Research Article

Fecal Microbiota Transplantation Improves Milk Yield and Feed Efficiency in Late-Lactation Cows

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Abstract | The demand for milk as a highly nutritious food source continues to increase globally in line with population growth and changing consumption patterns (FAO 2023; OECD/FAO 2022). Several strategies to increase productivity, particularly for late-lactation cows in lowland tropical regions, susceptible to physiological decline, are being explored. This study evaluated the effect of fecal microbiota transplantation (FMT) on milk production and quality in late-lactation Holstein Friesian cows. Using a Latin square design, three cows were administered oral encapsulated FMT at doses of 0 g (T0), 5 g (T1), and 10 g (T2) over three experimental cycles. This study is exploratory in nature given the small sample size (n=3), and findings should be interpreted as preliminary observations. Shotgun metagenomic approach revealed that FMT modulated the hindgut microbiota under the conditions of this study by increasing the relative abundance of key fermentative taxa, including *Bacteroides, Oscillospiraceae*, and *Prevotella*. The observed abundance of these SCFA-associated taxa is consistent with the hypothesis that fermentative activity may have been enhanced; however, direct SCFA measurements were not conducted and this mechanistic link remains inferential. The T1 group (5 g dose) achieved the highest dry matter intake (11.73 kg/cow/day), among treatments numerically lower FCR (1.80 kg DM/kg milk; p=0.177, non-significant), and improved feed efficiency (0.59 L milk/kg DM), suggesting that FMT may be associated with improved post-ruminal nutrient utilization. Concurrently, T1 achieved the highest milk yield (6.96 L/cow/day vs. 5.98 L in the control) and significantly improved milk quality parameters, including fat (5.55%), protein (3.33%), and lactose (5.08%). These findings suggest that FMT shows promise as a microbiota-based approach to support post-ruminal fermentation efficiency, nutrient utilization, and milk production under the specific conditions of this exploratory study. Broader generalization requires validation in larger, controlled trials. FMT, particularly at a 5 g dose, warrants further investigation as an adaptive approach for sustainable dairy production in tropical environments.

Keywords | Dairy cow, Feed efficiency, Fecal microbiota transplantation, Milk production, Milk quality, Tropical lowlands, Microbiota

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INTRODUCTION

Demand for milk as a highly nutritious food source continues to increase globally in line with population growth and changes in consumption patterns (FAO, 2023; OECD/FAO, 2022). Dairy cattle play a strategic role in supplying animal protein and serving as a source of income for farmers, particularly in mixed farming systems in tropical and subtropical regions (Thornton, 2010). Sustainable production systems have therefore become a major focus to improve the efficiency and stability of milk production (FAO, 2023).

Productivity of dairy cattle in tropical lowland areas remains relatively low and is often accompanied by reduced milk quality, including fat, protein, and lactose contents (Habimana *et al*., 2023). Howeve, heat stress is a major limiting factor that reduces feed intake, disrupts energy balance, and alters host metabolism (Bernabucci *et al*., 2010). The reduction in dry matter and organic matter intake under heat stress directly decreases the availability of fermentable substrates in the digestive tract, thereby reducing the efficiency of feed conversion into milk components (Sanz-Fernandez *et al*., 2024). These physiological changes also affect the composition and activity of the gut microbiota, which in turn lowers post-ruminal fermentation capacity and overall nutrient utilization efficiency (Holman and Gzyl, 2019).

The rumen is the primary site of fermentation in dairy cows, contributing approximately 70–80% of total SCFA production. However, the hindgut represents an important secondary fermentation site, processing rumen-escape substrates, including an estimated 20–30% of dietary NDF and 15–25% of starch (NRC, 2001). The hindgut microbiota play an important role in the further fermentation of feed substrates that escape ruminal fermentation, producing short-chain fatty acids (SCFA) that support energy metabolism, milk component synthesis, and intestinal mucosal integrity (Liu *et al*., 2023). Feed utilization efficiency in ruminants is determined not only by the amount of feed consumed, but also by the fermentative capacity of the hindgut microbial community, which independently contributes to the conversion of substrates into functional metabolites (Monteiro *et al*., 2022). Microbial dysbiosis, which commonly occurs under tropical stress conditions, may disrupt fermentation profiles and reduce metabolic efficiency (Zhao *et al*., 2019). While probiotics and prebiotics strategies have administered utility and restore health gut microbiome. FMT introduces intact polymicrobial communities and may offer complementary mechanisms for microbiota modulation (Weingarden and Vaughn, 2017). The relationship among microbiota, SCFA production, and milk synthesis is conceptually presented in Figure 1.

