International Journal of Scientific Engineering and Applied Science (IJSEAS) – Volume-3, Issue- 4, April 2017
ISSN: 2395-3470
www.ijseas.com

Characterization of goat milk protein and comparison of milk proteins using polyacrylamide gel electrophoresis

GARIMA SHARMA[1], DR. P.K. ROUT[2], DR. GAJENDRA SINGH[1]
1 IBMER Dept., Mangalayatan University, Beswah, Aligarh, Uttar Pradesh
2 Genetics and Breeding Division, Central Institute for Goat Research Centre, Makhdoom, Mathura, Uttar Pradesh

Abstract
Goat milk has been a rich source of proteins. The distinctive composition of goat milk, combined with its nutritional value, is linked to the liberation of protein fragments during digestion or technological processing, which are able to perform various biological activities. This has gain more attention due to its broad applications in the food, drug, and various other industries. Thus, here we characterized goat milk proteins to evaluate the protein function. This study is entitled to exemplify the proteome of goat milk of three Indian goat breeds Jamnapari (160), Barbari (110), Jakhrana (20) and to find the variants using SDS- PAGE. The electrophoretic pattern of goat milk samples showed the presence of four major casein variants, i.e., CSN1S1, CSN1S2, CSN2, CSN3 and two whey proteins, viz., β-lactoglobulin and α-lactalbumin. These major proteins exhibits variants i.e. CSN1S1*A 0.92 (Jamnapari), 0.83 (Barbari) 90 and 0.8 (Jakhrana), CSN1S1*B 0.1 in all the three breeds. CSN1S2*A 0.03 in barbari though was not found in jamnapari and jakhrana breeds. and CSN1S2*B 0.95 (Jamnapari), 0.78 (Barbari), 0.8 (Jakhrana). No polymorphism was observed at CSN2, CSN3 and α-lactalbumin locus in our study. However, frequency of β-LGA and β-LGB was 1 and 0,12 (Jamnapari), 1 and 0.1 (Barbari) and monomorphic in Jakhrana, respectively these studies also implied that variant A was present in all the breeds where as B 0.12 (jamnapari) and 0.1 (barbari) and in jakhrana B was not found. The major proteins in milks from bovine, caprine, and from humans were compared using a SDS PAGE method. Electrophoretic analysis of goat milk proteins showed the presence of variants. Cow milk and goat milk has shown approximately same protein composition where as human milk when compared to cow and goat milk has shown the absence of Alpha S1 casein protein which has a major role in milk protein allergies and β- lactoglobulin which is a major whey protein.

Keywords: Goat, comparison, Milk Characterization, Milk Proteome, Polymorphism, Proteomic Evaluation, SDS- PAGE

1. Introduction
India has been ranked second in goat population and produce first in goat milk production. Goat milk is known for its proteins and minerals contents and is comprised of 80% Casein and 20 % Whey protein. Characterization and identification of protein variants of milk proteins has been widely promoted in various species using electrophoretic techniques (Grosclaude *et al* 1987; Moioli *et al* 1998, Caroli *et al* 2009; Marletta *et al* 2007; Rando *et al 2000)* and thus showed casein has significant effect on milk production and technological properties and milk variants linked with the composition and biological properties of milk (Martin *et al* 2002; Vinesh *et al* 2013). Casein is the major protein segment of ruminant milk. It is the most valuable components due to its nutritional properties. They are a family of acidic, proline-rich phosphoproteins designed to form spherical, large, micellar structures in colloidal suspension with calcium phosphate. The casein fraction as first reported by Grosclaude *et al* 1987 encoded by four genes and is organized in a 250-kb genomic DNA segment in the following order: Casein Proteins**: α** S1-casein (CSN1S1), α-S2-casein (CSN1S2), β-casein (CSN2), κ-casein (CSN3) (Grosclaude *et al* 1987; Ferretti *et al* 1990; Threadgile and Womack 1990; Martin *et al* 2002), Whey Proteins: α- Lactalbumin, β- Lactglobulin**.** They have been mapped on chromosome 6 in cattle and goats (Hayes *et al*, 1993; Popescu *et al* 1996). β-casein known to be the most polymorphic protein and identified with 13 variants in cattles.

