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Effect Of Supplementing Bacterial Probiotics On Growth, Nutrient Digestibility, Milk Quality And Economics Of Lactating Crossbred Cattle In Nepal

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Abstract:
Background*: Nepal witnessed a significant growth over the last couple decades in meeting food and nutrition security through enhanced supply of milk across its ecological gradients. However, the challenge to mitigate the import of dairy products has attracted scientific consideration to elevate dairy cattle productivity through several disciplinary interventions, nutrition being the prominent of all.*
Materials and Methods*: A study to elucidate the effect of supplementing different probiotic strains on growth, digestibility of nutrients, milk yield, milk quality and overall economics of introducing probiotics to dairy cattle feeding was implemented. Altogether 12 crossbred lactating cows were kept in completely randomized design in metabolic crates for two months. Feed, feces and urine were analysed in the laboratory to test the digestibility while daily milk yield and quality were also monitored. Collected data were analysed using SPSS statistics and economics of supplementation was calculated using partial budgeting techniques.*
Results*: The study suggested that digestibility of dry matter and organic matter was significantly higher in probiotic supplemented groups ($p<0.05$) and, as a result, the net weight gain upon eight weeks of experimental period is also statistically higher ($p=0.03$). The change in milk yield and the quality parameters of milk affecting the price of the milk that determine the price remained unaffected. Likewise, owing to high price of supplement imported from abroad, the group not receiving any supplement performed better economically that those of supplemented groups.*
Conclusion: *Based on the partially promising results of the supplementing probiotics to the diet of lactating crossbred cows, farmers should be supported technically and in policy for such supplementation. The policy should also work on making these supplements available in the market for the commercial and semicommercial farmers at a cheaper price.*
Key Word*: Bacteria; benefit:cost; digestibility; milk quality; live weight gain*

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I. Introduction

The dairy industry in Nepal has long been playing crucial role in meeting the country's growing demand for milk and dairy products. However, this sector is also marked with suboptimal growth, poor nutrient utilization, low herd average and compromised milk quality. Past efforts generally were concentrated on improving the genetic makeup of indigenous and crossbred cattle and thereafter on improving the supply of green fodder to lactating dairy animals. Instilling in the achievements, current research strategies now revolve around improving dairy cattle performance by exploring the use of bacterial probiotics as a potential nutritional intervention.

Probiotics have shown enticing results in enhancing animal health, productivity[1,2,3,4] and farm economics[5,6]. Among these, bacterial probiotics including *Lactobacillus acidophilus, Bacillus subtilis,* and *Enterococcus faecium*, have been of particular interest due to their proven ability to positively influence rumen fermentation, nutrient utilization[1,4,7], and milk quality[8,9].

This study was designed to investigate the effects of bacterial probiotic supplementation on growth, nutrient digestibility, milk quality of lactating crossbred cattle and, at the same time, the economics of milk production. Given bacterial probiotics are found to positively impact growth, nutrient digestibility, milk quality, and the economics of lactating crossbred cattle, it could be extrapolated to improve the overall health and nutrition of cows across similar agroecology.

II. Material And Methods

Study location

The research was conducted at the experimental station of the National Cattle Research Program (NCRP) located in Rampur, Chitwan (27°39'N and 84°21'E), which is situated approximately 10 km west of the district headquarter, Bharatpur. The experiment was conducted during February-March of 2020 which is relatively cool season in the area. However, Chitwan is known for its tropical alike climate and temperatures after March start to rise kicking off hot summer in the area. Chitwan is also considered one of the significant dairy regions in Nepal. This is primarily due to the region's high demand for fluid milk, the convenient availability of feeds, straw, and veterinary services, which facilitate the growth of commercial dairy farming in the area.

Experimental design and housing

The experiment housed altogether 12 crossbred Jersey cattle at their parity between two and five and were within three months of their current lactation. The animals were randomized to fit into Completely Randomized Design (CRD) with four treatments and three replications. The list below is the description of treatments used for the experiment.

