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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(8): 1214-1219

www.thepharmajournal.com
Received: 16-06-2021
Accepted: 18-07-2021

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Evaluation and comparative study on the physico-chemical parameters of milk samples collected from Buffalo, cow, sheep and goat of north coastal Andhra Pradesh

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Abstract
The main aim of this study was to evaluate and compare the physico-chemical properties and proximate composition of milk samples obtained from different species mainly buffalo, cow, sheep and goat of north coastal area of Andhra Pradesh. The composition of milk may vary from place to place in species which depends upon several factors. The objective was to explore the variability of the nutritional characteristics of the original milk. The samples were analyzed for physico-chemical properties such as pH, specific gravity and titratable acidity, proximate composition like fat, protein, carbohydrate, ash and total solids. It was observed from the results that among all the species buffalo and goat milk showed higher levels of all the constituents than that of cow and sheep milk. Buffaloe milk showed higher specific gravity, titratable acidity, ash, and protein content than cow milk, but had a lower lactose level than goat and cow milk. All the tested parameters were similar in buffalo and goat milk except lactose which was higher in goat milk.

Keywords: physico-chemical composition, buffalo milk, cow milk, sheep milk and goat milk

Introduction
Ancient man after domestication of various species he learned to use the animals for the provision of milk. These animals include Cows, buffaloes, sheep, goats, and camels. Still these animals are utilized to produce milk for human use in various regions of the world. Milk is that the characteristic secretion of all mammals. All species of mammal's secrets milk from mammary gland to feed mammalian infant. It supplies nutrients, minerals and vitamins in proper form and amount to nourishment of the young. Milk antibodies plays an important role in protecting the young one against infectious diseases (Bylund, 1995) [9]. India's milk output grew from 146.3 million tons in 2014-15 to 198.4 million tons in 2019-20 of which 48% was contributed by buffaloes, 48% by cows, goat contribute 3% and less than 1% by other domestic species (Singh and Capita, 2018) [37]. In developing countries buffaloe milk plays a vital role in human nutrition because of valuable nutrient with high content of milk proteins, lipids, vitamin and other biologically active compounds (Mikailoglu *et al*., 2005) [25]. Buffalo milk contains high levels of fat and low level of cholesterol content which is beneficial for cardiovascular system. Buffalo milk contains 50% more protein, 40% more energy in calories, 40% more calcium, and 50% more natural antioxidants such tocopherol than cow milk. Because of all of these factors, it is heavily consumed by the majority of people on the continent. Cows have contributed significantly to human welfare by offering a variety of services such as draught power, milk, meat, skins, fuel, and a variety of other things (Hodgson, 1979) [20]. Cow milk has long been considered a valuable and nutritious source of nutrition for humans, and millions of people consume it in various forms every day (Heeschen, 1994) [19]. Sheep milk is a fantastic raw resource for the dairy sector, especially for cheese production (Park *et al*., 2007) [29]. Sheep milk has higher titratable acidity, specific gravity, refractive index, viscosity and lower freezing point than average cow milk (Haenlein and Wendorff, 2006) [15]. Goats have a unique role in the lives of smallholder farmers due to their small body size, which allows them to keep a large herd in a small space (Boylan *et al*., 1996) [7]. Goat has been referred as the "poor man's cow'' due to its great contribution to the health and nutrition of the landless and rural poor people (Dresch, 1988) [11]. Goat milk has a higher digestibility, alkalinity, and buffering capacity than cow or human milk (Park, 1994) [28].

The purpose of this research was to evaluate and compare the physicochemical properties of milk samples obtained from buffalo, cows, goats, and sheep in north coastal zone of Andhra Pradesh.

