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Effect of supplementation of probiotics on the performance of milk yield and economics of dairy cattle

Kumar Satendra*, Kumar Brajendu**

*Subject Matter Specialist (Veterinary Science) **Senior Scientist cum Head, Bihar Agricultural University Sabour, Krishi Vigyan Kendra, Khagaria, Bihar 851214, India.

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Bacillus Subtilis and *Bacillus Licheniformis*.

Abstract

Aims: A study was made to evaluate the effect of feeding different doses of probiotics on the milk yield and its composition of crossbred cows. *Settings and Design:* Twenty lactating crossbred cows were taken for the experiment and were randomly divided into four groups of five cows each. 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. *Methods and Material:* Cows were fed basal diet along with production ration. Milk sample collected weekly and corrected for 4% FCM. Milk, fat % and SNF were analyzed by using milk analyzer. *Statistical analysis used:* 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. The student 't' test (P= 0.05) was used to know the significant variation between two groups. *Results:* Significant (Pd"0.05) differences were observed in milk yield of cows fed multi-stain probiotics as compared to the control. The average milk production (4% FCM) was highest in 20gm probiotics supplemented cows is 7.89±0.21, followed by 7.05±0.19 and 6.52±0.07 in cows supplemented with 15gm and 10gm probiotics respectively, as compared to the control animals (5.73±0.06). In T_0,T_1, T_2 and T_3 group cows the increase in fat percentage was observed to be 4.24±0.03, 4.45±0.02, 4.52±0.09 and 4.78±0.08 respectively during the trial period where as mean SNF percentage of T_0, T_1, T_2 and T_3 increased to 8.01±0.14, 8.07±0.04, 8.23±0.08 and 8.75±0.08 percent respectively. *Conclusions:* It can be concluded that use of multi-stain probiotics is cost effective and increased the milk production by 29.13 % in case of 20gm probiotics supplementation followed by 15gm (17.70%) and 10gm supplementation (8.31%), respectively, in lactating crossbred cows. After supplementation of probiotics, mean fat percentage increase of T_1, T_2 and T_3 increased to 2.06, 5.36 and 10.65 percent respectively were as mean SNF percentage increase of T1, T_2 and T_3 increased to 0.75, 2.24 and 7.89 percent respectively. The net profit was estimated as Rs. 83.14, 85.43, 86.84and 93.15 per cow per day respectively in T_0, T_1, T_2 and T_3 groups and calculated cost-benefit ratio was 1: 1.42, 1: 1.47, 1: 1.53 and 1: 1.60 in T_0, T_1, T_2 and T_3 respectively.

Introduction

The mechanism of action of probiotic bacteria and their effect in combating digestive disorders, enhancing milk yield and its composition in animal has been demonstrated and supported in numerous scientific studies. Probiotics are defined as "live microorganisms that may beneficially affect the host upon ingestion by improving the balance of intestinal microflora" (Fuller, 1989). According to currently adopted definition by WHO, probiotics are "live organisms which when administered in adequate amount confers a health benefit on the host". Animal productivity can be enhanced by rumen manipulation by using many growth stimulants including hormones and antibiotics. But it has potential risk of developing antibiotic resistant gene and milk and meat antibiotic residue. Therefore best alternative for rumen manipulation is feeding microbes as probiotics (Aydin *et. al.*, 2009, Seo *et. al.*, 2010 and Rai *et. al.*,

Corresponding Author: Kumar Satendra, Subject Matter Specialist (Veterinary Science) , Bihar Agricultural University Sabour, Krishi Vigyan Kendra, Khagaria, Bihar 851214, India.
E-mail: drsatendra.kumar36@gmail.com
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2013). Probiotics may be lactic acid producing bacteria, lactic acid utilizing bacteria or other microorganisms. Yeast (*Saccharomyces cerevisiae*) was reported to increase nutritional value of poor quality forage and high grain diet, improve development of rumen lactate consuming bacteria, prevent accumulation of lactate and also results in rumen pH drops. It was also observed to stimulate cellulolytic bacteria in the rumen, to increase fiber digestion and flow of microbial protein from rumen and to improve feed intake and milk yield in dairy cows (Newbold *et.al.*, 1996, Beauchemin *et. al.*, 2003, Desnoyers *et. al.*, 2009, Dutta *et. al.*, 2009, Baiomy 2011, Srinivas *et. al.*, 2011, Mousa *et. al.*, 2012, Ayad *et. al.*, 2013, Diler *et. al.*, 2014 and Shreedhar 2016). Yeast is more effective when animals are particularly under heat stress (Huber, 1998 and Schingoethe *et. al.*, 2004). Hence, the present study was undertaken with the objective to evaluate the effect of feeding probiotics on the milk yield and its composition of lactating crossbred dairy cows as well as to evaluate the associated economic benefit to the dairy farmers.

