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Genetic polymorphism of milk protein and its effects on milk composition traits in Muzaffarnagari sheep

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Milk protein genetic polymorphisms have received considerable research interests in animal breeding because of their relationships with production traits, milk composition and milk quality (Mele *et al.* 2007, Dario *et al.* 2008). Milk protein genetic variants differ from breed to breed in their occurrence and frequency. Information on the gene frequencies of milk protein genetic variants in the population is required for breed characterization of any species at genetic level. There is no information available on milk protein variants of Muzaffernagari sheep. The present study was undertaken to determine/assess the various milk protein variants and to study the effect of various milk protein variants on casein and total protein content of milk in Muzaffarnagari sheep.

Individual milk samples from 86 lactating Muzaffarnagari ewes maintained at the Central Institute for Research on Goats, Makhdoom, Mathura, were collected and stored at –20°C after collection for further use. SDS-PAGE was carried out in skimmed milk samples and was analysed by means of SDS (Grosclaude and Martine 1997) and urea (Medrano and Sharrow 1989) PAGE and alkaline pH. Gels were stained with Coomassie Brilliant Blue. Milk protein variants were determined by the molecular weight in gel documentation system. The casein content in milk was estimated by Walker-formal casein test as described by Scott (1986). The total proteins in milk were estimated by Lowry method using Folin-ciocalteu's phenol reagent (Lowry *et al.* 1951). POPGENE software (Yeh *et al.* 1999) was used to estimate the allelic frequencies and to verify Hardy-Weinberg equilibrium. The standard error (SE) of the allele frequencies was calculated according to the formula as suggested by Weir (1996). To determine the effect of milk protein variants on total protein and total casein content of milk, the data were analyzed using the mixed model least-squares analysis for fitting constants (Harvey 1990) considering different fixed effects (viz. αs_1 casein, αs_2 casein and ê casein genotypes) in the model.

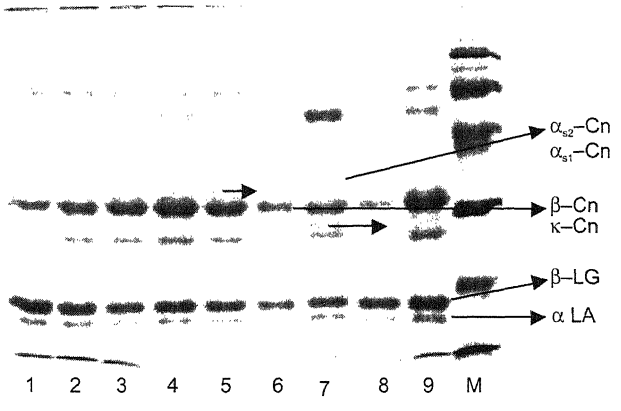

Fig. 1. SDS-polyacrylamide gel electrophoresis of milk protein variants of Muzaffanagari sheep, M = Marker

The electrophoretic pattern revealed that the milk sample of this breed contained four major casein variants, i.e., α_{S1}-casein, α_{S2} - casein, β-casein, and κ-casein and two whey proteins, viz. β-lactoglobulin and α-lactalbumin (Fig. 1). The frequencies of different genotypes and alleles of milk protein variants were estimated from SDS-PAGE data (Table 1). Three α_{S1}-casein variants namely α_{S1}-Cn^A, α_{S1}-Cn^E and α_{S1}-Cn^F were observed in this study. The predominant gene of α_{S1}-casein in this breed was α_{S1}-Cn^E with a frequency of 0.38±0.05, whereas the α_{S1}-Cn^A had a frequency of 0.24±0.09. The frequency of null allele (α_{S1}-Cn°) in the population was 0.37±0.06 in this study. In the genotypic frequencies (Table 1), homozygous EE accounted for 26.7% of the population, followed by heterozygous AE with 22%, homozygous AA with 11.6% and heterozygous AF with 2.3%. The rare α_{S1}-Cn^F variant occurred in the milk of this breed with a frequency of 0.01±0.05 (Table 1). Similarly, the presence of F allele as heterozygous AF form as well as existence of null allele in α_{S1} locus was also observed in Indian goat breeds (Kumar *et al.* 2008). Our study showed that the population was in genetic disequilibrium with regards

