Pongjongmit *et al.*
CABI Agriculture and Bioscience (2024) 5:13
https://doi.org/10.1186/s43170-023-00211-0

CABI Agriculture and Bioscience

RESEARCH

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Effect of manila palm (*Veitchia merrillii* Becc.) on rumen fermentation and microbial population in beef heifers

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Abstract

The aim of the study was to evaluate the influence of manila palm (MP) on rumen fermentation and rumen microbial ecology in beef cattle. Four Brahman crossbred heifers with an initial body weight (BW) of 250 ± 40 kg (1–1.5 years old) were randomly assigned according to a 4 × 4 Latin square design to the following treatments: (1) Control (0% concentrate powder MP), (2) 1% concentrate powder MP, (3) 3% concentrate powder MP, and (4) 5% concentrate powder MP. The manila palm contained 12.54% of condense tannin. Cow were fed with a rice straw ad libitum and concentrate at 1%BW. There were no treatments effect on ruminal pH, NH_3-N, microbial protein synthesis, nor ruminal temperature. Inclusion of MP did not affect ($P > 0.12$) blood urea N and hematocrit. However, ruminal concentration of acetic acid was linearly decreased ($P < 0.01$), while propionic acid was linearly increased ($P < 0.01$) as MP was increased in diet. In a such a manner that predicted ruminal CH_4 production was decreased ($P < 0.01$) as MP was increased in diet. Inclusion of MP in diet decreased linearly ($P = 0.02$) protozoal population as MP level increased. The present study suggests that manila powder has modulatory effects on ruminal fermentation, decreasing acetate to propionate ratio, reducing estimated CH_4 production without effects on ruminal pH, microbial synthesis nor blood parameters. Changes on ruminal fermentation parameters could be partially explained by decreases in the ruminal protozoa. Supplementation beyond 3% of concentrate portion (approximately 1.2% of total ration DM intake) did not increase significantly this positive effects, thus is recommended 1.2% of total ration as optimal use as an animal feed supplement.

Keywords Manila palm, Rumen fermentation, Microbial population, Beef cattle

Introduction

Although beef cattle production is commercially important in Thai agriculture, beef production efficiency is frequently suboptimal. This mainly is caused by a lack of protein and energy during the dry season, especially if the cattle are fed with a rice straw based rations. Therefore, further research looking for alternative ingredients with potential applications in feedlot feeds, mainly in terms of locally readily available supplies, could be beneficial. The Manila palm (*Adonidia merrillii* Becc.) is a type of palm tree that belongs to the *Iracaceae* family. Whole Manila palm fruit contains approximately 62.15% dry matter (DM), 4.51% crude protein (CP), 67.63% neutral detergent fiber (NDF), and 37.98% pure detergent fiber (ADF) (Bassey et al. 2017). Furthermore, MP seed contains the antiparasitic arecoline that kills tapeworm and nematodes and is rich in tannins (20 mg tannins/100 g DM) same that reduces ruminal protein degradation and modulate ruminal fermentation. In addition, numerous gallic acid moieties may be present connected by ester connections.

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These represent esters between gallic acid and sugars. These gallotannins are found in a variety of plant parts, most frequently in the bark, leaves, and fruits. Gallotannins have a lot of phenolic hydroxyl groups, which allows them to form stable protein–tannin complexes and interact with a range of protein targets in both microorganisms and mammals, as well as, can also be combined with catechins (Van Wyk et al. 2015). Those chemical properties of MP hypothetically help to promote better ruminal fermentation efficiency in cattle that are fed high fiber forage diets. The expected improvements are increases in N retention and the modulation of ruminal fermentation to favoring ruminal fermentation to propionic acid, with the concomitant reduction of CH_4 production (Moss et al. 2000). Commercial powders or pellets made from fruit peels are available on farms or in industrial canneries. Fruit peels in pellet or powder form can be added to cattle and buffalo feed. Suyitman et al. (2020) discovered that palm leaves and fronds supplemented with minerals and cassava leaf meal (PLFMC) may completely substitute native grass while also enhancing the performance and digestibility in beef cattle. However, the effects of phytocompounds in MP powder on rumen fermentation, microbial counts, and CH4 production in cattle have not been investigated. Therefore, the objective of this study was to assess how Manila palm (*Veitchia merrillii* Becc.) affects rumen fermentation, rumen ecology, CH4 production and microbial protein synthesis in cattle.