Material and Methods

A. Selection of Animal

The present investigation was undertaken at Krishi Vigyan Kendra, Khagaria. Twenty crossbred cows 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

All crossbred cows were provided with a basal diet comprising of dry fodder, green fodder and concentrates separately to meet the maintenance and production requirement (NRC, 2001).

The cows were fed with a basal diet of dry fodder 5 kg/day, green fodder 20 kg/day and maintenance concentrates ration of 1 kg/day. The production ration consisted of the concentrate mixture @ 1 kg/ 3L of milk production. The concentrate mixture was prepared within the farm and composed of maize (50 %), mustard seed cake (23 %), wheat (25 %), mineral mixture (2 %) and salt (1 %).

The cows were divided into four groups and each group consisted of 5 animals (T_0, T_1, T_2 and T_3) based on similar average milk yield and stage of lactation. T_0 acted as control group which was not fed with probiotics while T_1, T_2 and T_3 groups of cows were fed with probiotics at the rate 10gm, 15gm and 20 gm/ day/cow respectively. The experiment was carried out for seventy days.

The probiotics used in this experiment included *Saccharomyces cerevisiae, Lactobacillus acidophilus, Lactobacillus sporogenes, Bacillus subtilis* and *Bacillus licheniformis*, the compositions of which is given in Table 1.

C. Sampling and Analysis

Daily milk yield was recorded for pre-trial period of 2 weeks, trial period of 6 weeks and post-trial period of 2 weeks. Similarly, milk samples were drawn for analysis in morning and evening hours once in each week of the experimental period. Individual morning and evening milk yield of lactating cows were recorded weekly and corrected for 4% fat content (FCM) using the formula of 4% FCM = 0.4 x milk yield (kg) + 15 x fat yield (kg) as stated by Gains (1928). Milk, fat % and SNF were analyzed by using milk analyzer.

Majority of the farmers of the study were practicing intensive management systems. The feed cost was apparently high under this management system as the cut and fed method is practiced. The cost calculations are based on dairy enterprises that consist of the following elements: Total feed cost, Cost of probiotics, Average daily milk yield, cost of milk production, and Daily income on milk sale. The analysis results in a comparison of returns and total costs per liter of milk. The total expenses includes cost of dry fodder, green fodder, homemade concentrate mixture and probiotics while daily income on milk sale was calculated by multiplying average daily milk yield and cost of milk production.

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. The student 't' test (P= 0.05) was used to know the significant variation between two groups.

Results and Discussion

Effect of Feeding Probiotics on Milk Production

The effect of feeding probiotics on milk yield is presented in Table 2. It shows mean values of weekly average milk production for the pre trial period of two weeks, trial period of 6 weeks and post trial period of two weeks for the treatments T_0, T_1, T_2 and T_3. Before starting the experiment mean (±SEM) daily milk yield was observed to be 5.48±0.10 L/day for control group and 5.71 ± 0.09 L/day, 5.74 ± 0.16 L/ day and 5.83 ± 0.11 L/day for treatments T_1, T_2 and T_3

respectively. It indicates that there was no significant difference among all treatments before starting the experiment. The milk production improvement response for T_0 and the treatments supplied with different doses of probiotics was estimated throughout the experimental period. From the pre trial period to the trial period the average milk yield in T_0 cows increased from 5.48 ±0.10L/day to 5.53 ± 0.05 L/day which was not significant while in T_1, T_2 and T_3 groups, the enhancement in milk yield was observed to be from 5.71 ± 0.09 L/day to 6.11 ±0.07 L/day, 5.74 ±0.16 L/day to 6.53 ±0.15 L/day and 5.83 ±0.11 L/day to 7.04± 0.15 L/day respectively. From the studies it can be concluded that the enhancement in milk yield in T_2 and T_3 groups was significantly higher than in the control group. Hence, our study reveals a significant improvement in the milk production induced by incorporation of probiotics in the diet. Similar result was observed by Willian *et. al.*, (1991), Wohlt *et. al.*, (1991), Piva *et. al.*, (1993), Putnam *et. al.*, (1997), Yasuda *et. al.*, (2007), Dutta *et. al.*, (2008), Desnoyers *et. al.*, 2009, Dutta *et. al.*, 2009, Baiomy 2011, Srinivas *et. al.*, 2011, Yalein *et. al.*, (2011), Vibute *et. al.*, (2011), Mousa *et. al.*, 2012, Hossain *et. al.*, (2014), Ayad *et. al.*, 2013, Diler *et. al.*, 2014 and Shreedhar *et. al.* (2016). In our investigation production of milk during six weeks of trial period increased by an average of 0.4 L/day, 0.79 L/day

