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Screening of taurine and crossbred breeding bulls for A1/A2 variants of β-casein gene

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ABSTRACT

Amongst the 13 known allelic variants of b-casein gene, A1 and A2 are reported to be the most common forms in dairy cattle. A1 allele of β-casein has been implicated as a risk factor to several health disorders in human beings whereas milk with A2 allele, β-casein is considered safe for human consumption. Different studies in recent past have shown widespread presence of β-casein A1 allele in European *Bos taurus* cattle. In our country, extensive use of taurine germplasm under genetic improvement program could disseminate such undesirable allele in Indian native cattle breeds. The present study targeted several exotic (51 Holstein Friesian, 40 Jersey) and crossbred (89) bulls approved for genetic improvement programmes in India so as to determine their existing status of A1/A2. The frequency data indicated predominance of the desirable A2 allele across all cattle types studied with a mean frequency of 0.645. Among the genotypes, A1A2 was more common in Holstein Friesian and Jersey while A2A2 was at higher frequency in the crossbreds. The study suggested that screening of all breeding bulls for their A1/A2 variant status would be a promising way to check the flow of undesirable alleles in our native breeds. The study also revealed that moderate to high frequency of A2 allele among the crossbred bulls further favours the current belief that milk being marketed in India is safe for human consumption.

Key words: β-casein, Breeding bulls, Milk protein, PCR-RFLP

Bovine milk contains 3–5% proteins, of which 80% is casein and rest 20% is whey protein. β-casein is the second most abundant protein and crucial for casein micelle structure (Threadgill and Womack 1990). The polymorphic status of bovine β-casein is confirmed, and till date 13 allelic variants have been identified (Kaminski *et al.* 2007). Amongst these, A1 and A2 variants are reported to be the most common allelic variants of β-casein in dairy cattle (Farrell *et al.* 2004). The polymorphic nature and its association with milk, fat and protein yield attracted several efforts in evaluating this locus as a potential dairy trait marker (Ikonen *et al*. 1999,Caroli *et al.* 2004, Kucerova *et al.* 2006). Consumption of milk with A1 has been linked to increased risk of human diseases such as type I diabetes mellitus (Elliot *et al.* 1999, Thorsdottir *et al.* 2000), coronary heart disease (McLachlan, 2001), arteriosclerosis (Tailford *et al.* 2003), sudden infant death syndrome (Sun *et al.* 2003), schizophrenia and autism (Woodford 2008). However, A2 β-casein not been linked to any of such health issues (Kaminski *et al.* 2007). The A1 and A2 variants of bovine β-casein differ at amino acid position 67 with histidine in A1 and proline in A2 milk. This polymorphism leads to key conformational changes in the secondary structure of expressed β-casein protein (Elliot *et al.* 1999, McLachlan 2001). Due to presence of histidine at amino acid 67 position, digestion of A1 β-casein milk releases a 7 amino acid bioactive peptide called beta-casomorphin 7 (BCM-7) in small intestine, while proline in A2 milk at 67 position prevents the split at this particular site and generates peptide BCM-9 (Roginski 2003, Kostya *et al.* 2004). It is believed that generation of BCM-7 is the major causative factor associated with A1 milk related health disorders (Trompette *et al.* 2003).

In India, Holstein Friesian, Jersey and Brown Swiss cattle have been extensively used since 1960 for crossbreeding and genetic improvement programmes. The status of A1/A2 alleles in these taurine sire lines being used in artificial insemination (AI) programmes of the country have not been documented till date. Considering the widespread use of taurine germplasm in our crossbreeding programmes for years and fact that these cattle could be the potential source for undesirable A1 allele of β-casein warrants the need to

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analyze the status of β-casein A1/A2 alleles in these animals to draw a sound breeding policy and minimize the risk of disseminating the A1 allele in Indian cattle or buffaloes. In the present work, we report the distribution pattern of β-casein A1/A2 variants in a large number of taurine crossbred bulls being used in crossbreeding programmes in different parts of the country.

