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Research Article

EFFECT OF DIFFERENT TREATMENTS OF PRE-MILKING TEAT STIMULATION ON BACTERIOLOGICAL QUALITY OF RAW MILK IN CROSSBRED CATTLE

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Abstract- The objective of the study was to find out the effect of different treatments of pre-milking teat stimulation on bacteriological quality of raw milk in crossbred cattle. Pre-milking udder stimulation was done by milker's hand, latex made hand gloves, cotton duster and muslin cloth, respectively, in treatment T_1, T_2, T_3 and T_4. In each of the treatments, milk samples were evaluated for Standard plate count (SPC), Poteolytic bacterial count (PBC), Lactic acid bacterial count (LABC), Lipolytic bacterial count (LBC) and Coliform count (CC). SPC was found significantly higher in T_1, T_2 as compared to T_3 and T_4. However, no significant difference was found in PBC, LABC and LBC among all the four treatments. In each treatment, correlation was studied among different bacteriological quality parameters. In T_1 LBC and PBC were significantly correlated ($P<0.05$) with each other. In T_2, PBC was positively correlated with LABC ($P<0.05$), while LBC showed significantly ($P<0.05$) positive correlation with LABC and PBC. In T_4, LBC was positively correlated ($P<0.05$) with LABC and PBC.

Keywords- Pre-milking, Teat, Stimulation, Bacteriological Quality, Raw Milk

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Introduction

Dairying is acknowledged as one of the major instruments in bringing about socio-economic transforming of rural people in India [11]. The success of dairy farm depends on profitable production of high quality milk [12]. Milk and milk products have an immense value in human nutrition throughout the world and these products must be of high hygienic quality. The milking management is considered as one of the most important and crucial activities at dairy farm having a profound bearing on the farm production efficiency and profitability [12]. In less developed areas and especially in hot tropics, production of high quality of safe product is most important but not easily accomplished [1]. This is required since milk is very much suitable for microbial growth and development. The fluid or semi-fluid nature of milk and its chemical composition (containing the essential nutrients) renders it as one of the ideal culture media for microbial growth and multiplication [14]. Information on the bacterial content of a milk sample may reflect on the state of health of the cow, the conditions under which the milk is stored and distributed and its public health significance [3]. Various sources of contamination in raw milk arising from external environment are feed, barn, cow's udder, belly, hind quarters, milker's hand, methods of milking, milking utensils and methods of milk handling etc. Pre-milking tactile teat stimulation is important to enhance the activity of neuroendocrine mechanism in dairy cows [12]. Purpose of pre- milking udder stimulus is to activate the receptors in the teat, which are sensitive to touch, and warmth and this is usually done before the milking so called pre-stimulus. Proper milking routine should provide un-stressful environment for the cow and ensure that the pre-milking teat preparation is performed in the same sequence of events to facilitate milk ejection before the milking and to minimize the amount of milk that should be removed by stripping [10]. The contamination of microorganisms depends upon the condition at the time of production [6, 8]. It is therefore, apparent that the different treatments of udder stimulus activities have special physiological purposes. Adoption of pre-milking stimulation of cow's udder for two minutes prior to milking to activate the receptors in the teat may be recommended to dairy farmers as an effective routine management practice to obtain higher milk yield [11]. Proper pre-milking udder stimulation is essential for the optimum production of quality milk [12]. Considering the significance of udder stimulation in cows towards enhancement in milk yield and quality production of milk [12] the present investigation was carried out to find the effect of different treatments of pre-milking teat stimulation on bacteriological quality of raw milk in crossbred cattle.

Materials and Methods

The present experiment was conducted in dairy farm of Allahabad Agricultural Institute (SHIATS), Allahabad-Uttar Pradesh, India. All crossbred cows were subjected to California Mastitis Test (CMT) and positive reactors were discarded. Sixteen apparently healthy crossbred cows free from any noticeable injuries on udder and kept under similar management conditions were randomly selected. Sanitary measures like clipping of long hair on the udder and flank, cleaning of dairy barns, washing of udder and teats with clean water before milking were adopted [10]. All animals were maintained under scientific tail to tail feeding and

management system [10]. Cows were milked by dry full hand diagonal method of milking [13, 5].

Four different treatment groups (4 cows in each group) for experiment were as follows:

T_1 = Pre- milking udder stimulus with milker's hand as control.
T_2 = Pre- milking udder stimulus using latex made hand gloves.
T_3 = Pre -milking udder stimulus using cotton duster.
T_4 = Pre- milking udder stimulus using muslin cloth.

Representative samples of 200 ml raw milk were collected in sterile conical flasks of 250 ml. capacity and immediately plugged aseptically with cotton plugs. These samples were brought immediately to the laboratory for determination the total bacterial count per ml in raw milk by SPC and population density of four physiological groups of bacteria *viz.* LABC, LBC, PBC and CC. 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. All the bacteriological pipettes of 1 ml capacity were immersed in chromic acid solution overnight, washed with tap water and dried, they were wrapped in paper and sterilized in hot air oven at 120^0C for an hour. Test tubes were washed thoroughly with detergent and tap water; they were plugged with sterile absorbent cotton and then sterilized in autoclave at 120^0C at 1.2 kg /cm^2 for 20 minutes. Petri plates were thoroughly washed with detergent and then with 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 and sterilized in hot air oven at 120^0C for an hour.

