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Short Communication

β casein Polymorphism in Indigenous and Exotic Cattle Breeds of Pakistan

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ABSTRACT

Milk composition depends largely on the breed's genetics and ration fed. Different proteins can be found in milk. Beta-caseins are thought to be more important because some serious health-related issues in humans have been reported with the consumption of A1 milk (mutated casein variant). This study was planned to investigate the polymorphism in the beta-casein gene (CSN2) in Sahiwal (40), American Holstein Friesian (40) and the crossbred (Sahiwal × HF) (50). PCR-RFLP and conformational sequencing were performed to investigate the beta-casein polymorphism. Results of the present study showed that there was not any mutated genotype (A1A1) available in all of the three breeds. All three breeds possess dominant genotype A2A2 with genotypic frequency (0.925, 0.4, 0.64) respectively and we have also found some heterozygous genotypes A1A2 in all breeds with genotypic frequency (0.075, 0.6, 0.36) respectively. Findings of the present study revealed that A2 (the dominant allele) is present with a pretty much higher frequency (0.9625, 0.7, 0.82) in all the three studied breeds and the mutated allele A1 is present with very low allelic frequency (0.037, 0.3, 0.18).

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Key words
DNA sequencing, Beta casein, Genetic variations, PCR-RFLP, A1 and A2 Genotype

Milk proteins constitute 3% of the total chemical components of milk. Caseins constitute 80% of total milk proteins (Ho *et al*., 2014). Three major types of caseins are found in milk which include alpha, beta and kappa casein. Beta-caseins are the most important proteins found in milk and share the major part about 30% of the milk protein (Ho *et al*., 2014; Malarmathi *et al*., 2014; Boro *et al*., 2016). There are twelve different genetic forms of beta-casein available, among those two A1 and A2 are more common and have an important role regarding milk production and the human health aspect (Farrell *et al*., 2004). In cattle, the dominant variant for beta-casein protein was A2 beta-casein. Many years ago, a natural mutation appeared in cows from European breeds which have resulted in another casein variant A1 beta-casein and producing A1 milk (Boro *et al*., 2016). Casein protein is composed of 209 amino acids and a change at 67th amino acid (proline to histidine) can cause A2 milk to A1 milk. Proline (CCT) is responsible for A2 milk and histidine (CAT) is responsible for A1 milk (Ul-Haq *et al*., 2014; Ho *et al*., 2014; Singh *et al*., 2015). Some other variants like B, C, D, E, F, G, H1, H2, and I are also found but are not as importance as A1 and A2 (Farrell *et al*., 2004).

Beta-casein variants A1 is responsible for causing serious health problems for human such as ischemic heart disease, arteriosclerosis, type 1 diabetes (DM-I) and unexpected infant death syndrome (Laugesen and Elliott, 2003; Birgisdottir *et al*., 2006; Caroli *et al*., 2009; Massella *et al*., 2017). Currently increased consumption of dairy products has been considered to be associated with serious health risks and worst symptoms of some disorders like gastrointestinal malfunction, immunity related issues and heart diseases (Barnett *et al*., 2014; Ul-Haq *et al*., 2014). Some of these effects are linked with beta-casomorphin-7, a peptide associated with A1 milk consumption. Beta-casomorphin-7(BCM-7) adversely affects the digestibility, reduces the production of lymphocytes and ultimately affects the immunity (Trompette *et al*., 2003; Zoghbi *et al*., 2005). The present study aims at investigating the beta-casein polymorphism in indigenous cattle Sahiwal, exotic breed American Holstein Friesian (HF) and crossbred (Sahiwal × HF). This study will help in selection of superior milk and breed improvement programs.

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Materials and methods

Whole blood samples (5 ml each) were collected from three cattle breeds-Sahiwal (n=40), HF (n=40) and Crossbred (Sahiwal x HF) (n=50). Sampling from Sahiwal cattle and Holstein Friesian (HF) was done at two Research and Training Farms at the University of Veterinary and Animal Sciences, Ravi Campus, whereas sampling from crossbred was done at Livestock Experiment Station, Qadirabad, Sahiwal. All unrelated animals were used for this study. Bovine gDNA was extracted using a standard protocol (Grimberg *et al*., 1989). PCR product was amplified using the following reported pair of primers (Mclachlan, 2006).

F 5′- CCT TCT TTC CAG GAT GAA CTC CAG G- 3′
R 5′- GAG TAA GAG GAG GGA TGT TTT GTG GGA GGC TCT- 3′.

