

Effective Microorganisms-Supplemented Total Mixed Ration on Nutrient Utilization and Nitrogen Excretion in Lactating Dairy Cows

R. M. H. Shaill-Rathnayake¹, W. A. D. Nayananjalie^{2*}, A. M. J. B. Adikari², and U. L. P. Mangalika³

ABSTRACT

This study was conducted to evaluate the dietary effects of effective microorganisms (EM) supplementation on nutrient intake, digestibility, blood metabolites, and nitrogen (N) excretion in lactating dairy cows. Eight Jersey crossbred lactating dairy cows in the same parity were randomly assigned to two dietary treatments: control TMR (C-TMR) and an EM-supplemented TMR (EM-TMR) at a rate of 10 mL kg⁻¹ for 07 days in metabolic stalls, following a completely randomised design. Based on the observed results, EM supplementation significantly ($p < 0.05$) influenced the nutrient intake (DM: 15.17 $\pm$ 0.17, CP: 1.93 $\pm$ 0.02, CF: 2.81 $\pm$ 0.03, and ADF: 2.85 $\pm$ 0.03 kg, day⁻¹) compared to the control (DM: 14.42 $\pm$ 0.17, CP: 1.79 $\pm$ 0.02, CF: 2.61 $\pm$ 0.03, and ADF: 2.65 $\pm$ 0.03 kg, day⁻¹). Significantly ($p < 0.05$) lower faecal, urinary and total N excretion was observed in EM supplemented group (Faecal: 73.82 $\pm$ 4.76, Urine: 348.01 $\pm$ 21.41 and Total N: 421.82 $\pm$ 25.93 g, day⁻¹) compared to the control (Faecal: 97.43 $\pm$ 4.76, Urine: 461.81 $\pm$ 21.41 and Total N: 559.24 $\pm$ 25.93 g, day⁻¹), while blood metabolites did not show any significant difference ($p > 0.05$). In conclusion, EM supplementation significantly improved nutrient intake, digestibility, and N utilisation while reducing manure N excretion. These findings suggest that EM is a promising feed supplement for improving gut health and nutrient digestion in lactating dairy cows, while promoting sustainable farming through improved nutrient efficiency and reduced environmental footprint.

Keywords: Dairy cows, Feeds, Metabolism, Probiotics, Yeast.

¹ Postgraduate Programme, Faculty of Agriculture, Rajarata University of Sri Lanka, Anuradhapura, Sri Lanka.

² Department of Animal and Food Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka, Anuradhapura, Sri Lanka.

³ Department of Animal Production and Health, Getambe, Peradeniya, Sri Lanka.

*Corresponding author; e-mail: deepthin@agri.rjt.ac.lk

INTRODUCTION

The dairy industry currently faces a significant challenge of enhancing animal nutrition while improving milk production, economic profitability, animal health, and welfare. Feeds play a crucial role in this context, as it directly influences nutrition status, productive and reproductive performance, nutrient excretion to the environment, and overall farm profitability (NRC, 2021). However, the lower feed digestion and utilization efficiency may result in animal malnutrition, reduced overall productivity, and compromised health. Further, inefficient nutrient utilization increases nutrient excretion in manure, which contributes to environmental issues such as eutrophication and atmospheric pollution (Spek *et al.*, 2013). Therefore, an improved rumen microbial ecosystem and ruminal fermentation dynamics are essential for proper nutrient digestion and utilisation.

The rumen provides the ecosystem for the multispecies population of bacteria, protozoa, fungi, and archaea. They produce volatile fatty acids (VFAs) and microbial protein by degrading fibrous plant materials, which is essential for energy and protein availability (CGR/FAO, 2023). Consequently, rumen modification by introducing exogenous microorganisms has shown improved rumen fermentation dynamics and nutrient digestibility (Kholif *et al.*, 2024). Through various mechanisms, exogenous microbes influence nutrient utilisation by improving fiber digestion, stabilizing rumen pH, improving N utilisation, and enhancing microbial protein synthesis (Nalla *et al.*, 2022). Bach (2024) and Terré *et al.* (2024) showed that feed efficiency in dairy cows improved with direct-fed microbial strains. Further, Reuben *et al.* (2022) and Rathnayake *et al.* (2026a) showed that effective microorganisms (EM) have been improving nutrient utilisation in cattle by favourably modulating gastric fermentation. Rathnayake *et al.* (2026b) extensively reviewed the effectiveness of EM supplementation for beef and dairy cattle and concluded a positive effect on production performance. Some studies reported positive effects on dry matter intake (DMI), nutrient digestibility and milk production (Polyorach *et al.*, 2023; Tadesse *et al.*, 2024; Rathnayake *et al.*, 2026b), while others reported negative effects (Nalla *et al.*, 2022). These differences could be attributed to variation in EM composition, dosage levels, study duration, and diet composition (Miranda-Figueroa *et al.*, 2021).

When considering the metabolizable effect of dietary intervention in dairy nutrition, blood metabolites play a key role in giving a clear image of the effects of diet on nutritional status and metabolic function. Parameters such as serum glucose, urea N, and cholesterol are indicative of nutrient intake, absorption, hepatic metabolism, and tissue development (Nielsen *et al.*, 2007). Changes in these blood metabolites in response to microbial feed additives are a positive indicator for studying the post-absorptive nutrient metabolism and metabolic health of the cow (Ramirez-Garzon *et al.*, 2024). The limited studies on the evaluation of microbial supplementation on blood metabolites remain a gap to be addressed in dairy cattle.

