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Kaposvár University, Faculty of Animal Science, Kaposvár

Molecular characterization of k-casein allelic variants in Bulgarian buffalo breed

B. Beneduci[1], I. Krastanov[2], F. Maretto[1], N. Oblakov[3] , M. Cassandro[1]

[1]Department of Animal Science, University of Padova, 35020 Legnaro (PD), Agripolis - viale dell'Università 16., Italy
[2]Department of Cattle Breeding and Technologies, Agricultural Institute, Stara Zagora, Bulgaria
[3]Free Researcher Consultant, Sofia, Bulgaria

ABSTRACT

This study aimed to analyze the genetic polymorphism at the k-CN (CSN3) locus in the Murrah Bulgarian buffalo breed. A 221 bp fragment of exon IV of CSN3 gene was amplified and sequenced to detect polymorphisms in 215 animals. Genotyping, by means of sequence alignments, revealed the presence of one polymorphism at codon 135 that occurs with a substitution of a Thr (ACC) of the A genetic variant for an Ile (ATC) of the B genetic variant, while codon 148 resulted monomorphic for the presence of an Ala residue (GCT). Additionally a silent mutation Thr-Thr (ACC/ACT) occurs at 136 codon position. Genotypes frequencies at codon 135 were 0.56 (AA), 0.40 (AB) and 0.04 (BB) being the gene frequencies 0.76 and 0.24 for the A and B allele respectively. No significant deviation from Hardy-Weinberg Equilibrium was observed.
(Keywords: k-casein, genotyping, Murrah Bulgarian buffalo)

INTRODUCTION

Caseins are a family of milk proteins that approximately consitute 80% of total lactoproteins (*Otaviano et al*., 2005). They are largely present under micelle form, which consists of four phosphorylated fractions: αs1, αs2, β, k-casein. Post-translational modifications together with the presence of genetic variants are the factors which determine casein heterogeneity (*Ferranti et al.,* 1998). Casein genes have been studied intensively because, in domestic animals, their polymorphism has been associated to difference in milk composition, processing, quality and also yield characteristics.

Genotyping of milk proteins is extremely important for selection practice to improve industrial milk production. To date nine variants have been found in cattle for k-CN gene: A, B, C, E, F, G, H, A1 and J (*Dogru et al.,* 2009). A and B variants are the most common in cosmopolitan and local cattle breeds (*Chessa et al.,* 2007; *Pacini et al.,* 2008). The variant B has a significant direct effect on protein content, and on milk yield (*Ganagaraj et al.,* 2008): the milk from BB genotypes results in shorter rennet coagulation time, formation of a firmer curd and a greater cheese yield than milk from AA genotype (*Otaviano et al.,* 2005; *Comin et al.,* 2008). The k-CN A allele has a threonine (ACC) aminoacid at position 136 and an aspartic acid (GAT) at position 148 (*Farrell et al.,* 2004; *Chianese et al.,* 2009). In the k-CN B variant isoleucine (ATC) and alanine (GCT) substitute the aforementioned threonine and aspartic acid residues (GenBank: CAP12622.1). The aim of this work was to genetically characterize the k-CN gene in Murrah Bulgarian buffalo breed in order to investigate the different allelic variants present in the population.

