Haryana Vet. (June, 2017) **56 (1)**, 1-4

Research Article

STATUS OF β CASEIN GENE IN HARDHENU CROSSBRED CATTLE

RAMKARAN, ANITA GANGULY, SANDEEP GERA, SANDEEP KUMAR*, ANKIT MAGOTRA, AMAN KUMAR and MANINDER SHEORAN
Department of Veterinary Physiology and Biochemistry, College of Veterinary Sciences
Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125 004, India

Received: 28.01.2017; Accepted: 22.05.2017

ABSTRACT

The present study was undertaken to explore the existing variability in β-casein A1/A2 polymorphism in Hardhenu crossbred cattle. Genomic DNA was extracted from whole blood of randomly selected Hardhenu cows (n=50). Allele specific PCR for β-casein was carried out to explore A1 and A2 variants. A PCR product of 244 bp was amplified from exon 7 of β-casein gene. Genotype frequencies were found to be 0.32 (A1A1) and 0.68 (A1A2). The frequency of A1 and A2 allele was 0.66 and 0.34, respectively. In the present investigation, frequency of A2 allele (0.34) in Hardhenu cattle was found to be comparatively lower than the Frieswal population. Therefore, the existing genetic variability in β-casein (A1/A2) locus of Hardhenu population may be exploited for future genetic selection to minimize the negative effect of A1 milk.

Key words: Beta-casein, CASB, AS-PCR, A1 milk, A2 milk

Milk is one of the primary protein diets to the human population. Cow milk protein contains 25-30% β casein, which contains 209 amino acids. Bovine β casein (CASB) gene is located on chromosome number 6 and belongs to the cluster of 4 casein genes αS1, αS2, β and K (Rijnkels, 2002). Beta-casein (CSN2) is the most polymorphic milk protein gene with 13 known protein variants. Variants A1 and A2 of CSN2 are the most common and can be found in many dairy breeds (Farrell *et al*., 2004).

The difference between A2- and A1- β–casein variants is a single amino acid substitution (CCT→CAT:: Pro→His) at the 67th residue of 209 amino acid chain. This difference might generate conformational changes in the secondary protein structure and therefore change physical properties of casein micelles. The presence of histidine instead of proline at codon 67 makes A1 and B variants exclusively susceptible to gastrointestinal proteolysis digestion to liberate bioactive peptide, beta-casomorphin-7 (BCM-7) (De Noni, 2008) which may lead to adverse physiological effects. Investigations in mice and rabbits suggested the probable implication of bovine BCM-7 in potential risk of human ischemic heart diseases, insulin-dependent diabetes (Laugesen and Elliott, 2003), sudden infant death syndrome (Sun *et al*., 2003) and atherosclerosis (Tailford *et al*., 2003). CSN2-A2 decreases the level of serum cholesterol and LDL lipids which play an important role in prevention of a wide range of human vascular diseases (Kaminski *et al*., 2007).

*Corresponding author: drsandgupta@gmail.com

Mishra *et al.* (2009) reported the predominance of A2 allelic variant in 8 river buffaloes and 15 zebu cattle breeds in India. On contrary, crossbred Frieswal (HF X Sahiwal) cattle had been reported to have substantially higher proportion of A1 allele in heifers (0.32) and bulls (0.44) (Ganguly *et al.,* 2013). However, screening of most of the crossbred population of India has not yet been undertaken for CSN2-A1/A2 polymorphism.

The Hardhenu (Holstein Friesian X Sahiwal X Hariana) is a novel cattle introduced by LUVAS, Hisar for commercial exploitation, providing high productivity of Holstein Friesian and low repeat breeding of Sahiwal with sustainability and survivability of the Hariana breed. Keeping in view the increasing trend of crossbred cattle population in India as well as significant association of A1 and A2 beta-casein (CSN2) polymorphism with health promoting aspects and milk performance traits, the present study was undertaken to explore the status of A1/A2 polymorphism in Hardhenu (HF X Sahiwal X Hariana) cattle.

MATERIALS AND METHODS

Experimental Animals: The present study was conducted on Hardhenu cattle, maintained at Animal farm of LUVAS, Hisar with prior permission from Institutional Animal Ethics Committee. A total of 50 randomly chosen animals were used for the present study.

