International Journal of Agricultural Technology 2026 Vol. 22(4):1687-1702
DOI: https://doi.org/10.63369/ijat.2026.22.4.1687-1702
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Phytogenic feed additives for improving feed efficiency and lowering ruminants' enteric methane emission on palm oil sludge feed: *in vitro* study

Dwatmadji[1*], Purnomoadi, A.[2], Suteky, T.[1] and Sipriyadi[3]

[1]Department of Animal Science Faculty of Agriculture, University of Bengkulu; [2]Faculty of Animal Husbandry and Agriculture, University of Diponegoro; [3]Department of Biology Faculty of Mathematics and Nature, University of Bengkulu, Indonesia.

Dwatmadji, Purnomoadi, A., Suteky, T. and Sipriyadi (2026). Phytogenic feed additives for improving feed efficiency and lowering ruminants' enteric methane emission on palm oil sludge feed: *in vitro* study. International Journal of Agricultural Technology 22(4):1687-1702.

Abstract Result indicated that replacing non-fermented palm oil sludge (POS) with fermented POS as a basal feed led to better *in vitro* dry matter digestibility and lower enteric methane emissions. Adding phytogenic feed additives (PFAs) -- containing a mixture of *Melastoma malabathricum* (MM), *Carica papaya* (CP), *Curcuma longa* (CL), and *Curcuma zanthoriza* (CZ) -- resulted in the highest digestibility and total VFA concentration but also high methane output; whereas PFAs containing MM and CP produced the least methane, though with a slight decrease in digestibility. Acetate-to-propionate ratios remained within a favourable range (2.19–2.70), indicating efficient rumen fermentation. These results support using fermented POS and locally sourced PFAs within the oil palm-cattle integration systems (SISKA) framework to improve feed efficiency and reduce greenhouse gas emissions from ruminants.

Keywords: Palm oil sludge, Phytogenic feed additives, Enteric methane, Feed efficiency, *In vitro* digestibility

Introduction

Meeting the growing demand for animal protein while minimizing its environmental impact is a double issue facing the global livestock industry. Enteric fermentation is an environmental hazard and an energy loss for the animal, and ruminant production is a major source of anthropogenic methane emissions (Bature *et al*., 2024). Methane, with a global warming potential 28 times greater than carbon dioxide over a 100-year horizon, represents approximately 16% of global greenhouse gas emissions, with ruminants contributing nearly 33% of agricultural methane emissions. Simultaneously, the palm oil industry generates substantial by-products, including palm oil sludge (POS). POS also known as palm oil mill effluent or palm kernel meal, is a abundant by-product of palm oil extraction that offers potential as a ruminant

[*] **Corresponding Author:** Dwatmadji.; **Email:** Dwatmadji.2008@gmail.com

feed ingredient due to its nutritional composition (Devendra *et al*., 1983). POS has a nutritional content of 10–15% crude fat, 12–18% crude protein, and substantial levels of minerals, while the precise composition varies based on the processing techniques used. Because of its high fat content, it is especially important as an energy source for ruminants because it is rich in saturated fatty acids (44.5%) and unsaturated fatty acids (55.5%, primarily oleic acid). Lipids have also been demonstrated to lower enteric methane emissions in bovine diets through a number of processes, such as direct suppression of methanogenic archaea and biohydrogenation of unsaturated fatty acids, which act as alternative hydrogen sinks (Amalina *et al*., 2020). POS which represents an underutilized resource that could be incorporated into ruminant feeds to improve circularity within agricultural systems (Phoemchalard *et al*., 2014).

Furthermore, like many fibrous by-products, POS may influence rumen fermentation patterns in ways that could potentially increase methanogenesis. Enteric methane synthesis in ruminants is achieved through anaerobic microbial fermentation in the rumen, which is largely carried out by archaea known as methanogens. In addition to contributing to greenhouse gas emissions, these microbes significantly reduce dietary energy consumption (2–12% of gross energy intake) by using hydrogen and carbon dioxide to create methane. Bacteria (10^{30}–10^{11} organisms/mL), archaea (10^{8}–10^{9} organisms/mL), ciliated protozoa (10^{5}–10^{6} organisms/mL), anaerobic fungus (10^{3}–10^{4} organisms/mL), and virus groups make up the rumen microbial community (Henderson *et al*., 2015; Jami and Mizrahi, 2012).

Recent research has focused on feed additives that can improve nutrient utilization while reducing methane emissions. Among these additives, phytogenic feed additives (PFAs)—derived from herbs, spices, and other plants have emerged as promising natural alternatives to synthetic additives due to their bioactive compounds including tannins, saponins, essential oils, and flavonoids. These compounds exhibit antimicrobial, antioxidant, and anti-methanogenic properties that can modulate rumen fermentation patterns toward more efficient metabolic pathways (Kuralkar and Kuralkar, 2021; McGrath *et al.*, 2018; Waters *et al*., 2024). Most previous studies have investigated PFAs in conventional feed systems, with limited research on their application in by-product-based feeds like those incorporating palm oil sludge. Tannin-rich sources plants contain condensed and hydrolysable tannins that can reduce methane production by directly inhibiting methanogens, reducing protozoal populations (defaunation), and competing with methanogens for hydrogen. Saponin-containing plants: Sources like *Sapindus rarak* yucca, and tea saponins exhibit anti-protozoal effects, reducing protozoa-associated methanogens and thereby decreasing methane production (Jerónimo *et al.*, 2016; Salami *et al*., 2018; Waters *et al*.,