MATERIALS AND METHODS

Ear-punch samples were collected from 215 Bulgarian Murrah buffaloes. DNA was purified using Maxwell16® station and the Maxwell16®Tissue DNA Purification Kit (Promega). DNA was precipitated o/n with three volumes of 70% cold EtOH, 0.1 M sodium acetate (pH 5.2) and 2 µl of glycogen 2 mg/ml, pellet was then washed twice in 70% cold EtOH and resuspended in 50 µl of ddH_2O. DNA concentration was estimated using Qubit® fluorometer (Invitrogen). A 221 bp fragment of exon IV of the CSN3 gene was amplified using primers: Bub-CSN3-f 5'-TGCCAAGCCCAGCCAACTACC-3' and Bub-CSN3-r 5'-CGACGGTTGAAGTAACTTGGGCTG-3'. 40 ng of DNA were used for PCR amplification using 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 conditions used were: initial denaturation at 98 °C for 30 s followed by 40 cycles of 98 °C for 7 sec, 64 °C for 15 s and 72 °C for 20 s, with a final extension step at 72 °C for 7 min. PCR products were purified with Agencourt AMPure Purification System (Beckman Coulter) and sequencing reaction was performed using GenomeLab™ DTCS Quick Start Kit for Dye Terminator Cycle Sequencing following manufacturer's instructions. The Agencourt CleanSEQ Purification System (Beckman Coulter) was used for the purification of sequencing products. Sequencing was performed on a CEQ8000 Genetic Analysis System (Beckman Coulter) and single-nucleotide polymorphism (SNP) was performed with Genetic Analysis Software v.9.00 (Beckman Coulter). Descriptive statistics were calculated using Genepop v.4 (*Rousset,* 2007).

RESULTS AND DISCUSSION

A 221 bp fragment of exon IV of the CSN3 gene was amplified and sequenced in 215 buffaloes belonging to Murrah Bulgarian breed. Sequences were aligned and analyzed using BioEdit v. 7.0.5 (*Hall,* 1999) and a substitution of an Ile residue for Thr at 135 codon position was detected. A silent mutation at Thr^{136} was also observed (ACC/ACT) while no genetic variant of Ala^{148} was detected (*Table 1*).

Table 1

Positions and amino acid differences in genetic variants of k-casein in cattle and buffalo

	Position and amino acid in the protein		
	135	136	148
k-casein (*Bos taurus*)	Thr	Thr (*Ile*)	Asp (*Ala*)
k-casein (*Bubalus bubalis*)	Thr (*Ile*)	Thr	Ala

Between brackets are indicated the substitutions in cattle for the B allelic variant according to *Farrell et al.,* 2004. The B variant observed in this study in buffalo is indicated in *italics*.

Mitra et al. (1998) reported the same amino acid substitution T135I of the k-CN gene in buffalo defined as B variant. Genotypic frequencies were 0.56, 0.40 and 0.04 for AA, AB and BB genotypes and gene frequencies of allele A and B were 0.76 and 0.24 respectively. Deviation between observed genotypic frequencies and those expected under Hardy-Weinberg equilibrium were not significant ($P>0.05$) suggesting that the Murrah Bulgarian population is in equilibrium at the CSN3 locus.

Recent studies in buffaloes used a PCR-RFLP approach with restriction enzymes *Hind*III, *Hinf*I and *Taq*I (*Abbasi et al.,* 2009 and *Abdel Dayem et al.,* 2009). Such approach can detect polymorphism in cattle where k-CN A and B variants differ for T136I and D148A but it could not detect the different genetic variants in our buffalo population because cattle sequence variations are not conserved. These findings demonstrate the importance of the direct sequencing approach to detect k-CN polymorphisms in buffaloes. Since k-CN variant B is associated with higher fat, protein, and casein content in cattle milk and it has a significant influence on cheese making properties and superior rennet coagulation properties in comparison to A variant (*Heck et al.,* 2009), further studies are required in order to confirm such association also in buffalo to eventually implement, in a selection program scheme, a MAS (Marker Assisted Selection) approach based on CSN3 marker.

CONCLUSIONS

A portion of the buffalo CSN3 locus was successfully amplified and a polymorphism was observed at 135 codon position allowing the detection of A and B allelic variants. These results could be achieved with the use of direct sequencing approach while no polymorphism could be detected using PCR-RFLP technique implemented for k-CN genotyping in cattle.

Homozygote BB individuals result in lower frequencies than AA and AB genotypes but the gene frequency of the B allele is reasonably high to evalutate an effective strategy to increase this favorable genotypes. Moreover a study on functional aspects of the different genetic variant could be performed in buffaloes to confirm a positive effect of the B allele known in other livestock species.

