**A Comparative Study of Lipolytic Bacterial Count (LBC) and Proteolytic Bacterial Count (PBC) Quality of Buffalo and Cow Milk at Different Times**

**ABSTRACT**

This study evaluates the bacterial quality of raw milk from buffaloes and cows collected between January and March 2020 at LNCT University Bhopal (M.P.) focusing on microbial loads and antimicrobial susceptibility of common milk-borne bacteria. Rigorous sanitary measures were employed during milk collection to ensure cleanliness. All sanitary precaution was followed to produce clean milk. Milk samples were collected from three buffaloes and three cows over ten days, with each sampling time (morning, noon, and evening) and evaluated for the lipolytic bacterial count (LBC) (102) and proteolytic bacterial count (PBC) (102) per mililiter. Each animal samples were replicated ten times. Statistical analysis of the collected data revealed significant differences in the mean values of LBC and PBC between species and milking times. Morning samples of raw cow milk exhibited the lowest LBC, while buffalo milk showed comparatively lower PBC across all sampling times. Bacterial counts were highest in noon samples, followed by evening, with morning milk displaying the lowest counts. Notably, coliform bacteria were absent across all samples. These findings suggest that raw cow milk collected during morning milking is of superior microbial quality in terms of LBC, while buffalo milk generally maintains lower PBC levels.

**Key Words:** Cow, Buffalo, Raw milk, Bacterial quality, milking time.

**INTRODUCTION**

Milk, recognized as a nutrient-rich and wholesome food, is highly susceptible to contamination and spoilage if not managed under proper hygienic conditions. Studies by Mubarack*et al.* (2010) and Lingathurai and Vellathurai (2010) have identified the presence of pathogenic bacteria in milk as a significant public health concern, particularly among populations that consume raw milk. The presence of bacteria in raw milk not only reduces its shelf life but also poses health risks, as certain bacteria and their associated enzymes and toxins can survive pasteurization, potentially leading to serious health hazards (Salman and Hamad, 2011).

Milk-borne pathogens can infect humans through the consumption of contaminated raw or unpasteurized milk and its derivatives (Bertu *et al*., 2010). Instances of milk-borne zoonotic diseases such as brucellosis, tuberculosis, and enterotoxaemia have been documented in various studies, including research by Shirima *et al*., (2003). These risks underscore the importance of public health regulations discouraging the informal milk trade and the consumption of raw or unpasteurized milk (Kang’ethe *et al*., 2000). These bacteria, including *Clostridium spp.*, *Bacillus spp.*, and *Pseudomonas spp.*, produce proteases that remain active even after pasteurization (Fox et al., 2017). Research indicates that cow milk generally has a higher PBC than buffalo milk due to differences in protein composition and inherent antimicrobial peptides present in buffalo milk (Sharma et al., 2020). The proteolytic activity in milk increases with storage time, particularly under suboptimal refrigeration conditions, leading to significant quality losses in both buffalo and cow milk. . Buffalo and cow milk has shown that buffalo milk tends to have a lower LBC than cow milk due to its higher fat content and natural antimicrobial properties (Kumar & Singh, 2021). Studies have identified *Pseudomonas spp.*, *Bacillus spp.*, and *Staphylococcus spp.* as common lipolytic bacteria in raw and processed milk (Chambers, 2019). These bacteria can survive refrigeration and persist in dairy environments, leading to spoilage even under cold storage conditions. Patel *et al*. (2022) reported that the LBC and PBC in both types of milk increased with time, especially when stored at room temperature. Refrigeration significantly reduced bacterial proliferation, but psychrotrophic bacteria such as *Pseudomonas* species continued to grow, affecting milk quality over extended storage durations. Successfully running a dairy farm and making it more profitable for rural area farmers was helpful. Livestock provides farmers with regular, supplementary income to producers engaged in secondary and tertiary farming related to the livestock business. Besides providing manure, livestock is an important source of value-added byproducts. Small dairy farms are not properly processed and utilized as a commercial activity, but have immense future business potential (Singh *et al*., 2024).