Materials and Methods

The milk samples of buffalo, cow, sheep and goat milk (ten milk samples from each species) were obtained various districts of northern coastal area of Andhra Pradesh. The samples were analyzed for physico-chemical properties and proximate composition of milk. All the samples were analyzed in triplicate. The 'Analytical Reagent' (AR) grade chemicals and reagents were used for analytical work. All the reagents were freshly prepared before analysis.

pH

pH of milk samples was determined by potentiometric method using digital pH meter (Cyberscan 2500, Eutech Instruments). The pH meter was first calibrated using standard buffers of pH 4.0 and 9.2 and standardized using pH buffer of 7.0 at 20.0 ± 0.1 °C.

Specific gravity

The milk samples were warmed to about 40 °C for 5 min by placing in hot water bath. The samples were removed from water bath and mixed gently by inverting and rotating it (bottle) taking care to avoid frothing. Then cooled to a temperature close to that of the lactometer calibration temperature. The milk was poured into the cylinder and adjusted the level of the milk in the measuring cylinder so as to allow slight overflow when the lactometer is inserted. The lactometer reading was noted carefully avoiding parallax and calculated the specific gravity of milk.

Specific gravity of the given sample of milk = CLR / 1000 + 1

Titratable acidity

Titratable acidity of milk samples was determined as per IS: SP-18, Part XI, (1981).

Procedure

Ten millilitres of thoroughly mixed milk was pipetted into 150 mL of conical flask. Three to four drops of phenolphthalein indicator solution (0.5%) were added to the flask. The contents of flask were titrated against standard (0.1 N) NaOH solution added drop by drop from the burette until pink colour persists for at least 20 s and the volume of 0.1 N NaOH required to change the colour was noted down.

$$\text{Titratable acidity (as \% lactic acid)} = \frac{9NV_1}{V_2}$$

Where,
N = normality in mL of the 0.1 N NaOH solution
V_1 = volume in mL of the 0.1 N NaOH required for titration
V_2 = volume in mL of milk taken for the test

Total solids

Total solid (TS) content of milk samples was determined by the gravimetric method as described in IS: SP-18, Part XI, (1981). A clean, dry empty dish and lid were heated in oven (Falcon Scientific Co., Bengaluru, India) maintained at 100±2 °C for 1 h, cooled in a desiccator and weighed accurately. About 5 mL of sample was pipetted into the dish and weighed along with the lid. The dishes were placed without lid on a boiling water bath until the water was removed from the sample. The water under surface of the dish was wiped and placed along with the lid in the oven maintained at 100±2 °C for 3 h. They were then cooled in a desiccator and weighed accurately. Heating at 100±2 °C for 30 min, cooling and weighing were repeated until successive weights did not vary by more than 0.5 mg.

$$\text{\% TS in milk} = \frac{W3-W1}{W2-W1} \times 100$$

Where,
W1 = Weight of empty dish
W2 = Weight of empty dish + weight of sample
W3 = Weight of dish + weight of sample after drying

Fat

Fat content of milk samples were determined using volumetric method

Procedure

Exactly 10 mL of Gerber sulphuric acid was measured using automatic (tilt) and poured into the butyrometer. Then 10.75 ml of the well-mixed sample of milk and 1 ml amyl alcohol was added to the above butyrometer and tighten the stopper and mixed the contents by shaking the butyrometer at 45° angle until all the curd have been dissolved. The butyrometer was kept into the centrifuge machine. The samples were centrifuged at 1000– 1200 rpm for 5 min and observed the fat % by adjusting the fat column within the scale on butyrometer.

Protein

Protein content in milk samples was estimated by Kjeldahl method as per IS: SP-18, Part XI, (1981).

Procedure

Nitrogen content in milk samples was estimated by Kjeldahl method as per IS:SP-18 (1981). Milk samples were digested in H_2SO_4, using $CuSO_4.5H_2O$ as catalyst with K_2SO_4 as boiling point elevator, to release nitrogen from protein and retain nitrogen as ammonium salt. Concentrated NaOH was added to release NH_3, which was distilled, collected in H_3BO_3 solution, and titrated.