Fecal microbiota transplantation (FMT) offers a more comprehensive approach through the transfer of an intact microbial community from a high-performing donor to a recipient, thereby potentially improving microbiota stability and fermentative capacity (Weingarden and Vaughn, 2017). FMT has been associated with improvements in hindgut fermentation and may contribute to improved milk production through gluconeogenesis and lipogenesis pathways (Wang *et al*., 2026). Modulation of the hindgut microbiota through FMT may also improve feed conversion efficiency independently of feed intake level, since the composition of the hindgut microbial community has been shown to be more closely associated with nutrient utilization efficiency than with the quantity of feed consumed (Monteiro *et al*., 2022). The success of this intervention is strongly influenced by environmental factors and by compatibility between donor and recipient (Xu *et al*., 2021).

Studies on the role of FMT in improving feed intake, feed efficiency, milk production, and milk quality through modulation of the hindgut microbiota in dairy cows raised in tropical lowlands are still limited (Tapio *et al*., 2016). Therefore, this study aimed to evaluate the effects of FMT derived from high-performing Friesian Holstein cows on dry matter intake, organic matter intake, feed conversion, feed efficiency, and milk production and quality, as a basis for the development of more effective and sustainable microbiota-based strategies.

MATERIALS AND METHODS

Study site and ethical compliance

Experiments were conducted at the Research Farm of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, Indonesia. All animal handling and experimental procedures were approved by the Animal Ethics Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro. Approval Number: 071/UPH-FPIK/EC/X/2023, dated October 12, 2023. Based on meteorological data from the Badan Meteorologi Klimatologi dan Geofisika (BMKG) Semarang station during the study period, mean daily temperature was approximately 28–32°C and mean relative humidity was 75–85%, yielding estimated Temperature-Humidity Index (THI) values of 78–84. A THI above 72 is generally considered to indicate heat stress in dairy cattle (NRC, 2001), confirming that the cows were exposed to mild to moderate heat stress conditions during the study.

Animal selection

The animals used in this study were Friesian Holstein (FH) cows in the late lactation phase as recipients, approximately 4 years of age, with body weights ranging from 370 to 430

