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Milk protein polymorphism in Jamunapari Goats

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Abstract

In this study out of 27 samples were for casein estimation in which four ás1-casein variants namely A, B, E and F were observed. Out of total milk sample analyzed, 14 milk samples showed BE variants, 7 milk samples showed AF variants, 5 milk samples showed AB variant and only 1 milk sample showed AA variant. The predominant allele of ás1-casein in Jamunapari goat was ás1-cnB, with a frequency of 0.37; whereas the frequency of ás1-can, ás1-cnE and ás1-cnF were, 0.24, 0.25 and 0.12, respectively. The observed heterozygosity for ás1-casein in Jamunapari goat was 0.77 whereas the expected heterozygosity was 0.73.Out of 27 milk samples 21 milk samples showed AA variant and only 6 samples showed AB variant in ás2 casein. The predominant allele of ás2 casein locus in Jamunapari goat was A with a frequency of 0.88 whereas the frequency of B was 0.11. For ás2 casein, the values of obs. and exp. heterozygosity were 0.22 and 0.20, respectively. In the present investigation, carried out on 27 milk samples of Jamunapari goats no polymorphism of â-Cn locus was observed. Out of 27 samples all samples contained the â-Cn variant A and expressed in homozygous form in the population. Hence the allelic frequency of A variant for this locus was 1.00.In case of â-Cn the values of obs. and exp. heterozygosity was found 0.In the present study, no polymorphism of -Cn locus was observed. Out of 27 milk samples all samples contained the -Cn variant A and expressed in homozygous form in the population. The allelic frequency of a variant for -Cn locus was 1.00. In case of -Cn the values of obs. and exp. heterozygosity was found 0. In the present studies, the polymorphic pattern was observed in the â- LG locus for Jamunapari goats. Two variants namely, A and B were observed. The allele frequency of â- LG A variant was 0.87 whereas the allele frequency for â- LG B variant was 0.12. Results of the present study suggested that the milk protein variant in Jamunapari goat breed might be selection criteria for selecting high total protein content in milk.

Key Words:

Jamunapari Goat, Milk Protein, Genetic Polymorphism.

Introduction

The Jamunapari or Etawah goat (*Capra hircus*) is named after an area beyond the river Jamunawhere the breed was developed and is a dual purpose milk-and-meat goat breed which is mainly found in the undulated land of Chakarnagar between the ravines of theJamuna (Yamuna) and Chambal rivers in the Etawah districtof the Indian state of Uttar Pradesh (Acharya, 1982).The predominantly white-haired Jamunapari goat is probablythe tallest and most handsome of Indian goat breeds (Singh, 1966), the

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other distinguishing feature of the breedbeing a thick growth of long hair on the hindquarters. Theyhave a large body, long and wide pendulous ears, a pronouncedRoman nose and parrot mouth, and short and flathorns. The breed is a prolific and non-seasonal breeder(Devendra, 1985) and females possess large udders with big teats and are well known for milk production because they produce the highest amounts of milk of all Indian goat breeds, milk yield ranging from 1.13 to 3.63 kg/day or about 200 kg per lactation (Acharya, 1982, Rout *et al.*, 2004)

Goat milk contains a very heterogenous mixture of proteins. Milk proteins are divided in to two major groups i.e. casein and the whey protein. The goat milk contains four caseins (ás1-casein, ás2-casein, â-casein and ê-casein) and two whey proteins (â-lactalbumin and á-lactalbumin), which contributes 95% of the total milk proteins and are synthesized under complex hormonal system. There are four casein genes, namely ás1-casein, ás2-casein, â-casein, ê-casein and two whey protein genes, namely â-lactalbumin and á-lactalbumin; with their variants (Ferrantti et al., 1983).

The genetic variant of milk protein is a heritable trait and they are governed by co-dominant autosomal genes. These genetic variants are used as markers for the Mendelein segregation analysis (Grosclaude et al., 1987, Ferretti et al., 1990). The genetic variants of milk protein give considerable variation between species in their occurrence and frequency, which can be exploited for processing and human nutrition.

