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Effect of Milk Sources on Whey Protein and Fractions of Casein

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Milk from Cow, Buffalo and goat was used for the study of isolation and fractionation of casein and whey proteins. Buffalo and goat milk were subjected to physicochemical attribute studies and a detailed protein profile namely total protein, casein fractions α-, β- and κ casein and whey protein were separated by urea fractionation method. The aim of this study was to compare various casein fractions isolated from cows, Buffalo and Goat milk. The method of separating casein into its fractions is based on the solubility of the individual components in urea the solution. The separation of casein fractions was carried out in dissolving molar concentration of urea and the yield of fractions and total protein percentages were calculated. Buffalo and goat milk have comparatively higher total protein and whey protein content as compared to cow milk. Maximum

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whey proteins found in goat milk (20.58%) whereas cow milk had the lowest whey protein (19.29%). Among casein fractions, α fraction (16.64%) was found maximum in Buffalo milk followed by cow (14.92%) and goat milk (5.42%). The goat milk showed the highest level of β casein (17.81%) whereas, the lowest concentration of β casein was found in cow milk (9.38%).

Study Design: A significant contribution to the total milk production of India comes from buffalo milk and goat milk. Despite that, Buffalo milk and goat milk are not being utilized for many products because of difference in composition of different species milk and their inherent problems associated in the production of *products*.

Place and Duration of Study: Karnataka Veterinary Animal and Fisheries Sciences University (KVAFSU), Dairy Science College, Hebbal, Bangalore Karnataka, India

Materials: The Goat milk samples were collected from Sinchana Goat and Sheep farm, Marenahalli village (Bengaluru Rural Dist) and Buffalo milk was obtained from Country Delight Pvt. Ltd., J. P. Nagar, Bengaluru, Karnataka. Cow milk used in this investigation was collected from SEDP, Dairy Science College, Hebbal, Bengalore.. All the glassware used was soaked in chromic acid solution, repeatedly washed with water, rinsed with distilled water and dried before use. For microbiological analysis dried test tubes, conical flasks, pipettes were cotton plugged and sterilized in a hot air oven. The chemicals and reagents used in this study were mainly of analytical grade procured from Prince Laboratory Company Pvt. Limited, Bangalore. The protein molecular weight markers used for the electrophoretic study were procured from Bangalore Genei Pvt. Ltd.

Methodology: Standard urea fractionation method adopted by Hipp *et al* was used for isolation and fractionation of caseins. Skim milk was prepared by subjecting whole milk for centrifugation, the skim milk obtained was acidified using 1 % hydrochloric acid at 20^{0} C to obtain casein precipitate. Obtained whole casein was dissolved in 3.3 M urea at pH 7.5 and adjusted to pH 4.6 which precipitates the bulk of α casein and leaving k-caseins into soluble filtrates. Again the supernatant was adjusted to pH 4.9 diluted to 1.0 M urea and warmed to 30^{0} C precipitating the β-caeisn. Further, addition of ammonium sulphate was used to isolate k-casein from the precipitate.

Results: The sources of protein had a significant influence on the total casein, casein fractions and whey protein content of different species. The highest quantity of total caseins (34.30g/l) and whey protein (8.87 g/l) were observed in buffalo milk followed by cow (28.52 g/l) and lowest was observed in goat milk (28.45 g/l). Total protein, casein and whey protein contents were greatly affected by the source of milk.

Conclusion: The significant effect of source of milk on total protein, casein, its fractions and their yield was observed. highest yield of casein (54.31%) was observed in cow milk than buffalo milk (48.95 %) and lower yield was found in goat milk. Amongst the three species, highest per cent of β casein was noted in goat milk (54.05 %) followed by buffalo (36.03%) and cow milk (34.14%).

Recommendation: *Rasagulla* will be consumed universally by all age groups globally. Thus, the consumption of *Rasagulla* helps in providing overall nutritional requirements. However, the study has been conducted on a pilot scale. Moreover, commercial production may necessitate huge cost on enzymatic modification of proteins and separation peptides for value addition.

