International Journal of Agriculture and Biosciences 2026 15(4): 1828-1834.
https://doi.org/10.47278/journal.ijab/2026.090

RESEARCH ARTICLE
eISSN: 2306-3599; pISSN: 2305-6622

Potential use of Microencapsulated Tropical Plant-based Phytonutrient Compounds on *in vitro* Fermentation Characteristics and Methane Mitigation

Parichat Wadjeam [1], Bounnaxay Viennaxay [2], Maharach Matra [3], Kampanat Phesatcha [4] and Burarat Phesatcha [1]*

[1]Department of Applied Biology, Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan, Nakhon Ratchasima 30000, Thailand
[2]Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand
[3]Division of Animal Science, Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Maha Sarakham 44150, Thailand
[4]Department of Animal Science, Faculty of Agriculture and Technology, Nakhon Phanom University, Nakhon Phanom 48000, Thailand
*Corresponding author: burarat_kat@hotmail.co.th

ABSTRACT

The aim of this study was to evaluate the appropriateness of microencapsulated *Flemingia* and mangosteen peel extracts (mFMPE) as a potential provider of PC for in vitro rumen fermentation and methane production. The study was done with a completely randomized design (CRD). The treatments were added with mFMPE at concentrations of 0, 2, 4, and 6% of the total substrate. The inclusion of mFMPE substantially enhanced the *in vitro* dry matter degradability at 12 and 24 h. When 4% of the total substrate was added, the mFMPE showed the highest levels of propionate and total volatile fatty acid (VFA) production, while reducing the proportion of methane (CH4). Furthermore, ammonia-nitrogen concentration was significantly increased with mFMPE supplementation. Therefore, mFMPE exhibits significant promise as a valuable nutritional supplement for ruminant feed additives.

Keywords: Microencapsulation, Phytonutrient extracts, Tropical plant, Rumen ecology, Ruminants.

Article History
Article # 26-044
Received: 16-Jan-26
Revised: 23-Mar-26
Accepted: 25-Mar-26
Online First: 07-Apr-26

INTRODUCTION

Animal agriculture is recognized as a significant contributor to greenhouse gas (GHG) emissions, accounting for a substantial portion of all agricultural-sector emissions (McAllister et al., 2015; FAO, 2023). The majority of GHGs released directly by animal agriculture are methane (CH_4) and nitrous oxide (N_2O), which possess global warming potentials significantly higher than that of CO_2. Ruminants account for roughly 80% of livestock-sector emissions, primarily through enteric fermentation (Cieślak et al., 2016; Terry et al., 2020). In recent years, the urgency to mitigate these emissions has intensified due to accelerating climate change, prompting extensive research into sustainable nutritional interventions (Hristov et al., 2022; IPCC, 2022). Thus, reducing methane emissions from ruminants is highly advantageous not only for mitigating environmental impacts but also for improving feed energy efficiency, as enteric methanogenesis represents a 6–10% loss of gross dietary energy (Lileikis et al., 2023; Shinkai et al., 2024). Consequently, the scientific focus has shifted strongly toward identifying and implementing practical CH4 reduction techniques, including the use of plant secondary metabolites and phytonutrients in ruminant feeding systems (Malyugina et al., 2025; Phupaboon et al., 2025).

Tropical plants with high amounts of phytonutrient components (PCs), including phenolics and flavonoids, have emerged as compelling options for suppressing CH_4 production in ruminant feeding (Gunun et al., 2019; Bashir et al., 2025). Scientific studies have demonstrated that these tropical plants can improve rumen microbial balance and optimize protein utilization (Calabrò et al., 2015). One of the most interesting tropical plants, *Flemingia* (*Flemingia macrophylla*) is a captivating tropical plant that originates

Cite this Article as: Wadjeam P, Viennaxay B, Matra M, Phesatcha K and Phesatcha B 2026. Potential use of microencapsulated tropical Plant-based phytonutrient compounds on *in vitro* fermentation characteristics and methane mitigation. International Journal of Agriculture and Biosciences 15(4): 1828-1834. https://doi.org/10.47278/journal.ijab/2026.090

A Publication of Unique Scientific Publishers

from South and South-East Asia. These plants contain significant amounts of protein and PC (Andersson et al., 2006). Additionally, the peel of the Mangosteen fruit (*Garcinia mangostana*) is a by-product of tropical agriculture in certain countries. It contains PC, which has the potential to inhibit the growth of certain microorganisms in the rumen, hence reducing the formation of CH_4 in the digestive system. The utilization of mangosteen peel as a feed ingredient for ruminants has the potential to reduce CH4 synthesis and biohydrogenation, while maintaining a stable ruminal pH and volatile fatty acid levels. However, it may reduce the population of methanogenic microorganisms in the rumen (Ampapon et al., 2019). This study is focused on merging two plant species through the utilization of cutting-edge techniques named "microencapsulation". This approach has the potential to address technical issues in the fields of food, pharmaceuticals, and agriculture (Paulo & Santos, 2017). Microencapsulation is a process that involves trapping substances in a continuous matrix of wall materials, resulting in particles with a size distribution ranging from 1-5,000 µm. These substances can be in solid, liquid, or gaseous form (Sharif et al., 2020). Microencapsulated-PCs are an advanced technology employed in the nutrition of ruminants. The implementation of microencapsulation aims to provide environmental protection, regulate the release characteristics of coated substances to enhance their bioavailability, and improve the practicality of materials (Ozkan et al., 2019). Therefore, the objective of this work was to assess the suitability of mFMPE as a potential source of PC on *in vitro* rumen fermentation and methane production.

