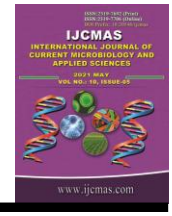
Int.J.Curr.Microbiol.App.Sci (2021) *10*(05): 400-408

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 10 Number 05 (2021)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2021.1005.047

Effect of Feeding Multi-Strain Probiotic on Milk Quality and Milk Quantity in Murrah Buffaloes

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ABSTRACT

Keywords

Murrah buffaloes, milk production performance, multi-strain probiotic, milk yield milk constituents, Cost economics

Article Info

Accepted:
12 April 2021
Available Online:
10 May 2021

Twelve Murrah buffaloes in their early lactation were divided into two groups of six buffaloes each on the basis of average daily milk yield in a completely randomized design. Group I was set as control and Group-II was supplemented with multi-strain probiotic @ 20gm/animal/day for a period of 90 days. The milk yield, 6% fat corrected milk yield, milk constituents yield, and the cost per kg milk produced were recorded during the experiment. The average daily milk yield and 6% fat corrected milk yield (kg) milk fat, SNF and total solids % were significantly ($P<0.05$) high in treatment group compared to control group of buffaloes. The cost per kg milk production was Rs 14.83 and Rs16.51 for treatment and control groups, respectively which saved Rs 1.68/liter milk production per day due to probiotic supplementation in buffaloes. The present research concluded that multi-strain probiotic supplementation @20gms/animal/day increased milk yield, milk constituent yield and decreased cost of milk production in Murrah buffaloes.

Introduction

Livestock sector plays an important role in Indian economy and is an essential part of Indian agriculture. Buffaloes contribute significantly towards milk production, meat production, draught power and manure production. Murrah buffaloes are found throughout the country due to their higher milk production potential coupled with adaptation to wide environmental conditions and feed conversion efficiency. Composition of milk is economically important to milk producers, important to dairy industries for producing better quality products and nutritionally important to milk consumers for their health. Nowadays along with fat percentage, other milk components such as solid not fat (SNF), total solids (TS), protein, lactose and ash are also measured (Malek dos Reis *et al.,* 2013). Milk fat represents chief constituent of buffalo milk followed by lactose and protein. Probiotics are live micro-organisms which when administered in adequate amounts confer a health benefit on the host (WHO). In dairy animals, probiotics are commonly used to improve ruminal and intestinal microflora populations, consequentially enhance the performance and health of animal, and boosts synthesis of protein and vitamins, as well as milk production and compositions. Most of commercial products use multi-strain probiotics, although the benefits of incorporating more strain and/or species in one product has not been clearly determined. Several researchers reported that milk yield, milk fat yield, milk protein yield, casein yield, lactose percentage, total solid and solid-not-fat were significantly improved with probiotic feeding (Iwanska *et al.,* 2000; Meeske *et al.,* 2002; Hossain, *et al.,* 2014).

Most of the literatures enlists that, modification of rumen microbial populations, enhancing feed digestibility and nutrient absorption, rumen pH regulation, colonization of gastro-intestinal tracts and competitive exclusion of pathogenic agents, production of antimicrobial substances and altering gene expression of pathogenic microorganisms were among common modes of action of probiotics in improving the production performance of dairy animals. Since there is paucity of information on the effect of feeding multi-strain probiotics on milk production performance in Murrah buffaloes, the present research was proposed with the following objectives to find effect of multi-strain probiotic supplementation on milk production, milk constituents and cost on milk production in Murrah buffaloes.

Materials and Methods

Twelve Murrah buffaloes in their early lactation were selected from Buffalo Research Station, Venkataramannagudem and divided into two groups of six buffaloes each on the basis on average daily milk yield in a completely randomized design (CRD). The average daily milk yield in each of the groups was similar before the start of the experiment. All the buffaloes were housed individually in a well-ventilated shed with a provision for individual feeding and watering facilities. The shed was provided with concrete flooring and asbestos roof with good ventilation facilities. The shed was cleaned and washed daily with water and disinfectant solution. All the buffaloes were fed with chopped green fodder (Hybrid Napier), dry fodder (paddy straw) and concentrate as the basal diet individually as per their nutrient requirements (ICAR 2013). The buffaloes were divided into two groups i.e. Group I is control and Group-II was supplemented with multi-strain probiotic containing 6 bacterial strains (*Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus returi, Lactobacillus lactis, Streptococcus faecium*) and 2 yeast strains (*Aspergillus oryzae, Saccharomyces cervisiae*) (2×10^9cfu/gm) @ 20gm/animal/day for a period of 90 days.