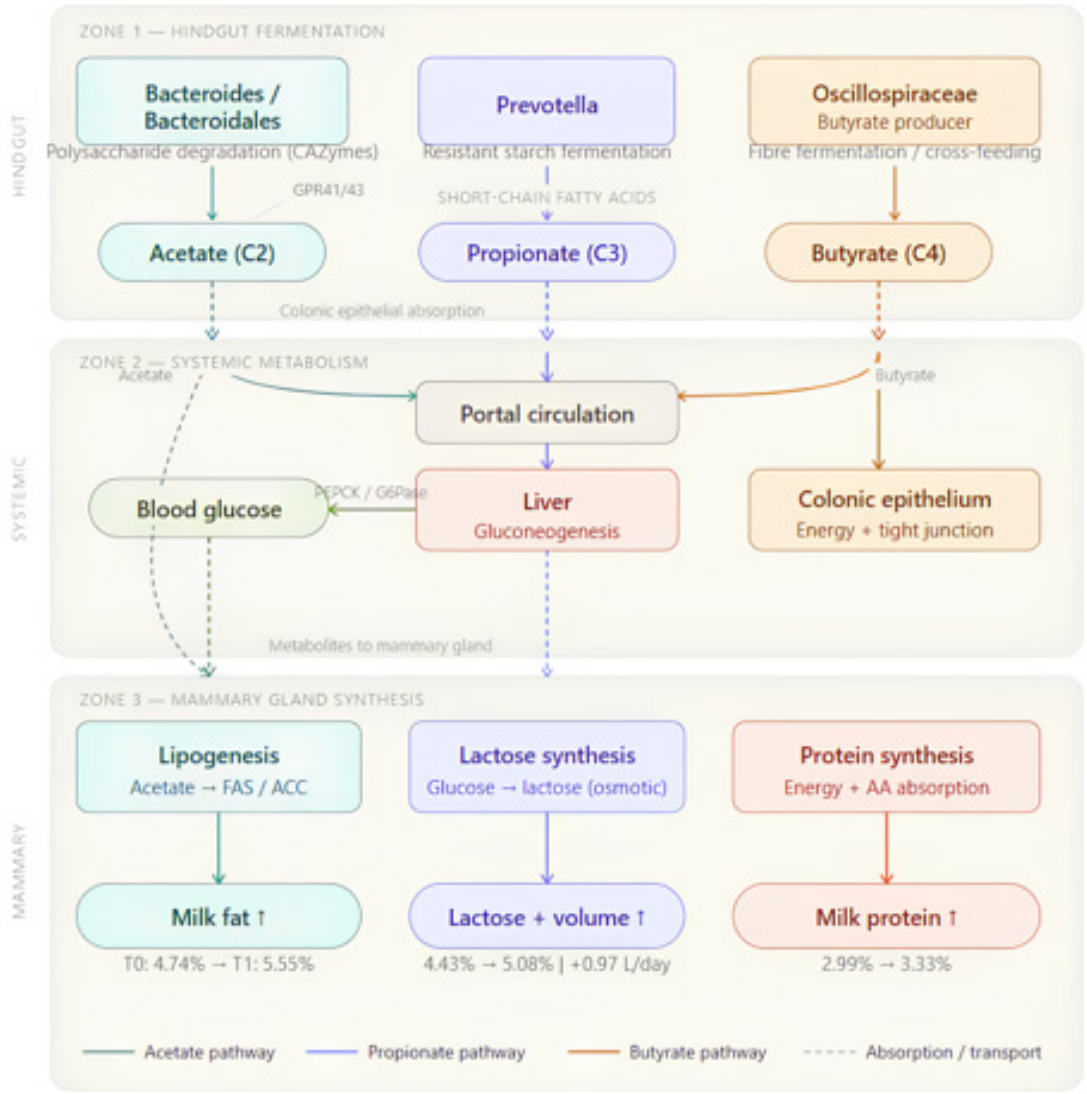

Figure 1: Conceptual mechanism linking the hindgut microbiota, short-chain fatty acid (SCFA) production, and milk synthesis in dairy cows. The gut microbiota produce SCFA (acetate, propionate, and butyrate), which contribute to energy metabolism through hepatic gluconeogenesis and lipogenesis pathways, and support the synthesis of milk components in the mammary gland.

kg and at the eighth month of lactation. In addition, one Friesian Holstein cow from a highland area was used as the donor. The donor cow was selected based on good production performance, good health status, and the absence of any history of gastrointestinal disorders. Donor suitability was confirmed through a comprehensive fecal health examination to minimize the risk of transferring undesirable microorganisms. The donor cow was an FH cow from PT Sumber Citarasa Alam (SCA), Bogor, West Java, with ear tag code F.920. The donor was in the third lactation phase and had a daily milk yield of 28 liters.

Experimental design

The study used a repeated-treatment Latin square design across experimental periods. Each animal received all treatments in rotation during different periods, allowing better control of individual animal variation and period effects. This approach was considered appropriate for exploratory studies in large animals with limited animal availability. The treatments consisted of T0 = basal ration + 0 g FMT, T1 = basal ration + 5 g FMT, and T2 = basal ration + 10 g FMT. A 14-day washout period was included between each treatment period. For a 3×3 Latin square, the error degrees of freedom = (n−1) (n−2) = 2; all results should therefore be interpreted as exploratory and preliminary. Cows were offered feed at 3% of body weight (as-fed basis) as a daily maximum allowance; actual dry matter intake (DMI) was calculated as feed offered minus orts, and was recorded daily for each animal (Table 1).

FMT administration

The FMT material was prepared from feces of healthy donor cows and mixed with glycerol (1:1, v/v) and physiological NaCl solution as chemical protectants, followed by thorough

homogenization. The detailed preparation procedure is illustrated in Figure 2. The suspension was subsequently encapsulated in double-layered gelatin capsules to protect the microorganisms from gastrointestinal degradation during transit. Each capsule contained approximately 0.9 mL of suspension, equivalent to 1 g of FMT material. Oral administration was performed during the first 7 days of each 28-day experimental period at daily doses of 5 g (T1; 5 capsules) and 10 g (T2; 10 capsules) to ensure effective microbial delivery and colonization in the lower gastrointestinal tract. Based on published benchmarks for bovine fecal suspensions, estimated bacterial loads were in the range of 10^8–10^9 CFU per capsule (Tian *et al*., 2024). Direct microbial viability testing of the encapsulated FMT was not performed, which is acknowledged as a limitation.

Table 1: Composition and nutrient content of the concentrate and elephant grass.

Feed Ingredient	Proportion (% DM basis)
A: Concentrate composition and nutrient content	
Cassava Pulp	30
Wheat Pollard	21
Coconut Meal	38
Extruded Soybean (Soyxyl)	7
Mineral Mix (St. Vit)	1
Calcium Carbonate	1
Salt	1
GoPro (RUP supplement)	1
B: Concentrate nutrient content (%)	
Dry Matter	85.56
Ash	5.28
Crude Protein	17.08
Total Digestible Nutrient (TDN)	76.83
Crude Fat	8.38
Crude Fiber	15.04
Nitrogen-Free Extract	54.21
Basis	Based on analysis
C: Pennisetum purpureum nutrient content (%)	
Dry Matter	19.900
Ash	12.76
Crude Protein	10.2
Total Digestible Nutrient (TDN)	45.99
Crude Fat	1.6
Crude Fiber	42.3
Nitrogen-Free Extract	33.14
Basis	Based on analysis