Moreover, environmental sustainability has become vital in dairy production, as dairy farms produce considerable amounts of waste and excreta. The dairy sector contributes to the environmental loading through the release of manure, nutrient pollutants, and greenhouse gases (Spanghero & Kowalski, 2021). These excretions consist of nutrients like N and P coming from the undigested portions of the ingested feeds. This excreted N subsequently contributed to ammonia (NH₃) volatilization, nitrate (NO₃⁻) leaching, and nitrous oxide (N₂O) emissions, which may pose significant threats to environmental quality. Urinary N had more environmental threats than faecal N. Therefore, the partition of faecal and urinary N excretions may help in the management of urinary N excretion, which could be beneficial for environmental sustainability through dietary intervention (Nennich *et al.*, 2006; Vasilev & Videv, 2023).

With the extensive survey of the existing studies related to the effect of EM on dairy nutrition, rigorous scientific evidence focusing on its effect on nutrient digestion and utilisation, blood chemistry, and manure excretion remains inadequate. Most existing studies primarily focused on production-related parameters (Tesfaye & Hailu, 2019), with limited focus on digestibility parameters. Thus, the present study aimed to provide scientific evidence of the effects of EM supplementation on nutrient intake, digestibility, blood metabolite concentrations, and manure N excretion in lactating dairy cows under controlled experimental conditions.

MATERIALS AND METHODS

Ethical Approval

Ethical approval was obtained from the Research Ethics Committee of the Uva Wellassa University of Sri Lanka (Ref. No: UWU/REC/2024/29).

Study Location

This study was conducted at the Neat Cattle Unit, NLDB Melsiripura (KG30050) Farm located in the Kurunegala district ($\sim 7.647764^\circ$ N, $\sim 80.516742^\circ$ E), North Western Province, Sri Lanka.

Experimental Design and Treatment Diets

Eight Jersey crossbred lactating cows (Age = 44.80 ± 5.88 months; BW = 403.78 ± 58.57 kg; Days in milk = 127.36 ± 28.05) in the same parity were randomly allocated into two dietary treatment groups using a completely randomized design (CRD). The study duration consisted of a seven-day adaptation period followed by a 12-week feeding trial. Cows were housed individually in a tie-up barn. Cows were fed with conventional total mixed ration (C-TMR) or EM-supplemented total mixed ration (EM-TMR). The ingredients and nutritional composition of the treatment diets are presented in Table 1 and Table 2, respectively. The commercially available EM1 solution (Sarvodaya SEEDS Limited, Colombo, Sri Lanka) was used as a supplement, and its composition is presented in Table 3.

Table 1. Ingredient composition on a dry matter (DM) basis to formulate 100 kg of fresh TMR.

Raw ingredients	Treatment diets (kg)	
	C-TMR	EM-TMR
EM1- Mixture	-	Added ¹
Guinea Grass (<i>Megathyrsus maximus</i>)	4.74	4.74
Corn (<i>Zea mays</i>) forage	3.60	3.60
Sorghum var. Hyb ² (<i>Sorghum bicolor</i>)	1.66	1.66
Hybrid Napier ³	1.67	1.67
Corn (<i>Zea mays</i>) silage	3.12	3.12
Corn (<i>Zea mays</i>) meal	1.74	1.74
Brewer's spent grain (<i>Oryza sativa</i>)	2.41	2.41
Commercially available cattle feed	9.07	9.07

¹ 10 mL of EM1[®] liquid mixed to 1 kg of TMR before feeding, ²Hybrid variety, strain sugar grace, ³*Pennisetum purpureum* $\times$ *Pennisetum americanum*, C-TMR- control, EM-TMR-treatment.

Total mixed ration (TMR) was prepared using the raw feed ingredients to meet the nutritional requirements of the lactating cows. For this, all fresh forages were chopped (~3-5 cm) using a slide-modified single-row mobile shredder (SHAKTHIMAN, Tirth Agrotechnology Ltd, India) operated by a dual-clutch 40 hp tractor connected by a three-point linkage and powered by a power takeoff (PTO) at ~630×g. TMR was freshly prepared by mixing all ingredients thoroughly, 2 hours before feeding. For the supplementation of EM, 10 mL of EM1 solution (approved for use as an animal feed supplement/probiotic under the Animal Feed Act no. 15 of 1986, Department of Animal Production and Health, Sri Lanka. Ref. No.: DAPH/VRA/7/2/2/S/4) was mixed into 1 kg of TMR ration immediately before feeding to the treatment group without allowing external fermentation.

Table 2. Proximate composition of the treatment diets.

