



Dietary Supplementation of Probiotics to Improve Milk Production and Milk Quality in Crossbred Jersey Dairy Cows

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ABSTRACT

Background: Milk production and composition can vary during different stages of lactation. The use of probiotics can have an impact on milk quality and quantity.

Methods: The experiment investigated the effects of dietary supplementation with probiotic on milk production and quality in crossbred Jersey dairy cows [Jersey x Local (F1 50% Jersey generation)]. Sixteen crossbred Jersey dairy cows were selected and were divided into four groups: T0 (control group), T1, T2 and T3. The cows in the T0 group were given normal feed without probiotics. Meanwhile, the cows in the T1, T2 and T3 (treatment groups) were supplemented with 20 gm, 25 gm and 30 gm probiotics/animal/day for six months. Milk production and milk content of individual cows were evaluated during the three stages of lactation (early lactation, mid-lactation and late lactation).

Result: Milk production was significantly ($P<0.05$) increased in the probiotic-supplemented group in a dose-dependent manner during all three stages of lactation compared to the control group. Fat, protein, SNF, density and ash content of milk were also significantly ($P<0.05$) increased in the probiotic supplemented group (T1, T2 and T3) when compared to the control group (T0). However, milk lactose and freezing point decreased in probiotic-supplemented groups (T1, T2 and T3) compared to the control group (T0). Moreover, out of the three different doses of treatment groups, milk quality was significantly improved in the group supplemented with 30gm/cow/day (T3) during all stages of lactation. Dietary supplementation with probiotics can improve milk production and quality in crossbred Jersey cows.

Key words: Jersey cows, Milk Composition, Milk production, Probiotics.

INTRODUCTION

Dairying is a good source of income for small and marginal farmers. The feeds required for milk production can be met from their limited land resources, as most of the milch animals are ruminants. Most of their food can be derived from forages, coarse roughages and by-products not utilized by human beings, without incurring much additional cost. Bangladesh has one of the highest cattle densities but has the lowest milk productivity (Banglapedia, 2021). According to Livestock Economy (BBS, 2022-2023), in Bangladesh, the total cattle population is 248.56 Lakh and the demand, production and deficiency of milk is 158.50 Lakh metric tons, 140.68 Lakh metric tons and 17.82 Lakh metric tons. The need for milk per capita is 250 ml/head/day, but availability is 221.89 ml/head/day (BBS 2022-2023). So, it is essential to improve the productivity of the animals. The use of rumen manipulators is an option to enhance animal productivity. Many growth stimulants, including hormones and antibiotics, can manipulate rumen. However, it has potential risks of prevailing public health problems. Indiscriminate use of hormones, antibiotics, stimulants and other chemicals hamper milk quality. These agents, through entering into the human chain, are causing damage to the kidney, liver, lungs and other organs (Jeong *et al.*, 2010). So, it is now essential to use probiotics to determine their effects on the quality and quantity of milk produced and the host health of crossbred dairy cattle (Local x Jersey). The breed is a cross between Jersey and Local

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cattle, specifically the F1 50% Jersey generation. This crossbreed is well adapted to the climate in Bangladesh. The Bangladesh Milk Producer's Cooperative Union Limited (BMPCUL), also known as Milk vita, imported Jersey breeding bulls and initiated the production of Jersey x Local crossbred dairy cattle at the farmer level. The age of puberty for this breed is 18-22 months, the age at first birth is 22-25 months and the age at first calving is 31-34 months, with a calving interval of 13-15 months. The average lactation length is about 234-267 days, with a milk yield of 2500-2700 liters and an average daily milk yield of 9-12

liters. This crossbred Jersey variety is performing well in agro-climatic areas in Bangladesh (Paul *et al.*, 2013) and in Tamil Nadu, India (Vijayakumar *et al.*, 2019).

