Effect of Probiotics Supplementation on Milk Yield and Its Composition in Lactating Holstein Fresien and Deoni Cross Bred Cows

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***Abstract*—Twenty four HFxDeoni cross bred cows were divided into four groups (6 cows in each group) on the basis of average milk yield, parity and stage of lactation. T_0 (control group) cows were not fed with probiotics. T_1, T_2 and T_3 (treatment groups) cows were fed with 10 gm, 15 gm and 20 gm probiotics per day, respectively, just before morning milking. The multi strain probiotic contained *Saccharomyces cervisiae* and *Lactobacillus sporogenes*. The animals were milked twice in a day; morning at 5.30 am and afternoon 3.30 pm. Daily milk yield was recorded in pre-trial period of 25 days and then during 60 days of experimental period. Milk samples from the individual cows were collected twice a week (in pre-trial period and in experimental period) and were analysed for fat, SNF, density, freezing point, protein, lactose and total ash using the milk analyzer. From pre-trial period to trial period, the milk yield increased from 8.31 L/day, 8.26 L/day and 8.48 L/day to 8.97L/day, 9.64L/day and 9.68L/day in T_1, T_2 and T_3 group (highly significant; $P<0.05$), respectively, compared to from 8.45 L/day to 8.57L/day in T_0 group. Milk were significantly higher in cows (T_1, T_2 and T_3) supplemented with probiotics than T_0. The freezing point decreased in T_1, T_2 and T_3 groups indicating increase in the total solids of milk compared to T_0. There were minor changes in ash content of milk by feeding probiotics. Economically, supplementing the diet with probiotic earned more profit and feeding @ 15 gm probiotic/day/animal was found more beneficial than feeding @ 10 and 20 g/day/cow.**

***Index Terms*—probiotic, *saccharomyces cervisiae*, *lactobacillus sporogenes*, milk yield, milk composition and economics**

I. Introduction

India stands number one in milk production in the world. However, the total milk production of India is contributed by a huge cattle and buffalo population [1]. Significant pressure on resources will occur as the global population is expected to increase to 9 billion by 2050 and because of deforestation and non availability of grazing land it will be difficult to manage the grazing land and fodder production with such a huge human and animal population. Therefore improving the productivity of the animals is important. Use of rumen manipulators is an option to enhance animal productivity. Rumen manipulation can be done by the use of many growth stimulants including hormones and antibiotics. However, it has potential risks of two prevailing public health problems such as the development of antibiotic resistance genes [2], [3] and milk & meat antibiotic residues [4]. The potential alternative is feeding of microbials as probiotics also called as directly fed microbes (DFM) [5].

The term probiotic, first introduced in 1953 by Kollath [6], is derived from the Greek language which means 'for life'. According to World Health Organization, probiotics denotes to live microorganisms that are administered in optimum quantity to confer a health benefit on the host and consumed as part of fermented foods with specially added active live cultures or as dietary supplements. Probiotics may be lactic acid producing bacteria (Spp. *Lactobacillus* and *Streptococci*), lactic acid utilizing bacteria, or other microorganisms which will have beneficial effects for the hosts and yeast products containing *Aspergillus* and *Saccharomyces* [2], [3].

In clinical trials, probiotics have been reported to enhance the growth of many domestic animals including cows, neonatal calves and piglets, broilers [7], and humans [8]. Yeast cultures, mainly *Saccharomyces cerevisiae*, may improve ruminal fermentation [9], [10] and therefore provide another alternative to feed additive. *Saccharomyces cerevisiae* has been considered as the promising probiotic culture for efficient nutrient utilization [5]. There are reports of beneficial effect of supplementing the animal feed with probiotics on milk yield, milk fat and milk protein content. The addition of

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yeast culture in the diet of Holstein cows was beneficial in improving milk production and milk fat, and some biochemical parameters of blood [11]. Kudrna *et al.*, (2007) [12] noted that yeast supplementation significantly improved the milk yield despite reducing the dry matter intake.