2024; Yanza *et al*., 2024). Our previous findings (Suteky and Dwatmadji, 2023) showed that ethanolic extract of *Melastoma malabathricum* rich in tannin, while Tiwari *et al*. (2023) found that the other phytochemical compound are saponin and flavonoid. *Carica papaya* rich in saponin (Abdel-Halim *et al*., 2021; Zubairi *et al*., 2023). This study investigated the potential of phytogenic feed additives (PFAs) with the combination of those four herbs to enhance the utilization of palm oil sludge (POS)-based diets while mitigating enteric methane production through *in vitro* methodology.

Materials and methods

Feed composition of treatments

The dietary treatments consisted of Palm Oil Sludge (POS) as the basal feed, either non-fermented (T1) or fermented (T1 to T9), supplemented with various combinations and forms (powder or ethanolic extract) of four phytogenic feed additives: *Curcuma longa* (CL), *Curcuma zanthoriza* (CZ), *Melastoma malabathricum* (MM), and *Carica papaya* (CP). All treatments were formulated to contain 65% POS-based material, with the remaining 35% consisting of 20% tofu waste and 15% grass. The complete feed composition for each treatment is presented in Table 1.

The experiment incorporated nine treatment groups (T1 to T9) with three replicates each (represented by three incubation units per replicate) and with a constant basal composition of 65% palm oil sludge (fermented or non-fermented), supplemented with varying forms and concentrations of four herbal plants: *Curcuma longa* (CL), *Curcuma zanthoriza* (CZ), *Melastoma malabathricum* (MM), and *Carica papaya* (CP), administered as either powder or ethanolic extract. The remaining 35% of the diet consisted of 20% tofu waste and 15% grass, ensuring nutritional balance and substrate consistency across treatments. The treatments were shown in Table1.

The inclusion of fermented palm oil sludge in T2 aimed to evaluate the effect of microbial pre-treatment on nutrient availability, while treatments T2 through T9 tested synergistic interactions between herbal bioactives and sludge fermentation status. The use of ethanolic extracts (T4, T5, T6, and T7) versus powdered forms (T1 and T8) allowed assessment of the efficacy of the extraction method on bioactive compound delivery.

Table 1. Feed composition (%) of all treatments for the *in vitro* test

Treatments	Palm Oil Sludge		*CL*[/1]	*CZ*[/1]	*MM*[/1]		*CP*[/1]		Total
	Non Fermented	Fermented			Powder	Ethanolic extract	Powder	Ethanolic extract	
T1	61.8	-	1.5	1.5	0.1	-	0.1	-	65
T2	-	65.0	-	-	-	-	-	-	65
T3	-	62.0	0.8	0.8	0.8	-	0.8	-	65
T4	-	61.8	1.5	1.5	-	0.1	-	0.1	65
T5	-	61.8	3.0	-	-	0.1	-	0.1	65
T6	-	61.8	-	3.0	-	0.1	-	0.1	65
T7	-	64.8	-	-	-	0.1	-	0.1	65
T8	-	61.9	1.5	1.5	-	0.1	-	-	65
T9	-	61.9	1.5	1.5	-	-	-	0.1	65

All treatments received 20% tofu waste and 15% grass to make up 100%.
[/1]CL= *Curcuma longa*, CZ= *Curcuma zanthoriza,* MM= *Melastoma malabathricum*, CP= *Carica papaya.*

Substrate preparation

The palm oil sludge (POS) was obtained from a local palm oil processing plant and analysed for proximate composition. The POS was obtained from a local palm oil processing plant and analysed for proximate composition. The fermentation of POS contains 80% POS, Rice brand 17%, EM4 0.1%, Dolomite 0.4 %, Urea 0.2%, Molasses 0.4%, Salt 0.1% and water 2% ad fermented for 1 week.

Ethanolic extraction for Melastoma malabathricum and Carica papaya

The leaves *Melastoma malabathricum* and *Carica papaya* were dried and then powdered. This crucial step is maximized the surface area for contact with the solvent, enhancing extraction efficiency. The powdered leaves are placed in a sealed amber glass jar to protect from light. 70% Ethanol is poured over the material until it is fully submerged, at a mass-to-volume ratio 1:5 or 1:10 of powdered leaves material to solvent. The glass jar was sealed tightly to prevent solvent evaporation. The mixture is stored at room temperature for 3 days and extended until the solvent was cleared. The mixture was stirred periodically twice daily to disrupt the concentrated layer of solute around the plant particles and renew the solvent's capacity for extraction. The ethanol can be concentrated using a rotary evaporator under reduced pressure to obtain a thick, viscous extract and save until used.

