



Nutrient Utilization in Bulls Fed Fermented Rations Containing Palm Decanter Cake and *Bacillus subtilis*

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ABSTRACT

Palm decanter cake (PDC) is an agro-industrial by-product with potential as an alternative feed resource for ruminants, although its relatively high fiber content may limit nutrient utilization. Incorporating PDC with probiotic *Bacillus subtilis* (PB) in fermented total mixed rations (FTMR) may provide a practical strategy for utilizing this by-product in beef cattle diets. This study evaluated animal responses to FTMR dietary regimens containing PDC and PB, with emphasis on feed intake, nutrient digestibility, rumen fermentation, microbial populations, and serum metabolites in beef cattle. Five Angus-crossbred bulls (initial body weight 477 ± 44 kg) were assigned to a 5×5 Latin square design. Dietary treatments consisted of a control FTMR without PDC or PB and four FTMR diets containing 10% or 20% PDC on a DM basis, supplemented with either 0.05% or 0.10% probiotic *B. subtilis*. Feed intake, apparent nutrient digestibility, rumen fermentation characteristics, microbial populations, and serum metabolites were determined. Dietary treatments did not affect dry matter intake, ruminal pH, $\text{NH}_3\text{-N}$ concentration, total volatile fatty acids, microbial populations, or serum metabolites ($p > 0.05$). Ether extract intake differed among dietary regimens ($p < 0.05$), with greater values in cattle receiving the diets containing 20% PDC with either PB level. Among the dietary regimens evaluated, the complete diet containing 20% PDC and 0.10% PB resulted in the greatest apparent digestibility of dry matter, organic matter, ether extract, neutral detergent fiber, and acid detergent fiber ($p < 0.05$). Sampling time significantly influenced rumen fermentation parameters, microbial populations, and serum metabolites ($p < 0.05$), reflecting normal postprandial changes after feeding. The digestibility response was attributable to the complete dietary regimen. The independent effects of PDC and PB and their interaction could not be isolated in this design. These results suggest that PDC can be included at up to 20% of dietary dry matter in FTMR containing *B. subtilis* as an alternative feed resource for beef cattle.

Keywords: agro-industrial by-product; beef cattle; nutrient utilization; probiotic

INTRODUCTION

The increasing demand for beef, driven by population growth, urbanization, and rising incomes, has intensified livestock production systems worldwide (Pulina *et al.*, 2021). Consequently, feed costs and competition for conventional feed resources have increased, particularly in tropical regions where feed availability and production efficiency are major concerns. Therefore, the use of locally available agro-industrial by-products as alternative feed resources has gained increasing attention for improving the sustainability and cost-efficiency of ruminant production systems (Shah *et al.*, 2025).

Palm decanter cake (PDC) is a solid by-product

generated during the crude palm oil extraction process in palm oil mills. During processing, fresh fruit bunches are sterilized and mechanically pressed to extract crude palm oil. The resulting slurry is then separated using a decanter centrifuge, and the solid fraction recovered from this process is referred to as PDC or decanter cake (Chan *et al.*, 2021). PDC production accounts for approximately 4%–5% of the fresh fruit bunches processed, resulting in substantial quantities being generated annually in palm oil-producing countries (Husin *et al.*, 2022). Despite its abundant availability, the utilization of PDC as a livestock feed ingredient remains relatively limited (Lim *et al.*, 2022). Because it originates from residual mesocarp tissues and oil-bearing solids, PDC contains moderate crude protein, relatively high

ether extract, and considerable fiber fractions, suggesting its potential as an alternative energy-rich feed ingredient for ruminants (Adebiyi *et al.*, 2020; Abdullah *et al.*, 2025). Recent studies have further demonstrated the potential of solid decanter cake as a ruminant feed ingredient. Santoso *et al.* (2025) reported that diets containing solid decanter cake at inclusion levels of 8.0% and 17.5% achieved dry matter digestibility values ranging from 62.62% to 65.71% and supported microbial fermentation when combined with readily fermentable carbohydrate sources. Based on these findings, PDC inclusion levels of 10% and 20% of dietary DM were selected as practical moderate and relatively high inclusion rates, respectively, within the complete FTMR dietary regimens evaluated in the present study. Abdullah *et al.* (2025) also found that a PDC-based diet supported weight gain in goats, providing additional evidence of its potential as an alternative feed resource for ruminants. More recently, an *in vitro* study using ruminal inoculum from beef cattle showed that solid decanter cake maintained dry matter degradability at 57.73% and increased total volatile fatty acid and propionate production as well as microbial protein synthesis (Yulistiani *et al.*, 2026). Nevertheless, the relatively high fiber and residual lipid contents of PDC may limit nutrient accessibility and lead to variable animal responses depending on its inclusion level, overall dietary composition, and processing method.

Fermented total mixed ration (FTMR) is widely used in ruminant production systems because of its benefits for feed preservation, palatability, and nutrient utilization. Although lactic acid bacteria (LAB) are commonly applied as silage inoculants to improve fermentation quality (Yin *et al.*, 2023), their effects on nutrient utilization and aerobic stability may be limited, increasing interest in alternative additives such as *Bacillus subtilis*. This bacterium is tolerant of adverse environmental conditions and can produce lignocellulolytic enzymes that enhance fiber degradation and improve silage quality (Guo *et al.*, 2022). In addition, *B. subtilis* produces antimicrobial compounds that suppress undesirable microorganisms, thereby reducing silage spoilage and improving aerobic stability (Bai *et al.*, 2020; Bonaldi *et al.*, 2021). As a primarily aerobic bacterium, *B. subtilis* may help establish favorable anaerobic conditions through oxygen consumption, thereby supporting microbial balance (Su *et al.*, 2020). Previous studies have further reported that *B. subtilis* supplementation can enhance rumen microbial activity, improve nutrient digestibility, and stabilize rumen fermentation (Meenongyai *et al.*, 2023).