The present study was carried out with an objective of finding the effects of feeding the lactating HFxDeoni cross bred dairy cows with probiotics on milk yield, milk composition and its economical benefit to the dairy farmers.

II. Materials and Methods

A. Selection of Animals

24 HFxDeoni cross bred cows from the Dairy Unit, Main Agricultural Research station, University of Agricultural Sciences, Raichur were selected for this study. The cows were multiparous (lactation number 2 and 3) and in early to mid lactation.

B. Experimental Design and Feeding

The cows in all the groups received a basal diet comprising of roughages and concentrates separately to meet the maintenance and production requirements. All the cows were fed with dry fodder ad libitum, 10kg/day of green fodder consisting of hybrid Napier DHN – 6 and maintenance concentrate ration of 0.5kg/cow. The production ration consisted of the concentrate mixture @1kg/3L of milk production. The concentrate mixture was prepared within the farm and composed of maize, cotton seed cake, wheat bran, rice bran, mineral mixture and salt. Concentrates were offered twice daily at the time of milking. The animals were milked twice daily at 5.30 am and 3.30 pm throughout the experimental period. The cows were divided into four groups each containing 6 animals (T_0, T_1, T_2 and T_3) based on the similar average milk yield, parity and stage of lactation. T_0 acted as control group and no probiotic was fed. T_1 cows were fed with probiotics Biobloom (Zydus AHL) @ 10 gm/day/cow, T_2 cows were fed with probiotics @ 15 gm/day/cow and T_3 cows were fed with probiotics @ 20 gm/day/cow. The experiment was carried out for 60 days.

C. Sampling and Analysis

Daily milk yield was recorded in pre-trial period of 25 days and then during 60 days of trial period. Similarly, milk samples from the individual cows were collected twice a week (in pre-trial period and in trial period) and were analysed for fat, SNF, density, freezing point, protein, lactose and total ash using the milk analyzer (Lactoscan®).

D. Statistical Analysis

In the present study, mean as a measure of central tendency and the standard error as a measure of random error were employed for the statistical analysis [13]. The students-t test (P= 0.05) was used to know the significant variation between the two groups.

III. Results and Discussion

A. Effect of Probiotic Feeding on Milk Yield

The effect of feeding of probiotic, Biobloom®, on milk yield is presented in Table I. From pre-trial period to the trial period the average milk yield in T_0 cows increased from 8.45 L/day to 8.57L/day which was not significant. In group T_1 cows it increased from 8.31 L/day to 8.97L/day. From pre-trial period to the trial period the average milk yield increased from 8.26 L/day to 9.64L/day in T_2 group and from 8.48 L/day to 9.68L/day in T_3 group. The increase in milk yield in T_1 cows was not significant. However, in T_2 and T_3 cows the increase in milk yield was very much significant ($P<0.05$).

TABLE I. Effect of Probiotic Supplementation on Milk Yield and Milk Composition (Mean ±Standard Error)