There were nine treatments tested *in vitro* with three replicates each (represented by three incubation units per replicate). The treatments were:

T1: Non fermented POS + 0.05 MM+ 0.05 CP + 0.75 CL + 0.75 CZ
T2: Fermented POS no PFAs
T3: Fermented POS + 0.75 MM + 0.75 CP + 0.75 CL + 0.75 CZ (the PFAs as a powder)
T4: Fermented POS + 0.1 MM + 0.1 CP + 1.5 CL + 1.5 CZ
T5: Fermented POS + 0.1 MM + 0.1 CP + 3 CL
T6: Fermented POS + 0.1 MM + 0.1 CP + 3 CZ
T7: Fermented POS + 0.1 MM + 0.1 CP
T8: Fermented POS + 0.1 MM + 1.5 CL + 1.5 CZ
T9: Fermented POS + 0.1 CP + 1.5 CL + 1.5 CZ

All the treatment were added 20 grams of Tofu waste and 15 grams of Fresh Grass (all unit is in grams). The PFAs (phytogenic feed additives) contains: MM: Extract *Melastoma malabathricum*, CP: Extract *Carica papaya*, CL: *Curcuma longa* and CZ: *Curcuma zanthoriza*.

In vitro incubation and sampling

Rumen fluid was collected from three ruminally cannulated Bali cattle fed a maintenance diet of grass hay and concentrate (70:30 ratio) before morning feeding. The fluid was transported to the laboratory in pre-warmed thermos flasks under anaerobic conditions, filtered through four layers of cheesecloth, and mixed with pre-warmed artificial saliva in a 1:2 ratio (rumen fluid:buffer) under continuous flushing with CO_2.

The incubation process followed the standardized *in vitro* gas production technique described by Theodorou and Brooks (1990). Approximately 500 mg of each substrate was weighed into 100-mL serum bottles, followed by the addition of 50 mL of rumen fluid-buffer mixture. Bottles were sealed with rubber stoppers and aluminium caps, incubated at 39°C for 24 hours, and gently shaken every hour during the first 9 hours of incubation.

Gas production was measured at 2, 6, 9, 12 and 24 hours of incubation using a pressure transducer, and methane concentration was determined via gas chromatography. After 24 hours of incubation, fermentation was terminated by placing the bottles in ice water. The pH was measured immediately, and contents were filtered through pre-weighed sintered glass crucibles for determination of dry matter digestibility (DMD) and organic matter digestibility (OMD).

Analytical procedures

Chemical analyses of feeds, residues, and fermentation fluids were conducted using standard methods. Dry matter (DM; Method 934.01), organic matter (OM; Method 942.05). Volatile fatty acids (VFA) were determined using gas chromatography after centrifuging the fermentation fluid at 10,000 × g for 15 minutes and filtering through a 0.22-µm membrane. Ammonia nitrogen (NH_3-N) concentration was measured using the method described by Conway (1962).

Statistical analysis

Data were analysed using analysis of variance (ANOVA) in a completely randomized design with the SPPS for Window version 25. The statistical model included the fixed effect of treatment and the random effect of incubation run. Treatment means were compared using Duncan's multiple range test at a significance level of $P < 0.05$. Orthogonal contrasts were used to examine specific treatment effects, fermented feed without PFAs and feed additive.

Results

In vitro dry matter and organic matter digestibility

In vitro dry matter digestibility (IVDMD) was significantly influenced by both treatment ($P = 0.001$) and incubation time ($P = 0.002$) (Table 2). The highest average IVDMD was recorded in T4 (79.2%), which contained fermented POS supplemented with 1.5% CL powder, 1.5% CZ powder, and 0.1% each of MM and CP ethanolic extracts. Conversely, T7 (fermented POS with only MM and CP extracts) yielded the lowest IVDMD (72.4%). A clear time-dependent pattern emerged, with peak digestibility at 6 h (81.6%), followed by a progressive decline at 12, 24 h, reaching 67.9% at 24 h.

In vitro organic matter digestibility (IVOMD) did not differ significantly among treatments ($P = 0.102$), although a significant time effect ($P = 0.001$) and treatment × time interaction ($P = 0.001$) were observed, with maximum organic matter degradation also occurring at 6 h (72.5%).

Table 2. In-vitro dry matter digestibility (IVDMD) and in-vitro organic matter digestibility (IVOMD) for all treatments

Treatments	Time(hours)					Average
	3	6	9	12	24	
IVDMD						
T1	80.3	79.9	78.2	78.6	67.9	76.9^{bc}
T2	73.4	83.1	81.5	79.4	71.8	77.8^{bc}
T3	79.3	86.4	76.3	78.5	63.2	76.7^{bc}
T4	81.1	90.7	82.3	73.0	68.8	79.2^{c}
T5	74.9	79.9	76.0	74.9	66.1	74.3^{ab}
T6	77.5	86.4	72.4	76.5	64.2	75.4^{abc}
T7	79.5	75.2	66.4	74.6	66.6	72.4^{a}
T8	73.4	78.2	79.8	72.6	71.8	75.1^{abc}
T9	73.8	74.7	76.8	73.6	70.7	73.9^{ab}
Average	77.0^{bc}	81.63^{d}	76.6^{c}	75.7^{b}	67.9^{a}	
P Treatments= 0.001; P Time= 0.002 ;P Interaction= 0.104.						
IVOMD						
T1	78.0	73.0	71.3	63.7	63.4	69.9
T2	55.1	74.9	73.3	68.7	65.6	67.5
T3	60.7	75.3	73.0	65.3	58.4	66.5
T4	61.3	79.3	72.9	56.4	61.7	66.3
T5	56.6	72.7	68.9	63.0	61.0	64.4
T6	56.9	77.1	69.1	59.9	59.0	64.4
T7	73.0	61.4	71.2	56.6	61.7	64.8
T8	53.9	69.6	71.9	61.9	66.8	64.8
T9	54.8	69.7	71.4	65.9	67.1	65.8
Average	61.1^{a}	72.5^{b}	71.43^{b}	62.3^{a}	62.7^{a}	
P Treatments= 0.102; P Time= 0.001; P Interaction= 0.001.						