Milk protein content is affected by season, stage of lactation, parity. A Seasonal variation has shown astonishing effect for milk protein in the lactating goats (Guo *et al*, 2001). The total protein in the goat milk has been found significantly ($p<0.05$) lower in premonsoon than the value of protein found during the season of post-monsoon (Bhatta *et al*, 2015). Generally it is known that milk protein (%) value is directly proportional with the milk fat (%) value. It is assumed that reduction in fat and protein concentration in goat milk has been a result of hot or warm ambient temperature (Marin *et al*, 2011). Many researchers has analyzed that hot and humid months (July and August) usually depresses protein content. There is a gradual increase of protein and fat in milk through the fall and peak levels occur in the colder months of winter. As temperatures increase through the spring, component levels are gradually decreased. These changes may be indicative of feed intake patterns, which are lower in summer due to changes in weather and temperature.

Milk protein genetic polymorphism has gained ample research interest in recent years because of strong linkage between milk protein and economically important traits in livestock. Thus this study is encompassed to characterize the milk proteomic structure in Indian goats at protein level.

2. Material and Methods

2.1 Sample Collection

Milk samples were collected from three homogenous lactating healthy goat Jamnapari (n=160), Barbari (n=110), Jakhrana (n=20) maintained at CIRG, Makhdoom, Mathura. Milk samples were transported to the laboratory. These samples were stored at -20°C for further analysis. The milk samples were centrifuged at 12,000 g for 10 min at -4°C. The fat layer was then carefully removed. The milk serum was extracted from below the creamy layer and was used for further for Protein characterization processes. The concentrations of protein of milk samples were estimated by Lowry *et al*., 1951.

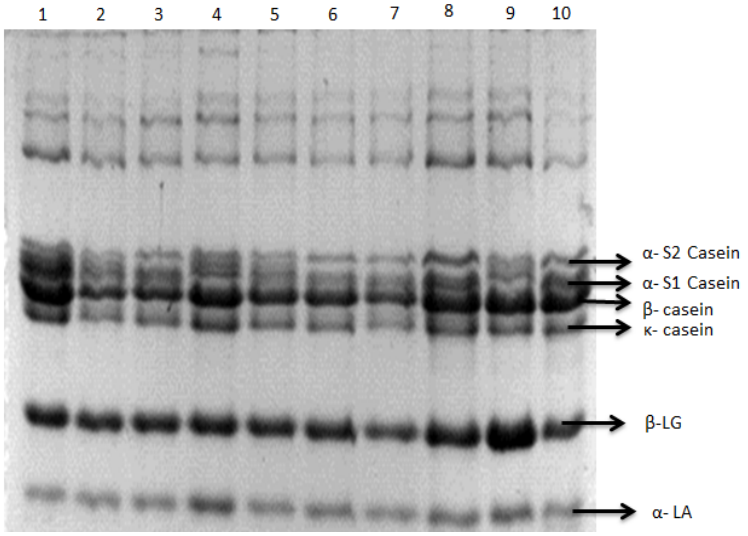
2.2 Analysis of Milk Protein variants by SDS- PAGE

SDS-PAGE technique was used for the evaluation of the protein structure of goat milk. This process was carried out in a Mini- Protean II electrophoresis cell (Bio- Rad Laboratories). Proteins band were analyzed on two gel compositions i.e. 5% (w/v) Stacking gel *(30% Degassed Acrylamide/ Bis acrylamide, 1M Tris pH 6.8, 10% SDS, 10% Ammonium persulphate, TEMED)* and 12% (w/v) Separating gel *(30% De- gassed Acrylamide / Bis acrylamide, 10% SDS, 1.5 M Tris pH 8.8, 8M Urea, 10% ammoinium persulpate, 50ul TEMED, distill water)* (laemmli, 1970). After the casting of gels, 8ul of protein samples (sample diluted with 8M Urea by 1:11 and then mix sample buffer with urea diluted samples at a ratio of 1:1 and heated at 95°c for 4 min) were injected into the wells. The gel was ran in the electrophoretic tank filled with running buffer pH 8.3 (Tris Base 250mM, Glycine 1.92M, SDS 1% and distill water) at 50 V for approximately 3 hours. The genetic variants were identified by their electrophoretic mobility as per the nomenclature of major and minor milk proteins. After electrophoresis, the SDS gels were stained with Commassie blue R- 250 for visualization of the protein bands. Milk protein variants were then determined with reference to standards in gel documentation system (SIGMAMARKER, M-62 4038).