Keywords: Rasagulla; chhana; buffalo milk; goat milk; milk protein; whey protein and casein.

1. INTRODUCTION

Milk production in India reached to 221.0 million tonnes in the year 2021-22 with a growth rate of 6.38 per cent per annum. The share of milk contribution by Cow, Buffalo and Goat to India's milk production is 51.85 per cent, 44.84 per cent and 2.93 per cent, respectively. Among the species, indigenous Buffaloes have the highest share of milk production in India with 32.13 per cent in the fiscal year 2022, followed by cross breed cows accounting for over 29.31 per cent of the total milk production in the country [1]. The richness of buffalo milk makes it highly suitable for processing if proper processing technologies are exploited. The earlier workers have reported about the fractionations of the cow and buffalo milk proteins but not much on goat milk protein fractions.

Buffalo milk, like cow's milk, can be utilized for the manufacturing of a wide variety of dairy products such as cream, butter, butter oil (clarified butter or ghee), UHT cream, ice cream, yoghurt and some cheeses without changing the equipment or processing strategies. However, processing technology and equipment designed for manufacturing cow milk product are often not

adequately suitable for the production of certain dairy products using Buffalo milk due to the differences in composition, physico-chemical, and functional properties. The variation in the composition of buffalo milk as compared to cow milk affects the processing and yield of various dairy products, as the machineries are standardized for cow milk. Out of total milk produced in India more than 50 per cent of milk is being utilized for the preparation of dairy products.

"Buffalo milk is often not considered as ideal for the manufacture of several types of cheeses, milk powders, evaporated & condensed milk, infant formulae and Chhana-based dairy sweets, due to the higher concentration of calcium, protein, fat and larger size of casein micelles, which produces undesirable quality, and causes textural defects in dairy products. Therefore, conventional processing technologies are often unsuitable and cannot be applied directly for the production of chhana and chhana based sweets out of buffalo milk. Pattern of milk consumption in India indicates that about 6 per cent of milk is converted into *Chhana and Chhana-based* products" [2].

"Generally, cow milk is preferred for chhana making as it produces soft body and smooth texture product which is highly suitable for the production of chhana-based sweets, particularly *rasagulla*. However, buffalo milk because of many inherent differences in physico- chemical make up as compared to cow milk, poses many technological problems in the preparation of good quality chhana and *rasagulla".* [2] Hence suitable modifications are required in buffalo milk in order to manipulate the composition which is similar to cow milk which ultimately makes it suitable to produce *chhana and chhana-based* sweets particularly *rasagulla.*

"Goat milk differs from cow or buffalo milk in having better digestibility, alkalinity, buffering capacity and certain therapeutic values. Goat milk is considered to be an ideal food for people suffering from cow milk allergies and other gastrointestinal ailments. Feeding goat milk to infants provides significantly higher digestibility as compared to cow milk. The children fed on goat milk surpassed those fed on cow milk in weight gain, skeletal mineralization and blood serum content of vitamin A, calcium, thiamin, riboflavin, niacin and hemoglobin" [3].

The protein fractions such as α-casein, β-casein, k- casein, β-lactoglobulin and α-lactalbumin are similar in concentration in goat milk and cow milk., but they differ widely in genetic polymorphisms and their frequencies in the goat population. Peptides formed from the enzymatic cleavage of caseins of goat milk have greater advantages than those from cow milk casein. Goat milk fat differs in contents of fatty acids profile significantly from average cow milk fat. Goat milk has a higher content of monounsaturated fatty acids, polyunsaturated fatty acids, medium chain fatty acids, than cow milk which are proven to be beneficial for cardiovascular disorders.