**MATERIALS AND METHODS**

The herd consisted of Buffaloes and cows of known breeds, and only healthy animals free from mastitis (as confirmed by a mastitis test) or any infection or injuries were included in this study. All animals were housed in a single barn prepared for milking thrice a day (morning at 5:00 clock, Noon at 12:30 and Evening at 6:30) and grouped as Buffaloes (M1) and cows (M2 and M3). Each group underwent ten replicates. Before milking, the udders were cleaned with a 2% potassium permanganate (KMnO₄) solution, and two streams of foremilk were discarded from each quarter. Milk samples were collected in sterile 250 ml conical flasks, aseptically plugged with cotton, and immediately transported to the laboratory for analysis. The total bacterial population was quantified, focusing on four physiological groups of bacteria: Proteolytic bacteria count (PBC), lipolytic bacteria count (LBC), and coliform bacteria count.

**Bacterial Analysis**

Samples were analyzed to determine the total viable count and specific bacterial groups. Procedures were carried out as described by (Chalmers, 2019).The PBC was measured using nutrient milk agar. The medium was prepared by adding 20 ml of sterilized skim milk to 200 ml of sterilized nutrient agar in a 250 ml conical flask just before pouring into Petri plates. Plates were incubated for 24 hours, and proteolysis was indicated by the appearance of clear hollow zones around bacterial colonies. LBC was determined using nutrient agar supplemented with 40 ml of melted butter fat and 10 ml of a 0.1% Nile blue sulphate solution (pH 7.0). The medium was sterilized by steaming for 30 minutes on three consecutive days. During use, the medium was vigorously shaken to emulsify fat globules. Lipolytic activity was indicated by bluish zones around colonies, while unhydrolyzed fat globules remained pink. Following were the bacterial parameters determined as per method of (Chalmers, 2019).

1. Proteolytic bacterial count (PBC)

2. Lipolytic bacterial count (LBC)

**Sterilization and Preparation of Materials**

Conical flasks were thoroughly cleaned, dried, and plugged with sterile absorbent cotton before being autoclaved at 120°C for one hour. Bacteriological pipettes (1 ml and 10 ml) were soaked in chromic acid overnight, washed, dried, wrapped in paper, and sterilized in a hot air oven at 120°C for one hour. Test tubes used for preparing 9 ml Ringer's solution blanks were washed, plugged with sterile cotton, and sterilized in an autoclave at 120°C and 1.2 kg/cm² for 20 minutes. Sterilized plates were stored in blocks of four, wrapped in paper.

**Ringer's Solution Composition (Prasad and Neeraj, 2004):**

* Sodium chloride (NaCl): 9 g
* Potassium chloride (KCl): 0.42 g
* Calcium chloride (CaCl₂): 0.24 g (0.48 g if hydrated salt, CaCl₂·6H₂O, is used)
* Sodium bicarbonate (NaHCO₃): 0.20 g
* Distilled water: 1000 ml

The prepared Ringer's solution was used for diluting milk samples to the desired ratio before plating. This detailed protocol ensured the accurate determination of bacterial counts, including PBC and LBC, and provided insights into the microbial quality of raw milk.

**RESULTS AND DISCUSSION**

The present investigation, titled ‘**A Comparative Study of Lipolytic and Proteolytic Bacterial Quality of Raw Buffalo and Cow Milk at Different Milking Times’,** was conducted during January. The study aimed to assess the bacterial quality of raw milk from buffaloes and cows collected at different milking times. Milk samples were obtained from three buffaloes and three cows over ten days, with each sampling time (morning, noon, and evening) treated as a replicate. The results of the investigation, including bacterial quality assessments, are presented in tabular format and graphically illustrated where necessary. The findings are organized under the following subheadings for clarity and detailed analysis:

1. **Lipolytic bacterial count/mℓ (LBC x 102)**

The data on the **Lipolytic Bacterial Count (LBC × 10² /ml)** in raw milk from buffaloes and cows at different milking times are presented in Table 1 and Figure 1. The results indicate that the mean LBC × 10² in buffalo milk during the three milking times—morning, noon and evening -was 37.12, 39.68, and 38.10, respectively, with an overall mean of 38.30. The differences among these mean values were statistically significant. Similarly, in cow milk, the mean LBC × 10² during morning, noon, and evening milking times was recorded as 37.74, 40.29, and 38.72, respectively, with an overall mean of 38.92. Statistical analysis revealed significant differences in these values due to the milking times, although variations due to replication were found to be non-significant (Table 1). Comparative analysis showed that LBC was consistently lower in buffalo milk than in cow milk. Among the milking times, morning milk exhibited the lowest LBC, while noon milk recorded the highest LBC, followed by evening milk. These results highlight temporal variations in bacterial loads and the superior microbial quality of morning milk. Research studies (Kumar & Singh, 2018; Smith *et al.,* 2020) suggest that cows generally exhibit higher metabolic efficiency than buffaloes, which could explain the observed variations. Additionally, environmental factors such as temperature, humidity, and feeding conditions might have contributed to these differences (Patel *et al.,* 2019).The notable variation in the M2 parameter across both groups may indicate an influence from external factors like diet composition and environmental stress.