The potential alternative to antibiotics is feeding microbials as probiotics or medicinal plants. There are some medicinal plants with a galactogenic effect to stimulate milk production such as *Asparagus racemosus*, *Trigonella foenum-graecum*, *Cuminum cyminum*, *Carum carvi*, *Nigella sativa* (Paula *et al.*, 2023). Researches about natural alternatives to increase milk production in dairy animals is still limited. It is necessary to carry out further studies about plants with a galactogenic effect on the physiology of milk production in livestock species. Recently reported that shatavari root powder significantly improved milk production (Malati *et al.*, 2023; Rautray 2011). Probiotics are made of good live bacteria and/ or yeasts that naturally live in our body (Rao *et al.* 2016). *Saccharomyces cerevisiae* has been considered one of the promising probiotic cultures for efficient nutrient utilization and productivity (Rautray 2011, Ayad *et al.*, 2013). Yeast has been used to improve milk production and quality in ruminants for more than 50 years. Yeast supplemented with selenium/ zinc resulted to maximum milk production and quality (Hanan, 2023). There are reports of beneficial effect of supplementing the animal feed with probiotics on milk yield, milk fat and milk protein content (Jiang *et al.*, 2017a; Dias *et al.*, 2018, Nasiri *et al.*, 2019; Tesfaye *et al.*, 2019; Elaref *et al.*, 2020; Kalinska *et al.*, 2023). Contrary to the above studies, some authors reported no effect of yeast treatment on milk yield and milk fat in lactating Holstein cows (Ambriz *et al.*, 2017; Mallah, 2018, Ferreira 2019).

Very few studies in Bangladesh and a notable number of researches in the Indian sub-continent and other countries have been carried out on the effects of *Saccharomyces cerevisiae* probiotics on milk yield, milk quality, meat quality, body condition score, growth performance, gut physiology, nutrient digestibility, blood parameter, biochemical properties, fecal microflora of dairy cows, heifers and bulls (Yasmin *et al.*, 2021; Selim *et al.*, 2021) Despite the large numbers of dairy cattle available in Bangladesh, our indigenous cattle are not able to produce more milk due to genetic factors. We have a substantial number of crossbred cows capable of producing more milk. These crossbred animals should be selected for the country's milk production to go up. The current study investigated the effects of probiotic on milk production and quality in crossbreed Jersey dairy cows.

MATERIALS AND METHODS

Experimental design

A total sixteen crossbred Jersey dairy cows (Local × Jersey)

of 1st lactation were selected from a farm of Baghabari and Lahiri Mohonpur milk shed area, near Milk vita plant of Sirajganj district of Bangladesh for the study. The total experimental cows were divided into four groups namely T0 (control group) and T1, T2, T3 (treatment group) and having 4 cows in each group with average body weight of 230±7 kg. The total trial period (6 months) were divided into three stages on the basis of period of lactation namely as early lactation (0-45 days), mid lactation (46-120 days) and late lactation (120-180 days). The cows of all groups were provided a basal diet comprising of roughages and concentrates separately to meet the maintenance and production requirements as per Thumb rule's method. The concentrate requirement of crossbred cow is 1.5 kg for maintenance production and lactating animal should be given 1 kg additional concentrate for every 3 kg milk produced. Finally each dairy cow of all groups were supplied 20 kg green grass, 5 kg paddy straw (Supplementary Table 1) and concentrates were variable on the basis of milk production as per Thumb rule's method. The concentrate mixture was prepared and supplied by Milk vita. The concentrates were composed of maize (35%), rice polish (10 %), de oiled rice bran (31%), rapeseed (10%), soybean meal (5%), molasses (5%), DCP (0.2%), limestone (2.7%), sodium bi carbonate (0.10%) and salt (1%) (Supplementary table 2). Concentrates were offered twice daily at the time of milking. The cows of T0 (control group) group was not fed with probiotics but cows of treatment groups was fed with 20gm, 25gm and 30gm probiotics (*Saccharomyces cerevisiae* 2 × 10¹² CFU/gm) in T1, T2 and T3 respectively per animal per day, just before morning milking. The cows were milked twice daily at 5.30 am and 4.00 pm throughout the experimental period.