Treatment 1 (CON): Animals receiving flour with no probiotics

Treatment 2 (LAP): Animals receiving 2.5 g Lactobacillus acidophilius per kg DM of feed

Treatment 3 (BST): Animals receiving 2.5 g/kg Bacillus subtilis per kg of DM of feed

Treatment 4 (EFC): Animals receiving 2.5 g/kg Enterococcus faecium per kg of DM of feed

The animals were randomized and housed in individual metabolic crates in NCRP, Chitwan. Animals were kept in the crates for adaptation for a period of a week where they were provided with control diet as stated in Treatment 1 (CON). After a week, animals were given treatment diets according to their allocation to treatments for two weeks. Upon adaptation to two weeks, total collection of feces and urine started. Collection lasted for a week.

Table 1: Composition and nutrient levels in treatment diets

Items	Proportion (%)
Composition	
Oat	16.2
Joint Vetch	9.1
Maize grain	33
Soybean meal	11.7
Rice bran	13.7
Rapeseed meal	7.2
Dal Chunnies	8.6
Vitamin Premix	0.5
Nutrient content	
Crude Protein	16.7
Crude Fat	2.7
NDF	34.5
ADF	9.3
Salt (NaCl)	1.7
Calcium	0.7
Phosphorus	0.4

The probiotics concentrations were evaluated in the microbiology laboratory and were found to be 5.4 x 109, 6.1x109 and 5.7x109 for L. acidophilus, B. subtilis and E. facecium respectively. The composition of the feed used for the experiment and their nutrient contents are presented in table 1.

Feeding, total collection and sampling

Feeding management: The animals were weighed at the initial day of the experiment before they were individually penned in metabolic crates. They were offered 2.5% dry matter (DM) on the body weight basis for maintenance and were provided with additional one kg DM for every three liters of milk produced in a day. Concentrate and roughage required for each animal were weighed individually in a bag on a daily basis. Half of the feed was offered in the morning at 09:00 AM while the remaining half was offered at 03:00 PM.

Samples of feed were collected at the time of preparation and sent to Animal Nutrition Laboratory in Khumaltar for nutrient analysis. Samples of refusal were collected, weighed and packed in a labelled Ziplock sac. Refusals were also dried and sent to Animal Nutrition Laboratory for analysis of nutrients.

Total collection: The feces and urine from individual pen were collected in the morning at 08:00 AM. Feces after weighing were mixed well with spatula and 10% on w/w basis were separated as samples every day for seven days. Likewise, urine was collected from individual animal in a plastic container and was weighed at 08:00 AM and 5% sample was collected after mixing each sample well.

Milk samples: Milk yield of individual animals were recorded for each milking while 50 mL sample of each cow was made on alternate days for morning and evening milking making one sample per cow per day.

Laboratory analysis

Feed and Urine: Feed and fecal samples collected were immediately dried in a hot air oven at 72°C until constant weight. The dried samples were then ground in a hammer mill using a mesh size of 1mm. Organic matter (OM) content of both feed and feces were obtained by combusting the samples in muffle furnace at 550°C for 3 hours. The ground samples were then subjected to analysis of Nitrogen using Kjeldahl method. Nitrogen content were multiplied by 6.25 to approximate the protein content in both feed and faeces. Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were analysed using the method explained by Goering and Van Soest10. Urine samples were dried and the residue were then subjected to Kjeldahal method for analysis of nitrogen.

Milk quality: The quality parameters of milk were analyzed using ultrasonic techniques with Lactoscan (Milkotronic Ltd, Nova Zagora, Bulgeria). The parameters tested were fat, SNF, protein, lactose, pH, conductivity and freezing point. Thirty samples of milk were tested for milk fat using Gerber centrifuge and pH using edge dedicated pH meter (Hanna Instruments, USA) and the corresponding values of fat and pH were regressed to obtain an equation to calibrate the ultrasonic automatic milk analyzer.

Statistical analysis

Data collected from the experiment were entered into Microsoft Excel™ and later imported to SPSS Statistics™ version 25 for Analysis of Variance (ANOVA). The model fitted was:

$Y_{ij} = \mu + \tau_i + \epsilon_{ij}$

Where,

- Y_{ij} is the j-th response for the i-th treatment
- τ_i i-th effect of supplementing probiotic strains
- μ constant component
- ϵ_{ij} independent random errors

Economic Analysis

The cost of each item involved in the feeding and overall management of cows were recorded while the pricing of milk has been done according to the government set regulation that considered volume, fat and solid-not-fat content of milk. Partial budgeting techniques was then applied to calculate the net profit, return per liter and overall benefit:cost ratio of supplementation.

