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Original Research

Effect of α S1-Casein Variants on Milk Production Traits in Sahiwal Cattle

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Abstract

The study was conducted to find the association of α S1-casein locus with the milk performance traits in Sahiwal cattle. The identification of genotypes of α S1-casein locus was made with ARMS-PCR in 107 Sahiwal cows maintained in NDRI, Karnal. The association of genotypes with milk production traits was carried out using least square analysis with two-way classification of ANOVA after adjustment of data for non-genetic factor i.e. parity. The C allele was found in higher frequency (0.61±0.04) than that of B allele (0.39±0.04) in the population. Among the α S1-casein genotypes, CC was observed in 54 (0.50) animals whereas BC genotype was observed in 22 (0.21) animals. The genotype BB was found in 31 (0.29) animals in the population. The α S1-casein genotypes showed a non-significant (P<0.05) effect on all the parameters under study viz. milk fat %, SNF%, SNF yield and milk yield. The α S1-casein locus is less informative in the selection of animals for higher yields of fat, SNF and milk in the population under study since there was no effect of genotypes in milk performance traits.

Keywords: α S1-casein, ARMS-PCR, Genotypes, Milk Production Traits, Sahiwal

Introduction

Milk protein variants which are mainly affected by genetic factors (Sanchez *et al*., 2017) have an important influence on milk production traits (Yang *et al*., 2020). There are six major milk protein components *viz.* αS1-Casein, αS2-Casein, β-Casein, κ-Casein, α-Lactalbumin and β -Lactoglobin in bovine milk (Sanchez *et al*., 2017). The αS1-casein consists of 199 amino acids residues is a fraction which forms up to 40% of bovine caseins in milk (Mercier *et al*., 1971). So far 8 alleles were identified within the αS1-casein: A, B, C, D, E, F, G and H (Farrell *et al*., 2004). However, the most frequent alleles are B and C found both in dairy and beef cattle (Litwińczuk *et al*., 2006). The allele B at the 192 position of the polypeptide chain encodes glutathione, whereas the allele C encodes glycine. Variant A occurs sporadically. Alleles C, D and E were created due to the mutation of allele B (Erhardt *et al*., 1998). The αS1-casein genetic polymorphisms have been reported in many species, including goat (Carillier-Jacquin *et al*., 2016), sheep (Gencheva *et al*., 2020), buffalo (Cosenza *et al*., 2011) and cattle (Erhardt *et al.,* 1998). The αS1-Casein is the calcium-sensitive casein as they are precipitated in the presence of low concentrations of calcium and stably maintained in a micelle suspension as a result of their interaction with k-casein (Mackinlay and Wake, 1971). They show high polymorphism and different polymorphic forms produce different quantities of caseins in milk. Like other caseins, αS1-Casein also influence the quantity and quality of milk.

India and its neighbouring countries are homeland and origin for many of the elite breeds developed and presently available. Among these, Sahiwal is one of the important milch breeds of zebu cattle originated and available in India. The present study was undertaken to find out association between αS1-casein variants and production traits in Sahiwal cattle.

Materials and Methods

The study was conducted on Sahiwal breed of cattle maintained at Cattle Yard, National Dairy Research Institute, Karnal (NDRI). The lactating 107 cows were selected and used for DNA analysis. Blood samples were collected aseptically from jugular vein of the animals and DNA was isolated from blood following the standard phenol chloroform extraction protocol (Sambrook and Russel, 2000). The identification of genotypes of αS1-caseins locus was made with Amplification Refractory Mutation System – Polymerase Chain Reaction (ARMS-PCR). The tetra-primer ARMS-PCR method encompasses a deliberate mismatch at position –2 from the 3'-terminus. An extra destabilizing mismatch has been found to increase the specificity of classical ARMS-PCR (Bathelier *et al*., 1998). A 'strong' mismatch (G/A or C/T mismatches) at the 3'-terminus of an allele-specific primer will likely require a 'weak' second mismatch (C/A or G/T) and vice versa, whereas a 'medium' mismatch (A/A, C/C, G/G or T/T) at the 3'-terminus will likely require a 'medium' second mismatch. The αS1-Casein was genotyped using the tetra primers comprising OUTER (forward: 5´- TGCATGTTCTCATAATAAACC - 3' ; reverse: 5´- GAAGAAGCAGCAAGCTGG- 3´) and INNER (forward: 5´- CATTCCATTTCCTGTATAATG AGGCA - 3´; reverse: 5´- AATTCTAAGGAGAGTTTACAACAAAGACGC - 3´) (Rincon and Medrano, 2003). The PCR amplification was carried out in a 25 µl volume containing 2.5 µl of 10X PCR buffer, 2mM of $MgCl_2$, 200 µM of each dNTP, 5 pM each of outer and 12 pM each of inner primers, 2 U Taq DNA polymerase and 60 ng genomic DNA. The following stages were applied: at 95°C for 5 min, followed by 35 cycles of 95°C for 30 sec, 59°C for 45 sec, 72°C for 30 sec and final synthesis at 72°C for 10 min. The amplified DNA products were separated in 3% agarose gel in 0.5X TBE containing 1.0 µ M ethidium bromide and visualized and photographed under UV Transilluminator.