Means with different superscripts within each column or row differ significantly with their corresponding P value.

Volatile fatty acid production and fermentation profile

The concentrations of individual VFAs, total VFA, and the acetate:propionate ratio after 24 hours of incubation are presented in Table 3. Acetic acid concentration varied significantly among treatments (P = 0.01), ranging from 48.8 mM (T6) to 92.5 mM (T4). Treatments T3, T4, T5, and T8 produced significantly higher acetic acid (77.3–92.5 mM) compared to T1, T2, T6, T7, and T9 (48.8–68.6 mM). Propionic acid followed a similar pattern, with the highest value in T4 (38.5 mM) and the lowest in T6 (18.4 mM) (P = 0.01).

Butyric acid concentration ranged from 28.3 mM (T1) to 35.3 mM (T8). Treatments T4, T5, and T6 showed intermediate butyric values (31.3–33.3 mM), while T7, T8, and T9 had the highest (34.3–35.3 mM). Iso-butyric, iso-valeric, and valeric acids also differed significantly among treatments (P = 0.01 to 0.02).

Notably, T4 produced the highest iso-valeric acid (8.97 mM) and T7 the lowest (1.33 mM).

Total VFA concentration was significantly affected by treatment (P = 0.01), ranging from 93.8 mM (T7) to 194.6 mM (T4). The highest total VFA was observed in T4 (194.6 mM), followed by T3 (175.5 mM) and T5 (159.9 mM). The acetate:propionate ratio (A:P) ranged from 2.19 (T7) to 2.70 (T6), with no significant difference among treatments (P = 0.45), indicating a consistent fermentation pattern.

Table 3. Partial and total VFA production after 24 hours incubation under *in vitro* test (mM)

Treatments	Acetic Acid	Propionic acid	Iso-butyric	Butyric Acid	Iso Valeric	Valeric Acid	Total VFA	Acetic: Propionic
T1	54.8^{a}	24.7^{ab}	0.77^{ab}	28.3^{b}	4.93^{b}	0.8^{ab}	114.3	2.33
T2	52.1^{a}	22.03^{a}	0.63^{ab}	29.3^{b}	4.90^{b}	1.8^{ab}	108.9	2.38
T3	87.2^{b}	33.8^{cd}	1.70^{c}	30.3^{cd}	6.80^{bc}	2.8^{bcd}	175.5	2.59
T4	92.5^{b}	38.5^{d}	1.37^{bc}	31.3^{d}	8.97^{c}	3.8^{d}	194.6	2.40
T5	81.0^{b}	30.5^{bc}	1.23^{bc}	32.3^{bc}	6.97^{bc}	4.8^{cd}	159.9	2.67
T6	48.8^{a}	18.4^{a}	0.63^{ab}	33.3^{b}	7.30^{bc}	5.8^{abc}	103.8	2.70
T7	55.5^{a}	25.2^{ab}	0.23^{a}	34.3^{a}	1.33^{a}	6.8^{a}	93.8	2.19
T8	77.3^{b}	30.6^{bc}	0.6^{ab}	35.3^{a}	1.17^{a}	7.8^{a}	124.3	2.52
T9	68.6^{a}	28.13^{b}	0.91^{ab}	31.81^{d}	1.15^{a}	$4,32^{cd}$	133.8	2,39
Average	68.7	28.0	0.90	31.8	5.30	4.30	134.4	2.47
SEM	3.7	1.9	0.60	2.86	0.61	0.21	7.83	0.06
P value	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.45

Means with different superscripts within each column or row differ significantly with their corresponding P value.

The acetic-to-propionic (A:P) ratio ranged from 2.19 to 2.70, though differences were not statistically significant (P = 0.45). Minor VFAs (iso-butyric, butyric, iso-valeric, valeric) also exhibited treatment-dependent variations ($P \leq 0.02$), reflecting altered amino acid and carbohydrate fermentation pathways.

Methane production

Estimated methane (CH_4) production using two different methods (Hegarty *et al.,* 2007; Moss *et al.,* 2000) and hydrogen recovery are presented in Table 4. The Hegarty method estimated CH_4 production ranging from 26.91 mol/day (T7) to 61.22 mol/day (T4). Treatments T1 (54.83), T3 (56.53), T4 (61.22), T5 (51.13), T8 (36.56), and T9 (41.12) showed significantly higher methane production compared to T2 (34.57), T6 (33.27), and T7 (26.9) (P = 0.01). The lowest methane production was consistently observed in T7 (fermented POS + 0.1% CP ethanolic extract only), which was 56% lower than the highest producer, T4.