Preparation of media for microbial examination of milk samples-

Ringer's solution-

It was needed for dilution of milk samples in desired ratio before planting.

Sodium chloride (Nacl)	:	9 g
Potassium chloride (Kcl)	:	0.42g
Calcium chloride ($Cacl_2$)	:	0.24g*
Sodium bicarbonate ($NaHCO_3$)	:	0.20g
Distilled water	:	1000ml

*0.48 in case of hydrated salt, ($CaCL_2.6H_2O$)

Standard plate count (SPC) for Total Bacteria

Agar-agar	:	15 g
Peptone	:	5 g
Sodium chloride	:	5 g
Beef extract	:	3 g
Distilled water	:	1000 ml
pH	:	7.2

Peptone, sodium 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 is indicator. Agar powder was dissolved in 900 ml distilled water by streaming for 15 minutes and filtrated Peptone, NaCl and beef extract were added, then dispensed into conical flasks, plugged and sterilized in autoclave at 1.25 kg/ cm^2 pressure for 20 minutes. The following procedure was used for SPC in milk.

1. Milk sample collected were shaken gently 25 times in back and motion on a leveled table, in a time of about 7 seconds.
2. Dilution of agitated samples of milk samples of milk were prepared with the help of sterilized 9 ml blank of ringer's solution such as 1:10, 1:100, 1:1000, care was taken to shake diluted sample as stated above.
3. Sterilized pipettes were used to measure quantity of 1ml suitable milk dilution and transfer to priority marked sterilized Petri plates in duplicates.
4. As the dilution was transferred into the Petri plates, the month of agar flasks were flamed safely and approximately 15ml of the nutrient agar medium was poured into each dish to cover about 3 mm deep.
5. Agar medium was mixed with the dilution by gently rotating and tilting the. The dishes. After agar medium become solid, the plates were inverted and incubated for two days at 37 ^{0}C.
6. After incubation, those plates were selected which had 30 to 300 colonies and counted with the help of Quebec counter. The average number of bacterial count on two plates was determined by multiplying it with dilution factor to determine bacterial number per ml of milk.

Lactic acid bacterial count (LABC)

LABC was determined in lactose agar medium.

Agar-agar	:	15 g
Peptone	:	5 g
Lactose	:	5 g
Beef extract	:	3 g
Andred's indicator	:	10 ml (Acid fuchsine 0.05% aq. Solution (i.e.50 mg acid fuchsine in 100 ml water)
Distilled water	:	1000 ml
Normal NaOH	:	10 ml

More sodium hydroxide was added until the solution becomes straw colored. It was allowed to stand for 24 hrs.

Proteolytic bacterial count (PBC)

PBC was determined in nutrient milk agar medium

Nutrient agar	:	1000 ml
Sterilized skim milk	:	1000 ml

Twenty ml sterilized skim milk was added to 200 ml of sterilized nutrient agar in conical flask of 250 ml just 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	:	10 ml
(0.1% aqueous solution) pH	:	7.0

Nutrient agar was prepared, melted butter fat and Nile blue sulphate indicator was added placed in 250 ml capacity flasks. The medium was streamed for 30 minutes on each of three successive days for sterilization. At the time of use, medium was shaken vigorously for emulsifying fat globules. Lipolytic bacteria hydrolyzed pink fat globules and produced a blush color around the unhydrolyzed pink fat globules and produced a blush color ground beneath the colonies. The hydrolyzed fat globules appeared pink due to the action of Nile blue sulphate.

Coliform count (CC)

Coliform were determined in Maconcky's bile salt agar medium

Agar-agar (powder)	:	15 g
Peptone	:	20 g
Lactose	:	10 gm
Sodium chloride (Nacl)	:	15 gm
1% aqueous solution of Bromocresol purpule	:	2.5 ml
Distilled water	:	1000 ml
pH	:	7.4

The sodium taurocholate, peptone and sodium chloride were dissolved in 1000 ml distilled water by steaming for 30 minute and adjusted to 7.4 at 60^0C. Then agar-agar powder was dissolved at 100 ^{0}C and filter. Lactose and bromocreasol powder purple indicator were added to the filter solution and then plugged and sterilized as mentioned earlier.

The incubation times for various physiological groups of bacteria are as follows:

Sr.No.	Bacterial group	Temperature (0^0C)	Incubation period
1	Standard plate count (SPC)	37	48 hrs.
2	Proteolytic bacterial count (PBC)	30	24 hrs.
3	Lipolytic bacterial count(LBC)	30	40 hrs.
4	Lactic acid bacterial count (LABC)	35	48 hrs.
5	Coliform count (CC)	37	40 hrs.