Nutrients	Treatment Diets ± SD*	
	C-TMR	EM-TMR
Dry matter (%)	35.34±0.45	35.75±1.13
Crude protein (%)	12.47±0.04	12.73±0.10
Crude fiber (%)	18.11±1.32	18.53±1.27
Ether extract (%)	2.98±0.67	2.66±0.48
Ash (%)	8.26±0.27	8.49±0.10
Acid detergent fiber (%)	18.43±0.28	18.85±0.08
Neutral detergent fiber (%)	45.68±0.93	42.85±0.69
Total digestible nutrients (%)	74.55±0.29	74.11±0.08
Nitrogen-free extracts (%)	58.13±1.08	57.59±1.83
Digestible energy (Mcal kg ⁻¹)	1.49±0.01	1.48±0.01
Metabolizable energy (Mcal kg ⁻¹)	1.22±0.01	1.21±0.01

*SD – Standard deviation, C-TMR – Control, EM-TMR – Treatment.

Note: Total digestible nutrients, nitrogen-free extract, digestible energy, and metabolizable energy were calculated using equations 3, 4, 5, and 6, respectively, as outlined in the calculations and statistical analysis section. These mathematical estimations were computed based on primary proximate composition values obtained through the laboratory analysis of feed samples in triplicate (n = 3).

Table 3. Laboratory parameters of EM1 solution.

Parameter	Value
pH	3.43
Oxidation-reduction potential (mV)	80
Brix	5.8
Lactic acid bacteria (CFU mL ⁻¹)	1.2×10 ⁸
Yeasts (CFU mL ⁻¹)	1.5×10 ³
Phototropic bacteria	Detected

Source: Lab report of Sarvodaya Seeds EM1 solution (Analyzed by Research Department, EMRO, Japan).

Digestibility Trial

The digestibility trial was started in the last week of the feeding trial of 12 weeks' duration. Before beginning the digestibility trial, a visiting veterinary surgeon inspected all experimental animals. Four healthy representative cows were randomly selected for each treatment group (total = 8 cows) and housed individually in metabolic stalls (W 120 × L 210 cm) for 07 days.

The C-TMR and EM-TMR were continuously offered as two equal meals in a day at 07:00 and 18:00 h, with each cow allowed *ad libitum* access to drinking water. During the digestibility trial, the offered feed and leftovers were recorded daily in the morning and evening before and after feeding. The feed intake (FI) was calculated by subtracting the leftovers from offered feed. The stall is equipped with a sprinkler system to use when the noon temperature increases above 28°C to minimize heat stress.

Total Faeces and Urine Collection

The total faeces and urine volume was collected daily. The urine collection was done by employing the bucket collection method as mentioned in Bailoni *et al.* (2025), and 50 mL representative samples were collected at 04:00 and 17:00 h each day. The urine samples were immediately acidified with 1 mL of 6N H₂SO₄ and stored at -18°C until being analyzed for urinary N. The faeces samples were collected at 04:00 and 17:00 h each day. The representative samples were collected by covering the floor of the cows' defecation area using a low-density polyethylene (LDPE) plastic sheet, and 250 g of faeces was transferred into the labelled, sterilised containers and then immediately stored at -18°C.

At the end of the digestibility trial, faeces samples were thawed and mixed to obtain a composite sample (~250 g) for each corresponding cow. The composite samples were dried in 60°C using a hot air oven (YCO-010, Germany), ground and sieved in 1mm sieve and stored at room temperature in labelled airtight containers until being analyzed for ash, ether extract (EE), crude fiber (CF), acid detergent fiber (ADF), neutral detergent fiber (NDF) and crude protein (CP) contents in accordance with AOAC and Van Soest (AOAC, 2019; Van Soest *et al.*, 1991). For ash content analysis, samples were burned at 550°C for 6 hours in a muffle furnace (DMF3514, Korea). For the EE, samples were analysed using a semi-

automated solvent extractor (VELP Scientifica-SER 148, Italy), and CF, ADF and NDF contents were analysed in a fibre extraction unit (VELP Scientifica-FIWE 6, Italy). To determine the N content, the Kjeldahl apparatus (digestor: SH220F, Kjeldahl distillation unit: K9840, Hanon, Hanon Instruments, China) was used, and then CP was calculated using the conversion factor ($CP=N \times 6.25$).

Blood Sampling

Blood samples were collected before the experiment began and on the last day of the experiment. Blood samples were drawn from the jugular vein of each cow using a disposable injection syringe attached to an 18-gauge sterile needle. Then, drawn blood samples were immediately transferred to a 10 mL aliquot into labelled, non-anticoagulant-added plain sterile glass tubes and placed at 4°C in an insulated box until dispatched to the laboratory.

Blood Samples Analysis

Blood samples were analyzed at the Animal Clinical Diagnostic Laboratory, Department of Farm Animal Production and Medicine, Faculty of Veterinary Medicine and Animal Sciences, University of Peradeniya, Sri Lanka, and the Animal Science Laboratory, Department of Animal and Food Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka. To separate the serum from the blood, samples were centrifuged at $\sim 450 \times g$ for 5 min (Z300, Hermle LaborTechnik GmbH, Germany). Then, two aliquots of serum from each sample were carefully transferred into the labelled 2 mL microcentrifuge tubes and stored at -20 °C until analysis.