PCR reaction mixture (15 µl) contained PCR grade water, dNTPs, $(NH_4)_2SO_4$ buffer, DNA, forward primer, reverse primer, and Taq polymerase. The following thermal cycle was followed: 94°C for 3 min; 94°C for 30 seconds, 30 repeats of 66°C for 30 second and 72°C for 30 second and final extension 72°C for 10 min.

For PCR-RFLP analysis, the PCR product (121 bp) was digested with 5 units of the *DdeI* enzyme overnight at 37°C. Agarose gel (4%) was used to visualize the digested fragments. Conformational sequencing was also performed to validate the RFLP results. Gene and genotypic frequencies were calculated using the pop gene 32 software (Yeh *et al*., 2000).

Results and discussion

Table I shows genotype frequencies of three breeds. It was observed that most of the animals in Sahiwal breed have A2A2 genotype but a few of these showed heterozygous genotype A1A2. We have not found any A1A1 genotype in the Sahiwal population. In the present study, the frequency of the A2A2 genotype was observed as 0.925, while A1A2 come up with a frequency of 0.075 in Sahiwal cattle. Whereas the allelic frequencies were 0.9625 for the A2 allele and 0.0375 for the A1 allele (Table I). The sequence result revealed that the selected herd of Sahiwal breed had no A1A1 genotype (Fig. 1). Some studies on their native breeds like. Kangeyam breed carried only A2 allele (Malarmathi *et al*., 2014), Zebu cattle carried A2 allele with frequency 0.987 (Mishra *et al*., 2009), Slovak spotted breed carried A2 with frequency 0.7072 (Miluchova *et al*., 2013).

RFLP analysis of HF shows that all the individuals possess two genotypes, dominant genotype A2A2 and heterozygous genotype A1A2 with the genotypic frequency of 0.4 (A2A2) and 0.6 (A1A2) respectively. We have not found any mutated A1A1 genotype for beta-casein protein

Table I. Genotypic frequencies of three cattle breeds.

Breeds	No. of samples	Genotyping frequencies			Allelic frequencies	
		A2A2	A1A2	A1A1	A1	A2
Sahiwal	40	0.925	0.075	0	0.037	0.9625
Holstein friesian	40	0.4	0.6	0	0.3	0.7
Crossbred	50	0.64	0.36	0	0.18	0.82

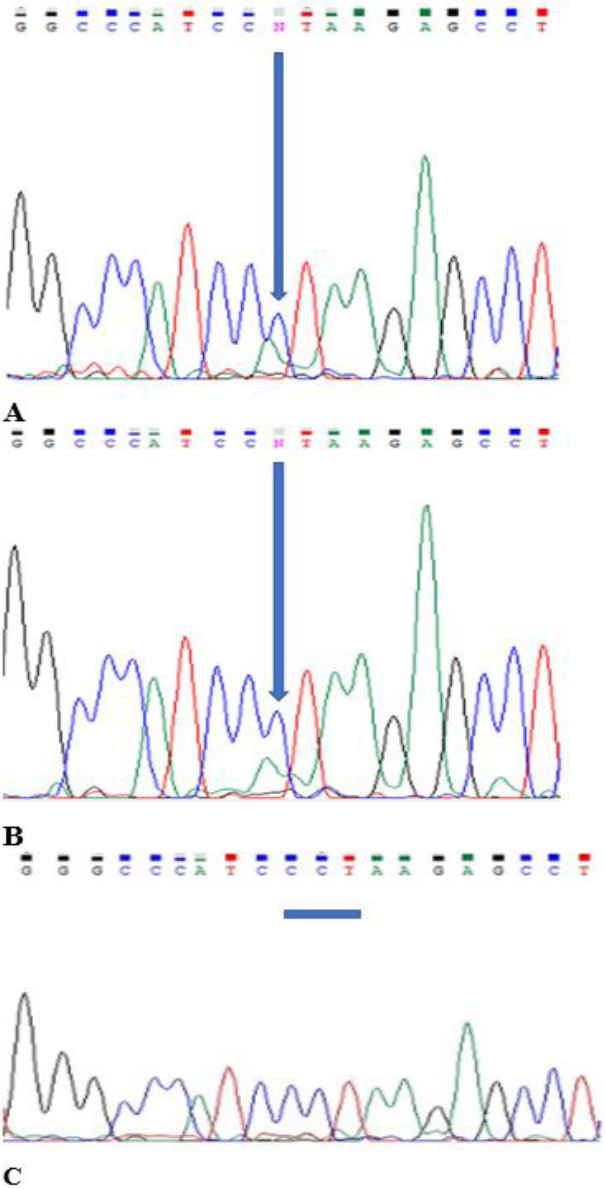

Fig. 1. Sequence confirmation of genotype of Sahiwal (A), Holstein Friesian (B), and Crossbred (Sahiwal × Holstein Friesian) (C). Chromatograms CSN2 gene showing genotype A1A2 (in A and B) and A2A2 (in C).