The observations on the yields of milk, fat and SNF were recorded and their percentages were estimated at day 50, 100 and 150 for the animals genotyped. These three test days represent the three stages of lactation. The average of these three test days were taken to find out the association of genotypes with the variation in production parameters. Subsequent to adjustment of data for non-genetic factor *i.e.*, parity, the association of genotypes with milk production traits was carried out using least square analysis with two-way classification of ANOVA according to Snedecor and Cochran (1996).

Results and Discussion

ARMS-PCR Genotyping of αS1-Casein

The genotypes of αS1-casein locus were detected from DNA by ARMS-PCR using two primer pairs of OUTER

and INNER. There were two mismatches at –2 in 3' ends in both primers. Two alleles were observed due to mutation on position 100 of amplicon. The C allele was produced due to mutation in nucleotide position 1956 from Argenine (A) to Guanine (G).

The amplified products of genotypes BB, BC and CC animals yielded two (310 and 236 bp), three (310, 236 and 130 bp) and two (310 and 130 bp) fragments, respectively (Figure 1). In the present study, C allele was found in higher frequency (0.61±0.04) than that of B allele (0.39±0.04) in the population (Table 1). As shown in table 1, among the αS1-casein genotypes, CC was observed in 54 (0.50) animals whereas BC genotype was observed in 22 (0.21) animals. The genotype BB was found in 31 (0.29) animals in the population. The present findings are in agreement with the reported predominance of the αS1-casein C allele in zebu population contrast with the high frequency of the αS1-casein B allele in *Bos taurus* breeds (Hristov *et al*., 2013; Mir *et al*., 2014). Baker and Manwell (1991), however, a higher frequency close to 0.9 was reported for the C variant in *Bos indicus* while in *Bos taurus* its frequency ranges from 0.2 to 0.4. Similarly, Silva and Del Lama (1997), through horizontal electrophoresis on starch gel, observed the frequency of αS1-casein C allele between 0.864 and 1.00 in zebu cattle raised in Brazil (Gir, Guzerat, Sindhi and Nellore). The fixation of αS1-casein C allele was reported in Nellore cattle. The αS1-casein B allele occurred with higher frequencies in taurine breeds (0.51 to 1.00), conversely, αS1-casein C allele was predominant in zebu breeds with the frequencies between 0.56 and 0.75 (Cerriotti *et al.*, 2004). Oner and Elmac (2006) found very high frequency (0.862) of αS1-casein B allele in Holstein cows. This is in agreement with our present finding. The other variant αS1-casein D allele was reported in Jersey cows from the Netherlands by Corradini (1969). This variant was not found in Sahiwal population. This asymmetric distribution in zebu and European cattle has been explained by the different processes of domestication to which these animals were submitted (Grosclaude *et al.*, 1974).

