Haryana Vet. (December, 2021) **60(2),** 220-222

Research Article

COMPARATIVE STUDY OF PHYSICO-CHEMICAL AND PROTEIN PROFILE OF MITHUN AND CATTLE MILK

ANATOLI ZHIMOMI[1], AKHILESH KUMAR*, LALCHAMLIANI and SOSANG LONGKUMER
[1]SRM University, Chennai, ICAR-National Research Centre on Mithun, Medziphema, Dimapur-797106, India
*Present address: ICAR-IVRI, Izatnagar, Bareilly-243122, India

Received: 31.03.2021; Accepted: 05.07.2021

ABSTRACT

The present study aimed to study the physical and chemical characteristics of mithun milk and to ascertain the differences in milk proteins between mithun and cattle. The findings of study indicated that the values of pH, specific gravity, corrected lactometer reading (CLR) and percentages of fats, solids-non-fat (SNF), total solids, total protein, ash and casein in mithun milk were significantly higher ($p \leq 0.05$) than cattle, whereas lactose content and acidity did not show any significant variation. The characterization of caseins (α-, β-, κ-caseins) and whey (lactoferrin, β- lactoglobulin, α-lactalbumin) proteins using Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE) and Urea-SDS-PAGE revealed the absence of any observable differences in the milk proteins of two species. The author concludes that mithun milk is nutritionally superior to other ruminant species. The high fat and protein content in mithun milk may be exploited for the preparation of value-added milk products. The animal also has a scope to be promoted as a moderately good milk animal for home consumption to address the widespread challenges of malnutrition in an economically poor section of society.

Keywords: Casein, Cattle, Milk, Mithun, Urea-SDS-PAGE, Whey

How to cite: Zhimomi, A., Kumar, A., Lalchamliani and Longkumer, S. (2021). Comparative study of physico-chemical and protein profile of mithun and cattle milk. *Haryana Vet.* **60(2)**: 220-222.

Mithun (*Bos frontalis*), a unique ruminant species, geographically limited to the North-Eastern Hilly states of Arunachal Pradesh, Nagaland, Manipur, and Mizoram in India is raised under free-range forest for meat production and often referred as "Ceremonial Ox" (Nath and Verma, 2000).

The casein micelles are constituted by different caseins subunits (αs1-CN, αs2-CN, β-CN) which are held together by calcium phosphate bridges and wrapped up in soluble κ-casein molecules (Vasbinder *et al.*, 2003). The major whey proteins are α-lactalbumin and β-lactoglobulin. Milk is also comprised of minor proteins, such as serum albumin, immunoglobulins, lactoferrin, transferrin, calcium binding protein, prolactin, folate-binding protein and protease-peptone (Park *et al.*, 2007).

There are very limited studies on mithun milk, however these studies did not compare physicochemical difference of cattle and mithun milk. Further, the only one published report of mithun milk protein profiling was also limited to casein fraction only (Nath, 2000). Keeping in view the availability of limited information on mithun milk characteristics, the present study aimed to study the physicochemical properties and protein profiling of mithun milk and its comparison with cattle.

MATERIALS AND METHODS

Animal: The milk samples were collected from mithun (n=11) maintained under semintestive system at the Institute Farm, Medziphema, Nagaland and Holstein Friesian cattle (n=15) from the organized dairy farm, Dimapur.

Sampling: Freshly collected milk samples were used within an hour of milking for analysis of pH and specific gravity. The remaining milk samples were stored under 4 °C for further use.

Determination of physicochemical properties of milk: Freshly collected milk samples were subjected to the analysis of pH, acidity, specific gravity, fat, total solid not fat (SNF), total solids (TS), total protein, casein, ash and lactose. The pH of the fresh milk samples was measured using a pH meter (EUTECH instruments pH 510, Thermo Fisher Scientific, USA). The paramaters, viz., milk fat, specific gravity, TS and Ash % was determined by method of ISI, 1961. The milk protein was determined by method of Pyne (1932) and Lactose % in milk was calculated using method of Lane and Eynon (1923).

The casein content in milk was determined using ISI (1961) method. The acidity in milk was determined by the sample titration against 0.1 N NaOH solution with 1% phenolphthalein as an indicator and acidity was expressed as a percentage of lactic acid (ISI, 1973).

