 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 215/0.8L 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

The data were processed with statistical software products SYSTAT 13 by Hypothesis Testing: Paired t-test and graphs were plotted in MS EXCEL.

Results and discussion

Figure 1 presents data about allele frequencies of kappa casein (CSN_3) in Brown cattle from a previous study of ours (Angelova et al., 2014). As seen from the figure, kappa casein (CSN_3) was described by three alleles: A, B and H, with predominance of allele B – 0.598. The presence of allele H though at a very low frequency, is interesting – 0.013. Thus, it determined other genotypes, which are not encountered in the results of scientists having investigated the milk polymorphism in Brown cattle breed.

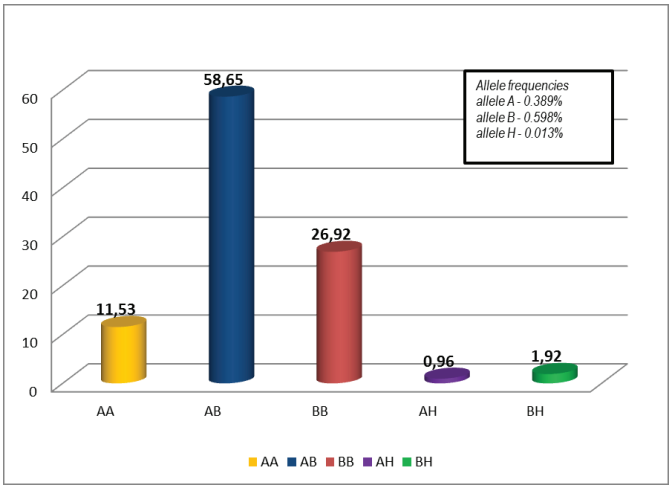

Figure 1. CSN_3 allele frequencies and genotypes

Established bovine genotypes with respect to kappa casein – CSN_3 demonstrated that the main proportion of animals were from the heterozygous AB genotype – 58.65%, followed by homozygous BB – 26.92%. As already mentioned, an H allele was detected, determining the presence of heterozygous AH and VH genotype with respective frequencies 0.96 and 1.92%. Cows from the AA genotype in this study were 11.53%.

The study of Dogru and Ozdemir (2009) with 93 blood samples from Brown Swiss cattle found out 3 genotypes of kappa casein. Allele frequencies of alleles A and B were 0.495 and 0.505, respectively, whereas the frequency of AA genotype was 0.1935, that of BB – 0.2043 and that of AB: 0.6022. Zepeda-Batista et al. (2017) have reported the same three genotypes – AA, AB and BB.

Table 1 shows that there is a highly reliable difference in the characteristics of fatty substances between different genotypes - AA and BB, AB and BB. The reliability of the differences with regard to the trait content of protein substances is not taken

into account, except for the genotypes BB and BH, in which a highly reliable difference has been established (p<0.001).

Figure 2 shows the average milk fat and protein contents in cows from different CSN_3 genotypes. The milk from heterozygous AB and AH cows was outlined with the highest fat percentage – 4.85% and 4.81%, respectively. Homozygous cows produced milk with the lowest fat content. It could be noted that three of the kappa casein genotypes: AH, BH and BB, had the same average milk protein content of 3.50%. Milk fat content of cows from genotype AA was 3.60%, whereas that of genotype AB was rather higher (5.00%). The fat percentage is a parameter varying within a broader range than milk protein. The animals carrying the AB genotype produced milk with the highest fat and protein contents – 4.85% and 5.00%, respectively.

Table 1. Statistical significance between genotypes of kappa casein (CSN_3) and chemical composition of milk in Brown cattle

Genotype	Variable					
	Fat, %			Protein, %		
	SD of Difference	t	p-Value	SD of Difference	t	p-Value
AA AB	1.199	-3.333	0.002**	0.316	1.388	0.173
AA AH	1.132	-2.458	0.023*	0.363	-0.788	0.440
AB AH	0.999	-0.681	0.504	0.414	-0.798	0.434
AA BB	1.334	-3.571	0.001***	0.335	1.820	0.077
AB BB	1.142	-4.458	0.000***	0.390	-0.783	0.434
AH BB	0.930	0.644	0.527	0.278	1.787	0.088
AA BH	0.877	-3.209	0.003**	0.401	-1.215	0.234
AB BH	1.027	-0.990	0.330	0.350	-2.574	0.015*
AH BH	0.944	0.377	0.710	0.353	-1.697	0.104
BB BH	0.886	-0.534	0.598	0.263	-3.626	0.001***

