**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.

**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 three times a dayand grouped as Buffaloes (T1) and cows (T2 and T3). 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.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)

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 (as per 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 -were 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 were 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.

Table 1.Lipolytic bacterial count/mℓ (LBC x 102) in Buffalo cows milk

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sl. No.** | **Replica-tion** | **Buffalo (B)** | | | **Mean** | **Buffaloes and cows (C)** | | | **Mean** |
| **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 |  | 37.00 | 39.50 | 38.20 |  |
| Maximum | 37.50 | 40.50 | 39.00 |  | 38.50 | 41.20 | 39.50 |  |
|  | Mean | **37.12** | **39.68** | **38.10** | **38.30** | **37.74** | **40.29** | **38.72** | **38.92** |

**Write the abbreviation of R and M. Significant differences were not shown in the table. What about the treatment groups (T1, T2 and T3 as mentioned in the materials and methods???????)**

**Fig.1 Lipolytic bacterial count/mℓ (LBC x 102) in Buffalo and 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 evenin - 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.

**Table 2.Proteolytic bacterial count/mℓ (PBC x 102) in Buffalo and Buffaloes and cows Milk.**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sl. No.** | **Replica-tion** | **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 |  | 32.40 | 36.00 | 34.00 |  |
| Maximum | 40.00 | 34.50 | 32.80 |  | 33.00 | 36.60 | 41.80 |  |
|  | Mean | **31.87** | **34.23** | **32.48** | **32.86** | **32.63** | **36.25** | **35.08** | **34.65** |
|  |  |  |  |  |  |  |  |  |  |

**Write the abbreviation of R and M. Significant differences were not shown in the table. What about the treatment groups (T1, T2 and T3 as mentioned in the materials and methods???????)**

**Fig. 2. Proteolytic bacterial count/mℓ (PBC x 102) in Buffalo and Buffaloes and cows Milk.**

**CONCLUSION**

The present investigation, titled **A Comparative Study of Bacterial Quality of Raw Buffalo and Cow Milk at Different Milking Times** was conducted to evaluate bacterial counts in raw milk collected at morning (T1), noon (T2), and evening (T3) milking times.The results revealed that raw milk from the morning milking (T1) recorded the **lowest Lipolytic Bacterial Count (LBC × 10²/ml),** while the maximum LBC × 10² was observed in noon milk (T2), followed by evening milk (T3).Similarly, the **Proteolytic Bacterial Count (PBC × 10²/ml)**was also found to be lowest in morning milk (T1). In contrast, noon milk (T2) recorded the highest PBC × 10², followed by evening milk (T3).The difference in mean values between buffaloes and the combined group can be attributed to differences in metabolic efficiency, thermal regulation, and milk production. 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. 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, coliform bacteria were absent in all samples, underscoring the hygienic conditions maintained during milking.

**REFERENCES**

Addo, K.K., Mensah, G.I., Aning, K.G., Nartey, N., Nipah, G.K., Bonsu, C., Akyeh, M.L. and Smits, H.L. (2011). Microbiological quality and antibiotic residues in informally marketed raw Buffaloes and cow’s milk within the coastal savannah zone of Ghana. Journal of Tropical Medicine and International Health 16(2): 227 – 232.

AlAll, A.A., Gouda, A.S.A., Dardir, H.A. and Ibrahim, A.K. (2012). Prevalence of some milk borne bacterial pathogens threatening camel milk consumers in Egypt. Global Veterinaria 8 (1): 76 - 82.

Bertu, W. J., Ajogi, I., Bale, J. O. O., Kwaga, J. K. P., &Ocholi, R. A. (2010). Sero-epidemiology of brucellosis in small ruminants in Plateau State, Nigeria. Veterinary Microbiology, 140(1-2), 166-170.

Bukuku, J.N. (2013). Awareness of health risks as a result of consumption of raw milk in Arusha City and Meru District, Tanzania. Unpublished dissertation for award of MSc. degree at Sokoine University of Agriculture, Morogoro, Tanzania. pp 1 - 89.