For the analysis, frozen serum samples were thawed at room temperature. Serum glucose (GLUC-PAP), urea (UR3825), cholesterol (CH8310), and β hydroxybutyrate (RB1007) were analyzed using a semi-automated biochemistry analyzer (CHEM-7, Erba Transasia Diagnostics, India) with commercially available assay kits (Randox Laboratories Ltd., UK). For the determination of serum non-esterified fatty acid (NEFA), Frozen serum samples were thawed at room temperature and analyzed using a microplate reader (51119700DP, USA) with a commercial colorimetric assay kit (abx294423, Free Fatty Acids/NEFA Assay Kit, Abbexa Ltd, UK).

Calculations and Statistical Analysis*Nutrient intake and apparent total tract digestibility*

Feed DMI, faecal DM output and the faecal outputs of individual nutrients (N_{out}) were calculated as described by Karunanayaka *et al.* (2022). Nutrient intake (N_{int}) and apparent total tract digestibility (ATTD) for each nutrient (C_n) were calculated using Equations 1 and 2 (Karunanayaka *et al.* 2022).

$$N_{int} = \text{DMI} \times C_n \text{ -----Equation 1}$$

$$\text{ATTD}\% = \left(\frac{N_{int} - N_{out}}{N_{int}} \right) \times 100 \text{ -----Equation 2}$$

Total digestible nutrients and feed energy value calculation

Feed total digestible nutrients (TDN), nitrogen-free extract (NFE), and energy values were calculated using the following equations (NRC, 2001).

$$\text{TDN}\% = 93.53 - (11.03 + \text{ADF}) \text{ -----Equation 3}$$

$$\text{NFE}\% = 100 - (\text{CP}\% + \text{EE}\% + \text{CF}\% + \text{Ash}\%) \text{ -----Equation 4}$$

$$\text{DE} = \text{TDN}\% \times 0.02002 \text{ -----Equation 5}$$

$$\text{ME} = \text{TDN}\% \times 0.01642 \text{ -----Equation 6}$$

Data analysis

Data analysis was performed using RStudio version 2025, 4.5.1, with a linear mixed-effects model, using a 95% confidence interval. Differences were considered statistically significant at $p \leq 0.05$.

RESULTS AND DISCUSSION**Proximate Evaluation of Experimental Diets**

The proximate composition of the formulated diets had no significant differences ($p > 0.05$) between the rations, with close values for all nutritional parameters, and calculated energy values showed that both diets are nutritionally equivalent. Dry matter, CP, CF, ADF, NDF, and calculated energy (ME) values are within the typical reference ranges in dairy cows' rations (NRC, 2001; Karunanayaka *et al.*, 2021). For the non-biased experimental results, nutritionally similar diets support uniform nutrient intake, while stabilizing the ruminal fermentation and minimizing the diet selection of cows. Therefore, when formulating diets in experimental settings, a similar chemical composition is essential (Kumar *et al.*, 2021).

The nutritional similarity between these two diets (C-TMR and EM-TMR) indicated that any animal-related response may be the result of biological effects of EM supplementation rather than nutritional effects. Furthermore, the existing studies of direct-fed microbial or fermented TMR diets fed to dairy cows showed that productivity and digestibility responses can occur even when basal nutrient composition remains comparable, because microbial additives mainly influence the rumen fermentation dynamics and digestion process rather than improving chemical nutrient levels (Ramirez-Garzon *et al.*, 2024; Wang *et al.*, 2024).

Nutrient Intake and Digestibility

This section presents the dietary effect of EM supplementation on nutrient intake and digestibility in lactating dairy cows, and supplementary data are summarized in Table 4. Nutrient intake and digestibility in lactating dairy cows fed

Table 4. Nutrient intake and digestibility in lactating dairy cows fed EM-supplemented vs conventional TMR diets.

Nutrients	Treatments		MSE (±)	p-Value
	C-TMR	EM-TMR		
Nutrient Intake				
Dry matter (kg, day ⁻¹)	14.42 ^b	15.17 ^a	0.17	0.0248
Crude protein (kg, day ⁻¹)	1.79 ^b	1.93 ^a	0.02	0.0056
Crude fiber (kg, day ⁻¹)	2.61 ^b	2.81 ^a	0.03	0.0045
Ether extract (kg, day ⁻¹)	0.43 ^b	0.40 ^a	0.01	0.0118
Ash (kg, day ⁻¹)	1.19 ^b	1.28 ^a	0.01	0.0042
Acid detergent fiber (kg, day ⁻¹)	2.65 ^b	2.85 ^a	0.03	0.0061
Neutral detergent fiber (kg, day ⁻¹)	6.58	6.44	0.07	0.2420
Nutrient Digestibility				
Dry matter (%)	59.81	63.01	1.80	0.2555
Crude protein (%)	66.22 ^b	76.06 ^a	1.41	0.0027
Crude fiber (%)	47.98 ^b	61.52 ^a	2.52	0.0091
Ether extract (%)	88.31	88.67	3.70	0.9471
Ash (%)	24.90	38.12	3.85	0.0515
Acid detergent fiber (%)	25.20	29.99	2.92	0.2907
Neutral detergent fiber (%)	43.66 ^b	52.99 ^a	2.47	0.0369

a and **b**, means within the same row with different superscripts, are significantly different ($p < 0.05$); C-TMR: control diet, EM-TMR: EM-supplemented diet, MSE: mean standard error.