An essential part of setting up fermentation byproducts for ruminant biosynthetic pathways is done by the rumen. The rumen needs to be in good health in order to provide an ideal environment for the following reasons: rumen microorganisms (bacteria, protozoa, fungus), pH, substrates (fiber, energy, active fibers, etc.), fermentation end products (NH3-N, VFA), microbial synthesis, etc. Specifically, NH3-N is a crucial nitrogen source for microbial protein synthesis, while propionate (C3), acetate (C2), and butyrate (C4) are significant energy sources that support the synthesis of glycogenic and lipogenic compounds (Cherdthong et al. 2014). In a variety of research publications and review papers, factors that in-fluence the generation and absorption of these chemicals have been identified (Li and Sangwon 2019). It was discovered that feed types and the ratio of roughage to concentrate had an impact on a developed rumen, which in turn changed the population of microorganisms and the fermentation patterns. Between temperate and tropical feed resources, there were notable changes in feed type and quality, which were expected to have an impact on rumen bacteria and the nutrient pool in the fermentation. Moreover, the rumen ecology in these areas would be impacted by the various feeding practices that are prevalent (Asizua et al. 2018).

Plants containing secondary metabolites, condensed tannins and saponins, have the ability to control rumen fermentation by increasing feed energy utilization while reducing the rumen's methanogenic capacity (Mao et al. 2010). Tannins' manner of action is solely dependent on both the type of bacteria present and their chemical makeup (Vasta et al. 2019). In a study with crossbred heifers, Montoya-Flores et al. (2020) found that the addition of *Pennisetum purpurum* pod meal along with *Samanea saman* decreased CH4 emissions.

Methods

Animals, nutrition, experimental design, and animal husbandry

Using a 4×4 Latin square design, four Brahman crossbred heifers (weighing 250 ± 40 kg at birth to 1.5 years old) were randomly allocated to undergo ruminal fermentation. Our study examined the impact of adding MP on rumen assessment, ecology, CH4 production, and microbial protein synthesis in cows fed a diet based on forage. Diets consisted in a concentrate, offered at 1% of BW registered at start of each period, and rice straw offered ad libitum. Manila palm (MP) powder was obtained from whole fruit of manila palm sun drying for 3 days and ground through a 1 mm sieve by using the cyclotech mill, tecator, Sweden. Chemical characteristics of ingredients and MP are shown in Table 1. Treatments were as follows: (1) Control (0% concentrate powder MP), (2) 1% concentrate powder MP, (3) 3% concentrate powder MP, and (4) 5% concentrate powder MP. Experimental periods lasted 21 days, with 14-d of treatment adaptation and 7-d collecting period. At final of each period cows were rested during 30-d in which heifers received control diet. Diets were offered twice at 08.00 and 17.00 h.

Table 1 Chemical compositions of manila palm (*Adonidia merrillii* Becc.), rice straw and concentrates

Items	Concentrates	Rice straw	Manila palm whole fruit
Dry matter, %	90.48	90.45	62.15
	% Dry matter		
Ash	11.76	12.04	3.26
Organic matter	88.23	87.96	96.73
Crude protein	16.54	2.79	4.51
Acid insoluble ash	2.13	11.43	0.39
Neutral detergent fiber	52.22	83.20	67.63
Acid detergent fiber	21.43	42.90	37.98
Condensed tannin, %	–	–	12.54

Data collection

Refusals were collected, weighed and sampled, to determine DM intake. Approximately 1 kg of feces, collected via rectum, was taken during 7 successive days at 05.00 h am. Blood and rumen samples were collected on the last day of each period at 0, 3, and 6 h after morning feeding. Rumen fluid, approximately 300 mL, was collected via esophagus using a vacuum pump; the first 100 mL of rumen fluid was discarded to avoid saliva contamination. The pH and temperature of the rumen fluid were immediately measured using a portable pH and thermometer, and the rumen fluid was filtered and acid was added. Approximately 10 ml of blood samples were collected via the jugular vein. Urine was collected (when and how) and preserved with 5% H_2SO_4 solution.

