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SUPPLEMENTING PROBIOTICS (*Saccharomyces cerevisiae*) IN MULTIPAROUS CROSSBRED COWS RATION PROVOKE MILK YIELD AND COMPOSITION

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ABSTRACT: Ten multiparous cows were selected to determine the effect of probiotics (*Saccharomyces cerevisiae*) on milk yield and composition. These cows were taken as control group before feeding probiotics and after feeding they were taken as treatment group. The cows were supplemented with 15g live yeast culture per head per day for a one month trial period. In the conducted experiment it was seen that there was significant (P<0.05) improvement in milk yield after supplementing probiotics (0.3 liter/ day/ animal which is 8.8% in average daily milk yield) to the cross breed dairy cows. It was observed that there was no significant improvement in butter fat percentage of milk (P>0.05) and acidity (%) between treatment group and control group, but significant improvement (P<0.05) was found in protein content and solids-not-fat content of milk.

Keywords: Crossbred Cows, Milk Yield, Milk Composition, Probiotics, *Saccharomyces cerevisiae*

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INTRODUCTION

Probiotics are defined as "live microorganisms that may beneficially affect the host upon ingestion by improving the balance of the intestinal micro flora" (Fuller, 1989). The concept of microbial manipulation in the gastro-intestinal tract was first appreciated by Metchnikoff (1907) who viewed the consumption of yoghurt by Bulgarian peasants as conferring a long span of life. There are many workers worked on to analyse the effect of probiotics on dairy cattle. Research on the effect of probiotics on milk yield and composition has been very limited. In a recently published paper, Chiquette et al. (2008) reported increased production of fermentation products and milk fat percentage when a newly isolated bacterial strain (*Prevotella bryantii* 25A) was fed to dairy cows from 3 weeks pre-partum to 7 weeks post-partum. But Raeth-Knight et al. (2007) failed to observe any effect on milk yield or composition or dry matter intake when feeding dairy cows (averaging 74 ± 32 days in milk) a combination of *Lactobacillus acidophilus* (1 x 109 cells/day) and *Propionibacterium freudenreichii* (2 x 109 cells/day).

A probiotic in terms of its beneficial effects, *S. cerevisiae* has many properties from the most basic to highly advanced. When ingested in a quantity of two tablespoons daily, the commercially prepared product known as "nutritional yeast" provides 52 percent of the recommended daily amount (RDA) of protein. The use of *Saccharomyces cerevisiae* as a probiotic, when added to feed in small amounts, began during 1940s and 1950s (Beeson and Perry, 1952). Products containing *S. cerevisiae* have been used to improve daily gain and milk production in ruminants (Wallace, 1993). The increasing concern regarding the use of antibiotics has led to even greater interest in probiotics as feed additives.

Various models have been designed to explain the effects of yeast in the rumen (Newbold et al., 1996). Data indicate that supplementation of yeast in the ruminant diet may improve feed intake (Robinson and Garette, 1999; Williams et al., 1991), milk production (Abdel-ghani, 2004; Wang et al., 2001), weight gain (Salama et al., 2002). The present investigation was conducted with major objective to study the effect of probiotics on milk production and milk composition of lactating cows.

MATERIALS AND METHODS

The experiment was conducted in Mithapukur upazilla of Rangpur district, in a small private dairy farm consisting of 10 lactating cows and 4 dry cows. During the period between15 December 2011to 15 January 2012 in winter period at the farm of Holstein–Friesian Black and White dairy cattle and the laboratory test was conducted at the quality control department of Rangpur Dairy and Food Products limited, Baldipukur, Mithapukur, Rangpur; to

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evaluate the effect of probiotics on quality & quantity of milk of crossbred dairy cow. To complete the research work following steps were followed.

Experimental design:

A total of 10 lactating cows were taken to treat with yeast culture (*Saccharomyces cerevisiae*) supplement. These cows were taken as control (marked as 'A') before treatment. And after treating with probiotics these were taken as treatment group (B) and there sample's test record was compared with the samples taken before treatment. Probiotics were fed at the morning period of each day during concentrate feeding. Milk samples were collected two times during experiment, before treatment and after finishing experiment. Samples were collected at 8 A.M in each time of collection & send to laboratory for testing. Before sending the milk sample it was properly mixed for proper sampling.