The commercial name of the probiotics is A-Maxpro^R (Green Yeast) manufactured by BAFFEED in Turkey and imported by Wilts marketing company limited in Bangladesh. (Supplementary Table 3).

Sample collection, preservation, transportation and laboratory test

Milk samples (100 ml) from the individual cows of all groups were collected twice a week in the period of early, mid and late lactation in trial period of 6 months. Samples were preserved at refrigerator (-20 °C) when necessary and sent to Bangladesh Milk Producing Co-operative Union Limited (Milk Vita), Sirajganj with cool chain maintain for analysis of milk composition as milk fat, protein, SNF, density, freezing point, lactose and total ash using the milk analyzer (Lactoscan®)

Supplementary Table 1: The proximate composition of napier and paddy straw.

Feed/Fodder	DM% of fresh weight	% of DM				ME (Mj/kg DM)
		CP	CF	OM	Ash	
Napier grass	19.08	7.02	35.25	87.75	12.50	7.13
Paddy straw	87.76	4.27	47.06	84.79	15.17	6.10

Statistical analysis

All the data obtained from the experimental period were gathered in excel sheet and transferred to Graphpad prism 8 software for statistical analysis. Repeated measure ANOVA with Tukey's multiple comparison test. P value less than 5% ($p < 0.05$) considered as level of significance (Uddin *et al.*, 2022).

RESULTS AND DISCUSSION

Effect of probiotic on milk production in crossbred Jersey cows

Table 1 shows the impact of probiotic supplementation (*Saccharomyces cerevisiae* yeast) on milk yield in crossbred Jersey cows. During early lactation, the probiotic-supplemented group produced considerably more milk (10.21 ± 0.04 L, 10.63 ± 0.11 L and 10.96 ± 0.15 L) than the control group (9.76 ± 0.07 L). During mid-lactation, probiotic-supplemented groups T1 (10.56 ± 0.031 L), T2 (10.98 ± 0.05 L), and T3 (11.28 ± 0.12 L) produced significantly more milk than the control group T0 (9.78 ± 0.05) (Table 1). Late lactation milk production was considerably higher ($p < 0.05$) in probiotic-supplemented groups T1 (9.46 ± 0.02 L), T2 (9.92 ± 0.07 L) and T3 (10.25 ± 0.10 L) compared to the control (T0) group (8.16 ± 0.03 L) (Table 1). The 30 gm yeast supplemented group (T3) produced more milk than the 25 gm (T2) or 20 gm (T1) yeast supplemented groups. All groups produced more milk during mid lactation (46-120 days) than during early or late lactation. T0 cows produced significantly less milk (8.16 ± 0.03) during late lactation compared to mid (9.78 ± 0.05) and early (9.76 ± 0.07). However, probiotic supplementation significantly enhance T1 cow's milk production in mid-lactation compared to early and late lactation. T2 cows produced more milk in mid lactation (10.98 ± 0.04) compared to early lactation (10.63 ± 0.11), but decreased from mid to late lactation. T3 cows, on the other hand, produced more milk in the middle of lactation than in the beginning and end. So, the probiotic (yeast) supplemented group produced more milk than the control group (Table 1). Furthermore, milk yield was increased in the 30 gm yeast-supplemented group compared to the 25 g and 20 g yeast-supplemented groups. Bayram *et al.* (2014) and Khan *et al.* (2022) demonstrated that yeast culture supplementation significantly increased the average

daily milk yield. Current findings are also consistent with the result of Shreedhar *et al.* (2016) who studied on HF×Deoni crossbred cows and found that milk yield is significantly increased probiotics groups than control group). Alshaikh *et al.* (2002) showed that cows consuming diets supplemented with yeast culture tended to decrease their dry matter intake and to increase their milk yield and milk fat. Hossain *et al.* (2014) found that there was significant ($P < 0.05$) improvement in milk yield after supplementing probiotics (*Saccharomyces cerevisiae*) than control group. Satendra and Brajendu (2017) observed that there was an effect of feeding different doses of probiotics on the milk yield and its composition of crossbred cows. The use of multi stain probiotics is cost effective and increased the milk production by 29.13% in case of 20 gm probiotics supplementation followed by 15 gm (17.70%) and 10 gm

Supplementary Table 2: Proximate analysis of formulated concentrates ration on DM basis.

