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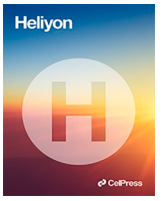

Research article

Interaction of a source rich in phytonutrients (fruits peel pellets) and polyunsaturated oil (Tung oil) on *in vitro* ruminal fermentation, methane production, and nutrient digestibility

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ABSTRACT

Tropical fruit peels from mangosteen, rambutan, and banana are rich in phytonutrients. Several studies reported that the phytonutrients improved rumen fermentation. Nevertheless, the combination of phytonutrients and essential fatty acids on rumen fermentation have not yet been investigated. Hence, the aim of this research was to investigate the influence of fruit peel pellets (mangosteen, rambutan, and banana peel; MARABAC) containing phytonutrients and tung oil supplementation on rumen fermentation and the degradability of nutrients. Four levels of MARABAC (0, 2, 4, and 6 %) and four levels of tung oil (0, 2, 4, and 6 %) were supplemented with concentrate according to a 4 × 4 factorial arrangement in a completely randomized design (CRD). Rumen fermentation parameters, including gas production, ammonia nitrogen (NH_3–N), volatile fatty acids (VFA), nutrient degradability (IVDMD and IVOMD), and *in vitro* methane (CH_4) production were determined. The results showed that there were no interactions between MARABAC and Tung oil treatments for all terms of kinetic gas and cumulative gas, IVDMD and IVOMD, and *in vitro* ammonia-nitrogen (NH_3–N). However, when combining MARABAC and tung oil beyond the 4 % level, VFA and *in vitro* CH_4 production was severely affected. The supplementation of MARABAC and tung oil decreased gas production and rumen nutrient degradability ($p < 0.05$). Acetate (C_2) and propionate (C_3) production were significantly affected by the level of MARABAC supplementation. NH_3–N was dropped when levels of MARABAC and tung oil supplementation were increased. There were interactions between MARABAC and tung oil on total VFA and *in vitro* CH_4 production at 8 h (h). In addition, *in vitro* CH_4 production decreased ($p < 0.05$) with higher levels of MARABAC supplementation. It could be concluded that MARABAC and tung oil supplementation significantly contributed to improving the production of gas and could be applied to decrease rumen CH_4 production, thereby reducing the emission of greenhouse gases.

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1. Introduction

Currently, researchers around the world have been interested in the effects of rumen CH_4 from the enteric fermentation of ruminants, contributing to global warming. Mitigation of rumen CH_4 production essentially those of practical innovations and cost-effective means are essentially required. Currently, researchers have shown more interest in manipulating the enteric fermentation through strategic supplementation with feeds comprising of phytonutrients, especially saponins, condensed tannins, and flavonoids [1–7], to shift fermentation pattern, type of rumen microbiomes and hence, opportunities to mitigate rumen methane production.

Reduction of methane gas from fermentation end-products was achieved by feeding strategies supplemented with their secondary compounds that impacted on rumen protozoa without affecting the rumen biomass as a whole [8]. Additionally, it was increased rumen fermentation characteristics, including the enhancement of microbial protein synthesis, reduced methane emissions, and modulation of ruminal pH, depending on the type of tropical plants and the dose of phytonutrients [1,9]. For instance, fruit peels for utilizing as a ruminant enhancer included mangosteen (*Garcinia mangostana* L. [10]), rambutan (*Nephelium lappaceum* L. [11]), and banana flower (*Musa sapientum* L. [12]). Numerous studies have reported that the effect of feed supplemented with fruit peels to enhances rumen ecology and mitigates the production of methane in Thai beef cattle [10,11,13,14]. However, their ability to reduce CH_4 production in the rumen is dependent on both the level and source of phytonutrients [15].

Another strategy for reducing of ruminal CH_4 production is the use of dietary supplemental oils. Due to the biohydrogenation process of unsaturated FA by ruminal bacteria, oils can serve as hydrogen ion (H^+) sink reducing CH_4 methane formation. There are several studies that have revealed that feeding of plant-rich oils, particularly n-3 polyunsaturated fatty acids (PUFAs), unsaturated oils, for example, oleic acid (rapeseed), linoleic acid (cottonseed or sunflower), and saturated sources, such as palm oil or tung oil, which are effective in reducing CH_4 production [16,17]. Especially, tung oil was extracted from nut of the tung tree (*Vernicia fordii* and *Vernicia montana*) [18]. The compositions contained a high oil content (47–63 %) and fatty acid profiles consist of 5.5 % palmitic acid, 4.0 % oleic acid, 8.5 % linoleic acid, 82.0 % alpha-eleostearic acid (n-5 PUFA), and a novel classified trienoic fatty (ESA, 18:3Δ9cis, 11trans,13trans) [19]. Previous research investigated the use of tung oil in the animal diet of tilapia [20] and laying hens [21], who reported the potential to be used as an energy source for growth and increased conjugated linoleic acid (CLA), non-toxicity, and other essential oil content in their muscular tissue. However, supplementation with CT and saponins can alter the biohydrogenation of fatty acids and digestibility in rumen [22].

