

Strategic Supplementation of *Flemingia* Silage to Enhance Rumen Fermentation Efficiency, Microbial Protein Synthesis and Methane Mitigation in Beef Cattle

Bounnaxay Viennasay

Department of Animal Science, Khon Kaen University.

Metha Wanapat (✉ metha@kku.ac.th)

Khon Kaen University <https://orcid.org/0000-0002-7633-052X>

Research article

Keywords: Fodder silage, Feed utilization, Rumen metabolism, Methane production, Phytonutrients

Posted Date: May 4th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-24054/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at BMC Veterinary Research on December 9th, 2020. See the published version at <https://doi.org/10.1186/s12917-020-02703-x>.

Abstract

Background: Good quality protein as an on-farm feed resources has been in great demand to support the productivity of ruminants. A digestion trial using beef cattle crossbreds was conducted to assess the four dietary treatments of *Flemingia macrophylla* silage (FMS) supplementation at 0, 0.2, 0.4 and 0.6 kg dry matter (DM)/day in a 4 × 4 Latin square design. Feed DM intakes were measured during the 14 days and sample of feeds, feces, urine, as well as rumen fluid, blood were collected during the 7 days while the animals were on metabolism crates.

Results: Based on this experiment revealed that strategic supplementation of FMS increased ($P < 0.05$) nutrients digestibility (organic matter, crude protein, and acid detergent fiber) enhanced rumen total volatile fatty acid production especially propionic acid (C_3), $C_2:C_3$ ratio while, remarkably promoted the microbial protein synthesis (MPS) by increasing N-balance and retention of purine derivatives.

Conclusion: Under this experiment, the results revealed the potential use of FMS as a good-quality feed to improve nutrients digestibility, rumen fermentation, microbial protein synthesis, and to mitigate methane production. FMS supplementation at 0.6 kg DM/day exhibited the best result.

Background

Feed resources for ruminants are important in the livestock feeding systems for small-scale tropical farmers; particularly in the dry season [1]. *Flemingia* is a multipurpose legume shrub that yields fresh biomass of about 55 tons/ha/year and thrives well in diverse conditions [2]. It contains high levels of crude protein (17–26%), condensed tannins (CT) 6–11% and saponins (SPN) [3–5]. The presence of these phytonutrients in feed resources has been shown to enhance the rumen fermentation efficiency and greatly reduce rumen methane (CH_4) production [6]. Fagundes et al. [7] also reported that supplementation of *Flemingia* at 125 g of dry matter intake in goats did not affect adversely the feed intake and milk production. In addition [8], reported that supplemented *Flemingia* hay meal at 150 g/head/day increased digestibility of nutrients, rumen fermentation and microbial protein synthesis. Moreover, Kang et al. [4] confirmed that *Flemingia* leaves supplementation improved rumen fermentation and reduced the CH_4 production. Conserving of feed in the form of silage has been a good practice especially for dry season feeding [9]. Silage quality can be enhanced by addition of urea and molasses in fodder crop silage [10–12].

However, there was limited information about the utilization of *Flemingia macrophylla* silage on rumen fermentation. Hence, the aim of this experiment was to investigate the impact of *Flemingia macrophylla* silage on nutrient digestibility, rumen fermentation and microbial protein synthesis in beef cattle.

Results

Nutritive value, feed intake and nutrient digestibility

Concentrate supplement was formulated using cassava chip and agricultural by-products. The nutritive values of the feeds were in good ranges especially the FMS which had a good characteristic of silage both physically and chemically.

Strategic supplementation of FMS did not influenced the total feed intake ($P > 0.05$), however nutrients digestibilities of DM, OM, CP, NDF, and ADF were significantly increased ($P < 0.001$) (Table 2). In addition, the supplementation of FMS at 0.6 kg/head/day was the highest in nutrients digestibilities.

Table 2
Effect of *Flemingia macrophylla* silage (FMS) on feed intake and nutrients digestibility

