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Analysis of the milk protein polymorphism in Muzaffarnagari sheep

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Abstract

In the present investigation, Whole milk was taken out in 50 ml in a sample. Total 26 milk samples were taken. The predominant allele of ás1-casein in Muzaffarnagari sheep was á$_{s1}$-cn^{B}, with a frequency of 0.288, whereas the frequency of ás1-cn^{A} and á$_{s1}$-cn^{E} were, 0.634, 0.076, respectively. The observed heterozygosity for ás1-casein was 0.269 whereas the expected heterozygosity was 0.518. Two variants A and B were observed. The allele frequency of -cn^{A} variant was 0.884 whereas the allele frequency for -cn^{B} variant was 0.115, for -cn the values of observed and expected heterozygosity were 0.230 and 0.208, respectively. In á-LA the observed and expected heterozygosity was similar (0.038). In the present investigation, the monomorphic pattern was observed in the â-LG locus. All samples showed AA variant. Hence, the allele frequency of A variant for â-LG locus was 1.00 and the observed and expected heterozygosity was 0.000. The overall least-square mean for total protein content, fat content, SNF, density, lactose and ash were 3.89±0.12%, 7.26±0.39%, 11.24±0.35%, 36.31±1.22%, 6.03±0.19% and 0.88±0.03%, respectively. The animals having ás$_1$-cn-BE variant showed significantly higher total protein content their milk than those of animals having ás$_1$-Cn-AA and AB variants.

Key words:

Muzaffarnagari sheep, polymorphism and milk protein

Introduction

Sheep is one of the important species of livestock because of its multi-facet utility for wool, mutton, milk, skin and manure. Sheep rearing contributes greatly to the agrarian economy, especially in areas where crop and dairy farming are not economical and play an important role in the livelihood of a large proportion of small farmers and landless laborers. Gel Electrophoresis (SDS-PAGE) technique uses random primers to amplify unknown genomic sequences. The above technique also finds applications in marker assisted selection, parentage analysis, breed identification and population genetic studies. The knowledge of genetic similarity is useful in a breeding program because it facilitates efficient sampling and utilization of germplasm resources. The breeder can use genetic similarity information to make informed decisions regarding the choice of genotypes to cross for the development of breeds or to facilitate the identification of diverse parents to cross in hybrid combinations in order to maximize the expression of heterosis. The information about genetic similarity within a sheep population can be useful for the farmers because by using such information they can raise genetically superior animals with improved production traits and in turn increase their profitability. Muzaffarnagari sheep is one of the tallest and heaviest mutton breeds of India. It produces

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comparatively heavier carcass witch fetches higher price in the market.

Materials and Methods

The present investigation was carried out in the Genetics and Breeding Division, Central Institute for Research on Goats, Makhdoom, Farah-281122, Mathura, (U.P.).

Experimental animal

Muzaffarnagari sheep is one of the tallest mutton breeds of India. The animals of this breed are primarily maintained for mutton purpose, though it also produces fleece of coarse carpet quality. The breed id known for faster growth rate and mothering ability. The overall body weight of flock under semi-intensive feeding management is 32.0kg at 12 month of age. However, the average body weight elite rams being used in the breeding programmed of the project weighing above 50.0kg. Few breeding rams are also being utilized in the project, which are weighing 70.0kg weight. The average greasy fleece weight is around 1.1kg. Annually this is less than in other sheep breed of this region i.e. northwestern arid and semi arid region. The average age at first breeding of male was 10 -14 months and a breeding life 5-6 years Average age at first lambing in ewes was 16-18 months. Lambing rate ranged from 60-95% with lambing interval of 6-8 months. Muzaffarnagari ewes produce 8-12 lambs in their lifetime. The milk yield was 300-500 ml in lactation length of 120-180 days .The breed purity has been diluted to nearly 40 %, mainly due to the non-availability of good breeding rams and lack of grazing land. The current situation and status of the breed, warrants its in situ conservation.