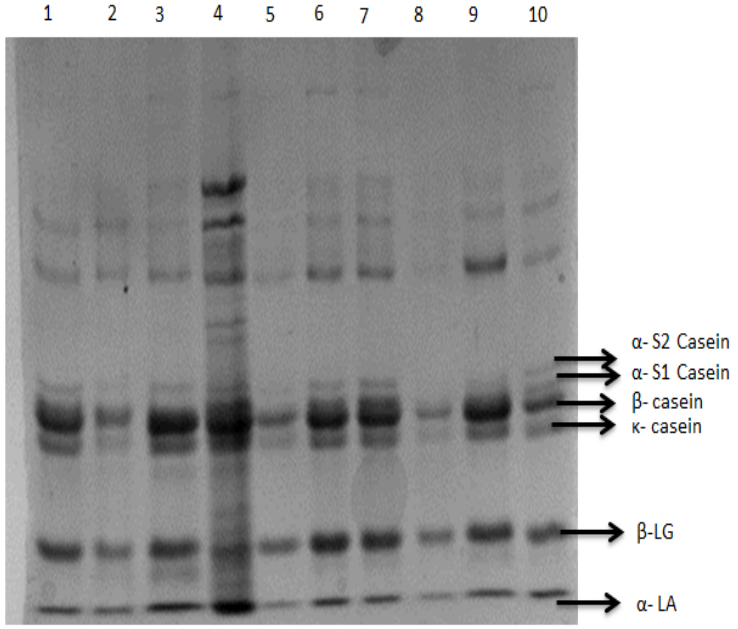
3. Results

3.1 Electrophoretic separation of variants in milk protein

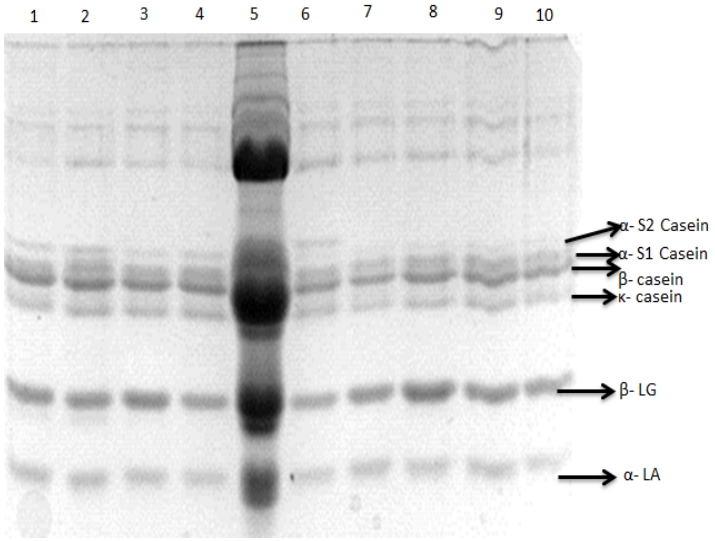
The electrophoretic pattern of goat milk samples showed the presence of four major casein variants, i.e., αS1- casein, αS2 - casein, β-casein, and κ- casein and two whey proteins, viz., β-lactoglobulin and α-lactalbumin. A much defined separation of variants of casein and whey proteins has been analyzed on alkaline urea gel. Figure 1 shows the fine separation of milk proteins and their variants on the basis of which the variant frequency has been calculated and listed in table 1.