Daily DM intake, CP, CF, ash, and ADF showed significant ($p < 0.05$) improvement in EM-supplemented cows, compared to the control (Table 4). This improvement may be due to the

enhanced ruminal degradation kinetics rather than simple changes in diet palatability. Multistrain microbial consortia presented in the EM favourably alter the ruminal fermentation dynamics by enhancing lignin and cellulose particle breakdown while increasing digestion rate. This rapid digestion process increases voluntary feed intake by suppressing tension-receptor signals, which are responsible for feeling the physical gut-fill effect in the reticulorumen (Polyorach *et al.*, 2023). Moreover, microbial fermentation of feed materials synthesises beneficial organic acids that enhance ration's organoleptic properties, which improve voluntary consumption without affecting NDF intake (Tadesse *et al.*, 2024). Further, EM supplementation enhanced feed palatability through the production of volatile metabolites by lactic acid bacteria and yeast (Ma *et al.*, 2023). Moreover, improved digestibility of CP and NDF, such as structural carbohydrates, leads to improved practical breakdown. This faster degradation may enhance the ruminal passage rate by reducing the physical gut fill effect, while enhancing feed consumption. This enhanced feed consumption of CP and CF, and NDF digestibility further highlights the EM effect on optimizing the rumen microenvironment. Rather than simply adding nutrients, EM acts in mutually with the rumen native microbes. Yeast contained in the EM consortium utilize trace O₂ by optimizing anaerobic conditions for the native cellulolytic bacteria to degrade feed particulates (Ogunade *et al.*, 2019). Moreover, EM secretes some exogenous enzymes and essential growth promoters, such as organic acids and B-vitamins, that helps to fibrolytic activity and breaking down the chemical structure of lignocellulosic bonds (Uyeno *et al.*, 2015). Similarly, the enhanced CP digestibility could be evident in the more efficient breakdown of structural proteins into peptides essential for microbial protein synthesis. Overall, EM improves lactating cows' nutritional digestibility by enhancing rumen ecology, enzyme-substrate interactions, and overall nutrient absorption efficiency, aligning with known probiotic modes of action in ruminants (Ma *et al.*, 2023; Uyeno *et al.*, 2015).

Blood Metabolites

Blood metabolites provide a clear image of nutrient utilisation and metabolic homeostasis of lactating cows. The supplementation of EM on blood metabolites was not significantly ($p>0.05$) different among the treatments (Table 5). Though numerically higher serum

glucose, cholesterol, and numerically lower SUN were observed in the EM-supplemented group compared to the control.

Table 5. Blood metabolites in lactating dairy cows fed EM-supplemented vs conventional TMR diets.

Parameters	Treatments		MSE ($\pm$)	p-Value
	C-TMR	EM-TMR		
*SUN (mg dL ⁻¹)	12.14	11.92	1.15	0.0788
Serum Glucose (mg dL ⁻¹)	43.69	53.56	4.12	0.0967
Serum Cholesterol (mg dL ⁻¹)	36.70	45.36	4.86	0.4969
**BHB (mmol L ⁻¹)	0.72	0.70	0.05	0.4727
***NEFA (mmol L ⁻¹)	0.045	0.037	0.01	0.3886

MSE- Mean standard error; *SUN – Serum urea nitrogen; **BHB- β hydroxybutyrate ***NEFA- Serum non-esterified free fatty acids; C-TMR: control diet, EM-TMR: EM-supplemented diet.

In lactating dairy cows, glucose circulation primarily occurs through the hepatic gluconeogenesis process while using ruminal propionate as the principal substrate. The slightly elevated serum glucose level suggests that EM supplementation may have enhanced total volatile fatty acid yield and propionate molar proportions, which produce more energy to support milk synthesis (Niknia *et al.*, 2025). This enhanced glucogenic status lowers the need for utilizing deposited peripheral adipocyte fat for energy generation. Consequently, the concurrent downward trends in NEFA and BHB show reduced triacylglycerol lipolysis and attenuated hepatic β -oxidation, pointing toward an energy-sparing metabolic state that mitigates subclinical metabolic stress during lactation (Karunanayaka *et al.*, 2022).

The decreasing trend in SUN further highlights an optimized ruminal N balance. Elevated SUN concentrations in the control group typically reflect excess ruminal ammonia production (NH₃-N) resulting from asynchronous carbohydrate and protein degradation, which necessitates energy-expensive hepatic ureagenesis. The numerically lower SUN levels in EM-supplemented cows point toward improved microbial N capture and enhanced microbial protein synthesis (Tadesse *et al.*, 2024). By synchronizing fermentable energy availability with degradable N in the rumen, EM additives appear to foster a metabolic milieu that satisfies the energetic demands of lactation while preserving metabolic stability.

Nitrogen Excretion

In dairy farming, N metabolism and circulation are one of the most important indicators of dietary N utilisation and environmental N footprint. The faecal and urinary N excretion of

cows receiving the two dietary treatments is presented in Table 6. Observed values for cows receiving the two different treatments are comparable with the studies conducted by Vasilev and Videv (2023), Terré *et al.* (2024) and Oyebade *et al.* (2023).

Table 6. Faecal and urinary outputs of lactating dairy cows fed with EM-supplemented vs conventional TMR diets.