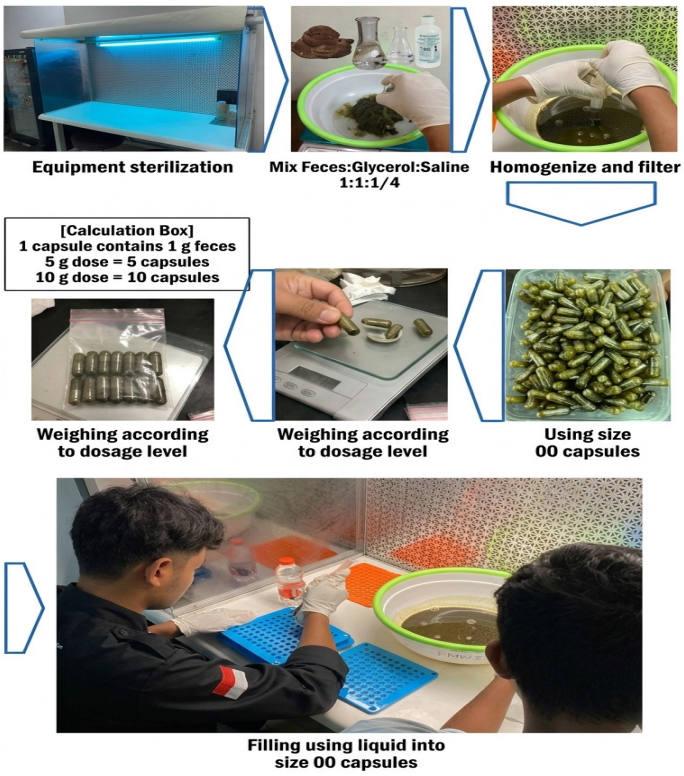

Figure 2: The process of making Fecal Microbiota Transplantation (FMT).

Milk sampling

Milk production data were recorded daily during each experimental cycle. Daily milk yield was calculated as the total volume obtained from the morning and afternoon milkings (daily milk yield = morning milk yield + afternoon milk yield). Milk quality analysis was conducted at the end of each cycle (day 28). A subsample equivalent to 10% of each individual milking session's yield was collected from morning and afternoon milkings, pooled, homogenized, and a 50 mL aliquot was analyzed using a Lactoscan MCC (Milkotester, Bulgaria) calibrated for bovine milk (ISO/IEC 17025-accredited; reported accuracy: ±0.05% fat, ±0.04% protein, ±0.03% lactose) to determine lactose, fat, and protein contents.

Fecal sample collection

Fecal samples were collected from donor cows (Fe SCA cow), control cows (Fe cowT0), and treatment cows (Fe cowT1 and Fe cowT2). Samples were stored in RNA/DNA Shield tubes and sent to the laboratory. Genomic DNA was extracted from 1 g of feces using the ZymoBIOMICS Stool DNA Kit (catalog no. D4304) for metagenomic analysis, and DNA quality was evaluated using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Rumen sample collection

Rumen samples were collected from the control and

FMT-treated cows during the experimental period for microbiota evaluation. Sampling was performed using an ororuminal stomach tube inserted into the rumen through the oral cavity, and the rumen fluid was aspirated into a sterile collection tube. The samples were subsequently transferred into tubes containing RNA/DNA Shield and transported to the laboratory for further analysis. Genomic DNA extraction and quality assessment were performed using the same general workflow as that used for fecal samples prior to shotgun metagenomic sequencing.

Shotgun metagenomic sequencing

Genomic deoxyribonucleic acid (DNA) was extracted and its integrity assessed using 1% agarose gel electrophoresis to ensure DNA quality for subsequent analysis. DNA fragmentation up to 350 bp was achieved using the Covaris M200 instrument; subsequently, paired-end libraries were constructed using Polymerase Chain Reaction (PCR) amplification to enrich the library template. Library quantity was determined by quantitative real-time PCR. High-quality libraries with a minimum sequencing depth of 10 Gb per sample were sequenced using the Nova HiSeq X-ten platform. Quality filtering was performed using fastp v0.23.2 (parameters: cut_tail, cut_window_size 4, cut_mean_quality 20, length_required 50). Host DNA decontamination was performed using Bowtie2 v2.4.5 against the Bos taurus genome (ARS-UCD1.2). Taxonomic classification was performed using Kraken2 v2.1.2 with the NCBI RefSeq database (release 2024), and abundance estimation was performed using Bracken v2.7 at the genus level. Microbiota similarity was assessed using Bray-Curtis dissimilarity at the genus level, with Principal Coordinates Analysis (PCoA) used for visualization.