All the experimental buffaloes were identified using ear tags. All the animals were vaccinated against HS and FMD and were dewormed one week prior to the start of the experiment. All the animals were allowed to have adaptation period for 15 days before the actual trail, during which they were fed with basal diet. After the adaptation period animals were fed with their respective treatment diets for 90 days. The diet of the experimental animals included chopped Hybrid Napier as roughage source and concentrate mixture. The feeding and watering of experimental animals was done individually and the amount of concentrate mixture and fodder was offered as per their nutrient requirement (ICAR 2013). The multi-strain probiotic Lactose Plus® was procured from International Health Care Limited, Vijayawada, Andhra Pradesh. The probiotic was mixed with concentrate mixture and offered as per the dose rate. Concentrate mixture was offered daily before milking the animals and green fodder at 10:00 h and 15:00 h. Clean drinking water was made available to animals round the clock. Daily feed offerings and refusals were recorded prior to the morning feeding to obtain feed intake of each animal. The leftover of the concentrate mixture, if any was weighed one hour after it was offered. Milking of buffaloes was done by hand milking by milkers twice daily at 4.00 AM and 4.00 PM at milking parlour. The individual milk yields were recorded in Kg at each milking by digital weighing machine. Milking was done twice daily (AM and PM) and milk yield records were maintained throughout the experimental period (90 days after start of experimental period). Daily milk yield was obtained by adding AM and PM yields. Milk samples were collected to analyze fat, SNF and total solids by automatic milk analyzer at Buffalo Research Station, Venkataramannagudem.

About 70 ml of milk sample from individual animals after each milking was collected weekly in a sterile milk sample collection bottle. The sample so collected was then kept at 4°C till analysis was done. Milk was kept for two hours after milking and then stirred well for minimum 5 minutes by vertical and circular slow movements. Milk constituents i.e. fat, SNF and total solids, were estimated by automatic milk analyzer at Buffalo Research Station, Venkataramannagudem. The milk sample was pre warmed at 39-40°C before analysis. Then the milk sample was poured slowly from one vessel to another vessel by slightly tilting the vessel and using the side walls of the vessel to avoid formation of foam for the equal distribution of fat within the sample. The total solids content of the milk was arrived by the addition of fat and SNF percentages.

Total Solids % = Fat % + SNF %

From the milk yield and butter fat yield of animals, the 6 per cent fat corrected milk yield (FCM) was calculated (Rice *et al.*, 1970).

6% FCM = 0.308 x Total Milk Yield (kg) + 11.54 x Total Fat Yield (kg)

Cost of feed was calculated based on the cost of feed ingredients in the local market plus the additional cost of probiotic in the experimental group. Milk yield and cost of feed were calculated on a daily basis. Cost per kg milk produced (₹) was calculated based on the market value of milk and total feeding cost for the month. The properly classified and tabulated data collected during the experimental period were subjected to statistical analysis by adopting appropriate methods of analysis of variance as described by Snedecor and Cochran (1994). The data obtained was analyzed according to statistic computer program SPSS version (15.01) (SPSS, 2006).

Results and Discussion

Effect on daily milk yield (kg) and 6% fat corrected milk yield

Effect of probiotic supplementation on average daily milk yield (kg) and 6% fat corrected milk yield in Murrah buffaloes is represented in the Table 1. From 2nd week onwards, the average daily milk yield started to increase in probiotic supplemented buffaloes compared to the control buffaloes and the difference was statistically significant ($P<0.05$) from 8th week onwards till the end of the experiment. From 2nd week onwards, the