*p< 0.05; **p< 0.01; ***p< 0.001

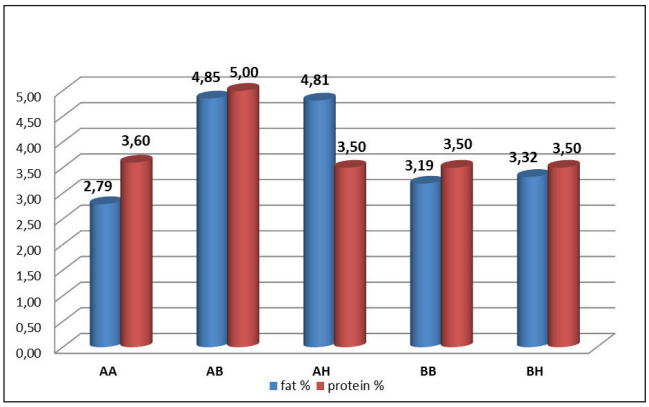

Figure 2. Average milk fat and protein content in cows from different CSN_3 genotypes

Table 2 shows that there is a highly reliable difference in the rennet coagulation time and the curd firmness between the different genotypes - AB and BB, as well as between BB and BH. Significance of differences in coagulation tightening time was not reported, except between genotypes AA and BH, and BB and BH, where a significant difference was found (p<0.05).

There were substantial variations in milk coagulation traits in the five CSN_3 genotypes (Figure 3). The milk of cows from genotype AH exhibited the shortest rennet coagulation time (8.19 min) and the highest curd firmness (37.00 mm), whereas k_{20} of this genotype was 1.01 min. Three of the genotypes had similar milk coagulation properties, as follows – genotype BH: RCT-13.74 min, a_{30} – 37.00 mm, k_{20} – 0.97 min, BB: RCT-14.43 min, a_{30} – 30.07 mm, k_{20} – 1.47 min and genotype AA: RCT – 14.93 min, a_{30} – 34.25 mm, k_{20} – 2.20 min. Cows from the AB genotype produced milk with the longest RCT of 18.04 min, average curd firmness 32.00 mm, and curd firming time 2.99 min. Cows carrying the H allele were characterized by milk with the highest curd firmness – 37.00 mm. The longest RCT was found out in heterozygous AB cows – 18.04 min. Losi et al. (1973) found out that the milk of cows from the BB kappa casein genotype reacted faster to addition of chymosin and that higher coagulation time was considerably shorter than that of cows from the AA genotype, while the values of RCT in the milk of heterozygous cows were intermediate.

Table 2. Statistical significance between genotypes of kappa casein (CSN_3) and coagulation properties of milk in Brown cattle

Genotype	Variable								
	RCT, min			A30, mm			K20, min		
	SD of Difference	t	p-Value	SD of Difference	t	p-Value	SD of Difference	t	p-Value
AA AB	9.853	-0.746	0.461	17.685	0.945	0.351	1.577	1.616	0.115
AA AH	9.855	-0.585	0.565	17.622	1.379	0.182	1.491	1.907	0.070
AB AH	9.771	-1.530	0.141	17.537	2.225	0.037*	1.206	1.566	0.132
AA BB	7.289	2.108	0.042*	14.972	-2.904	0.006**	1.657	0.965	0.341
AB BB	9.671	5.256	0.000***	17.629	-5.077	0.000***	1.380	-0.519	0.604
AH BB	11.063	1.486	0.152	18.880	-3.422	0,003**	1.005	-1.644	0.115
AA BH	7.320	-3.571	0.001***	14.158	3.172	0.004**	1.061	2.485	0.019*
AB BH	8.349	-2.472	0.020*	16.621	2.021	0.053	1.007	1.017	0.317
AH BH	12.023	-0.839	0.411	20.100	-0.032	0.975	1.128	-0.527	0.603
BB BH	8.373	-5.154	0.000***	16.880	5.235	0.000***	1.400	2.047	0.050*