2. MATERIALS AND METHODS

The Goat milk samples were collected from Sinchana Goat and sheep farm, Marenahalli village (Bengaluru Rural Dist) and Buffalo milk was obtained from Country Delight Pvt. Ltd., J. P. Nagar, Bengaluru, Karnataka. Cow milk used in this investigation was collected from SEDP, Dairy Science College, Hebbal, Bengalore. Commercially available pure Neutrase enzyme was purchased from DSM Nutritional Products India Pvt. Ltd, Bangalore. All the experiment values are obtained from the average of three trails and statistical analysis was carried out to know the degree of variance among the samples. Glassware's used was soaked in chromic acid solution, repeatedly washed with water, rinsed with distilled water and dried before use. For microbiological analysis dried test tubes, conical flasks, pipettes were cotton plugged and sterilized in hot air oven. The chemicals and reagents used in this study were mainly of analytical grade procured from Prince Laboratory Company Pvt. Limited, Bangalore. The protein molecular weight markers used for the electrophoretic study was procured from Bangalore Genei Pvt Ltd. All the necessary reagents were prepared in distilled or double glass distilled water for all analytical purposes and freshly prepared reagents were used in the study. Standard procedures (IS 1479) 2001 were followed for analysis milk.

2.1 Isolation of Whole Casein and Whey Proteins

Whole casein and whey proteins were isolated by coagulation of buffalo and goat skim milk separately at pH 4.6 using 10 per cent dilute hydrochloric acid. Then the suspension was cooleddown to room temperature and was left for 5 min. Afterwards, it was filtered through muslin cloth and casein precipitate was washed 2 to 3

times with cold distilled water to remove traces of acid. The resultant product was freeze dried [4]. Whey proteins were separated by precipitation and filtration of whey. The protein was estimated by Kjeldahl Method.

2.2 Fractionation of Caseins by Urea Solubility Method

Casein fractions were separated based on their differential solubility in urea solution as per the method outlined [4].

2.3 Analysis of Casein Fractions by SDS-PAGE

SDS-PAGE was carried out to assess the molecular weight ranges of casein fractions [5]. The following reagents were employed for analysis.

3. RESULTS AND DISCUSSION

Effect of source of milk protein on yield of caseins and whey proteins Casein and whey proteins were isolated from cow, buffalo and goat milk. The yield of total protein, casein, and whey protein are presented in Table (1). As observed from the table, buffalo milk resulted in a significantly higher total protein content (42.50 g/l) followed by goat milk (35.82 g/l) and cow milk (35.76 g/l). The respective casein content of cow, buffalo and goat milk were observed to be 28.52 g/l, 34.30g/l, and 28.45 g/l. The casein yield was highest in buffalo milk (80.71 %) followed by cow milk (79.76 %) and goat milk (79.42 %). The protein content of cow, buffalo and goat milk was observed to be 3.58, 4.25 and 3.68 per cent respectively. The whey protein yield was observed to be 7.24, 8.20 and 7.37 g/l, respectively, for cow, buffalo and goat milk. Whey protein yield varied between 19.29 to 20.58 per cent as against casein yield which varied between 79.42 to 80.71 percent.

The cow, buffalo and goat milk used in this investigation were analysed for total protein, casein and whey proteins content, and the results are presented in Table (1). It is pertinent to note that buffalo milk resulted in highest yield of total protein (42.50 g/l) as compared to cow's (35.76 g/l) and goat milk (35.82 g/l). Higher yield of total protein in buffalo milk is mainly attributed to higher initial protein content in buffalo milk. The buffalo milk resulted in higher yield of casein (34.30 g/l) as compared Cow (28.52 g/l) and goat milk (28.45g/l). Buffalo milk yielded not only higher casein but also higher whey protein content. In case of buffalo milk the yield of whey protein was higher (8.20 g/l) as against cow's milk (7.24 g/l) and goat milk (7.37 g/l), these results are in agreement with the earlier workers [6,7,5]. The Buffalo milk possessed higher casein and whey protein content as compared to cow's milk and goat milk.