Milk samples were warmed at 38±1 °C and 5±0.1 g was weighed into Kjeldahl tube. Five grams of digestion mixture and 12.5 mL of concentrated sulphuric acid were added to the flask. The contents were digested in Kjeldhal digestion unit (Gerhardt Analytical Systems, Germany) until clear bluish-green digest was obtained. About 30 mL of distilled water was added to the tube along the sidewalls. The tube was placed in Kjeldahl distillation unit. Auto measured quantity (30 mL) of 50% (w/v) standard NaOH solution was added to it to make the solution alkaline. The contents were steam distilled and liberated ammonia was collected in 25 mL of saturated boric acid solution containing 2-3 drops of mixed indicator (equal volumes of a 0.1% saturated solution of methyl red in 95% ethanol and 0.1% solution of methylene blue in 95% ethanol). After completion of distillation, the distillate was titrated against 0.1 N standard sulphuric acid to an end point of pink colour. A blank test was carried

simultaneously using all the reagents and 0.5 g pure analytical grade sucrose in place of the test material. The total nitrogen content was calculated using following formula:

$$\% \text{ Nitrogen} = \frac{14.007 \times (Vs - Vb) \times \text{Normality of sulphuric acid}}{\text{Weight of sample}} \times 100$$

Where,
Vs = mL of 0.1 N H_2SO_4 titrant used for test portion
Vb = mL of 0.1 N H_2SO_4 titrant used for blank
% Protein content in milk = % Nitrogen × 6.38

Total ash
Total ash content was determined as per the method described in IS: SP-18, Part XI, (1981). About 1000 milligram of sample was weighed accurately in a previously heated, cooled and weighed silica crucible. It was carefully charred on a heater or flame and then the sample was kept in a muffle furnace (Murophy Scientific, Bengaluru, India) maintained at a temperature not more than 550 °C until white ash was obtained. Care was taken not to exceed the temperature of the muffle furnace beyond 550 °C to avoid evaporation the evaporation of certain metal chlorides. The crucibles were cooled and stored in a desiccator until the final weight was taken.

$$\% \text{ Ash in milk} = \frac{W_1}{W} \times 100$$

Where,
W = Weight of the sample
W_1 = Weight off the residue after heating

Lactose
Lactose content was estimated by Lane –Eynon method

Procedure
Twenty-five ml of milk samples were taken in to a 500 mL conical flask and diluted with distilled water to about 200 mL. Exactly 3.75 mL of 10% acetic acid was added and boiled. It was cooled and transferred to a 250ml volumetric flask and make up the volume to mark with distilled water. The solution was filtered through a dry filter paper, and filled into the burette. Five mL of each Fehling's Solution A and Fehling's solution B was pipetted in to 250 ml conical flask and preliminary titration was done by adding the filtrate containing lactose, from the burette, to the Fehling's solution kept boiling till the blue color changed to red. Another 10ml of Fehling's solution A and B was added further to it and heated to boiling. About 5 drops of methylene blue indicator was added to the boiling mixture and titrated by additions of 4 – 6 drops of the filtrate until the blue color was changed to colorless supernatant with the formation of brick red precipitate.

$$\text{Weight of lactose present in 100ml of milk} = \frac{W}{V} \text{ X } 250 \text{ X } \frac{100}{25} \text{ mg.}$$

Where;
Volume of milk filtrate required for complete reduction of 10ml of Fehling's solution = V ml
Lactose equivalent in mg for ml = W g.
V ml of filtrate lactose = W mg

Therefore, 250ml of filtrate w/v X 250mg lactose = 25ml of milk

Statistical Analysis
The statistical analysis was carried out using SPSS program (Statistical Package for Social Sciences version 16). The significant differences between means were calculated by one-way Analysis of Variance (ANOVA).