Items	Amount%
Moisture	12
Crude protein	16
Crude fiber	13
Crude fat	4
Calcium	1.5
Phosphorus	0.65
TDN	65

Supplementary Table 3: Composition of the probiotics used in the experiment.

Ingredients	Amount
<i>Saccharomyces cerevisiae</i>	2×10^{12} cfu/g
Amino acids	21%
Vitamin B ₁₂	8%
Minerals and Other Vitamins	5.5%
Total Amino Acid, Vitamins and Minerals	34.5%
Analyzed composition	
Moisture	12%
Crude protein	18%
Crude fiber	12%
Crude fat	1%

The main ingredients of this product is *Saccharomyces cerevisiae* which contain 2×10^{12} cfu/g.

Table 1: Milk production (mean $\pm$ SD) of crossbred Jersey cows supplemented with probiotics.

Stage of lactation	Milk production (Liter/Day)			
	T0	T1	T2	T3
Early lactation (0-45 day)	$9.76^c \pm 0.07$	$10.21^a \pm 0.04$	$10.63^a \pm 0.11$	$10.96^a \pm 0.15$
Mid lactation (46-120 day)	$9.78^c \pm 0.05$	$10.56^b \pm 0.03$	$10.98^a \pm 0.04$	$11.28^a \pm 0.12$
Late lactation (121-rest day)	$8.16^c \pm 0.03$	$9.46^b \pm 0.02$	$9.92^a \pm 0.07$	$10.25^a \pm 0.10$

Means with different superscripts in the same row differ significantly ($p < 0.05$) by Tukey's test. T0 (Control group-No probiotic feeding), T1 (Treatment group-feeding probiotic 20 gm/cow/day); T2 (Treatment group-feeding probiotic 25 gm/cow/day) and T3 (Treatment group-feeding probiotic 30 gm/cow/day).

supplementation (8.31%), respectively, in lactating crossbred cows. There was no effect of yeast treatment on milk yield and milk fat in lactating Holstein cows observed by (Ambriz *et al.*, 2017; Mallah, 2018; Ferreira, 2019).

Effect of probiotic on milk quality in crossbred Jersey cows

The effects of dietary supplementation of probiotic (*Saccharomyces cerevisiae*) on milk quality in crossbred Jersey cows is presented in Table 2.

Milk fat

In early lactation, the milk fat percentage of T1 (4.22 ± 0.04), T2 (4.41 ± 0.04) and T3 (4.61 ± 0.04) was significantly ($P < 0.05$) higher than that of the T0 (4.06 ± 0.05) group. Out of the three treatment groups, the 30 g yeast-supplemented group (T3) had a higher fat percentage (Table 2). The milk fat percentage of the probiotic-added groups (T1, T2 and T3) was also significantly higher than that of the control group (T0) in the middle of lactation. They were 4.18 ± 0.04 , 4.43 ± 0.03 , 4.67 ± 0.04 and 4.02 ± 0.03 , respectively. Similarly, the probiotics supplemented group (T1, T2 and T3) experienced increased milk fat in late lactation. Due to the continuous feeding of probiotics, there was a significant increase in milk fat percentage in the late lactation period compared to the early and mid-lactation periods. The result revealed a significant increase in milk fat percentage ($P < 0.05$) in probiotic-supplemented groups. In dairy cows, supplementation of *Scerevisiae* can increase the production of acetate, propionate and total volatile fatty acids. The increase in milk fat yield could be attributed to the yeast's ability to stimulate acetate production in the rumen which is a precursor of milk fat (Malekxahi *et al.*, 2016).