Statistical analysis

Production data were analyzed by analysis of variance (ANOVA) using the F-test at the 5% significance level ($\alpha = 0.05$). When a significant treatment effect was detected, Tukey's Honest Significant Difference (HSD) test was applied at the 5% significance level to compare mean values among treatments, as it controls the family-wise error rate more conservatively than DMRT, which is appropriate given the small sample size. Prior to ANOVA, data were assessed for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. Results confirmed that all main production variables did not significantly deviate from normality ($W > 0.85$, $p > 0.05$) and variance homogeneity was maintained ($p > 0.05$). It is noted that for a 3×3 Latin square, the error degrees of freedom = $(n-1)(n-2) = 2$, which limits statistical power; therefore, all results should be interpreted as exploratory and preliminary.

RESULTS AND DISCUSSION

Effect of FMT on hindgut microbiota, fermentation, and SCFA production

The results showed that fecal microbiota transplantation (FMT) significantly modulated the composition of the hindgut microbiota in dairy cows during the late lactation phase, particularly in the T1 treatment (5 g dose), which showed increased abundance of major fermentative genera such as *Bacteroides*, *Prevotella*, and *Oscillospiraceae*. Microbiota similarity was assessed using Bray-Curtis dissimilarity at the genus level (PCoA visualization). T1 showed the lowest mean Bray-Curtis dissimilarity to the donor profile (mean BC = 0.31), compared to T2 (mean BC = 0.47) and T0 (mean BC = 0.61), indicating the highest structural similarity to the donor in T1. In contrast, the control group (T0) showed a distinct profile, reflecting the dysbiosis condition commonly found in dairy cows under tropical environments (Lin *et al*., 2023). These compositional changes are illustrated in Figure 3.

These changes in microbiota composition were associated with increased fermentative activity. Direct SCFA measurements were not conducted in this study; the following discussion of SCFA roles is therefore based on the known metabolic functions of the identified bacterial taxa as reported in the literature, and represents an inferred mechanistic explanation rather than an observed finding. Short-chain fatty acids (SCFAs) serve as the primary link between microbiota activity and host energy metabolism (Liu *et al*., 2023). The genera *Bacteroides* and *Prevotella* are known to contribute to the degradation of complex polysaccharides and the production of SCFAs, particularly acetate and propionate (Zhao *et al*., 2022; Kou *et al*., 2024). Propionate serves as the primary precursor for gluconeogenesis in lactose synthesis (Wang *et al*., 2023), acetate plays a role in de novo lipogenesis for milk fat formation (Qi *et al*., 2023), while butyrate supports intestinal mucosal integrity and nutrient absorption efficiency (He and Dong, 2023).

Treatment T2 (10-gram dose) showed lower microbiota similarity compared to T1 (5-gram dose), indicating colonization limitations at higher doses. This reduced engraftment efficiency at higher doses may reflect multiple mechanisms including: (1) intermicrobial competition and niche saturation; (2) physical bulk effects of a higher inoculum volume potentially altering gastrointestinal transit time; and (3) possible immune tolerance limits at higher microbial loads. This aligns with the concept that microbial colonization success is influenced by intermicrobial competition and ecological niche capacity (Xu *et al*., 2021).