Result and Discussion
Physico-chemical properties
pH Value
At the time of sampling itself milk samples from different species of animal pH was determined. The results showed that the pH values were in the range of 6.61-6.99 in buffaloe milk, 6.59-6.67 in cow milk, 6.46-6.64 in sheep milk and 6.54 -6.69 in goat milk samples. pH values of buffaloe milk were significantly (P<0.001) higher than that of cow, sheep and goat milk (Table 1). The results showed that the pH values of cow and goat were non significantly (P>0.05) different from each other. The pH value of buffalo milk ranges from 6.57 to 6.84 and is not affected by season of calving, month or lactation number but correlated with solid-not-fat and lactose contents (Minieri *et al*., 1965) [26]. Lingathurai *et al*. (2009) [23], Han & Ding, 1994 and Fundora *et al*. (2001) [17, 13] also reported the average pH of raw cow milk were (6.44±0.25) which is similar to present report. Gervilla *et al*. (1997) [14] also reported that the pH value of bovine milk 6.66, sheep milk pH 6.58 and goat milk pH 6.59 which were in support with present study.

Specific gravity
The values of specific gravity of milk samples collected from buffaloe, cow, sheep and goat samples were given in the Table (1). It was observed from results that the specific gravity was found in the range of 1.034-1.035 in buffaloe milk, 1.028-1.031 in cow milk, 1.029-1.031 in sheep milk and 1.035-1.036 in goat milk. Specific gravity of buffaloe and goat milk was higher than that of cow and sheep milk at highly significant level (P<0.001). There was non significant (P>0.05) difference between cow and sheep milk, buffaloe and goat milk. Haggag *et al*. (1991) [16] research findings regarding specific gravity are in line with the present findings (1.035) for the specific gravity of normal buffalo milk. Buffalo milk had a lower specific gravity of in some clinical and subclinical cases, 1.014 and 1.028 respectively. The goat milk specific gravity reported in the present study was in support with Singh *et al*. (2015) [38]. The increase specific gravity in goat milk than cow and sheep milk might be due to stage of the lactation or the basal diet.

Titratable acidity
The titratable acidity values of fresh milk samples from all species were calculated immediately after they were collected and the data is shown in the Table 1. Titratable acidity was found in the range of 0.16-0.19% in buffaloe milk, 0.14-0.17% in cow milk, 0.14-0.17 in sheep milk and 0.16-0.19% in goat milk. The values of titratable acidity of buffaloe and goat was found significantly (P<0.001) higher than cow and sheep. Difference between the values of buffaloe and goat milk; cow and sheep milk were non-significant (P>0.05). Lactic acid accounted for 25% of total acidity in fresh milk. In

buffalo milk, acidity was correlated with fat and solid-to-fat ratios, but not in cow milk. The values of the buffalo milk titratable acidity were in accordance with the findings Rehman and Salaria (2005) [33]. The values of titratable acidity in cow milk were in support with that reported by Enb *et al*. (2009) [12]. The goat milk titratable acidity values of were similar to the findings of Sawaya *et al*. (1984) [36]. The values of sheep milk titratable acidity were similar to that reported by Haenlein and Wendorff (2006) [15]. Lactic acid, citric acid, and phosphoric acid all contribute to the acidity of milk (Bylund, 1995) [9].

Table 1: Physico-chemical properties of different species of milk

Species	pH values	Specific gravity	Titratable Acidity (%LA)
Buffaloe	6.85±0.11^{c}	1.036^{b}	0.17±0.01^{b}
Cow	6.63±0.02^{b}	1.029^{a}	0.16±0.01^{a}
Sheep	6.56±0.06^{a}	1.030^{a}	0.16±0.01^{a}
Goat	6.64±0.05^{b}	1.036^{b}	0.17±0.01^{b}

The Values bearing different alphabets significantly differ (***P<0.001, **P<0.01, *P< 0.05) among the rows