Importantly, it shows the interest in phytonutrients in various fruit peels consisting of mangosteen, rambutan, and banana peel that were formulated into MARABAC and tung oil extracts for finding alternate sources to control rumen pH, such as bacteria, to substitute antibiotics or chemicals, as well as to reduce production costs and increase the animal production economy. The purpose of supplementation of fruit peels mixed with seed oil in this experiment was predicated on the hunch that they would increase *in vitro* ruminal pH, fermentation characteristics, nutritional degradability, and proteolytic bacteria synthesizing protein supply to ruminants. Therefore, the current study aimed to investigate the effects of combining, in different proportions, a rich source of phytonutrients (fruit peel pellet, MARABAC) with a polyunsaturated oil (tung oil) on rumen fermentation end-products, including *in vitro* nutrient degradability, volatile fatty acid, ammonia nitrogen (NH_3–N), and methane mitigation.

Table 1
Chemical composition of concentrate, rice straw, and MARABAC pellet used in the experiment.

Items	Concentrate	Rice straw	MARABAC
Cassava	56		
Dried brewery grain	16		
Rice bran	7		
Palm meal	8		
Soybean meal	8		
Urea	2.5		
Molasses	1		
Sulphur	0.5		
Mineral mixed	0.5		
Salt	0.5		
Chemical composition (% Dry matter)			
Dry matter	84.4	89.4	90.0
Organic matter	90.3	85.4	95.57
Crude protein	15.3	2.4	10.0
Neutral detergent fiber	36.7	78.9	58.11
Acid detergent fiber	11.6	52.6	42.42
Condensed tannins (g/kg DM)			105.1
Saponins (g/kg DM)			117.3
Antioxidant activity			
%DPPH			85.88
%ABTS			91.55
FRAP (g TROE/kg DE)			6.55

DPPH inhibition = DPPH radical-scavenging activity; ABTS inhibition = ABTS radical-scavenging activity; FRAP capacity = ferrous ion reducing power; GAE = gallic acid equivalent; TROE = Trolox equivalent; MARABAC = fruit peel pellet (mangosteen, rambutan, and banana peel); DM = Dry matter.

2. Materials and methods

The rumen fluidic of animals used in this experiment was approved by the Institutional Animal Care and Use Committee of Khon Kaen University and carried out by the Institute of Animals for Scientific Purpose Development (IAD), Thailand (record no. 0201.2.11/73).

2.1. Treatments diets and experimental design

The phytonutrient source used was elaborated based on the fruit peel of mangosteen, rambutan, and banana flower (MARABAC). The preparation of the pellet was made following the method of Wanapat et al. [6]. Briefly, the fruit peel powder (45 % mangosteen, 30 % rambutan, and 15 % banana flower) and cassava starch (10 %) were well mixed with water. The mixture was processed into pellets by machine (Kakiuchi Co., Ltd., Nankoku, Kochi, Japan) and reduced moisture by being sun-dried enough to reach at least 90 % dry matter. The source of polyunsaturated oil used was tung oil, which was obtained from the local market in Khon Kaen of Thailand. The substrate utilized was prepared based on rice straw and a concentrate mixed in a proportion of 40:60. The ingredients and chemical composition of rice straw and concentrate are shown in Table 1. The study was planned using a completely randomized design (CRD) in a 4 × 4 factorial arrangement, with the first treatment diet factor using four levels of MARABAC supplementation (0, 2, 4, and 6 % of total DM substrate) and the second factor using four levels of tung oil supplementation (0, 2, 4, and 6 % of total DM substrate). *In vitro* gas production including, blank (medium only), was run in triplicates, while *in vitro* degradability and *in vitro* fermentation end-products were run duplicate.

2.2. Animal donors and medium solution preparation

The ruminal fluid was obtained from four Thai-crossbreed dry cows (450 ± 30 kg BW) adapted to a diet consisting of concentrate containing 18 % CP (offered at 1 % BW) and rice straw (offered *ad libitum*). Approximately 800 mL of rumen fluid from each cattle was collected before the morning feeding by an esophageal tube connected to a vacuum pump. The tube, made from rubber, was inserted through the cattle mouth and into the esophagus, and then down into the rumen for rumen fluid collection. The rumen fluid was filtered through four layers of cheesecloth and transferred to pre-warmed thermos flasks (39 °C) in closed containers before moving to the laboratory. The method of rumen inoculum was prepared according to Menke et al. [23], as described by Kang et al. [24]. Briefly, artificial saliva (6000 mL) was prepared by distilled water (2850 mL), rumen buffer solution (1440 mL; 70 g of $NaHCO_3$ and 8 g of NH_4HCO_3 were dissolved with distilled water made up to 2 L), macro-mineral solution (1440 mL; 12.4 g of KH_2PO_4, 11.4 g of Na_2HPO_4, 4.44 g of NaCl, and 1.2 g of $MgSO_4.7H_2O$ were dissolved with distilled water made up to 2 L), reduction solution (297 mL; freshly prepared; 2.016 g of Na_2S. $9H_2O$,and 12 mL of 1 M NaOH were dissolved with distilled water made up to 300 mL), resazurine (7.32 mL; 0.1 g was dissolved with distilled water made up to 100 mL), and micro-mineral solution (0.72 mL; 10.0 g of $MnCl_2$. $4H_2O$, 13.2 g of $CaC1_2$. $2H_2O$,1 g of $CoCl_2$.$6H_2O$, 8.0 g of $FeC1_3$. $6H_2O$ were dissolved with distilled water made up to 100 mL). Then, rumen fluid (3000 mL) was combined with 6000 mL of the artificial saliva (2:1; artificial saliva: rumen fluid), which kept stirring at 39 °C on a hotplate magnetic stirrer and continuous flushing with carbon dioxide.