Combining PDC with *B. subtilis* in FTMR may provide complementary functions beyond those expected from either component alone. PDC supplies residual lipids, proteins, and structural carbohydrates, whereas *B. subtilis* may improve the fermentation environment by consuming oxygen, suppressing undesirable microorganisms, and producing extracellular enzymes. These microbial activities could increase the accessibility of nutrients associated with the fibrous PDC fraction. However, these proposed mechanisms were not measured directly in the present study and should be regarded as a biological rationale rather than evidence of synergistic activity.

Although PDC and *B. subtilis* have independently demonstrated potential as feed ingredients and microbial additives for ruminants, respectively, limited information is available on animal responses to FTMR containing both components. In particular, it remains unclear whether complete dietary regimens containing PDC and *B. subtilis* can improve nutrient utilization while maintaining favorable ruminal fermentation, microbial populations, and metabolic status in beef cattle under tropical production conditions.

Therefore, this study evaluated the effects of complete FTMR dietary regimens containing PDC and *B. subtilis* on feed intake, nutrient digestibility, ruminal fermentation characteristics, ruminal microbial populations, and serum metabolites in Angus crossbred bulls. We hypothesized that, compared with the control FTMR, the dietary regimens containing PDC and *B. subtilis* would increase the apparent digestibility of dry matter, organic matter, and fiber fractions. We further hypothesized that these regimens would maintain ruminal pH within the physiological range, increase volatile fatty acid production, and support favorable ruminal microbial populations without adversely affecting feed intake or serum metabolite profiles.

MATERIALS AND METHODS

Animals, Dietary Treatments, and Data Collection

All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee (IACUC) of Kasetsart University, Thailand (Approval No. ACKU67-CSC-007). Five Angus-crossbred bulls (50% Angus, 25% Charolais, 12.5% Brahman, and 12.5% Thai native), aged 24–28 months, with an average body weight of 477 ± 44 kg, were assigned to a five-period crossover experiment arranged as a 5×5 Latin square design. Each animal received each of the five dietary treatments once, while the treatment sequence was balanced across animals and periods according to the Latin square arrangement. Each experimental period lasted 21 days and consisted of 14 days of adaptation followed by 7 days of sample collection. No separate washout period using the basal diet was implemented between experimental periods. Instead, the 14-day adaptation phase allowed the animals to adjust to the newly assigned diet and was intended to minimize potential carryover effects from the preceding treatment. Before the experiment, all animals were treated with a commercial anthelmintic (Ivomec-F, Vet Inter Pharma Co., Ltd., Samut Prakan, Thailand). Bulls were housed individually in concrete-floored pens (3×4 m) equipped with individual feed troughs and an automatic drinker. A 30-day pre-experimental adaptation period was provided before the study, and body weight was recorded at the beginning and end of each period.

The experimental treatments consisted of five distinct dietary regimens: T1, 0% PDC + 0% PB (control); T2, 10% PDC + 0.05% PB; T3, 10% PDC + 0.10% PB; T4, 20% PDC + 0.05% PB; and T5, 20% PDC + 0.10% PB. Because the control treatment did not include PDC or PB and the design did not contain all possible combinations of the two factors, the treatments were evaluated as

five distinct dietary regimens rather than as a complete factorial arrangement. Diets were formulated to contain 12.0% CP and 65.0% TDN to meet the nutrient requirements of growing–fattening beef cattle with approximately 450–500 kg body weight according to NASEM (2016). The PDC was used to partially replace rice bran and naturally fermented cassava pulp as alternative energy and lipid sources. Because of the relatively high crude protein content of PDC, urea inclusion was reduced as the PDC level increased to maintain isonitrogenous diets. Palm decanter cake was obtained from a commercial palm oil processor (Isan Palm Industry Development Company Limited, Sakon Nakhon, Thailand). Fresh cassava pulp was naturally fermented without the addition of fungal or bacterial inoculants. The cassava pulp was stored in sealed 200-L blue plastic drums (Kunshan Zhida Plastic Products Co., Ltd., Jiangsu, China) and allowed to ferment under anaerobic conditions at ambient temperature (28–35 °C) for at least 21 days through the activity of indigenous microorganisms before being used in the experimental diets. The probiotic product (Sporezyme-Forte, AMCOVET Co., Ltd.) contained *B. subtilis* at a minimum viable concentration of greater than 1×10^{11} CFU/g. The product was included at 0.05% or 0.10% of dietary DM, equivalent to 0.5 or 1.0 g/kg DM, respectively. Based on the observed mean DMI for each treatment, the estimated daily intakes of the probiotic product were 4.55 and 8.65 g/animal for the diets containing 10% PDC with 0.05% and 0.10% probiotic, respectively. The corresponding values were 4.71 and 9.15 g/animal for the diets containing 20% PDC with 0.05% and 0.10% probiotic, respectively. These intakes provided estimated minimum daily doses of greater than 4.55×10^{11} , 8.65×10^{11} , 4.71×10^{11} , and 9.15×10^{11} CFU/animal, respectively. These estimates were calculated from the minimum viable concentration declared by the manufacturer and the mean DMI recorded for each treatment. The PB inclusion levels were selected based on the manufacturer's recommendations and previous studies evaluating probiotic supplementation in beef cattle (Meenongyai *et al.*, 2023).

Preparation and Fermentation of the FTMR

For each dietary treatment, five independent batches of FTMR were prepared, corresponding to the five experimental periods. Each batch was prepared 21 days before the start of its respective period, divided among eight 200 L high-density polyethylene drums, and sealed to maintain anaerobic conditions throughout fermentation. The DM content of each ingredient was determined before mixing to achieve the target dietary DM content. The original TMR mixtures contained approximately 33% DM before fermentation. No additional water was added to the TMR mixture; moisture was supplied primarily by naturally fermented cassava pulp, which contained approximately 85% moisture.