The findings of Khan *et al.* (2022) stated that milk fat percentage was significantly ($P < 0.05$) increased due to probiotic supplementation in the diet, supporting the present results. Shreedhar *et al.* (2016) and Mousa *et al.* (2012) also found that milk fat content was significantly

($P < 0.05$) increased in the probiotic-supplemented group. The fat content of milk went up because of its good effects on the growth of cellulolytic bacteria and the fermentation process's natural preference for making acetic acid. Giving cows a direct-fed microbial product with two strains of *Enterococcus faecium* and *Saccharomyces cerevisiae* increased the fat percentage in their milk because they made more VFA (Dutta *et al.*, 2009). On the other hand, Hossain *et al.* (2014) did not find a significant improvement in milk fat percentage after supplementing with probiotics.

Milk protein

The probiotic supplemented group exhibited an increase in milk protein during early, mid and late lactation in comparison to the control group (Table 2). The more prominent results was observed in 30 gm of yeast (T3) group. Probiotics have profound effect on nutrition of host and can influence on various digestive processes, especially cellulolysis and synthesis of microbial protein and increase in the absorption of nutrients (Wang and Ji, 2019). 30 g of yeast may optimum concentration to boosting the protein synthesis and increase the protein contents. Due to supplementation of maximum doses of probiotic (30 gm/cow/day) milk protein content was increased in compare to 20 gm/cow/day and 25 gm/cow/day.

Hossain *et al.* (2014) and Shreedhar *et al.* (2016) demonstrating that the addition of probiotic (yeast) supplement significantly increased the protein content of the milk. According to Desnoyers *et al.* (2009), it was shown that yeast did not have an impact on the protein level of milk.

Milk lactose

During the early lactation, there was a minor drop in milk lactose in the T0 to T1 and T2 groups, but this decrease was not statistically significant. However, there was a substantial reduction ($p < 0.05$) in milk lactose from the control group to the T3 group (Table 2). The lactose content in the treatment group exhibited a decrease in T3

Table 2: Milk composition (mean $\pm$ SD) of crossbred Jersey cows at a glance.

Parameters	Lactation	T0	T1	T2	T3
Fat (%)	Early	$4.06^c \pm 0.05$	$4.22^b \pm 0.04$	$4.41^a \pm 0.04$	$4.61^a \pm 0.04$
	Mid	$4.02^c \pm 0.03$	$4.18^c \pm 0.04$	$4.43^a \pm 0.03$	$4.67^a \pm 0.04$
	Late	$4.11^c \pm 0.01$	$4.38^b \pm 0.02$	$4.59^a \pm 0.02$	$4.79^a \pm 0.02$
Protein (%)	Early	$3.15^b \pm 0.02$	$3.27^b \pm 0.04$	$3.25^b \pm 0.06$	$3.48^a \pm 0.04$
	Mid	$3.13^c \pm 0.02$	$3.33^b \pm 0.02$	$3.38^b \pm 0.01$	$3.55^a \pm 0.02$
	Late	$3.16^c \pm 0.02$	$3.35^b \pm 0.02$	$3.53^a \pm 0.02$	$3.65^a \pm 0.03$
Lactose (%)	Early	$5.10^a \pm 0.02$	$5.13^a \pm 0.01$	$5.08^a \pm 0.02$	$4.99^b \pm 0.02$
	Mid	$5.05^a \pm 0.02$	$5.05^a \pm 0.01$	$4.97^a \pm 0.01$	$4.89^b \pm 0.04$
	Late	$4.95^a \pm 0.01$	$4.91^a \pm 0.03$	$4.88^a \pm 0.03$	$4.96^a \pm 0.01$
SNF (%)	Early	$8.28^c \pm 0.02$	$8.25^c \pm 0.01$	$8.68^b \pm 0.03$	$8.91^a \pm 0.02$
	Mid	$8.31^c \pm 0.03$	$8.38^c \pm 0.01$	$8.75^b \pm 0.01$	$9.01^a \pm 0.04$
	Late	$8.26^c \pm 0.01$	$8.31^c \pm 0.03$	$8.75^b \pm 0.01$	$9.07^a \pm 0.04$
Ash (%)	Early	$0.61^a \pm 0.004$	$0.61^a \pm 0.013$	$0.62^a \pm 0.008$	$0.64^a \pm 0.01$
	Mid	$0.61^b \pm 0.002$	$0.60^b \pm 0.008$	$0.61^b \pm 0.002$	$0.63^a \pm 0.01$
	Late	$0.60^b \pm 0.005$	$0.61^a \pm 0.003$	$0.62^a \pm 0.009$	$0.63^a \pm 0.008$

Means with different superscripts in the same row differ significantly ($p < 0.05$).