Parameter		T_0,	T_1,	T_2	T_3
Average milk yield (L)/day	**Pre-trial period**	8.45 ±0.15	8.31 ±0.61	8.26 ±0.38	8.28 ±0.20
	Trial period	8.57 ±0.24	8.95 ±0.15	9.64 ±0.07	9.68 ±0.46
Milk yield on 61st day of experiment		8.70 L	9.25 L	11.20 L	11.50 L
% Increase in milk yield		1.42%	7.70%	16.71%	16.90%
Fat %	**Pre-trial period**	3.93 ±0.15	3.89 ±0.21	3.81 ±0.50	3.95 ±0.42
	Trial period	3.95 ±0.26	4.38 ±0.53	4.85 ±0.06	4.91 ±0.41
SNF %	**Pre-trial period**	8.10 ±0.01	8.09 ±0.12	8.01 ±0.28	8.09 ±0.37
	Trial period	8.09 ±0.07	8.30 ±0.24	8.95 ±0.40	9.11 ±0.05
Density (g/cub. cm)	**Pre-trial period**	27.73 ±0.14	27.81 ±0.11	27.65 ±0.09	27.69 ±0.19
	Trial period	27.71 ±0.51	27.93 ±0.39	28.55 ±0.23	29.15 ±0.01
Freezing point (-)°C	**Pre-trial period**	-0.519 ±0.10	-0.501 ±0.16	-0.521 ±0.11	-0.532 ±0.17
	Trial period	-0.518 ±0.01	-0.530 ±0.02	-0.573 ±0.04	-0.607 ±0.09
Protein %	**Pre-trial period**	2.90 ±0.01	2.98 ±0.10	2.85 ±0.14	2.89 ±0.08
	Trial period	2.91 ±0.05	2.95 ±0.02	3.05 ±0.15	3.07 ±0.03
Lactose %	**Pre-trial period**	5.15 ±0.33	5.11 ±0.12	5.18 ±0.10	4.95 ±0.11
	Trial period	5.18 ±0.03	4.63 ±0.23	4.38 ±0.25	4.40 ±0.04
Ash %	**Pre-trial period**	0.59 ±0.20	0.61 ±0.16	0.60 ±0.41	0.57 ±0.28
	Trial period	0.60 ±0.61	0.61 ±0.01	0.63±0.03	0.60 ±0.04

The percent of increase of milk yield in control group was 1.42% and in T_1 group it was 7.70%. Whereas in T_2 and T_3 it was 16.71% and 16.90% which exemplifies the significant increase of milk yield because of feeding of probiotics. The milk yield on 61st day of experiment was 8.70 L, 9.25 L, 11.20 L and 11.50 L, respectively. The milk yield in T_2 and T_3 cows was significantly higher than the control group. The weekly improvement in the milk yield because of supplementing with probiotics is depicted in the Fig. 1.

Our results are supported by the results of the many researchers. Yasuda *et al.*, (2007) [14] reported 3-16% increase in milk production in HF cows by supplementing the diet with probiotics. Vibhute *et al.*, (2011) [15] reported that feeding the probiotics, *Lactobacillus*, *Saccharomyces* and *Propionibacterium* spp. increased the milk production by 4.65-5.41 L in crossbred cows in Akola, Maharashtra. Total daily milk productions of the cows fed with probiotics were 12.7% and 11.5% higher than those of animals in the control group [16].

B. Effect of Probiotic Feeding on Milk Composition

The effect of supplementing the diet with probiotics on milk composition of HFxDeoni crossbred cows is presented in Table I. In the T1 group cows the fat percentage increased from 3.89 to 4.38. In T_2 group cows the fat percentage increased from 3.81 to 4.85% and in T_3 group cows the fat% increased from 3.95 to 4.91%. Compared to control group (T_0) the fat percentage increased in the treatment groups. In T_1 group fat % increased by 0.49 % in absolute terms. In T_2 group cows the fat percentage increased by 1.04% and in T_3 group cows the fat% increased by 0.96% which was highly significant ($P<0.05$).

Increase in milk fat was observed at the second and third week (p=0.045, p=0.003), respectively after supplementation with yeast [11]. In ewes, feeding of yeast culture resulted in increased milk yield, milk fat, protein, lactose and total solids [17], [18]. In a study conducted in Pakistan, 1.22-1.45% of increase in milk fat % was recorded in buffaloes [19]. As per Dutta et al., (2009) [20] review, supplementation with direct-fed-microbial product consisting of two strains of *Enterococcus faecium* and *Saccharomyces cerevisiae* increased fat percentage in the milk from cows due to increased VFA production.