2.3. Fermentation substrates in vitro

Substrate (rice straw and concentrate mixture ratio; 40:60) was ground to pass through a 1-mm mesh using a Cyclotech Mill (Tecator, Höganäs, Sweden) and weighed at 500 mg into 50-mL serum bottles and then added with MARABAC (0, 2, 4, and 6 % DM) and tung oil (0, 2, 4, and 6 % DM) for each bottle treatment. Three incubation batches were conducted for gas measurement at 10 incubation points, consisting of 51 samples (16 treatments, 3 replications, and +3 blanks). The 68 bottles (16 treatments, 2 replications, 2 times of sampling at 4 and 8 h of incubation, and +2 blanks) were separately prepared for NH_3–N, VFA, and methane prediction. Another 68 bottles (16 treatments, 2 replications, 2 times of sampling at 12 and 24 h of incubation, and +2 blanks) were separately prepared for *in vitro* dry matter degradability (IVDMD) and *in vitro* organic matter degradability (IVOMD). The bottles, which were closed with a rubber stopper and covered by an aluminium cap, were arranged and flushed with carbon dioxide and pre-heated to 39 °C for 12 h in an incubator (Memmert IN160, Schwabach, Germany) before adding rumen inoculum.

2.4. Sampling procedures, data collection and chemical analysis

Gas productions from *in vitro* fermentation were measured at intervals of incubation at 1, 2, 4, 6, 8, 12, 24, 48, 72, and 96 h in an incubator by using a syringe glass pressure.

An additional 68 bottles underwent separation at 4 and 8 h of incubation. From each of these bottles, 18 mL of fluid sample was kept in a plastic bottle that contained 2 mL of 1 M H_2SO_4 to discontinue the fermentation process and then stored at −20 °C prior to NH_3–N and VFA analyses.

Additionally, at 12 and 24 h, another 68 bottle samples *(34* samples per time of sampling*)* were collected and stored at −20 °C for nutrient degradability determinations (IVDMD and IVOMD). The data of cumulative gas products was subjected to the equation of Orskov and Mcdonald [25] as follows:

$$Y = a + b\left[1 - e^{(-ct)}\right]$$

where Y is the gas volume that was produced at time t (mL); a is the gas volume that was produced from the immediately soluble fraction (mL); b is the gas volume that was produced from the insoluble fraction is the gas production from the insoluble fraction (mL); c is the gas production rate constant for the insoluble fraction (mL/h); t is the incubation time; and a+b is the potential extent of gas production.

The fluid samples were centrifuged by using microcentrifuges (Eppendorf, 5415 R, Radnor, Pennsylvania, United States) at 10,000×*g* for 15 min at 4 °C, and the supernatant was collected for NH_3–N and VFA analysis. The ammonia nitrogen (NH_3–N) was analyzed by using a UV spectrophotometer (T80 + UV/Vis, PG Instruments Ltd., Leicestershire, UK) according to the method of Fawcett and Scott [26]. The volatile fatty acid concentrate (acetic acid, propionic acid, and butyric acid) detections were performed with minor modifications as described by Yamamoto-Osaka et al. [27]. Briefly, 0.5 mL of the supernatant sample was mixed with 0.15 mL of 50 % H_2SO_4, 0.5 mL of 5 mmol/L 2-5-methylvaleric acid (Sigma Aldrich, St. Louis, MO, USA) for internal standard, 1 mL of diethyl ether, and then vortexed, placed in ice baths for 3 min, and centrifuged at 3000 rpm for 10 min at 4 °C. After that, 0.8 mL of the ether layer was transferred to a micro-centrifuge tube containing approximately 0.1 g of anhydrous ($CaCl_2$) and left to stand in ice baths for 5 min and the ether extract was transferred to a vial bottle (1.5 mL). Qualitative measurements of volatile fatty acid concentrations were performed by gas chromatography (Nexis GC-2030, Shimadzu, Kyoto, Japan). The estimation of methane (CH_4) production (mmol/L) was performed according to the equation of Moss et al. [28] as follows: CH_4 production = 0.45 (acetate) − 0.275 (propionate) + 0.4 (butyrate).

In vitro degradability values of the sample were performed by filtering through pre-weighed gooch crucibles, which use vacuum pressure through a vacuum pump. The crucibles were dried overnight at 100 °C by using a hot air oven to measure the DM content for the determination of IVDMD. After that, the crucibles and residue left were burned at 550 °C for 6 h to determine IVOMD. The weight of DM content was calculated for IVDMD and IVOMD according to the equation of Van Soest et al. [29]. Substrates (rice straw, concentrate diet, and MARABAC) were dried at 60 °C for 72 h and ground to pass through a 1-mm mesh using a Cyclotech Mill (Tecator, Höganäs, Sweden) prior to proximate analysis. All samples were analyzed for nutritive values following to the procedure of AOAC [30], which includes DM, CP analyzed by the Kjeldahl method, and ash. The detergent fiber was assessed using sodium sulfite and amylase according to Van Soest et al. [29] procedures.