Test Parameters:

Two types of milk record were collected, one for quality of the milk, another is quantity. Qualitative record consist of composition of milk such as fat%, protein%, solids not fat% (SNF %), corrected lactometer reading (CLR) and acidity%. Quantitative record is the average yield of milk per head per day.

Experimental diet:

All cows were administered similar ration during experiment consist of concentrate mixture and roughage. Each cow given 6kg of roughage and 3kg of concentrate mixture per day which was divided into two parts and given at two times per day, morning and evening. Concentrate mixture consisted of wheat bran 250g/kg, rice polish 400g/kg, kheshary 200g/kg, mastered oil cake 140g/kg and vitamine-mineral premix 10g/kg. Roughage feed consisted of straw. Yeast culture supplemented as 15g/cow/day

Composition of supplemented probiotics:

The supplemented probiotics named (RUMISAC) marketed by Prime Care, Bangladesh and produced by Zeus Biotech ltd. India. Rumi Sac contains live yeast cell 3000 million per gram with enzymes, vitamins, amino acids, organic minerals and oligosaccharides.

Laboratory test:

Fat%: Fat% was determined by 'Garber centrifuge method'. In this method at first 10 ml of conc. H_2SO_4 was taken in butyrometer, then 10ml milk was added slowly by the side, then added 1ml amyl alcohol. Then closed the butyrometer by cock stopper then shaking slowly to mix the content. After proper mixing butyrometer was placed in the centrifuge machine and centrifuged it for 5 minutes at rate of 2000 RPM. After completing centrifugation then the scale was read.

Acidity%: For determination of acidity 18gm of milk sample was taken in a small container then 2-3 drops of phenolphthalein was added to milk sample as indicator. Then the container was placed under the burette containing 0.1N NaOH solution which was allowed to mix with the milk drop by drop until the pink color appear. The percentage of acidity was calculated by the following formula:

$$\% \text{ of acidity} = \{\text{ml of NaOH X normality } (0.1) \text{ X } 0.09 \text{ X } 100\} \div \text{wt of milk}$$

Protein%: Protein percentage was determined by Milk Scanner; The machine which automatically revealed the protein percentage of milk.

Solids Not Fat (S.N.F%): SNF was calculated by the following formula:

S.N.F = (Fat%÷5)+(C.L.R÷4)+0.14

C.L.R (Corrected Lactometer Reading: Corrected Lactometer Reading calculated by the following formula:

Corrected lactometer reading (CLR) = LR + CF

Where CF for Quevennes lactometer

CF (+) = 0.1 x difference in temperature above 60^0F

CF (-) = 0.1 x difference in temperature below 60^0F

Statistical Analysis

The recorded data were analyzed statistically between control group and treatment group using paired t-test method with the help of t-test calculator.

RESULTS & DISCUSSION

The experiment was conducted to study the effects of probiotics on the quality and quantity of cross breed lactating cows. So, to study the effects of probiotics on quantity; average daily milk yield was recorded before treatment and after treatment and results are shown in Table 1. And for effect on quality, milk samples were tested two times, before treatment and after treatment and results are shown in Table 2.

Effects on Milk Yield:

There was positive response of supplementing Probiotics (Live yeast cell culture) on crossbreed dairy cows as observed in the results shown in table-1. The average daily milk yield of cows before supplementing Probiotics were 3.5, 3.0, 2.5, 3.0, 3.5, 2.75, 3.0, 3.25, 3.5 and 3.0 liter /day respectively, and the mean of all cows was 3.1±0.10.

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The average daily milk yield of cows after supplementing Probiotics were 4.0, 3.5, 3.5, 3.0, 3.5, 3.0, 3.0, 3.5, 4.0 & 3.0 liter/day respectively& the mean was 3.4±0.124. So in comparison between two results it is shown that there was slight increase (0.3 liter/ day/animal which is 8.8%) in average daily milk yield after feeding Probiotics. Lehloenya et al. (2007) reported a 9% increase in milk yield when a mixture of yeast and *Propionibacterium* was fed to dairy cows from 2 weeks pre-partum to 30 weeks post-partum, which is similar with the obtained result.