Table 1. Effect of source of milk on yield of total protein, caseins and whey proteins

Source of Milk	Total Protein (g/l)	Protein (%)	Caseins (g/l)	Casein Yield (%)	Whey Proteins (g/l)	Whey Proteins Yield (%)
Cow	35.76^{a}	3.58^{a}	28.52^{a}	79.76^{a}	7.24^{a}	20.24^{a}
Buffalo	42.50^{b}	4.25^{b}	34.30^{b}	80.71^{a}	8.20^{b}	19.29^{a}
Goat	35.82^{a}	3.68^{a}	28.45^{a}	79.42^{a}	7.37^{ac}	20.58^{a}
CD (p<0.05)	0.57	0.51	0.55	0.53	0.53	0.58

- *All the values are average of three trails.*
- *Similar superscripts indicate non-significant at corresponding critical difference (CD)*

Table 2. Effect of source of protein on yield of various fractions of casein

Source of Casein Fractions	Total Casein (g/l)	Yield (%)	Total α-Casein (g/l)	Yield (%)	Total β-Casein (g/l)	Yield (%)	Total k-Casein (g/l)	Yield (%)
Cow	27.47^{a}	76.83^{a}	14.92^{a}	54.31^{a}	9.38^{a}	34.14^{a}	3.17^{a}	11.53^{a}
Buffalo	34.13^{b}	76.70^{a}	16.64^{b}	48.75^{b}	12.30^{b}	36.03^{b}	5.19^{b}	15.20^{b}
Goat	26.62^{c}	72.33^{b}	5.42^{c}	20.36^{c}	17.85^{c}	54.05^{c}	3.35^{ac}	20.49^{c}
CD (p<0.05)	0.53	0.54	0.47	0.60	0.56	0.50	0.55	0.49

- *All the values are average of three trails.*
- *Similar superscripts indicate non-significant at corresponding critical difference (CD)*