Data were statistically analyzed by one-way ANOVA and results were expressed as mean ± SE. Means were compared using Tukey's multiple comparisons test.

The statistical package of Graph pad prism, San Diego, USA was used for analyzing the data.

Results and Discussion

The present study was designed to study the effect of different treatment of pre milking teat stimulation on bacteriological quality of raw milk in cows. Average standard plate count (SPC) in treatments T_1, T_2, T_3 and T_4 was 37.2±3.39, 33.9±3.03, 31.6±3.33 and 32.6±1.50, respectively. SPC was significantly (P<0.05) higher in T_3 and T_4 than T_1 and T_2.The Observations with regard to SPC/ml in raw milk are in agreement with [2, 4].

The mean lactic acid bacterial count (LABC) was 27.4±1.83, 26.5±1.71, 25.1±2.51 and 26.1±2.99, respectively, in T_1, T_2, T_3 and T_4. There was no significant difference in mean LABC among all the four treatments. The observation with regard to LABC/ ml in raw milk is in agreement with [2]. Mean proteolytic bacterial count (PBC) was 23.9±2.84 in T_1, 21.3±2.58 in T_2, 22.7±4.21 in T_3 and 23.9±3.10 in T_4. However, no significant difference in mean values of PBC was found among treatments T_1, T_2, T_3 and T_4.The observations with regard to PBC/ml in raw milk are in agreement with [9]. Average lipolytic bacterial count (LBC) was 29.8±2.48, 28.5±3.24, 27.7±2.83 and 27.3±3.88, in T_1, T_2, T_3 and T_4, respectively. However, among all treatments, no significant difference in average LBC was observed. Observations with regard to LBC/ml in raw milk are in agreement with [9, 7]. Mean Coliforms count (CC) 10 per ml was recorded as nil in T_1, T_2, T_3, and T_4; the differences in the values of coliform in raw milk under different treatments were found non-.significant. [9] also did not find presence of this group of bacteria in raw milk. Correlation among various bacteriological quality parameters in T_1 revealed that LBC and PBC were significantly correlated (P<0.05; r=0.71) while as other parameters did not show any significant correlation among themselves. In treatment T_2, PBC was positively correlated with LABC (P<0.05; r=0.83), while as LBC showed significantly (P<0.05) positive correlation with LABC (r=0.88) and PBC (r=0.81). In treatment T_3, LBC was positively correlated (P<0.05) with LABC (r=0.73). In T_4, LBC was positively correlated (P<0.05) with LABC (r=0.76) and PBC (r=0.75).

Table-1 *Effect of different treatments of pre-milking teat stimulation on bacteriological quality of raw milk in crossbred cattle*

PARAMETERS	T_1	T_2	T_3	T_4
SPC (10^3)/ml.	37.2±3.39^A	33.9±3.03^A	31.6±3.33^B	32.6±1.50^B
LABC (10^2)/ml.	27.4±1.83	26.5±1.71	25.1±2.51	26.1±2.99
PBC (10^2)/ml.	23.9±2.84	21.3±2.58	22.7±4.21	23.9±3.10
LBC (10^2)/ml.	29.8±2.48	28.5±3.24	27.7±2.83	27.3±3.88
CC (10)/ml.	-	-	-	-

Means bearing different superscripts (A and B) in row differ significantly at 5% (P<0.05).
SPC=Standard plate count (10^3)/ml; LABC=Lactic acid bacterial count (10^2)/ml;
PBC=Proteolytic bacterial count (10^2)/ml; LBC=Lipolytic bacterial count (10^2)/ml;
CC=Coliforms count (10)/ml.

Table-2 *Correlation among bacteriological quality parameters in T_1*

	SPC	LABC	PBC
SPC			
LABC	-0.45		
PBC	0.09	0.22	
LBC	0.38	-0.12	0.71*

Correlation is significant at 5 % (P<0.05)

Table-3 *Correlation among bacteriological quality parameters in T_2*

	SPC	LABC	PBC
SPC			
LABC	-0.16		
PBC	-0.34	0.83*	
LBC	0.00	0.88*	0.81*

Correlation is significant at 5 % (P<0.05)

Table-4 *Correlation among bacteriological quality parameters in T_3*

	SPC	LABC	PBC
SPC			
LABC	0.01		
PBC	0.39	0.42	
LBC	0.05	0.73*	0.25

Correlation is significant at 5 % (P<0.05)

Table-5 *Correlation among bacteriological quality parameters in T_4*

	SPC	LABC	PBC
SPC			
LABC	0.42		
PBC	-0.34	0.39	
LBC	0.07	0.76*	0.75*

Correlation is significant at 5 % (P<0.05)

Conclusion

From the study it is concluded that standard plate count (SPC) was significantly (P<0.05) reduced in milk when teat was stimulated by cotton duster and muslin cloth as compared to when stimulated by milker's hand, latex made hand gloves. Pre-milking udder stimulation had no significant effect on PBC, LABC and LBC.

Conflict of Interest: None declared

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