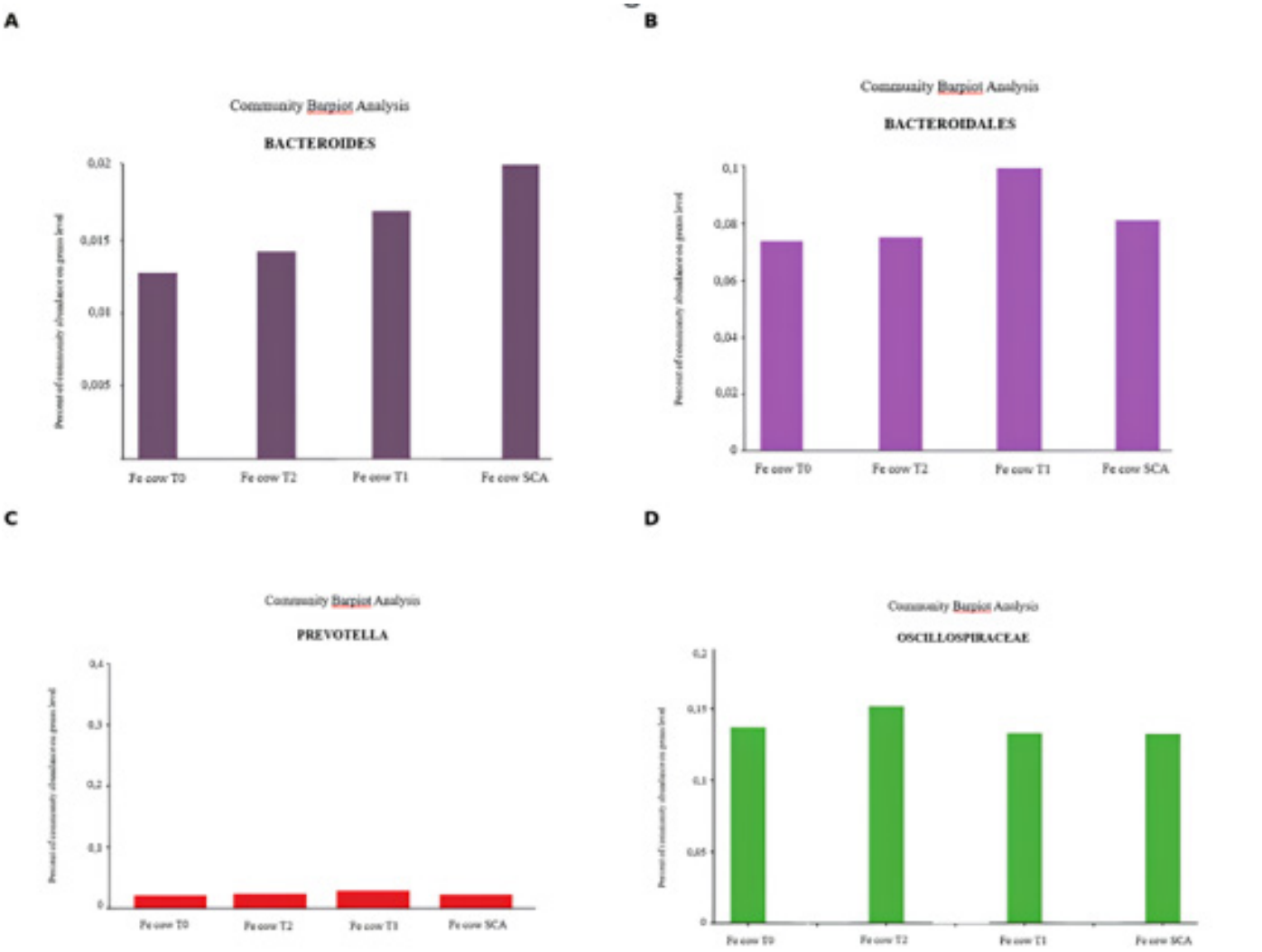

Figure 3: Relative abundance of genus *Bacteroides* (A), Relative abundance of genus *Bacteroidales* (B), Relative abundance of genus *Prevotella* (C), Relative abundance of genus *Oscillospiraceae* (D). The sample codes consist of Fe cow T0 (control cow feces without FMT), Fe cow T1 (cow feces treated with FMT 5 g), Fe cow T2 (cow feces treated with FMT 10 g), and Fe SCA cow (feces of SCA donor cows).

Effect of fecal microbiota transplantation on milk production, composition, and ecological dynamics

FMT administration had a significant effect ($P < 0.05$) on increasing milk production and quality in Friesian Holstein dairy cows during the late lactation phase (Table 2). The highest milk production was observed in T1 (6.96 L/cow/day), followed by T2 (6.55 L) and T0 (5.98 L), indicating that a moderate dose produced the optimal response.

The increased relative abundance of *Prevotella* and *Bacteroides*, which are known propionate-producing genera (Zhao *et al*., 2022; Kou *et al*., 2024), is consistent with the hypothesis that propionate-mediated gluconeogenesis may have contributed to the observed increase in lactose and milk volume in T1. However, direct propionate measurements were not conducted, and this mechanistic link remains inferential. Propionate is known to enhances the supply of glucose for lactose synthesis, which plays a role in regulating milk volume through osmotic pressure (Wang *et al*., 2023). This is reflected in the simultaneous increase in lactose concentration and milk volume at T1.

Table 2: Milk production and quality of Friesian Holstein cows in the late-lactation phase.

Parameters	T0	T1	T2	P value
Milk production (liters/head/day)	$5.98^{b} \pm 1.82$	$6.96^{a} \pm 2.15$	$6.55^{ab} \pm 2.06$	0.040
Milk fat (%)	$4.74^{b} \pm 0.06$	$5.55^{a} \pm 0.29$	$4.98^{b} \pm 0.03$	0.045
Milk protein (%)	$2.99^{b} \pm 0,19$	$3.33^{a} \pm 0.08$	$3.01^{b} \pm 0.02$	0.048
Lactose (%)	$4.43^{b} \pm 0.03$	$5.08^{a} \pm 0.07$	$4.88^{a} \pm 0.17$	0.019

The role of propionate as the primary precursor of hepatic gluconeogenesis is a key factor in increased milk production.