Parameters	Treatments		MSE ($\pm$)	p-Value
	C-TMR	EM-TMR		
Faeces volume DM basis (kg, day ⁻¹)	5.81	5.60	0.29	0.6430
Faecal N (g, day ⁻¹)	97.43 ^a	73.82 ^b	4.76	0.0129
Faecal N% of N intake	5.40 ^a	3.82 ^b	0.23	0.0034
Urine volume (L, day ⁻¹)	9.41 ^a	7.65 ^b	0.40	0.0212
Urinary N (g, day ⁻¹)	461.81 ^a	348.01 ^b	21.41	0.0095
Urinary N% of N intake	7.53 ^a	8.09 ^b	0.08	0.0037
Total N (g, day ⁻¹)	559.24 ^a	421.82 ^b	25.93	0.0093
Total N% of N intake	31.05 ^a	21.88 ^b	1.28	0.0026

a and **b**, means within the same row with different superscripts, are significantly different ($p < 0.05$); C-TMR: control diet, EM-TMR: EM-supplemented diet, MSE: mean standard error.

The results showed that all the assessed and calculated N excretion parameters were significantly different ($p < 0.05$) between the treatments (Table 6). This significant reduction in total faecal N excretion and faecal N expressed as a percentage of N intake shows improved total-tract N digestion and improved ruminal amino acid profile. The consortia of EM facilitates the cross-feeding networks within the rumen by optimizing the rapid conversion of degradable dietary protein into microbial biomass (Lu *et al.*, 2019; Reuben *et al.*, 2022). This synchronized N assimilation minimizes the passage of unfermented peptide fractions into the lower gastrointestinal tract, while reducing faecal N loss without affecting total faecal DM output (Table 6).

Concurrently, total urinary N output significantly decreased by 24.6% in EM-supplemented cows, while a significant drop in daily urine volume. In ruminants, urinary N consists primarily of urea produced by the liver from excess NH₃-N. By aligning carbohydrate fermentation with protein degradation, EM provided the consortia of microbes that reduce unabsorbed ruminal NH₃-N accumulation, as a result of this reducing hepatic urea synthesis and subsequent renal excretion (Nalla *et al.*, 2022; Ramirez-Garzon *et al.*, 2024; Terré *et al.*, 2024). However, the significant increase in urinary N as a percentage of N intake shows a metabolic repartitioning between excretion routes. As faecal N losses were reduced to a larger percentage, the urinary N excretion accounted for a slightly larger proportion of the

remaining N pool, a shifting pattern observed when N utilization efficiency increases overall (Spek *et al.*, 2013; Spanghero & Kowalski, 2021).

At the animal level, EM supplementation reduced total N excretion from 31.05% to 21.88% of total N intake. Obtaining a nearly 10% drop in relative N loss confirms that EM supplementation enhanced greater metabolic N retention into productive outputs (such as milk protein) rather than excretion. From an environmental management perspective, this significant reduction of total N excretion, specifically urinary urea, cut the substrate available for environmental NH₃ volatilization, atmospheric N₂O formation, and NO₃ leaching, offering a practical nutritional tool for sustainable dairy farming.

CONCLUSIONS

In conclusion, dietary EM supplementation enhanced voluntary DMI and improved the digestibility of key nutrients, such as CP, CF, and NDF, without affecting the blood metabolites. Therefore, it can be suggested as a viable nutritional strategy to improve nutrient utilisation efficiency, support metabolic health, and mitigate environmental N footprint in dairy farming. These findings highlight EM's potential as a feed additive that supports both improved productivity and sustainable dairy farming practices. Future research should investigate the long-term effects of EM supplementation on milk yield and reproductive performance under commercial dairy conditions across management systems and age groups to fully establish its role in enhancing sustainable dairy nutrition.

ACKNOWLEDGEMENTS

This research was financially supported by the National Science Foundation (NSF/RD/06/RG/2024/AG/01) in Sri Lanka, as well as Mr. Laksmen Bandaranayake, Chairman of the Sarvodaya Sarvasara Agro Research and Technology Centre, for assisting in facilitating the EM solution used in this study.

REFERENCES

1. AOAC, 2019 Official Methods of Analysis of the Association of Official Analytical Chemists: Official Methods of Analysis of AOAC International. 21st Edition, AOAC, Washington, DC.

