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# Comparative Quantitative Analysis of Collected Milk Samples from Northern Region of Bangladesh

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## Abstract

**Objectives:** This study aimed to compare the physico-chemical and microbial quality of local milk samples from buffalo (B), cow (C), goat (G) and sheep (S).

**Methods:** Milk samples (B, C, G, S, 1 litre each) were collected in sterile bottles from Rajshahi, the northern part of Bangladesh. The samples were analyzed to compare various physico-chemical parameters including pH, specific gravity, moisture content, total solid content, protein, and fat using common methods. Microbiological analysis was performed in MRS agar to count the number of facultative anaerobic microbs (*Lactobacillus*) using serial dilution technique.

**Findings:** The pH and specific gravity of B, C, G and S at the time of collection were 6.95±0.03, 6.67±0.01, 6.56±0.03, and 6.63±0.02, and 1.013±0.01, 1.020±0.06, 1.032±0.03, and 1.011±0.02, respectively; moisture contents were 90.23±0.05, 90.43±0.07, 89.44±0.01, and 88.94±0.09% and total solid contents were 9.77±0.07, 9.57±0.09, 10.56±0.08, and 11.06±0.10%, respectively. Protein and fat contents of B, C, G and S were 3.23, 5.30, 3.41, and 5.59% and 0.00034±0.03, 0.00043±0.01, 0.00054±0.02, and 0.00018±0.03 g/ml, respectively. Colony (*Lactobacillus*) count after 48h incubation (CFU/mL) of freshly collected samples from B, C, G, and S were $7.4\times10^9\pm0.14$, $4.0\times10^9\pm0.20$, $1.8\times10^9\pm0.21$, $2.0\times10^9\pm0.13$.

**Conclusions:** pH and specific gravity of all the tested samples were similar with no significant differences. Highest moisture content (90%) was found in cow and Buffalo milk and the total solid content of sheep milk was higher than other followed by goat milk. Fat content of goat was highest followed by cow milk, whereas the protein content of sheep and cow milk was higher than buffalo and goat milk. Buffalo milk contains highest number of viable cells (7 billion) followed by cow milk (4 billion) indicating the effectiveness of buffalo milk with respect to parameters studied.

**Keywords:** Milk; Viable Microorganism; Lactobacillus; Nutrition; Protein and fat contents

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## Introduction

Milk is a nutrient-rich white liquid food that can be obtained from a variety of mammals such as cows, goats, sheep and buffalo, as well as humans. It is the key supply of nourishment for infant mammals (including humans). Early-lactation milk contains colostrum, which carries the mother's antibodies to its young as well as can decrease the danger of various diseases. It holds a lot of other nutrients [1] including protein and lactose. Interspecies use of milk is not exceptional, mainly with humans, many of whom drink the milk of other mammals [2, 3] and an important part of the diet of billion inhabitants. The entire milks hold the similar type of ingredients except in changeable quantity. Inside a certain variety (cow, goat, sheep and buffalo), inherent features and ecological environment such as the weather and the phase of lactation control the composition [4-6].

In Bangladesh, every year milk requirement is 16.49 million tons and according to Department of livestock service, Bangladesh produces 9.4 million tons of milk in a year mainly by cows which is 63% of the whole necessity [7]. Cow's milk has long been considered a highly nutritious and valuable human food and is

 

consumed by millions daily in variety of products [8]. In addition to cow, goats also play a special role in the life of small holder farmer due to his large donation to the health and diet of the landless and poor. Their small sizes build it feasible for cultivators to keep a large herd in small area [9]. Goat milk vary from cow or human milk in having improved digestibility, alkalinity as well as buffering ability [10]. Sheep milk is an outstanding fresh material for the milk manufacturing industry particularly in cheese creation [11]. It has elevated specific gravity, viscosity, refractive index, titratable acidity, and lower freezing point than regular cow milk [12]. Buffalo is the most important mammal and is being very liked by the inhabitants of the sub-continent. Buffalo milk is favored more than the cow's milk due to taste, high content of milk proteins, lipids, vitamin and other biologically energetic material [13].

Lactic acid bacteria are extensively distributed in the plants, insects, birds, humans, animals, water, which they used for the fermentation and preservation of a wide range of milk, meat and vegetable foods [14]. Lactic acid bacteria produce lactic acid as their key product and are proven valuable effects on human health including improvement of lactose intolerance, prevention and healing of diarrhea, preservation of regular intestinal flora, antagonism not in favor of pathogens and inspiration of the immune system, anticarcinogenic action and declining of serum cholesterol level [15]. There has been a growing interest in the microbial content in milk since the quality of milk is influenced by the viable microbial contents [15].