MATERIALS & METHODS

Preparation of mFMPE

Fresh Flemingia (*Flemingia macrophylla*) leaves were collected from the experimental farm of Khon Kaen University, and mangosteen (*Garcinia mangostana*) peels were procured from a local fruit market in Khon Kaen, Thailand. The botanical identification and authentication of both plants were formally performed by Tropical Feed Resources Research and Development Center, Department of Animal Science, Faculty of Agriculture, Khon Kaen University, prior to further processing. Subsequently, the fresh plants were sun-dried at 60°C for approximately 2-3 days. The dried plant was ground using a Cyclotech Mill (Tecator, Hoganas, Sweden) with a 1-mm sieve. The powder was combined with deionized water and subjected to microwave heating at 60°C. After 35 minutes, the particles were separated by filtration. The liquids were mixed with the wall materials (cricket meal and tween 80), as explained in Nouri (2019). Then, the process involved the use of a Bŭchi B-191 Mini Spray Dryer (Kurek & Pratap-Singh, 2020), to create spray-dried microencapsulated PC extracts from *Flemingia* and mangosteen peel, as detailed in Phupaboon et al. (2022).

Chemical Analyses

The dry matter (DM), ash, and crude protein (CP) content of concentrate, rice straw, and mFMPE was chemically evaluated (AOAC, 2012); as described in numbers 967.03, 942.05, and 984.13, respectively. The Ankom A200i Fiber Analyzer (Ankom Technology Co., New York, USA) was used to determine the fiber, specifically neutral-detergent fiber (NDF) and acid-detergent fiber (ADF) (Van Soest et al., 1991). Moreover, the mFMPE samples were analyzed for the presence of total phenolic compounds (TPC) using the Folin-Ciocalteu reagent and measuring the absorbance at 765 nm (Al-Duais et al., 2009) and the total flavonoid compounds (TFC) were determined using the method described by Topçu et al. (2007). The colorimetric changes were measured at 415 nm using a 10% aluminum chloride solution. The mFMPE sample was evaluated for its antioxidant capacity using ABTS (Re et al., 1999), DPPH (Gali & Bedjou, 2019) and FRAP (Benzie & Strain, 1996). Briefly, for the ABTS assay, the ABTS radical cation was generated by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate in the dark for 12-16 h before use. The solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. Then, 0.1 mL of the extract was mixed with 2.9 mL of the diluted ABTS solution. After 6 minutes of incubation, the absorbance was recorded at 734 nm. For the DPPH assay, 0.1 mL of the sample extract was mixed with 2.9 mL of a 0.1 mM DPPH radical solution in methanol. The mixture was shaken vigorously and left to stand in the dark at room temperature for 30 minutes, and the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. For the FRAP assay, the working FRAP reagent was prepared daily by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl, and 20 mM $FeCl_36H_2O$ in a 10:1:1 (v/v/v) ratio. A volume of 0.1 mL of the extract was incubated with 2.9 mL of the FRAP reagent in the dark for 30 minutes. The absorbance of the colored product (ferrous tripyridyltriazine complex) was then measured at 593 nm.

Rumen Inoculum Preparation

Ruminal fluids were obtained from four Holstein-crossbred dairy cows, weighing 400 ± 10 kg, that were fed a total mixed ration (TMR) consisting of 60% roughage and 40% concentrate on a dry matter basis. The primary roughage source in the TMR was chopped rice straw. The diet was designed to meet the cows' nutrient needs as specified by the National Research Council (2001). Rumen fluid samples were collected by inserting a tube connected to a vacuum pump through the mouth and into the middle of the rumen, where they were then transferred into a plastic flask. The filtered samples were placed in a thermally insulated bottle at 39°C after four layers of folded cheesecloth were used for filtration. The preparation of the medium solution includes micro-mineral solution, resazurine, reduction solution, macro-mineral solution, buffer solution, and distilled water. The rumen fluid was mixed with the prepared artificial saliva medium at a 1:2 (mL/mL) ratio, as described by Matra et al. (2023).

Experimental Design and Treatments

The study was conducted using a completely

randomized design (CRD). The dietary substrates, consisting of a mixture of rice straw and concentrate (the ingredients and chemical composition of which are detailed in (Table 1)) in a 60:40 ratio on a dry matter basis, were weighed at 0.5 g and placed into 60 mL bottles. The treatments were then supplemented with mFMPE at 0, 2, 4, and 6% of the total substrate.

Table 1: Feed ingredients and chemical composition

Items	Concentrate	Rice straw	mFMPE
Feed Ingredients (% as fed)			
Cassava chip	54.0		
Rice bran	17.0		
Palm meal	13.0		
Soybean meal	10.5		
Urea	2.5		
Sulfur	1.0		
Mineral mixed	1.0		
Salt	1.0		
Chemical composition			
Dry matter (%)	92.5	89.4	91.3
	----------% DM----------		
Organic matter	92.6	85.4	94.2
Crude protein	14.6	2.4	4.5
Neutral detergent fiber	20.5	76.9	50.4
Acid detergent fiber	8.2	52.4	27.1
Antioxidant capacity			
TPC (mg GAE/g DM)			2871.8
TFC (mg QUE/g DM)			436.7
DPPH radical inhibition (%)			35.5
ABTS radical inhibition (%)			16.0
FRAP (mg TROE/g DM)			17.9

mFMPE, microencapsulated *Flemingia* and mangosteen peel extracts; DM, dry matter; TPC, total phenolic content; TFC, total flavonoid content; GAE, gallic acid equivalent; QUE, quercetin equivalent; DPPH, 2, 2-diphenyl-1-picrylhydrazyl as DPPH radical scavenging activity; ABTS, 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) as ABTS radical scavenging activity; FRAP, ferric reducing antioxidant power; TROE, trolox equivalent.

