Archives of Agriculture and Environmental Science 8(3): 352-357 (2023)
https://doi.org/10.26832/24566632.2023.0803012

e-ISSN: 2456-6632

This content is available online at AESA

Archives of Agriculture and Environmental Science

Journal homepage: journals.aesacademy.org/index.php/aaes

ORIGINAL RESEARCH ARTICLE

Effect of supplementing bacterial probiotics on microbial protein synthesis, blood indices and rumen ciliate protozoa population of lactating crossbred cattle in Nepal

Tulasi Prasad Paudel[1,2*], Dainik Bahadur Karki[2], Naba Raj Devkota[2], Megh Raj Tiwari[1] and Ujjal Tiwari[2]

[1]Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur, NEPAL
[2]Agriculture and Forestry University (AFU), Rampur, Chitwan, NEPAL
[*]Corresponding author's E-mail: tulasi.paudel@narc.gov.np

ARTICLE HISTORY

Received: 25 June 2023
Revised received: 29 July 2023
Accepted: 14 August 2023

Keywords

Biochemistry
Hematology
Microbial protein synthesis
Probiotics
Rumen protozoa

ABSTRACT

In recent times, the dairy industry in Nepal has been recognized as a burgeoning agro-industry, successfully meeting the domestic demand for fluid milk. Nevertheless, further efforts are necessary to achieve self-sufficiency in dairy products. To enhance milk production, alongside breed improvement and health management initiatives, nutritional interventions have been identified as crucial. In this context, a study was conducted in Rampur, Chitwan, focusing on the supplementation of diverse strains of bacterial probiotics in lactating crossbred cattle to evaluate their effects on microbial protein synthesis, animal health assessed through blood indices, and changes in rumen fauna. The findings of the study revealed that the inclusion of bacterial probiotics resulted in notable improvements in the overall excretion of purine derivatives. Specifically, the individual excretion of allantoin, xanthine, and hypoxanthine significantly increased ($p<0.01$) in the group supplemented with *Lactobacillus acidophilus*, while uric acid levels remained unchanged. Consequently, microbial nitrogen supply and absorption also showed a significant increase within the same group. However, blood hematological and biochemical parameters remained unaffected across all treatment groups. Moreover, the supplementation did not induce any notable changes in the individual or overall population of rumen ciliate protozoa. The study underscores that the use of *L. acidophilus* can enhance the overall protein economy of the rumen, thus potentially reducing production costs by substituting expensive sources of dietary protein for lactating crossbred dairy cows.

Citation of this article: Paudel, T. P., Karki, D. B., Devkota, N. R., Tiwari, M. R., & Tiwari, U. (2023). Effect of supplementing bacterial probiotics on microbial protein synthesis, blood indices and rumen ciliate protozoa population of lactating crossbred cattle in Nepal. *Archives of Agriculture and Environmental Science*, *8*(3), 352-357, https://dx.doi.org/10.26832/24566632.2023.0803012

INTRODUCTION

Dairy cattle serve a crucial role in ensuring food security by supplying a reliable source of milk and its products. Hence, maximizing the productivity and health of lactating cows becomes paramount to meet the growing demands. In recent times, there has been an increasing interest in exploring the potential benefits of probiotics as a nutritional intervention to enhance the performance and well-being of dairy cattle (Adjei-Fremah *et al*., 2018; Mahesh *et al*., 2021; Wang *et al*., 2023; Nalla *et al*., 2022). In Nepal, the optimization of lactating crossbred cattle's productivity holds great significance in meeting the rising milk and dairy product requirements (CASA, 2020; MoALD, 2022). However, several challenges hinder the full realization of dairy cattle's potential in this region, such as suboptimal rumen fermentation, inefficient nutrient utilization, and health problems. As a result, it becomes essential to investigate the impact of bacterial probiotic supplementation on key aspects of dairy cow nutrition to address these challenges effectively. Probiotics exert their beneficial effects by influencing the microbial population in the rumen. Thus, by supplementing specific probiotic strains, the modulation of the rumen microbial community shows promise in improving nutrient digestion, microbial protein synthesis, and overall rumen function in lactating cows