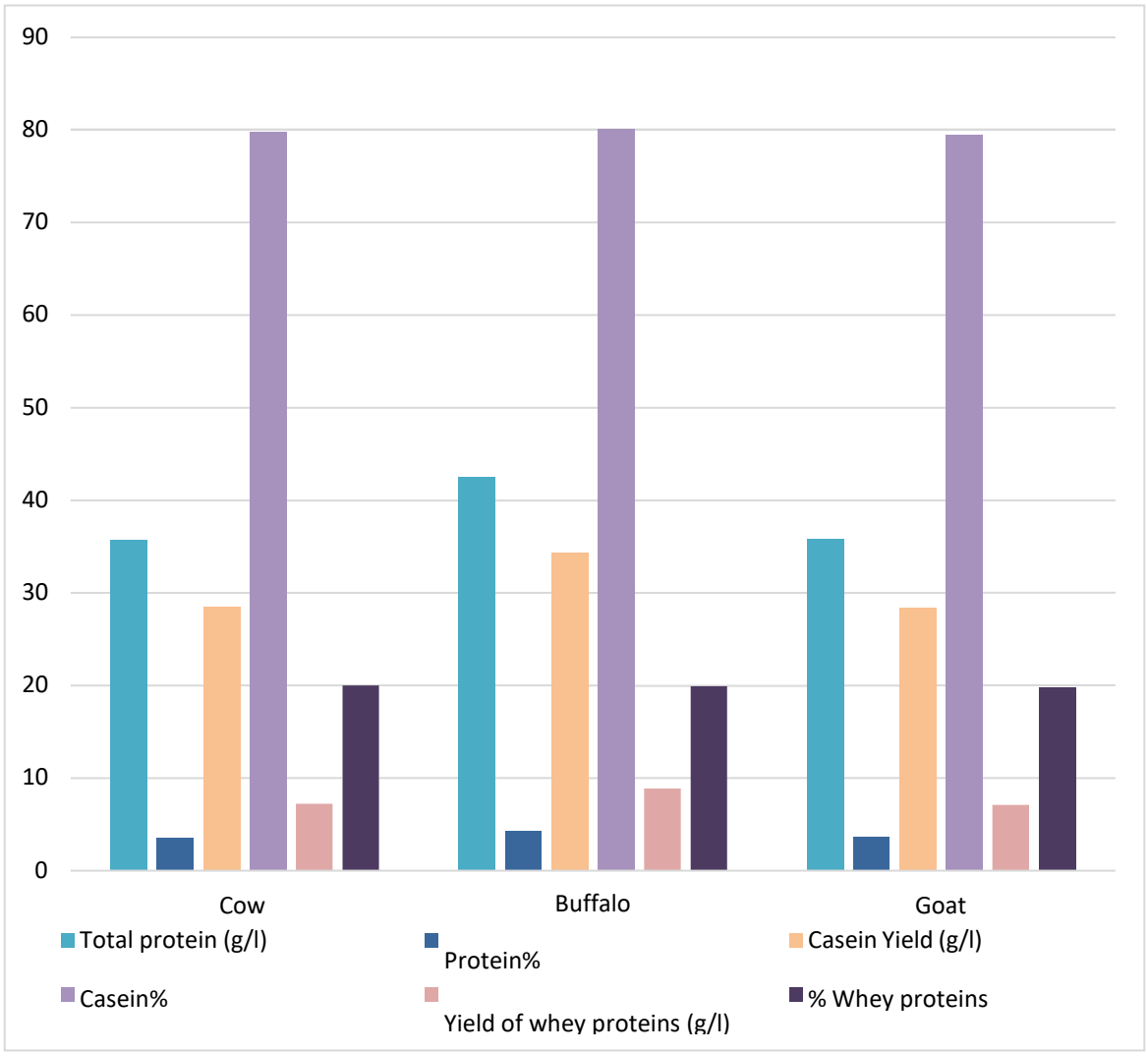

Fig. 1. Effect of source of milk protein on yield of caseins and whey protein

3.1 Effect of Source of Protein on Yield of Various Fractions of Casein

The effect of source of milk protein on yield of various fractions of protein is presented in Table (2) and Fig (2). The total casein content of cow, buffalo and goat milk was recorded to be 27.47 g/l,34.13g/l, and 26.62 g/l, respectively. The total α casein content in buffalo milk (16.64 g/l) and cow milk (14.92 g/l) were significantly higher as compared to goat milk (5.42 g/l). Goat milk had significantly higher β casein (17.85 g/l) as compared to buffalo (12.30 g/l) and cow milk (9.38 g/l,). It is observed from the Table (3) that the concentration of k- casein was much higher in buffalo milk (5.19 g/l) as compared to goat milk (3.35 g/l) and cow milk (3.17 g/l). There was a significant difference in k-casein content of buffalo milk (5.19 g/l) as compared to cow (3.17 g/l) and goat milk (3.35 g/l). Significant difference was observed in the protein content amongst Cow, Buffalo and Goat.

The effect of source of milk proteins on yield of various fractions of casein was studied and results are presented in Table (2). The casein obtained from various sources was subjected to fractionation and the yield of various fractions viz α casein, β casein and k casein were estimated. It is pertinent to note that buffalo milk resulted in higher yield of total α casein and k-casein (16.64 and 5.19 g/l) as compared to cow (14.92 and 3.17 g/l) and goat milk (5.42g/l and 3.35 g/l). This could be due to genetic inheritance of buffalo

