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EFFECT OF DIFFERENT HYGENIC CONDITION DURING MILKING ON BACTERIAL COUNT OF COWS' MILK

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Abstract

Effect of three hygienic conditions (T_1- washing and disinfection of udder and teats, milker hands, and a sanitary rinse of milking pails using calcium hypochlorite solution with 200 ppm Cl; T_2- washing of udder and teats, milker hands and milking pails with normal water; and T_3- without any kind of preparation of udder and teats, milker hands and milking pails) before milking were studied. Fifteen "Red Chittagong" milking cows were selected and divided into the three treatment groups (5 in each) maintaining under identical management conditions icluding floor hygiene, feeding and post-milking equipment sanitation. Initial bacterial quality as measured by Standard Plate Count (SPC), Coliform Count (CC) and Methylene Blue Reduction Time (MBRT) of milks obtained under T_1, T_2 and T_3 were significantly ($P<0.01$) different. On the basis of SPC, CC and MBRT initial bacterial load was highest for T_2- milk and lowest for T_1- milk Initial SPC of T_1 was 86% and 64% lower than that of T_2 and T_3 respectively, whereas CC of T_1 was 89% and 69% lower than that of T_2 and T_3, respectively. Standard Plate Count and CC of T_3 were respectively 43% and 65% lower than those of T_1. The initial MBR time were in the order $T_1 = T_3 > T_2$ ($P<0.01$). Results of the present study revealed that milking hygiene has significant effect on initial bacterial count of milk.

Key words: Milking hygiene, Red Chittagong cows, Bacterial count

Introduction

Milk is considered as a universal food. The nutritive pefection of milk has made it not only a unique food for human being but also a medium most favourable for microbial growth. Microorganisms which may gain entry into milk can multiply and bring about spoilage of milk and milk products and render them unsafe due to potential health hazards. It is doubtless that milk is secreted by the cells of the udder in a sterile state but soon become contaminated by bacteria normally present in the udder. During the normal milking operation however, milk is subjected to contamination from the exterior of the udder and adjacent areas, dairy utensils, milk contact surfaces, the hands of milkers or dairy workers, air of the milking parlour, water used and flies. Normally these sources would contributes diversified microbes and might be belonging to pathogens or spoilage microorganisms.

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The hygienic measures taken during and after milking essentially determine what foreign microorganisms enter the milk, including human pathogens. This applies also to their numbers. The count of properly drawn mixed milk from healthy cows is about 100000 ml^{-1}, sometimes even less. If however, the hygienic standards during milking are poor, freshly drawn mixed milk can have a much higher count, upto one million ml^{-1} (Walstra *et al.,* 1999). Since the number and type of organisms in milk usually get increased by contamination, it is necessary right from milking such hygienic conditions should be maintained that minimum number of microorganisms gain entrance into milk. Several investigators suggested that milking hygiene could significantly reduce the microbial count in milk (Petrovic *et al.,* 2006; Koshy and Prasad, 1993 and Sakya and Srivastava, 1982).

Milk production in Bangladesh is characterized by low yield non-descript cows, small producers, production in small lots and under poor hygienic conditions. As a result, milk reaching into market usually with higher microbial load and making it difficult to enforce standards relating to the hygienic quality of milk. Proper milking hygiene to obtain milk of lower initial bacterial load should be concentrated. The present study was taken up with a view to study the effect of milking hygiene on initial bacterial count and the effectiveness of calcium hypochlorite solution (200 ppm Cl) as a sanitary agent for hygienic milking.

Materials and Methods

This study was conducted at Dairy Farm and Dairy Microbiology Laboratory of the Department of Dairy Science, Bangladesh Agricultural University, Mymensingh, during the period of September to October; 2007.

Preparation of calcium hypochlorite solution

A stock solution was prepared by making a smooth watery paste of 100 gram of commercial chloride of lime (Bleaching powder) containing 30 percent available chlorine (Cl), and then adding water slowly and stirring thoroughly until the solution amounts to 2 liters. Then it was allowed to settle and strained into a tightly closed glass bottle. The stock solution was kept in a cold dark room. For the final use 250 ml of this stock solution was added to every 20 liters of water and mixed thoroughly. So, prepared final solution contains approximately 200 ppm (187 parts of available chlorine to 1,000,000 parts of water) chlorine, which is the strength recommended for farm use. This final solution was immediately used. Used solution was thrown away and new one mixed daily. The stock solution was used for maximum 7-8 days and new stock solution was prepared once a week.

Experimental procedure

Fifteen milking cows were selected from the elite herd of *Red Chittangong* Cattle Project maintained at BAU Dairy Farm. Cows were of similar yield characteristics, and randomly divided into three treatment groups containing five (5) cows in each. All the milking cows were maintained under identical managemental conditions including floor hygiene,

feeding and post milking equipment sanitation. Udder and teats of five cows were washed with 200 ppm chlorine solution (previously made), milking pails and milkers' hands were also sanitized with the same solution before milking. Three hygenic conditions represents are T_1, T_2 and T_3 respectively. In T_1 group, calcium hypochlorite solution (200 ppm Cl) was used as a sanitary agent for pre-milking washing and disinfection of udder and teats, hands of milker, and milking pails; in T_2 group, milking was done in wet condition after washing udder and teats, hands of milker, and milking pails with normal water; and in T_3 group, udder and teats, hands of milker and milking pails were not prepared by any means.