III. Result

Dry matter Intake, digestibility of nutrients and daily weight gain

Dry matter intake of cows across all treatment groups were similar statistically regardless of inclusion of any of the bacterial probiotic strains. Nevertheless, as presented in table 2, the digestibility of dry matter(p=0.04), organic matter (p=0.01), and neutral detergent fiber (p=0.03) were significantly affected. In the meanwhile, the digestibility of crude protein and acid detergent fiber; ADF did not respond to the strains of probiotics used in the experiment. However, the resultant change in final body weight of the cows remained unaffected. Interestingly, the change in body weight after eight weeks of experimental period, the net gain in body weight of the experimental animals across treatments showed some impact of inclusion of different bacterial strains (p=0.03).

Table no 2: Effect of supplementing different bacterial probiotic strains on dry matter intake, digestibility of nutrients and daily weight gain of lactating dairy cows

	Treatments					
Parameters	CON	LAP	BST	EFC	SEM	p-Value
DM Intake	11.56	12.95	12.00	11.20	0.24	0.66
Digestibility of major nutrients						
Dry matter	68.3	71.2	64.6	66.5	1.24	0.04
Organic matter	69.4	71.7	62.7	69.6	0.96	0.01
Crude protein	62.3	62.9	60.6	61.4	1.31	0.16
NDF	38.4	42.5	39.9	40.7	1.13	0.03
ADF	62.9	64.2	62.2	61.9	1.37	0.09
Initial Weight	304.00	333.00	363.00	294.33	12.99	0.25
Final Weight	321.00	351.33	359.67	320.00	11.23	0.54
Net weight gain	17.00	18.33	-3.33	25.67	4.06	0.03

CON: Control; LAP: Lactobacillus acidophilus; BST: Bacillus subtilis; EFC: Enterococcus faecium; DM: Dry matter; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber

Milk yield and quality parameters

The difference in daily milk yield of cows before the experiment were tested and were statistically non-significant across treatment groups (Table 3). Similar pattern of statistical significance after completion of the experiment was recorded and found out that none of the bacterial strains had significant impact on improving the milk yield.

Likewise, some quality parameters of milk were also tested to assess the effect of inclusion of supplementation of bacterial probiotics on them (Table 3). All parameters tested were statistically non-significant indicating there was no effect of any particular strain on the quality parameters of milk.

Table no 3: Effect of supplementing different bacterial probiotic strains on daily milk yield (kg) and quality parameters of milk of lactating dairy cows

	Treatments					
Parameters	CON	LAP	BST	EFC	SEM	p-Value
Before Treatment	6.35	6.19	6.19	6.44	0.17	0.95
After Treatment	6.30	6.17	5.98	6.53	0.17	0.77
Increase in Yield	-0.05	0.07	-0.21	0.10	0.13	0.90
Milk Quality						
Fat%	6.71	6.18	6.55	6.34	0.27	0.93
Solid-not-fat%	9.11	9.25	9.19	8.84	0.11	0.60
Protein%	3.32	3.37	3.34	3.21	0.04	0.60
Lactose%	5.00	5.08	5.05	4.85	0.06	0.61
Salt%	0.74	0.75	0.74	0.72	0.01	0.66
Conductivity (milliSiemens, mS)	4.16	4.59	4.48	4.59	0.11	0.50
pH	6.40	6.40	6.24	6.37	0.05	0.59
Freezing Point °C	-0.61	-0.61	-0.58	-0.61	0.01	0.61

CON: Control; LAP: Lactobacillus acidophilus; BST: Bacillus subtilis; EFC: Enterococcus faecium;

Economic analysis of supplementation

The benefit of supplementation of different bacterial probiotic strains over the cost incurred during supplementation, feeding and management of experimental animals (Table 4). The analysis of benefits over the cost indicate that supplementation has substantially increased the cost of feeding and hence the control group receiving none of the supplementation provided better economic return. The B:C ratio of 1.29 of the control group over other groups receiving either L. acidophilus, B. subtilis or E. faecium.