The phytonutrients content of MARABAC were analyzed according to the method of Phupaboon et al. [31]. The mixture was shaken and incubated overnight at room temperature. The extract was centrifuged at 6000 rpm at 3 °C for 15 min, and the supernatants were removed for analysis. Plant secondary compounds namely condensed tannin, were determined by vanillin-hydrochloric acid (HCl) method [32] and saponins were determined by the procedure of Kwon et al. [33]. The antioxidant activity of extract was modified by

Table 2
Effect of MARABAC and tung oil supplementations on gas kinetics, cumulative gas production.

MARABAC (%)	Tung oil (%)	Gas kinetics					Cumulative gas (mL) produced at 96 h
		a (mL)	b (mL)	c (mL/h)		a + b (mL)	
0	0	5.37	112.42	0.048		117.79	122.46
	2	5.78	106.13	0.049		111.90	117.29
	4	4.27	98.49	0.058		102.76	108.36
	6	6.03	100.29	0.050		106.32	111.32
2	0	4.83	104.77	0.046		109.61	113.16
	2	7.03	103.44	0.046		110.47	113.66
	4	4.52	96.99	0.051		101.52	106.62
	6	7.54	102.18	0.039		109.72	112.56
4	0	8.12	110.35	0.041		118.48	120.92
	2	3.95	97.31	0.045		101.27	103.39
	4	4.03	93.91	0.053		97.94	102.09
	6	6.44	104.03	0.045		110.47	113.42
6	0	6.86	105.27	0.038		112.14	113.62
	2	4.96	100.95	0.044		105.91	108.69
	4	3.48	93.63	0.052		97.11	100.42
	6	2.89	99.09	0.037		101.98	102.22
SEM			1.292	2.880	0.003	3.574	3.798
Comparison							
MARABAC		0.40	0.18	0.01		0.19	0.02
Tung oil		0.08	<0.01	<0.01		<0.01	<0.01
Interaction		0.19	0.50	0.88		0.31	0.29
Orthogonal polynomials							
MARABAC (linear)		0.37	0.03	<0.01		0.04	<0.01
MARABAC (quadratic)		0.22	0.75	0.43		0.85	0.93
MARABAC (cubic)		0.98	0.58	0.20		0.65	0.64
Tung oil (linear)		0.31	<0.01	0.41		<0.01	<0.01
Tung oil (quadratic)		0.05	<0.01	<0.01		<0.01	<0.01
Tung oil (cubic)		0.29	0.09	<0.01		0.08	0.18

MARABAC = fruit peel pellet (mangosteen, rambutan, and banana peel); SEM = standard error of the mean; a = the gas volume that was produced from the immediately soluble fraction (ml); b = the gas volume that was produced from the insoluble fraction is 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.

Phupaboon et al. [34] to evaluate by using FRAP (ferric reducing antioxidant power), DPPH (2, 2-diphenyl-1 picryl hydrazyl) and ABTS (3-ethylbenzothiazoline-6-sulphonic acid) according to the methods of Brand-Williams et al. [35], Dudonné et al. [36] and Benzie and Strain [37], respectively.

2.5. Statistical analysis

The factorial arrangements (4 × 4) in a completely randomized design (CRD) were used to evaluate the effects of MARABAC and tung oil supplementation. Type III fixed effects of MARABAC treatments, tung oil levels, and their interactions were analyzed using the Mixed Procedure of SAS (version 9.4; SAS Institute, Cary, NC, USA). The sample bottle (replication) was a random effect, and gas production was a repeated measure. The least squares mean was separated via the PDIFF option and considered significant at $p < 0.05$. Orthogonal polynomial contrasts were used to analyze linear, quadratic, and cubic responses to supplementation of MARABAC and tung oil, with the level of significance at $p < 0.05$.

3. Results

The chemical composition of concentrate, rice straw, and MARABAC are presented in Table 1. Concentrate, rice straw, and MARABAC contained 15.3, 2.4, and 10.0 % CP, respectively. The average of CT and saponin content in MARABAC were 105.1 mg/g dry weight and 117.3 mg/g dry weight, respectively.

3.1. In vitro cumulative gas production and parameters of gas kinetics

The effects of MARABAC and tung oil on gas kinetics and cumulative gas are presented in Table 2. There was no interaction between MARABAC and tung oil treatments for all terms of kinetic gas and cumulative gas. There were no significant effects of MARABAC and tung oil supplementation on gas kinetics in terms of the immediately soluble fraction (a). Gas kinetics in terms of insoluble fraction (b), extent rate (c), potential extent of gas production (a + b) and cumulative gas were affected by tung oil supplementation ($p < 0.01$), while MARABAC supplementation had an effect on extent rate (c) and cumulative gas ($p < 0.05$). Increasing levels of MARABAC supplemented had a linear decrease ($p < 0.05$) in terms of insoluble fraction (b), extent rate (c), potential extent of gas production (a + b), and cumulative gas, while increasing levels of tung oil up to 6 % DM had a quadratic decrease ($p < 0.01$) in terms of extent rate (c), potential extent of gas production (a + b), and cumulative gas.

