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A comparative study of standard plate count (SPC) and lactic acid bacterial count (LABC) quality of 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 where 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 standard plate count/mℓ (SPC) (10^4), lactic acid bacteria count/mℓ (LABC) (10^3), 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 SPC/10^4 mℓ, LABC/10^3 mℓ. In view of the finding and result presented above, it may be concluded that the raw Cow milk of morning T1 was found best in terms of minimum standard plate count/mℓ (SPC) (10^4), lactic acid bacterial count/mℓ (LABC) (10^3).

Keywords: Cow, raw milk, bacterial quality, milking time

Introduction
Milk is an important source of nutrients to human and animals. It is meant to be the first and the only food for the offspring of mammals as is almost complete food. Almost 87% of milk is composed of water and the remaining part comprises total solids (carbohydrates, fat, proteins and minerals) contained in a balanced form and digestible elements for building and maintaining the human and animal body. Milk meant for human consumption must be free from any pathogenic organisms. Microbial contamination in milk may cause milk-borne diseases to humans while others are known to cause milk spoilage. Many milk-borne epidemics of human diseases are spread through milk contamination. Sources of microbial contamination in milk include primary microbial contamination from the infected or sick lactating animal. The secondary causes of microbial contamination occurs along the milk value chain which may include contamination during milking by milers, milk handlers, unsanitary utensils and/or milking equipments and water supplies used in sanitary activities. Other secondary sources of microbial contamination occur during milk handling, transportation and storage. There is tertiary microbial contamination which occurs mainly due to re-contamination of milk after being processed due to unhygienic conditions and/or poor or improper handling 2 and storage of milk during consumption. The quality of milk is determined by its composition and overall hygiene. Demand for livestock production has increased because of the rise in the world's population. Raw milk (RM) often contains microorganisms which may cause food borne diseases Milk producers, processors, regulators and consumers share a common objective: the production and sale of safe, high quality milk and dairy products. Bacterial enumeration of bulk raw milk samples forms the basis of many dairy regulatory programs. The risk assessment of raw cow milk brings together information on the public health risks associated with the consumption of raw cow milk, and estimates the resulting burden of illness that may occur under current Indian production and marketing conditions. Raw milk can often be contaminated with pathogens, either directly through organisms shed as a result of udder infection or indirectly. Indirect contamination may arise from (i) a cow's own faecal matter contaminating the udder and teats, (ii) faecal matter of other cows contaminating the udder (iii) milking clusters contacting surfaces with faecal contamination, and (iv) post-harvest environmental contamination. An intensive search of published and unpublished literature shows that internationally, raw cow milk is often contaminated with pathogens and, whilst data is scarce for Australia, the data which is available confirms that raw cow milk is a source of low levels of pathogenic microorganisms.

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. Milk samples were tested for determining the total bacteria determined by standard plate bacteria count and population density of four physiological group of bacteria *viz*. lactic acid bacteria count.

Samples were collected from the milking pail separately in sterile 250 ml conical flasks and plugged aseptically with cotton plug. The samples were brought immediately to laboratory for determination of total viable count as standard plate count (SPC) and their four physiological groups *viz*. lactic acid bacterial count (LABC), Distribution of Cow_1, Cow_2, Cow_3. Parameters of Study were the bacterial parameters Determined as per method of Standard plate count/ml (SPC) for total bacterial and Lactic acid bacterial count (LABC) Prior to use all the conical flasks were thoroughly cleaned, dried, plugged with absorbent type cotton and then sterilized in an autoclave at 120 °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 °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 °C for an hour. Ringer's solution was needed for dilution of milk samples in desired ratio before plating as per.

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, ($CaCl_2.6H_20$)

Standard plate count (SPC)-Nutrient Agar medium, Agar-Agar – 15 g, Peptone – 5 g, Sodium chloride – 5 g, Beef extract – 3 g, Distilled water – 1000 ml, pH - 7.2 peptone, sod. Chloride (NaCl) and beef extract were dissolved in 1000 ml distilled water and pH was adjusted to 7.2 at 60 ^{0}C using Bromothymol blue as indicator. Agar power was dissolved in 900 ml distilled water by steaming for 15 minutes and filtered peptone NaCl and beef extract were added, then dispensed in to conical flasks, plugged and sterilized in autoclave at 1.25 kg/cm^2 for 20 minutes.