Laboratory analyses

Feed, refusals, and feces were ground to pass through a 1 mm screen by using the cyclotech mill, tecator, Sweden after being dried at 60 °C for 48 h. After being ground, the samples were examined using the following methods: Van Soest et al. (1991), Schnieder and Flat (1975), and AOAC (1990) for dry matter (DM), organic matter (OM), ash, ether extract (EE), and crude protein (CP). Van Soest et al. (1991) and Schnieder and Flat (1975) for acid insoluble ash (AIA). The first portion was mixed with 5 ml of 1 M H_2SO_4, centrifuged at 16,000 *g* for 15 min, and subsequently used for NH_3-N analysis using an Kjeltech Auto 1030 Analyzer and the Kjeldahl methods according to AOAC (2012) guidelines. The total volatile fatty acids (VFA) and VFA profiles were analysed using HPLC according to the method of Samuel et al. (1997).

Blood urea nitrogen and hematocrit (Hct) were determined by Crocker (1967; Kaneko et al. 1997) Urine was analyzed for purine derivative excretions using (Lapierre and Lobley 2001).

Calculations

Rumen fermentable digestible organic matter (DOMR) was calculated as follows: DOMR (kg/day) = digestible organic matter intake (DOMI, kg/day) × 0.65, where DOMI = [organic digestibility (kg/kg DM) × organic matter intake (kg/day)]/100, 1 kg DOMI = 15.9 MJ ME/kg (ARC 1984; Kearl 1982).

For determining microbial population, protozoa and fungus were enumerated under a microscope and a hemocytometer (Galyean 1989). Quantities was expressed as cell/mL.

The excretion of purine derivatives was used to estimate the microbial purine concentration and microbial N synthesis efficiency using the equation that Chen and Gomez devised (Chen and Gomez 1992).

$$Y = 0.12X + (0.20\ BW0.75)$$

Microbial N synthesis was estimated by urinary estimated by urinary excretion of purine derivatives (PD) according to the equation of Chen and Gomez (1992):

$$MN(g/d) = 70X/(0.116 \times 0.83 \times 1000) = 0.727X$$

where X and Y are, respectively, absorption and excretion of PD in mmol/d. Efficiency of microbial N synthesis (EMNS) was calculated using the following formula:

$$EMNS = \text{microbial N}(g/d)/DOMR$$

where DOMR = digestible OM apparently fermented in the rumen.

Quantification of microbial population

The repeated bead beating plus column (RBB + C) technique developed by Yu and Morrison (Yu and Morrison 2004) was used to extract community DNA from 0.5 g of rumen fluid and digesta, and it was found to considerably enhance DNA yields. Sixteen samples in all, from four treatments, four time periods, and two rumen fluid sampling times, were collected (0 and 6 h post-feeding). The agarose gel electrophoresis and spectrophotometry methods were also used to assess the quantity and quality of all these DNA samples (Yu and Morrison 2004). The target 16S rDNA of each species was amplified using particular primers to create a quantitative test (Table 2), and the purified DNA was measured using a spectrophotometer and multiple dilutions. The target DNA was measured using sequential ten-fold dilutions of the previously measured DNA standards, ranging from 10^3 to 10^9 DNA copies. Using a Chromo 4™ system (BioRad, USA),

Table 2 PCR primers for amplifying target populations

Target species	Primer sequence
Total bacteria[a]	F5′-CGGCAACGAGCGCAACCC-3′
	R5′ CCATTGTAGCACG TGTGTA GCC-3′
Protozoa[c]	F5′-GCTTTCGWTGGTAGTGT TT-3′
	R5′-ACTTGCCCTCYAATCGTWCT-3′
F.succinogenes[a]	F5′-GTTCGGAATTACTGGGCGTAAA-3′
	R5′-CGCCTGCCCCTGAACTATC-3′
R.flavafaciens[a]	F5′-CGAACGGAGATAATTTGAGTTTACTTAGG-3′
	R5′-CGGTCTCTGTATGTTATGAGGTATTACC-3′
R.albus[b]	F5′-CCCTAAAAGCAGTCTTAGTTCG-3′
	R5′-CCTCCTTGCGGTTAGAACA-3′

[a] Denman and McSweeney (2006)

[b] Koike and Kobayashi (2001)

[c] Sylvester et al. (2004)

real-time PCR amplification and detection were carried out.