(Cangiano *et al*., 2020; Anee *et al*., 2021). A study conducted by Bouchard *et al.* (2019) revealed that cows receiving bacterial probiotics experienced improved dry matter intake, higher milk protein content, and an increased antibody response to vaccination compared to control cows. These findings underscore the potential advantages of specific probiotic strains. Nevertheless, it is crucial to note that the effectiveness of probiotics may vary depending on factors such as the strain used, dosage administered, and the duration of supplementation. Moreover, as probiotics can influence the rumen microbial population and enhance rumen fermentation, they can contribute to enhanced performance in lactating cows. Therefore, the current study aims to assess the effects of different bacterial probiotic strains on various production and health-related parameters of lactating dairy cattle, particularly in the Chitwan region.

MATERIALS 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.

The probiotics concentrations were evaluated in the microbiology laboratory and were found to be 5.4 × 109, 6.1×109 and 5.7×109 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.

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

NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; NaCl: Sodium Chloride.

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. A separate sample for nitrogen analysis was made while a separate sample from each cow was kept in a Schott bottle acidified with sulfuric acid in order to make the final pH of 3 for analysis of purine derivatives.

Blood samples: Blood samples on the other hand were collected at the end of the collection period with needle and vacutainers from the jugular vein. Two separate vials for hematological and biochemical parameters were kept. The serum from non-heparinized vacutainer was extracted and sent to laboratory for biochemic parameters.

Rumen samples: Rumen liquor samples were collected using stomach tube from four cows one each from four treatments selected randomly. The contents were mixed well and sieved through clean muslin cloth and preserved in a Hungate tube for enumeration of protozoa.

Laboratory analysis

Feed: 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 (Zaklouta *et al.*, 2011).

Urine: Samples of urine was acidified for future analysis of purine derivatives. The acidified samples were then subjected to estimation of purine derivatives namely allantoin, xanthine, hypoxanthine and uric acid using the method explained by Chen and Gomes (1992).

Blood hematology and biochemistry: Blood samples collected after the digestibility experiments were subjected to ALTA Hematology Analyzer (Athenesedx, India) and biochemical parameters were tested using Biochemistry Analyzer GSI-2100 (Globe Scientific Instruments, India).

Protozoa: The rumen samples collected using stomach tube and stored in Hungate tubes were processed in the microbiology laboratory. A drop of rumen liquor was placed on a Sedgewick Rafter counting chamber. Manual published by Dehority (1993) under 100X magnification was used to enumerate and classify the ciliates.

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:

$$Yij = \mu + \tau i + \epsilon ij$$

Where,
Yij is the j-th response for the i-th treatment
τi i-th effect of supplementing probiotic strains
μ constant component
ϵij independent random errors

RESULTS AND DISCUSSION

Synthesis of microbial protein

Excretion of all purine derivatives except uric acid in the urine across the treatment groups as an effect of the supplementation of bacterial probiotics were significantly different ($p<0.01$). Likewise, the total PD excreted in a day across the treatment groups as also statistically different ($p<0.01$). The resultant supply of microbial protein in the rumen as well as absorption thereof were also statistically highly significant across treatments ($p<0.01$) as shown in Table 2. Such improvement in the amount of microbial protein to be supplied to the animal and that absorbed by them is in line with the findings made by Xie *et al.* (2019). Such improvement is largely attributable to enhanced microbial population in the host animal due to the influx of probiotics through feed. Such inclusion not only improves the quantity of microbial protein supplied to the host animal but also their quality (Nalla *et al.*, 2022).

Table 2. Effect of supplementing different probiotic strains on purine derivatives and synthesis and supply of microbial crude protein (g/day) in the rumen of lactating dairy cows.

Parameters	Treatments				SEM	p-Value
	CON	LAP	BST	EFC		
Allantoin (g/day)	173.22	228.79	171.58	129.22	13.71	<0.01
Xanthine+Hypxanthine	8.25	14.97	16.30	12.07	11.07	<0.01
Uric acid	2.76	9.52	5.22	4.20	1.06	0.33
Total PD Excretion	184.23	253.29	193.10	145.48	1.32	<0.01
Microbial Nitrogen Supply	183.44	261.22	193.14	138.21	12.30	<0.01
Microbial Nitrogen Absorbed	133.94	184.15	140.39	105.77	13.97	<0.01

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

Table 3. Effect of supplementation of different bacterial probiotic strains on blood hematological parameters of lactating dairy cows.