Statistical analysis showed that there was significant difference ($P<0.05$) within the daily milk yield of experimental cows before treatment and after treatment. Similar results were observed by Williams et al. (1991), Wohlt et al. (1991), Piva et al. (1993), Dutta et al. (2008), Yalçın et al. (2011), Vibhute et al. (2011). Bruno et al. (2009) reported that Cows fed yeast culture produced 1.2 kg/d more milk. Jacquette et al. (1988) and Ware et al. (1988) reported increased milk yield (1.8 kg/day) when feeding cows *Lactobacillus acidophilus* (2 x 109 cells/day) compared with the control group. Gomez-Basauri et al. (2001) observed an increase in milk production (0.73 kg/day) when feeding cows a mixture of *L. acidophilus*, *L. casei* and *Enterococcus faecium*. More recently, Stein et al. (2006) reported an 8.5% increase in 4% fatcorrected milk in cows receiving 6 x 1010 *Propionibacterium*/day from 2 weeks pre-partum to 30 weeks post-partum. More recent studies have looked at the combination of yeasts and bacteria.

But some authors observed contradict result, that there was no positive response on milk yield by supplementing Probiotics (Erdman and Sharma, 1989; Arambel and Kent, 1990; Swartz et al., 1994). Putman et al. (1997) found that milk yield of dairy cows was increased with addition of yeast but only when protein content was deficient in the diet. Some other authors found a positive response in primiparous cows but not in multiparous cows (Robinson and Garret, 1991).

In a large animal study (366 cows), Oetzel et al. (2007) did not observe any effect of Enterococccus faecium + S. cerevisiae on milk yield or composition when fed to cows from 10 days prepartum to 23 days post-partum. However, Nocek et al. (2003) observed an increased dry matter intake (2.6 kg/day) and increased milk yield (2.3 kg/day) with the same combination of probiotics offered from 3 weeks pre-partum to 10 weeks post-partum. Similar results were obtained by Nocek and Kautz (2006) in a very similar trial using 44 Holstein cows.

Table 1 - Effects of Probiotics on milk yield of experimental cows

Experimental Cow	Average milk yield in liter/day		
	A		B
Cow-1	3.5		4.0
Cow-2	3.0		3.5
Cow-3	2.5		3.5
Cow-4	3.0		3.0
Cow-5	3.5		3.5
Cow-6	2.75		3.0
Cow-7	3.0		3.0
Cow-8	3.25		3.5
Cow-9	3.5		4.0
Cow-10	3.0		3.0
Mean ± S.D	3.1±0.10		3.4±0.124
t-value		2.8823	
p-value		0.0181	
Significance		*	

NS: Not significant; * = 5% level of significance; A = Control group; B = Treatment group

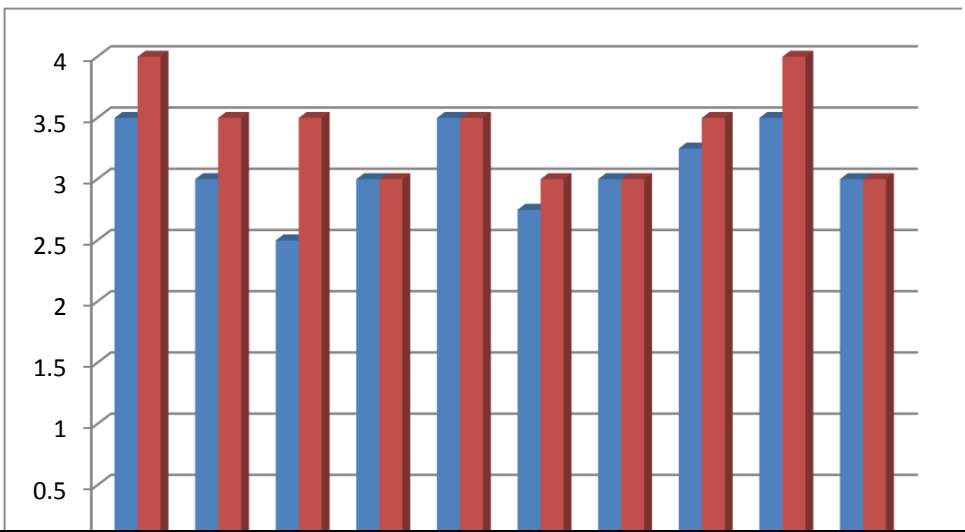

Figure 1. Comparison between average daily milk yield in kg/day between control group and treatment group.