Table no 4: Benefit: Cost analysis of supplementation of different bacterial probiotic strains

Parameters	CON	LAF	BST	EFC
Revenues				
Milk	438.50	411.44	411.63	434.00
Manure	47.64	37.72	36.76	42.00
Total	486.14	449.16	448.39	476.00
Cost				
Feed	224.40	224.40	237.60	228.80
Grass	25.18	25.44	26.56	25.72
Straw	44.00	44.00	46.40	44.80
Labour	75.40	89.40	89.40	89.40
Electricity	8.64	8.64	8.64	8.64
Supplement	0.00	6.00	6.00	6.00
Total	377.62	397.88	414.60	403.36
Net Profit	108.52	51.28	33.79	72.64
Returns/Liter	17.23	8.31	5.65	11.12
B:C Ratio	1.29	1.13	1.08	1.18

CON: Control; LAP: Lactobacillus acidophilus; BST: Bacillus subtilis; EFC: Enterococcus faecium

IV. Discussion

The experiment did come up with statistically similar dry matter (DM) intake across the treatments. Similar DM intake in this experiment across the probiotic supplemented treatments is consistent with the study carried out in growing lambs[11] and very close to Erasmus et. al.[12]. The inclusion of different strains of bacterial probiotics usually is associated with the change in the rumen microbial ecology of the host animals. The added number of microflora from outside the rumen would gradually takeover and either start by themselves or assist the existing microflora and fauna to digest the feed that has been offered to animal. Similar pattern was observed in our experiment too. The inclusion of L. acidophilus has increased the DM as well as OM digestibility in the experimental cows. Similar claims have also been made by other studies too[3,13,14]. Such symbiosis usually is associated with supplemented microbials help assist the rumen to keep the buffer in place[4,15].

Growth of cows responded to such supplementation which also supports the claims made by several similar studies[11,16]. Similar studies reported that this could be explained by the enhanced production of digestion enzymes upon introduction of the new probiotic microflora in the rumen environment[1,4,7]. Roodposhti and Dabiri[2] also found the inclusion of probiotics in combination with prebiotics to have the daily weight gain improved in newborn female calves. However, the final weight of the animals did not exhibit any significant differences. This could be attributable to observation made over relatively shorter period of time for weight gain and focused more on digestibility of nutrients in the experiment. In principle, enhanced digestion efficiency always result in better availability of nutrients to the host animal and hence the improved growth performance.

The yield and quality parameters were statistically indifferent across all treatment groups. These findings differ from studies carried out elsewhere[8,9] where improvements in both quantity and quality of milk were observed. These improvements could be ascribed to improved rumen microbiome thereby improving the supply and absorption of microbial crude protein. However, some other studies suggest increase in the daily milk yield of cows without altering any quality parameters[17,18].

The economic study indicated in general that the supplementation cost was higher than that of the benefit received from these interventions. These results are in consistence with the report of Várhidi et. al.[6] where they surveyed the Hungarian dairy farmers on their perception of use of probiotics on dairy animals and concluded that there were meagre improvements in milk yield and quality thereby less return in comparison to the investment they would have to make for procurement of probiotics to their dairy animals. However, in Indian conditions where inputs could be procured at relatively cheaper rates, supplementing lactating crossbred cows with probiotics could exert better benefits to dairy farmers[5].

V. Conclusion

Introducing bacterial probiotics to the routine dietary intake of lactating crossbred dairy cows have demonstrated partially promising results. The supplementation has particular impact on the digestibility of dry matter, organic matter and fiber fraction of the feed that has been offered to supplemented group. However, the impact on the milk quality and overall milk yield was not evident. The short span of the experimental period merely of eight weeks could probably not suffice to exhibit the impact on these parameters. Hence, the extended use of probiotic supplementation on dairy animals starting from weaning could be a strategy as suggested by several literatures. In the meanwhile, this experiment did not take into consideration the synergy and interaction of combination of other probiotics or the prebiotics in this experiment, which should be tested for their superiority in the near future. The resultant impact on digestibility of nutrients, milk yield and milk quality parameters could yield to a better milk production economics for the promising dairy farmers of Nepal.

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