In vitro Incubation Procedures

Gas production was monitored at specific time intervals (1, 2, 4, 6, 8, 12, 24, 48, 72, and 96 hours) during the incubation period. Each treatment consisted of three bottles. The samples were individually collected for pH, VFA, and NH_3-N, respectively. The samples were measured pH (pH meter; HANNA Instruments HI 8424 microcomputer, Singapore). The rumen fluid samples were centrifuged at 16,000 × g for 15 minutes after filtration through cheesecloth. Subsequently, VFA profiles were analyzed using an HPLC machine (ETL Testing Laboratory, Inc., Cortland, NY, USA) (Samuel et al., 1997), and the NH_3-N content was examined using micro-Kjeldahl methods (Van Soest et al., 1991). The supernatant was stored at -20°C. The measurements of methane production were conducted using a Gas chromatography (GC) instrument (GC2014; Shimadzu Co Ltd., Kyoto, Japan) equipped with a thermal conductivity detector (TCD) and a packed column. The operational temperatures were maintained at 70 °C for the column oven, 200 °C for the injector, and 250°C for the detector. High-purity helium was utilized as the carrier gas with a flow rate of 30 mL/min. Moreover, the assessment of nutrient degradability was conducted using an independent set at 12 and 24 h of incubation. At the end of each specified incubation period, the fermentation process was stopped, and the contents of the bottles were filtered through pre-weighed Gooch crucibles. The residual substrates were washed thoroughly with warm distilled water and then oven-dried at 60°C for 48 h to determine the *in vitro* dry matter degradability (IVDMD).

Statistical Analyses

The data were analyzed employing the general linear model approach according to the method of SAS (2013). The statistical model used for the analysis was: $Yij = \mu + Ti + eij$ where Yij is the observation of the dependent variable, μ is the overall mean, Ti is the effect of the mFMPE supplementation level and eij is the random residual error. Tukey's test was used to compare the mean values of the experimental treatments. Treatment means were deemed significantly different whether their p-values were below 0.05 and 0.01, respectively. The analysis of mFMPE supplementation responses was conducted using orthogonal polynomials to identify trends.

RESULTS

Nutritional Composition of mFMPE

As presented in Table 1, the chemical composition of mFMPE was as follows: 91.3% DM, and on a dry matter basis, 94.2% OM, 4.5% CP, 50.4% NDF, and 27.1% ADF. Moreover, The PC present in mFMPE had a TPC of 2,871.8 mg GAE/g DM and TFC of 436.7 mg QUE/g DM. The antioxidant capacity is comprised of 35.5% DPPH, 16.0% ABTS, and 17.9 mg TROE/g DM in FRAP, respectively.

Gas Production Kinetics and Feed Degradability

The gas production, specifically gas *a* and *b*, exhibited significant variation (linear effect; $P<0.05$), while gas *c* and *a+b* were significantly influenced (quadratic effect; $P<0.05$) when mFMPE supplementation was implemented. Furthermore, cumulative gas at 96 h was improved (quadratic effect; $P<0.01$) by mFMPE feeding. In part of *in vitro* DM degradability, both 12 and 24 h after incubation were significantly increased, especially when supplemented at 4% mFMPE. The findings on gas production and feed degradability are given in Table 2. Fig. 1 illustrates that cumulative gas production increased with mFMPE supplementation, with the 4% level yielding the highest gas volume throughout 1–96 h of incubation, indicating enhanced fermentation efficiency.

Table 2: Effect of mFMPE on gas production kinetics and *in vitro* DM degradability

mFMPE (% of total substrate)	Gas production kinetics[1]				Cumulative gas (mL) at 96 h[2]	IVDMD (%)	
	a	*b*	*c*	*a+b*		12 h	24 h
0	-4.12[a]	82.85[a]	0.033[a]	78.73[a]	47.8[a]	74.62[a]	67.56[a]
2	-3.91[b]	57.52[b]	0.036[a]	53.61[b]	65.7[b]	79.79[b]	69.38[a]
4	-3.25[b]	69.15[b]	0.027[b]	65.90[c]	75.1[c]	81.38[c]	75.05[b]
6	-3.45[b]	56.12[b]	0.022[b]	52.67[b]	68.3[b]	79.81[b]	72.50[b]
SEM	0.51	2.38	0.01	1.92	0.20	0.47	1.38
Orthogonal polynomials							
Linear	0.02	0.02	0.03	0.02	<0.01	0.02	0.01
Quadratic	0.30	0.16	0.02	0.03	<0.01	0.01	0.02
Cubic	0.44	0.26	0.07	0.23	0.22	0.16	0.24

mFMPE, microencapsulated *Flemingia* and mangosteen peel extracts; IVDMD, *in vitro* dry matter degradability; [1]Gas production kinetics, *a*, the gas production from the immediately soluble fraction (mL); *b*, the gas production from the insoluble fraction (mL); *c*, the gas production rate constant for the insoluble fraction (mL/h); *a+b*, the potential extent of gas production (mL); [2]Cumulative gases at 96 h (mL/0.2 g DM substrate); [a-c] Means within the same column with different letters are significantly different at $P < 0.05$.