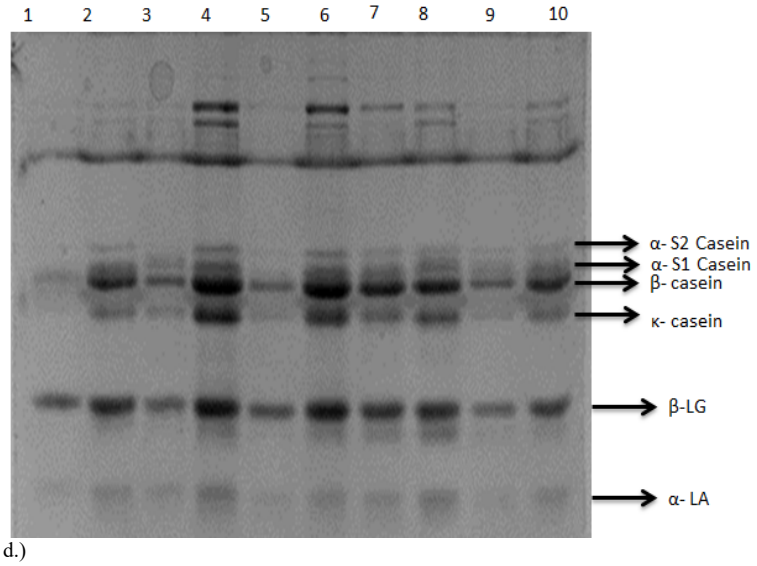
a.)



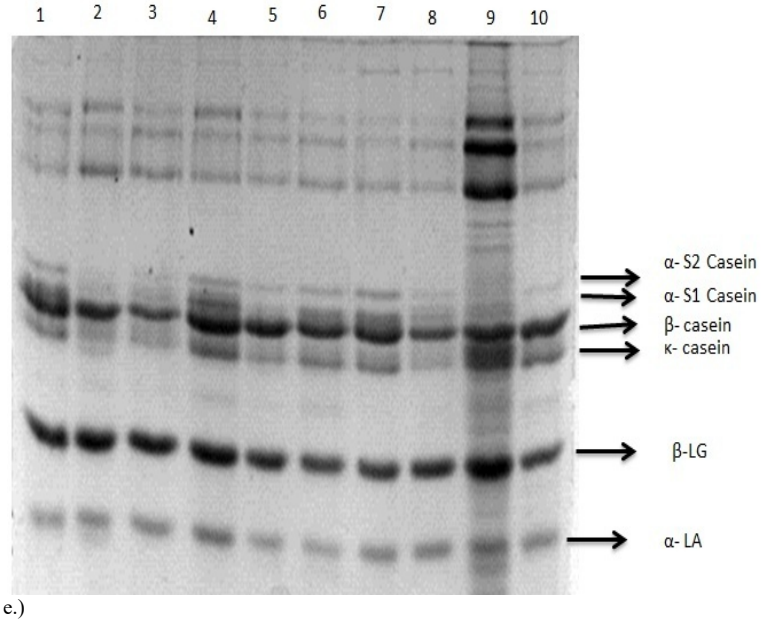
b.)



c.)



d.)



e.)

Figure 1: Electrophoretic pattern obtained by SDS- PAGE in a.) and b.) Jamnapari c.) Barbari d.) and e.) Jakhrana goats.

Protein Variants present in Different Goat breeds					
Goat Breed	**Number of Goats (n)**	**Proteins**	**A**	**B**	**0**
Jamnapari	160	CSN1S1	0.92	0.1	0.075
		CSN1S2	-	0.95	0.05
		CSN2	0.96	-	0.03
		CSN3	1	-	-
		A- LA	1	-	-
		B- LG	1	0.12	-
Barbari	0	CSN1S1	0.83	0.1	0.17
		CSN1S2	0.03	0.78	0. 17
		CSN2	0.99	-	0.1
		CSN3	1	-	-
		A- LA	1	-	-
		B- LG	1	0.1	-
Jakhrana		CSN1S1	0.8	0.1	0.2
		CSN1S2	-	0.8	0.2
		CSN2	1	-	-
		CSN3	0.95	-	0.05
		A- LA	1	-	-
		B- LG	1	-	-

Table 1: Showing the protein variants in the Goat breeds: Jamnapari, Barbari, Jakhrana.

In the current study, two variants were identified in αS1- casein protein viz. CSN1S1*A, CSN1S1*B. The predominant variant of αS1- casein was CSN1S1*A, with a frequency of 0.92 (Jamnapari), 0.83 (Barbari) and 0.8 (Jakhrana), whereas the frequency of CSN1S1*B variant was 0.1 in all the three breeds. The αS1- casein protein also showed the presence of null allele. The frequency of null allele for this locus (CSN1S1*O) was 0.075 (Jamnapari), 0.17 (Barbari), 0.1 (Jakhrana). The CSN1S2 locus is characterized by the presence of two variants