Variables	Treatments				SEM	p- value
	CON	LAP	BST	EFC		
WBC(10^3uL)	14.5	16.8	20.6	14.6	1.6	0.58
LYM (10^3uL)	7.3	7.8	10.5	6.3	1.0	0.58
GRAN (10^3uL)	5.3	6.9	6.6	6.2	0.4	0.65
RBC (10^6uL)	5.4	5.5	5.3	5.8	0.2	0.89
HGB (g/dL)	12.3	12.7	11.7	12.5	0.6	0.95
HCT%	32.5	31.3	30.3	23.9	2.8	0.76
MCV (fL)	59.4	56.4	57.6	56.8	0.8	0.63
MCHC (g/dL)	37.6	40.6	38.4	38.0	0.5	0.10
RDW%	16.0	16.0	16.4	15.9	0.3	0.90
PLT (10^3uL)	183.0	292.0	195.3	234.3	32.7	0.70
MPV (fL)	7.6	8.4	8.2	7.4	0.2	0.35
PDW%	8.8	8.4	8.8	7.9	0.2	0.60
P_LCR%	11.6	19.0	11.9	10.3	1.4	0.08

CON: Control; LAP: Lactobacillus acidophilus; BST: Bacillus subtilis; EFC: Enterococcus faecium; WBC: White blood cells; LYM: Lymphocytes; GRAN: Granulocytes; RBC: Red blood cells; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean cell volume; MCHC: Mean Corpuscular Hemoglobin Concentration; RDW: RBC distribution width; PLT: Platelets; MPV: Mean platelet volume; PDW: Platelet distribution width; P_LCR: Platelet large cell

Table 4. Effect of supplementation of different bacterial probiotic strains on blood biochemical parameters of lactating dairy cows.

Parameters	Treatments				Total	p-value
	CON	LAP	BST	EFC		
Glucose (mg/dL)	79.00	77.67	79.67	77.00	78.33	0.79
Urea (mg/dL)	30.67	34.33	34.33	31.33	32.67	0.91
Creatinine (mg/dL)	0.80	0.75	0.68	0.68	0.73	0.27
Uric acid (mg/dL)	2.70	2.43	2.17	2.60	2.48	0.63
Total protein (g/L)	7.03	6.90	6.50	7.07	6.88	0.86
Albumin (g/L)	3.00	3.37	3.03	2.93	3.08	0.82
Cholesterols (mg/dL)	178.67	138.33	158.67	156.67	158.08	0.76
SGPT (mg/dL)	23.67	24.33	27.33	26.00	25.33	0.56
SGOT (mg/dL)	51.00	69.33	58.00	69.33	61.92	0.39
Alkaline Phosphatase (mg/dL)	55.33	89.33	55.33	62.67	65.67	0.88

CON: Control; LAP: Lactobacillus acidophilus; BST: Bacillus subtilis; EFC: Enterococcus faecium; SGPT: Serum glutamic-pyruvic transaminase; SGOT: Serum glutamic-oxaloacetic transaminase.

Blood hematological parameters

All blood cell types RBC, WBC and platelets remained unaffected due to supplementation of different bacterial probiotic strains to the cows. In the same line, other parameters like hemoglobin, hematocrit, granulocytes, mean cell volume and others (Table 3) too did not differ as an effect of supplementation of different bacterial probiotic strains to the diet of cows. These findings do not agree with the findings reported by Yasmin *et al.* (2021) and Anee *et al.* (2021) in which the probiotic supplemented group had higher HGB, HCT, RBC and MCV. In our study, the animals were exposed to probiotics supplementation for relatively shorter period of only eight weeks which might not be sufficient for the animals to retain more minerals in the blood which could facilitate the synthesis of these blood elements.