Collection of milk samples

The milk samples were collected at morning as well as in evening. Whole milk was taken out in 50 ml of sample tubes after washing the udder and teats with potable water and moping with cotton cloth. A total of 26 milk samples collected from Muzaffarnagari sheep flock maintained under Network Project on Sheep Improvement at Central Institute for Research on Goats, Makhdoom. The collected milk samples were stored at -20 ºC for the further used. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) method is used to detect genetic variants in milk. Milk protein band pattern were studied on 14% Polyacrylamide gel electrophoresis (Laemmli, 1970).

Statistical analysis

The current version (version 1.30) is designed specifically for the analysis of co-dominant and dominant markers using haploid and diploid data. It performs most types of data analysis encountered in population genetics and related fields. It can be used to compute the summary statistics (e.g., allelic frequency, gene diversity, genetic distance, F-statistics, multi-locus structure etc.) for i) single-locus, single population; ii) single-locus, multiple populations; iii) multi-locus, single population and multi-locus, multiple populations. Alternatively, to verify the results obtained by using POPGENE software, the allelic frequencies of different variants were calculated by the following formula as described by Mahe and Grosclaude (1993).

Results and Discussion

The results of the present investigation have been presented and discussed in the following orders as to confirm the objective of the study. Milk Samples of 26 Muzaffarnagari sheep were analyzed to study the genetic variants of milk protein of this breed. The separation of genetic variants of $á_{s1}$- casein, $ás_2$-casein, â-casein, 8-casein, â-lacto globulin and á-lactalbumin in milk of Muzaffarnagari sheep were carried out by SDS-PAGE (Sodium dodecyl sulphate polyacrylamide gel electrophoresis) method. The distribution of genetic variants of milk protein are presented in Table 2.

Table 1. Heterozygosity (obs. and exp.) at different locus.

Locus	Heterozygosity	
	Observed	Expected
CAS 2	0.5000	0.4487
CAS 1	0.2692	0.5181
CAS-B	0.2308	0.2081
CAS-K	0.2308	0.2081
α-LA	0.0385	0.0385
β-LG	0.0000	0.0000

Table 2. Genotype distribution and Hardy-Weinberg equilibrium test for the different milk protein loci.

Locus	Genotypes	Heterozygosity		χ^2-value
		Observed	Expected	
CAS 2	AA	11	11.66	0.0381
	AB	13	11.66	0.15
	BB	2	2.66	0.16
CAS 1	AA	15	10.35	2.08
	AB	3	9.70	4.63
	BB	4	2.05	1.83
	BE	4	1.17	6.77
CAS-β	AA	20	20.29	0.00
	AB	6	5.41	0.06
CAS -κ	AA	20	20.29	0.00
	AB	6	5.41	0.06
β-LA	AA	25	25.00	0.00
	AB	1	1.00	0.00
α-LG	AA	1	1.00	1.00

Table 3. Least squares means of different milk contents.

Parameter	Least Square Mean
Fat	7.26 ± 0.39%
SNF	11.24± 0.35%
Density	36.31± 1.22%
Protein	3.89± 0.12%
Lactose	6.03± 0.19%
Ash	0.88± 0.03%
Temp.	28.63± 1.30%

Casein region

In the present study, three ás1-casein variants namely A, B and E were observed. Out of 26 milk samples 15 milk samples showed AA variants, 4 milk samples showed BB variants, 4 milk samples showed BE variant and 3 milk samples showed AB variants. The predominant allele of ás1-casein was $á_{s1}$-cn^B, with a frequency of 0.288, whereas the frequency of ás1-cn^A and $á_{s1}$-cn^E were, 0.634, 0.076, respectively. The observed heterozygosity for ás1-casein was 0.269 whereas the expected heterozygosity was 0.518.