MATERIALS AND METHODS

For the present work, blood/semen samples of 51 Holstein Friesian, 40 Jersey and 89 crossbreds were collected from different breeding station/semen distribution units. These stations either procure or produce frozen semen straws of the exotic or crossbred elite bulls and further distribute them to state agricultural universities, animal husbandry departments, dairy co-operatives and non-governmental organizations for dissemination among the local cows. The stations selected for sampling were located in different geographical and agroclimatic zones, viz. Kerala (South-coastal region), Rajasthan (North-western plain region), Himachal Pradesh (Northern-hilly region), and Haryana and Western Uttar Pradesh (resource-rich plain region).

Genomic DNA was extracted by enzymatic digestion using proteinase K followed by routine phenol-chloroform extraction method (Sambrook *et al.* 1989). Primers and PCR-RFLP protocol that were employed to amplify the 251 bp fragment of exon 7 of β-casein gene and distinguish the A1/A2 variants is similar to that reported by Lien *et al*. (1992). PCR was performed using 150–200 ng of genomic DNA in a reaction volume of 25 µl containing 5 pmol of each primer, 200 µM dNTPs, 1.5 mM $MgCl_2$, and 1 U of Taq DNA polymerase. PCR was carried out in a thermal cycler using the following cycling programme: 95°C for 5 min, followed by 30 cycles at 94°C for 30 sec, 63°C for 40 sec and 72°C for 20 sec with a final extension at 72°C for 3 min. The purified PCR products were digested with 5 U of *Taq* I restriction enzyme at 65°C for 3 h. The digested products were resolved on 3.5% agarose gel in 1X TAE buffer and genotypes were recorded as the fragment size (Table 1).

RESULTS AND DISCUSSION

Digestion of PCR amplicons with *Taq*I restriction enzyme yielded fragments in three combinations: A1A1 genotype with two bands (213 and 38 bp), A1A2 genotype with two bands (251 and 213 bp) and A2A2 genotype with a single fragment (251 bp) (Figure 1). The gene and genotype frequencies of *Taq*I variants are furnished in Table 2. Among the two alleles, A2 was predominant across all the animals with a mean value of 0.645 and ranged from 0.559 (Holstein) to 0.702 (crossbred). On the other hand, the frequency of A1 allele ranged from 0.298 (crossbred) to 0.441 (Holstein) with a mean of 0.355. The heterozygote A1A2 genotype was more common in Holstein (0.451) and Jersey (0.600), while A2A2 genotype was at higher frequency among the crossbreds (0.506). The frequency data that we observed for A1/A2 variants in Holstein and Jersey bulls being used in India are similar to the earlier findings reported in different taurine cattle (Kaminski *et al.* 2007).

Table 1. Fragment size corresponding to different beta casein genotypes

Genotype	Fragment size (bp) after digestion with *Taq* I
A1A1	213, 38
A1A2	251, 213
A2A2	251

The gene and genotypic frequency was calculated by direct counting method.

The relatively higher prevalence of desirable A2 allele (0.645) observed across all the bulls corresponded to the existence of high frequency of heterozygous genotype A1A2 (0.506).

In one of our study (Mishra *et al.* 2009), status of A1/A2 alleles was reported for the first time in a large number of Indian native cattle (*Bos indicus:* 15 breeds; n, 618) and buffalo (*Bubalus bubalis:* 8 breed; n, 231) breeds. Our data suggested predominance or near fixation of A2 allele in Indian native cattle whereas its complete fixation in buffaloes. Further, existence of high mean frequency of A2A2 (0.974 in native cattle and 1.00 in buffaloes), strongly indicated preponderance of the preferred genotype and absence of undesirable A1A1 genotype of β-casein in naturally evolved native cattle and buffalo breeds of India. Based on this finding we carried out screening of the exotic bulls being used in large scale crossbreeding programme for milk productivity enhancement in India. Our previous study (Mishra *et al.* 2009) and present results in crossbred bulls suggest that in India the scenario related to A1 β-casein associated health issues is on the safer side. The moderate to high frequency of A2 allele among the crossbred bulls further favours the current belief that milk being marketed in India is safe for human consumption.