The protein content of the milk of T_0 cows was 2.90% before the trial and was 2.91% after the trial. Whereas the protein content of the milk of T_1, T_2 and T_3 cows was 2.98%, 2.85% and 2.89% respectively and it increased to 3.05 and 3.07% respectively in T_2 and T_3. The lactose content of the milk was 5.15, 5.11, 5.18 and 4.95% respectively in T_0, T_1, T_2 and T_3 before the trial. And after the trial of feeding the probiotics for 60 days the lactose percentage was 5.18, 4.63, 4.38 and 4.40% in T_0, T_1, T_2 and T_3, respectively (Table I).

The protein content of the milk increased in T_1, T_2 and T_3 cows compared to T_0 cows though not significant. The result obtained is in accordance with the findings of Singh and Kumar (1996), [21] who found that protein content of the milk increased by 11.90, 21.43 and 21.43% by feeding probiotic Yes-Sacc® @ 5, 10 and 15 g/day/buffalo respectively. In T_1 group cows there was slight decrease in protein content and was 2.95%, 0.03% less than the pre-trial period. Desnoyers et al., (2009) [22] found that yeast supplementation increased milk yield (+1.2 g/kg of BW) but had no influence on milk protein content. However, Yeast supplementation increased daily yields of milk by 0.9 L, protein by 0.31% in dairy cows fed with *Saccharomyces cerevisiae* CNCM I-1077 in a study done by Luciene *et al.*, (2011) [23] which supports our results. The lactose content of the milk increased by 0.03% in T_0 and it decreased in the treatment groups T_1, T_2 and T_3. The decrease in treatment groups could be explained by inverse relation between lactose and the protein and fat content of milk.

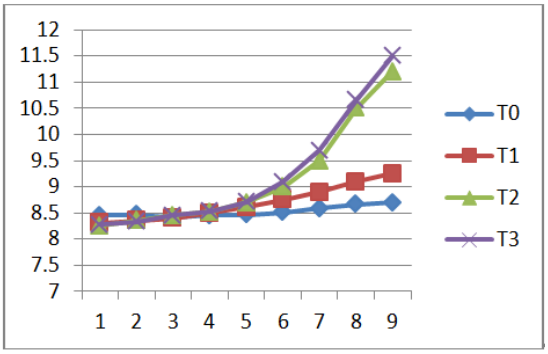

Figure 1. Weekly improvement in milk yield because of feeding of probiotics

The SNF of the milk in T_0 cows and was 8.10 and 8.09% respectively before the trial and after the trial. The SNF of the milk before the trial was 8.09, 8.01 and 8.09% in T_1, T_2 and T_3, respectively. It increased to 8.30, 8.95 and 9.11% respectively in T_1, T_2 and T_3 after feeding the probiotics (Table I). There was no change in the SNF of the milk in T_0 cows. Whereas in T_1, T_2 and T_3, groups the SNF increased is significantly ($P<0.05$) after feeding the probiotics. The increase in SNF could be due to increase in protein content of the milk in the treatment groups. Significant improvement ($P<0.05$) was found in protein content and solids-not-fat content of milk in a study conducted in multiparous cows fed with *Saccharomyces cerevisiae* [24].

The density of the milk was 27.73 and 27.71 g/cm^3 in T_0 cows respectively before the trial and after the trial. It was 27.81, 27.65 and 27.69 g/cm^3, respectively in T_1, T_2 and T_3 before the trial. It increased to 27.93, 28.55 and 29.15 g/cm^3 respectively in T_1, T_2 and T_3 after feeding with probiotics. The significant increase in the density of the milk of the cows that have been fed with probiotics could be explained by the increase in the fat and the protein content of the milk. The freezing point of the milk was -0.501, -0.521 and -0.532^{0}C respectively in T_1, T_2 and T_3 before the trial. It decreased to -0.530, -0.573 and -0.607^{0}C because of feeding of probiotics for 60 days. The readings for ash content of the milk before and after the trial are presented in the Table I. Again increase in the fat and protein content of the milk in the treatment groups gave rise to decrease in the freezing point of the milk. The freezing point of the milk decreased significantly after feeding of probiotics for 60 days. There were very minor changes in the ash content of the milk due to supplementation of the diet with probiotics.