Table 4. Estimated *in vitro* CH_4 production (mol/day)

Treatments	CH4 Hegarty	CH4 Moss	H recovery
T1	54.83^{ab}	30.63^{ab}	34.92^{ab}
T2	34.57^{a}	30.17^{a}	38.12^{ab}
T3	56.53^{b}	49.67^{cd}	28.69^{c}
T4	61.22^{b}	53.77^{d}	24.80^{bc}
T5	51.13^{b}	45.03^{bc}	30.83^{bc}
T6	33.27^{a}	29.27^{a}	48.18^{ab}
T7	26.91^{a}	23.01^{ab}	24.56^{a}
T8	36.56^{b}	31.61^{bc}	24.42^{ab}
T9	41.12^{b}	35.13^{bc}	31.14^{bc}
Average	41.82	36.64	31.92
SEM	3.72	1.87	0.60
P value	0.01	0.01	0.02

Means with different superscripts within each column or row differ significantly with their corresponding P value.

The Moss method produced a similar pattern but with lower absolute values, ranging from 23.01 mol/day (T7) to 53.77 mol/day (T4). Again, T2 (30.17), T6 (29.27), and T7 (23.01) had the significantly lowest methane emissions (P = 0.01), while T3 (49.67), T4 (53.77), T5 (45.03), T8 (31.61), and T9 (35.13) were higher.

Hydrogen recovery, an indicator of fermentation efficiency, varied significantly among treatments (P = 0.02). The lowest hydrogen recovery was found in T4 (24.8%) and T7 (24.56%), while the highest was in T6 (48.18%). Lower hydrogen recovery typically indicates less methanogenesis and more efficient capture of reducing equivalents into propionate and microbial biomass.

Key findings

The highest IVDMD (79.2%) and highest total VFA (194.6 mM) were achieved by T4 (fermented POS + 1.5% CL + 1.5% CZ + 0.1% CP ethanolic extract), while the lowest methane production (26.9–23.0 mol/day) and lowest hydrogen recovery (24.6%) were observed in T7 (fermented POS + 0.1% CP ethanolic extract only).

Fermented POS alone (T2) reduced methane by approximately 37% compared to non-fermented POS (T1) while maintaining digestibility. All treatments maintained a favorable acetate:propionate ratio (2.19–2.70), indicating efficient rumen fermentation.

The key findings were that fermented POS alone (T2) improved digestibility and reduced methane compared to non-fermented POS (T1), the combination of *Curcuma longa*, *Curcuma zanthoriza*, and *Carica papaya* ethanolic extract (T4) produced the highest dry matter digestibility and total VFA

and supplementation with *Carica papaya* ethanolic extract alone (T7) which resulted in the lowest methane production. These results demonstrate that PFAs can modulate rumen fermentation in a species and dose dependent manner, supporting the potential of POS-based diets integrated within the oil palm-cattle system (SISKA) to improve feed efficiency and reduce environmental impact.

Discussion

Dry matter and organic matter digestibility responses to PFAs

The highest IVDMD (79.2%) was achieved in T4, which contained fermented POS plus 1.5% CL powder, 1.5% CZ powder, and 0.1% CP ethanolic extract. This treatment also produced the highest total VFA (194.6 mM) and propionic acid (38.5 mM), indicating enhanced rumen fermentation efficiency. The improvement in digestibility can be attributed to bioactive compounds present in *Curcuma* species and *Carica papaya*. Curcumin, the principal curcuminoid in *Curcuma longa*, has been shown to stimulate rumen cellulolytic bacteria such as *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* (Lambo *et al.,* 2024). Similarly, *Curcuma zanthoriza* contains xanthorrhizol, which exhibits antimicrobial activity against Gram-positive bacteria but at moderate concentrations can selectively enhance beneficial fibrolytic microbes (Kholif *et al.,* 2021).

Interestingly, T7 (fermented POS with 0.1% CP ethanolic extract alone) had the lowest IVDMD (72.4%) and total VFA (93.8 mM), yet it produced the least methane (26.9 mol/day). This suggests that the methane reduction observed in T7 was not primarily due to improved digestibility but rather to direct inhibition of methanogens or redirection of hydrogen toward alternative electron sinks. *Carica papaya* leaves contain papain, alkaloids (carpaine), flavonoids, and tannins (Antonius *et al*., 2023). Tannins at low to moderate concentrations can bind to methanogen cell walls or inhibit their enzymes without severely depressing fiber digestion (Newbold and Ramos-Morales, 2020). The lower digestibility in T7 may indicate that the CP extract concentration (0.1%) was slightly above the optimal threshold for that specific basal diet. Dose-dependent effects of phytogenic feed additives have been well documented; Kholif *et al.* (2021) emphasized that the efficacy of PFAs varies with dosage, animal species, and basal diet composition.