To ensure uniform distribution, the PB product (*B. subtilis*; $>1 \times 10^{11}$ CFU/g; Sporezyme-Forte, AMCOVET Co., Ltd.) was first premixed with a small amount of rice

bran and soybean meal before being incorporated into the total mixed ration. The ingredients were mixed using a vertical mixer (Çelikel, Konya, Turkey) for 15 min. The resulting mixture was packed into 200 L high-density polyethylene drums at approximately 190 kg per drum and compacted to minimize residual air. The drums were then sealed to establish anaerobic conditions. The FTMR was fermented for at least 21 days through the activity of indigenous microorganisms present in the feed ingredients together with the supplemented *B. subtilis*. During fermentation, the drums were maintained at an ambient temperature of 31.5 °C, with a recorded range of 28–35 °C.

At the end of the fermentation period, three drums were randomly selected from each dietary treatment within each experimental period for pH determination. One representative composite sample was obtained from each selected drum by combining equal amounts of material collected from the upper, middle, and lower portions using a stainless-steel sampling probe. Thus, three drum samples were analyzed for each treatment within each period, resulting in 75 drum samples across the five treatments and five experimental periods. For pH determination, a 25 g portion of each composite sample was mixed with 100 mL of distilled water, homogenized for 2 min, and filtered through two layers of cheesecloth. The pH of the filtrate was measured using a calibrated waterproof digital pH meter (HI 98127, Hanna Instruments, Inc., Woonsocket, RI, USA). The instrument was calibrated before measurement using standard buffer solutions at pH 4.01 and 7.01. The pH values obtained from the three selected drums were averaged to provide one representative mean for each treatment within each period. Across the 25 independent fermentation batches, the mean final pH was 4.22 ± 0.16 , with batch mean values ranging from 4.01 to 4.65. The present study was designed primarily to evaluate animal responses to FTMR containing PDC and *B. subtilis*. Therefore, final pH was the only FTMR fermentation characteristic determined. The diet was offered ad libitum twice daily at 08:00 and 17:00, with feed allowance adjusted to maintain approximately 10% refusals. Refusals were recorded daily to calculate dry matter intake (DMI). Feed ingredients and chemical composition are presented in Table 1.

Sample Collection and Laboratory Analysis

Feed samples were collected throughout each experimental period. Fecal samples were collected directly from the rectum of each animal on days 15 to 18 at 0, 6, 12, and 18 h after feeding. Samples were composited by animal and stored at –20 °C until analysis. Feed and fecal samples were dried at 60 °C for 72 h, ground to pass through a 1-mm screen using a laboratory mill (Polymix® PX-MFC 90 D, Kinematica AG, Lucerne, Switzerland), and stored in zip-lock plastic bags until chemical analysis.

Analyses of dry matter (DM), crude protein (CP), ether extract (EE), and ash were performed according to Latimer (2023). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using an automated fiber analyzer (ANKOM 200/220, ANKOM

Table 1. Feed ingredient composition and chemical composition of fermented total mixed rations containing different inclusion levels of palm decanter cake (PDC) and probiotic *Bacillus subtilis* (PB)

Item	Control	10% PDC		20% PDC		PDC
		0.05% PB	0.10% PB	0.05% PB	0.10% PB	
Cassava chip	4.00	9.30	9.60	7.20	7.00	
Rice bran	13.60	9.10	8.60	4.00	4.00	
Palm kernel meal	4.00	4.00	4.00	4.00	4.00	
Soybean meal	9.00	6.60	6.70	10.10	10.30	
Urea	1.00	1.00	1.00	0.20	0.10	
Vitamin-mineral premix	0.30	0.30	0.30	0.30	0.30	
Salt	0.50	0.50	0.50	0.50	0.50	
Dicalcium phosphate	0.70	0.70	0.70	0.50	0.50	
Sulfur	0.10	0.10	0.10	0.10	0.10	
PDC	0.00	10.00	10.00	20.00	20.00	
Fermented cassava pulp	33.60	28.35	28.40	23.05	23.10	
Probiotics	0.00	0.05	0.10	0.05	0.10	
Rice straw	33.20	30.00	30.00	30.00	30.00	
Total	100.00	100.00	100.00	100.00	100.00	
Chemical composition (% DM)						
DM	31.19	30.78	31.34	32.65	32.01	22.36
CP	12.02	11.63	12.24	11.92	12.25	15.97
OM	91.22	90.03	90.89	90.00	90.13	81.87
EE	2.75	3.47	3.75	4.49	4.36	12.13
NDF	47.88	49.92	48.99	48.77	48.72	57.19
ADF	32.60	34.21	32.93	33.02	32.69	44.56
Ash	8.78	9.97	9.11	10.00	9.87	18.13

Note: Chemical composition values represent analyzed mixed diets. Ingredient proportions are expressed as % of total diet (DM basis); DM is expressed as % fresh matter; other chemical components are expressed as % DM. The last column (Palm decanter cake) provides the chemical composition of the PDC ingredient. DM = dry matter; CP = crude protein; OM = organic matter; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber.

Technology Corp., Fairport, NY, USA) following Van Soest *et al.* (1991). Acid-insoluble ash (AIA) was used as an internal marker to estimate apparent total tract nutrient digestibility (Arbaoui & de Vega, 2025). Apparent nutrient digestibility (%) was calculated as follows: Apparent digestibility (%) = $100 \times [1 - (\text{marker in feed} / \text{marker in feces}) \times (\text{nutrient in feces} / \text{nutrient in feed})]$.

Rumen Fluid Collection and Analysis

Rumen fluid samples were collected at 0 and 2 h after morning feeding during each experimental period using a flexible esophageal probe connected to a vacuum suction system. To minimize salivary contamination, the initial 200 mL of rumen fluid was discarded, and approximately 200 mL was subsequently collected from each animal. Ruminal pH was measured immediately using a waterproof digital pH meter (HI 98127, Hanna Instruments, Inc., Woonsocket, RI, USA).