Table 3
Effect of MARABAC and tung oil supplementations on *in vitro* dry matter degradability (IVDMD) and *in vitro* organic matter degradability (IVOMD) from *in vitro* incubation with rumen fluid.

MARABAC (%)	Tung oil (%)	IVDMD, %		IVOMD, %	
		12h	24h	12h	24h
0	0	72.14	57.79	89.84	94.14
	2	70.92	58.55	91.73	94.29
	4	69.13	63.98	92.65	93.08
	6	64.52	65.57	93.24	93.73
2	0	66.30	56.57	92.90	93.93
	2	70.24	63.68	93.11	93.13
	4	72.51	59.51	92.20	94.30
	6	71.62	69.41	93.10	92.79
4	0	68.68	60.52	93.31	93.16
	2	71.36	58.79	92.49	93.94
	4	70.94	67.99	92.96	93.24
	6	76.06	70.49	91.18	92.89
6	0	70.97	61.51	92.41	92.69
	2	72.00	69.82	92.38	91.35
	4	72.96	72.14	92.48	91.35
	6	71.70	76.92	92.25	91.04
SEM		2.459	1.862	0.664	0.462
Comparison					
MARABAC		0.54	<0.01	0.28	<0.01
Tung oil		0.82	<0.01	0.80	0.21
Interaction		0.52	0.08	0.07	0.44
Orthogonal polynomials					
MARABAC (linear)		0.16	<0.01	0.51	<0.01
MARABAC (quadratic)		0.78	0.04	0.20	0.02
MARABAC (cubic)		0.76	0.68	0.41	0.24
Tung oil (linear)		0.50	<0.01	0.54	0.04
Tung oil (quadratic)		0.51	0.64	0.59	0.89
Tung oil (cubic)		0.92	0.71	0.95	0.81

MARABAC = fruit peel pellet (mangosteen, rambutan, and banana peel); SEM = standard error of the mean.

3.2. In vitro nutrient degradability

As shown in Table 3, there was no interaction between MARABAC and tung oil treatments for IVDMD and IVOMD. The IVDMD at 12 h of incubation was affected by MARABAC and tung oil supplementation ($p < 0.01$). Increasing levels of MARABAC and tung oil had a quadratic increase in IVDMD at 12 h ($p < 0.01$). The IVOMD at 24 h of incubation was affected by MARABAC supplementation ($p < 0.01$). Increasing levels of MARABAC had a quadratic decrease in IVOMD at 24 h ($p < 0.05$), while increasing levels of tung oil had a linear decrease ($p < 0.05$) at 24 h of incubation.

3.3. In vitro rumen fermentation end-products

The effects of MARABAC and tung oil on volatile fatty acids (VFA) are presented in Table 4. There was interaction ($p < 0.01$) between MARABAC and tung oil treatments at 8 h for butyric acid (C_4) and total volatile fatty acid (Table 5). On 0, 2, and 4 % of MARABAC supplementations at 8 h, the butyric acid showed no differences with increased levels of tung oil supplementations. While at 6 % MARABAC, the butyric acid was linearly increased ($p < 0.05$) with increased levels of tung oil supplementation.

At 6 % of MARABAC supplementations in the 8 h incubation, the total VFA was linearly decreased ($p < 0.05$) with increasing levels of tung oil, but at 0 % MARABAC, the total VFA was decreased when there were tung oil supplementations. However, at 2 and 4 % of MARABAC supplementations, there were not affected ($p > 0.05$) by the increasing level of tung oil. While the mean value of total VFA at 0, 2, and 4 % MARABAC were increased with increased levels of tung oil. However, at 6 % of MARABAC supplementation, the total VFA decreased with increased levels of tung oil supplementation.

There was no interaction between MARABAC and tung oil treatments ($p > 0.05$) for acetate (C_2), propionate (C_3), butyric acid (at 4 h) and acetate to propionate ratio (Table 5). Acetate (C_2) at 4 h of incubation was affected by MARABAC ($p < 0.05$) and tung oil supplementation. There was a quadratic decrease ($p < 0.01$) with increased levels of MARABAC and a linear increase with increasing levels of tung oil ($p < 0.01$). There was a quadratic decrease ($p < 0.01$) with increased levels of MARABAC. The propionate (C_3) at 4 h incubation had response at quadratic increase ($p < 0.05$) with increased levels of MARABAC supplementation. The butyrate (at 4 h) was affected by MARABAC ($p < 0.01$) and tung oil ($p < 0.01$) supplementation. Increasing levels of MARABAC had a quadratic increase in butyrate ($p < 0.01$) and increasing levels of tung oil had a linear increase ($p < 0.01$). The acetate to propionate ratio at 4 h incubation had a quadratic decrease ($p < 0.05$) with increased levels of MARABAC supplementation.

Table 4
Effect of MARABAC and tung oil supplementations on *in vitro* volatile fatty acids (VFAs).