Out of 26 milk samples, 13 milk samples showed AB variant and 11 milk samples showed AA variant and only 2 milk samples showed BB in $á_{s2}$ casein. The predominant allele of $á_{s2}$ casein locus was A with a frequency of 0.673 where as the frequency of B was 0.326. For $á_{s2}$ casein, the values of observed and expected heterozygosity were 0.500 and 0.448, respectively. In the present study, the polymorphic pattern was observed in the â-cn locus for Muzaffarnagari sheep. Two variants namely, A and B were observed. The allele frequency of â- cn^A variant was 0.884 whereas the allele frequency for â- cn^B variant was 0.115. In â-cn the observed and expected heterozygosity of â-cn were 0.230 and 0.208, respectively. In the present Investigation, the polymorphic pattern was observed in the ê-cn locus for Muzaffarnagari sheep. Two variants A and B were observed. The allele frequency of ê-cn^A variant was 0.884 whereas the allele frequency for ê-cn^B variant was 0.115, for ê-cn the values of observed and expected heterozygosity were 0.230 and 0.208, respectively.

Discussion

Casein region

Regarding the allelic variants at $á_{s1}$-cn locus, there are 16 co dominant alleles, A, B_1, B_2, B_3, B_4, C, D, E, F, O_1 and O_2 (Grosclaude and Martin, 1997), H, I, L (Chianese *et al., 1997),* M (Bevilacqua *et al.,* 2002) and N (Ramunno *et al., 2005)* were reported in different breeds of goats. In a study, Di-Stasio *et al.* (1983) analyzed 990

milk sample of Sardinian goat and reported the gene frequencies of á$_{s1}$-Cn^{A}, á$_{s1}$-Cn^{B} and á$_{s1}$-Cn^{C} as 0.64, 0.16 and 0.20, respectively. Grosclaude *et al.* (1987) reported that the amount of total casein in caprine milk was positively correlated with the amount of á$_{s1}$-casein and á$_{s1}$-casein was highest in case of A, B and C allele. The á$_{s1}$-cn F variant was discovered by Grosclaude *et al.* (1987) and suggested that the á$_{s1}$-cn F allele was responsible for the production of low á$_{s1}$-casein in goat milk. The frequency of á$_{s1}$-Cn^{F} locus was lower in Spanish breeds (0.08, 0.04, 0.0 and 0.0 for Murciano-Granadina, Malaguena, Payoya and Canaria, respectively) while the E allele was predominant in Murciano- Granadina (0.59), Malaguena (0.65) and Payoya (0.76) breeds (Jordana *et al.*, 1996). The gene frequencies for of á$_{s1}$-Cn^{A} and á$_{s1}$-Cn^{F} in local goat in the present study was well comparable with the findings observed by Prakash *et al.* (2002) and Kumar (2005) in different Indian goat breeds. Not much work has been done in Indian sheep breeds.

On the other hand, Sacchi *et al.* (2005) identified association of eight alleles with three synthesis levels. Three variants (A, B, C) were described by Boulanger *et al.* (1984) and Bouniol *et al.* (1994), which differed by amino acid substitution (Martin and Addeo, 1995). Variants A, B, C, E (Lagonigro *et al.*, 2001), F (Ramunno *et al.*, 2001) and G (Erhardt *et al.*, 2002) are associated with normal synthesis levels, whereas D and O are associated with lower and null synthesis level. Boulanger *et al.* (1984) has described á$_{s2}$- Cn^{A} and á$_{s2}$-Cn^{B} allele in goats. The á$_{s2}$-Cn^{A} was found to be predominant in Alpine (0.85) and Saanen (0.87) breeds. Russo *et al.* (1986) reported that the two alleles (á$_{s2}$-Cn^{A} and á$_{s2}$-Cn^{B}) polymorphism was widely distributed in goat population. In a study on milk protein polymorphism in Indian goat breeds, Prakash *et al.* (2002) reported that in á$_{s2}$-Cn locus, the most prevalent variant was A in all the Indian goat breeds and mostly observed as homozygous AA. In his study, he observed that the genotypic frequency of á$_{s2}$-Cn^{A} was almost similar in three breeds i.e. 0.75, 0.78 and 0.72 in Barbari, Marwari and Sirohi goats, respectively. However, it was observed lower in case of Jamunapari (0.65) and Jakhrana goat (0.57). The other á$_{s2}$-Cn variant B was found as homozygous (BB) in Jamunapari while it was in heterozygous (AB) form in Barbari and Marwari goat breeds. The C variant of á$_{s2}$-Cn was rare and expressed as heterozygous (AC) in Jamunapari goat. The heterozygous BC was also observed in Marwari, Kutchi and Jakhrana goat. Similarly, Kumar (2005) observed two alleles at á$_{s2}$-Cn locus in Indian goat breeds and variant á$_{s2}$-Cn^{A} was predominant in all the Indian goat breeds. The frequency of á$_{s2}$-Cn^{A} allele in Jamunapari, Barbari, Marwari, Sirohi, Jakhrana, Beetal, Ganjam and non-descript goats from U.P. and M.P. was 0.78, 0.65, 0.96, 0.73, 0.96, 0.82, 0.85, 0.95 and 0.53, respectively. The distribution of ás2-Cn^{B} allele was very low in all the Indian goat breeds.