*p<0.05; **p<0.01; ***p<0.001

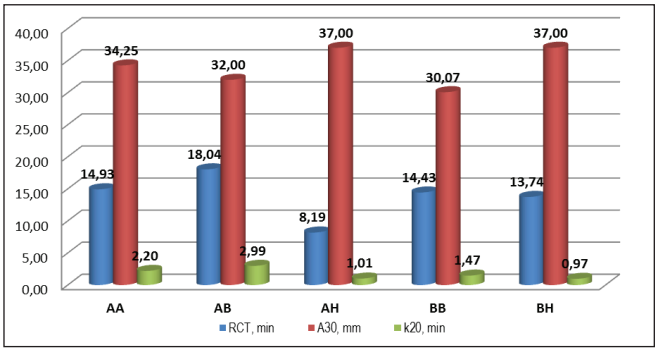

Figure 3. Rennet coagulation time, curd firmness and curd firming time in milk of cows from different CSN_3 genotypes

Malacarne et al. (2006) commented the results from a study aimed at comparison of the proximate analysis, distribution of main milk protein fractions, milk coagulation properties and yield in production of Parmigiano-Reggiano cheese from the milk of Italian Brown and Holstein herds. The animals included in this study were housed in farms with similar management, size, location, number of lactation and days in lactation. The milk of Italian Brown cattle was characterised with higher casein content than milk of Holstein cows (27.1 g/kg and 23.7 g/kg, respectively). Curd firming time of milk from Italian Brown cows was considerably shorter, presuming a higher extent of casein micelles aggregation. The coagulum of milk from Italian Brown cows showed better rheological properties and lower fat loss in cheese. The yield of Parmigiano-Reggiano cheese was higher by +0.99 kg cheese per 100 kg milk.

Researchers from Parma affirmed that the Brown breed was superior to Holsteins by 16% regarding the yield of cheese per 1 kg milk. This parameter is very important for dairy sector economy in countries like Bulgaria, where more than 75% of milk is processed into cheeses. The primary reason for the superiority of the Brown cattle breed is milk composition and milk protein quality, as the very favourable protein/fat ratio of milk. The real cause is that 78% of milk protein in this breed is casein – the most important protein for good technological properties of raw milk during its processing into cheese. Casein content in the Brown cattle breed is 26.2 g/kg on the average vs standard value of 22.5 g/kg. Casein is outlined with a certain diversity, kappa casein being the most important for coagulation (12.67% in the milk of Brown cattle; vs standard of 11.25%) (brownswiss.org.nz).

Conclusion

Based on the results obtained, it was found that: (i) kappa casein (CSN_3) is characterized by five genotypes – AA, AB, BB, AH and BH, the frequency of which varied within various ranges; (ii) the differences between the BB and BH genotypes are highly reliable in the studied traits, with the exception of the percentage of fatty and curd firming time; (iii) the milk of cows with genotype AB was outlined with the highest content of fat and protein: 4.85% and 5.00%, respectively; (iv) the milk of heterozygous cows from genotype AB demonstrated the longest rennet coagulation time – 18.04 min; (v) the animals

carrying the H allele produced milk with the highest curd firmness – 37.00 mm.

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References

Angelova T, Yordanova D, Karabashev V, Kalaydhziev G, Laleva L, Cassandro M, Krastanov J, Popova Y and Oblakov N, 2014. Polymorphism and allelic frequency of CSN_3 in other widely used and indigenous breeds of cattle raised in Bulgaria. 21 Rencontres Recherches Ruminants, Paris, p. 266.

Azevedo ALS, Nascimento CS, Steinberg RS, Carvalho MRS, Peixoto MGCD, Teodoro RL, Verneque RS, Guimarães SEF and Machado MA, 2008. Genetic polymorphism of the kappa-casein gene in Brazilian cattle. Genetics and Molecular Research, 7, 623-630.

Brownswiss.org.nz/downloads/news/Cheese%20protein%20power.pdf.

Caroli AM, Chessa S and Erhardt GJ, 2009. Invited review: Milk protein polymorphisms in cattle: Effect on animal breeding and human nutrition. Journal of Dairy Science, 92, 5335-5352.

Dogru U and Ozdemir M, 2009. Genotyping of Kappa-Casein Locus by PCR-RFLP in Brown Swiss Cattle Breed. Journal of Animal and Veterinary Advances, 8, 779-781.