The collected rumen fluid was divided into subsamples for analysis. For ammonia nitrogen ($\text{NH}_3\text{-N}$) and volatile fatty acid (VFA) determination, 45 mL of rumen fluid was mixed with 5 mL of 1 M H_2SO_4 and centrifuged at $9,000 \times g$ for 15 min at 2°C . The supernatants were stored at -20°C until analysis. Ammonia nitrogen concentration was determined using an automated distillation unit (VAPODEST 500, C. Gerhardt GmbH & Co. KG, Königswinter, Germany) following Latimer (2023). Volatile fatty

acids were analyzed using high-performance liquid chromatography (HPLC; Agilent 1200 Series, Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a diode array detector and a ZORBAX Eclipse XDB-C18 column (4.6×150 mm, $5 \mu\text{m}$). The mobile phase consisted of 0.1 M KH_2PO_4 with the pH adjusted to 2.4 using phosphoric acid, and the injection volume was 20 μL . Predicted methane (CH_4) production was estimated using the stoichiometric equation of Moss *et al.* (2000) as follows: $\text{CH}_4 = 0.45 \times \text{acetate} - 0.275 \times \text{propionate} + 0.40 \times \text{butyrate}$. Carbon dioxide (CO_2) production was calculated as follows: $\text{CO}_2 = (\text{acetate} / 2) + (\text{propionate} / 4) + (1.5 \times \text{butyrate})$.

The remaining rumen fluid was used to analyze total microbial populations. Samples were preserved in 10% formalin at a ratio of 1:9 (v/v; rumen fluid:formalin). Total populations of bacteria, protozoa, and fungal zoospores were determined using a hemocytometer (BOECOTM, Hamburg, Germany) following Galyean (2010). Microorganisms were counted under a light microscope at $400\times$ magnification for protozoa and fungal zoospores and at $1,000\times$ magnification with oil immersion for bacteria.

Blood Sample Collection and Analysis

Blood samples (10 mL) were collected from the jugular vein at 0 and 2 h after morning feeding on the last day of each experimental period. The samples were collected into tubes without anticoagulant and allowed

to clot at room temperature for 30 min. Serum was separated by centrifugation at $3,000 \times g$ for 10 min and stored at -20°C until analysis. Serum concentrations of glucose, urea, and triglycerides were determined using an automated clinical chemistry analyzer (UniCel Dx C 800 Synchron Clinical System, Beckman Coulter, Inc., CA, USA).

Statistical Analysis

All statistical analyses were performed using the PROC MIXED procedure of SAS OnDemand for Academics (SAS Institute Inc., Cary, NC, USA). Dietary treatment was analyzed as a single fixed effect representing the five dietary regimens. Because the treatment structure was not a complete factorial arrangement, the independent effects of PDC and PB and their interaction could not be estimated. Variables measured once per period, such as feed intake and nutrient digestibility, were analyzed using the following mixed model:

$$Y_{ijk} = \mu + T_i + P_j + A_k + \varepsilon_{ijk}$$

where Y_{ijk} is the observed response variable, μ is the overall mean, T_i is the fixed effect of dietary treatment ($i = T1, T2, \dots, T5$), P_j is the fixed effect of period ($j = 1, 2, \dots, 5$), A_k is the random effect of animal ($k = 1, 2, \dots, 5$), and ε_{ijk} is the residual error term. Variables measured repeatedly over time (e.g., rumen fermentation parameters and serum metabolites) were analyzed using a repeated-measures mixed model:

$$Y_{ijkl} = \mu + T_i + P_j + \text{Time}_l + (T \times \text{Time})_{il} + A_k + \varepsilon_{ijkl}$$

where Time_l is the fixed effect of sampling time ($l = 0$ h, 2 h), and $(T \times \text{Time})_{il}$ is the interaction between treatment and time. Other terms are as previously defined. A compound symmetry covariance structure was selected based on the lowest Akaike information criterion. Degrees of freedom were adjusted using the Kenward–Roger method. Least squares means were compared using the PDIF option with Tukey's adjustment. Differences among treatments were considered significant at $p < 0.05$.

RESULTS

Chemical Analysis of FTMR and Palm Decanter Cake

The chemical compositions of PDC and the experimental FTMR diets are presented in Table 1. Palm decanter cake contained 22.36% DM, 15.97% CP, 12.13% EE, 81.87% OM, and 18.13% ash, with relatively high fiber contents of 57.19% NDF and 44.56% ADF. The DM content of the FTMR diets ranged from 30.78% to 32.65% and increased slightly with PDC inclusion. Organic matter remained high across treatments (90.00%–91.22%), whereas CP concentrations were similar among diets (11.63%–12.25% DM), indicating that the diets were isonitrogenous. Ether extract increased with increasing PDC inclusion, from 2.75% in the control diet to 3.47%–3.75% in the 10% PDC diets and 4.36%–4.49% in the 20% PDC diets. Neutral detergent fiber, ADF, and

ash contents also increased slightly with increasing PDC inclusion.

Dry Matter and Nutrient Intakes

Dry matter and nutrient intakes are presented in Table 2. Dietary treatments did not affect DMI, DMI/BW, OM intake, CP intake, or NDF intake ($p > 0.05$), although cattle fed 20% PDC with 0.05% PB showed numerically greater DMI and NDF intake than those fed 10% PDC with 0.10% PB. Ether extract intake increased with increasing PDC inclusion, and cattle receiving 20% PDC at both PB levels had greater EE intake than those in the other treatments ($p < 0.05$). Similarly, cattle fed 10% PDC with PB had greater EE intake than the control cattle ($p < 0.05$). Acid detergent fiber intake was also greater in cattle fed diets containing 10% or 20% PDC with 0.05% PB than in those fed 10% PDC with 0.10% PB ($p < 0.05$).