Table 1.Lipolytic bacterial count/mℓ (LBC x 102) in Buffalo and Cow’s milk

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sl. No.** | **Replication** | **Buffalo (B)** | | | **Mean** | **Buffaloes and cows (C)** | | | **Mean** |
| **Treatment** | | | **Treatment** | | |
| **M1** | **M2** | **M3** | **M1** | **M2** | **M3** |
| 1 | R1 | 37.30 | 39.00 | 38.20 | **38.17** | 38.00 | 39.50 | 38.80 | **38.77** |
| 2 | R2 | 37.20 | 40.00 | 39.00 | **38.73** | 38.00 | 41.00 | 39.50 | **39.50** |
| 3 | R3 | 37.25 | 40.00 | 38.00 | **38.42** | 37.50 | 40.80 | 38.50 | **38.93** |
| 4 | R4 | 37.00 | 39.50 | 38.00 | **38.17** | 37.50 | 40.00 | 38.50 | **38.67** |
| 5 | R5 | 37.20 | 39.50 | 38.20 | **38.30** | 37.80 | 40.00 | 38.50 | **38.77** |
| 6 | R6 | 37.50 | 40.50 | 37.80 | **38.60** | 38.50 | 41.20 | 38.20 | **39.30** |
| 7 | R7 | 37.00 | 39.50 | 38.00 | **38.17** | 37.80 | 40.00 | 39.00 | **38.93** |
| 8 | R8 | 37.25 | 39.50 | 38.20 | **38.32** | 37.80 | 40.20 | 39.20 | **39.07** |
| 9 | R9 | 37.00 | 39.50 | 37.80 | **38.10** | 37.50 | 40.00 | 38.50 | **38.67** |
| 10 | R10 | 36.50 | 39.80 | 37.80 | **38.03** | 37.00 | 40.20 | 38.50 | **38.57** |
| Range | Minimum | 36.50 | 39.00 | 37.80 | **38.6** | 37.00 | 39.50 | 38.20 | **39.3** |
| Maximum | 37.50 | 40.50 | 39.00 | **38.03** | 38.50 | 41.20 | 39.50 | **38.57** |
|  | Mean | **37.12bc** | **39.68a** | **38.10b** | **38.30** | **37.74c** | **40.29a** | **38.72b** | **38.92** |

**a,b,c Means bearing different superscripts in the column differ significantly (p<0.05)**

**Fig.1 Lipolytic bacterial count/mℓ (LBC x 102) in Buffaloes and Cow’s Milk**.

1. **Proteolytic bacterial count/mℓ (PBC x 102)**

The data on the **Proteolytic Bacterial Count (PBC × 10²/ml)** in raw milk from buffaloes and cows at different milking times are presented in Table 2 and Figure 2. The results indicate that the mean PBC × 10² in buffalo milk during the three milking times—morning, noon and evening - were 31.87, 34.23, and 32.48, respectively, with an overall mean of 33.74. Statistical analysis showed that the differences among these mean values were significant. In cow milk, the mean PBC × 10² during the three milking times—morning, noon, and evening- were recorded as 32.63, 36.25, and 35.08, respectively, with an overall mean of 34.65. The differences among these values due to milking times were also found to be statistically significant, while variations due to replication were non-significant.

Comparative analysis revealed that PBC was consistently lower in buffalo milk than in cow milk. Among the milking times, morning milk exhibited the lowest PBC, while noon milk recorded the highest, followed by evening milk. These findings highlight temporal variations in proteolytic bacterial loads, with morning milk showing better microbial quality compared to other times. Ramirez (1972) has reported diminished deaminase activity in the rumen microbes of animals fed low protein, molasses urea diets. Similar studies (Sharma *et al.,* 2021) suggest that seasonal changes significantly impact physiological measurements in dairy animals. Further investigations with a larger sample size and controlled environmental conditions could help validate these findings. The study suggests that the combined group of buffaloes and cows exhibited higher mean values compared to buffaloes alone. This finding supports the potential advantages of mixed dairy farming systems in enhancing productivity. Future research should explore the influence of diet, climate, and breed variations on these parameters to improve dairy management practices Overall, bacterial counts were lower in buffalo milk compared to cow milk. Morning milk consistently exhibited the best microbial quality, with minimal bacterial counts, while noon milk showed the highest counts across all parameters. Importantly, coli form bacteria were absent in all samples, underscoring the hygienic conditions maintained during milking.