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Effects on milk composition:

Effects on Butter Fat%: Table 1 shows the composition of av. milk fat% of 10 cows before treatment were 6.3, 4.7, 5.6, 3.8, 4.1, 4.3, 3.9, 3.6, 4.1 & 5.3 respectively. And the mean value of fat% was 4.57±0.28.Table-2 shows that the av. milk fat% of 10 cows after treatment were 5.7, 4.8, 5.2, 3.9, 4.2, 4.0, 4.2, 3.8, 4.3& 5.1 respectively. And the mean value of fat% after treatment 4.52±0.2. Statistical analysis showed that there was no significant difference (P>0.05) between the Fat% of treatment group and control group. In agreement with this some studies with lactating animals found no response by supplementing Probiotics in the composition of milk (Erdman and Sharma ,1989; Arambel and Kent, 1990; Swartz et al., 1994; Dutta et al., 2008).But some authors such as Williams (1989), Gunther (1989), Piva et al. (1993), Chiquette (1995), Vibhute et al. (2011) obtained reverse result, that there was significant improvement in butter fat % after supplementation with probiotics. But in some individual cows such as Cow-2, Cow-4, Cow-7 there was slight improvement in the butter fat%.

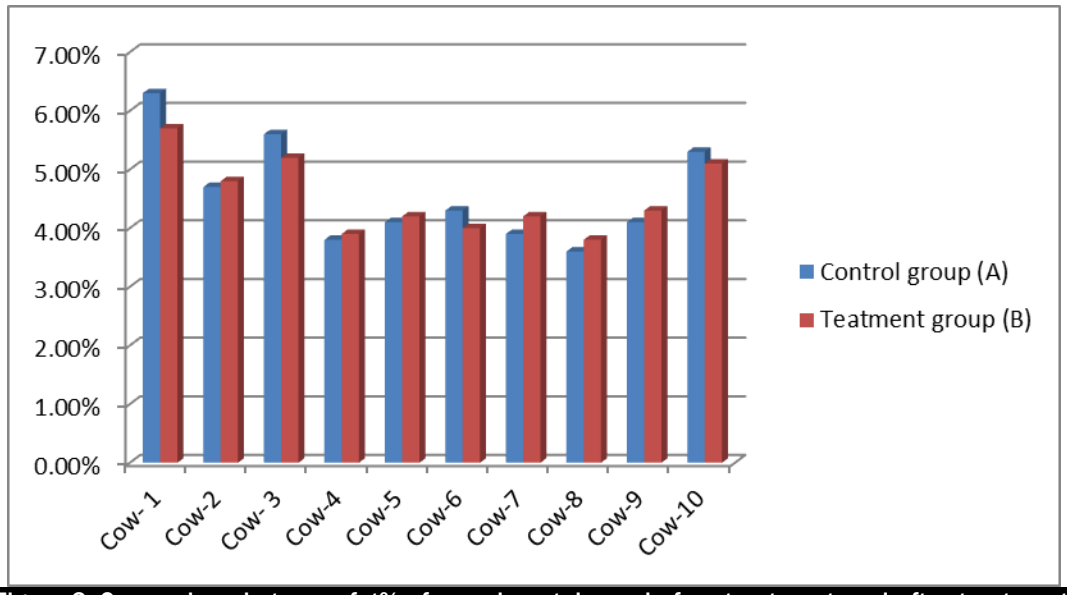

Figure 2. Comparison between fat% of experimental cow before treatment and after treatment.

Effects on Protein%: Table-1 shows the composition of av. Protein % of 10 cows before treatment were 3.25, 3.12, 3.21, 3.5,3.4, 3.13, 3.26, 3.14, 3.45 and 3.41 respectively and the mean protein% obtained before treatment 3.29±0.04. Table-2 shows that milk protein% of 10 cows after treatment were 3.5, 3.55, 3.41, 3.25, 3.5, 3.35, 3.32, 3.30, 3.65 and 3.45 respectively and the mean protein% obtained after treatment was 3.43±0.039. From the table-1&2 it is shown that there is slight improvement among mean protein% obtained before treatment 3.29±0.14 and after treatment 3.352±0.1. Significant difference (P<0.05) was found in protein% between control group and treatment group. Which was similarly showed previously by several authors such as Kobayashi *et al.* (1995), Oetzel et al. (2007), Brunoa et al. (2008), Yalçın et al. (2011), Vibhute et al. (2011). But some authors showed that there was no significant influence of probiotics on milk composition as well as protein %. (Erdman and Sharma, 1989; Arambel and Kent, 1990; Swartz et al., 1994; Dutta et al., 2008)