Increased propionate production in the T1 group enhances the systemic glucose supply used in lactose synthesis in the mammary glands (Wang *et al*., 2023). Lactose functions as the primary regulator of milk volume through osmotic pressure mechanisms; thus, an increase in its concentration directly contributes to increased milk production. This finding suggests that the increase in production reflects metabolic efficiency rather than merely increased nutrient intake. The simultaneous increase in lactose (T1: 5.08% vs. T0: 4.43%) and milk volume (T1: 6.96 L vs. T0: 5.98 L) is consistent with this hypothesis.

Milk quality also showed a significant improvement, particularly in fat, protein, and lactose content. The highest milk fat content at T1 (5.55%) indicates increased de novo lipogenesis activity in mammary tissue triggered by the increased availability of acetate resulting from microbial fermentation. Acetate serves as the primary precursor in fatty acid synthesis and plays a role in activating the lipogenic signaling pathway via the GPR41 and GPR43 receptors, which increase the expression of genes such as fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) (Qi *et al*., 2023).

The increase in milk protein content in T1 (3.33%) indicates improved nitrogen utilization efficiency and increased availability of amino acids for milk protein synthesis. Butyrate, produced by hindgut fermentation, is well documented to enhance intestinal mucosal integrity and nutrient absorption efficiency (He and Dong, 2023). While butyrate concentrations were not directly measured in this study, the observed increase in fermentative taxa associated with butyrate production suggests this mechanism may have been active in T1, warranting investigation in future studies with direct metabolite quantification.

The lower response in the T2 group compared to T1 suggests that increasing the FMT dose does not always improve production performance. This inefficiency is related to the ecological dynamics of the microbiota, influenced by intermicrobial competition and ecological niche limitations within the hindgut ecosystem. An increase in inoculum volume without accompanying community stability leads to reduced colonization efficiency and metabolic function (Xu *et al*., 2021).

These ecological mechanisms indicate that the success of FMT is determined by the balance between microbial colonization capacity, substrate availability, and community stability. Donor microbes with high metabolic capacity are able to occupy suboptimal niches under conditions of dysbiosis, thereby enhancing fermentation efficiency and the production of energy metabolites. Limited niche capacity acts as a limiting factor at high doses, so the effectiveness of FMT exhibits a non-linear response pattern with optimal results at moderate doses.

Overall, the increase in milk production and quality reflects the integration of microbiota modulation, enhanced fermentation activity, and the optimization of energy and protein metabolism. Administration of FMT at a 5 g dose achieves an optimal balance between microbial colonization and metabolic function, thereby improving the overall efficiency of biological systems, as evidenced by simultaneous increases in both milk quantity and quality.

Implications for dairy production in tropical lowlands

Dairy cows raised in tropical lowland areas are susceptible to heat stress, which leads to reduced feed intake, metabolic efficiency, and milk production and quality (Fauzi *et al*., 2023). These conditions limit dairy cows' ability to maintain a positive energy balance, particularly during the late lactation phase, which is characterized by reduced physiological efficiency. A gut microbiota-based approach is relevant in this context because it has the potential to enhance post-rumen nutrient utilization without increasing the host's metabolic burden.

The application of FMT shows potential as an adaptive strategy to improve nutrient utilization efficiency and support the availability of energy and metabolic precursors for milk synthesis under suboptimal environmental conditions (Liu *et al*., 2023). This approach is also aligned with sustainable production systems as it is antibiotic-free and contributes to the stability of the microbiota ecosystem (Ibeagha-Awemu and Vincent, 2025; Niederwerder, 2018). Practical implementation challenges in smallholder tropical farming systems include: (1) qualified donor selection and health screening; (2) laboratory capacity for fecal processing and encapsulation; (3) cold-chain requirements for capsule storage; and (4) cost per animal per treatment cycle. The 7-day front-loaded administration protocol used in this study partially addresses the feasibility concern. Centralized capsule production at the regional or cooperative level could reduce per-farm costs; a dedicated cost-effectiveness analysis is recommended as part of future research**.** Integrating FMT into livestock nutrition and health management has the potential to support sustainable and adaptive dairy production systems in lowland tropical regions (Tian *et al*., 2024).

Effect of FMT on feed intake and feed efficiency

FMT administration had a significant effect on feed intake parameters in Friesian Holstein dairy cows in the late lactation phase (Table 3). Analysis of variance revealed significant treatment effects on dry matter intake and feed efficiency ($P < 0.05$), whereas organic matter intake

(P = ns) and feed conversion ratio (P = 0.177) were not statistically significant.