Collection of Blood and DNA Isolation: Ten ml of blood was aseptically collected from the jugular vein in vacutainer tube containing EDTA as anticoagulant. The

samples were properly labeled and were stored in deep freezer at -20^{0}C temperature till the isolation of genomic DNA. Genomic DNA was isolated by phenol-chloroform method following standard protocol (Sambrook and Russell, 2001).

Evaluation of Quality and Concentration of DNA: Genomic DNA quality was checked to ensure the presence of intact DNA by running the isolated DNA on agarose gel [0.8 % (w/v)]. The purity of the genomic DNA was assessed by UV spectrophotometer by checking the optical density (OD) value at 260 and 280 nm. The samples having OD ratio (260 nm/ 280 nm) 1.7 to 1.9 were used for further experiment. The concentration of DNA was calculated by using the following formula: DNA concentration (µg/µl) = OD260 x (Dilution factor) x 50/ 1000. Finally, DNA was diluted in distilled water at the concentration of 50ng/µl. In the PCR reaction 2 µl diluted DNA was used.

Allele Specific PCR to Explore β-casein (A1/A2) Polymorphism: To explore A1/A2 β-casein polymorphism, allele-specific PCR (AS-PCR) was carried out as described by Ganguly *et al.* (2013). Briefly, one forward primers with either A (5' CTT CCC TGG GCC CAT CCA 3') or C (5' CTT CCC TGG GCC CAT CCC 3') at the 32 end and a common reverse (CR) primer (5' AGA CTG GAG CAG AGG CAG AG 3') were used to amplify a 244 bp fragment (Fig. 1). Primer pairs A with CR and C with CR were anticipated to produce histidine (A1) and proline (A2) specific amplicon, respectively. The PCR reaction was carried out in a 0.2 ml PCR tubes in a thermal cycler (T 100 thermal cycler, BIO-RAD). For the PCR reaction, a premixed ready-to-use solution, GoTaq® G2 Hot Start Green Master Mix (Promega, Madison, WI, USA) was used following manufacturer instruction. Briefly, PCR was carried out from a starting template of approximately 100 ng of genomic DNA (2µl) in a final reaction volume of 25 µl containing 12.5µl GoTaq® G2 Hot Start Green Master Mix (2X), 0.50 µl of each 10 µM primer and nuclease-free water up to25µl. PCR condition were followed as described by Ganguly *et al.* (2013) where initial denaturation was carried out at 94°C for 5 min; followed by 5 cycles of 94°C for 30s, 66°C for 30s and 72°C for 30s; thereafter 30 cycles of 94°C for 30s, 64°C for 30s and 72°C for 30s and a final extension 72°C for 5 min. The amplified PCR products were electrophoresed in 1% agarose gel at 70 V for 45 minutes along with molecular marker (100bp) and visualized under Gel Documentation System (UVP Benchtop UV Transilluminators, Fisher Scientific).

Calculation of Gene and Genotypic Frequencies: Gene and genotypic frequencies were calculated as given by Falconer and Mackay (1996).

RESULTS AND DISCUSSION

In the present investigation variability in β-casein A1/A2 polymorphism was explored in the crossbred Hardhenu cattle utilizing AS-PCR protocol. PCR product of 244 bp was amplified from exon 7 of β-casein gene (Fig. 1). Initially amplification was carried out in higher annealing temperature (66°C) for five cycles, followed by 30 cycles at 64°C. This was followed to increase the stingency of amplified product for the primer having exact match with template strand and to reduce further the chance of mismatch primer's opportunity to get amplified in the initial cycling stage. Two genotypes A1A1 and A1A2 were observed in the randomly selected samples (n=50) of Hardhenu cattle population with the frequencies of 0.32 and 0.68, respectively. The frequency of A1 and A2 allele was 0.66 and 0.34, respectively (Table 1). However, no animal with A2A2 genotype was observed. The two genotypes viz., A1A1, A1A2 were efficiently distinguished and identified in this population by this technique (Fig. 1).