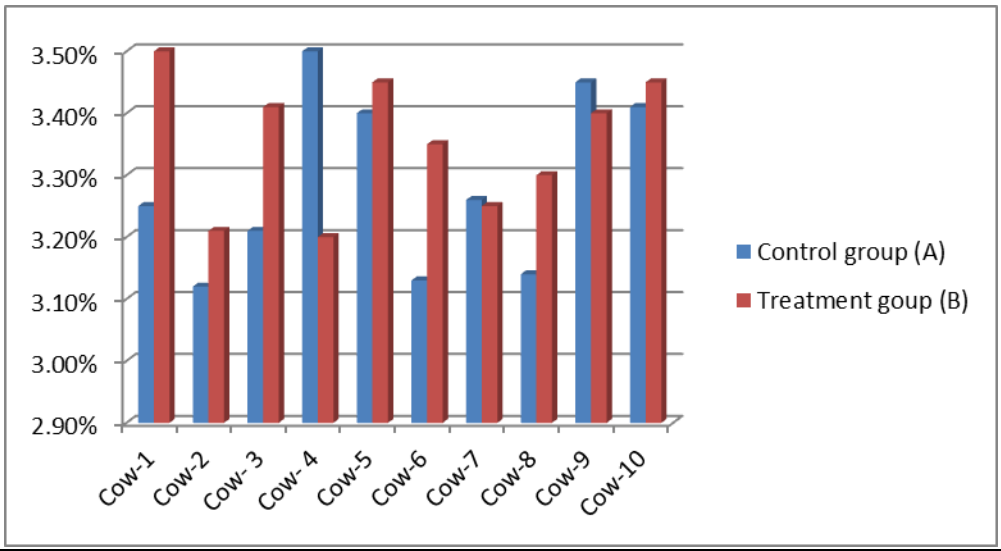

Figure 3. Comparison between the protein% of milk of experimental cows before treatment and after treatment

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Effects on Solids Not Fat%:

From Table-1 it is shown that before feeding probiotics, observed SNF% of experimental cows were 8.90, 7.83, 8.51, 8.4, 8.21, 8.25, 8.17, 7.86, 8.46 & 8.20 respectively and the mean SNF% of 10 cows was 8.28±0.99. After feeding probiotics, observed SNF% of experimental cows were 9.03, 8.60, 8.43, 8.67, 8.73, 8.44, 8.23, 8.65, 8.50 & 8.41 respectively and the mean SNF% of 10 cows after feeding probiotics was 8.57±0.069. So in comparison between control result and treatment result it is shown that there was improvement in the SNF% of milk after feeding Probiotics. Statistical analysis also showed that there was significant difference ($P<0.05$) within the SNF% between control group and treatment group, as observed earlier by Brunoa et al. (2008), Vibhute et al. (2011), Ahmad et al. (2011). The results are also in agreement with Yasuda et al. (2007), they found that the amount of solids-not-fat in milk of treated group was significantly increased in comparison those of control group.