Milking was done by hand and in open pails. Mixed milk samples of 250 ml from individual treatment groups were taken into three sterile bottles and kept in ice box. Milk samples were brought to laboratory for analysis immediately after milking. Milk samples were collected at an interval of 3-4 days for analysis during the study period. The milk samples were analyzed for initial microbial quality with the help of Standard Plate Count (SPC), Coliform Count (CC), Methylene Blue Reduction Time (MBRT) test. All the parameters were studied as per the procedure described in the Standard Methods for the Examination of Dairy Products (APHA, 1998).

The statistical analyses were done by plotting experimental data in Completely Randomized Design (CRD). Statistical package MSTAT-C was used for analytical purpose. In case of significant difference LSD test was carried out to find out the significant difference among different treatment means.

Results and Discussion

In the present study, milk samples were collected from cows maintained under three different hygienic conditions (T_1, T_2 & T_3) before milking. The initial (immediately after milking) microbiological quality was determined by performing Standard Plate Count (SPC), Coliform Count (CC), Methylene Blue Reduction Time (MBRT) test. The results of trials conducted along with necessary discussions are presented below:

Standard plate count

Results of Standard Plate Count (SPC) as obtained from milks of different hygienic groups are presented in Table 1. The mean values of initial SPC were 57 ± 13, 411 ± 75 and 161 ± 37 thousand cfu/ml for T_1, T_2 and T_3 milk, respectively. The mean SPC values were significantly different ($P<0.01$). The mean initial SPC was in the order of $T_1<T_3<T_2$ Thus the lowest and highest initial bacterial load was found in the hygiene group T, and T_2 respectively.

Results obtained from each trial represented that wet milking (T_2) is a major cause of high initial bacterial count. This may be due to dripping of surplus water (that contains enormous number of bacteria) from the udder and adjacent areas into milk and may also be due to the fact that higher degrees of wetness of udder and milker hands help leaching of dirt and bacteria from teats, udder and hands into milk. Thus, making the

milk unclean with increased bacterial count. Similar comments have been reported from Galton *et al.* (1984) and Hogan *et al.* (1979) in their studies on milking hygiene. Both of them reported that higher degree of wetness of udder surface and teats is associated with increased number of bacteria in milk. In contrast to T_2, the initial bacterial load of T_3 was reduced by as much as 61% and representing that milking in dry condition significantly reduces bacterial count. It is because no surplus water remains in the surface of the udder to drip into the milk and due to less chance of leaching dirt and bacteria from udder, teats and hands into milk. Thus merely cleaning and washing is not sufficient, rather drying of udder and hands is important to obtain clean milk.

Table 1. Standard plate counts (×103 cfu/ml) under different hygienic conditions

Hygiene groups	SPC at different observation days									Mean ± SD	CV	Level of sig.
	1	2	3	4	5	6	7	8	9			
T_1	36	67	53	49	57	8-	55	47	70	57±13[c]		
T_2	510	340	285	390	430	470	360	500	410	411±75[a]	23	**
T_3	115	150	143	165	229	160	157	209	121	161±37[b]		

** (P<0.01)
[abc] means values in the same column with different superscripts differ significantly

In T_1 group initial SPC was far too lower than T_2 and T_3. This may be due to the effect of disinfection of udder and milkers hands and sanitary rinse of milking pails prior to milking. Initial SPC of T_1 was as much as 86% and 65% (64.59) less than the counts obtained respectively from T_2 and T_3 group. Schoken-Iturrino *et al*. (1982) studied the effect of several sanitary measures during milking on the microbial count of milk. Samples were taken during milking from 16 friesian cows milked by hand (i) Without any preparation or with (ii) udder preparation with sodium hypochlorite solution with active CI at 5 ppm (iii) preparation of udder and milkers' hand or (iv) preparation of udders, hands and teat cups. Bacterial count in milk decreased in the order (i)> (ii) = (iii)> (iv) (P>0.05). Differences in the mean bacterial count among milking were significant at 1% level. Sakya and Srivastava (1982) reported a 79.7% and 83.6% reduction (P<0.01) in mean bacterial count by washing and disinfection (200 ppm CI) of milker hands and cows' udder respectively. Petrova and Petroava (1985) showed that bacterial counts were halved by washing udder with a disinfectant as opposed to using only a dry cloth for cleaning. Results of the present study are in agreement with the findings of previous investigators. The drastic reduction in initial SPC in T_1, compared to T_2 and T_3 may be attributed to combined cleaning and sanitizing effect of chlorine solution. Results of individual observation days as presented in the Table 1 showed considerable variation within each treatment group.