MARABAC (%)	Tung oil (%)	C_2 (mol/100mol)		C_3 (mol/100mol)		C_4 (mol/100mol)	
		4 h	8 h	4 h	8 h	4 h	8 h
0	0	69.16	67.18	16.59	20.02	14.25	12.80
	2	70.95	65.60	17.68	19.69	11.37	14.70
	4	70.73	70.18	18.18	17.55	11.09	12.28
	6	67.75	64.96	20.57	20.22	11.68	14.83
2	0	63.71	73.62	19.85	15.17	16.44	11.21
	2	64.78	62.04	20.89	21.20	14.33	16.76
	4	65.95	69.08	19.41	19.03	14.64	11.89
	6	70.61	70.89	16.89	15.13	12.51	13.98
4	0	62.65	65.84	21.61	19.27	15.74	14.89
	2	66.88	62.45	19.75	21.85	13.38	15.70
	4	65.27	61.53	20.11	22.95	14.62	15.52
	6	67.52	65.83	18.71	19.46	13.9	14.71
6	0	69.05	69.18	19.03	18.5	11.91	12.32
	2	73.14	69.91	17.57	17.07	9.82	13.02
	4	69.45	63.12	17.74	20.07	12.82	16.81
	6	74.24	58.29	15.13	22.52	10.63	19.19
SEM		1.489	0.953	1.291	3.352	0.523	1.467
Comparison							
MARABAC		0.02	0.14	0.20	0.32	<0.01	0.04
Tung oil		0.05	0.24	0.84	0.72	<0.01	<0.01
Interaction		0.41	0.13	0.67	0.49	0.08	<0.01
Orthogonal polynomials							
MARABAC (linear)		0.67	0.18	0.69	0.48	0.87	0.05
MARABAC (quadratic)		<0.01	0.84	0.04	0.87	<0.01	0.83
MARABAC (cubic)		0.85	0.10	0.34	0.08	0.20	0.30
Tung oil (linear)		<0.01	0.17	0.37	0.54	<0.01	0.03
Tung oil (quadratic)		0.59	0.39	0.79	0.34	0.55	0.64
Tung oil (cubic)		0.36	0.37	0.84	0.81	0.14	0.10

MARABAC = fruit peel pellet (mangosteen, rambutan, and banana peel); SEM = standard error of the mean; C_2 = acetate; C_3 = propionate; C_4 = butyrate.

Table 5
Effect of MARABAC and tung oil supplementations on acetate to propionate ratio and total volatile fatty acids (VFAs).

MARABAC (%)	Tung oil (%)	C_2/C_3		Total VFA (mmol/L)	
		4 h	8 h	4 h	8 h
0	0	4.17	3.43	21.53	46.33
	2	4.01	3.42	36.50	34.16
	4	3.90	4.00	35.31	36.51
	6	3.30	3.27	37.48	36.29
2	0	3.23	4.85	18.3	36.65
	2	3.12	2.93	25.45	32.67
	4	3.42	3.91	23.44	35.05
	6	4.23	4.82	27.69	32.23
4	0	2.91	3.43	22.26	28.37
	2	3.39	2.92	29.69	32.18
	4	3.27	2.86	24.72	36.84
	6	3.71	3.38	32.98	33.42
6	0	3.64	3.80	34.61	40.80
	2	4.23	4.10	31.55	40.66
	4	3.92	3.15	24.82	24.54
	6	5.22	2.68	31.47	19.70
SEM		0.384	0.586	3.833	4.336
Comparison					
MARABAC		0.15	0.13	<0.01	<0.01
Tung oil		0.47	0.58	<0.01	<0.01
Interaction		0.70	0.30	0.12	<0.01
Orthogonal polynomials					
MARABAC (linear)		0.40	0.34	0.77	0.03
MARABAC (quadratic)		0.03	0.59	<0.01	0.51
MARABAC (cubic)		0.44	0.04	0.14	0.79
Tung oil (linear)		0.15	0.51	0.02	0.02
Tung oil (quadratic)		0.60	0.32	0.74	0.94
Tung oil (cubic)		0.51	0.57	0.03	0.79

MARABAC = fruit peel pellet (mangosteen, rambutan, and banana peel); SEM = standard error of the mean; C_2/C_3 = acetate/propionate.

Table 6
Effect of MARABAC and tung oil supplementations on ammonia-nitrogen (NH_3–N) concentration and methane production.

MARABAC (%)	Tung oil (%)	NH_3–N (mg/dl)			CH_4 production (mmol/L)		
		4 h	8 h	Mean	4 h	8 h	Mean
0	0	15.47	19.78	17.63	6.94	13.75	10.34
	2	14.67	18.98	16.83	11.56	10.20	10.88
	4	15.2	18.59	16.90	11.05	11.56	11.30
	6	13.88	18.34	16.11	11.06	10.77	10.92
2	0	14.5	18.05	16.28	5.43	12.25	8.84
	2	13.61	17.78	15.70	7.41	9.41	8.41
	4	13.03	16.95	14.99	7.07	10.73	8.90
	6	12.93	16.79	14.86	8.84	10.74	9.79
4	0	13.12	17.53	15.33	6.31	8.59	7.45
	2	12.06	17.41	14.74	8.93	9.10	9.02
	4	12.66	16.43	14.55	7.33	9.94	8.63
	6	12.46	16.08	14.27	9.83	10.07	9.95
6	0	12.66	15.73	14.20	10.59	12.66	11.63
	2	11.38	14.85	13.12	9.96	13.00	11.48
	4	11.7	14.33	13.02	7.82	7.26	7.54
	6	10.85	14.46	12.66	10.56	5.44	8.00
SEM		0.326	0.186	0.230	1.141	1.391	1.587
Comparison							
MARABAC		<0.01	<0.01	<0.01	<0.01	<0.01	0.02
Tung oil		0.01	<0.01	<0.01	<0.01	<0.01	0.66
Interaction		0.86	0.89	0.94	0.15	<0.01	0.05
Orthogonal polynomials							
MARABAC (linear)		<0. 01	<0.01	<0.01	0.89	0.03	0.13
MARABAC (quadratic)		0.43	0.07	0.95	<0.01	0.50	0.02
MARABAC (cubic)		0.76	<0.01	0.55	0.23	0.50	0.84
Tung oil (linear)		<0.01	<0.01	0.07	0.01	0.01	0.82
Tung oil (quadratic)		0.41	0.19	0.73	0.74	0.59	0.87
Tung oil (cubic)		0.06	0.26	0.77	0.02	0.76	0.29