To deal with the perseverance of under nutrition in Bangladesh, multiple evidence-based, nutrition-specific involvements have been in place for a couple of decades. Cereals and rice are the main food in Bangladesh with just about two-thirds of the everyday diet consisting of rice, some vegetables, a little quantity of pulses and minimal amount of protein. Animal-sourced foodstuff such as meat, milk, eggs, and cheese still make up moderately small percentage of a usual Bangladeshi family diet, balanced with grains and cereals. Milk, milk products, and meat are taken rarely and in very minute quantity. Consequently, conventional ingestion behaviors frequently do not turn into a balanced nutritious diet [16]. A large number of factors directly affect the consumption expenditure such as income, prices of individual commodities, volume and composition of household, etc. [17]. There is only just proof that the recent increase in production has increased milk consumption by the poor, particularly by women and children. And, as noted, the increase is comparatively little in relation to potential demand. The cost of milk differs in different place of Bangladesh. In rural milk-producing region, the producers sold milk at $ 0.4/L, people in city were paying $ 0.7/L for pasteurized milk and $ 0.9-1.0 /L for fresh milk.

To increase people's awareness of the use of dairy food, beneficial effects of microbes and for application them in food processing especially in rural areas, the present study was carried out to measure and compare the content of fat, protein, solid, and viable microbes in cow, goat, sheep and buffalo milk samples collected from Rajshahi, the northern part of Bangladesh.

# Material and Methods

The present investigation was undertaken at the Department of Pharmacy, University of Rajshahi, Bangladesh during 2018-19.

## Collection of Samples

Four fresh milk samples were collected separately in sterile bottles from buffalo (B), cow (C), goat (G) and sheep (S) and were immediately preserved in refrigerator at $4^0$ C.

# Physico-chemical properties of raw milk

## pH

The pH of different milk samples was determined by using a digital pH meter (Hana pH meter No. 211, China). The pH meter was standardized by pH 4.0 and pH 7.0 buffer solution. pH was measured over several days.

## Specific gravity

Specific gravity or relative density was determined by using pycnometer as described by AOAC, 2003 [18]. In brief, the mass of the flask was measured, cleaned, and dried. Then milk samples were filled and measured. The difference between two mass is the actual mass of samples (m). The following formula was used: Specific gravity or relative density= m/v where v = volume.

## Moisture and total solid content

To determine moisture content 5 g of the each milk sample (Mo) was positioned in petri dishes and reserved in the Gallen kamp oven at 130°C for 1 h. The dehydrated samples were subsequently chilled in desiccators and weighed (M1). The average mass of the three masses was used to determine the moisture content of the samples. The moisture content [18] uttered as a % was calculated as follows:

Moisture content (%) = Mo-M1/Mo ×100.

Total solids content [18] was determined by the following formula:

Total solid (%) = M1/Mo ×100

## Protein

Protein contents of milk samples were determined by method described by Scott et al., 1984 [19].

## Fat

Fat content was determined (on wet weight basis) by Soxhlet's method using the formula: % Fat = g of fat in dry sample/g fat of in wet sample× 100 [20].

## Viable Microbes analysis (Total viable count)

The microbiological analysis of milk samples was carried out for total viable count by "serial dilution pour plate technique" [21].

In brief, specific amount of milk samples were transferred into testube containing 1 ml sterile PBS (pH 7.2) and 10-fold dilutions were made in PBS. Subsequently, 100 µl of each dilution was smeared on the surface of MRS agar and was incubated at 37°C for 24-48 h to count the viable bacteria (CFU).

## Statistical Analysis

All experimental data were used in triplicate and expressed as mean ± SEM. One-way ANOVA followed by Dunnett post hoc test using SPSS 16 software was used for significance test between control and treatment group. Significance level was tested at 5% (p* value < 0.05), 1% (p** value < 0.01) and 0.1% (p*** value < 0.001), respectively.

# Results and Discussion

## pH

The pH of different milk samples is revealed in **Table 1**. In every case, pH slightly decreased during storage. Observed pH at day '0' (collection day) for B, C, G and S was 6.95, 6.67, 6.56, and 6.63, respectively, while at the day '21' (stored at 4°C temperature) pH was 6.61, 6.50, 6.51, and 6.58, respectively. The result is in line with previous report by Samel *et al*. [22] indicating decline in pH during storage of milk. A decreasing trend in pH in storage may be documented to enhance in acidity of milk which increases the fatty acid and lactic acid concentration. These results could be related with the work of Rehman *et al*. [23] who studied that storage period cause a decline in pH and increase in acidity of milk to facilitate raise lactic acid concentration due to degradation of lactose.