Proximate composition of milk

Total Solids

The concentration of total solids in milk samples collected from buffaloe, cow, sheep and goat were given in the Table 2. Results illustrated that the concentration of total solids was in the range of 16.87-19.11% in buffaloe milk, 12.12-15.22% in cow milk, 12.25-13.31% in sheep milk and 17.80-18.60 in goat milk. The concentration of total solids in buffaloe milk was higher than that of sheep and goat milk at significant (P<0.001) level. The concentration of total solids in goat milk was also higher than that of cow and sheep milk at significant (P<0.001) level. Statistical analysis showed non- significant (P>0.05) difference between the concentration of total solids in buffaloe and goat milk. The concentration of total solids found in the buffalo milk was similar to that reported by Ahmad *et al*. (2007) [2], Menard *et al*. (2010) [24] and Han *et al*. (2012) [18]. Total solids content in milk of various species of cow like Ayrshire, Brown Swiss, Holstein, Jersey and Zebu were found to be 13.1%, 13.3%, 12.2%, 15.0%, 14.7%, respectively (Altman and Dittmer 1961) [3]. The present findings of total solids in cow milk samples range were found lower than that of Ceballos *et al*. (2009) [10] who reported 11.36% total solids in cow milk. The total solids concentration in goat milk was similar to that determined by Kanwal *et al*. (2004) [21]. Talevski *et al*. (2009) [39] observed that the concentration of total solids in sheep milk was similar to the present findings.

Fat content

Fat content in milk samples collected from buffaloe, cow, sheep and goat are given in the Table 2. Results revealed that fat content was in the range of 6.98-8.89% in buffaloe milk, 3.39-4.98% in cow milk, 3.14-4.67% in sheep milk and 6.10-6.82% in goat milk. The amount of fat content in buffaloe milk was higher than the milk of other species at highly significant (P<0.001) level. The amount of fat content in goat milk was significantly (P<0.001) significantly higher than sheep and cow milk but lower than that in buffaloe milk at a significant level (P<0.001). There was non- significant (P>0.05) difference found between the amount of fat content in cow and sheep milk. Buffaloe milk is almost twice as rich in fat as compared to cow milk. Han *et al*. (2012) [18] reported range of fat content between 6.57% and 7.97% in buffalo milk. The values of buffaloe milk were in support with Varrichio *et al*. (2007) [40] who reported the average value of buffaloe milk fatwas 8.3% and reaches high in normal healthy condition. The fat content in cow milk was slightly higher than the findings of Kula (2016) [22] and Barreto *et al*. (2019) [6] but in agreement with the findings of Zhang *et al*. (2004) [41] and Lingathurai *et al*. (2009) [23]. Mixed southern milk has better nutritious contents than western milk, according to research findings (Han & Ding, 1994; Amerjit & Tshihiko, 2003) [17, 4]. Sheep although produces less milk than cow but the fat content was more than in cow milk. The fat content of sheep in the present study was lower than the findings reported by Kula. (2016) [22] and Barreto *et al*. (2019) [6]. The amount of fat content found in goat milk during this investigation was similar to that cited by pal *et al*., 2011 and higher than that reported by Singh *et al*. (2014). The variation in fat content might be due to quality and quantity of the feed, stage of lactation, genetical variation.

Protein content

From the Table 2 it was observed that the protein concentration of the milk samples was in the range of 3.82-4.57%, 3.19-3.78%, 2.34-3.48% and 4.56-5.62% for buffaloe milk, cow milk, sheep milk and goat milk respectively. The amount of protein content in goat milk was significantly higher than (P<0.001) buffaloe, cow and sheep. The amount of protein content in buffaloe milk was higher than milk of cow and sheep but lower than goat milk at highly significant level (P<0.001). There was no significant difference (P>0.05) found between the protein content in cow and sheep milk. Buffalo milk has a higher protein concentration than cow milk (Ahmad *et al*., 2007) [2]. The research finding of buffaloe milk protein content was in support with Kula. (2016) [22] and Barreto *et al*. (2019) [6]. Braun and Stefanie (2008) [8] reported high protein content in buffaloes than the research findings. The range of cow milk protein was similar to that of the research finds of Kula. (2016) [22] and Barreto *et al*. (2019) [6]. Protein content of various breeds of cow such as Ayrshire, Brown Swiss, Guernsey, Holstein, Jersey, and Zebu were found as 3.6%, 3.8%, 3.9%,4.7%, 4.9%, 4.9% (Altman *et al*., 1961) [3]. The sheep milk protein content was found lower than that reported by Pavic *et al*. (2002) [30], Kula 2016 [22] and Barreto *et al*., 2019 [6]. Goat milk protein content range was found higher than the research findings of Arora *et al*. (2013) [5]. The variation in the protein content might be due breed difference, stage of lactation and health status of the udder.