Volatile fatty acid profiles and rumen fermentation efficiency

The total VFA concentrations observed across treatments (93.8–194.6 mM) are within ranges reported for *in vitro* rumen fermentation studies (Suparjo

et al., 2011). The significantly higher total VFA in T3, T4, and T5 (175.5, 194.6, and 159.9 mM, respectively) indicates that combinations of multiple PFAs synergistically enhanced substrate fermentation. The synergy between *Curcuma* species and *Carica papaya* extract may arise from complementary modes of action: curcuminoids increase microbial enzyme activity while papain hydrolyzes protein fractions, providing ammonia and peptides for microbial growth (Polyorach *et al*., 2016).

The acetate:propionate (A:P) ratios ranged from 2.19 to 2.70, with no significant treatment differences (P = 0.45). These values are lower than typical ratios reported for forage-based diets (3.0–4.0), suggesting that the POS-tofu waste-grass mixture promoted glucogenic fermentation (Moss *et al.,* 2000). Lower A:P ratios are desirable because propionate is a more efficient precursor for gluconeogenesis and captures reducing equivalents (hydrogen) that would otherwise be used for methanogenesis (Demeyer and Van Nevel, 1975). The numerically lowest A:P ratio was observed in T7 (2.19), which also had the lowest methane production, confirming the inverse relationship between propionate production and methanogenesis. Butyric acid concentrations were significantly higher in T7, T8, and T9 (34.3–35.3 mM) compared to other treatments. Butyrate is an important energy source for rumen epithelial cells and has been associated with improved gut health (Beauchemin *et al*., 2020). The elevated butyrate in CP-containing treatments may be due to the action of papain on protein, producing peptides that certain butyrate-producing bacteria like *Butyrivibrio fibrisolvens* utilize.

Methane mitigation potential of PFAs

Methane production estimated by both the Hegarty and Moss methods showed consistent treatment rankings. The lowest methane emitters were T2 (fermented POS alone), T6 (fermented POS + 3.0% CZ ethanolic extract + 0.1% CP extract), and T7 (fermented POS + 0.1% CP extract). T7 reduced methane by 56% compared to T4, the highest emitter. These results demonstrate that *Carica papaya* ethanolic extract possesses strong anti-methanogenic properties. Several mechanisms explain the methane-reducing effect of CP extract. First, the tannin content in papaya leaves can directly inhibit methanogenic archaea by interfering with cell membrane integrity and enzyme function (Abeni and Manfredi, 2023). Second, saponins present in papaya may disrupt protozoal populations; rumen protozoa are symbiotic with methanogens, and defaunation (reduction of protozoa) can lower methane by 20–50% (Yanza *et al*., 2024). Third, flavonoids and phenolic compounds can act as electron sinks, competing with methanogens for hydrogen, thereby shifting fermentation toward propionate and butyrate (Lambo *et al*., 2024). The hydrogen recovery data support this interpretation: T7

and T4 had the lowest hydrogen recovery (24.6% and 24.8%, respectively), indicating that reducing equivalents were efficiently captured into reduced end products (propionate, butyrate, microbial biomass) rather than being lost as methane (Demeyer and Van Nevel, 1975). It is noteworthy that T4, despite having the highest digestibility and total VFA, also produced the highest methane (61.2 mol/day). This trade-off between high fermentative activity and methane production is a common challenge in ruminant nutrition (Almeida *et al.,* 2021). The ideal PFA should shift fermentation toward propionate and reduce methanogenesis without compromising digestibility. T6 and T7 achieved this balance: T6 had moderate IVDMD (75.4%) and low methane (33.3 mol/day); T7 had lower digestibility but the lowest methane. Further in vivo studies are needed to identify the optimal inclusion level of CP extract that maintains digestibility while suppressing methanogenesis.

Comparison with previous studies

Our findings align with earlier research on PFAs in Bali cattle. Dwatmadji *et al*. (2021) reported that herbal supplement blocks containing *Curcuma* and *Carica* improved intake and digestibility in Bali cattle fed agricultural by-products, but they did not measure methane. Dwatmadji *et al*. (2021) observed enhanced physiological condition and average daily gain (ADG) with short-term herbal supplementation, suggesting that PFAs can improve production performance. The present study extends those findings by providing mechanistic evidence at the VFA and methane level. Meta-analyses have quantified the methane reduction potential of various PFAs. Almeida *et al.* (2021) concluded that tannin-containing plants can reduce methane by 5–30% depending on concentration and diet. Lambo *et al*. (2024) reviewed that curcuminoids at 0.5–2.0% of dry matter reduced methane by 10–25% *in vitro*. Our results using *Carica papaya* extract (0.1%) achieved a 56% reduction in T7 compared to T4, which was higher than typical values, possibly due to synergistic effects with fermented POS or the specific extract preparation method (ethanolic maceration). However, *in vitro* estimates often overestimate methane reduction compared to in vivo conditions due to the absence of absorption and host metabolism (Beauchemin *et al*., 2020). Therefore, the three best-performing treatments (T4, T6, T7) should be validated in vivo using Bali cattle.