For *F. succinogenes*, standard PCR conditions are as follows: 9 min of denaturation during the first cycle, followed by 10 min of extension during the final cycle. The amplification of the remaining two 16S rRNAs was carried out in a comparable fashion, with the exception of using 55 °C as the annealing temperature. A prior publication by Yu and Morrison (2004) quantified primer numbers, status, and total bacterial counts. Four sample-derived standards were created using the DNA processing pool set from the community. For every real-time PCR test, DNA standards were created from samples using routine PCR. After that, the PCR products were measured using a spectrophotometer and purified using the QIA Rapid PCR Purification Kit (QIAGEN, Inc., Valencia, CA). The length and mass concentration of the PCR product were used to calculate the copy number concentration for each standard produced from the sample. Ten-fold serial dilutions were carried out in Tri-EDTA prior to real-time PCR (Yu and Morrison 2004). Four real-time PCR standards in all were developed. The same parameters that applied to the conventional PCR previously discussed also applied to the target gene real-time PCR experiment. For real-time PCR amplification, Biotools QuantiMix EASY SYG KIT (B&M Labs, S.A., Spain) was utilized. Every PCR was run in duplicate.

Statistical analysis

All 4×4 Latin square design data from the experiment were analyzed using the SAS (1996) GLM procedure according to the following model:

$$Y_{ijk} = l + M_i + A_j + P_k + B_t + e_{ijkt}$$

where Y_{ijk} is the observed value of animal j, receiving diet i, in period k; l is the overall average; M_i is the treatment effect (i = 1–4); A_j is the animal effect (j = 1–4); P_k is the period effect (k = 1–4); B_t is the time effect (t = 1–4) and e_{ijkt} is the residual effect. Significance was determined at $p < 0.05$. All the data were submitted to variance analysis. Using orthogonal polynomial contrasts, the treatment trends were statistically contrast-ed (linear and quadratic). The Tukey's test was performed to find differences between treatment means, and $p < 0.05$ was regarded as statistically significant.

Results and discussions

Chemical composition of straw and MP was according to composition reported by Gunun et al. (2019). Concentrate composition was in close agreement with the warranty analysis posted in the product label (Table 1).

Voluntary feed intake (kg/d) was not affected by MP inclusion averaging 6.345 kg. This absence of the effect was noted when daily intake was estimated as %BW and g/Kg BW^{75}. As planned concentrate feed intake was around 1% of BW (0.91%), similarly, ad libitum Intake of rice straw did not change by treatments. Similar results led to the conclusion that supplementation with Delonix regia seed meal had no effect on diet and estimated feed intake ($P > 0.05$) (Supapong et al. 2017). Similar results were reported by Gunun et al. (2019), who discovered that CT containing seed meal from Antidesma Thwaites Anum Muell. Arg. may be used as a supplement in feed without having an adverse impact on the feed consumption of dairy cows.

Table 3 Effect of manila palm (*Veitchia merrillii* Becc.) on voluntary feed intake

Items	Level of Manila palm in Diet (%)				SEM	Contrast	
	0	1	3	5		Linear	Quadratic
Rice straw intake							
kg	3.93	3.29	4.11	3.83	0.58	0.84	0.76
%BW	1.21	1.28	1.46	1.46	0.21	0.33	0.87
g $kg^{-1}BW^{0.75}$	51.34	51.19	59.80	58.74	8.38	0.43	0.96
Concentrate intake							
kg	2.68	2.26	2.72	2.56	0.41	0.96	0.75
%BW	0.83	0.88	0.97	0.98	0.15	0.43	0.89
g $kg^{-1}BW^{0.75}$	35.10	35.11	39.54	39.27	5.91	0.54	0.98
Total DM intake							
kg	6.61	5.55	6.83	6.39	0.98	0.89	0.75
%BW	2.04	2.15	2.43	2.44	0.35	0.37	0.88
g $kg^{-1}BW^{0.75}$	86.44	86.29	99.34	98.01	14.20	0.47	0.97