LABC was determined in lactose agar medium- Agar-Agar - 15 g, Peptone - 5 g, Lactose - 20, Beef extract - 3 g, Andred's indicator - 10 ml, Distilled water - 1000ml, pH - 7.0 Andred's indicator- Acid fuschsin (0.05% aq. soln) (50 mg in 100 ml water).

Results and Discussion

The present investigation entitled "A comparative study of Standard plate count (SPC) and Lactic acid count (LABC) bacterial quality of row cow milk at different milking times" was carried out during January 2019 to 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:

1. Standard plate count/mℓ (SPC) (10^4)

The data showing standard plate count/mℓ (SPC) (10^4) in the raw milk of Cows is presented. The following observations were made: In general, the SPC/mℓ (10^4) in raw milk at three milking time, replicated ten times, ranged between150.00 – 234.20. The SPC/mℓ (10^4) in the raw milk at three milks time T_1, T_2 and T_3 in ten replications ranged from 150.00 - 180.00, 202.40 - 234.20, and 168.40 - 205.40, respectively.

The mean SPC/mℓ (10^4) in the raw milk of Cows at three milking time T_1, T_2 and T_3 (average of ten replications) was recorded as 170.29, 218.05 and 192.32, respectively, with overall mean of 193.55. The mean SPC/mℓ (10^4) in the raw 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 197.83, 200.70, 194.87, 198.73, 200.27, 198.27, 185.78, 194.47, 182.80 and 180.87, respectively.

The minimum SPC/mℓ (10^4) (170.29) was found in morning T_1, whereas, the maximum was recorded in the noonT_2 (218.05) followed by evening T_3 (192.32).

The difference between the mean values of SPC/mℓ (10^4) of raw milk due to different milking time was significant, while the effect due to replication was non-significant. Table.1 furnish the data on standard plate count/mℓ (SPC) (10^4) in raw milk of three Cows at three milking time, in ten replications. The results obtained showed that three milking time T_1, T_2 and T_3 registered mean SPC/mℓ (10^4) as 170.29, 218.05 and 192.32, respectively, with overall mean of 193.55. The difference in these values due to milking time was significant, however, due to replication; the difference was non-significant. Morning milking time T_1 recorded minimum SPC while Noon milking T_2 recorded the maximum followed by evening milking time T_3.

Table 1: Standard Plate Count/mℓ (SPC x 10^4) in milk of Cow at different milking time

Replication		Milking Time			Range		Mean
		T_1	T_2	T_3	Minimum	Maximum	
R_1		170.80	221.70	201.00	170.80	222.70	197.83
R_2		175.70	224.00	202.40	175.70	224.00	200.70
R_3		174.00	212.20	198.40	174.00	212.20	194.87
R_4		172.80	218.00	205.40	172.80	218.00	198.73
R_5		171.40	227.00	202.40	171.40	227.00	200.27
R_6		171.00	234.20	189.60	171.00	234.20	198.27
R_7		150.00	222.00	185.20	150.00	222.00	185.73
R_8		180.00	207.00	199.40	180.00	207.00	195.47
R_9		168.00	212.00	168.40	168.00	212.00	182.80
R_{10}		169.20	202.40	171.00	169.20	202.40	180.87
Range	Minimum	150.00	202.40	168.40			
	Maximum	180.00	234.20	205.40			
	Mean	170.29	218.05	192.32			193.55
					F- test		S
					S.Ed.(±)		4.09
					C. D. (P = 0.05)		8.60

2. Lactic Acid bacterial count/mℓ (LABC) (10^3)

The data showing lactic acid bacterial count (LABC) (10^3) of raw milk of Cows is presented. The following observations were made: In general, the LABC/mℓ (10^3) in raw milk at three milks time, replicated ten times, ranged between 35.50-40.40.

The LABC/mℓ (10^3) in the milk at three milking time T_1, T_2 and T_3in ten replications, ranged from 35.50 - 37.50, 36.00 - 40.40, and 36.40 - 40.00, respectively.