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Table1. Genotypic and allelic frequencies of milk protein variants in Muzaffarnagari sheep

Locus	Genotyping frequency						Allelic frequency				
	AA	AE	EE	AF	AB	OO	A	E	F	B	O
a_{S1} Cn	0.12(10)	0.22(19)	0.27(23)	0.02(02)	-	0.37(32)	0.24[0.09]	0.38[0.05]	0.01[0.05]	-	0.37[0.04]
a_{S2} Cn	0.33(20)	-	-	-	-	0.77(66)	0.23[0.07]				0.77[0.04]
b Cn	1.00(86)	-	-	-	-	-	1.00				
k Cn	0.51(44)	-	-	-	-	0.49(42)	0.51[0.05]				0.49[0.05]
b LG	-	-	-	-	1.00(86)	-	0.50			0.50	
a LA	1.00(86)	-	-	-	-	-	1.00				

"O" represents the absent of allele (null allele)

Figures in parentheses and brackets are number of animals and estimates for standard errors, respectively.

to αs_1-Cn locus and it may be ascribed to small number of observation. No evidence of polymorphism with respect to α_{S2}-, β- and κ-casein locus was observed in the present study. A number of polymorphism studies on ovine in different breeds have been reported by various workers (Pirisi *et al.* 1999, Amigo *et al.* 2000, Moioli *et al.* 2007). The electrophoretic pattern of β-lactoglobulin showed the presence of A and B variants on the gel but expressed in the heterozygous form in our study. Ivankovic and Dovc (2004) also observed A and B variants of β-lactoglobulin in other sheep breeds. The monomorphic form of α-lactalbumin variant existed in all the samples under study and it was observed in homozygous (AA) state. All the loci except β-Cn, β-lactoglobulin and α- lactalbumin locus in the population showed absence of allele (null allele) in the present investigation.

The overall least-squares means of casein and total protein content in milk of this breed for different casein protein variants was 5.03±0.09% and 3.02±0.13 g/litre, respectively. The frequency of a_{S1}-Cn^{AF} genotype was too small and hence excluded from the analysis. The present study showed that only α_{S1} casein had significant (P<0.01) effect on casein content of milk in Muzaffarnagari sheep. The ewes having a_{S1} Cn^{AE} variant had higher casein content in their milk as compared to ewes having a_{S1}-Cn^{AA} variant ((5.25±0.12% vs. 4.64±0.16%, respectively). Significantly (P<0.01) higher casein content was also observed in ewes not having a_{S1} variant (a_{S1}-Cn°°) as compared to those having a_{S1}-Cn^{AA} variant. EE milk was also higher in casein content than AE milk (4.86±0.12% vs. 5.25±0.12%, respectively). However, no significant (P>0.05) differences in casein content of milk between animals having a_{S1}-Cn°° and a_{S1}-Cn^{AE} or a_{S1}-Cn^{EE} variant were observed. Significant effect of a_{S1} casein locus on casein content of milk was reported in other breeds of sheep (Pirisi *et al.* 1999). None of these milk protein variants under study had significant effect on total protein content of milk in Muzaffarnagari sheep.

SUMMARY

The electrophoretic pattern revealed the presence of three α_{S1}- casein alleles, viz. α_{S1}-Cn^{A}, α_{S1}-Cn^{E} and α_{S1}-Cn^{F} in the milk samples of Muzaffarnagari sheep. The frequency of predominant α_{S1}-Cn^{E} locus and rare α_{S1}-Cn^{F} variant in this population were 0.38 and 0.01, respectively, where the frequency of null allele for this locus (α_{S1}-Cn°) was 0.37. All the loci except β-Cn, β-lactoglobulin and α- lactalbumin locus in the population showed the presence of null allele. No polymorphic patterns were observed at α_{S2}-, β- and κ - casein locus in this study. The β- lactoglobulin loci showed the presence of A and B variants on the gel. The α-lactalbumin loci revealed the monomorphic form in the population. The study also showed that α_{S1}- casein variant had only significant effect on casein content of milk and none of these milk protein variants had significant effect on total protein content of milk in this breed.

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