Genetic polymorphism in the milk protein is usually a consequence of mutation which results in changes in nucleotide sequence of particular gene involved and there by different amino acid sequence will result. The specific functions of milk protein, such as water binding, gelatin, and emulsification and foaming, are directly related to the physio-chemical characteristics of the protein. Caseins, a coagulable milk protein, are the most abundant milk proteins in ruminants. Caseins polymorphism is directly related with the quality, quantity and properties of proteins as well as milk production. The milk casein loci are highly polymorphic in nature. The ás1-casein is responsible for curd firmness, for cheese making, improver thermal stability and increases calcium content. Besides, genotyping of milk ás1- casein will act as marker for producing desired type of milk by genetic manipulation (Leroux, 1990). Manipulation of milk protein gene improves the nutrition quality of milk. The â-casein is used as a raw material for producing â-casomorphin (a hepto-peptide), which is similar to opiates used in sheep in insulin secretions (Maubois, 1984). Similarly in ê-casein concentration enhances stability of casein aggregates, decreases micelle size and decreased coagulation. Milk protein genetic polymorphisms have received

considerable research interests in recent years because of possible associations between milk proteins genotypes and economically important traits in dairy animals.

Many research reports have indicated the possible relationship between the genetic variants of milk proteins genes and milk related traits (Amigo et al., 2000; Bevilacqua et al., 2002; Feligini et al., 2005 and Kumar et al., 2008). Therefore, milk protein genes might be useful as genetic markers for additional selection criterion in dairy animal's breeding. The possibilities for genetically improving the population for a single gene are determined in part by the frequencies of the alleles. In our country there is no literature is available on milk polymorphism in Jamunapari goats. Therefore, the present study has been designed in this direction with the following objective:

To analyze the milk protein polymorphism in Jamunapari goats.

Materials And Methods

Collection of sample

The milk samples were collected from 27 doe of Jammunapari goats, maintained at Central Institute for research on goats (CIRG), Makhdoom, Farah, Mathura. The whole milk was taken out in 50 ml of sample tubes after washing the udder with

potable water and moping with cotton cloth. The precaution was taken during collection of sample, so that the milk of one sample should not get mixed with other samples. The milk samples were stored at -20°C after collection for further use.

SDS-PAGE

The genetic variants in milk samples were detected by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS – PAGE) method. The milk protein variant were analysed after staining with Coomassie blue. The SDS-PAGE was carried out in Mini protein gel (Bio-Red) and protein vareint was analyzed in Gel Documentation system (Alpha Innotech Corp., USA).

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 Protein Genotyping By Sds-page

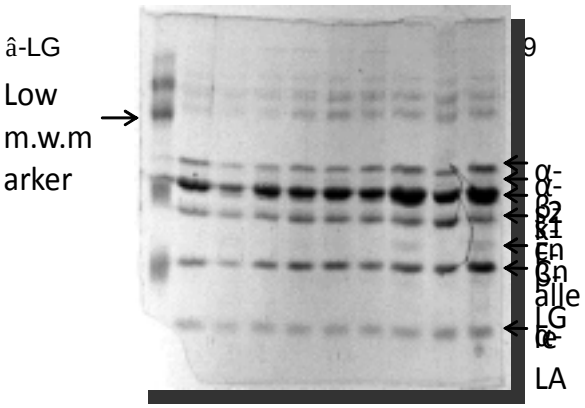
Milk Samples of 27 Jamunapari goats were collected to analyze the genetic variants of milk protein of this breed. The separation of genetic variants of ás1- casein, ás2-casein, â-casein, 8-casein, â-lactoglobulin and á-lactalbumin in milk of Jamunapari goats were carried out by SDS- PAGE (Sodium dodecyl sulphate polyacrylamide gel electrophoresis) method.

Different genetic variants of milk protein in Jamunapari goats

Locus	Allele	Frequency
CAS 2	A	0.8891
	B	0.1108
CAS 1	A	0.2405
	B	0.3706
	E	0.2588
	F	0.1299
CAS-â	A	1.000
CAS-ê	A	1.000
á - LA	A	1.00
â - LG	A	0.8694
	B	0.1306

Heterozygosity (obs. and exp.) at different locus in Jamunapari goats

Locus	Heterozygosity	
	Obs.	Expec.
CAS 2	0.2220	0.2013
CAS 1	0.7780	0.7345
CAS-â	0.0000	0.0000
CAS-ê	0.0000	0.0000
á-LA	0.0000	0.0000
â-LG		9



Casein Region

In this study out of 30 milk samples, 27 samples were used for casein estimation in which four ás1-casein variants namely A, B, E and F were observed. Out of total milk sample analyzed, 14 milk samples showed BE variants, 7 milk samples showed AF variants, 5 milk samples showed AB variant and only 1 milk sample showed AA variant. The predominant allele of ás1-casein in Jamunapari goat was ás1-cnB, with a frequency of 0.37; whereas the frequency of ás1-can, ás1-cnE and ás1-cnF were, 0.24, 0.25 and 0.12, respectively. The observed heterozygosityfor ás1-casein in

Jamunapari goat was 0.77 whereas the expected heterozygosity was 0.73.