namely CSN1S2*A and CSN1S2*B and the frequencies of the variant CSN1S1*A was 0.03 in barbari though was not found in jamnapari and jakhrana breeds. The frequency for CSN1S2*B variant was 0.95 (Jamnapari), 0.78 (Barbari), 0.8 (Jakhrana) respectively. Previous study by (Ramunno *et al.* 2001) reported that the homozygous genotypes are associated with good quality of milk protein. No polymorphism was observed at CSN2, CSN3 and α-lactalbumin locus in our study. However, in their earlier studies the monomorphic pattern (CSN3A) in CSN3 locus in Barbari goats was reported by Kumar *et al* 2002 and Kumar *et al* 2005. The electrophoretic pattern of β-lactoglobulin (β-LG) showed the presence of two genetic variants at β-lactoglobulin locus (viz., A and B) and the frequency of β-LGA and β-LGB was 1 and 0,12 (Jamnapari), 1 and 0.1 (Barbari) and monomorphic in Jakhrana, respectively. The presence of β-LGA and β-LGB at this locus was reported in Saanen and Alpine goat (Boulanger *et al*1976) and in Jamunapari, Barbari, Sirohi and Jakhrana breeds (Kumar *et al* 2002) and these studies also implied that variant A was dominant over variant B which is corroborated with the present findings. Figure 1 explains the electrophoretic separation of casein and whey proteins in SDS PAGE in Jamnapari, Barbari and Jhakrana goat breeds.

3.2 Comparative analysis of Goat milk protein with Human and Cow milk proteins

In the present study, we have compared the SDS PAGE pattern of human, goat and cow milk proteins. In the figure 2, human milk protein has shown the absence of Alpha S1 casein protein which is the predominant factor causing milk protein allergies whereas cow and goat milk showed an abundant presence similar to Zicarelli et al study in 2004. In the electrophoretic separation of milk variants of Human milk showed the presence of Alpha S2 casein and β-casein and hence support the findings of Sood etal in 1997 and many other researchers. Additionally human milk showed the absence of β-lactoglobulin, an outranged protein for infants who are intolerant of cow milk. Human milk, cow and goat milk all contain alpha-lactoalbumin, but with slightly different structures. The lactoalbumin in human milk is best consumed, but people who are allergic to the cow lactoalbumin may still consume goat milk.

In cases where breast feeding is not possible, cow milk is commonly acted as a substitute for human milk (El-Agamy, 2007). Figure 3 explains the seperartion of cow and goat milk proteins using SDS PAGE. Here, cow milk and Goat milk doesn't show any major difference in protein content. Lara-Villoslada and others (2005) explain further that the lower allergenicity of goat milk compared to cow milk is due to the fact that a lower share of αs1-casein reduces the sensitivity to the other allergen protein such as β-lactoglobulin.

CSN3 and α-lactalbumin locus in our study. However, in their earlier studies the monomorphic pattern (CSN3A) in CSN3 locus in Barbari goats was reported by Kumar *et al* 2002 and Kumar *et al* 2005. The electrophoretic pattern of β- lactoglobulin (β-LG) showed the presence of two genetic variants at β-lactoglobulin locus (viz., A and B) and the gene frequency of β-LGA and β-LGB was 1 and 0,12 (Jamnapari), 1 and 0.1 (Barbari) and monomorphic in Jakhrana, respectively. The

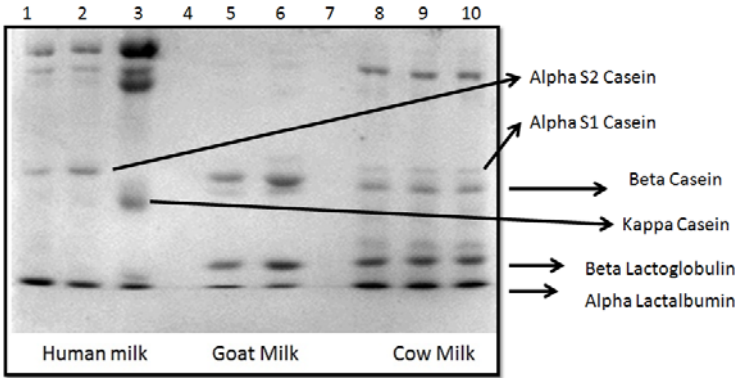


Figure 2: Showing the separation of Human, Goat and Cow milk proteins using SDS- PAGE and is comassiee blue stained to visualize the protein bands.