Separation of the casein and whey proteins: The protein components (casein and whey) in milk were separated using Isoelectric Precipitation method as described by Si-Ahmed *et al.* (2012) with some modifications. The casein

*Corresponding author: dr_akhil2005@yahoo.co.in

was weighed and stored at room temperature for further study. The supernatant (whey proteins) was dialyzed with distilled water and freeze-dried and kept at -20 ºC until use.

Characterization of casein and whey proteins: Sodium Dodecyl Sulfate- Polyacrylamide Gel Electrophoresis (SDS-PAGE) was performed as per Ng-Kwai- Hang and Kroeker (1983) with some modifications. The Protein bands on gels were screened on a UV transilluminator (Biometra TI 3, Germany).

SDS-PAGE and Urea-PAGE: SDS-PAGE protocol was optimized and 12% and 15% acrylamide concentration has successfully discriminated the whey proteins to their respective markers in mithun and cattle milk but 15 % polyacrylamide resulted in optimal resolution (Fig. 1 and 2). The optimum casein separation with good resolution has been found with Urea-PAGE having 15% polyacrylamide and 4M Urea concentration. The milk samples were analyzed using optimized protocol of SDS-PAGE and Urea- PAGE.

Statistical analysis: Data were analyzed statistically using the independent Sample t-test (Snedecor and Cochran, 1994) and ($p \leq 0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

There was a significant difference ($p \leq 0.05$) in the pH, specific gravity, corrected lactometer reading, fats, solids-not-fat, total solids, total protein, ash and casein contents between the milk of mithun and cattle (Table 1). However, no significant differences were observed in lactose content and acidity. The present study attempted comparative evaluation of physicochemical and proteins profiling of mithun and cattle. The findings of the study suggests the superiority of mithun milk than that of cattle milk which are in concordance of previous studies (Nath and Verma, 2000; Mondal *et al.*, 2001; Mech *et al.*, 2008). The higher level of total solids, fats, SNF, protein and ash contents in mithun milk as compared to cattle milk as well as other ruminants could be attributed to the unique genetic makeup of this species with low average milk yield (Mech *et al.*, 2008).

The findings of electrophoretic mobility patterns of whey proteins in mithun and cattle milk using optimized SDS-PAGE protocol (15% Polyacrylamide) are presented in Fig. 2. The optimized protocol of Urea-PAGE has resulted appreciable separation of good resolution of casein proteins in mithun and cattle milk samples (Fig. 3). As SDS dissociates proteins into their constituent polypeptide chains and has been used for separation of the proteins according to their molecular weight, the protein

Table 1

Physico-chemical propoerties (Mean±SE) of mithun (n=11) and cattle (n=15) milk

Parameters	Mithun	Cattle
pH	6.65±0.03[a]	6.53±0.03[b]
Specific gravity	1.04±0.00	1.03±0.00
CLR	36.82±0.65[a]	29.87±0.49[b]
Caseins	2.46±0.08[a]	1.34±0.07[b]
Fats (%)	7.76±0.54[a]	3.42±0.36[b]
SNF (%)	11.53±0.21[a]	8.88±0.13[b]
Total solids (%)	19.39±0.80[a]	12.36±0.41[b]
Total Protein (%)	7.76±0.54[a]	7.58±0.41[b]
Lactose content	4.46±0.05	4.85±0.15
Acidity (%)	0.17 ±0.00	0.15±0.00
Ash (%)	0.83 ±0.03[a]	0.61±0.03[b]

*a, b indicates values with different subscripts within row differ significantly ($p \leq 0.05$).

with lower molecular weight would show higher mobility, hence, the remaining band would be of β-caseins and κ-caseins with repect to the standard marker of α-casein.