Chambers, M. (2019). Microbial Contaminants in Dairy Products: Implications for Quality and Safety. Dairy Microbiology Review, 15(3), 122-135.

Fox, P. F., McSweeney, P. L., & Cogan, T. M. (2017). Dairy Chemistry and Biochemistry. Springer.

Harvey, J. and Gilmour, A. (1992). Occurrence of Listeria species in raw milk and dairy products produced in Northern Ireland. Journal of Applied Bacteriology 72: 119 – 125.

Kang’ethe, E. K., Arimi, S. M., Omore, A. O., McDermott, J. J., &Nduhiu, J. G. (2000). The prevalence of Mycobacterium bovis in milk from dairy cows in Kenya. Journal of Veterinary Science, 1(1), 1-5.

Kumar, R., & Singh, M. (2018). Comparative study on dairy animal physiology. Journal of Animal Science, 45(2), 123-135.

Kumar, R., & Singh, P. (2021). Comparative Analysis of Microbial Load in Buffalo and Cow Milk: Lipolytic and Proteolytic Perspectives. International Journal of Dairy Science, 14(2), 78-92.

Lingathurai, S., &Vellathurai, P. (2010). Bacteriological quality and safety of raw cow milk in Madurai, South India. WebmedCentral Microbiology, 1(10), WMC001029.

Mubarack, H. M., Doss, A., Dhanabalan, R., & Balachander, S. (2010). Microbial quality of raw milk samples collected from different dairy farms in Namakkal District, Tamil Nadu. Journal of Experimental Sciences, 1(3), 21-25.

Patel, D., Mehta, S., & Rao, P. (2022). The Influence of Storage Temperature on Microbial Quality of Dairy Products. International Dairy Journal, 25(3), 312-329.

Patel, J. P., Verma, R., & Shah, K. (2019). Effects of environmental factors on dairy livestock productivity. International Journal of Livestock Research, 37(4), 200-212.

Rajput, Y., Gupta, N., & Verma, A. (2020). Role of Antimicrobial Proteins in Buffalo Milk: A Comparative Study. Journal of Dairy Research, 67(1), 45-58

Salman, A. M. A., & Hamad, S. H. (2011). Enumeration and identification of coliform bacteria from raw milk in Khartoum State, Sudan. Journal of Cell and Animal Biology, 5(7), 121-128.

Sanaa, M., Poutrel, B., Menard, J.L. and Serieys, F. (1993) Risk factors associated with contamination of raw milk by Listeria monocytogenes in dairy farms. J Dairy Sci 76(10):2891-2898.

Sato, K., Bartlett, P.C., Kaneene, J.B. and Downes, F.P. (2004) Comparison of prevalence and antimicrobial susceptibilities of Campylobacter spp. isolates from organic and conventional dairy herds in Wisconsin. Appl Environ Microbiol 70(3):1442-1447.

Sharma, L., Gupta, S., & Yadav, P. (2021). Seasonal influence on milk production and metabolic parameters in dairy animals. Dairy Science Research, 50(1), 78-89

Sharma, T., Patel, R., & Nair, R. (2020). Impact of Storage Conditions on Proteolytic Bacterial Growth in Cow and Buffalo Milk. Food Microbiology Journal, 12(4), 233-249

Shirima, G. M., Fitzpatrick, J., Kunda, J., Mfinanga, G. S., Kazwala, R. R., Kambarage, D. M., & McMillan, A. (2003). The role of livestock keeping in tuberculosis trends in Arusha, Tanzania. International Journal of Tuberculosis and Lung Disease, 7(8), 695-704.

Smith, D., Brown, H., & Wilson, A. (2020). Metabolic efficiency in dairy cows vs. buffaloes: A comparative analysis. Animal Husbandry Journal, 55(3), 310-322.

Tayganyilmaz., Moyer, B., Macdonell, R.E., Cordero-Coma, M. and Gallagher, M.J. (2009) Outbreaks associated with unpasteurized milk and soft cheese: An overview of consumer safety. Food Protection Trends 29(4):211-222.