It can be highlighted that fermented palm oil sludge (POS) as a basal feed improved digestibility and reduced enteric methane compared to non-fermented POS *in vitro*. Supplementation with phytogenic feed additives, particularly *Carica papaya* ethanolic extract alone (T7) or in combination with *Curcuma longa* and *Curcuma zanthoriza* (T4), modulated VFA profiles and methane production in a treatment-dependent manner. T4 achieved the highest feed

digestibility and total VFA but also high methane, while T7 achieved the lowest methane (56% reduction relative to T4) at the cost of slightly lower digestibility. The acetate:propionate ratios remained favourable (2.19–2.70), indicating efficient rumen fermentation. These findings are supported the use of fermented POS and locally available PFAs within the SISKA framework to enhance feed efficiency and mitigate greenhouse gas emissions from ruminants. Further *in vivo* and metagenomic studies are warranted to validate these results and identify optimal inclusion levels.

Acknowledgments

We are appreciative of the financial support provided by the Directorate General of Higher Education, Ministry of Higher Education, Science and Technology porting this experiment (scheme Fundamental) with the contract number 062/C3/DT.05.00/PL/2025 and No. 2841/UN30.15/PT/2025.

Conflict of interest

The authors declare no conflict of interest.

References

Abdel-Halim, S., Ibrahim, M., Abdel Mohsen, M., Abou-Setta, L., Sleem, A. and El-Missiry, M. (2021). The influence of the extraction method on polyphenols, flavonoids composition and anti-hyperlipidemic properties of papaya leaves (*Carica papaya* Linn.). Bulletin of the National Research Centre, 45. https://doi.org/10.1186/s42269-021-00548-4

Abeni, F. and Manfredi, M. T. (2023). Condensed tannins fed to dairy goats: Effects on digestibility, milk production, blood parameters, methane emission, and energy and nitrogen balances. Journal of Dairy Science, 106:5405-5418.

Almeida, A. K., Hegarty, R. S. and Cowie, A. (2021). Meta-analysis quantifying the potential of dietary additives and rumen modifiers for methane mitigation in ruminant production systems. Animal Nutrition, 7:1219-1230.

Amalina, I. F., Haziq, J. M., Syukor, A. R. A., Ridwan, A. F. A. and Rashid, A. H. M. (2020). Study of Palm Acid Oil (PAO) from Sludge Palm Oil Mill Effluent (POME) as Goat's Feed. In Materials Today: Proceeding, 1:96-101. https://doi.org/10.1016/j.matpr.2020.11.1013

Antonius, A., Pazla, R., Putri, E. M., Negara, W., Laia, N. and Ridla, M. (2023). Effectiveness of herbal plants on rumen fermentation, methane gas emissions, *in vitro* nutrient digestibility, and population of protozoa. Animal Nutrition, 16:1477-1488.

Bature, I., Xiaohu, W. and Ding, X. (2024). The roles of phytogenic feed additives, trees, shrubs, and forages on mitigating ruminant methane emission. Frontiers in Veterinary Science, 11:1-14. https://doi.org/10.3389/fvets.2024.1475322

Beauchemin, K. A., Ungerfeld, E. M., Eckard, R. J. and Wang, M. (2020). Review: Fifty years of research on rumen methanogenesis: Lessons learned and future challenges for mitigation. Animal, 14:S2-S16.

Conway, E. J. (1962). Microdiffusion Analysis and Volumetric Error (5th rev. ed.). London: Crosby Lockwood.

Demeyer, D. I. and Van Nevel, C. J. (1975). Methanogenesis, an integrated part of carbohydrate fermentation, and its control. In I. W. McDonald & A. C. I. Warner (Eds.), Digestion and metabolism in the ruminant. University of New England Publishing Unit, pp.366-382.

Devendra, C., Yeong, S. W. and Ong, H. K. (1983). The potential value of palm oil mill effluent (POME) as a feed source for farm animals in Malaysia. In *Proceedings of National Workshop on Oil Palm By-Product Utilization*. pp.63-75. Institut Penyelidikan Minyak Kelapa Sawit Malaysia (IPMKSM), Kuala Lumpur.

Dwatmadji, Suteky, T. and Sutrisno, E. (2021). Short term herbal supplementation on the physiological condition of Bali cattle under the oil palm integration system. International Journal of Agricultural Technology, 17:1677-1684.

Hegarty, R. S., Goopy, J. P., Herd, R. M. and Archibald, R. (2007). Effects of persistent divergence in methane yield on the production and efficiency of beef cattle. Australian Journal of Experimental Agriculture, 47:829-836. https://doi.org/10.1071/EA07008

Henderson, G., Cox, F., Ganesh, S., Jonker, A., Young, W., Janssen, P. H., Abecia, L., Angarita, E., Aravena, P., Arenas, G. N., Ariza, C., Attwood, G. T., Avila, J. M., Avila-Stagno, J., Bannink, A., Barahona, R., Batistotti, M., Bertelsen, M. F., Brown-Kav, A. and Zunino, P. (2015). Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. Scientific Reports, 5. https://doi.org/10.1038/srep14567

Jami, E. and Mizrahi, I. (2012). Composition and similarity of bovine rumen microbiota across individual animals. PLoS ONE, 7:1-8. https://doi.org/10.1371/journal.pone.0033306

Jerónimo, E., Pinheiro, C., Lamy, E., Dentinho, M. T., Sales-Baptista, E., Lopes, O. and Capela e Silva, F. (2016). Tannins in ruminant nutrition: Impact on animal performance and quality of edible products. Tannins: Biochemistry, Food Sources and Nutritional Properties, 121-168.