- 355 2. Bach, A. 2024. 156 Probiotics (direct fed microbials) and feed efficiency in dairy cows. J.
356 Anim. Sci. 102:394-395. <https://doi.org/10.1093/jas/skae234.448>.
- 357 3. Bailoni, L., S. Arango, N. Guzzo, S. Currò, E. Bianco, E. Simonetti, E. Zerbinati, S. Rainis,
358 and C. Sartori. 2025. Effect of a daily outdoor access on the urination and defecation
359 behaviors and nitrogen excretion by lactating cows. Front. Vet. Sci. 12:1429638.
360 <https://doi.org/10.3389/fvets.2025.1429638>
- 361 4. CGR/FAO (Commission on genetic resources for food and agriculture) 2023.
362 Microorganisms relevant to ruminant digestion. Item 9.2 of the Provisional Agenda of the
363 United Nations of the Food and Agriculture. Available online with updates at
364 [https://openknowledge.fao.org/server/api/core/bitstreams/b6f5771a-15a3-466e-ae0a-](https://openknowledge.fao.org/server/api/core/bitstreams/b6f5771a-15a3-466e-ae0a-962efe32d0f6/content)
365 [962efe32d0f6/content](https://openknowledge.fao.org/server/api/core/bitstreams/b6f5771a-15a3-466e-ae0a-962efe32d0f6/content).
- 366 Karunanayaka, W., D. Nayananjalie, R. Appuhamy, J. Adikari, V. Weerasingha, A. Kumari,
367 S. Somasiri, R. Liyanage, P. Mangalika, and Sundarabarathy T. 2022. Effect of TMR
368 briquettes on milk production, nutrient digestibility, and manure excretions of dairy cows in
369 the dry zone of Sri Lanka. Animals 12:932-944. <https://doi.org/10.3390/ani12070932>.
- 370 5. Karunanayaka, W., D. Nayananjalie, S. Somasiri, J. Adikari, W. Weerasingha, A. Kumari,
371 U. Mangalika, S. Dissanayake, and T. Sundarabarathy. 2021. Nutritional and keeping quality
372 of total mixed ration briquettes produced for lactating dairy cows. SLJAE. 3:46-66.
373 <https://doi.org/10.4038/sljae.v3i1.60>.
- 374 6. Kholif, A.E., A. Anele, and U.Y. Anele. 2024. Microbial feed additives in ruminant
375 feeding. AIMS Microbiol. 10:542. <https://doi.org/10.3934/microbiol.2024026>.
- 376 7. Kumar, S., M.K. Rajput, and P.K. Yadav. 2021. Essentials of laboratory animal science:
377 principles and practices. In P. Nagarajan, Ramachandra R. Gudde, R. Srinivasan (eds.),
378 Laboratory Animal Nutrition. Springer Singapore. pp.373-404. [https://doi.org/10.1007/978-](https://doi.org/10.1007/978-981-16-0987-9_16)
379 [981-16-0987-9_16](https://doi.org/10.1007/978-981-16-0987-9_16).
- 380 8. Lu, Z., Z. Xu, Z. Shen, Y. Tian, and H. Shen. 2019. Dietary energy level promotes rumen
381 microbial protein synthesis by improving the energy productivity of the ruminal
382 microbiome. Front. Microbiol. 10:847-861. <https://doi.org/10.3389/fmicb.2019.00847>.
- 383 9. Ma, L., L. Wang, Z. Zhang, and D. Xiao. 2023. Research progress of biological feed in
384 beef cattle. Animals. 13:2662. <https://doi.org/10.3390/ani13162662>

- 385 10. Miranda-Figueroa, A., C. Gutiérrez-Aguilar, M. González-Ronquillo, A. Romero-Pérez,
386 C.C. Márquez-Mota, J.M. Talamantes-Gómez, J. Aviles-Nieto, L. Vallejo-Hernández, and L.
387 Corona. 2021. PSX-B-1 Effect of exposure time and dose of effective microorganisms
388 (EM®) on feed in vitro rumen fermentation and digestibility. *J. Anim. Sci.* 99:455-457.
389 <https://doi.org/10.1093/jas/skab235.810>.
- 390 11. Nalla, K., N.K. Manda, H.S. Dhillon, S.R. Kanade, N. Rokana, M. Hess, and A.K.
391 Puniya. 2022. Impact of probiotics on dairy production efficiency. *Front. Microbiol.*
392 13:805963. <https://doi.org/10.3389/fmicb.2022.805963>.
- 393 12. Nennich, T.D., J.H. Harrison, L.M.V. Wieringen, N.R. St-Pierre, R.L. Kincaid, M. A.
394 Wattiaux, and E. Block. 2006. Prediction and evaluation of urine and urinary nitrogen and
395 mineral excretion from dairy cattle. *J. Dairy Sci.* 89:353-364.
396 [https://doi.org/10.3168/jds.S0022-0302\(06\)72101-4](https://doi.org/10.3168/jds.S0022-0302(06)72101-4).
- 397 13. Nielsen, N.I., N.C. Friggens, T. Larsen, J.B. Andersen, M.O. Nielsen, and K.L.
398 Ingvarsen. 2007. Effect of changes in diet energy density on feed intake, milk yield and
399 metabolic parameters in dairy cows in early lactation. *Animals* 1:335-346.
400 <https://doi.org/10.1017/S1751731107683815>.
- 401 14. Niknia, A.D., R. Vakili, A. Mokhtarpour, and V. Palangi. 2025. Using yeast-based
402 probiotic as dietary supplement during transition period to improve performance of dairy
403 cows. *J. Hellenic. Vet. Med. Soc.* 76:8721-8728. <https://doi.org/10.12681/jhvms.37189>.
- 404 15. NRC. (2001). Nutrient requirements of dairy cattle, 7th ed. National Academies Press.
405 United States of America.
- 406 16. NRC. (2021). Nutrient requirements of dairy cattle, 8th ed. National Academies Press,
407 United States of America.
- 408 17. Ogunade, I., H. Schweickart, M. McCoun, K. Cannon, and C. McManus. 2019.
409 Integrating 16S rRNA sequencing and LC-MS-based metabolomics to evaluate the effects
410 of live yeast on rumen function in beef cattle. *Animals.* 9:28.
411 <https://doi.org/10.3390/ani9010028>
- 412 18. Oyeade, A.O., S. Lee, H. Sultana, K. Arriola, E. Duvalsaint, C.N. De Guzman, I.F.
413 Marenchino, L.M. Pacheco, F. Amaro, L.G. Ghizzi, and D. Vyas. 2023. Effects of direct-fed