Our results are well corroborated by the findings of other researchers. The average milk yield, 6% fat corrected milk (FCM) yield, solids not fat (SNF) percent and total solids increased (P>0.05) in Yeast Supplemented group of buffaloes [25], [26]. In a study done by Vibhute et al., (2011) [15] milk yield, fat, protein and SNF content tended to be higher in cows supplemented with probiotic preparation. In a study carried out in ruminants, the 4% fat corrected milk yield, total solids(%), protein (%), fat yield, protein yield and lactose yield were significantly higher (P<0.05) in *Saccharomyces cerevisiae* supplemented groups than control one [27]

The scientific reason for improving the productivity of the animals could be explained by the findings of other researchers. Supplementation of early lactation dairy cows with probiotic altered the rumen fermentation patterns in favour of propionate, with potential benefits for energy balance and animal productivity [28]. The stimulation of lactic acid-utilising bacteria could account for *Saccharomyces cerevisiae*-induced decrease in lactic acid production [29] and hence corresponding stabilization of ruminal pH. Stabilization of ruminal pH improves propionic acid production [9]. Feeding of yeast allows the maintenance of the cellulolytic flora [30] and enhances the degradation of plant fibers, and therefore, the digestibility of the diet. Probiotics also improve the immune mechanism against the gastrointestinal pathogens and hence more productivity [31], [32]. Yeast culture (YC) supplements containing *Saccharomyces cerevisiae*, are known to be rich source of enzymes, vitamins, other nutrients and important co-factors, have been reported to produce a variety of beneficial production responses [3].

When we worked out the economics of supplementing the diet with the probiotic Biobloom® to the lactating animals the daily cost of milk production was Rs. 48.60, 52.95, 55.80 and 58.65 per cow, respectively in T_0, T_1, T_2 and T_3 group cows. And the daily earning was Rs. 325.66, 334.02, 366.32 and 367.84 per cow, respectively in T_0, T_1, T_2 and T_3 group cows. Therefore the daily profit comes to Rs. 277.06, 281.07, 310.52 and 309.19 per cow, respectively in T_0, T_1, T_2 and T_3 (Table II.).

TABLE II. ECONOMICS OF FEEDING OF PROBIOTICS IN THE DIET OF CROSS BRED HFXDEONI COWS

Parameters	**T0**	**T1**	**T2**	**T3**
Total feed cost* (Rs/animal/day)	48.60	49.95	51.30	52.65
Cost of probiotics (Rs/animal/day)**	0.00	3.00	4.50	6.00
Total expenses(Rs/animal/day)	48.60	52.95	55.80	58.65
Average daily milk yield(kg/animal)	8.57	8.79	9.64	9.68
Cost of milk production (Rs/kg)	5.67	5.68	5.32	5.43
Daily income on milk sale* (Rs/animal/day)**	325.66	334.02	366.32	367.84
Profit (Rs/day)	277.06	281.07	310.52	309.19

* Includes the cost of roughage and concentrate
** The cost of Biobloom = Rs. 300/kg
*** The cost of milk in this region Rs. 38/L

Therefore by the findings of our study, it can be concluded that supplementing the feed with probiotic Biobloom® increased the quantity of the milk and improved the composition and quality of the milk. It signifies that the productivity of the animal has improved and hence each animal earns more income. Compared to T_0 group, in T_1, T_2 and T_3 groups each cow earned Rs. 4.01, 33.46 and 32.13/day more profit, respectively (Table II). Our results also concludes that, though supplementing the feed with probiotic Biobloom® @ 20g/day/cow increased the milk yield and improved its composition, it is less economical than feeding of Biobloom® @ 15g/day/cow.

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