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A comparative study of lipolytic & Proteolytic bacterial quality of raw cow milk at different milking times

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Abstract
The experiment was conducted at the Livestock production and management (unit), MGCGV Chitrakoot-Satna (M.P.). To complete the research work following steps were followed by during January to February 2019. All sanitary precaution was followed to produce clean milk. The sample of the raw milk of three animals each were replicated ten time and tested to determine the lipolytic bacteria count/mℓ (LBC) (10^2) proteolytic bacteria count/ (PBC) mℓ (10^2) in the raw milk. The data obtained for the aforesaid tests were subjected to statistical analysis. The result of the statistical analysis showed that the differences in mean values of LBC/10^2, and PBC/mℓ 10^2. In view of the finding and result presented above, it may be concluded that the raw Cow milk of morning T_1 was found best in terms of minimum lipolytic bacterial count/mℓ (LBC) (10^2), photolytic bacterial count/mℓ (PBC (10^2).

Keywords: Cow, raw milk, bacterial, quality

Introduction
Milk is considered an outstanding food source, as it is rich in proteins, fats, carbohydrates, minerals and vitamins. Yet the quality of the milk produced is often a major barrier to its marketing (Smith *et al*., 2005). Milk considered to be of good quality must have satisfactory microbiological and physical-chemical characteristics. Due to its constitution, Milk composition is affected by a number of factors including genetic and environmental factors. The microbial load of milk is a major factor in determining its quality. It indicates the hygienic level exercised during milking, that is, cleanliness of the milking utensils, condition of storage, manner of transport as well as the cleanliness of the udder of the individual animal. Milk from a healthy udder contains few bacteria but it picks up many bacteria from the time it leaves the teat of the cow until it is used for further processing.
Milk and dairy products are important components of the diet worldwide. The quality and shelf life of liquid milk as well as dairy products are often compromised by flavor, odors, and visual defects arising from the bacterial growth and activities of heat-stable enzymes produced by psychotropic bacteria before processing (Techer *et al*., 2014).
Contamination of milk and milk products by pathogenic microorganisms is a global health concern; however, its fatal impact on human and animal health in the developing countries including in Bangladesh has not yet been extensively resolved except a few research works (1, 14). Since the constituents of milk and milk based products are adequate enough to support the microbial growth and replication, the dairy foods intensely demand a careful microbiological examination for the quality assurance for the sake of consumer safety.

Materials and Methods
The heard consociated of breed cow and only healthy cows free from mastitis as detected by mastitis test and suffering from any infection or injuries were selected for this experiment. All were housed in one barn prepared for milking almost at three times was divided groups *viz*. cow, T_1, T_2, T_3. In all ten replications were made under each group. Udders were washed with 2 per cent potassium per magnate ($KMNO_4$) and two streams of fore milk from each quarter of s. Milk samples were tested for determining the total bacteria determined by population density of four physiological group of bacteria *viz*. Proteolytic bacteria count, lipolytic bacteria count and coliform bacteria count.
Samples were collected from the milking pail separately in sterile 250 ml conical flasks and plugged a septically with cotton plug.

The samples were brought immediately to laboratory for determination of total viable count as and their four physiological groups proteolytic-bacteria count (PBC), lipolytic bacterial count (LBC).
Following were the bacterial parameters determined as per method of (Chalmers, 1953)

1. Proteolytic bacterial count (PBC)
2. Lipolytic bacterial count (LBC)

Prior to use all the conical flasks were thoroughly cleaned, dried, plugged with absorbent type cotton and then sterilized in an autoclave at 120 ^{0}C for an hour.
Prior to use all the bacteriological pipettes of 1 ml and 10 ml capacity were immersed in chromic acid solution over night, washed with tap water and dried. They were wrapped in paper and sterilized in hot air oven at 120 ^{0}C for an hour. Test tubes were washed thoroughly with detergent and tap water. Then test tubes were used for preparing 9ml blanks of Ringer's solution for dilution of the sample. They were plugged with sterile absorbent cotton and then sterilized in autoclave at 120 ^{0}C at 1.2 kg/cm^2 for 20 minutes. These were thoroughly washed with detergent then tap water and kept on a clean table in inverted position for drying. Dried plates were wrapped in paper in block of 4 in each. These were sterilized in hot oven at 120 ^{0}C for an hour. It was needed for dilution of milk samples in desired ratio before plating as per (Prasad and Neeraj, 2004).