Blood biochemical parameters

The effect of supplementation of bacterial probiotic strains on the blood biochemical parameters were also tested and results are presented in Table 4. The parameters tested are glucose, urea, creatinine, uric acid, total protein, albumin, cholesterol, serum glutamic-pyruvate transaminase, serum glutamic-oxaloacetic transaminase and alkaline phosphatase. All parameters during the test did not confirm the statistical differences across the treatment designed and tested. These findings also align to the findings of Yasmin *et al.* (2021) where they reported statistical indifference among the gradient of probiotic supplementation. Likewise, our results are in agreement with the compiled findings of several studies by Wang *et al.* (2023). The study, however, reported changes in some immunological and antioxidant indices which was beyond the scope of this study. The hypothesis of improvement in the health of animal is that improved biochemical and hematological indices would improve the immune system and thereby the health of the supplemented animal (Wang *et al.*, 2023).

Enumeration of rumen ciliate protozoa

The inclusion of different bacterial probiotic strains on overall count of rumen ciliate protozoa were counted. The count values of *Entodinium*, *Diplodinium*, *Eudiplodinium*, *Isotricha* and *Dysotricha* did not demonstrate any effect on enumerated values as an effect of inclusion of different strains of probiotic bacteria (Table 5). Likewise, the overall population of ciliates across all treatment groups also remained statistically similar.

Table 5. Effect of supplementation of different bacterial probiotic strains on number of rumen protozoa species of lactating dairy cows.

Protozoa species	Treatments				SEM	p-value
	CON	LAP	BST	EFC		
Entodinium	587200.00	427733.33	478400.00	428266.67	34299.29	0.39
Diplodinium	8000.00	6400.00	8000.00	10666.67	1597.98	0.37
Eudiplodinium	10666.67	11733.33	8000.00	6933.33	1866.67	0.85
Isotricha	6933.33	3200.00	8533.33	0.00	2258.09	0.13
Dysotricha	1600.00	1066.67	6400.00	0.00	1327.87	0.37
Total	614400.00	450133.33	509333.33	445866.67	36032.82	0.39

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

The indifference in the enumeration result of ciliate protozoa is in agreement with the result obtained by Dagnaw Fenta *et al.* (2023) where they also found no significant difference in population of rumen ciliate protozoa. The competition between flora and fauna of the rumen for substrate and space as defined by microbiological principles would require bacterial probiotics to effectively colonize the substrate material and then multiply. The amount administered in this experiment could be insufficient for improved digestion efficiency but not at the level to compete with the ciliate protozoa and thereby change their population in the rumen.

Conclusion

The addition of probiotics to lactating crossbred dairy cows has shown promising outcomes. This includes the potential to replace costly sources of dietary protein traditionally provided to enhance the health and productivity of these cows, as evidenced by the excretion of purine derivatives and the supply and absorption of microbial nitrogen in the rumen. Such a substitution could lead to improved economics in milk production, ultimately enhancing the competitiveness of the dairy sector in the country. In the present study, the effectiveness of single-strain probiotics was evaluated. However, the combined impact of two or more probiotics has yet to be explored. Therefore, future research should focus on investigating the synergy achieved through probiotic combinations, with or without prebiotics, to establish a more sustainable system. Moreover, the authors suggest that administering these probiotics from calf growth to the production stage could further enhance overall digestion and production efficiency in dairy animals. This longitudinal approach may yield additional benefits and is worth exploring.
Additionally, there is a need for more research into rumen ecology concerning the inclusion of probiotics. Understanding and manipulating the rumen environment could help mitigate the emission of enteric methane, presenting a novel area for improvement in the field.

ACKNOWLEDGEMENTS

This journal article is a product of an experiment conducted as part of the PhD dissertation. The authors would like to thank the support they received from Agriculture and Forestry University (AFU), Rampur, Nepal Agricultural Research Council (NARC), Khumaltar, Agriculture and Food Security Project (AFSP) funded by the World Bank. The team of the National Cattle Research Program (NCRP) are highly acknowledged for their contribution in providing shed, experimental animals and assistance in making observations.