SEM standard error of mean

Table 3 displays the effects of treatment on rumen fermentation, blood urea nitrogen, Hct concentration, and CH4 generation. The addition of MP had no effect ($P > 0.05$) on butyric acid, NH_3-N, pH, temperature, or total rumen VFA proportions. However, adding MP resulted in an increase ($P < 0.05$) in propionic acid and a decrease ($P < 0.05$) in ruminal acetate proportions. The 3% and 5% inclusion levels showed the greatest impact of this effect. This could be as a result of the diet containing more manila palm, which facilitates improved rumen digestion and absorption and raises propionic acid levels. These increases may be explained by the crude protein found in manila palms, which the rumen may use to synthesize propionate, which the rumen ferments quickly. So, it would be intriguing to carry out additional study on how the availability of plants that contain tannin in the tropics affects rumen efficiency. Interestingly, total VFA was not affected by treatments even if the increase of diet NDF due to the addition of Manila palm (NDF: 67.63%). According to Calabrò et al. (2002) this results could be attributed to the higher NDF digestibility in the experimental diets. According to Phesatcha et al. (Phesatcha et al. 2021), *Flemingia macrophylla* supplementation increased the proportion of C3 while decreasing the fraction of C2. The predicted shift in the VFA profile from C2 to C3 was due to the switch from CH_4 to H_2, which is advantageous for the host's energy supply. In contrast to the control group, the manila palm supplementation resulted in a decrease ($P < 0.05$) in CH4. Condensed tannin (CT) found in manila palms may be the cause of this. The fact that CT can lower the number of protozoa may be connected to these decreases. This showed that there will be no detrimental effects on rumen fermentation when using manila palm as a feed supplement for beef cattle. used volatile fatty acid as a variable to calculate methane production, which varied between treatments. Furthermore, Moss et al. (2000) demonstrated that the changing hydrogen itinerary in rumen ecology may be explained by predicting methane synthesis using the amount of volatile fatty acid. The estimated CH4 generation of beef cattle given CT was decreased, which is consistent with a prior study of Kongmun et al. (2009). By enhancing rumen propionic generation, lowering protozoa, and thus reducing methane production, diet containing CT and SP also had a more notable impact on rumen fermentation (Ampapon et al. 2019) (Table 4).

BUN and Hct concentrations were not significant different among treatments. Blood urea levels could indicate whether or not adequate N is available to the rumen microbial population. According to numerous research, a BUN value of 7–20 mg/dl is considered normal (Hammond 1983). According to Preston et al. (Preston et al. 1998), BUN readings indicate the amount of ammonia produced in the rumen and are strongly connected with protein consumption. This would suggest that the rumen might employ and/or absorb the available NH_3-N for further synthesis. Lapierre and Lobley (2001) reported that the amount of ammonia absorbed from the rumen was correlated with the BUN content in circulation. As a result, a high BUN content suggests either an overabundance of protein or low calories. Ruminal NH3-N concentrations are low and the amount of N recycled back into the rumen as urea is elevated when there is a protein shortage in the diet. BUN and ruminal NH3-N have a strong correlation because of these metabolic exchanges (Hennessy and Nolan 1988).