Apparent Digestibility

The effects of PDC supplementation combined with PB in FTMR on apparent nutrient digestibility are presented in Table 2. Cattle fed diets containing 20% PDC with 0.10% PB had greater DM and OM digestibility than those fed diets containing 10% PDC with either PB level ($p < 0.05$). Crude protein digestibility was greater in cattle fed 10% PDC with 0.10% PB than in those fed 10% PDC with 0.05% PB ($p < 0.05$). In addition, cattle receiving 20% PDC with 0.10% PB showed the highest EE digestibility among treatments ($p < 0.05$). Similarly, NDF and ADF digestibility were greater in cattle fed 20% PDC with 0.10% PB than in those fed 10% PDC with either PB level ($p < 0.05$).

Rumen Fermentation

The effects of PDC supplementation combined with PB in FTMR on rumen fermentation characteristics are presented in Table 3. Dietary treatments did not affect ruminal pH, ammonia nitrogen ($\text{NH}_3\text{-N}$), total volatile fatty acids (total VFA), the acetate-to-propionate ratio (C2:C3), or predicted CO_2 and CH_4 production ($p > 0.05$), and no treatment $\times$ sampling time interaction was observed ($p > 0.05$). However, sampling time significantly affected rumen fermentation parameters ($p < 0.05$). Ruminal pH decreased from 6.89–7.13 before feeding to 6.50–6.69 at 2 h after feeding, whereas $\text{NH}_3\text{-N}$ concentrations increased from 6.72–7.73 to 8.34–11.03 mg/dL. Similarly, total VFA, acetate, and butyrate concentrations increased after feeding ($p < 0.01$), with total VFA increasing from 111.71–117.13 mmol/L at 0 h to 122.42–147.50 mmol/L at 2 h after feeding. In contrast, propionate concentration was not affected by sampling time ($p = 0.26$). The C2:C3 ratio decreased after feeding ($p < 0.05$), although no differences among treatments were observed for total VFA, individual VFA concentrations, or the C2:C3 ratio ($p > 0.05$). A tendency for a treatment $\times$ sampling time interaction was observed for predicted CH_4 production ($p = 0.06$), whereas no interaction was detected for predicted CO_2 production ($p = 0.44$). Predicted CO_2 and CH_4

Table 2. Least squares means for dry matter intake, nutrient intake, and apparent nutrient digestibility in cattle fed fermented total mixed rations containing different inclusion levels of palm decanter cake (PDC) and probiotic *Bacillus subtilis* (PB)

Item	Control	10% PDC		20% PDC		SEM	p-value
		0.05% PB	0.10% PB	0.05% PB	0.10% PB		
DMI							
kg/d	9.33	9.09	8.65	9.41	9.15	0.58	0.053
%BW	1.72	1.71	1.62	1.76	1.66	0.10	0.056
Nutrient intake (kg/d)							
OM	8.51	8.18	7.86	8.47	8.25	0.52	0.060
CP	1.12	1.06	1.06	1.12	1.12	0.07	0.069
EE	0.26 ^c	0.32 ^b	0.32 ^b	0.42 ^a	0.40 ^a	0.02	<0.001
NDF	4.47	4.54	4.24	4.59	4.46	0.28	0.077
ADF	3.04 ^{ab}	3.11 ^a	2.85 ^b	3.11 ^a	2.99 ^{ab}	0.19	0.028
Apparent digestibility, %							
DM	58.07 ^{ab}	49.10 ^c	54.33 ^{bc}	56.23 ^{ab}	59.78 ^a	2.00	0.003
OM	61.97 ^{ab}	53.22 ^c	57.82 ^{bc}	59.89 ^{ab}	63.45 ^a	2.18	0.008
CP	57.44 ^{ab}	47.01 ^b	58.98 ^a	53.76 ^{ab}	56.89 ^{ab}	2.97	0.044
EE	88.65 ^c	89.70 ^{bc}	89.05 ^c	92.40 ^b	94.20 ^a	1.42	0.010
NDF	45.76 ^{ab}	37.27 ^b	39.51 ^b	42.95 ^{ab}	50.60 ^a	3.16	0.011
ADF	45.81 ^{ab}	38.30 ^b	39.74 ^b	41.63 ^{ab}	49.39 ^a	2.61	0.007

Note: ^{a-c} Means within a row with different superscripts differ significantly ($p < 0.05$); SEM = standard error of the mean; DMI = dry matter intake; %BW = as percent of body weight; DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber.

Table 3. Least squares means for rumen fermentation variables and predicted gas production in cattle fed fermented total mixed rations containing different inclusion levels of palm decanter cake (PDC) and probiotic *Bacillus subtilis* (PB)

Item	Control	10% PDC		20% PDC		SEM	p-value		
		0.05% PB	0.10% PB	0.05% PB	0.10% PB		TRT	Time	TRT × Time
Rumen pH									
0 h	7.08	7.08	7.13	6.89	7.11	0.10	0.28	<0.01	0.67
2 h	6.51	6.69	6.53	6.50	6.60				
NH ₃ -N (mg/dL)									
0 h	7.73	7.11	6.72	7.00	7.00	1.04	0.77	<0.01	0.35
2 h	8.34	8.90	10.81	11.03	8.96				
Acetic acid (C ₂) (mmol/L)									
0 h	81.98	78.95	79.87	84.32	85.93	7.65	0.49	<0.01	0.27
2 h	100.33	106.95	82.78	86.56	99.56				
Propionic acid (C ₃) (mmol/L)									
0 h	22.77	24.11	22.47	20.44	20.10	3.89	0.48	0.26	0.98
2 h	25.65	25.68	28.14	26.20	21.58				
Butyric acid (C ₄) (mmol/L)									
0 h	9.51	12.52	9.36	11.47	11.10	1.33	0.23	<0.01	0.75
2 h	14.07	14.87	11.49	13.86	12.42				
Total VFA (mmol/L)									
0 h	114.26	115.59	111.71	116.23	117.13	10.18	0.55	<0.01	0.76
2 h	140.05	147.50	122.42	126.62	133.56				
C2:C3 ratio									
0 h	5.05	4.78	5.61	4.67	5.02	0.58	0.79	0.01	0.50
2 h	3.98	4.35	3.39	3.60	4.74				
Predicted carbon dioxide (mmol/L)									
0 h	60.95	64.29	59.60	64.47	64.64	5.13	0.36	<0.01	0.44
2 h	77.68	82.20	65.67	70.62	73.81				
Predicted methane (mmol/L)									
0 h	34.44	33.91	33.51	36.91	37.58	3.36	0.36	<0.01	0.06
2 h	43.72	47.02	34.11	37.29	43.84				