The mean LABC/mℓ (10^3) in the milk at three milking time T_1, T_2 and T_3 (average of ten replications) was recorded as 36.42, 38.92 and 37.63, respectively, with overall mean of 37.66.

The mean LABC/mℓ (10^3) 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 37.33, 37.33, 37.17, 37.50, 36.33, 37.73, 38.43, 37.97, 38.27, and 38.50, respectively. The minimum LABC/mℓ (10^3) (36.42) was recorded in morning milking T_1, while the maximum was recorded in noon milking T_2 (38.92) followed by evening milking T_3 (37.63). The difference between the mean values of LABC/mℓ (10^3) of row milk due to difference milking time was significant, while the effect due to replication was non-significant. Contain the data on lactic acid bacterial count/mℓ (LABC) (10^3) in raw milk of Cow sat three milking time, each replicated ten times. The results obtained showed that milking time T_1, T_2 and T_3 registered mean LABC/mℓ (10^3) as 36.42, 38.92 and 37.63, respectively, with overall mean of 37.66. The difference in these values due to milking time was significant, whereas, due to replication, the difference was found non-significant. T_1 (morning) recorded minimum LABC while T_2 (noon) recorded the maximum followed by T_3 (evening).

Table 2: Lactic Acid bacterial count/mℓ (LABC) (10^3) in milk of Sahiwal Cow at different milking time

Replication		Milking Time			Range		Mean
		T_1	T_2	T_3	Minimum	Maximum	
R_1		37.50	37.00	37.50	37.00	37.50	37.33
R_2		36.50	39.00	36.50	36.50	39.00	37.33
R_3		35.50	39.60	36.40	35.50	39.60	37.17
R_4		36.00	39.50	37.00	36.00	39.50	37.50
R_5		36.50	36.00	36.50	36.00	36.50	36.33
R_6		35.50	40.20	37.50	35.50	40.20	37.73
R_7		37.50	38.80	39.00	37.50	39.00	38.43
R_8		36.00	40.40	37.50	36.00	40.40	37.97
R_9		37.20	39.20	38.40	37.20	39.20	38.27
R_{10}		36.00	39.50	40.00	36.00	40.00	38.50
Range	Minimum	35.50	36.00	36.40			
	Maximum	37.50	40.40	40.00			
	Mean	36.42	38.92	37.63			37.66
					F- test		S
					S.Ed.(±)		0.51
					C. D. (P = 0.05)		1.06

Standard plate count/mℓ (SPC) (10^4) the data on standard plate count/mℓ (SPC) (10^4) in raw milk of three Cows at three milking time, in ten replications. The results obtained showed that three milking time T_1, T_2 and T_3 registered mean SPC/mℓ (10^4) as 170.29, 218.05 and 192.32, respectively, with overall mean of 193.55. The difference in these values due to milking time was significant, however, due to replication, the difference was non-significant. Morning milking time T_1 recorded minimum SPC while Noon milking T_2 recorded the maximum followed by Evening milking time T_3.

Lactic acid bacterial count/mℓ (LABC) (10^3) contain the data on lactic acid bacterial count/mℓ (LABC) (10^3) in raw milk of Cow sat three milking time, each replicated ten times. The results obtained showed that milking time T_1, T_2 and T_3 registered mean LABC/mℓ (10^3) as 36.42, 38.92 and 37.63, respectively, with overall mean of 37.66. The difference in

these values due to milking time was significant, whereas, due to replication, the difference was found non-significant (morning) recorded minimum LABC while T_2 (noon) recorded the maximum followed by T_3 (evening).

Conclusion

The present investigation entitled a comparative study of standard plate count (SPC) and lactic acid bacterial count (LABC) quality of row 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). Minimum standard plate count/mℓ (SPC) (10^4) was recorded in the raw milk of morning T_1 and the maximum in noon T_2 followed by evening T_3. Lactic acid bacterial count/mℓ (LABC) (10^3) was recorded minimum in the raw milk of morning T_1 and maximum in Noon T_2 followed by evening milking T_3.

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