Table 1: Composition of Probiotics used in the diet of Crossbred Cow

S. No.	Composition (Each 125gm of Probiotic Contains)	Quantity
1	*Saccharomyces cerevisiae*	1000 billion CFU
2	*Lactobacillus acidophilus*	30000 billion CFU
3	*Lactobacillus sporogenes*	30000 billion CFU
4	*Bacillus subtilis*	60000 billion CFU
5	*Bacillus licheniformis*	60000 billion CFU
6	Fructo Oligo Saccharid (FOS)	5000 mg
7	Mannan Oligo Saccharid (MOS)	5000 mg

CFU- Colony Forming Unit

Table 2: Effect of Probiotics supplementation on milk yield & milk composition (Mean±Standard Error)

Parameters	Trial (Weeks)	Technology option			
		T_0: Farm feeding Schedule (Farmers Practice)	T_1: Farm feeding schedule + probiotics @ 10 gm / day	T_2: Farm feeding schedule + probiotics @ 15 gm / day	T_3: Farm feeding schedule + probiotics @ 20 gm / day
Average milk yield (4%FCM)	Pre-trial period	5.66±0.14	6.02±0.10	5.99±0.23	6.11±0.14
	Trial period	5.73±0.06^{NS}	6.52±0.07*	7.05±0.19*	7.89±0.21*
	Post-trial period	5.80±0.12^{NS}	6.63±0.09*	7.26±0.27*	8.47±0.21*
Fat %	Pre-trial period	4.22±0.07	4.36±0.06	4.29±0.16	4.32±0.09
	Trial period	4.24±0.03^{NS}	4.45±0.02*	4.52±0.09*	4.78±0.08*
	Post-trial period	4.27±0.07^{NS}	4.49±0.04*	4.60±0.15*	4.93±0.12*
SNF %	Pre-trial period	7.99±0.24	8.01±0.11	8.05±0.12	8.11±0.12
	Trial period	8.01±0.14^{NS}	8.07±0.04*	8.23±0.08*	8.75±0.08*
	Post-trial period	8.02±0.23^{NS}	8.12±0.07*	8.29±0.12*	8.79±0.10*

*Significance ($P \leq 0.05$)
NS=Non significance ($P \geq 0.05$)

Table 3: Economics of feeding Probiotics in the diet of Crossbred Cow

Sl. No.	Parameters	T_0	T_1	T_2	T_3
1.	Total feed cost* (Rs/animal/day)	136.50	137.95	140.12	143.75
2.	Cost of probiotics** (Rs/animal/day)	0.00	8.80	13.20	17.60
3.	Total expenses (Rs/animal/day)	136.50	146.75	153.32	161.35
4.	Average daily milk yield (Kg/animal)	5.78	6.11	6.32	6.71
5.	cost of milk production (Rs/Kg)	23.62	24.02	24.26	24.05
6.	Daily income on milk sale*** (Rs/animal/day)	219.64	232.18	240.16	254.98
7.	Profit (Rs/day)	83.14	85.43	86.84	93.15
8.	B:C Ratio	1.42	1.47	1.53	1.60

* Includes the cost of roughage and concentrate
**The cost of probiotics = Rs. 880/kg
***The cost of milk in this region Rs. 38/L

and 1.21 L/day in T_1, T_2 and T_3 respectively. Piva *et. al.* (1993) and Erasmus *et. al.* (1992) reported relatively lower response while Erdwan and Sharma (1989), Arambel and Kent (1990), and Swartz *et. al.*, (2004) did not find significant enhancement in milk yield by supplementation of probiotics. Response to probiotics was found to be quite variable among the studies of different authors which may be related to the types and doses of diet provided, types of probiotics and animals tested (Willian *et. al.*, 1991).

Effects of Probiotic Feeding on Milk Fat Percentage

The effect of supplementing the diet with probiotics on milk fat percentage of crossbred cows is presented in Table- 2. The average fat percentage of pre trial period of four treatments T_0, T_1, T_2 and T_3 was observed to be 4.22±0.07, 4.36±0.06, 4.29±0.16 and 4.32±0.09 respectively. This means that there was no significant difference among all four treatments before starting the experiment. There was no significant increase in fat percentage of T_0 group during the trial period as the enhancement in milk yield was observed from 4.22±0.07 to 4.24±0.03 during the period. In T_1, T_2 and T_3 groups, increase in fat percentage was observed to be from 4.36±0.06 to 4.45±0.02, 4.29±0.16 to 4.52±0.09 and 4.32±0.09 to 4.78±0.08 respectively during the trial period. Compared to control group (T_0) fat percentage increase can be estimated as 0.09 percent, 0.23 percent and 0.46 percent in T_1, T_2 and T_3 groups respectively.