Table 4 Impact of the manila palm (*Veitchia merrillii* Becc.) on the generation of CH4, HCT concentration, blood urea nitrogen, and rumen fermentation in beef cattle

Items	Level of Manila palm in Diet (%)				SEM	Contrast	
	0	1	3	5		Linear	Quadratic
Ruminal, pH	6.93	6.85	6.81	6.80	0.000000026	0.52	0.71
Ruminal temperature, °C	38.32	38.25	38.01	38.07	0.63	0.73	0.92
Blood urea nitrogen (BUN), mg/dL	10.77	10.98	11.08	10.69	0.18	0.87	0.12
Hematocrit (Hct), %	25.75	24.46	23.50	23.96	1.81	0.45	0.64
NH_3-N, mg/dL	15.76	16.15	18.49	16.74	1.74	0.52	0.55
Total VFA, mmol/l	127.31	129.53	130.62	118.54	4.41	0.23	0.14
	VFA profiles, mol/100mol						
Acetic acid	68.28[a]	65.06[b]	60.64[c]	61.10[c]	0.86	< 0.01	0.06
Propionic acid	21.82[c]	26.21[b]	29.96[a]	30.36[a]	1.15	< 0.01	0.12
Butyric acid	9.89	8.73	9.40	8.55	0.65	0.28	0.82
CH_4 production[A]	28.68[a]	25.56[b]	22.81[c]	22.56[c]	0.81	< 0.01	0.11

[abc] Values on the same row with different superscripts differed ($P < 0.05$) NH_3-N = amonia nitrogen, VFA = volatile fatty acids, SEM = standard error of mean, [A]Calculated according to Moss et al. (2000) CH_4 production = 0.45 (acetic acid) − 0.275 (propionic acid) + 0.4 (butyric acid)

Ahola et al. (2006) reported that hematocrit is a measure of the concentration of erythrocytes. This is in line with what Roza et al. found (Roza et al. 2015) The quantity of erythrocytes affects the hematocrit levels. The values of hematocrit and erythrocytes are parallel to one another. Hematocrit of beef cattle in this study (19.6–26.7%) is within the normal range as determined between 20.00 and 38.00% (Jain 1993).

There was no change in the population's total direct count of bacterial and fungal zoospores by MP inclusion (Table 5); however, protozoal population was linearly ($P < 0.01$) decreased by MP. However, microbial population study by using real-time PCR technique found that total bacteria, *F.succinogenes*, *R.flavefaciens* and *R.albus* were not significant different among treatment ($P > 0.05$). While, protozoa was decrease in beef cattle fed with manila palm powder ($P < 0.05$). Nonetheless, Russell and Rychlik (2009) discovered that the microbial ecology will change in accordance with the rations supplied to ruminants. Poungchompu et al.'s earlier research (Poungchompu et al. 2009) shown that MPP and soapberry fruit significantly decreased the number of rumen protozoa. Additionally, herbs with tannins may reduce protozoa (Bhatta et al. 2009). According to Wanapat and Cherdthong (2008) most *F. succinogenes* population were found higher in rumen digesta than in rumen fluid. Conversely, supplementation with mangosteen peel 3% did not affect microbial populations. According to Salais et al. (1977), the type of fiber and protein level of the forage have significant roles in influencing how an individual reacts to a diet. It is commonly recognized that NH3 is necessary for the growth of cellulolytic bacteria (Bryant 1973). Without NH3, they could not develop on other nitrogen sources. The authors of the study by Koike et al. (2003) postulated that cell proliferation on the straw could be the primary cause of the observed increase in attached cell numbers. However, it was observed that the numbers of attached cells of the three species increased gradually and peaked at 24 or 48 h (10^9 per gram dry matter (DM) for *F. succinogenes* and 10^7 per gram DM for *R. flavefaciens*, respectively, and 10^6 per gram DM for *R. albus*). The increased cell populations four hours after feeding may be due to both cell growth following feeding and more bacterial adherence from the liquid phase or other particles.

Table 5 Effect of manila palm (*Veitchia merrillii* Becc.) on microbial population in beef cattle

Items	Level of Manila palm in Diet (%)				SEM	Contrast	
	0	1	3	5		Linear	Quadratic
Total direct count (cells/mL)							
Protozoa ($\times 10^5$)	4.65[a]	4.42[b]	4.39[b]	4.37[b]	0.07	0.02	0.17
Anaerobic fungi ($\times 10^7$)	5.54	5.61	5.51	5.60	0.04	0.60	0.69
Bacteria ($\times 10^{11}$)	6.07	6.02	6.01	5.98	0.06	0.37	0.86
Real-time PCR technique, copies/ml of rumen content							
Total bacteria, $\times 10^9$	16.18	25.22	19.18	21.21	3.02	0.51	0.26
Protozoa, $\times 10^6$	6.59[a]	4.99[ab]	4.71[ab]	3.39[ab]	0.67	0.01	0.84
F.succinogenes, $\times 10^6$	4.66	4.33	4.39	4.26	0.77	0.75	0.91
R.flavefaciens, $\times 10^6$	27.61	29.61	31.85	29.43	3.39	0.62	0.53
R.albus, $\times 10^6$	4.16	3.39	4.17	3.31	1.35	0.84	0.75