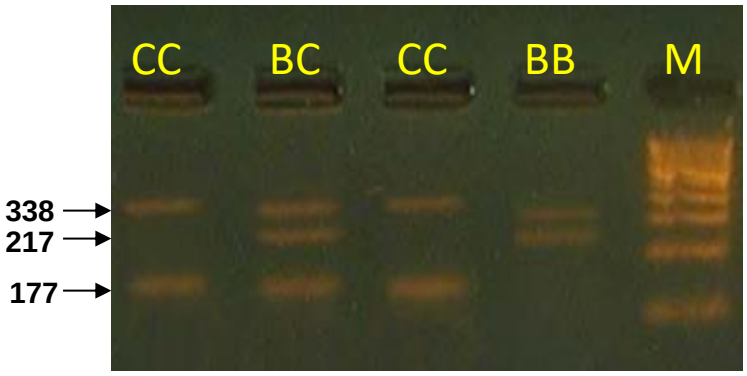

Figure 1: Genotypes of αS1-casein gene revealed by ARMS-PCR in 3% agarose gel

The genotypic frequencies observed in αS1-casein locus in the population were not within the Hardy-Weinberg expectation.

Association of αS1-Casein Genotypes on Milk Production Traits

Data analysis was done using least square technique with three-way classification of analysis of variance for non-genetic factors viz. season and parity. The effect of season was classified into four groups as: Winter (Nov- March), Summer (April-June), Rainy (July- August) and Autumn (September-October). The effect of parity on milk production parameters was accounted up to fourth lactation.

Table 1: Allele and genotypic frequencies of αS1-casein in Sahiwal cattle

	Type	Number	Frequency	Observed Heterozygosity	Expected Heterozygosity	Chi-square value
Allele	B	83	0.39±0.04	0.21	0.48	26.07*
	C	131	0.61±0.04			
Genotype	BB	31	0.29			
	BC	22	0.21			
	CC	54	0.5			

**significant at p>0.05*

The F ratios for the least square ANOVA for the means of three test days i.e., 50th, 100th and 150th showed significant effect on fat percentage and non-significant effect on rest of the milk performance traits (Table 2). Further analysis for fat percentage was conducted separately for test days whereas mean record was used for SNF percentage, and yields of fat, SNF and milk. The raw data for fat, SNF and milk yield were adjusted for the non-genetic factor *i.e.*, parity which show the significant effect on fat, SNF and milk yield (P<0.05).

Table 2: F ratios for the least square ANOVAs for the means of three test days (TD: 50th, 100th and 150th days) milk yield and milk compositions in Sahiwal cattle

SV	df	Fat%	SNF%	Fat Yield	SNF Yield	Milk yield
TD	2	4.840**	0.532	1.852	1.807	1.803
Season of Calving	3		1.938	2.248	2.008	2.19
Parity	3		1.867	2.914*	3.322*	3.357*
αS1-Genotype	2	0.619 (50 days), 0.233 (100 days), 0.251 (150 days)	0.519	0.089	0.136	0.1

**P<0.05; **P<0.01*

The effect of milk protein genotypes on production traits was carried out using least square analysis with two-way classification of ANOVA. The statistical analysis showed a non-significant (P<0.05) effect of αS1-casein genotypes on all the parameters under study *viz*. milk fat%, SNF%, SNF yield and milk yield. Further, there were no significant differences between αS1-casein genotypes and fat percentage for three test days *i.e.*, 50th, 100th and 150th. Similar to present finding, in Sahiwal cattle of Pakistan all three genotypes (BB, BC and CC) at αS1-casein locus had no effect (p > 0.05) on milk 1st lactation, 2nd lactation (Mir *et al*., 2014). In the previous study, αS1-casein BB genotype correlated with higher milk production than those with either AB or BC genotype (Ng-Kwai-Hang *et al.*, 1984; Aleandri *et al*., 1990). The findings of Hristov *et al*. (2013) also agree with the dominance of the B allele over the C allele in relation to milk production in Bulgarian Rhodopean cattle. On the other hand, Miciński *et al*. (2007) reported superiority of the heterozygous BC genotype with reference to milk yield in Jersey cows.

There was no significant association between αS1-casein genotypes and milk performance traits. Therefore, αS1-casein locus is less informative in the selection of animals for higher yields of fat, SNF and milk in the population under study. However, it may be further confirm using more number of animals and the locus may be tested for association with other milk performance traits.

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Conflict of Interests

There is no conflict of interest.

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