Coliform count

Results of coliform count (CC) for T_1, T_2 and T_3 are presented in Table 2. The mean values of CC were 38 ± 20, 359 + 90 and 123 + 20 cfu/ml for T_1, T_2 and T_3, respectively. The mean values of CC showed significant difference (P<0.01). The mean CC was in the order of T_2>T_3>T_1. Highest mean coliform count (359 /ml) was found in T_2 and the mean values of T_3 (123/ml) and T_1 (38/ml) were reduced respectively by

65% and 89% compared to T_2. The mean CC of T_1 was reduced by 69 % compared to T_3. Results of individual observation days as presented in the Table 2 showed considerable variation within each treatment group and such variation is not unusual.

Table 2. Coliform Counts (cfu/ml) under different hygienic conditions

Hygiene groups	CC at different observation days									Mean ± SD	CV	Level of sig.
	1	2	3	4	5	6	7	8	9			
T_1	0	45	41	35	38	69	53	0	62	38±20[c]		
T_2	470	300	184	410	330	360	390	480	310	359±90[a]	32	**
T_3	96	107	105	124	154	142	135	149	98	123±20[b]		

** (P<0.01)
[abc] means values in the same column with different superscripts differ significantly

It is worth mentioning here that the initial coliform count was negative for 2 observations under T_1, indicating that proper cleaning and disinfection of cows' udder, milkers' hands and milking equipment results great reduction in coliform count. The initial coliform count of each trial under T_1 was below the coliform standard (100/ml) for raw milk. Percentage reduction of CC was higher than the reduction of SPC. It may be due to the fact that unsanitary conditions help increased contamination of milk by coliform, and contamination originating from udder, hands, equipment and also water used associated with coliform groups. Henderson (1971) stated that organisms associated with dirt such as manure, bedding, and soil are often of gas forming Escherichia-acrobacter group and may fall from the belly, flanks, tail, and udder into the milk pail and thus carry a considerable number of bacteria into the milk. It may also be attributed to faster germicidal action of hypochlorite and its more effectiveness against the coliforms. The reduction of mean CC under T_3 compared to T_2 essentially determine the effectiveness of dryness of udder surface during milking and also indicate that udder surface is an important sources of coliform bacteria.

From the results it was found that disinfection of udder and teats, milkers' hands, and sanitary rinse of pails all had significant effect to the reduction of coliform count in freshly drawn milk samples. Hogan *et al.* (1984) attributed higher coliform count to the degree of wetness of udder; Petrova and Petrova (1985) showed that udder disinfection (Cl 5 ppm) can half the coliform count in milk; but Sakya and Srivastava (1982) showed non-significant effect of udder disinfection (200 ppm Cl) on coliform count; and Kantona and Szita (1982) attributed increased coliform count to the improper equipment sanitation. The present experiment design does not support to make such specific distinction but results are in overall agreement with the previous findings and suggest that proper hygienic measure during milking significantly reduce the incidence of coli counts in milk.

Methylene blue reduction time test

Results of Methylene Blue Reduction Time (MBRT) test are presented in Table 3. The mean MBRT of T_1, T_2 and T_3 groups were 693 $\pm$ 23, 513 $\pm$ 85 and 650 $\pm$ 45 minutes,

respectively. The mean values of MBRT were highest in T_1. There was non significant difference between T_1 and T_3 but difference between T_1 and T_2, and T_2 and T_3 were significant (P<0.01).

Table 3. Methylene blue reduction time (minute) under different hygienic conditions

Hygiene groups	MBRT at different observation days									Mean ± SD	CV	Level of sig.
	1	2	3	4	5	6	7	8	9			
T_1	720	690	690	720	720	660	690	660	690	693±23[a]		
T_2	360	510	570	600	540	510	600	390	540	513±85[b]	9	**
T_3	720	660	660	630	600	630	630	600	720	650±45[a]		

** (P<0.01)
[abc] means values in the same column with different superscripts differ significantly

Day to day observation showed less variation (CV-9.27%) in MBRT compared to SPC and CC. Thus the results of MBRT fail to determine the initial microbial quality of milk compared to SPC in the case where the contamination level is low. MBRT places all the milk samples in the Good grade where SPC places T_1 and T_3 in the Very good grade and T_2 in the good grade, according to Prasad (1997) and IS (1962) recommendations, respectively. Similar finding have been reported from Sinha *el al.* (1968), Lakhani and Singh (1998) and Marutirum and Singh (1967). Grag and Mandokhot (1997) observed that MBRT places milk samples in a higher grade than it is placed by 5°C.

Conclusion

The study revealed that milking clean cows in dry condition gives better initial bacterial quality of milk compared to washing of udder, milker hands and milking pails with normal water before milking. Washing and disinfection of udder and milkers' hands, and sanitary rinse of milking pails just before milking significantly improved initial bacterial quality of milk. The calcium hypochlorite solution (200 ppm Cl) can be used as an effective sanitizer in milking hygiene operations to improve initial bacterial quality of milk.

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