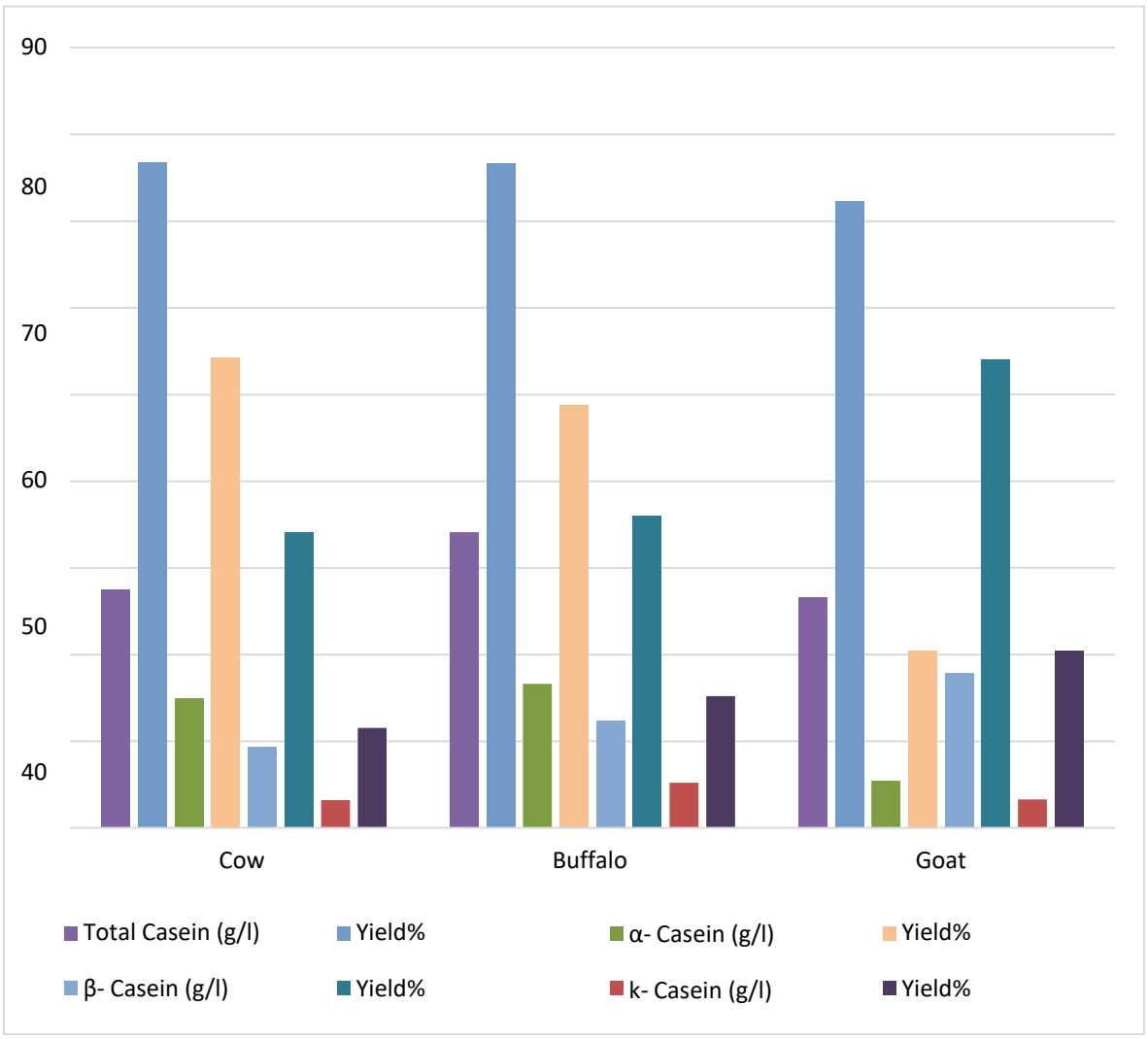

Fig. 2. Effect of source of protein on yield of various fractions of casein

milk, which carry higher proportion of α casein and k-casein than cow and goat milk. The results are in agreement with earlier workers [8,5]. Similarly, β casein content was also higher in buffalo milk (12.30 g/l) as compared to cow milk (9.38 g/l). But in goat milk β casein (17.85 g/l) content was significantly higher as compared to cow (9.38 g/l) and buffalo milk (12.30 g/l), though goat milk yielded lowest per cent of α-casein as compared to cow and buffalo milk. This may be due to variation from species specific [9,3].

4. CONCLUSION

Casein obtained from cow, buffalo and goat milk were fractionated by urea solubility method and the fractions were quantified. The significant effect of source on protein yield and fractions of caseins was observed. Higher yield of α -casein (54.31 %) was observed in cow milk than buffalo milk (48.95 %) and lower yield (20.36 %) was found in goat milk. Amongst the three species, highest per cent of β casein was noted in goat milk (54.05 %) followed by buffalo (36.03 %) and cow milk (34.14 %), The highest molecular weight of α-casein (23.82 kDa) was observed in cow milk followed by goat milk (23.61KDa) and buffalo milk (22.74 kDa). Whereas the molecular weight of β casein in cow milk was 24.31, and it was 23.84 for buffalo milk and 23.82 for goat milk. There was no wide variation in molecular weight of k-casein irrespective of source of milk. The molecular weight of k- casein varied between 19.15 to 19.38kDa.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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