| Items | FMS kg/d of dry mater | | | | SEM | P-value |
|--|------------------------------|-------------------------------|-------------------------------|------------------------------|------|---------|
| | 0 | 0.2 | 0.4 | 0.6 | | |
| Roughage Intake (DM basis) | | | | | | |
| Kg/d | 3.13 ± 0.84 | 3.23 ± 0.97 | 3.40 ± 0.89 | 3.58 ± 0.70 | 1.00 | 0.47 |
| Body weight (%) | 1.81 ± 0.25 | 1.97 ± 0.22 | 1.89 ± 0.34 | 2.14 ± 0.11 | 0.60 | 0.44 |
| Concentrate Intake | | | | | | |
| kg/d | 0.90 ± 0.17 | 0.90 ± 0.17 | 0.90 ± 0.17 | 0.90 ± 0.17 | 0.00 | 0.00 |
| Body weight (%) | 0.50 ± 0.03 | 0.50 ± 0.03 | 0.50 ± 0.03 | 0.50 ± 0.03 | 0.00 | 0.00 |
| Total Intake | | | | | | |
| Kg/d | 4.00 ± 0.98 | 4.30 ± 1.13 | 4.10 ± 1.03 | 4.50 ± 0.85 | 1.00 | 0.48 |
| Body weight (%) | 2.32 ± 0.24 | 2.48 ± 0.22 | 2.41 ± 0.35 | 2.60 ± 0.21 | 0.06 | 0.44 |
| Digestibility (%) | | | | | | |
| Dry matter | 54.11 ± 1.49 ^a | 58.61 ± 0.68 ^b | 62.30 ± 2.57 ^c | 63.62 ± 2.49 ^c | 0.21 | 0.001 |
| Organic matter | 58.20 ± 0.46 ^a | 64.40 ± 0.42 ^b | 68.40 ± 2.10 ^c | 71.45 ± 0.66 ^d | 0.29 | 0.001 |
| Crude protein | 53.80 ± 0.65 ^a | 55.60 ± 0.32 ^b | 57.10 ± 0.17 ^c | 58.70 ± 0.32 ^d | 0.10 | 0.001 |
| Neutral detergent fiber | 50.00 ± 0.81 ^a | 51.90 ± 0.92 ^{ab} | 54.00 ± 0.56 ^{ab} | 55.40 ± 1.89 ^c | 0.12 | 0.05 |
| Acid detergent fiber | 45.80 ± 0.28 ^a | 47.30 ± 0.54 ^b | 49.60 ± 0.88 ^c | 52.80 ± 0.54 ^d | 0.15 | 0.001 |
| a,b,c,d Means in the same row with different superscripts differ ($P < 0.01$), SEM Standard error of the mean. | | | | | | |

Table 3
Effect of *Flemingia macrophylla* silage (FMS) on rumen ecology and fermentation

| Items | FMS kg/d of dry mater | | | | SEM | P-value |
|--|------------------------------|--------------------------------|--------------------------------|-------------------------------|------|---------|
| | 0 | 0.2 | 0.4 | 0.6 | | |
| NH ₃ -N (mg/ml) | 19.31 ± 0.12 ^d | 18.32 ± 0.13 ^c | 17.53 ± 0.11 ^b | 16.91 ± 0.08 ^a | 0.50 | 0.001 |
| BUN (mg/dl) | 10.11 ± 0.13 | 10.03 ± 0.21 | 10.21 ± 0.43 | 10.03 ± 0.38 | 0.02 | 0.83 |
| Total VFA (mM/L) | 99.50 ± 4.61 ^a | 102.90 ± 4.61 ^{ab} | 107.70 ± 3.52 ^{bc} | 112.50 ± 4.23 ^c | 2.82 | 0.004 |
| VFA (mol/100 mol) | | | | | | |
| Acetic acid | 73.90 ± 0.67 ^d | 73.00 ± 0.36 ^c | 71.70 ± 0.94 ^b | 70.20 ± 0.98 ^a | 0.81 | 0.001 |
| Propionic acid | 16.50 ± 0.20 ^a | 17.70 ± 0.19 ^b | 19.30 ± 0.59 ^c | 20.90 ± 0.74 ^d | 0.95 | 0.001 |
| Butyric acid | 9.70 ± 0.53 ^c | 9.20 ± 0.33 ^b | 9.00 ± 0.39 ^{ab} | 8.90 ± 0.26 ^a | 0.18 | 0.002 |
| Acetic acid to Propionic acid | 4.50 ± 0.08 ^d | 4.10 ± 0.06 ^c | 3.80 ± 0.16 ^b | 3.40 ± 0.16 ^a | 0.23 | 0.001 |
| Methane (mol/100 mol) | 32.60 ± 0.16 ^d | 31.68 ± 0.14 ^c | 30.59 ± 0.45 ^b | 29.38 ± 0.55 ^a | 0.69 | 0.001 |
| pH | 6.82 ± 0.03 | 6.87 ± 0.02 | 6.84 ± 0.03 | 6.89 ± 0.02 | 0.02 | 0.13 |
| Protozoa (× 10 ⁵ Cell/ml) | 8.08 ± 0.07 ^c | 6.65 ± 0.05 ^b | 4.98 ± 0.07 ^a | 4.40 ± 0.09 ^a | 0.83 | 0.009 |
| ^{a,b,c,d} Means in the same row with different superscripts differ ($P < 0.01$), <i>SEM</i> Standard error of the mean, <i>BUN</i> Blood urea nitrogen, <i>VFA</i> Volatile fatty acid, Methane production = 0.45 (acetic acid) – 0.275 (propionic acid) + 0.4 (butyric acid) | | | | | | |

Rumen fermentation efficiency and blood urea nitrogen

FMS supplementation increased total VFA and propionic acid (C3) ($P < 0.001$), among treatments. While the concentrations of NH₃-N, acetic acid (C3), butyric acid (C4), acetic acid to propionic acid (C2 to C3), CH₄ production, and protozoal population were decreased ($P < 0.001$) among treatments, respectively. However, ruminal pH and BUN were not changed.