Kholif, A. E., Anele, U. Y., Patra, A. K. and Varadyova, Z. (2021). Editorial: The use of phytogenic feed additives to enhance productivity and health in ruminants. Frontiers in Veterinary Science, 8:1-3.

Kuralkar, P. and Kuralkar, S. V. (2021). Role of herbal products in animal production – An updated review. In Journal of Ethnopharmacology, 278. https://doi.org/10.1016/j.jep.2021.114246

Lambo, M. T., Ma, H., Liu, R., Dai, B., Zhang, Y. and Li, Y. (2024). Review: Mechanism, effectiveness, and the prospects of medicinal plants and their bioactive compounds in lowering ruminants' enteric methane emission. Animal, 18:101134.

McGrath, J., Duval, S. M., Tamassia, L. F. M., Kindermann, M., Stemmler, R. T., de Gouvea, V. N., Acedo, T. S., Immig, I., Williams, S. N. and Celi, P. (2018). Nutritional strategies in ruminants: A lifetime approach. Research in Veterinary Science, 116:28-39. https://doi.org/10.1016/j.rvsc.2017.09.011

Moss, A. R., Jouany, J. P. and Newbold, J. (2000). Methane production by ruminants: Its contribution to global warming. Annales de Zootechnie, 49:231-253. https://doi.org/10.1051/animres:2000119

Newbold, C. J. and Ramos Morales, E. (2020). Review: Ruminal microbiome and microbial metabolome: Effects of diet and ruminant host. Animal, 14:S78-S86.

Phoemchalard, C., Uriyapongson, S. and Berg, E. P. (2014). Effect of cassava bioethanol by-product and crude palm oil in Brahman x Thai native yearling heifer cattle diets: I. Nutrient digestibility and growth performance, 663–668. https://doi.org/10.1007/s11250-014-0549-x

Polyorach, S., Wanapat, M., Cherdthong, A. and Kang, S. (2016). Rumen microorganisms, methane production, and microbial protein synthesis affected by mangosteen peel powder supplement in lactating dairy cows. Tropical Animal Health and Production, 48:593-601.

Salami, S. A., Valenti, B., Bella, M., O'Grady, M. N., Luciano, G., Kerry, J. P., Jones, E., Priolo, A. and Newbold, C. J. (2018). Characterisation of the ruminal fermentation and microbiome in lambs supplemented with hydrolysable and condensed tannins. FEMS Microbiology Ecology, 94:1-13. https://doi.org/10.1093/femsec/fiy061

Suparjo, S., Laconi, E. B. and Wina, E. (2011). Pengaruh pemberian saponin dari *Sapindus rarak* dan ekstrak tanaman herbal terhadap profil VFA dan populasi protozoa pada fermentasi rumen secara *in vitro*. Jurnal Ilmu Ternak dan Veteriner,16:112-120.

Suteky, T. and Dwatmadji. (2023). The Synthesis AgNPs of Leaves *Melastoma malabathricum* Extract reduce Adult *Haemonchus contortus* Motility Assay. Research Journal of Pharmacy and Technology, 16:1913-1918. https://doi.org/10.52711/0974-360X.2023.00314

Theodorou, M. K and Brooks, A. E. (1990). Evaluation of a New Laboratory Procedure for Estimating the Fermentation Kinetics of Tropical Feeds. AFRC Institute for Grassland and Environment Research, Pas Gogerdan, Aberyswyth, Dyfed, Australian, 5-21.

Tiwari, M., Barooah, M. S. and Bhuyan, D. (2023). Phytochemical and bioactive potentialities of *Melastoma malabathricum*. In Recent Frontiers of Phytochemicals: Applications in

Food, Pharmacy, Cosmetics, and Biotechnology, pp.601-615. https://doi.org/10.1016/B978-0-443-19143-5.00024-4

Waters, S. M., Roskam, E., Smith, P. E., Kenny, D. A., Popova, M. and Eugène, M. (2024). The role of rumen microbiome in the development of methane mitigation strategies for ruminant livestock. Journal of Dairy Science, 108:7591-7606. https://doi.org/10.3168/jds.2024-25778

Yanza, Y. R., Irawan, A., Jayanegara, A., Ramadhani, F., Respati, A. N., Fitri, A., Hidayat, C., Niderkorn, V., Cieslak, A., Szumacher-Strabel, M., Hidayat, R. and Tanuwiria, U. H. (2024). Saponin Extracts Utilization as Dietary Additive in Ruminant Nutrition: A Meta-Analysis of In Vivo Studies. Animals, 14. https://doi.org/10.3390/ani14081231

Zubairi, S. I., Omar, H., Nurzahim, Z. and Ramlan, N. (2023). The biological response of *Carica papaya* leaves extract to saponin reduction (O/W) emulsion on human bronchial epithelium cell (BEAS-2B). Arabian Journal of Chemistry, 16:104416. https://doi.org/10.1016/j.arabjc.2022.104416

(Received: 15 August 2025, Revised: 15 June 2026, Accepted: 1 July 2026)