6% fat corrected milk yield started to increase in probiotic supplemented buffaloes compared to the control buffaloes and the difference was statistically significant (P<0.05) from 9th week onwards till the end of the experiment. The results obtained in the present study is consistent with the findings of El-Bordeny *et al.,* (2019) who reported increased milk production in Egyptian buffaloes supplemented with yeast-based probiotics. Similar results of increased milk yield and 4% fat corrected milk yield (P<0.05) was reported in probiotic supplemented buffaloes compared to the control (Azzaz *et al.,* 2015). A significant (P>0.05) increase in 6% fat corrected milk (FCM) yield was also observed by Kumar *et al.,* (2011b) in probiotic supplemented buffaloes over to the control group. Increase in 4% fat corrected milk in probiotic supplemented cows reported by several earlier studies (Sretenovic *et al.,* 2008; Alshaikh *et al.,* 2002; Moallem *et al.,* 2009) corroborated with present research. In a similar study, milk yield was increased from third week onwards in cows supplemented with multi-strain probiotic (Vibhute *et al.,* 2011) is consistent with present findings. Parallel to the present findings, Shreedhar *et al.,* (2016) reported that multi-strain probiotic supplementation increased milk production in crossbred cows. A similar result of increased milk production was also reported by Moallem *et al.,* (2009) and Phondba *et al.,* (2009) in cows supplemented with yeast. Responses to probiotc supplementation in lactating animals are depending on several factors, such as stage of lactation, age, DMI, feed composition, and probiotic dose (Desnoyers *et al.,* 2009; Ayad *et al.,* 2013 and Rossow *et al.,* 2018). In the present study, multi-strain probiotic supplementation increased 15.53% milk yield in Murrah buffaloes compared to the control. Similar results of 11.5 and 12,7% higher daily milk yield was reported with probiotic feeding in cows (Diler *at al.,*2014). Parallel to the present findings Yasuda *et al.,* (2007) reported 3-16% increase of milk production in Holstein Friesian cows with probiotics supplementation. Increase in milk yield of Holstein Friesian cows by 1.1 kg/d (8%) higher compared to control group reported by Maamouri *et al.,* 2014 corroborated with present findings. The increased milk production with multi-strain probiotic supplementation in buffaloes in the present study might be due to the altered rumen fermentation patterns in favour of propionate, (Aikman *et al.,* 2011) and stabilization of ruminal pH which improved propionic acid production resulted increased milk production (Beauchemin *et al.,* 2003). Further, the yeast in the multi-strain probiotic supplementation may increase the cellulolytic bacteria and fibre digestion in the feed (Denev *et al.,* 2007).

Yeast supplements containing *Saccharomyces cerevisiae*, are known to be rich sources of enzymes, vitamins, other nutrients and important co-factors, have been reported to produce a variety of beneficial production responses (Rai *et al.,* 2013). Probiotics may also improve the immune mechanism against the gastrointestinal pathogens and hence more productivity (Walker, 2008). However, Raeth-knight *et al.,* (2007) observed no significant effect of direct fed microbials in the milk yield of Holstein dairy cows.

Milk fat

Effect of multi-strain probiotic supplementation on mean milk fat (%) in Murrah buffaloes is represented in the Table 2. From 2nd week onwards, the average milk fat % starts to increased in probiotic supplemented buffaloes compared to the control buffaloes and the difference was statistically significant (P<0.05) from 9th week onwards. Similar results of increased fat % (P<0.05) was reported in probiotic supplemented buffaloes compared to the control (Azzaz *et al.,* 2015).

Table.1 Effect of probiotic supplementation on daily milk yield and 6% fat corrected milk in Murrah buffaloes

	Week	C	T
Daily milk yield	1	7.58±0.5	7.61±0.31
	12*	9.14±0.1[a]	11.78±0.36[b]
	Overall mean	8.24±0.34[a]	9.52±0.14[b]
6% fat corrected milk	1	8.51±0.16	8.52±0.39
	12*	8.94±0.14[a]	10.47±0.23[b]
	Overall mean	8.74±0.23[a]	9.56±0.41[b]

Means with different superscripts in a row differ significantly (*P<0.05).

Table.2 Effect of probiotic supplementation on milk fat% in Murrah buffaloes

	Week	C	T
Milk fat%	1	8.51±0.16	8.52±0.39
	12*	8.94±0.14[a]	10.47±0.23[b]
	Overall mean	8.74±0.23[a]	9.56±0.41[b]
Milk SNF	1	10.26±0.31	10.28±0.33
	12*	10.53±0.19[a]	10.91±0.24[b]
	Overall mean	10.34±0.21[a]	10.56±0.33[b]
Milk total solids	1	18.77±0.81	18.84±0.19
	12*	19.47±0.13[a]	21.41±0.22[b]
	Overall mean	19.08±0.11[a]	20.12±0.21[b]

Means with different superscripts in a row differ significantly (*P<0.05)

Table.3 Effect of probiotic supplementation on cost per kg milk produced (₹) in Murrah buffaloes

Cost of feeding (₹)		
Parameters	**C**	**T**
Total feed cost (Rs/animal/day)	136.10 ±2.32	137.2±3.41
Cost of probiotics (Rs/animal/day)	0	4
Total expenses(Rs/animal/day)	136.10± 2.11	141.2±4.1
Average daily milk yield (kg/animal/day)	8.24±0.34	9.52±0.51
Cost of milk production (Rs/kg)	16.51±1.21	14.83±2.38

Parallel to the present findings, multi-strain probiotic supplementation increased fat content in the cows (Shreedhar *et al.*, 2016; Alshaikh *et al.*, 2002 and Vibhute *et al.*, 2011)). Several earlier studies (El-Din, 2015; Zhang *et al.*, 2013; Ayad *et al.*, 2013; Dehghan *et al.*, 2012; Yalcin *et al.*, 2011) also reported that probiotic and yeast supplementation improved fat yield in cows. Increased milk fat yield in buffaloes might be due to increased number of cellulolytic bacteria and fibre digestion resulted increased acetate production and butter fat in buffaloes supplemented with probiotic (Denev *et al.*, 2007). Contrary to our findings Morsy *et al.*, (2014) and Raeth-knight *et al.*, (2007) observed no significant effect of probiotics on milk fat percentage in cows (Denev *et al.*, 2007).