REFERENCES

Abbasi, B., Fayazi, J., Beigi Nasiri, M.T., Roshanfekr, H.A., Mirzadeh, Kh., Sadr, A.S. (2009). Analysis of Kappa Casein Gene Polymorphism by PCR-RFLP in Buffalo Population in Khouzestan Province. Research Journal of Biological Science. 4. 10. 1073-1075.

Abdel Dayem, A.M.H., Mahmoud, K.Gh.M., Nawito, M.F., Ayoub, M.M., Darwish, S.F. (2009). Genotyping of kappa-casein gene in Egyptian buffalo bulls. Livestock Science. 122. 286-289.

Chessa, S., Chiatti, F., Ceriotti, G., Caroli, A., Consolandi, C., Pagnacco, G., Castiglioni, B. (2007). Development of a single nucleotide polymorphism genotyping microarray platform for the identification of bovine milk protein genetic polymorphisms. J. Dairy Sci., 90. 451-464.

Chianese, L., Quarto, M., Pizzolongo, F., Calabrese, M.G., Caira, S., Mauriello, R., De Pascale, S., Addeo, F. (2009). Occurrence of genetic polymorphism at the αs1-casein locus in Mediterranean water buffalo milk. International Dairy Journal. 19. 181-189.

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 k-Casein Genotypes on Milk Coagulation, Quality, and Yield Traits in Italian Holstein Cows. Journal of Dairy Science. 91. 4022-4027.

Dogru, U., Ozdemir, M. (2009). Genotyping of K-Casein Locus by PCR-RFLP in Brown Swiss Cattle Breed. Journal of Animal and Veterinary Advances. 8. 4. 779-781.

Farrell, 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, K.F., Swaisgood, H.E. (2004). Nomenclature of the Proteins of Cows' Milk-Sixth Revision. Journal of Dairy Science. 87. 1641-1674.

Ferranti, P., Scaloni, A., Caira, S., Chianese, L., Malorni, A., Addeo, F. (1998). The Primary Structure of Water Buffalo As1- and B-casein: Identification of Phosphorylation Sites and Characterization of a Novel B-casein Variant. Journal of Protein Chemistry. 17.

Gangaraj, D.R., Shetty, S., Govindaiah, M.G., Nagaraja, C.S., Byregowda, S.M., Jayasankar, M.R. (2008). Molecular characterization of kappa-casein gene in buffaloes. Science Asia. 34. 435-439.

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis. Nucl. Acids. Symp., 41. 95-98.

Program for Windows 95/98/NT.

Heck, J.M., Schennink, A., van Valenberg, H.J., Bovenhuis, H., Visker, M.H., van Arendonk, J.A., van Hooijdonk, A.C. (2009). Effects of milk protein variants on the protein composition of bovine milk. Journal of Dairy Science. 92. 3. 1192-1202.

Mitra, A., Balakrishnan, C.R. (1998). Kappa-casein polymorphisms in Indian dairy cattle and buffalo: A new genetic variant in buffalo. Animal Biotechnology. 9. 81-87.

Otaviano, A.R., Tonhati, H., Desidério Sena, J.A., Cerón Muñoz, M.F. (2005). Kappa-casein gene study with molecular markers in female buffaloes *(Bubalus bubalis).* Genetics and Molecular Biology. 28. 2. 237-241.

Pacini, F., Fellin, A., Andrighetto, C., Dal Zotto, R., De Marchi, M., Cassandro, M., Lombardi, A. (2008). Detection of B κ-casein variant (CSN3*B) in Burlina dairy cattle by PCR-TTGE. Dairy Science Technology. 88. 217-223.

Rousset, F. (2008). Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Molecular Ecology Resources. 8. 103-106.

Corresponding authors:

Barbara Beneduci
Department of Animal Science, University of Padova
I-35020 Legnaro (PD), Agripolis - viale dell'Università 16., Italy
Tel.: +39 049 827 2614, fax: +39 049 827 2633
e-mail: barbara.beneduci@live.it