## Specific gravity

The specific gravity of milk measured at 15-20 ºC is normally 1.028-1.033 kg/L. It depends on the protein and fat content. The specific gravity of fat is 0.93, solids-non-fat is 1.6 and water is 1.0 kg/L. Specific gravity lower than 1.01 kg/L is an indication of extraneous water. The specific gravity of different milk samples is exposed in **Table 2**. All samples possess similar specific gravity under experimental condition.

## Total solid and Moisture content

The data regarding the influence of storage time on the total solids of the different milk showed in the **Table 3**. Total solid contents of B, C, G and S at "0" day were 9.77, 9.57, 10.56, and 11.06 %, respectively, indicating sheep possesses highest solid contents. After three weeks of storage (stored at 4°C temperature) the value raised to 12.11, 11.65, 13.82, and 17.65%, respectively, as shown in **Table 3**. The existence of significant difference in all milk samples might be owing to sedimentation as well as fat partition. The outcome of current study can be related to the finding of Datta *et al*. [24] who relates the increase in total solids content to sedimentation and fat separation at all temperature by the course of time.

Moisture contents of B, C, G and S at "0" day were 90.23, 90.43, 89.44, and 88.94%, respectively, while at "21" day the values were 87.89, 88.35, 86.18 and 82.35%, respectively. The reduction in moisture content of milk sample might be due to increase in total solids of milk during storage. The current study can be supported by Datta *et al*. [24] who expressed that while the entire solids increased ultimately moisture will be decreased.

## Protein

Protein content in milk samples is given in **Figure 1**. According to the result, protein contents of B, C, G and S at "0" and "21" day were 3.23, 5.30, 3.41 and 5.59 % and 4.29, 6.29, 4.87, and 6.12 %, respectively. The outcomes pointed out that the % of protein content was increased in all the milk samples on day 21 stored at 4°C temperature. According to Law and Haandrikman [25], the proteolytic method of lactic acid bacteria is important for their development in milk. The increase in protein contents in milk samples depends on the proteolytic activity of Lactic acid bacteria which hydrolyses proteins into peptides and amino

**Table 1:** pH of different milk samples.

| Name of milk samples | pH | |
|---|---|---|
| | at day '0' | at day '21' |
| Cow | 6.67±0.01 | 6.50±0.02 |
| Goat | 6.56±0.03 | 6.51±0.04 |
| Sheep | 6.63±0.02 | 6.58±0.01 |
| Buffalo | 6.95±0.03 | 6.61±0.03 |

**Table 2:** Specific gravity of different milk samples.

| Name of milk samples | Specific Gravity | |
|---|---|---|
| | at day '0' | at day '21' |
| Cow | 1.020±0.06 | 1.021±0.04 |
| Goat | 1.032±0.03 | 1.034±0.03 |
| Sheep | 1.011±0.02 | 1.012±0.08 |
| Buffalo | 1.013±0.01 | 1.015±0.01 |

**Table 3:** Total solid and moisture content of different milk samples.

| Name of milk samples | Total solid (%) | | Moisture (%) | |
|---|---|---|---|---|
| | at day '0' | at day '21' | at day '0' | at day '21' |
| Cow | 9.57±0.09 | 11.65±0.08 | 90.43±0.07 | 88.35±0.09 |
| Goat | 10.56±0.08 | 13.82±0.01 | 89.44±0.01 | 86.18±0.01 |
| Sheep | 11.06±0.10 | 17.65±0.11 | 88.94±0.09 | 82.35±0.08 |
| Buffalo | 9.77±0.07 | 12.11±0.06 | 90.23±0.05 | 87.89±0.07 |

acids. Lactic acid bacteria require a wide range of amino acids for growth and their proteolytic enzyme complement is able to split most types of peptide bonds [26]. Probably, the free amino acid present in samples was the result of hydrolysis of protein under the influence of proteolytic enzymes. During the storage period these free amino acids again combine to form the peptide bonds that transform into protein. Hence, the protein contents of milk samples increased during storage [27].