MARABAC = fruit peel pellet (mangosteen, rambutan, and banana peel); SEM = standard error of the mean; NH_3–N = ammonia nitrogen; CH_4 = methane.

3.4. In vitro ammonia-nitroge and methane production

The effects of MARABAC and tung oil on ammonia-nitrogen (NH_3–N), and methane (CH_4) production are presented in Table 6. There was no interaction ($p > 0.05$) between MARABAC and tung oil treatments for NH_3–N and CH_4 production (at 4 h and mean value).

The *in vitro* NH_3–N in all periods of incubation was affected by MARABAC ($p < 0.01$) and tung oil ($p < 0.01$) supplementation. The increasing levels of MARABAC and tung oil supplementation, the NH_3–N had a linear decrease ($p < 0.01$).

The *in vitro* CH_4 production (at 4 h incubation) was affected by MARABAC ($p < 0.01$) and tung oil ($p < 0.01$) supplementation. The increasing levels of MARABAC had a quadratic decrease ($p < 0.01$) to CH_4 production at 4 h and the mean value, and it had a linear increase ($p < 0.05$) with increased levels of tung oil.

However, there was an interaction effect ($p < 0.01$) between MARABAC and tung oil treatments for methane production at 8 h of incubation. On 0 and 2 % of MARABAC supplementations, the methane production decreased had tung oil supplementations. While at 4 % MARABAC, the CH_4 was linearly increased with increased levels of tung oil supplementation. However, at 6 % MARABAC, the methane was linearly decreased with increased levels of tung oil supplementation.

4. Discussion

4.1. Effect on gas production parameters and nutrient degradability

The production of ruminal gas usually occurs as the result of the anaerobic fermentation process that is conducted by the rumen microbe. The fermentation process of the dietary substrates might have a significant impact on the total rate of gas production in the rumen [38]. The production of gas was profoundly influenced by the types of roughages and the amount of carbohydrates present in the feed substrates [39]. Under this study, increasing MARABAC levels that are rich in plant secondary compounds (CT and saponins) up to 6 % of the total substrate are linearly decreasing in all term of gas kinetics and cumulative gas. These results were similar to previously reported that using mangosteen peel powder (contained CT 2–6%; [10]), grape pomace (contained CT 12.3 %; [40]), and rambutan fruit peel powder (contained CT 12 %; [11]) reduced the cumulative gas production at 96 h.

This may be caused by the antimicrobial properties of plant secondary compounds against bacteria, protozoa, and fungi, which are widely recognized to reduce anaerobic fermentation process [1,41–43]. The rumen microorganisms ability to release the enzymes required for feed digestion may be reduced and inhibited by the high quantity of CT from MARABAC [43,44]. Their interfering ability in the bacterial cell membrane to dissolve membrane structures and induce ion leakage is considered to be the main potentiality of their antimicrobial mode of action, whether microcidal or microstatic activities [45]. However, the cumulative gas at 2 and 4 % of MARABAC supplementation was not different between the control groups. In addition, Gunun et al. [13] reported that rambutan peel powder (11 % CT) supplementation did not alter gas production or in *in vitro* degradability.

Tung oil supplementation had an impact on gas kinetics, particularly the gas production kinetics, which decreased at 2 and 4 % supplementation. This can be attributed to reduce rumen degradable by antimicrobial effect of essential oils, which may have been caused by the tung oil's most potential adverse impact on the NDF digestion by covering the substrate coat [46].

The DM and OM degradability were significantly increased after 12 and 24 h of fermentation. Both MARABAC and tung oil supplementation levels had profound effects. The *in vitro* degradability of DM and OM were linearly decreased with an increasing level of MARABAC and tung oil up to 6 %. However, at 2 and 4 % had no difference with the control group.

In support, Ampapon and Wanapat [11] reported that rambutan fruit peel powder supplementation at 6 % reduced the *in vitro* digestibility, while 2 and 4 % supplementation of substrate did not have any effect. In addition, Wanapat et al. [6] reported that supplementation of fruit peel pellet (MARABAC) at 200 g/day in the diet (3.3 % substrate) does not impact on nutrient digestibility of cattle. This could be due to the high dose of CT causing a corresponding decrease in microbes, which then decreased degradability [47,48]. In support, CT and saponin can bind with the structure of proteins and polysaccharides, including cellulose, hemicelluloses, and pectin, leading to slow digestion of both, and CTmight also interfere with digestion by binding microbial enzymes, leading to a net decrease in ruminal protein degradability and plant cell wall digestion [1,49,50]. In addition, essential oils in tung oil might suppress amylolytic and proteolytic bacteria, leading to slow digestion of readily degradable substrates without impairing the digestion of fiber [45,51].