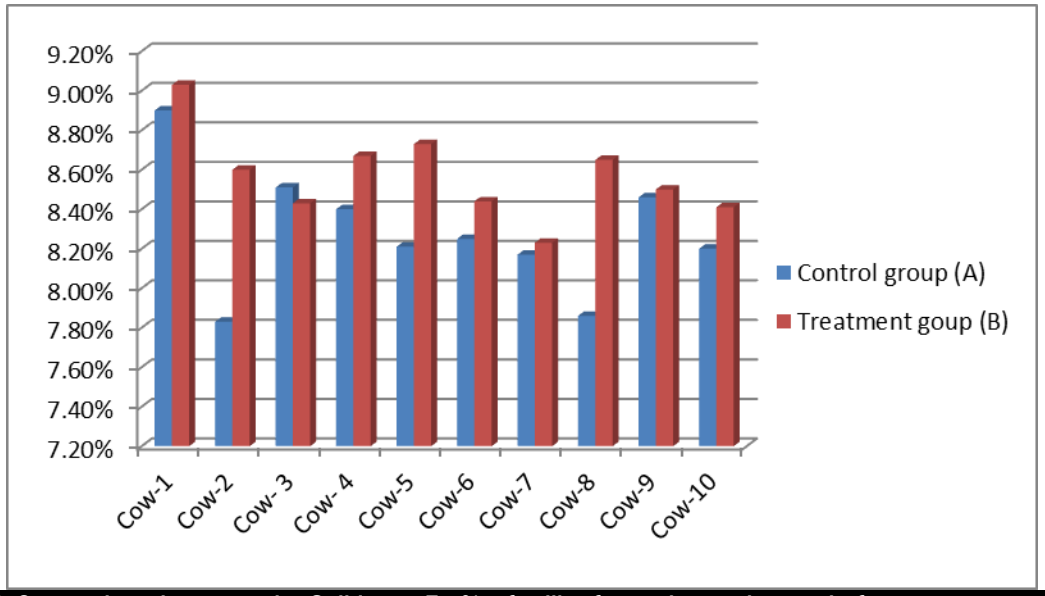

Figure 4. Comparison between the Solids-not-Fat% of milk of experimental cows before treatment and after treatment

Table 2 - Quality of milk of experimental cow before and after treatment

Experimental Cow	Parameters									
	Fat%		Protein%		SNF%		Acidity		C.L.R	
	A	B	A	B	A	B	A	B	A	B
Cow-1	6.3	5.7	3.25	3.5	8.90	9.03	0.15	0.15	30	31
Cow-2	4.7	4.8	3.12	3.55	7.83	8.60	0.14	0.16	27	30
Cow-3	5.6	5.2	3.21	3.41	8.51	8.43	0.15	0.15	29	29
Cow-4	3.8	3.9	3.5	3.25	8.4	8.67	0.16	0.14	30	31
Cow-5	4.1	4.2	3.4	3.5	8.21	8.73	0.14	0.15	29	31
Cow-6	4.3	4.0	3.13	3.35	8.25	8.44	0.16	0.16	29	30
Cow-7	3.9	4.2	3.26	3.32	8.17	8.23	0.14	0.14	29	29
Cow-8	3.6	3.8	3.14	3.30	7.86	8.65	0.15	0.15	28	31
Cow-9	4.1	4.3	3.45	3.65	8.46	8.50	0.15	0.16	30	30
Cow-10	5.3	5.1	3.41	3.45	8.20	8.41	0.14	0.14	28	29
Mean±SE	4.57±0.28	4.52±0.2	3.29±0.04	3.43±0.039	8.28±0.99	8.57±0.069	0.148±0.0025	0.15±0.0026	28.9±0.31	30.1±0.28
t-value	0.522		2.5266		3.023		0.6124		3.3425.	
p-value	0.614		0.0324		0.014		0.55		0.0086	

NS: Non significant; * = 5% level of significance; A = Control group; B = Treatment group

Effects on Acidity of milk:

From Table-2 it is shown that before feeding probiotics, observed acidity of experimental cows were 0.15, 0.14, 0.15, 0.16, 0.14, 0.16, 0.14, 0.15, 0.15, and 0.14 respectively and mean value of acidity was 0.148±0.0025. After feeding probiotics, observed acidity of experimental cows were 0.15, 0.16, 0.15, 0.14, 0.15, 0.16, 0.14, 0.15, 0.16 and 0.14 respectively and mean value of acidity was 0.15±0.002. Statistical analysis revealed that there was no significant ($P>0.05$) variation in acidity of milk between control group and treatment group. No other study was conducted on the effect of Probiotics on acidity of milk previously by any scientist.

Effects on Corrected Lactometer Reading (CLR):

Table-2 shows that the Corrected Lactometer Reading of experimental cows before treatment with Probiotics was 30, 27, 29, 30, 29, 29, 29, 28, 30 and 28 respectively, and the mean value was 28.9±0.31. Table- 2 shows

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that the Corrected Lactometer Reading of experimental cows after treatment was 31, 30, 29, 31, 31, 30, 29, 31, 30, 29 respectively and the mean value was 30.1±0.28. In comparison between the results of before treatment group and after treatment group, there was slight variation. Statistical analysis showed that there was significant difference ($P<0.05$) within the corrected lactometer reading between control group and treated group. No other study was conducted on the effect of Probiotics on Corrected Lactometer Reading of milk previously by any scientist.

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