Out of 27 milk samples 21 milk samples showed AA variant and only 6 samples showed AB variant in ás2 casein. The predominant allele of ás2 casein locus in Jamunapari goat was A with a frequency of 0.88 where as the frequency of B was 0.11. For ás2 casein, the values of obs. and exp. heterozygosity were 0.22 and 0.20, respectively.

In the present investigation, carried out on 27 milk samples of Jamunapari goats no polymorphism of â-Cn locus was observed. Out of 27 samples all samples contained the â-Cn variant A and expressed in homozygous form in the population. Hence the allelic frequency of A variant for this locus was 1.00.Incase of â-Cn the values of obs. and exp. heterozygosity was found 0.

In the present study, no polymorphism of ê-Cn locus was observed. Out of 27 milk samples all samples contained the ê-Cn variant A and expressed in homozygous form in the population. The allelic frequency of A variant for ê-Cn locus was 1.00. Incase of ê-Cn the values of obs. and exp. heterozygosity was found 0.

Whey Protein

In the present studies, the polymorphic pattern was observed in the â- LG locus for Jamunapari goats. Two variants namely, A and B were observed. The allele frequency of â- LG A variant was 0.87 whereas the allele frequency for â- LG B variant was 0.12.

In the present investigation, the monomorphic pattern was observed in the á-LA locus for Jamunapari goats. Out of 27 milk samples all samples showed AA variant. Hence, the allele frequency of A variant for á-LA locus was 1.00.

Discussion

Casein protein-

Regarding the allelic variants at ás1-cn locus, there are 16 co dominant alleles, A, B1, B2, B3, B4, C, D, E, F, O1 and O2 (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 ás1-CnA, ás1-CnB and ás1-CnC 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 ás1-casein and ás1-casein was highest in case of A, B and C allele. The ás1-cn F variant was discovered by Grosclaude et al. (1987) and suggested that the ás1-cn F allele was responsible for the production of low ás1-casein in goat milk. The frequency of ás1-CnF 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 ás1-CnA and ás1-CnF 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.

On the other hand, Sacchi et al. (2005) identified that eight alleles have been associated 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.,2001a)and G (Erhard et al.,2002) are associated with normal synthesis levels, whereas D and O are associated with lower and null synthesis level (Ramunno et al.,2001b). Boulanger et al.,(1984) has described ás2- CnA and ás2-CnB allele in goats. The ás2-CnA was found to be predominant in Alpine (0.85) and Saanen (0.87) breeds. Russo et al., (1986) reported that the two alleles (ás2-CnA and ás2-CnB) 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 ás2-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 ás2-CnA was almost similar in three breeds i.e. 0.75, 0.78 and 0.72 in Barbari, Marwai and Sirohi goats, respectively. However, it was observed lower in case of Jamunapari (0.65) and Jakhrana goat (0.57). The other ás2-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 ás2-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 ás2-Cn locus in Indian goat breeds and variant ás2-CnA was predominant in all the Indian goat breeds. The frequency of ás2-CnA allele in Jamunapari, Barbari, Marwari, Sirohi, Jakhrana, Beetal, Ganjamand 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-CnB 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; 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-CnA variant was dominated in goat breeds of India and the frequency of â-CnA 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-CnB was not observed in any milk samples of Indian goats. The â-CnO (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; Caroli et al., 2001). Russo et al., (1990) 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-CnA 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.