The whey proteins (α-Lactalbumin, β- Lactoglobulin Lactoferrin) of mithun and milk samples have successfully discriminated to their respective markers at 12% and 15% polyacrylamide concentrations, whereas casins were distinctly separated at 15% polyacrylamide in SDS and Urea-PAGE. The inability of a distinct separation of caseins at 12% polyacrylamide concentration could be attributed to the high presence of proteins with similar molecular weights. The similar finding has also been reported by Zagorchev *et al.*, 2013. Further due to the similar molecular weights of α, β and κ-caseins, and to the fact that caseins run anomalously on SDS-PAGE and caseins associate with each other in solution mainly through hydrophobic interactions, separation of the isolated molecules by electropheresis requires the addition of an agent, usually urea, that disrupts these interactions. The best resolution of casein fractionation by urea-PAGE has also been documented in another study (Huma *et al.*, 2018). The electrophoretic pattern of cattle, buffalo, caprine, camel and equine milk casein demonstrated that the principal bands in species corresponded to αs1-casein, β-casein with exception of κ-casein of equine milk with comparable migration for αs1-, β- and κ-casein in cattle, buffalo, caprine and equine milk where as camel milk showed slower migration rate (Merin *et al.*, 2001; El-Agamy *et al.*, 2009). In another study, the electrophoretic mobility pattern of αs1-in cattle, buffalo and sheep was

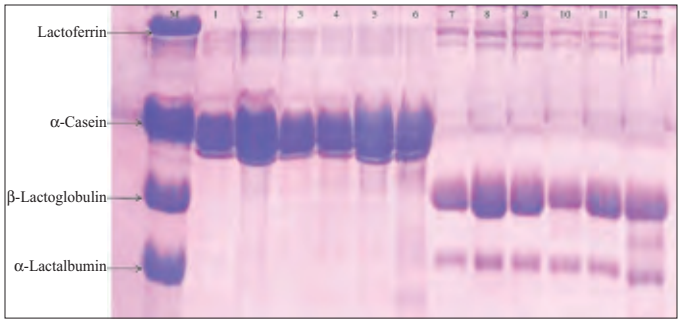

Fig. 1. SDS-PAGE profile of milk proteins (12% Acrylamide). M- Markers, Lanes 1-3: Mithun caseins, Lanes 4-6: Cattle caseins, Lanes 7-9: Mithun whey proteins, Lanes 10-12: Cattle whey proteins.

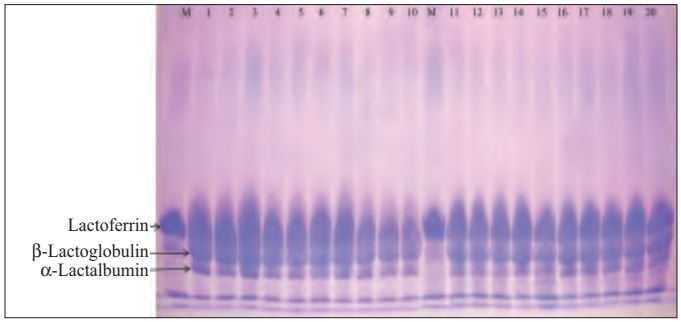

Fig. 3. SDS-PAGE profile of casein fractions (15% Acrylamide, 4M Urea). M- Marker (α-Casein), Lanes 1-10: Mithun caseins and Lanes 11-20: Cattle caseins.

found similar whereas αs2-casein was found absent in buffalo milk. The most predominat casein protein reported in sheep and goats showed that the αs1-, β- casein and αs1-, αs2 and β-casein, respectively (Huma *et al.*, 2018). The similar migration pattern of mithun and HF milk in present study suggests the presence of similar protein components.

CONCLUSION

The results indicated that the percentages of fats, solids-not-fats (SNF), total solids, total protein, ash and casein in mithun milk were significantly higher ($p \leq 0.05$) than in HF milk, whereas the milk protein constituents did not show any variability in both species.

ACKNOWLEDGMENT

The authors sincerely acknowledge the Director, ICAR-NRC on Mithun, Medziphema, Nagaland for conducting the study.

REFERENCES

El-Agamy E.I., Nawar, M., Shamsia, S.M,, Awad, S. and Haenlein, G.F.W. (2009). Are camel milk proteins convenient to the nutrition of cow milk allergic children. *Small Rumin. Res.* **82**: 1-6.