The fat percentage increase in T_3 can be termed as significant (P<0.05) which showed that the treatment T_3 was significantly superior to remaining treatments. Though there was no significant difference between T_1 and T_2, both were moderately significant over control group T_0. The difference was calculated to be 0.54, 0.33, 0.26 and 0.07 percent between treatments T_3 and T_0, T_3 and T_1, T_3 and T_2, T_2 and T_1 respectively. Statistical analysis showed that there was significant difference (P>0.05) between the fat percentage of treatment groups and control group. This is in agreement with this some studies made on the lactating animal in which significant response was observed in the composition of milk by supplementing probiotics in diet. (Gunter, 1989, Willaim, 1991, Piva *et. al.,* 1993, Chiquette, 1995, Yasuda and Fukata, 2004, Srinivas *et. al.,* 2011, Yalein *et. al.*, 2011, Vibhute *et. al.,* 2011, Mousa *et. al.,* 2012, and Shreedhar *et. al.*2016,). But some authors such as Erdman and Sharma, (1989), Arambel and Kent, (1990), Swartz *et. al.,* (1994), Dutta *et. al.,* (2008), and Hossain *et. al.,* (2014) did not find significant improvement in milk fat percentage after supplementing with probiotics. Improvement of milk fat in dairy cows supplemented with probiotics is associated with positive effect of the stimulation of cellulolytic bacteria and preferred orientation of fermentation to acetic acid production.

Effect of Probiotics Feeding on Solid Not Fat percentage

As per the Table 2 the average SNF percent during pre-trial period was 7.99±0.24, 8.01±0.11, 8.05±0.12 and 8.11±0.12 for control group (T_0) and treatment groups T_1, T_2 and T_3 respectively which indicated that there was no significant difference among all the four treatment groups before starting the experiment. After 6 weeks of trial period the mean SNF in T_0 was found to be to 8.01±0.14 while in T_1, T_2 and T_3, it increased to 8.07±0.04, 8.23±0.08 and 8.75±0.08 percent respectively.

There was no significant change in the SNF of the milk in T_0 cows whereas in T_1, T_2 and T_3 treatment groups the increase in SNF was observed to be significant (P< 0.05). The table-1 shows that there was average higher solid not fat percent for T_3 than other treatment groups. So it can be concluded that supplementation of probiotics resulted in improvement in the solid not fat percentage. Statistical analysis also indicated significant difference (P< 0.05) within the solid not fat percent between control group and treatment groups. These results are in agreement with the observations of Yasuda *et. al.* (2007), Brunoa *et. al.* (2009), Vibhute *et. al.* (2011), Ahmad *et. al.* (2011), Mousa *et. al.*, 2012, Hossain *et. al.* (2014) and Shreedhar *et. al.,* (2016).

Economics of Probiotics Supplementation

Economics of supplementing diet with the probiotics is given in Table 3. During the pre-trial period of 2 weeks, trial period of 6 weeks and post-trial period of 2 weeks, cow were fed with roughages like maize, berseem, wheat straw and concentrate. Therefore calculation of feeding cost includes the cost of roughages, concentrate and probiotics. The cost of milk production was estimated to be Rs. 136.50, 146.75, 153.32 and 161.35 per cow per day in T_0, T_1, T_2 and T_3 group cows respectively.

The daily earning was calculated as Rs. 219.64, 232.18, 240.16 and 254.98 per cow in T_0, T_1, T_2 and T_3 group cows respectively. Hence the daily profit was estimated as Rs. 83.14, 85.43, 86.84 and 93.15 per cow respectively in T_0, T_1, T_2 and T_3 groups. The cost-benefit ratio was 1: 1.42, 1: 1.47, 1: 1.53 and 1: 1.60 in T_0, T_1, T_2 and T_3 respectively. It signifies that the productivity of the animals has improved which provided more earning to the farmer.

Conclusion

The results of our study indicated that supplementing multi-stain probiotics to the diet of lactating cows is cost effective and increased the milk production by 29.13 % in case of 20gm probiotics supplementation followed by 15gm (17.70%) and 10gm supplementation (8.31%), respectively, in lactating crossbred cows. After supplementation of probiotics, mean fat percentage increase of T_1, T_2 and T_3 increased to 2.06, 5.36 and 10.65 percent respectively were as mean SNF percentage increase of T1, T_2 and T_3 increased to 0.75, 2.24 and 7.89 percent respectively. It signified that the productivity of the animals had improved resulting in more earning to the farmer. Therefore, inclusion of probiotics in dairy animal's diet should be encouraged.

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Key Messages

The multi-stain probiotics containing *Saccharomyces cerevisiae, Lactobacillus acidophilus, Lactobacillus sporogenes, Bacillus subtilis* and *Bacillus licheniformis* were fed @ 20gm/day/animal improves milk yield as well as fat % and SNF. Increased quantity and quality of milk enhance cost-benefit ratio of dairy farming.

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