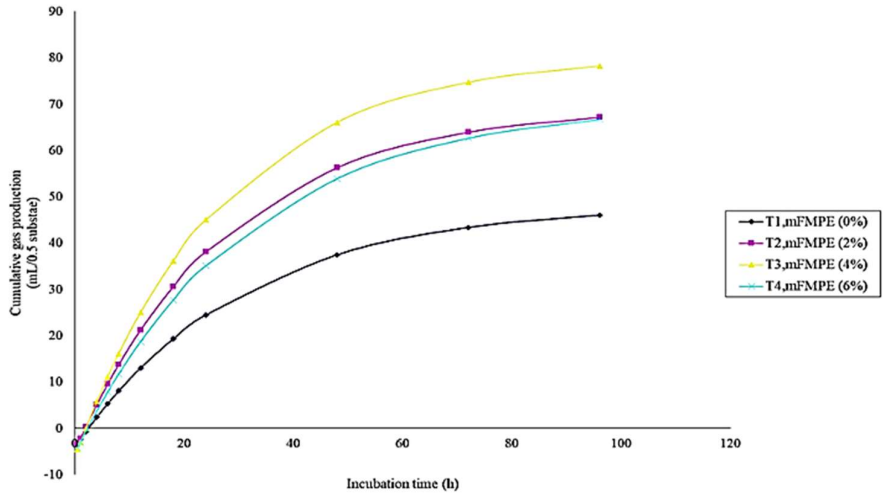

Fig. 1: Effect of mFMPE on cumulative gas production curves after 1-96 h of incubation.

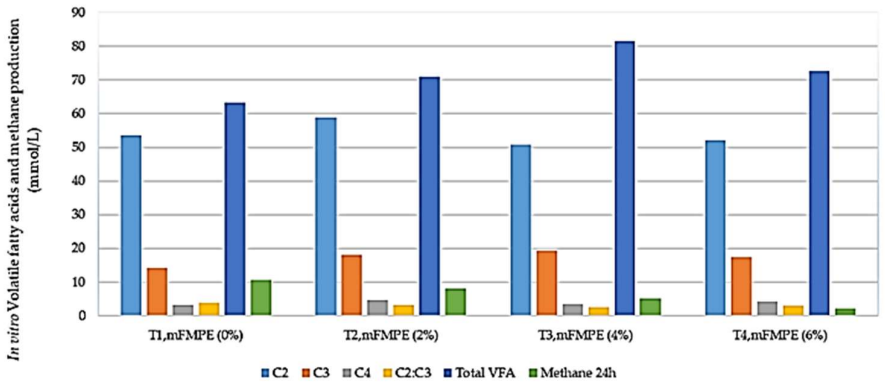

Fig. 2: Effect of mFMPE on rumen volatile fatty acids (VFAs) and methane production.

Volatile Fatty Acids and Methane Profiles

Table 3: The mFMPE treatment indicated significant linear effects on acetate ($P<0.05$), propionate ($P<0.05$), the acetate-to-propionate ratio ($P<0.01$), and total VFA production ($P<0.05$) compared to the control treatments. Additionally, the 4% mFMPE supplementation had the greatest propionate ($P<0.01$) and total VFA content ($P<0.05$). While, the concentration of butyrate showed no significant difference ($P>0.05$) with the addition of mFMPE. In addition, the production of methane declined linearly ($P<0.05$) after 12 and 24 h of fermentation as the level of mFMPE increased, especially at 4-6% of total substrate. As illustrated in Fig. 2, mFMPE supplementation markedly increased propionate and total VFA concentrations while linearly decreasing methane production.

Table 3: Effect of mFMPE on *in vitro* gas production, volatile fatty acids and methane production

mFMPE (% of total substrate)	VFA[1] (mol/100 mL)				Total VFA (mmol/L)	CH_4 production (%)	
	C_2	C_3	C_4	C_2:C_3		12 h	24 h
0	53.44^{a}	14.27^{a}	3.23	3.74^{a}	63.30^{a}	1.98^{a}	10.60^{a}
2	58.85^{b}	18.08^{b}	4.73	3.25^{a}	70.94^{b}	1.59^{b}	8.05^{b}
4	50.66^{c}	19.38^{c}	3.26	2.61^{b}	81.67^{c}	1.50^{b}	4.97^{b}
6	52.01^{a}	17.41^{d}	4.32	2.98^{c}	72.73^{d}	1.22^{b}	2.18^{c}
SEM	1.32	0.28	0.45	0.05	0.82	0.35	0.44
Orthogonal polynomials							
Linear	0.03	0.02	0.92	<0.01	0.01	0.02	0.01
Quadratic	0.88	0.41	0.96	0.76	0.20	0.58	0.08
Cubic	0.16	0.25	0.18	0.37	0.12	0.44	0.82

mFMPE, microencapsulated *Flemingia* and mangosteen peel extracts; CH_4, methane; [1]VFA, volatile fatty acid production: C_2, acetate; C_3, propionate; C_4, butyrate; [a-d] Means within the same column with different letters are significantly different at $P < 0.05$.

Ruminal pH and Ammonia-Nitrogen Concentration

There was no significant effect on ruminal pH (12 and 24h) when the level of mFMPE was increased ($P>0.05$). Whilst the ammonia nitrogen content at 12 and 24 h showed a quadratic increase ($P<0.05$) when mFMPE was used at a concentration of 6% of the total substrate (Table 4).

Table 4: Effect of mFMPE on rumen pH and ammonia-nitrogen concentration

mFMPE (% of total substrate)	pH		Ammonia nitrogen (mg/dL)	
	12h	24h	12h	24h
0	7.0	7.0	18.46^{a}	19.04^{a}
2	7.0	6.9	18.92^{a}	20.44^{b}
4	6.9	6.9	17.84^{b}	21.84^{c}
6	6.9	6.9	19.62^{c}	19.53^{a}
SEM	0.44	0.02	0.07	0.14
Orthogonal polynomials				
Linear	0.43	0.27	0.39	0.07
Quadratic	0.71	0.39	0.03	0.04
Cubic	0.43	0.68	0.12	0.06

mFMPE, microencapsulated *Flemingia* and mangosteen peel extracts; [a-c] Means within the same column with different letters are significantly different at $P < 0.05$.