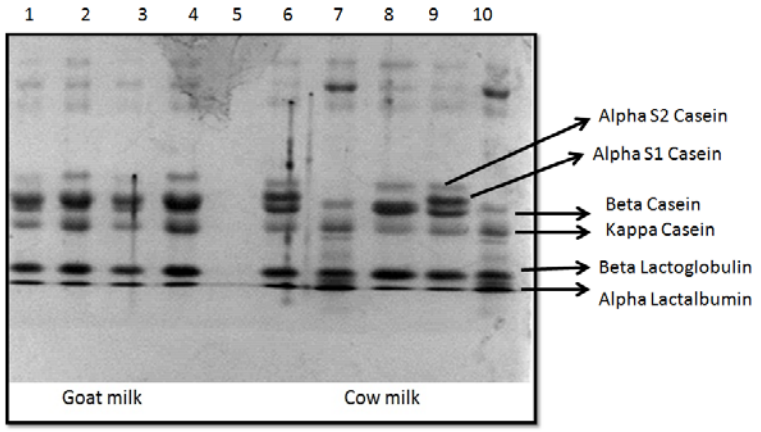


Figure 3: Showing the separation of Goat and Cow milk proteins using SDS- PAGE and is coomassie brilliant blue stained to visualize the protein bands.

4. Discussion

Regarding the allelic variants at αS1-Cn locus, different variants at this locus were reported in various goat breeds (Grosclaude and Martin 1997; Moili *et al*1998; Kusza *et al* 2007). The gene frequencies of CSN1S1*A, CSN1S1*B in different breeds of goats in the present study were well comparable with the findings observed by Kumar *et al*2002, Kumar *et al* 2008 in different Indian goat breeds. The polymorphism in the αS1- Casein genotype (Rout *et al* 2007) has important impacts on goat milk fat, and its fatty acids composition (Chillard *et al* 2006). Previous study by (Ramunno *et al.* 2001) reported that the homozygous genotypes are associated with good quality of milk protein. No polymorphism was observed at CSN2, presence of β-LGA and β-LGB at this locus was reported in Saanen and Alpine goat (Boulanger *et al*1976) and in Jamunapari, Barbari, Sirohi and Jakhrana breeds (Kumar *et al* 2002) and these studies also implied that variant A was dominant over variant B which is corroborated with the present findings.

The comparative analysis of caprine, bovine and human milk has shown the importance and efficacy of milk proteins. It's most

important allergens are considered to be β-lactoglobulin (absent in human milk) and αs1-casein. It contains considerably more whey proteins (35-50%) than cow milk (about 20%), and the concentration of the most allergenic casein fraction αs1 is 1.5-2.5 g/l. In comparison, the content of αs1-casein in cow milk is about 10 g/l (Pastuszka et al., 2016). Goat milk components have a higher degree of assimilability as compared to cow milk. Its main protein is β-casein, with total protein content depending on the αs1-casein genetic variant. Goats with the '0' variant do not synthesize this allergenic protein. Clinical and immunochemical studies indicate, however, that it cannot be a substitute for cow milk without the risk of an anaphylactic reaction.

5. References

A. Boulanger. Etude biochimiqueetgenetique des proteins du lait de chevre (caprahircus). Thesis,University of Paris, VII. 1976.
5368. 2011.

A. Kumar. Molecular Characterization of milk protein polymorphism in Indian goats. Ph.D. Thesis submitted to Central Institute for Research on Goats, Makhdoom, Farah, Mathura, U.P. 149pp. 2005.

A.M. Caroli, S. Chessa, G.J. Erhardt. J. Dairy Sci. Milk protein genetic variation in cattle: impact on animal breeding and human nutrition. 92: 5335–5352. 2009.

A.M. Marín, P. Gómez-Cortés, A.G. Castro, M. Juárez, L.P. Alba. J Dairy Sci. Animal performance and milk fatty acid profile of dairy goats fed diets with different unsaturated plant oils. 94(11): 5359.