4.2. Effect on rumen fermentation end-products

Although the effects of CT and saponins on VFA synthesis are diverse, most studies show that CT and saponins increase the proportion of propionate and decrease the proportion of acetate, butyrate, and branched-chain VFA [42]. In the current research, supplementation at 0, 2, and 4 % of MARABAC showed an increase in total VFA when increasing levels of tung oil. In addition, propionate (C_3) concentration was trending to quadratic increase by the highest at 4 % MARABAC supplementation. There was no significant difference in the concentration of butyrate (C_4) among the supplementation groups. In support, Wanapat et al. [6] reported that supplementation of fruit peel pellet (MARABAC) at 3.3 % substrate improved the C_3 ratio and total VFA. Furthermore [11], reported that increasing levels of rambutan fruit peel increased propionate and total VFA. Previous studies [52,53] reported that propionate (C_3) and total VFA concentration were profoundly increased by bamboo grass (contained 2.5 % CT) supplementation in the diet. Ruminal VFA produced in the rumen is the major source of energy as it is absorbed via the rumen epithelium and transported to the liver, where gluconeogenesis is commonly metabolized to yield glucose and ATP. The influence of both MABABAC and tung oil on

increased VFA would enhance more energy and ATP, which would be useful for the ruminant hosts.

Changes in the synthesis of fermentation end products such as ammonia and VFA in the rumen are caused by the impacts of plant secondary compounds on bacterial, protozoal, and fungi populations. Thus, CT, saponins, and essential oils commonly decrease the quantity of NH_3–N released in the rumen, improving ruminants' ability to absorb amino acids [54,55]. This reduced ammonia content results from less protein breakdown in the feed and is frequently accompanied by a decrease in the generation of isoacids [56]. In addition, ammonia concentrations in the rumen are probably related to a reduction in protozoal populations, which play a crucial part in the process of ruminal protein breakdown [57]. The limitation of protein metabolism in the rumen may be due to two additive processes, the first of which is a reduction in the breakdown of proteins into peptides [58], and the second of which is a particular suppression of microorganisms such as "hyperammonia-producing bacteria" and their deaminase activity [59]. These mechanisms may work in conjunction with one another to provide the observed effect. Therefore, the current study found that supplementing with enhanced levels of MARABAC and tung oil led to a drop in NH_3–N. In support, supplemented plant substrates that contained high doses of plant secondary compounds such as bamboo grass [52,53], mangosteen peel [10] and fruit peel pellets [6] in the cattle diet had decreased rumen NH_3–N.

The effect of CT and saponins in MARABAC that depress methane production was consistent with effects found in earlier research [6], whereby supplemented fruit peel pellets were fed to cattle, and it was reported that they mitigated rumen CH_4 production and reduce protozoal population. Ampapon and Wanapat [11] reported that the addition of rambutan fruit peel powder to cattle diets mitigates CH_4 production and suppresses the protozoal population. Similar findings had also been reported by Viennasay et al. [52], Suriyapha et al. [53], and Anantasook et al. [60]. To have the greatest impact on CH_4, these plant secondary compounds can have a primary and selective effect, preferably by directly inhibiting methanogenic archaea and/or depressing the metabolic pathways of rumen microbes involved in methanogenesis [45]. It is currently unclear how CT exerts its effects. Some ruminal microbes may be inhibited by CT, suggesting they are antimicrobial substances [50]. In particular, studies have shown that CT may suppress the development or activity of rumen methanogens by binding proteins and enzymes of the rumen microbe, engaging in bactericidal or bacteriostatic activities [8,61]. Some ruminal protozoa are directly inhibited by CT, whereas the related methanogens are influenced by the compound indirectly. Also, saponins show considerable antiprotozoal activity by building complexes with sterols in the cell membranes of protozoa, and they diminish CH_4 synthesis by inhibiting protozoa as well as the accompanying symbiotic methanogens, which account for a significant amount of overall CH_4 production [1,41].

5. Conclusions

The results of this study suggested that supplementation of MARABAC and tung oil at a rate of up to 4 % of the total dietary DM substrate could significantly enhance the *in vitro* fermentation process. The phytonutrients in MARABAC increased the C_3 concentration and mitigated rumen CH_4 production. However, further research is needed for *in vivo* experiments imposing MARABAC and tung oil supplementation in the growth performance and lactating trials to determine potential beneficial impacts and their practical implementation.

Ethics statement

The current study and the use of all cattle were approved by the Khon Kaen University Committee of Animal Care and Use for Research permit no.0201.2.11/73.

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Ronnachai Prommachart: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Srisan Phupaboon:** Validation, Methodology, Formal analysis, Data curation. **Maharach Matra:** Validation, Methodology, Formal analysis, Data curation. **Pajaree Totakul:** Methodology. **Metha Wanapat:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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