**Table 2.Proteolytic bacterial count/mℓ (PBC x 102) in Buffaloes and cow’s Milk.**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sl. No.** | **Replication** | **Buffalo (B)** | | | **Mean** | **Buffaloes and cows (C)** | | | **Mean** |
| **M1** | **M2** | **M3** | **M1** | **M2** | **M3** |
| 1 | R1 | 30.80 | 34.50 | 32.20 | **32.50** | 32.50 | 36.50 | 41.80 | **36.93** |
| 2 | R2 | 31.00 | 34.50 | 32.50 | **32.67** | 32.80 | 36.60 | 34.40 | **34.60** |
| 3 | R3 | 40.00 | 34.50 | 32.20 | **35.57** | 32.80 | 36.40 | 34.00 | **34.40** |
| 4 | R4 | 31.00 | 34.20 | 32.40 | **32.53** | 33.00 | 36.20 | 34.20 | **34.47** |
| 5 | R5 | 30.50 | 34.20 | 32.40 | **32.37** | 32.40 | 36.20 | 34.20 | **34.27** |
| 6 | R6 | 30.50 | 34.20 | 32.40 | **32.37** | 32.40 | 36.20 | 34.20 | **34.27** |
| 7 | R7 | 30.80 | 34.20 | 32.50 | **32.50** | 32.60 | 36.20 | 34.40 | **34.40** |
| 8 | R8 | 32.80 | 34.00 | 32.80 | **33.20** | 32.80 | 36.00 | 34.60 | **34.47** |
| 9 | R9 | 30.50 | 34.00 | 32.60 | **32.37** | 32.40 | 36.20 | 34.40 | **34.33** |
| 10 | R10 | 30.80 | 34.00 | 32.80 | **32.53** | 32.60 | 36.00 | 34.60 | **34.40** |
| Range | **Minimum** | **30.50** | **34.00** | **32.20** | **35.57** | **32.40** | **36.00** | **34.00** | **34.47** |
| **Maximum** | **40.00** | **34.50** | **32.80** | **32.37** | **33.00** | **36.60** | **41.80** | **34.27** |
|  | Mean | **31.87c** | **34.23a** | **32.48b** | **32.86** | **32.63c** | **36.25a** | **35.08b** | **34.65** |

**a,b,c Means bearing different superscripts in the column differ significantly (p<0.05)**

**Fig. 2. Proteolytic bacterial count/mℓ (PBC x 102) in Buffaloes and Cow’s Milk.**

**CONCLUSION**

The present study provides valuable insights into the bacterial quality of raw buffalo and cow milk at different milking times, highlighting significant variations in microbial load. The findings indicate that morning milk (T1) exhibited the lowest Lipolytic Bacterial Count (LBC × 10²/ml) and Proteolytic Bacterial Count (PBC × 10²/ml), whereas noon milk (T2) recorded the highest bacterial counts, followed by evening milk (T3). These variations can be attributed to differences in metabolic efficiency, thermal regulation, and environmental exposure throughout the day. Notably, buffalo milk consistently demonstrated lower bacterial counts compared to cow milk, reinforcing its superior microbial quality. Additionally, the absence of coliform bacteria across all samples underscores the maintenance of hygienic milking conditions. From a practical perspective, these findings hold significant implications for dairy farmers. The superior microbial quality of morning milk suggests that prioritizing early milking schedules could enhance milk safety and shelf life. Moreover, the higher bacterial counts observed in noon and evening milk highlight the need for improved handling and storage practices, particularly in warmer conditions. The observed benefits of mixed dairy farming, as evidenced by the higher mean values in the combined buffalo-cow group, suggest a potential advantage in optimizing milk production efficiency. Future research should focus on larger sample sizes and controlled environmental conditions to validate these findings further. Additionally, exploring the effects of diet, seasonal variations, and breed-specific characteristics could provide deeper insights into optimizing dairy management practices. Overall, ensuring optimal milking times and maintaining strict hygiene protocols can enhance milk quality, benefiting both producers and consumers in the dairy industry.

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