Whey protein

Boulanger (1976) observed A and B variant of â- LG in 5 Alpine and Saanen goat breeds. In another study, Pena et al., (2000) detected and characterized two new variants for the goat â- LG gene at cDNA level and confirmed at the genomic level. Similarly, Prakash et al., (2002) identified two variants of â- LG A and B in Jamunapari, Barbari, Sirohi and Jakhrana breeds. They also reported that variant A was observed to be dominant over Variant B for this locus. In another study, Kumar (2005) analyzed the milk samples of different Indian goats for milk protein polymorphism and reported that two

allele were present at â- lacto globulin locus and variant â- LGA allele in Jamunapari, Barbari, Marwari, Sirohi, Jakhrana, Beetal, Ganjam, Local U.P. and M.P. goat breeds were 0.813, 0.895, 0.500, 0.800, 0.838, 1.00, 1.00, 0.982 and 0.950, respectively. The B variant was very low in all breeds under study.

Maes et al., (1976) reported the existence of two variant for this locus in goat milk. Later on, Chifola and Micari (1987) also reported two variants of á-LAA and the rare á-LAB in three Italian breeds of sheep and their frequencies were .004, 0.012, respectively. Prakash et al., (2002) also identified two variants of á-LA where A variant was dominant over variant B. On the contrary, in study of Kumar (2005) on milk protein polymorphism of different Indian goats, it was observed that á-LA locus exhibited monomorphic pattern in all Indian goat breeds except Sirohi and Jakhrana. The á-LAA variant was predominant in all the goat breeds. The frequencies of á-LAA allele in Jamunapari, Barbari, Marwari, Sirohi, Jhakhrana, Beetal, Ganjam, Local U.P. and Local M.P. goat breeds were 1.00, 1.00, 1.00, 0.993, 0.971, 1.00, 1.00, 1.00 and 1.00, respectively. The B variant was not observed in Jamunapari, Barbari, Beetal, and Ganjam and non-descript breed from U.P. and M.P.

Summary and Conclusion

Goats play a vital role in the livelihood security of the small and marginal farmers and landless laborers especially in arid, semi-arid and mountainous regions of the country. They are hardy, disease resistant and widely adapted. They thrive well and reproduce well in tropical, cold, humid as well as dry regions. Their small size compared to cattle and buffaloes permit them to be maintained on a limited area. Goats consume a wide variety of grasses, weeds, bushes, shrubs, tree leaves and crop residues that would otherwise go waste and cause pollution. Goat is an efficient converter of low quality grasses and other grazing materials into milk, meat, skin, fibre and manure while utilizing traditional and under employed manpower. Goat milk is more beneficial than other livestock species to children, old and sick people as it is easily digestible due to smaller fat globules and certain medicinal values.

The Jamunapari goat is mainly found in the undulated land of Chakarnagar between the ravines of the Jamuna (Yamuna) and Chambal rivers in the Etawah district of the Indian state of Uttar Pradesh. The predominantly white-haired Jamunapari goat is probably the tallest and most handsome of Indian goat breeds, the other distinguishing feature of the breed being a thick growth of long hair on the hindquarters. They have a large body, long and wide pendulous ears, a pronounced Roman nose and parrot mouth, and short and flat horns. The breed is a prolific and non-seasonal breeder and females possess large udders with big teats and are well known for milk production because they produce the highest amounts of milk of all Indian goat breeds.

Goat milk has significance in human nutrition in two major areas (1) Feeding more starving and malnourished people in the most disadvantage place in our country (2) Treating people afflicted with cow milk allergies and gastro-intestinal disorders. Moreover the new concept of tailor making food to better fit human needs has not been applied to goat milk. India possess a great diversity in terms of goat wealth however a very little is known about genetic of milk protein in Indian goats.

Milk protein contains six main proteins, which includes the four caseins genes (ás1-cn, ás2-cn, â-cn, 8-cn) and two whey proteins genes (â-LG & á-LA) and it contribute 95% of the total milk protein , while casein has 80% of total protein. The milk proteins are synthesized completely under hormonal system. The genetic variants of milk proteins are heritable trait and they are governed by co-dominant autosomal genes.

As milk casein loci are highly polymorphic in nature, therefore characterization of milk casein loci of

Jamunapari goats will be an aid for breed characterization. Milk samples of 27 lactating goats from the flock maintained at the farm (Central Institute for Research on Goats). The experiment for this study was conducted at the Genetics and Breeding Division, Central Institute for Research on Goats, Makhdoom, Mathura, (U.P.).