Note: SEM = standard error of the mean; TRT = treatment; TRT × Time = treatment × sampling time interaction; NH₃-N = ammonia nitrogen; VFA = volatile fatty acid. The C2:C3 ratio was calculated for each individual observation by dividing the acetate concentration by the corresponding propionate concentration before statistical analysis.

production were significantly affected by sampling time ($p < 0.01$), increasing from 59.60–64.64 and 33.51–37.58 mmol/L at 0 h to 65.67–82.20 and 34.11–47.02 mmol/L at 2 h after feeding, respectively. However, neither predicted CO_2 nor CH_4 production was affected by dietary treatment ($p > 0.05$).

Rumen Microbial Population

The effects of PDC supplementation combined with PB in FTMR on rumen microbial populations are presented in Table 4. Total bacterial, protozoal, and fungal zoospore populations were not affected by dietary treatment ($p > 0.05$), and no treatment $\times$ sampling time interaction was detected for any microbial population measured ($p > 0.05$). Sampling time affected total bacterial and fungal zoospore populations ($p < 0.05$). In contrast, protozoal populations were not influenced by sampling time ($p > 0.05$).

Serum Metabolites

The effects of PDC supplementation combined with PB on serum metabolites are presented in Table 5. Dietary treatments did not affect serum glucose, serum urea nitrogen (SUN), or triglyceride concentrations ($p > 0.05$). However, sampling time significantly affected serum metabolites ($p < 0.05$). Serum glucose concentrations increased from 60.40–63.40 mg/dL before feeding to 65.20–70.60 mg/dL at 2 h after feeding.

Similarly, SUN concentrations increased from 5.00–7.20 to 7.40–9.60 mg/dL after feeding. Serum triglyceride concentrations also increased after feeding ($p < 0.05$), from 71.20–73.80 to 72.60–76.40 mg/dL.

DISCUSSION

The relatively high CP and EE contents of PDC reflect residual protein and lipid fractions remaining after palm oil extraction, whereas the elevated NDF and ADF contents indicate its fibrous nature. The high OM concentration further suggests that PDC may provide fermentable substrates for rumen microbial activity. Inclusion of up to 20% PDC with PB in FTMR maintained nutrient balance and satisfied the nutrient requirements of beef cattle. The slight increase in dietary DM likely reflected the lower moisture content of PDC, whereas residual oil contributed to the increased EE content and potentially greater dietary energy density (Abdullah *et al.*, 2025; Adebisi *et al.*, 2020). However, dietary EE concentrations remained within the recommended range for finishing cattle (NASEM, 2016). Fiber fractions increased only slightly with PDC inclusion, suggesting minimal effects on rumen fill and feed intake. Although elevated dietary NDF may reduce intake because of rumen fill limitations and slower digestion (Shi *et al.*, 2023), the changes observed in the present study were minimal. Increased ash content reflected the mineral composition of PDC without affecting overall OM concentration. Given its low

Table 4. Least squares means for total rumen microbial populations in cattle fed fermented total mixed rations containing different inclusion levels of palm decanter cake (PDC) and probiotic *Bacillus subtilis* (PB)

Item	Control	10% PDC		20% PDC		SEM	p-value		
		0.05% PB	0.10% PB	0.05% PB	0.10% PB		TRT	Time	TRT $\times$ Time
Bacteria ($\times 10^9$ cells/mL)									
0 h	1.14	1.08	0.96	0.90	0.91	0.24	0.18	<0.01	0.51
2 h	2.23	1.58	1.50	1.32	1.30				
Protozoa ($\times 10^5$ cells/mL)									
0 h	2.39	3.17	3.61	3.22	3.67	0.59	0.82	0.14	0.50
2 h	2.94	2.28	2.78	3.00	2.33				
Fungi ($\times 10^6$ cells/mL)									
0 h	1.49	1.67	2.05	2.15	1.79	0.75	0.75	<0.01	0.59
2 h	4.68	4.10	3.11	3.13	2.84				

Note: SEM = standard error of the mean; TRT = treatment; TRT $\times$ Time = treatment $\times$ sampling time interaction.

Table 5. Least squares means for serum metabolites in cattle fed fermented total mixed rations containing different inclusion levels of palm decanter cake (PDC) and probiotic *Bacillus subtilis* (PB)

Item	Control	10% PDC		20% PDC		SEM	p-value		
		0.05% PB	0.10% PB	0.05% PB	0.10% PB		TRT	Time	TRT $\times$ Time
Glucose (mg/dL)									
0 h	60.80	61.00	60.40	63.40	60.40	2.69	0.50	<0.01	0.49
2 h	67.60	70.60	68.00	67.20	65.20				
Urea nitrogen (mg/dL)									
0 h	7.00	7.20	6.40	5.00	6.00	0.85	0.26	<0.01	0.76
2 h	9.60	9.20	8.20	7.40	7.60				
Triglycerides (mg/dL)									
0 h	73.60	71.20	73.80	73.60	71.60	0.99	0.11	<0.01	0.50
2 h	75.60	72.60	76.40	74.80	73.80				

Note: SEM = standard error of the mean; TRT = treatment; TRT $\times$ Time = treatment $\times$ sampling time interaction.

inclusion level (0.05% or 0.10% of dietary DM), PB would be expected to contribute little directly to the nutrient composition of the FTMR. However, its independent effect on chemical composition could not be determined with this design.