DISCUSSION

Ruminants possess a distinctive digestive system that enables them to break down plant components, such as cellulose, hemicellulose, and lignin, which other animals cannot efficiently utilize (Shinkai et al., 2024). Tropical plants contain notable amounts of structural carbohydrates, such as cellulose and hemicellulose, while having relatively low crude protein levels. An inherent limitation that restricts anaerobic fermentation of organic matter and causes

prolonged digesta retention in the reticulo-rumen, resulting in significant enteric methane (CH4) emissions (Ali et al., 2019; Goopy et al., 2020). Considering these conditions, it has been argued that PCs found in many plants can reduce CH_4 emissions by affecting the rumen microbiome through several pathways (Vasta et al., 2019). In the recent investigation, nutrient degradability was enhanced with mFMPE supplementation. This phenomenon could be attributed to an increase in the microbial population, leading to greater feed breakdown. This breakdown is a crucial function of the BC present in mFMPE. Flavonoids and phenolics have a variety of biological properties that can influence ruminal microorganisms, thereby enhancing feed degradation in the rumen (Zhan et al., 2017).

The current findings are highly consistent with recent advances demonstrating the efficacy of PCs in modulating rumen fermentation and mitigating enteric methane emissions. Recent studies have highlighted that tropical plant extracts rich in tannins, saponins, and flavonoids, such as those derived from mangosteen peel and *Flemingia macrophylla*, actively disrupt the cell membranes of methanogenic archaea and ciliate protozoa (Antonius et al., 2023; Prachumchai et al., 2024). Because ruminal protozoa act as primary hosts for ecto- and endo-symbiotic methanogens, a reduction in the protozoal population directly limits methanogenesis. Furthermore, the inclusion of polyphenol-rich extracts has been recently shown to shift the volatile fatty acid (VFA) profile toward propionate production, which serves as a competitive alternative hydrogen sink in the rumen, thereby reducing the metabolic hydrogen available for methane synthesis (Amalyadi, 2025). More importantly, the application of microencapsulation techniques, similar to the one employed for mFMPE in the present study, has been recently proven to enhance the stability, bioactivity, and targeted release of these active phytonutrients in the rumen environment. This smart delivery mechanism maximizes their anti-methanogenic effects while minimizing the anti-nutritional impacts on fiber-degrading bacteria, ensuring that nutrient degradability remains uncompromised (Prachumchai et al., 2024; Amalyadi, 2025). Therefore, our results corroborate the growing body of recent evidence that strategic supplementation with protected plant extracts is a practical and sustainable strategy for advanced ruminant nutrition.

A ruminant's digestive system consists of four sections: the reticulum, rumen, omasum, and abomasum. The rumen, which can hold 50-70 liters in cattle, is the primary site where the resident microbial population ferments ingested plant material. This fermentation process produces volatile fatty acids (VFAs), which serve as a source of energy for the ruminant (Russell, 2002). According to Patra & Saxena (2009), PC also has the potential to alter propionate production in the presence of excessive hydrogen. Hydrogen is used to form propionate rather than serving as the primary substrate in the route that generates methane (Newbold et al., 2005). In the current experiment, the mFMPE supplementation resulted in a significant increase in propionate production, accompanied by a reduction in methane generation. Accordingly, Matra et al. (2023) demonstrated that microencapsulated-PCs from *Mitragyna* leaf extracts can regulate rumen fermentation, specifically by increasing propionate levels and decreasing methane production. Phesatcha et al. (2025a) demonstrated that supplementing diets with phytonutrient-rich combinations, such as Azolla leaf meal and turmeric powder, significantly enhances *in vitro* nutrient degradability and shifts the volatile fatty acid profile toward propionate production while effectively mitigating methane emissions. Furthermore, a recent *in vivo* study using phytonutrient-based pellets containing mangosteen peel and lemongrass, alongside insect protein, revealed a similar mechanism: the presence of condensed tannins and saponins successfully suppressed protozoal populations and methanogenesis without compromising ruminal pH or nutrient utilization in Thai native beef cattle (Phesatcha et al., 2025b). These contemporary findings strongly corroborate our results, highlighting that the strategic processing and encapsulation of tannin- and flavonoid-rich plants, such as Flemingia and mangosteen peel, provide a stable, highly effective delivery system to modulate the rumen microbiome and sustainably reduce methane emissions. Totakul et al. (2024) found that tropical plant-based PCs, a combination of fruit peel and lemongrass powder, effectively reduced methane emissions by improving rumen fermentation, particularly by increasing propionate concentration. Importantly, plant-based PC have significant biological effects on rumen fermentation, which are important for herbivory. PC can also influence the growth rate of the rumen microbial population, leading to changes that reduce CH4 production in ruminant digestive systems (Antonius et al., 2023). Additionally, recent comprehensive reviews and in vitro studies further confirm that nutritional manipulation using plant-derived active compounds is one of the most effective strategies to mitigate enteric methane without compromising ruminal fermentation (Ammar et al., 2024; Dayoub et al., 2024)

Ruminal pH is a vital factor influencing digestion in ruminants. It is essential to maintain a nearly neutral pH in the rumen in order to support the degradative activity of microorganisms and facilitate nutrient absorption. The rumen pH is generally between 6 and 7 when animals are fed forage diets. However, it can decrease further when an easily fermentable starch is present (Grünberg & Constable, 2009). In the present study, increasing the level of mFMPE did not significantly affect ruminal pH, which remained within the range 6.9-7.0. This could be attributed to the fact that mFMPE did not have a deleterious influence on microorganisms, hence maintaining an adequate level of rumen microbial activity. Similarly, Matra et al. (2023) found that pH remained unchanged as the level of supplementation with microencapsulated Mitragyna leaf extracts increased. Moreover, the ammonia nitrogen content was improved when mFMPE added. The plant-PCs extract increased ruminal NH3-N concentration, possibly by enhancing proteolysis. Rumen bacteria can acquire and utilize this nitrogen source to synthesize their own protein. (Wang et al., 2018; Marcos et al., 2020).