REFERENCES

Adjei-Fremah, S., Ekwemalor, K., Asiamah, E. K., Ismail, H., Ibrahim, S., & Worku, M. (2018). Effect of probiotic supplementation on growth and global gene expression in dairy cows. *Journal of Applied Animal Research*, *46*(1), 257–263

Anee, I. J., Alam, S., Begum, R. A., Shahjahan, R. M., & Khandeker, A. M. (2021) The role of probiotics on animal health and nutrition. *The Journal of Basic & Applied Zoology*, 82: 52, https://doi.org/10.1186/s41936-021-00250-x

Bouchard, D. S., Seridan, B., Saraoui, T., Rault, L., Germon, P., & Gonzalez-Moreno, C. (2019). Effect of a probiotic yeast on the fecal microbiota of dairy cows subjected to a subacute ruminal acidosis model. *Microorganisms*, 7(7), 211.

Cangiano, L., Yohe, T., Steele, M., & Renaud, D. (2020). Invited review: strategic use of microbial-based probiotics and prebiotics in dairy calf rearing. *Applied Animal Science*. 36: 630-651

CASA (2020). Dairy Sector Strategy-Nepal. Commercial Agriculture for Smallholder Agribusiness. Department for International Development (DFID). Kathmandu.

Chen, X. B., & Gomes, M. J. (1992). Estimation of Microbial Protein Supply to Sheep and Cattle Based on Urinary Excretion of Purine Derivatives - an Overview of the Technical Details. International Feed Resources Unit, Rowett Research Institute. Aberdeen, UK

Dagnaw Fenta, M., Gebremariam A. A., & Mebratu A. S. (2023). Effectiveness of Probiotic and Combinations of Probiotic with Prebiotics and Probiotic with Rumenotorics in Experimentally Induced Ruminal Acidosis Sheep. *Veterinary Medicine* (Auckl), 18: 63-78, https://doi.org/10.2147/VMRR.S396979

Dehority B. A. (1993). *Laboratory Manual for Classification and Morphology of Rumen Ciliate Protozoa*. CRC Press Boca Raton, USA

Goering, H. K. & Van Soest, P. J. (1970). Forage Fiber Analysis (Apparatus, Reagents, Procedures and Some Applications). Agriculture Handbook. United States Department of Agriculture, Washington DC.

Mahesh, M. S., Mohanta, R. K., & Patra, A. K. (2021). Probiotics in livestock & poultry nutrition and health. In G. Goel & A. Kumar (Eds.), *Advances in Probiotics for Sustainable Food and Medicine* (pp. 149–179). Springer Singapore.

MoALD (2022). *Statistical Information on Nepalese Agriculture*. Ministry of Agriculture and Livestock Development, Singhadurbar, Kathmandu

Nalla, K., Manda, N. K., Dhillon, H. S., Kanade, S. R., Rokana, N., Hess, M., & Puniya, A. K. (2022). Impact of probiotics on dairy production efficiency. *Frontiers in Microbiology*, 13: 805963. https://doi.org/10.3389/fmicb.2022.805963

Wang, L., Sun, H., Gao, H., Xia, Y., Zan, L., & Zhao, C. (2023). A meta-analysis on the effects of probiotics on the performance of pre-weaning dairy calves. *Journal of Animal Science and Biotechnology*, 14(3). https://doi.org/10.1186/s40104-022-00806-z

Xie, Y., Wu, Z., Wang, D., & Liu, J. (2019). Nitrogen partitioning and microbial protein synthesis in lactating dairy cows with different phenotypic residual feed intake. *Journal of Animal Science and Biotechnology*, 10, 1–8. https://doi.org/10.1186/s40104-019-0356-3

Yasmin, F., Alam, J., Kabir, E., Maruf, A., Islam, A., & Hossain, M. (2021). Influence of probiotics supplementation on growth and haemato-biochemical parameters in growing cattle. *International Journal of Livestock Research*, *11*(6), 36-42, https://doi.org/10.5455/ijlr.2021040 1123825

Zaklouta, M., Hilali, M., Nefzaoui, A., & Haylani M. 2011. *Animal Nutrition and Product Quality Laboratory Manual*. ICARDA, Aleppo, Syria.