- 414 microbial supplementation on performance and immune parameters of lactating dairy cows.
415 J. Dairy Sci. 106:8611-8626. <https://doi.org/10.3168/jds.2022-22898>.
- 416 19. Polyorach, S., R. Nampukdee, M. Wanapat, S. Kang, A. Cherdthong, O. Pongchompu,
417 P. Gunun, N. Gunun, S. Foiklang, A. Thinowong, and T. Norrapoke. 2023. Microbial
418 fermented liquid supplementation improves nutrient digestibility, feed intake, and milk
419 production in lactating dairy cows fed total mixed ration. Animals. 13:933.
420 <https://doi.org/10.3390/ani13050933>.
- 421 20. Ramirez-Garzon, O., D. Barber, L. Meneses, and M. Soust 2024. Effect of gestational
422 direct-fed microbials supplementation on the metabolic profile in periparturient dairy
423 cows. Animals. 14:2928. <https://doi.org/10.3390/ani14202928>.
- 424 21. Rathnayake Shaill R.M.H., W.A.D. Nayananjali, A.M.J.B. Adikari, and U.L.P.
425 Mangalika. 2026a. Effective microorganisms (EM): A promising approach to sustainable
426 dairy production: a review. Agric. Rev. 47:227-237. <https://doi.org/10.18805/ag.RF-393>.
- 427 22. Rathnayake Shaill R.M.H., W.A.D. Nayananjali, A.M.J.B. Adikari. 2026b. Role of
428 effective microorganisms (EM) in livestock nutrition and performance: a review. Agric. Rev.
429 47:1-6. <https://doi.org/10.18805/ag.RF-406>.
- 430 23. Reuben, R.C., M.M. Elghandour, O. Alqaisi, J.W. Cone, O. Márquez, and A.Z. Salem.
431 2022. Influence of microbial probiotics on ruminant health and nutrition: sources, mode of
432 action and implications. J. Sci. Food Agric. 102(4):1319-1340.
433 <https://doi.org/10.1002/jsfa.11643>.
- 434 24. Spanghero, M. and Z.M. Kowalski 2021. Updating analysis of nitrogen balance
435 experiments in dairy cows. J. Dairy Sci. 104:7725-7737. [https://doi.org/10.3168/jds.2020-](https://doi.org/10.3168/jds.2020-19656)
436 19656.
- 437 25. Spek, J.W., J. Dijkstra, G. Van Duinkerken, and A. Bannink. 2013. A review of factors
438 influencing milk urea concentration and its relationship with urinary urea excretion in
439 lactating dairy cattle. J. Agric. Sci. 151:407-423.
440 <https://doi.org/10.1017/S0021859612000561>.
- 441 26. Tadesse, E.T., G.A. Minneeneh, Y.Y. Mummed, M. Dejene, and E. Kebreab, 2024. Effect
442 of effective microorganisms treated wheat bran supplementation on intake, digestibility and

- 443 milk yield and composition of lactating cows fed natural pasture hay as a basal diet. LRRD.
444 36:11. <http://www.lrrd.org/lrrd36/2/3611eden.html>.
- 445 27. Terré, M., N. Prat, D. Sabrià, O. Queiroz, J.N. Joergensen, G. Copani, and B.I.
446 Cappellozza. 2024. Supplementing a *Bacillus*-based direct-fed microbial improves feed
447 efficiency in lactating dairy cows. Transl. Anim. Sci. 8: txae110.
448 <https://doi.org/10.1093/tas/txae110>.
- 449 28. Tesfaye, A. and Y. Hailu. 2019. The effects of probiotics supplementation on milk yield
450 and composition of lactating dairy cows. J. Phytopharmacol. 8:12-17.
- 451 29. Uyeno, Y., S. Shigemori, and T. Shimosato 2015. Effect of probiotics/prebiotics on cattle
452 health and productivity. Microbes Environ. 30:126-132.
453 <https://doi.org/10.1264/jsme2.ME14176>
- 454 30. Van Soest, P. V., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral
455 detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci.
456 74:3583-3597. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2)
- 457 31. Vasilev, V. and E. Videv. 2023. Fecal nitrogen excretion in dairy cows supplemented with
458 feed additive containing active dry yeast. Bulg. J. Anim. Husb. 60:29-35.
459 <https://doi.org/10.61308/VHQU9624>
- 460 32. Wang, L., S. Jin, P. Wang, X. Li, C. Liu, S. Sun, G. Zhang, J. Chang, Q. Yin, H. Zhang,
461 and Q. Zhu. 2024. Fermented total mixed ration enhances nutrient digestibility and
462 modulates the milk components and fecal microbial community in lactating Holstein dairy
463 cows. Front. Vet. Sci. 11:1408348. <https://doi.org/10.3389/fvets.2024.1408348>