Composition
Sodium chloride (NaCl) – 9 g
Potassium chloride (KCl) - 0.42 g
Calcium chloride ($CaCl_2$) - 0.24 g
Sodium bicarbonate ($NaHCO_3$) - 0.20 g
Distilled water - 1000 ml
0.48 in case of hydrated salt, ($CaCl_2.6H_20$)
Proteolytic bacterial count PBC was determined in nutrient milk agar medium Nutrient agar – 1000 ml, Sterilized skim milk – 100 ml, 20 ml sterilized skim milk was added to 200 ml of sterilized nutrient agar in conical flask of 250 ml just prior to pouring in Petri-plates. After incubation for 24 hours the development of clean hollow zone around the colonies in medium indicated the proteolysis by bacteria.
Lipolytic bacterial count (LBC)-Nutrient agar – 1000 ml, Melted butter fat – 40 ml, Nile blue sulphate indicator (0.1% - 10 ml aqueous solution) pH - 7.0.
Nutrient agar was prepared melted butter fat and Nile blue sulphate indicator was added and placed in 250 ml capacity flasks. The medium was steamed for 30 minutes on each of three successive days for sterilization. At the time of use, medium was shaken vigorously and emulsifying fat globules. Lipolytic bacteria hydrolysed pink fat globules and produced a bluish colour around the beneath the colonies. The unhydrolysed fat globules appeared pink due to the action of Nile blue sulphate.

Results and Discussion
The present investigation entitled "A Comparative Study of Lipolytic and Proteolytic bacterial quality of raw Cow milk at different milking times" was carried out during January study the bacterial qualities of milk of raw cow milk at different milking times. Three Cows were selected for the investigation. Their milk was taken for ten days as replicates at morning, noon and evening. The results of the investigation regarding the bacterial qualities of milk have been presented in tables and graphically illustrated, wherever required. The findings have been divided into the following sub-headings:

Lipolytic bacterial count/ mℓ (LBC x 10^2)
Proteolytic bacterial count/ mℓ (PBC x 10^2)
Lipolytic bacterial count/mℓ (LBC) (10^2) the data showing Lipolytic bacterial count/mℓ (10^2) in the raw milk of Cows is presented in Table 1. The following observations were made:

1. In general, the LBC/mℓ (10^2) in raw milk of Cows at three milking time, replicated ten times, ranged between 34.20-40.00.
2. The LBC/mℓ (10^2) in the milk of Sahiwal Cow sat three milking time T_1, T_2 and T_3 in ten replications, ranged from 34.20-37.00, 36.40-40.00, and 35.20-38.40, respectively.
3. The mean LBC/mℓ (10^2) in the milk of Cow sat three milking time T_1, T_2 and T_3 (average of ten replications) was recorded as 35.67, 38.08 and 36.49, respectively, with overall mean of 36.75.
4. 4.The mean LBC/mℓ (10^2) in the milk for ten replications, R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_9 and R_{10} was 36.70, 36.83, 36.00, 36.53, 36.13, 37.33, 38.47,36.33, 36.33 and 36.80, respectively.
5. The minimum LBC/mℓ (10^2) (35.67) was recorded in morning T_1, while the maximum was recorded in the noon milking T_2 (38.08) followed by evening milking C_2 (36.49).
6. The difference between the mean values of LBC/mℓ (10^2) of raw milk due to different Cows was significant, while the effect due to replication was non-significant.

The data on Lipolytic bacterial count/mℓ (LBC) (10^2) in raw milk of Sahiwal Cows at three milking time is furnished. The results contained in the Table showed that milking time T_1, T_2 and T_3 registered mean LBC/mℓ (10^2) as 35.67, 38.08 and 36.49, respectively, with overall mean of 36.75. The differences in these values due to milking time were found significant, but the effect due to replication was non-significant. Morning milk T_1 recorded minimum LBC while Noon milk T_2 recorded the maximum followed by Evening milk T_3.

Table 1: Lipolytic bacterial count/mℓ (LBC) (10^2) in milk of Sahiwal Cow at different milking time

Replication		Milking Time			Range		Mean
		T_1	T_2	T_3	Minimum	Maximum	
R_1		35.00	37.50	37.60	35.00	37.60	36.70
R_2		35.00	38.50	37.00	35.00	38.50	36.83
R_3		34.20	38.40	35.40	34.20	38.40	36.00
R_4		35.40	37.00	37.20	35.40	37.20	36.53
R_5		35.80	36.40	36.20	35.80	36.40	36.13
R_6		37.00	39.00	36.00	36.00	39.00	37.33
R_7		37.00	40.00	38.40	37.00	40.00	38.47
R_8		35.80	38.00	35.20	35.20	38.00	36.33
R_9		36.50	37.00	35.50	35.50	37.00	36.33
R_{10}		35.00	39.00	36.40	35.00	39.00	36.80
Range	Minimum	34.20	36.40	35.20			
	Maximum	37.00	40.00	38.40			
	Mean	35.67	38.08	36.49			36.75
					F- test		S
					S. Ed. (±)		0.41
					C. D. (P = 0.05)		0.85