Conclusion

Based on the results, adding mFMPE at a level of 4% of

the total substrate improved nutrient degradability, increased the generation of propionate content, and reduced the presence of methane production. Therefore, mFMPE has the potential to be a beneficial nutritional supplement for ruminant feed additives.

DECLARATIONS

Funding: This research has received funding support from the NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B13F660133]

Acknowledgment: We would like to express our appreciation to the Department of Applied Biology, Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan, Nakhon Ratchasima, Department of Animal Science, Faculty of Agriculture and Technology, Nakhon Phanom University, Thailand, and Bounthavyxay Cattle Farm Sole Co., LTD. Bounneua village, Bounneua distic, Phongsaly province, Laos

Conflict of Interest: The authors declare no conflict of interest with any organization.

Ethics Statement: This study was approved by the Animal Care and Use Committee of Rajamangala University of Technology Isan, Thailand (approval no. 01-67-002). According to Thailand's National Research Council's Ethics of Animal Experimentation, approval was required to collect rumen fluid from animals for this study's main objective, which comprised laboratory examination of ruminant feeds.

Author's Contribution: PW and BP conceived and designed the experiment. PW, BV, MM and KP performed the study and conducted lab analyses. PW supervised, BV and MM coordinated the experiments and KP provided feed formula. PW performed statistical analyses of experimental data and prepared the manuscript format. PW and BP prepared the draft of the manuscript. All authors critically revised the manuscript and approved the final version.

Animal Ethics: This study was approved by the Animal Care and Use Committee of Rajamangala University of Technology Isan, Thailand (approval no. 01-67-002). According to Thailand's National Research Council's Ethics of Animal Experimentation, approval was required to collect rumen fluid from animals for this study's main objective, which comprised laboratory examination of ruminant feeds.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

Publisher's Note: All claims stated in this article are exclusively those of the authors and do not necessarily represent those of their affiliated organizations or those of the publisher, the editors, and the reviewers. Any product that may be evaluated/assessed in this article or claimed by its manufacturer is not guaranteed or endorsed by the publisher/editors.

REFERENCES

Al-Duais, M., Müller, L., Böhm, V., & Jetschke, G. (2009). Antioxidant capacity and total phenolics of *Cyphostemma digitatum* before and after processing: use of different assays. *European Food Research and Technology*, 228, 813-821. https://doi.org/10.1007/s00217-008-0994-8

Ali, A.I., Wassie, S.E., Korir, D., Goopy, J.P., Merbold, L., Butterbach-Bahl, K., & Schlecht, E. (2019). Digesta passage and nutrient digestibility in Boran steers at low feed intake levels. *Journal of Animal Physiology and Animal Nutrition*, 03, 1325-1337. https://doi.org/ 10.1111/jpn.13158

Amalyadi, R. (2025). Smart Tannin Nanoparticles: A Novel Approach to Overcome Rumen Anti-Nutritional Barriers in Ruminant Nutrition. Thai *Journal of Nanoscience and Nanotechnology*, 10(2), 1-14. https://doi.org/10.55003/tjnn10120256

Ammar, H.A., Hanim, C., Kurniawati, A., Muhlisin, M., & Astuti, A. (2024). The effects of sappanwood (*Caesalpinia sappan*) addition on *in vitro* methane mitigation, gas production, and ruminal fermentation parameters: *Veterinary Integrative Sciences*, 22(3), 1103-1112. https://doi.org/10.12982/VIS. 2024.074

Ampapon, T., Phesatcha, K., & Wanapat, M. (2019). Effects of phytonutrients on ruminal fermentation, digestibility, and microorganisms in Swamp buffaloes. *Animals*, 9(9), 671. https://doi.org/10.3390/ani9090671

Andersson, M.S., Lascano, C.E., Schultze-Kraft, R., & Peters, M. (2006). Forage quality and tannin concentration and composition of a collection of the tropical shrub legume *Flemingia macrophylla*. *Journal of the Science of Food and Agriculture*, 86(7), 1023-1031. https://doi.org/10.1002/jsfa.2433

Antonius, A., Pazla, R., Putri, E.M., Negara, W., Laia, N., Ridla, M., Suharti, S., Jayanegara, A., Asmairicen, S., Marlina, L., & Marta, Y. (2023). Effectiveness of herbal plants on rumen fermentation, methane gas emissions, in vitro nutrient digestibility, and population of protozoa. *Veterinary World*, 16(7), 1477. https://doi.org/10.14202/vetworld.2023.1477-1488

AOAC (2012). Official methods of analysis, 19th ed.; Gaithersburg, MD, USA: Association of Official Analytical Chemists.

Bashir, A., Munir, A., Hira, H., Akram, R., Ghafoor, A., Mustafa, A., Bisma, & Jameel, M. (2025). Green guardians: plant-derived antioxidants and their role in oxidative stress control. *Agrobiological Records*, 22, 114-128. https://doi.org/10.47278/journal.abr/2025.054

Benzie, I.F., & Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, 239(1), 70-76. https://doi.org/10.1006/abio.1996.0292

Calabrò, S., Cutrignelli, M.I., Lo Presti, V., Tudisco, R., Chiofalo, V., Grossi, M., & Chiofalo, B. (2015). Characterization and effect of year at harvest on the nutritional properties of three varieties of white lupine (*Lupinus albus* L.). *Journal of the Science of Food and Agriculture*, 95, 3127-3136. https://doi.org/10.1002/jsfa.7049

Cieślak, A., Zmora, P., Matkowski, A., Nawrot-Hadzik, I., Pers-Kamczyc, E., El-Sherbiny, M., & Szumacher-Strabel, M. (2016). Tannins from Sanguisorba officinalis affect *in vitro* rumen methane production and fermentation. *Journal of Animal & Plant Sciences*, 26(1), 54.