Although the function of milk proteins in relation to human health remains debatable, however, there are several evidences linking A1 beta-casein to a series of illnesses by preferentially releasing an opioid peptide called beta-casomorphin-7 (BCM-7) upon digestion. The A1 allele frequency in different exotic breeds reported to vary between 0.01-0.06 (Guernsey), 0.09-0.22 (Jersey), 0.31–0.66 (Holstein), 0.43-0.72 (Ayrshire) and 0.71 (Danish Red) (Kaminski *et al.*, 2007). In India, predominance of A2 allelic variant has been observed in 15 zebu cattle breeds and eight breeds of river buffaloes (Mishra *et al.*, 2009). All the breeds were reported to

Table 1
Genotype and allele frequency of A1/A2 β-Casein gene in Hardhenu crossbred cattle

Breed	Genotype frequency			Total	Allele frequency	
	A1A1	A1A2	A2A2		A1	A2
Hardhenu crossbred cattle	0.32 (16)	0.68 (34)	0 (0)	50	0.66	0.34

Numbers of animals are stated within parenthesis

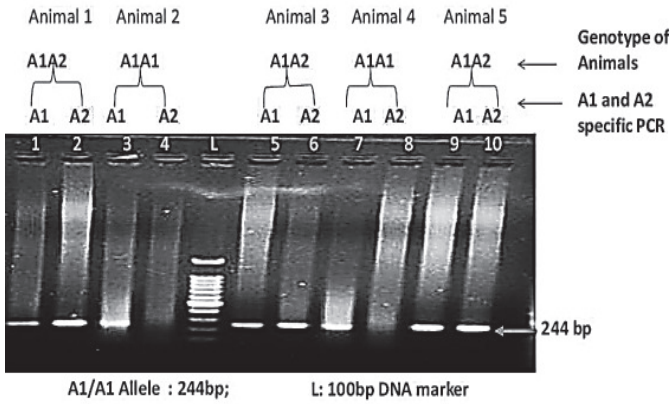

Fig 1. Agarose gel electrophoresis pattern of allele-specific PCR products of beta-casein genotypes.
Lanes 1, 2, 5, 6, 9 and 10=A1A2 genotype; Lanes 3, 4, 7 and 8=A1A1 genotype; Lane L:100 bp marker

carry fixed A2 allele, except two cattle breeds (Malnad Gidda and Kherigarh). These two breeds were found to carry A1 allele, mostly in A1/A2 heterozygous condition, with a frequency of 0.096 and 0.109, respectively (Mishra *et al*., 2009). On the contrary, substantially higher proportion of A1 allele has been reported in the Frieswal crossbred heifers (0.32) and bulls (0.44) (Ganguly *et al.*, 2013). Recently, Malarmathi *et al.* (2014) also observed A2 allele frequency to be 0.595 and 1.0 in HF crossbred (n=63) and Kangeyam cattle (n=22). Interestingly, in the present investigation frequency of A2 allele (0.34) in Hardhenu cattle was found to be comparatively lower than the Frieswal population. Recently, Rahimi *et al*. (2015) observed relatively higher frequency (38.2%) of beta-casein A1 allele in native cattle of the Kermanshah Province, Western Iran.

Earlier studies carried out in Finnish Ayrshire (Ikonen *et al.,* 1999; Ikonen *et al.,* 2001), New Zealand Ayrshire (Winkelman and Wickham, 1997) and Polish HF (Olenski *et al.,* 2010) showed a positive relationship of A2 β-casein variant with milk performance traits especially of protein and milk yield. Alternatively, Lipkin *et al.* (2008) observed negative influence of A1 variant on performance traits in Israeli HF. Nilsen *et al.* (2009) also observed an association of casein haplotypes in Norwegian Red Cattle and reported that C allele (determining A2 protein variant) of β-casein as a potential marker for higher protein and milk yield.

Beta-casein A2 milk produces four times less BCM-7 than the A1 milk upon hydrolysis (Cieslinska *et al.,* 2007). Moreover, a positive relationship of A2 variant with milk performance traits in different cattle breeds has been observed. Therefore, the existing genetic variability in β-casein (A1/A2) locus of Hardhenu population may be exploited for future genetic selection to minimize the negative effect of A1 milk. It is to mention that the sample size of the present investigation was not large enough. Therefore, to have a clear picture on A1/A2 polymorphic status of Hardhenu population, future studies may be directed to screen more number of cows and breeding bulls. Moreover, association of A2 variant needs to be established with performance traits in this population.

ACKNOWLEDGEMENTS

The authors express their sincere sense of gratitude to the Vice Chancellor, LUVAS, Hisar for providing research facilities and financial support. We acknowledge the support of Dr. Indrajit Ganguly, Senior Scientist, ICAR-NBAGR in drafting the manuscript.

REFERENCES

Cieslinska, A., Kamiñski, S., Kostyra, E. and Sienkiewicz-Szlapka, E. (2007). Betacasomorphin7 in raw and hydrolyzed milk derived from cows of alternative β-casein genotypes. *Milchwissenschaft* **62**: 125-127.