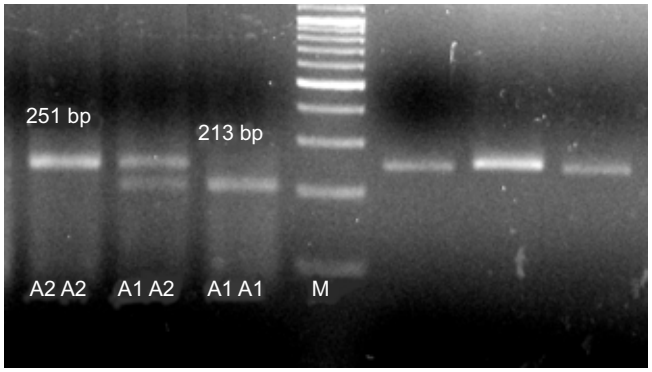

Fig. 1. Genotypes of b-casein in breeding bulls (3.5% agarose gel); M: 100 bp DNA Ladder

Table 2. Gene and genotype frequency of b-casein in breeding bulls

Breed	Genotype frequency			Gene frequency	
	A1A1	A1A2	A2A2	A1	A2
Holstein (51)	0.216 (11)	0.451 (23)	0.333 (17)	0.441	0.559
Jersey (40)	0.025 (01)	0.600 (24)	0.375 (15)	0.325	0.675
Crossbred (89)	0.101 (09)	0.393 (35)	0.506 (45)	0.298	0.702
Mean	0.114	0.481	0.405	0.355	0.645

Figures in parentheses are number of observation

Worldwide several studies were conducted to understand the allelic distribution of β-casein locus in taurine cattle and their association with production traits (Baranyi *et al.* 1997, Ehrmann *et al.* 1997, Winkelman and Wickham 1997, Caroli *et al.* 2004, Kaminski *et al.* 2006). A review by Kaminski *et al*. (2007) highlighted that there is variable extent in the context of associating these variants with production traits and also reported existence of wide range of variability (0.01 to 0.72) in the frequency distribution of A1 allele across different cattle types. Similar investigations on status of A1/A2 β-casein variants in bulls was also reported with the aim of developing herds of cows producing A2 milk (Iggman *et al.* 2003, Keating *et al.* 2008). Results from these studies revealed the frequency of β-casein A1 and A2 alleles in Holstein bulls as 0.402 and 0.598, respectively (Kaminski *et al.* 2006) while in Czech Fleckvieh bulls the corresponding values were 0.177 and 0.809 (Kucerova *et al.* 2006).

Although evidence for a clear link between A1 β-casein and a disease state has not been demonstrated, it may be necessary to monitor the status of A1/A2 alleles in our dairy animals as a cautionary measure. The presence of large crossbred cattle population and higher proportions of A1 allele in taurine bulls observed in the present study stipulate the need for careful screening of sire lines being used in breeding programmes for their A1/A2 status and putting selection pressure to drift herds towards A2. Such an approach would be an effective measure to prevent the dissemination of undesirable A1 allele in our existing A2 predominant cattle populations. Similar strategy of systematic monitoring of β-casein alleles in bulls and cows is being followed in Poland (Kamiski *et al*. 2006). In New Zealand, a DNA based kit has been developed to screen the cattle herds and A2 milk is marketed as a premium brand (A2 Corporation 2006).

Further in the regime of WTO, if the undesirable effects of β-casein A1 allele on health are validated and demands for A2 milk increases, our native cattle and buffalo breeds wherein A2 variant is naturally predominant, would have an upper edge over their taurine counterparts in meeting the global demands for A2 milk in the international dairy sector.

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REFERENCES

A2 Corporation 2006. http://www.a2corporation.com/index.php/ps_pagename/corporate. 16 July, 2008.

Baranyi M, Bosze Z, Buchberger J and Krause I. 1997. Genetic polymorphism of milk proteins in Hungarian cattle breeds and PCR amplification of betalactoglobulin exon 5 to identify genetic variant by RFLP. *Milk protein polymorphism. Proceedings of the IDF Seminar held in Palmerston North, New Zealand. International Dairy Federation, 87–92.*

Caroli A, Chessa S, Bolla P, Budelli E and Ganging G C. 2004. Genetic structure of milk protein polymorphism and effects on milk production traits in local dairy cattle. *Journal of Animal Breeding and Genetics* **121**: 119–27.

Elliott R B, Harris D P, Hill J P, Bibby N J and Wasmuth H E. 1999 Type I (insulin dependent) diabetes mellitus and cow milk: casein variant consumption. *Diabetologia* **42**: 292–96.