Dayoub, W., Riaz, M., Asnan, M., & Hamid, M.M.A. (2024). Nutritional manipulation as a strategy to mitigate enteric methane emissions from livestock – A review. *International Journal of Agriculture and Biology*, 32(6), 550-560. https://doi.org/10.17957/IJAB/15.2235

FAO (2023). Food and Agriculture Organization of the United Nations. Pathways towards lower emissions: A global assessment of the greenhouse gas emissions and mitigation options from livestock agrifood systems. Rome, Italy. pp: 1-77. https://doi.org/10.4060/cc9029en

Gali, L., & Bedjou, F., (2019). Antioxidant and anticholinesterase effects of the ethanol extract, ethanol extract fractions and total alkaloids from the cultivated Ruta chalepensis. *South African Journal of Botany*, 120, 163-169. https://doi.org/10.1016/j.sajb.2018.04.011

Goopy, J.P., Korir, D., Pelster, D., Ali, A.I.M., Wassie, S.E., Schlecht, E., & Butterbach-Bahl, K. (2020). Severe below-maintenance feed intake increases methane yield from enteric fermentation in cattle. *British Journal of Nutrition*, 123(11), 1239-1246. https://doi.org/10.1017/S0007114519003350

Grünberg, W., & Constable, P.D. (2009). Function and dysfunction of the ruminant forestomach. In Current Veterinary Therapy. Elsevier: Amsterdam, The Netherlands, pp: 12-19.

Gunun, P., Gunun, N., Khejornsart, P., Ouppamong, T., Cherdthong, A., Wanapat, M., Sirilaophaisan, S., Yuangklang, C., Polyorach, S.,

Kenchaiwong, W., & Kang, S. (2019). Effects of *Antidesma thwaitesianum* Muell. Arg. pomace as a source of plant secondary compounds on digestibility, rumen environment, hematology, and milk production in dairy cows. *Animal Science Journal*, 90(3), 372-381. https://doi.org/10.1111/asj.13147

Hristov, A.N., Melgar, A., Wasson, D., & Arndt, C. (2022). Symposium review: Effective nutritional strategies to mitigate enteric methane in dairy cattle. *Journal of Dairy Science*, 105(10), 8543-8557. https://doi.org/10.3168/jds.2021-21398

IPCC (2022). Climate Change 2022: Mitigation of Climate Change. Contribution of Working Group III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK and New York, NY, USA. https://doi.org/10.1017/9781009157926

Kurek, M.A., & Pratap-Singh, A. (2020). Plant-based (hemp, pea and rice) protein–maltodextrin combinations as wall material for spray-drying microencapsulation of hempseed (*Cannabis sativa*) oil. *Foods*, 9(11), 1707. https://doi.org/10.3390/foods 9111707

Lileikis, T., Nainienė, R., Bliznikas, S., & Uchockis, V. (2023). Dietary ruminant enteric methane mitigation strategies: current findings, potential risks and applicability. *Animals*, 13(16), 2586. https://doi.org/10.3390/ani13162586

Matra, M., Phupaboon, S., Totakul, P., Prommachart, R., Shah, A.A., Shah, A.M., & Wanapat, M. (2023). Microencapsulation of Mitragyna leaf extracts to be used as a bioactive compound source to enhance in vitro fermentation characteristics and microbial dynamics. *Animal Bioscience*, 37(1), 74. https://doi.org/10.5713/ab.23.0200

Malyugina, S., Holik, S., & Horky, P. (2025). Mitigation strategies for methane emissions in ruminant livestock: a comprehensive review of current approaches and future perspectives. *Frontiers in Animal Science*, 6, 1610376. https://doi.org/10.3389/fanim.2025.1610376

Marcos, C.N., Carro, M.D., Fernández-Yepes, J.E., Arbesu, L., & Molina-Alcaide, E. (2020). Utilization of avocado and mango fruit wastes in multi-nutrient blocks for goats feeding: In vitro evaluation. *Animals*, 10(12), 2279. https://doi.org/10.3390/ani10122279

McAllister, T.A., Meale, S.J., Valle, E., Guan, L.L., Zhou, M., Kelly, W.J., Henderson, G., Attwood, G.T. & Janssen, P.H. (2015). Ruminant nutrition symposium: use of genomics and transcriptomics to identify strategies to lower ruminal methanogenesis. *Journal of Animal Science*, 93(4), 1431-1449. https://doi.org/10.2527/jas.2014-8329

National Research Council (NRC) (2001). Nutrient requirements of dairy cattle, 7th Ed.; National Research Council. Washington, DC, USA: The National Academies Press.

Newbold, C.J., López, S., Nelson, N., Ouda, J.O., Wallace, R.J., & Moss, A.R. (2005). Propionate precursors and other metabolic intermediates as possible alternative electron acceptors to methanogenesis in ruminal fermentation *in vitro*. *British Journal of Nutrition*, 94(1), 27-35. https://doi.org/10.1079/BJN20051445

Nouri, A. (2019). Chitosan nano-encapsulation improves the effects of mint, thyme, and cinnamon essential oils in broiler chickens. *British Poultry Science*, 60(5), 530-538. https://doi.org/10.1080/ 00071668. 2019.1622078

Ozkan, G., Franco, P., De Marco, I., Xiao, J., & Capanoglu, E. (2019). A review of microencapsulation methods for food antioxidants: Principles, advantages, drawbacks and applications. *Food Chemistry*, 272, 494-506. https://doi.org/10.1016/j.foodchem.2018.07.205