## Fat

Fat contents in milk samples collected from cow, goat sheep and buffalo are shown in **Table 4**. Results illustrated that fat content was increased on day 21 storage periods at 4°C temperature. The increased value for B, C, G and S was 0.00036, 0.00054, 0.00063, and 0.00024 g/ml from 0.00034, 0.00043, 0.00054, and 0.00018 g/ml, respectively. The data is in line with the previously reported data by Connell et al. [28]

## Microbiological analysis

Raw milk is an example of an environment that contains a diverse and complex microbial population [29]. In our study, we counted viable microbial population in MRS agar which is most favorable media for *Lactobacillus* strains. The microbial populations were counted in terms of CFU/ml at day 0, 7, 14 and 21 (stored temperature at 4°C) after 48 hours incubation. The result showed in **Table 5**. The highest number of CFU was counted in buffalo milk samples with increasing numbers with time (**Table 5**). Kaur *et al* [30] also reported highest microbial contents of buffalo milk indicating the highest nutritive value of the milk.

# Conclusion

Since the pH and moisture content of milk were decreased with increased solid content, protein, fat, specific gravity and microbial contents with time, we could conclude that the chemical constituents of milk are altered with time, hence collection, storage and distribution of milk is very important. Considering the overall quality parameter, buffalo milk might be considered superior to other milk products.

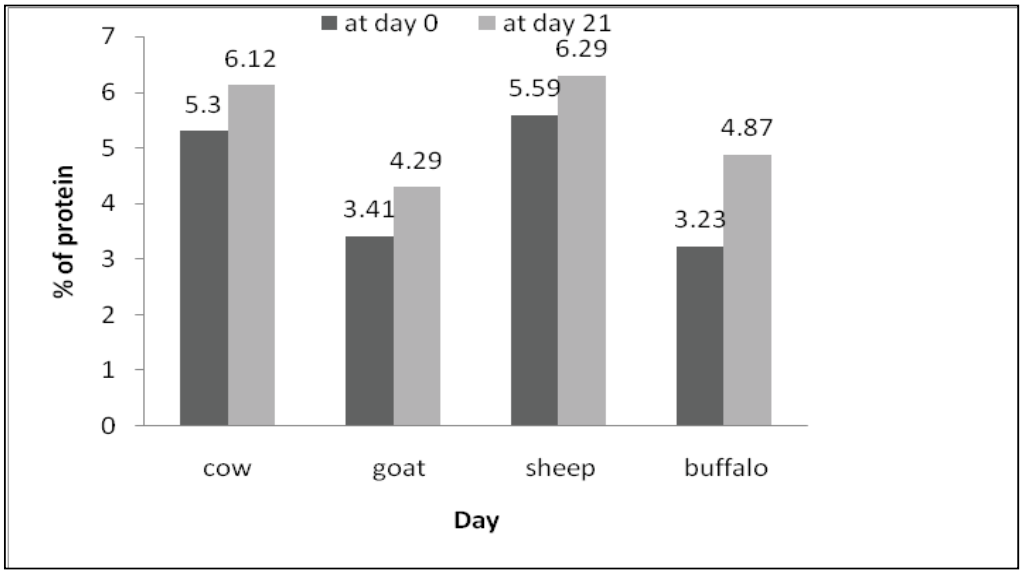


**Figure 1** Percentage of protein in different Milk samples at different day.

**Table 4:** Fat content in different Milk samples.

| Name of milk samples | Fat (g/ml) | |
|---|---|---|
| | at day '0' | at day '21' |
| Cow | 0.00043±0.01 | 0.00054±0.02 |
| Goat | 0.00054±0.02 | 0.00063±0.02 |
| Sheep | 0.00018±0.03 | 0.00024±0.01 |
| Buffalo | 0.00034±0.03 | 0.00036±0.02 |

**Table 5:** Colony of lactobacillus count in different milk samples.

| Name of milk samples | Colony (*lactobacillus*) count after 48 h incubation (CFU/ml) | | | |
|---|---|---|---|---|
| | at day 0 | at day 7 | at day 14 | at day 21 |
| Cow | $4.0\times10^9\pm0.20$ | $5.2\times10^9\pm0.19$ | $18.8\times10^9\pm0.32$ | $46.0\times10^9\pm0.12$ |
| Goat | $1.8\times10^9\pm0.21$ | $4.0\times10^9\pm0.23$ | $6.1\times10^9\pm0.18$ | $32.0\times10^9\pm0.22$ |
| Sheep | $2.0\times10^9\pm0.13$ | $4.3\times10^9\pm0.18$ | $8.0\times10^9\pm0.15$ | $11.0\times10^9\pm0.31$ |
| Buffalo | $7.4\times10^9\pm0.14$ | $9.2\times10^9\pm0.27$ | $48.0\times10^9\pm0.19$ | $60.0\times10^9\pm0.12$ |

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## Conflicts of interest

The authors declare no conflict of interest.

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