Huma, N., Ghaffar,F., Rafiq, S., Pasha, I., Sameen, A., Hayat, I. and Hussain, I. (2018). Characterization of milk proteins from different animal species through gel electrophoresis. *Pakistan J. Zool.* **50(5)**: 1983-1986.

ISI (1961). IS:1479, Part II. Methods of test for dairy industry. II. Chemical analysis of milk. Bureau of Indian Standards, New Delhi, India

ISI (1973). IS 1166. Specification for condensed milk. Bureau of Indian Standards, New Delhi.

Lane, J.H. and Eynon, L. (1923).Volumetric determination of reducing sugars by means of Fehling's solution, with methylene blue as internal indicator. *ISI.* **XXV** : 143-149.

Mech. A., Dhali. A., Prakash. B. and Rajkhowa, C.(2008). Variation in milk yield and milk composition during the entire lactation period in mithun cows (*Bos frontalis*). *Livest Res. Rural. Dev.* **20(5)** : 1-8.

Merin. U., Bernstein. S., Bloch-Damti. A., Yagil. R., van Creveld, C., Lindner and Gollop. N. (2001). A comparative study of milk serum proteins in camel and bovine colostrums. *Livest. Prod. Sci.* **67**: 297–301.

Mondal, S.K., Pal, D.T., Singh. G. and Bujarbaruah, K.M. (2001). Physico-chemical properties of mithun milk. *Indian J .Anim. Sci.* **71**: 1066-1068.

Nath, N.C. (2000) . Casein polymorphism in the milk of mithun. *Indian J .Anim. Sci.* **70**: 603.

Nath, N.C. and Verma, N.D. (2000). Biochemical evaluation of mithun milk for human consumption. *Indian Vet. J.* **77**: 418-423.

Ng-Kwai- Hang, K.F. and Kroeker. E.M. (1983). Rapid separation and quantification of major caseins and whey proteins of bovine milk by polyacrylamide gel electrophoresis. *J. Dairy Sci.* **67**: 3052-3056.

Park, Y.W., Juarez, M., Ramos, M. and Haenlein, G.F.W. (2007). Physicochemical characteristics of goat and sheep milk. *Small Rum. Res.* **68**: 88-113.

Pyne, G.T. (1932). The determination of milk-proteins by formaldehyde titration. *Biochem. J.* **26(4)**: 1006–1014.

Si-Ahmed, Z.S., Almi. D., Senoussi, C., Boudjenah, H.S and Mati, A. (2012). Separation and characterization of major milk proteins from Algerian Dromedary (*Camelus dromedarius*). *J. Food Agric.* **25**: 283-290.

Snedecar, G.W. and Cochran, W.G. (1994). Statistical Methods, (10th Edn.), Iowa State University Press, Ames, Iowa.

Vasbinder, A.J., Rollema, H.S, Bot, A. and de Kruif, C.G. (2003). Gelation mechanism of milk as influenced by temperature and pH; studied by the use of transglutaminase cross-linked casein micelles. *J. Dairy Sci.* **86**: 1556–63.

Zagorchev, L., Dimitrova, M., Odjakova, M., Teofanova, D. and Hristov, P. (2013). Electrophoretic characterization of milk proteins from Bulgarian Rhodopean cattle. *Bulg. J. Agri. Sci.* **19**: 197–200.

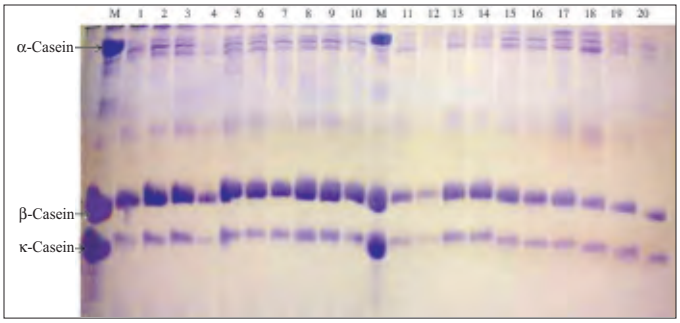

Fig. 2. SDS-PAGE profile of milk proteins (15% Acrylamide). M- Markers, Lanes 1-10: Mithun whey proteins, Lanes 11-20: Cattle whey proteins.