in the selected herd (Table II). Allelic frequency for beta-casein in Friesian cattle was observed as 0.3 (A1) and 0.7 (A2) (Table I). DNA sequence also revealed the absence of the A1A1 genotype in the selected herd of HF but the other two genotypes are available (A2A2, A1A2) (Fig. 1). Our results are partially supported by the findings of some researchers who reported that HF carried A2 as the abundant allele with frequency of 0.6322 but they also reported that HF carried all the three genotypes for beta-casein (A2A2, A1A2, and A1A1) with frequency of 0.4023, 0.4598, 0.1379 respectively (Miluchova *et al*., 2014).

Table II. Fragment size of different beta-casein (CSN2) genotypes after digestion of the PCR product (121 bp) with the *DdeI* restriction enzyme.

Geno-types	Fragment size	No. of genotypes		
		Sahiwal (n=40)	Holstein friesian (n=40)	Cross-bred (n=50)
A2A2	86 bp and 35 bp	37	16	32
A1A2	121 bp, 86 bp and 35 bp	3	24	18
A1A1	121 bp	0	0	0

A study by Malamarthi *et al*. (2014) reported that HF carried a high frequency of A2 allele (0.595) and low frequency of A1 allele (0.405). A study on German Friesian and Hungary Friesian partially supported the findings of the present investigation reported that German Friesian and Hungary Friesian carried allele A2 with a frequency of 0.496 and 0.470 (Mishra *et al*., 2009). A study also reported that Italian Friesians carried the highest frequency of A2 followed by A1. Dutch HF carried the highest frequency of A2 (0.692) followed by A1 (0.285), A3 (0.001) and B (0.022). In Swedish HF A2 (0.60) was predominant but A1 and B present in the lowest frequencies (0.34, 0.06) (Caroli *et al*., 2016). A study on Chinese Holstein revealed that these cattle have the highest frequency of A1A2 (0.353) genotype and the lowest frequency of A1 (0.030). The allele A1 and A2 present with frequencies 0.432 and 0.459 (Dai *et al*., 2016).

Our results showed that most of the Crossbred animals possess dominant beta-casein genotype A2A2. No animal comes up with mutated casein genotype A1A1 (Table II), a few heterozygous animals (A1A2) are present in selected crossbred herd. RFLP analysis of crossbred cattle showed that in cross-bred genotypic frequency of A2A2 type was 0.64 and for A1A2 type was 0.36, whereas the allelic frequencies for A2 allele was observed 0.82 and for allele, A1 was 0.18 (Table I). Conformational DNA Sequence results revealed that A1A1 genotype is not present in the selected herd of crossbred, but the other two genotypes are available (A2A2, A1A2) (Fig. 1). These results are partially supported by the findings of some researchers who reported that crossbred (Sahiwal × HF) possesses high frequency of A2 allele (0.65) and A1 present in low frequency (0.35). Ganguly *et al*. (2013) showed that Frieswal had three genotypes A2A2 (0.44), A1A2 (0.41) and A1A1 (0.15). Another study reported that in HF crossbred there was the superiority of A1 allele (0.6383) and A2 allele was present in lower frequency (0.3617). This crossbred carried two genotypes A1A1 and A1A2 with frequencies 0.28 and 0.72 (Shende *et al*., 2017). Frieswal heifers carried high frequency of A2 (0.68) and had all three genotypes A2A2, A1A2, A1A1 with frequencies 0.48, 0.40, 0.12 (Ganguly *et al*., 2013). Another study revealed that Brazil breeds Gir and Guzerá carried 0.98 and 0.97 A2 allele, 0.96 and 0.93 A2A2 genotype (Rangel *et al*., 2017). HF and Braunvieh maintained at Northern Italy (Emilia Romagna) carried an abundance of A2 allele (0.546) and A1A2 genotype (0.403) (Massella *et al*., 2017).

Conclusion

Beta casein is one of the major proteins found in milk and it has two important variants A1 and A2. The results from the present study showed that A2 was the dominant allele found in the studied population and we did not find any mutated genotype (A1A1). The majority of the studied animals from all three breeds possessed dominant allele A2 and dominant genotype A2A2.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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