Milk SNF

Effect of multi-strain probiotic supplementation on mean milk SNF (%) in Murrah buffaloes. is represented in the Table 2. From 2nd week onwards, the average SNF % started to increase in probiotic supplemented buffaloes compared to the control buffaloes and the difference was statistically significant (P<0.05) from 8th week onwards. Our results were consistent with the findings of Kumar *et al.*, (2011b) who observed a significant increase (P<0.05) in SNF percent in Graded Murrah buffaloes supplemented with yeast culture. Azzaz *et al.*, (2015) also reported significantly higher (P<0.05) SNF content in yeast culture supplemented buffaloes. The increased SNF content in the milk with probiotic supplementation reported by El-Din, (2015) and Vibhute *et al.*, (2011) in cows corroborated with present findings. Improved SNF content in the probiotic supplemented buffaloes might be due to increased digestibility of crude protein, neutral detergent fiber and acid detergent fiber (Boyd *et al.*, 2011).

The probiotics thought to increase the beneficial microorganisms in the gut, thereby increased nutrient digestibility in the buffaloes (Nocek *et al.*, 2006). Contrary to the present findings Morsy *et al.*, (2014) and Raeth-knight *et al.*, (2007) observed no significant effect of probiotics on milk SNF percentage in cows (Denev *et al.*, 2007).

Milk total solids

Effect of probiotic supplementation on mean milk total solids (%) in Murrah buffaloes is represented in the Table 2. From 2nd week onwards, the average total solids (TS) % started to increase in probiotic supplemented buffaloes compared to the control buffaloes and the difference was statistically significant (P<0.05) from 8th week onwards. Similar to our findings Azzaz *et al.*, (2015) observed significantly high (P<0.05) milk total solids % in lactating buffaloes when supplemented with probiotics. Kumar *et al.*, (2011b) also observed a significant increase (P<0.05) in milk total solids percent in Graded Murrah buffaloes supplemented with yeast culture. Parallel to the present findings, Vibhute *et al.*, (2011) and El-Din, (2015) observed increased milk total solids % in cows supplemented with probiotics and yeast. Multi-strain probiotic 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. Contrary to our findings, Raeth-knight *et al.*, (2007), Maamouri *et al.*, (2014) and Morsy *et al.*, (2014) observed no significant increase in the milk total solids (%) with probiotic supplementation

Cost per kg milk produced (₹)

Effect of multi-strain probiotic supplementation on cost per kg milk produced (₹) Murrah buffaloes is represented in the Table 3. The cost of feeding per day was Rs 137.2 and Rs 136.10 for the treatment and control groups, respectively. The higher cost of feeding for the treatment group is due to addition of cost of probiotic. However, the cost per kg milk production was Rs 14.83 and Rs 16.51 for treatment and control groups, respectively. The decreased cost per kg milk production for the treatment group was due to

9.52 kg/day higher milk production with probiotic supplementation compared to the control which saved Rs 1.68 per day due to probiotic supplementation. The results of the present study was consistent with the findings of Shreedhar *et al.*, (2016) who reported that probiotic supplementation @ 10, 20 and 30g/day resulted an additional income of Rs 4.01, 33.46 and 32.13/day, respectively in cows due to increased milk production. Similar to the present findings, Vibhute *et al.*, (2011) also reported that supplementing the diet with probiotic increased milk production, resulted increased net income in treatment groups compared to the control.

Multi-strain probiotic may be supplemented to the buffaloes @ 20gms/animal/day for economic milk production. Milk quantity and quality in terms of its constituents may be improved particularly under roughage-based buffalo productions system.

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How to cite this article:

Yaswanth Naidu, Y., K. Ananda Rao, Ch. Venkata Seshaiah, D. Srinivas Kumar and Sri Lekha, M. 2021. Effect of Feeding Multi-Strain Probiotic on Milk Quality and Milk Quantity in Murrah Buffaloes. *Int.J.Curr.Microbiol.App.Sci.* 10(05): 400-408.
doi: https://doi.org/10.20546/ijcmas.2021.1005.047