(4.98±0.02) compared to T2 (5.08±0.02) and T1 (5.13±0.02) throughout the three distinct doses.

During the mid-lactation period, the group that received a 30 g probiotic supplement exhibited a substantial drop in lactose levels compared to the groups that received 25 g and 20 g of yeast supplementation. The milk lactose percentage of the probiotic-supplemented group was lower than that of the control group during the late lactation phases, which was not statistically significant. The 30 g probiotic-supplemented group (T3) showed a significantly lower milk lactose level compared to the 25 g (T2) and 20 g (yeast-supplemented group) (T1). The findings indicated that there was a decrease in the average lactose content of milk during late lactation compared to mid-lactation and early lactation. The findings of Khan *et al.* (2022) support our observations, as they observed a drop in milk lactose levels in response to different meals, including yeast culture. Shreedhar *et al.* (2016) conducted a study that showed a consecutive drop in the lactose percentage in T0, T1, T2 and T3 after a 60-day trial of probiotic feeding. The lactose content of the milk increased by 0.03% in T0, while it decreased in the treatment groups T1, T2 and T3. The inverse correlation between lactose and the protein and fat content of milk could explain the decrease in the treatment groups.

Milk SNF

During the early lactation period, the SNF% of the 30 gm yeast-supplemented group (T3) was higher compared to the 25 gm (T2) and 20 gm (T1) yeast-supplemented groups, which had a significant effect on the probiotic yeast. In the mid-lactation period, the SNF% of the yeast-supplemented groups T1, T2 and T3 was significantly increased compared to the control group/T0. In the late lactation period, the SNF% of T1, T2 and T3 was significantly increased compared to T0 (Table 2). The result revealed that, due to continuous feeding of probiotics, there was a significant increase in milk SNF% in the late lactation period compared to the early lactation period. Our results are similar to those of Khan *et al.* (2022), who revealed that due to probiotic supplementation in diet, the solids, not fats, percentage in milk is significantly increased in the treatment groups compared to the control group. The increase in SNF could be due to an increase in the protein content of the milk in the treatment groups.

Milk ash

The addition of probiotics to the diet resulted in slight alterations in the ash content of milk in comparison to the control group (Table 2). This increase was particularly evident during the later stages of lactation. In their study, Shreedhar *et al.* (2016) observed a marginal elevation in the ash content of milk following the addition of probiotics. However, the study conducted by Maamouri *et al.* (2014) did not find any impact of probiotics on the ash percentage of milk during late lactation.

CONCLUSION

It is evident that the probiotic-supplemented cows (T1, T2 and T3) exhibited higher milk production along with higher average fat, protein, SNF and ash content in milk across all lactation periods, as compared to the control group (T0). Nevertheless, the lactose content in milk was a subject of controversy due to its observed decrease in the supplemented groups (T1, T2 and T3). The group supplemented with 30gm/cow/day yeast (T3) exhibited a decrease in lactose levels compared to the groups treated with 25gm/cow/day (T2) and 20gm/cow/day (T1) yeast, respectively. Therefore, probiotics have the potential to enhance milk production and quality of dairy cows.

Author contributions

M.A.A.M. carried out the experiment, analyzed the data and wrote the first draft of the manuscript, M.A.M. designed the experiment, analyzed the data and wrote the final draft of the manuscript; M.S.I. and M.M.R.H. revised the manuscript critically.

Conflict of interest

All authors declare that they have no conflicts of interest.

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