The genetic variants of ás1-cn, ás2-cn, â-cn, 8-cn, â-LG & á-LA genotypes were identified by Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) method. The milk protein band patterns were studied on 14% Polyacrylamide gel electrophoresis.

The electrophoretic pattern revealed that Jamunapari goat milk sample contained four major casein variants, i.e., ás1-cn, ás2-cn, â-cn and 8-cn and two whey proteins i.e., â-LG & á-LA. Further, ás1-cn locus showed four variants such as

A, B, E & F. The E and F variants were found as heterozygous form, i.e., BE & AF in majority number of samples. In the present study ás2-cn locus showed two variants A and B. B was found in heterozygous form, i.e., AB. No polymorphic pattern was observed at â and 8 casein locus. All the samples showed presence of A allele at â and 8 casein locus and they are found as homozygous AA form. Two variants were observed at â-LG locus such as A and B. The electrophoretic pattern of á-LA locus showed presence of A allele at á-LA locus and they are found as homozygous AA form.

In the present study four ás1-casein variants namely A, B, E & F were observed. The predominant allele of ás1-casein in Jamunapari goat was ás1-cn B, with a frequency of 0.37, whereas the frequency of ás1-cn-A, ás1-cn-E and ás1-cn-F alleles were 0.24, 0.25, and 0.12 respectively. In the genotypic frequencies, heterozygous BE accounted for 52% and AF accounted for 26% of the total population followed by homozygous AA with 11% and BB with 11% in this study. The E and F variants were present as heterozygous AF and BE state. The frequency of ás1-Casein BEwas high as compared to AA, BB and AF Variants with respect to ás1-casein locus. The observed and expected genotypic frequencies for different alleles showed highly significant difference in this study. Therefore the population under study was in Hardy-Weinberg disequilibrium with respect to ás1-casein as the ÷2 were estimated as 47.26 with 6 degree of freedom. The genetic disequilibrium for this locus may be discribed to small number of observation in the present investigation.

In the present investigation, carried out on 27 milk samples of Jamunapari goats, 2 variants were observed for ás2- Cn locus. Out of 27 milk samples, 21 samples contained the ás2- Cn variant A expressed in homozygous form in the population, whereas 6 samples contained the ás2- Cn variant B expressed in heterozygous form in the population. The allelic frequency of ás2-cn-A variant was 0.88 whereas the frequency of ás2-Cn B, variant was0.12. In the genotypic frequencies homozygous AA accounted for 80% of the total population followed by heterozygous AB with 20% in this study. The B variant was present as heterozygous AB state. The frequency of ás2-Cn AA was high as compared to ás2-Cn AB variant. With respect to ás2-Cn locus, the observed and expected genotypic frequencies for different alleles showed non significant differences in this study. Therefore the population under study was in Hardy-Weinberg equilibrium with respect to ás2-Cn locus as the ÷2 values was estimated as 0.34 with one degree of freedom.

No polymorphism of â-cn locus was observed in the present study. All samples under study contained the â-cn variant A and expressed in homozygous (AA) form in the population. The gene frequency of â-cnA variant was 1.00. The genotypic frequency of â-cn AA was also 1.00.

The monomorphic pattern was observed in the 8-Cn locus for Jamunapari goat. All samples contained the 8-Cn variant A expressed in homozygous (AA) form in the population. The allelic and genotypic frequencies for this locus were similar, i.e., 1.00.

Two genetic variants of â-LG, A and B were obtained in the present study (Table 2). The gene frequency of â-LG A and â-LG B was 0.870 and 0.129 respectively. The variant A was observed to be dominant over B variant for this locus. In this study, the population was in Hardy-Weinberg equilibrium with respect to â-LG locus as the estimated ÷2 value was of 8.27 with 1 degree of freedom.

For this investigation 30 milk samples were collected and results are based on 27 milk samples of Jamunapari goats. In this study no polymorphism of á-LA locus was observed. Out of 27 samples, all samples contained the á-LA variant A and expressed in homozygous form in the population. The allelic frequency of A variant for this locus was 1.00.The genotypic frequency of á-LA AA was 1.00.

Results of the present study suggested that the milk protein variant in Jamunapari goat breed might be selection criteria for selecting high total protein content in milk. As this experiment was conducted based on small number of samples, hence further research may be carried out with large number of samples to confirm the results and to draw valid conclusion in this aspect.

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