De Noni, I. (2008). Release of β-casomorphins 5 and 7 during simulated gastro-intestinal digestion of bovine β-casein variants and milk-based infant formulas. *Food Chem.* **110**: 897-903.

Falconer, D.S. and Mackay, T.F.C. (1996). Introduction to Quantitative Genetics. (4th edn.), Longman Group Ltd, Essex, UK.

Farrell, H.M., Jr 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. and Swaisgood, H.E. (2004). Nomenclature of the proteins of cows' milk-sixth revision. *J. Dairy Sci.* **87**: 1641-1674.

Ganguly, I., Gaur, G. K., Singh, U., Kumar, S., Kumar, S. and Mann, S. (2013). Beta-casein (CSN2) polymorphism in Ongole (Indian zebu) and Frieswal (HF×Sahiwal crossbred) cattle. *Ind. J. Biotech*. **12**: 195-198.

Ikonen, T., Bovenhius, H., Ojala, M., Ruottinen, O. and Georges, M. (2001). Associations between casein haplotypes and first lactation milk production traits in Finnish Ayrshire cows. *J. Dairy Sci.* **84**: 507-514.

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

Kaminski, S., Cieslinska, A. and Kostyra, E. (2007). Polymorphism of bovine beta casein and its potential effect on human health. *J. Appl. Genet.* **48**: 189-198.

Laugesen, M. and Elliott, R. (2003). Ischaemic heart disease, type 1 diabetes, and cow milk A1 beta-casein. *N. Z. Med. J.* **116**: 1-19.

Lipkin, E., Tal-Stein, R., Friedman, A. and Soller, M. (2008). Effect of quantitative trait loci for milk protein percentage on milk protein yield and milk yield in Israeli Holstein dairy cattle. *J. Dairy Sci.* **91**: 1614-1627.

Malarmathi, M., Kumar, T.M.A.S., Parthiban, M., Muthuramalingam, T. and Palanisammi, A. (2014). Anlysis of β-casein gene for

A1 and A2 genotype using allele specific PCR in Kangayam and Holstein Friesian crossbred cattle in Tamil Nadu. *Ind. J. Vet. Anim. Sci. Res.* **43(4)**: 310-315.

Mishra, B.P., Mukesh, M., Prakash, B., Sodhi, M., Kapila, R., Kishore, A., Kataria, R.R., Joshi, B.K., Bhasin, V., Rasool, T.J. and Bujarbaruah, K.M. (2009). Status of milk protein, β-casein variants among Indian milch animals. *Ind. J. Anim. Sci.* **79(7)**: 722-725.

Nilsen, H., Olsen, H.G, Hayes, B., Sehested, E., Svendson, M., Nome, T., Meuwissen, T. and Lien, S. (2009). Casein haplotypes and their associations with milk production traits in Norwegian Red cattle. *Genet. Select. Evol.* **41(24)**: 1-12.

Olenski, K., Kamiñski, S., Szyda, J. and Cieslinska, A. (2010). Polymorphism of the beta-casein gene and its associations with breeding value for production traits of Holstein–Friesian bulls. *Livestock Sci.* **131**: 137-140.

Rahimi, Z., Gholami, M., Rahimi, Z. and Yari, K. (2015). Evaluation of beta-casein locus for detection of A1 and A2 alleles frequency using allele specific PCR in native cattle of Kermanshah, Iran. *Biharean. Biol*. **9(2)**: 85-87.

Rijnkels, M. (2002). Multispecies comparison of the casein gene loci and evolution of casein gene family. *J. Mammary Gland Biol. Neoplasia* **7**: 327-345.

Sambrook, J. and Russell, D.W. (2001). Molecular Cloning: A Laboratory Manual. (3rd edn.), Cold Spring Harbour Laboratory Press, New York.

Sun, Z., Zhang, Z., Wang, X., Cade, R., Elmer, Z. and Fregly, M. (2003). Relation of beta-casomorphin to apnea in sudden infant death syndrome. *Peptides* **24**: 937-943.

Tailford, K.A., Berry, C.L., Thomas, A.C. and Campbell, J.H. (2003). A casein variant in cow's milk is atherogenic. *Atherosclerosis* **170**: 13-19.

Winkelman, A.M. and Wickham, B.W. (1997). Associations between milk protein genetic variants and production traits in New Zealand dairy cattle. *Int. Dairy Fed*. 38-46.