Franzoi M, Niero G, Visentin G, Penasa M, Cassandro M and De Marchi M, 2019. Variation of detailed protein composition of cow milk predicted from a large database of mid-infrared spectra. Animals, 9, 176. doi: 10.3390/ani9040176

Golijow CD, Giovambattista G, Poli M, Dulout FN and Lojo MM, 1996**.** Kappa-casein gene frequencies support subdivision and historical origin of Argentine Creole cattle. Brazilian Journal of Genetics 19, 583-586.

Gurses A, Yuce H, Etem EO and Patir B, 2016. Polymorphisms of Kappa-Casein Gene and their effects on milk production traits in Holstein, Jersey and Brown Swiss Cattle. Animal Production Science, 58, 778-784.

Ilie DE, Magdin A, Sălăjeanu A, Neamt R and Vintilă I, 2009. Influence of CSN_3 marker on milk composition in Romanian Brown and Romanian Simmental cattle from S.C.D.C.B. Arad. Scientific Papers Anim. Husbandry and Biotech., Timisoara, Romania, 42, 54-57.

The International Committee for Animal Recording (ICAR), 2019. www.icar.org.

Lein S, Kantanen J, Olsaker I, Holm LE, Eythorsdottir E, Sandberg K, Dalsgard B and Adalsteinsson SA, 1999. Comparison of milk protein allele frequencies in Nordic cattle breeds. Animal Genetics, 30, 85-91.

Losi G, Castagnetti GB, Grazia L, Zambonetti C, Mariani P and Russo V, 1973. Influenza delle varianti genetiche della caseina κ sulla formazione e sulle caratteristiche della cagliata. Sci. Technol. Alim., 3, 373-374.

Malacarne M, Summer A, Fossa E, Formaggioni P, Franceschi P, Pecorari M and Mariani P, 2006. Composition, coagulation properties and Parmigiano-Reggiano cheese yield of Italian Brown and Italian Friesian herd milks. Journal of Dairy Research, 73, 171-177.

Mota M, Pegolo LF, Bisutti V, Bittante G and Cecchinato A, 2020. Genomic Analysis of Milk Protein Fractions in Brown Swiss Cattle. Animals (Basel), 10, 336. doi: 10.3390/ani10020336.

Özdemir M and Doğru Ü, 2005. Relationship between kappa-casein polymorphism and production traits in Brown Swiss and Holstein. Journal of Applied Animal Research, 27, 101-104.

Petrovska S, **Jonkus D**, **Zagorska J and Ciprovica I**, 2017. The influence of kappa-casein and beta-lactoglobulin genotypes on milk coagulation properties in Latvia dairy breed. Research for rural development, 2, 74-80. doi:10.22616/rrd.23.2017.052.

Rachagani S and Ishwar G, 2008. Bovine kappa-casein gene polymorphism and its association with milk production traits. Genetics and Molecular Biology, 31, 893-897. doi:10.1590/S1415-47572008005000001.

Robitaille G, Britten M, Morisset J and Petitclerc D, 2002. Quantitative analysis of beta-lactoglobulin A and B genetic variants in milk of cows beta-lactoglobulin AB throughout lactation. Journal of Dairy Research, 69, 651-654.

Ron M, Yofee E, Ezra E, Medrano JF and Weller JI, 1994. Determination of effects of milk protein genotype on production traits of Israeli Holsteins. Journal of Dairy Science, 77, 1106-1113.

Sulimova GE, Abani Azari M, Rostamzadeh J, Mohammadabadi M and Lazebnyĭ OE, 2007. Allelic polymorphism of kappa-casein gene (CSN_3) in Russian cattle breeds and its informative value as a genetic marker. Genetika, 43, 88-95.

The European Brown Swiss journal, 2020. https://media.wix.com/ugd/8225cc_a2ec9bf4660e4d93ab822d167980a242.pdf

Zepeda-Batista JL, A.Saavedra-Jiménez L, Ruíz-Flores A, Domínguez R and Ramírez-Valverde R, 2017. Potential influence of κ-casein and β-lactoglobulin genes in genetic association studies of milk quality traits. Asian-Australasian Journal of Animal Science, 30, 1684-1688.

Zanon T, Costa A, De Marchi M, Penasa M, Koenig S and Gauly M, 2020. Milk yield and quality of Original Brown cattle reared in Italian alpine region. Italian Journal of Animal Science, 19, 1158-1170. doi: 10.1080/1828051X.2020.1825997