A. Rando, L. Ramunno, P. Masina. Nutr. Anim. Mutations in casein genes.Zootech. 26: 105–114. 2000.

B. Moioli, F. Pilla, C. Tripaldi. Small Rumin. Res. Detection of milk protein genetic polymorphism in order to improve dairy traits in sheep and goats: a review. 27: 185–195. 1998.

C.P. Popescu, S. Long, P. Riggs, J. Womack, S. Schmutz, R. Fries, D. Marletta, A. Criscione, S. Bordonaro, A.M. Guastella, G. D'Urso. Casein polymorphism in goat's milk. Lait 8: 491–504. . 2007.

D.S. Gallagher. Cytogenetics and Cell Genetics. Standardization of cattle karyotype nomenclature: report of the committee for the standardization of the cattle karyotype. 74:259-261. 1996.

D.W. Threadgile, J.E. Wamack. Nucleic Acids Research. Genomic analysis of major bovine milk protein genes.18: 6935- 6942. 1990.

F. Grosclaude, M.F. Mahe, G. Brignon, L. DiStasio, R. Jeunet. Genetic Selection Evolution. A Mendelian Polymorphism underlying quantitative variations of goat αS1 Casein. 19: 399- 412. 1987.

H. Hayes, E. Petit, C. Bouniol, P. Popeseu. Genetics. Localisation of alpha S2 casein gene to the homologous cattle, sheep and goat chromosome 4 by insitu hybridization.Cytogenetics and Cell. 64: 282-285. 1993.

L. Ferretti, P. Leone, V. Sgaramella. Linkage of the four bovine casein genes as demonstrated by pulsed field gel electrophoresis. Nucleic Acids Research. 18: 6829-6833. 1990.

L. Ramunno, G. Cosenza, M. Pappalardo. Animal Genetics. Characterization of two new alleles at the goat CSN1S2 locus. 32(5): 264-268. 2001.

M. Bhatta, D. Das, P.R. Ghosh. J Dairy Vet Anim Res. Influence of Seasonal Variation in the General Composition of Black Bengal Goat (Capra aegagrus hircus). Milk. 2(4): 00046. DOI: 10.15406/jdvar. 015.02.00046. 2015.

M.R. Guo, P.H. Dixon, Y.W. Park, J.A. Gilmore, P.S. Kindstedt. J Dairy Sci. Seasonal Changes in the chemical Composition of Commingled Goat Milk. 84(1): E79-E83. 2001.

O.H. Lowry, N.J. Rose brough, A.L. Farr, R.J. Randall. Journal of Biological Chemistry.193: 265 (The Original Method). 1951.

P. Kumar, P.K. Rout, R.N. Shukla, A. Mandal, R. Roy. Anim. Prod.Soc. (AAAP). Genetics of milk protein variants in different Indian goats.10th Int. Cong. Asian-Aust. Assoc., pp.168. 2002.

P. Martin, C. Bevilacqua, P. Ferranti, G. Garro, C. Valtri, R. Leronigro, C. Leroux, F. Pietrola, F. Addeo, F. Pilla, L. European Journal of Biochemistry. Chianese. Interallelic recombination is likely responsible for the occurrence of the new rate (αS1 casein variant in the goat species). 269: 1293-1303. 2002.

P.K. Rout, A. Kumar, A. Mandal. In: Food and bio process Engineering. Genetic Variability of milk casein loci in Indian goats and their effect on milk quality. 456-460, Anamaya Publishers, New Delhi. 2007.

P.V. Vinesh, B.B. Kaur, R. Datta, T.K. Goswami., S.L. De, Sachinandan. Gene. Characterization of β- casein gene in Indian riverine buffalo. 527 (2): 683–688. 2013.

R. Aschaffenburg, J. Drewry. Nature. Occurrence of different b-lactoglobulin in cow's milk. 76: 218–221. 1955.

R. Pastuszka, J. Barłowska, Z. Litwińczuk. Postepy Hig Med Dosw (online). Allergenicity of milk of different animal species in relation to human milk. 70: 1451-1459 e-ISSN 1732-2693. 2016.

U.K. Laemmli. Nature. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. 227(5259):680-5. 1970.

Y. Chilliard, J. Rouel, C. Leroux. Animal Feed Science and Technology. Goat's alpha-S1 casein genotype influences its milk fatty acid composition and delta-9 desaturation ratios. Volume 131, Issues 3–4: Pages 474–487. 2006.