Final pH was the only fermentation characteristic determined in the present study. Although the mean final pH of 4.22 indicated acidification of the FTMR, the concentrations of lactic, acetic, and butyric acids, ammonia-N, yeast and mold counts, and aerobic stability were not evaluated. Consequently, the fermentation quality and preservation stability of the FTMR could not be characterized comprehensively. The extent to which the fermentation process contributed to nutrient availability and subsequent animal responses also could not be determined. Therefore, the observed animal responses reflect the final fed dietary regimens rather than direct evidence of improved FTMR fermentation quality, aerobic stability, or silage fermentation kinetics. Without organic acid profiles or measurements during ensiling, the pathways and rate of acidification could not be established from final pH alone.

The numerically greater DMI observed in cattle fed 20% PDC with 0.05% PB may be related to differences in dietary physical characteristics. The greater bulk density of PDC may have increased dietary density and reduced rumen fill limitations, thereby allowing greater intake (Lee *et al.*, 2021). Moderate PB supplementation may also have influenced ruminal fermentation and digesta kinetics, which can affect feed intake (Silva *et al.*, 2024). Despite these numerical differences, OM and CP intakes were unaffected, indicating that PDC inclusion did not compromise overall nutrient intake. These results suggest that PDC can be included at up to 20% DM without adverse effects on feed intake, indicating acceptable palatability and dietary adaptation in FTMR systems. In contrast, the greater EE intake observed in cattle fed dietary regimens containing PDC was consistent with the higher lipid content of PDC (Adebiyi *et al.*, 2020). Similarly, greater NDF and ADF intakes in cattle fed diets containing 10% or 20% PDC with 0.05% PB were consistent with the numerically greater DMI and may be associated with the previously reported fibrolytic activity of *B. subtilis* (Bontà *et al.*, 2024).

Among the dietary regimens evaluated, the diet containing 20% PDC and 0.10% PB resulted in the greatest apparent digestibility of DM, OM, EE, NDF, and ADF. This response may reflect complementary functions of the greater amount of fibrous substrate supplied by PDC and the higher nominal dose of *B. subtilis* provided by PB. Although the fibrous matrix and residual lipid content of PDC may restrict nutrient accessibility, its structural carbohydrates provide substrates for microbial degradation. Potential mechanisms reported for *B. subtilis* include the production of extracellular enzymes such as cellulases, xylanases, and proteases, which may partially disrupt the feed matrix and facilitate the degradation of fiber and protein fractions (Rosazza *et al.*, 2023; Bontà *et al.*, 2024). During ensiling, *B. subtilis* may also consume residual oxygen and produce antimicrobial compounds that suppress undesirable microorganisms, potentially creating conditions that favor preservation

and subsequent nutrient utilization (Su *et al.*, 2020; Bai *et al.*, 2020; Bonaldi *et al.*, 2021). These potential microbial and enzymatic processes are hypotheses drawn from previous studies and were not tested here. Therefore, the greater apparent nutrient digestibility observed in cattle fed the diet containing 20% PDC and 0.10% PB should be interpreted as a response to the complete dietary regimen.

However, enzyme activity, viable *B. subtilis* counts after fermentation, and fermentation quality were not measured in the present study. These mechanisms should therefore be regarded as biologically plausible explanations rather than processes demonstrated by the current results. Moreover, because the treatment structure did not independently separate the effects of PDC and PB, the greater digestibility cannot be attributed conclusively to *B. subtilis* or to a synergistic PDC-by-PB interaction. Instead, it should be interpreted as an animal response to the complete dietary regimen containing 20% PDC and 0.10% PB. A complete factorial study incorporating measurements of fermentation quality, viable microbial counts, and extracellular enzyme activities would be required to verify the proposed complementary mechanism.

The absence of treatment effects on rumen fermentation indicates that PDC inclusion (10%–20% DM) with PB did not alter overall fermentation, likely because of the similar fermentable substrate composition across diets. Ruminal $\text{NH}_3\text{-N}$ concentrations were also unaffected, consistent with the isonitrogenous formulation of the diets, reflecting balanced nitrogen supply and microbial utilization (Zhang *et al.*, 2026). Although CP digestibility increased in cattle fed 10% PDC with 0.10% PB (Table 2, $p < 0.05$), this was not accompanied by an increase in $\text{NH}_3\text{-N}$, suggesting efficient microbial assimilation of released nitrogen. $\text{NH}_3\text{-N}$ concentrations remained within the optimal range for microbial activity (5.0–20.0 mg/dL), consistent with high-performance feeding conditions (Niazifar *et al.*, 2024).

Natural LAB in fermented cassava pulp may interact synergistically with supplemented *B. subtilis*. As a facultative anaerobe, *B. subtilis* can scavenge residual oxygen, thereby promoting anaerobiosis and supporting LAB and obligately anaerobic rumen microorganisms (Gresse *et al.*, 2025). The combined use of *B. subtilis*, LAB, and yeast has also been reported to enhance utilization of fibrous feed and modulate the rumen microbiota (Wei *et al.*, 2025). However, the absence of treatment effects on total and individual VFA concentrations indicates that PDC combined with PB did not alter the overall ruminal fermentation profile, possibly reflecting comparable fermentable substrate availability among diets (Tanuwiria *et al.*, 2025). Thus, no statistically detectable differences in ruminal fermentation profiles were observed among the complete dietary regimens. Synergistic or antagonistic interactions between PDC and PB could not be evaluated with this design.