Patra, A.K., & Saxena, J. (2009). The effect and mode of action of saponins on the microbial populations and fermentation in the rumen and ruminant production. *Nutrition Research Reviews*, 22(2), 204-219. https://doi.org/10.1017/S0954422409990163

Paulo, F., & Santos, L. (2017). Design of experiments for microencapsulation applications: A review. *Materials Science and Engineering: C*, 77, 1327-1340. https://doi.org/10.1016/j.msec.2017.03.219

Phesatcha, B., Phesatcha, K., Ampapon, T., & Wanapat, M. (2025a). Using Azolla (*Azolla microphylla*) leaf meal and phytonutrient powder on rumen fermentation efficiency and nutrient degradability using in vitro technique. *Animal Bioscience*, 38(4): 673-681. https://doi.org/10.5713/ab.24.0379

Phesatcha, B., Phesatcha, K., Matra, M., Ampapon, T., & Wanapat, M. (2025b). Potential use of insect protein and phytonutrient-based tropical plant supplementation on rumen fermentation characteristics and microbial protein synthesis in Thai native beef cattle. *Animal Bioscience*, 39(1), 250166. https://doi.org/10.5713/ab.25.0166

Phupaboon, S., Matra, M., Prommachart, R., Totakul, P., Supapong, C., & Wanapat, M. (2022). Extraction, characterization, and chitosan microencapsulation of bioactive compounds from *Cannabis sativa* L., *Cannabis indica* L., and *Mitragyna speiosa* K. *Antioxidants*, 11(11), 2103. https://doi.org/10.3390/antiox 11112103

Phupaboon, S., Matra, M., Prommachart, R., Totakul, P., & Wanapat, M. (2025). In Vitro fermentation studies through synergistic effects of antioxidant phytonutrients derived from encapsulated medical plants. International *Journal of Agriculture and Biosciences*, 14(6), 1113-1121. https://doi.org/10.47278/journal.ijab/2025.089

Prachumchai, R., Suriyapha, C., Dagaew, G., Sommai, S., Matra, M., Phupaboon, S., Phasuk, Y., & Wanapat, M. (2024). Microencapsulation of lemongrass and mangosteen peel as phytogenic compounds to gas kinetics, fermentation, degradability, methane production, and microbial population using in vitro gas technique. *Plos One*, 19(6), e0304282. https://doi.org/10.1371/journal.pone.0304282

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9-10), 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Russell, J.B. (2002). Rumen microbiology and its role in ruminant nutrition; Cornell University Press: Ithaca, NY, USA.

Samuel, M., Ceballos-Baumann, A.O., Blin, J., Uema, T., Boecker, H., Passingham, R.E., & Brooks, D. J. (1997). Evidence for lateral premotor and parietal overactivity in Parkinson's disease during sequential and bimanual movements. A PET study. *Brain: A Journal of Neurology*, 120(6), 963-976. https://doi.org/10.1093/brain/ 120.6.963

Sharif, N., Khoshnoudi-Nia, S., & Jafari, S.M. (2020). Nano/microencapsulation of anthocyanins; a systematic review and meta-analysis. *Food Research International*, 132, 109077. https://doi.org/ 10.1016/j.foodres.2020.109077

Shinkai, T., Takizawa, S., Fujimori, M., & Mitsumori, M. (2024). The role of rumen microbiota in enteric methane mitigation for sustainable ruminant production. *Animal Bioscience*, 37(2), 360-369. https://doi.org/10.5713/ab.23.0301

Statistical Analysis System (SAS) (2013). User's guide: Statistic. Cary, NC, USA: SAS Inst. Inc.

Terry, S.A., Romero, C.M., Chaves, A.V., & McAllister, T.A. (2020). Nutritional factors affecting greenhouse gas production from ruminants: implications for enteric and manure emissions. In Improving rumen function (pp: 505-546). Burleigh Dodds Science Publishing. https://doi.org/10.19103/AS.2020.0067.16

Topçu, G., Ay, M., Bilici, A., Sarıkürkcü, C., Öztürk, M., & Ulubelen, A. (2007). A new flavone from antioxidant extracts of *Pistacia terebinthus*. *Food Chemistry*, 103(3), 816-822. https://doi.org/10.1016/j.foodchem.2006.09.028

Totakul, P., Matra, M., Sommai, S., Viennasay, B., & Wanapat, M. (2024). Combination effects of phytonutrient pellet and lemongrass (*Cymbopogon citratus*) powder on rumen fermentation efficiency and nutrient degradability using in vitro technique. *Tropical Animal Health and Production*, 56(2), 97. https://doi.org/10.1007/s11250-024-03936-w

Van Soest, P.V., Robertson, J.B., & Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74(10), 3583-3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Vasta, V., Daghio, M., Cappucci, A., Buccioni, A., Serra, A., Viti, C., & Mele, M., (2019). Invited review: plant polyphenols and rumen microbiota responsible for fatty acid biohydrogenation, fiber digestion, and methane emission: experimental evidence and methodological approaches. *Journal of Dairy Science,* 102(5): 3781-3804. https://doi.org/10.3168/jds.2018-14985

Wang, R., Wang, M., Ungerfeld, E.M., Zhang, X.M., Long, D.L., Mao, H.X., Deng, J.P., Bannink, A., & Tan, Z.L. (2018). Nitrate improves ammonia incorporation into rumen microbial protein in lactating dairy cows fed a low-protein diet. *Journal of Dairy Science*, 101(11), 9789-9799. https://doi.org/10.3168/jds.2018-14904

Zhan, J., Liu, M., Su, X., Zhan, K., Zhang, C., & Zhao, G. (2017). Effects of alfalfa flavonoids on the production performance, immune system, and ruminal fermentation of dairy cows. *Asian-Australasian Journal of Animal Sciences*, 30(10), 1416. https://doi.org/10.5713/ajas.16.0579