Proteolytic bacterial count/mℓ (PBC) (10^2) the data showing proteolytic bacterial count/mℓ (PBC) (10^2) in the milk of

Cows are presented in Table 2. The following observations were made:

1. In general, the PBC/mℓ (10^2) in raw milk at three milking time, replicated ten times, ranged between 31.40-37.00.
2. The PBC/mℓ (10^2) in the milk of Cows at three milking time T_1, T_2 and T_3 in ten replications, ranged from 31.40-34.60, 33.80-37.00 and 33.00-36.00, respectively.
3. The mean PBC/mℓ (10^2) in the milk of Cow sat three milking time T_1, T_2 and T_3 (average of ten replications) was recorded as 32.87, 35.66 and 34.51, respectively with overall mean of 34.35.
4. The mean PBC/mℓ (10^2) in the milk for ten replications, R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_9 and R_{10} was 34.07, 34.27, 35.00, 34.97, 33.43, 34.07, 34.67, 34.80, 34.47 and 33.73, respectively.
5. The minimum PBC/mℓ (10^2) (32.87) was recorded in morning milking T_1, while the maximum was recorded in noon milking T_2 (35.66) followed by evening milking T_3 (34.51).
6. The difference between the mean values of PBC/mℓ (10^2) of raw milk due to different milking time was significant, while the effect due to replication was non-significant.

Presents the data on proteolytic bacterial count in raw milk of Cows under study at three milking time. The results contained in the Table showed that three milking time T_1, T_2 and T_3registered mean PBC/mℓ (10^2) as 32.87, 35.66 and 34.51, respectively, with overall mean of 34.35. The differences in these values due to milking time, was significant, whereas, due to replication, the differences were found non-significant (Table 2). T_1 (morning milking) recorded minimum PBC while T_2 (noon milking) recorded the maximum followed by T_3 (evening milking).

Table 2: Proteolytic bacterial count/mℓ (PBC) (10^2) in milk of Sahiwal Cow at different milking time

		Milking Time			Range		Mean
		T_1	T_2	T_3	Minimum	Maximum	
R_1		34.00	34.60	33.60	33.60	34.60	34.07
R_2		34.60	35.20	33.00	33.00	35.20	34.27
R_3		32.50	37.00	35.50	32.50	37.00	35.00
R_4		34.40	37.00	33.50	33.50	37.00	34.97
R_5		32.60	34.20	33.50	32.60	34.20	33.43
R_6		31.60	36.00	34.60	31.60	36.00	34.07
R_7		32.40	36.60	35.00	32.40	36.60	34.67
R_8		32.80	36.20	35.40	32.80	36.20	34.80
R_9		31.40	36.00	36.00	31.40	36.00	34.47
R_{10}		32.40	33.80	35.00	32.40	35.00	33.73
Range	Minimum	31.40	33.80	33.00			
	Maximum	34.60	37.00	36.00			
	Mean	32.87	35.66	34.51			34.35
					F- test		S
					S. Ed. (±)		0.53
					C. D. (P = 0.05)		1.11

Conclusion

The present investigation entitled A Comparative study of bacterial quality of raw Cow milk at different milking times was carried out during January 2019 study the bacterial qualities of milk of three Cows. The data collected for milk of three Cows, for ten days, with three milking times *viz*., morning, noon and evening on different parameters, were subjected to statistical analysis, applying the technique of analysis of variance (F-test). The results of the investigation regarding the bacterial qualities of milk of Sahiwal Cows have been presented in tables, graphically illustrated, and discussed in the preceding chapters.

1. Raw milk of morning milking T_1 recorded minimum lipolytic bacterial count/mℓ (LBC) (10^2). The maximum LBC/mℓ (10^2) was found in the noon milking T_3 followed by evening milking T_3.
2. Morning milk T_1 recorded lowest proteolytic bacterial count/mℓ (PBC) (10^2) in the raw milk, whereas, noon milk T_2 recorded the highest followed by evening milk T_3.
3. In all the parameters, the difference in the mean values due to milking time was found significant, but the difference due to replication was non-significant. In view of the findings and results presented above, it may be concluded that the raw Cow milk of morning T_1 was found best in terms of minimum lipolytic bacterial count/mℓ (LBC) (10^2), proteolytic bacterial count/mℓ (PBC (10^2).

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