Total VFA, acetate, and butyrate concentrations increased at 2 h post-feeding, consistent with enhanced postprandial ruminal fermentation and the accompanying decline in ruminal pH (Viquez-Umana *et al.*, 2025). In contrast, propionate concentration was unaffected by sampling time, indicating that the increase

in total VFA was primarily attributable to acetate and butyrate. Although the $C_2:C_3$ ratio decreased after feeding, this response should be interpreted cautiously because propionate did not increase significantly. Therefore, the lower $C_2:C_3$ ratio alone does not confirm a shift in ruminal fermentation toward propionate production.

Dietary treatment did not affect predicted CO_2 or CH_4 production. However, the tendency for a treatment $\times$ sampling time interaction for predicted CH_4 ($p = 0.06$) indicated numerical variation in postprandial responses among treatments. The postprandial increases in predicted CO_2 and CH_4 were consistent with enhanced ruminal fermentation and the associated changes in VFA production (S.-R. Lee *et al.*, 2021). Because CH_4 was estimated from VFA stoichiometry rather than measured directly, these results should not be interpreted as evidence of altered enteric methane emissions.

Direct microscopic counting revealed no statistically detectable treatment differences in total bacterial, protozoal, or fungal zoospore populations. Although treatment effects were not significant, total bacterial and fungal counts declined numerically in the diets containing PDC combined with PB. This numerical decline may partly reflect the greater dietary lipid supply from PDC because dietary fatty acids can disrupt microbial membranes and reduce microbial attachment to fibrous feed particles, potentially affecting fibrolytic bacteria and anaerobic fungi (Sun *et al.*, 2022). However, VFA concentrations were unaffected by dietary treatment, indicating that the numerical decline in microbial counts did not substantially alter the overall ruminal fermentation profile.

Sampling time affected total bacterial and fungal zoospore counts, indicating temporal variation in these broad microbial groups after feeding, consistent with previously reported temporal changes in ruminal microbial populations (Torres-Velázquez *et al.*, 2025). Greater nutrient digestibility may occur without a corresponding increase in total microbial counts because digestibility may be associated with changes in microbial community composition or substrate accessibility rather than total microbial abundance alone. Wang *et al.* (2024), for example, reported greater apparent total tract nutrient digestibility in cows fed FTMR together with changes in fecal microbial community composition. Fermentation of the FTMR may also have modified the feed matrix and increased nutrient availability before ingestion, thereby facilitating subsequent digestion without increasing total ruminal microbial counts (Bai *et al.*, 2024). Furthermore, apparent total tract digestibility represents nutrient disappearance throughout the gastrointestinal tract and therefore cannot be explained solely by ruminal microbial abundance. The direct microscopic method used in the present study quantified only broad microbial groups and could not specifically quantify fibrolytic or amylolytic bacteria, methanogens, or the supplemented *B. subtilis*. It also could not characterize microbial community composition or metabolic function, which would require molecular and functional approaches (Cammack *et al.*, 2018). Although *B. subtilis* has been reported to produce extracellular enzymes that may

contribute to nutrient degradation (Rosazza *et al.*, 2023; Bontà *et al.*, 2024), neither viable *B. subtilis* counts nor microbial enzyme activities were measured in the present study. Consequently, the greater nutrient digestibility observed with the complete dietary regimen containing 20% PDC and 0.10% PB cannot be mechanistically linked to changes in the ruminal microbiota based on the present data. Instead, it may reflect improved substrate accessibility or microbial functions that were not captured by total microscopic counts. Further studies using quantitative molecular methods, microbial community profiling, and direct measurements of fibrolytic and proteolytic activities are required to determine whether *B. subtilis* alters specific ruminal microbial populations or their metabolic functions.

Stable serum glucose concentrations likely reflected similar propionate supply among treatments, indicating comparable energy metabolism (Rathert-Williams *et al.*, 2023). The postprandial increases in glucose and SUN represented normal metabolic responses to feeding and ruminal protein degradation (Khan *et al.*, 2026). However, SUN concentrations remained within the normal physiological range, and the absence of treatment effects suggests similar nitrogen metabolism among diets, likely because of the isonitrogenous formulation and balanced nitrogen utilization (NASEM, 2016). Despite differences in dietary fat content, serum triglyceride concentrations did not differ among the complete dietary regimens. This likely reflects the moderate dietary fat levels and extensive ruminal lipid metabolism, resulting in similar fatty acid availability for absorption (Jiang *et al.*, 2024; Bionaz *et al.*, 2020). The treatment structure should be considered when interpreting these findings. Because diets containing PDC without PB and PB without PDC were not included, the independent contributions of PDC and *B. subtilis* could not be distinguished. Therefore, the observed responses should be attributed to the complete dietary regimens rather than to either component independently. The use of five animals may have limited the statistical power to detect modest treatment differences. Therefore, nonsignificant results should be interpreted as an absence of statistically detectable differences rather than evidence of biological equivalence. Further studies using a complete factorial design and a larger number of animals are warranted. Among the regimens evaluated, the diet containing 20% PDC and 0.10% PB resulted in greater apparent digestibility of several nutrient fractions without statistically detectable differences in ruminal fermentation characteristics, microbial populations, or serum metabolites.

CONCLUSION

Under the conditions of this study, FTMR containing up to 20% PDC and supplemented with *B. subtilis* did not adversely affect feed intake, ruminal fermentation characteristics, microbial populations, or serum metabolites in Angus crossbred bulls. Among the dietary regimens evaluated, the regimen containing 20% PDC and 0.10% PB resulted in the greatest apparent digestibility of DM, OM, EE, NDF, and ADF. These findings indicate that PDC may be incorporated at up to

20% of dietary DM in *B. subtilis* supplemented FTMR as an alternative feed resource for beef cattle in oil palm-producing regions. However, the independent and interactive effects of PDC and *B. subtilis* require confirmation using a complete factorial design.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this article.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

AI tool was used only during the initial drafting of this manuscript to improve the readability and language of the text. The final manuscript was subsequently reviewed and professionally edited by a certified English language editing service before submission. The authors reviewed and approved all revisions and take full responsibility for the content of the manuscript.

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