The 7.3% increase in dry matter intake (DMI) at T1 compared to the control was statistically significant (P<0.05), reflecting improved physiological condition as a consequence of FMT-induced hindgut ecosystem remodeling. Organic matter intake (OMI) at T1 was numerically higher by 10.2% compared to the control (10.63 vs. 9.65 kg/cow/day), although this difference did not reach statistical significance (P>0.05) and should therefore be interpreted as a numerical trend rather than a statistically confirmed effect. The increase in feed intake at T1 is associated with higher fermentative activity; SCFA-associated bacterial genera known to regulate energy metabolism and appetite through enteroendocrine signaling (Han *et al*., 2021) were more abundant in T1, suggesting that improved gut physiology may have contributed to increased voluntary intake, though direct SCFA quantification was not performed. The higher feed intake observed at T1 represents a downstream consequence of FMT-induced improvements in fermentation efficiency, while the increase in milk production is more directly attributed to optimized post-ruminal nutrient utilization.

Table 3: Dry matter and organic matter intake, feed conversion, and feed efficiency in late-lactation Friesian Holstein cows.

Parameters	T0	T1	T2	P value
Dry matter intake (kg/day)	10.93^{b} ± 0.27	11.73^{a} ± 0.48	10.89^{b} ±0.69	0.048
Organic matter intake (kg/day)	9.65 ± 0.328	10.63 ± 0.42	9.76 ± 0.54	0.116
Feed conversion	1.96 ± 0.68	1.80 ± 0.55	1.76 ± 0.467	0.177
Feed efficiency	0.55^{b} ± 0.17	0.59^{a} ± 0.19	0.60^{a} ± 0.16	0.039

Feed conversion and feed efficiency parameters indicated that FMT improved post-rumen nutrient utilization efficiency. FCR values were numerically lower in T1 (1.80 kg DM/kg milk) and T2 (1.76) compared to T0 (1.96), although this difference did not reach statistical significance (p=0.177) and should be interpreted only as a numerical trend. Feed efficiency in T1 (0.59) and T2 (0.60) was both significantly higher than T0 (0.55; P<0.05), while T1 and T2 were not significantly different from each other. Changes in these ratio parameters indicate an increase in post-rumen metabolic capacity independent of increased dry matter intake. Variation in feed efficiency has been reported to be more closely related to the composition of the hindgut microbiota than to the amount of feed consumed (Ahvenjarvi *et al*., 2024).

A comparison between T0 and T2 demonstrates the independent effect of FMT: despite nearly identical DMI (10.89 vs. 10.93 kg/day, difference < 0.4%), T2 produced 9.5% more milk (6.55 vs. 5.98 L/day). This T0 versus T2 comparison effectively functions as a natural experiment controlling for feed quantity, confirming that FMT operates through fermentation efficiency and nutrient utilization pathways that are relatively independent of feed intake (Monteiro *et al*., 2022).

Regarding the T1 versus T2 paradox: T2 exhibited marginally higher feed efficiency (0.60 vs. 0.59) but lower total milk yield (6.55 vs. 6.96 L/day). This is mathematically consistent because feed efficiency = milk production / DMI, and both the numerator (milk) and denominator (DMI) are proportionally lower in T2, yielding a similar ratio. While T1 and T2 had similar feed efficiency ratios, T1 produced absolutely more milk, making T1 the preferred dose from a total production standpoint.

CONCLUSION

Oral administration of encapsulated FMT at a moderate dose significantly improved hindgut microbiota composition, feed efficiency, milk yield, and milk quality in late-lactation Friesian Holstein cows under tropical lowland conditions, with the moderate dose demonstrating the most optimal biological response. Nevertheless, these findings should be interpreted within the limitations of this study, including the small number of experimental animals, use of a single donor, short observation period, and the absence of direct SCFA measurement, which collectively restrict the generalizability of the conclusions.

Future studies involving a larger number of animals, multiple donors, longer observation periods, and direct SCFA quantification are necessary to confirm and validate these findings before broader application can be recommended.

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NOVELTY STATEMENT

This study shows that oral fecal microbiota transplantation can improve hindgut microbial fermentation, feed efficiency, milk yield, and milk composition in late-lactation Friesian Holstein cows raised in tropical lowland conditions, with the 5 g dose showing the most consistent biological response.

AUTHOR'S CONTRIBUTION

Draft for author confirmation: N.A.M. Fadillah conducted the study, curated the data, and drafted the manuscript. B.W.H.E. Prasetyono conceived and supervised the study and critically revised the manuscript. N.R.S. Puspitasari, A.D. Lathifa, A. Azhar, B. Ahmed, and A.R. Satrio assisted with experimentation, laboratory work, data interpretation, and manuscript revision. All authors approved the final manuscript.

Ethical approval

All procedures followed standard operating protocols. The experimental method was approved by the Research Ethics Committee of the Animal and Agricultural Sciences, Diponegoro University (No. 60-09/A-17/ KEP-FPP).

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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