The â-Cn had for a long time been considered to be monomorphic until different authors indicated the existence of a null phenotype in some breeds of goats (Dall'Olio *et al.*, 1989 and Mahe and Grosclaude, 1993). Further studies indicated that three variants A, B (Mahe and Grosclaude, 1993) and C (Neveu *et al.*, 2002), differing by a single amino acid substitution, are associated with normal â-casein content. Dall' Olio *et al.* (1989) did not found â-casein bands in some of the individual of Garganica breed. Milk lacking of â-casein was described by Ramunno *et al.* (1995), using polyacrylamide gel electrophoresis (SDS-PAGE). The evidence of a null allele for â-casein was also noted in Cores goats and in Creole goats of the Guadalupe (Mahe and Groscluade, 1993). Prakash *et al.*, (2002) reported that the AA variant was observed as homozygous AA for â-casein locus in all the five Indian goat breeds. They also reported that the gene frequency of â-casein O ("null") allele in Jamunapari, Barbari, Sirohi and Jakhrana goats were 0.22, 0.11, 0.30 and 0.15, respectively. Kumar (2005) observed that at â-casein locus, â-Cn^{A} variant was dominated in goat breeds of India and the frequency of â-Cn^{A} in Jamunapari, Barbari, Marwari, Sirohi, Jakhrana, Beetal, Ganjam and non-descript goats from U.P. and M.P. was 1.00, 0.98, 1.00, 0.94, 1.00, 1.00, 0.98 and 1.00, respectively. Allele â-Cn^{B} was not observed in any milk samples of Indian goats. The â-Cn^{O} (null alleles) was observed in Barbari, Sirohi, and non-descript goats from U.P.

The structural analysis of the ê-casein locus in different goat breeds showed the existence of a biallelic polymorphism (Russo *et al*; 1986, Di Luccia *et al.*, 1990 and Caroli *et al.*, 2001). Russo *et al.* (1986) reported that two variants (A and B) exist in the N- terminal region of Para ê-casein in different goat breeds. Prakash *et al.* (2002) reported the homozygous (AA) form of 8-Cn locus in Jamunapari, Barbari, Sirohi and Jakhrana, Ganjam and non-descript goats from U.P. The frequency of variant ê-Cn^A was observed in Barbari, Marwari, Beetal and M.P. local goat as 0.97, 0.96, 0.97 and 0.96, respectively. The frequency of null allele for this locus was highest in U.P. local goat and lowest in Jakhrana goat.

Conclusion

In present investigation the overall least-square mean for total protein content, fat content, SNF, density, lactose and ash were 3.89±0.12%, 7.26±0.39%, 11.24±0.35%, 36.31±1.22%, 6.03±0.19% and 0.88±0.03%, respectively. The animals having $ás_1$-cn-BE variant showed significantly higher total protein content their milk than those of animals having $ás_1$-Cn-AA and AB variants. The findings of present study suggested that the milk protein variant in Jakhrana goat breed might be selection criteria to high total protein content in milk. As the experiment was conducted based on small number of animals hence, further research may be carried out with large number of samples to confirm the results and to draw valid coclusion in this aspect.

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