Ehrmann S, Bartenschlager H and Geldermann H. 1997. Quantification of gene effects on single milk proteins in selected groups of dairy cows. *Journal of Animal Breeding and Genetics* **114**: 121–32.

Farrell H M Jr, Jimenez-Flores R, Bleck G T, Brown E M, Butler J E and Creamer L K. 2004. Nomenclature of the proteins of cows' milk-sixth revision. *Journal of Dairy Science* **87**: 1641–74.

Iggman D, Birgisdottir B, Ramel A and Thorsdottir I. 2003. Differences in cow's milk composition between Iceland and the other Nordic countries and possible connections to public health. *Scandinavian Journal of Nutrition* **47**: 194–98.

Ikonen T, Ojala M and Ruottinen O. 1999. Associations between milk protein polymorphism and first lactation milk production traits in Finnish Ayrshire cows. *Journal of Dairy Science* **82**: 1026–33.

Kaminski S, Rusc A and Cieslinska A. 2006. A note on frequency of A1 and A2 variants of bovine beta-casein locus in Polish Holstein bulls. *Journal of Animal and Feed Science* **15**: 195–98.

Kaminski S, Cieslinska A and Kostyra E. 2007. Polymorphism of bovine beta-casein and its potential effect on human health. *Journal of Applied Genetics* **48**: 189–98.

Keating A F, Smith T J, Ross R P and Cairns M T. 2008. A note on the evaluation of a beta-casein variant in bovine breeds by allele-specific PCR and relevance to b-casomorphin. *Iris Journal of Agricultural and Food Research* **47**: 99–104.

Kostyra E, Sienkiewicz-Szapka E, Jarmoowska B, Krawczuk S and Kostyra H. 2004. Opioid peptides derived from milk proteins. *Polish Journal of Nutrition Science* **13/54**: 25–35.

Kucerova J, Matejicek A, Jandurova O M, Sorensen P, Nemcova E, Stipkova M, Kott T, Bouska J and Frelich J. 2006. Milk protein genes CSN1S1, CSN2, CSN3, LGB and their relation to genetic values of milk production parameters in Czech Fleckvieh. *Czech Journal of Animal Science* **51**(6): 241–47.

Lien S, Alestrom P, Hubgland H and Rogne S. 1992. Detection of

multiple beta-casein (CASB) alleles by amplification created restriction sites (ACRS). *Animal Genetics* **23**: 333–38.

McLachlan C N. 2001. Beta-casein A1, ischaemic heart disease mortality, and other illnesses. *Medical Hypotheses* **56**: 262–72.

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, b-casein variants among Indian milch animals. *Indian Journal of Animal Sciences* **79** (7): 722–25.

Roginsky H. 2003. Encyclopedia of Dairy Sciences. Academic Press, London

Sambrook J, Fritsh E F and Maniatis T. 1989. *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor 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**: 937–43.

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.

Thorsdottir I, Birgisdottir B E, Johannsdottir I M, Harris D P, Hill J, Steingrimsdottir L and Thorsson A V. 2000. Different (beta-casein) fractions in Icelandic versus Scandinavian cow's milk may influence diabetogenicity of cow's milk in infancy and explain low incidence of insulin dependent diabetes mellitus in Iceland. *Pediatrics* **106**: 719–24.

Threadgill D W and Womack J E. 1990. Genomic analysis of the major bovine milk protein genes. *Nucleic acid Research* **18** (23): 6935–42.

Trompette A, Claustre J, Caillon F, Jourdan G, Chayvialle J A and Plaisancie P. 2003. Milk bioactive peptides and b-casomorphins induce mucus release in rat jejunum. *The Journal of Nutrition* **133**: 3499–503.

Winkelman A M and Wickham B W. 1997. Associations between milk protein genetic variants and production traits in New Zealand dairy cattle. *Milk protein polymorphism. Proceedings of the IDF Seminar held in Palmerston North, New Zealand. International Dairy Federation* pp. 38–46.

Woodford K. 2008. A1 Beta-casein, Type 1 Diabetes and Links to other Modern Illnesses. *An invited plenary paper to the International Diabetes Federation Western Pacific Congress, Wellington, 2 April 2008.*