Table 2: Proximate composition of different species of milk

Species	Total solids (%)	Fat (%)	Protein (%)	Lactose (%)	Total Ash (%)
Buffaloe	18.38±0.96^{c}	8.12±0.50^{c}	4.24±0.25^{b}	4.57±0.19^{a}	0.88±0.03^{c}
Cow	14.14±1.13^{b}	3.91±0.72^{a}	3.39±0.16^{a}	4.81±0.37^{a}	0.69±0.09^{a}
Sheep	12.99±0.40^{a}	4.07±0.47^{a}	3.16±0.42^{a}	4.63±0.23^{a}	0.80±0.06^{b}
Goat	15.92±0.30^{c}	6.73±0.22^{b}	5.21±0.37^{b}	5.07±0.24^{b}	0.86±0.07^{c}

The Values bearing different alphabets significantly differ (***P<0.001, **P<0.01, *P< 0.05) among the rows

Lactose content

Lactose content in milk samples collected from buffaloe, cow, sheep and goat milk were given in the Table 2. Results illustrated that lactose content was in range of 4.21-4.91% in buffaloe milk, 4.10-5.20% in sheep milk and 4.47-5.31 in goat milk. The amount of goat milk lactose content was significantly (P<0.001) higher than buffaloe, cow and sheep

milk. A non- significant difference ($P<0.05$) was found between the amount of lactose content in buffaloe, cow and sheep. Buffalo milk is richer source of lactose than cow, sheep and goat milk so it is a good of source of energy for body activities particularly of brain. Rao and Nagarcenkar. (1997) [32] reported that lactose content in murrah (Indian) was 5.1% which is higher than the present research findings. The lactose content of cow milk discovered in this investigation was similar to that found by Samia *et al*. (2009) [35] and Lingathurai *et al* (2009) [23]. Lactose content in milk of various species of cow like Ayrshire, Brown Swiss, Holstein, Jersey, Zebu are found to be 4.7, 5.0, 4.9, 4.9 and 5.1%, respectively (Altman and Dittmer, 1961) [3]. Sheep milk lactose content was similar to that reported by Pavic *et al*. (2002) [31]. Lactose content in goat milk was in accordance with that reported by Bhosale *et al*. (2009).

Total Ash

Ash content in milk samples collected from buffaloe, cow, sheep and goat were shown in the Table 2. The ash level of buffaloe milk was found to be in the range of 0.84-0.94 percent, according to the findings, 0.56-0.81% in cow milk, 0.72-0.89% in sheep milk and 0.75-0.98% in goat milk. Amount of ash content in cow milk was lower than in goat and buffaloe milk at highly significant level ($P<0.001$). There was significant difference found between ($P<0.01$) between the amount of ash content in cow and sheep milk. There was non- significant difference ($P>0.05$) found between the ash content in the milk samples collected from goat and buffaloe milk samples. The amount of ash content present in the buffaloe milk was in line with Rao and Nagarcenkar. (1997) [32] findings who reported the ash content in Indian murrah buffaloe was 0.8%. The research findings of cow milk are in support with the findings of Enb *et al*. (2009) [12]. The results of ash content in sheep milk were similar to that reported by Adewumi and Olorunnisomo (2009) [1]. Goat milk ash content was in support with Sachdeva *et al*. (1974) [34] who reported the goat milk in the range of 0.82-0.9%.

Conclusion

Collectively our results indicated that physico-chemical properties of buffaloe and goat milk were higher than sheep and cow milk. It implies that buffaloe and goat milk could act as complete source of nutritive value in comparison with cow and sheep milk.

Acknowledgement

The authors are highly thankful to the Heads of the departments to the concerned disciplines for providing facilities to conduct this research.

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