SEM standard error of mean

[abc] Values on the same row with different superscripts differed ($P < 0.05$)

Table 6 Effect of manila palm (*Veitchia merrillii* Becc.) on microbial protein synthesis in beef cattle

Items	Level of Manila palm in Diet (%)				SEM	Contrast	
	0	1	3	5		Linear	Quadratic
Microbial protein synthesis							
PD excreted (mmol/day)	23.98	24.91	24.86	23.36	1.17	0.72	0.32
PD absorbed (mmol/day)	80.64	105.90	96.82	100.35	8.15	0.20	0.21
MNS (g N/day)	58.63	76.99	70.39	72.95	5.92	0.20	0.21
EMPS (g N/kg OMDR)	22.69	34.61	32.34	32.32	3.57	0.13	0.13

SEM standard error of mean, *PD* purine derivative, *MNS* microbial nitrogen supply, *EMPS* efficiency of microbial protein synthesis, *OMDR* organic matter digestible in the rumen

Table 6 shows the effect of manila palm (*Veitchia merrillii* Becc.) on microbial protein synthesis in beef cattle. Purine derivative absorbed, purine derivative excreted, microbial nitrogen supply and efficiency of microbial protein synthesis (EMPS) were not different among treatments ($P > 0.05$). This might be because manila palm hasn't the potential to improve the ruminal fermentation toward maximizing microbial protein synthesis. Interestingly, the amount of PD excreted by native Thai beef cattle ranges between those reported for buffalo and Freisian cattle (Cutrignelli et al. 2007). A previous study has shown that synthesis of microbial protein was affected by dietary protein (Cecava et al. 1991). However, enhanced microbial protein synthesis may be the cause of this study's increased nutritional digestibility. Furthermore, dietary sulfur, feeding frequency, rumen dilution rate, and the pace at which nitrogen and carbohydrate sources are broken down in the rumen further influence the synthesis of microbial proteins (Stern and Hoover 1979).

Conclusion

Manila powder has modulatory effects on ruminal fermentation, decreasing acetate, increase propionate, reducing estimated CH_4 production without effects on ruminal pH, microbial synthesis nor blood parameters. Changes on ruminal fermentation parameters could be partially explained by decreases in the ruminal protozoa. Supplementation beyond 3% of concentrate portion (approximately 1.2% of total ration DM intake) did not increase significantly these positive effects, thus is recommended 1.2% of total ration as optimal level for use as animal feed supplementation. However, more investigation is needed to fully understand how the manila palm affects the variety of methanogenic archaea.

Acknowledgements
The authors would like to express their most sincere thanks to Thailand Science Research and In-novation (TSRI) or TSRI Fund for providing financial support for this research.

Author contributions
TP, TN and KP: Investigation, Methodology, Data curation, Formal analysis, Software, and Project administration, Conceptualization, Methodology, and Project administration, Funding acquisition, Resources, Supervision, Validation; Visualization; TP: Roles/Writing—original draft; TP, TN and KP: Writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding
Not applicable.

Availability of data and materials
The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All of the experimental animals and methodology involved in this research were approved by the Animal Ethics Committee under the Institutional Guidelines approved the animal care and experimental procedures (approval on: Protocol #17062019). All procedures were carried out in compliance with the applicable rules and guidelines, according to our investigation. The research was conducted adhering to the ARRIVE standards.

Consent for publication
Yes.

Competing interests
The authors declare that they do not have any competing interests.

Received: 22 July 2023 Accepted: 27 December 2023
Published online: 06 February 2024

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