Nitrogen balance, excretion of purine derivatives and microbial nitrogen supply

Table 4. Shows nitrogen intake ranged from 34.81 ± 1.23 to 48.01 ± 2.12 g/d and was increased ($P < 0.001$), while N excretion was similar among treatments ($P > 0.05$). However, N absorbed and N retained were increased ($P < 0.001$), respectively. The FMS supplementation affected on allantoin, uric acid, PD, purine absorb, microbial nitrogen supply and EMNS ($P < 0.001$). Moreover, FMS affected on percent of MNS and EMNS were increased significantly among treatments ($P < 0.001$), respectively.

| Items | FMS kg/d of dry mater | | | | SEM | P-value |
|--|-------------------------------|-------------------------------|-------------------------------|-------------------------------|------|---------|
| | 0 | 0.2 | 0.4 | 0.6 | | |
| Nitrogen utilization (g/d) | | | | | | |
| Intake | 34.81 ± 1.23 ^a | 41.02 ± 1.54 ^b | 44.03 ± 1.98 ^c | 48.01 ± 2.12 ^d | 2.79 | 0.001 |
| N excretion (g/d) | | | | | | |
| Fecal N | 15.20 ± 2.32 | 16.00 ± 1.98 | 16.20 ± 1.67 | 13.90 ± 1.59 | 0.52 | 0.54 |
| Urinal N | 13.00 ± 1.41 | 13.40 ± 1.50 | 11.80 ± 1.83 | 10.50 ± 1.92 | 0.65 | 0.25 |
| N balance (g/d) | | | | | | |
| Absorbed N | 19.61 ± 1.04 ^a | 25.02 ± 1.67 ^b | 28.01 ± 1.91 ^b | 34.10 ± 1.98 ^c | 3.02 | 0.001 |
| Retained N | 6.70 ± 0.54 ^a | 11.61 ± 0.946 ^b | 17.63 ± 1.32 ^c | 22.30 ± 1.27 ^d | 3.41 | 0.001 |
| Allantoin (mM/d) | 108.92 ± 3.54 ^a | 122.61 ± 4.21 ^b | 130.67 ± 5.33 ^b | 148.71 ± 8.12 ^c | 8.30 | 0.001 |
| Uric acid (mM/d) | 26.11 ± 1.34 ^a | 29.42 ± 2.30 ^b | 31.33 ± 1.11 ^b | 35.56 ± 1.72 ^c | 1.99 | 0.001 |
| PD (mM/d) | 130.67 ± 3.43 ^a | 147.22 ± 2.31 ^b | 156.78 ± 3.16 ^b | 178.43 ± 2.81 ^c | 9.96 | 0.001 |
| Purine absorb (mM/d) | 109.3 ± 2.43 ^a | 125.7 ± 2.56 ^b | 135.4 ± 2.11 ^b | 157.0 ± 2.43 ^c | 9.96 | 0.001 |
| MNS (g N/d) | 79.41 ± 2.21 ^a | 91.4 ± 3.11 ^b | 98.4 ± 4.01 ^b | 114.1 ± 4.87 ^c | 7.24 | 0.001 |
| MNS increased (%) | 0.00 ± 0.00 ^a | 15.43 ± 1.65 ^b | 24.12 ± 1.93 ^b | 44.01 ± 2.88 ^c | 9.20 | 0.002 |
| EMNS (g N/kg OMDR) | 9.60 ± 0.13 ^a | 11.31 ± 0.46 ^b | 14.13 ± 0.72 ^c | 14.12 ± 0.54 ^c | 1.01 | 0.003 |
| EMNS increased (%) | 0.00 ± 0.00 ^a | 18.92 ± 1.63 ^b | 39.78 ± 1.97 ^b | 49.01 ± 2.41 ^c | 10.9 | 0.001 |
| ^{a,b,c} Means in the same row with different superscripts differ ($P < 0.05$), <i>SEM</i> Standard error of the mean, <i>N</i> Nitrogen, <i>MNS</i> Microbial nitrogen supply, <i>PD</i> Purine derivatives. Microbial N (g N/d) = $(X \times 70) / (0.116 \times 0.83 \times 1,000) = 0.727 \times X$ (where, X = total absorption of purine derivatives). EMNS = Efficiency of microbial nitrogen supply (g N/kg OMDR). OMDR (kg) = 65% of organic matter digestible in total tract | | | | | | |

Table 4

Effects of *Flemingia macrophylla* silage (FMS) on nitrogen balance, excretion of purine derivatives and microbial nitrogen supply

Discussion

Nutritive value of experimental feeds, feed intake and nutrients digestibility

As presented in Table 1, crop-residue such as rice straw was abundantly available and used as a roughage source during the long dry season, despite its' low level of CP (2.80%) and high in fibrous fractions (71.70% NDF, 47.70% ADF). This value was similar to those reported by Wanapat et al. [13]. Details of rice straw and its' treatment had been illustrated by Wanapat et al. [14]. *Flemingia* is a fodder shrub that can grow well in many diverse areas and the young plant including leaf, branch and stem can be harvested for ruminant feeding. It contains high level of crude protein, condensed tannins and saponins. The feed can be ensiled as silage for long time feeding, details are shown in Table 1, FMS contains 18.10% CP, 95.60% OM, 47.50% NDF, 37.20% ADF, and 10.20% CT, as a high-quality silage with pH of 4.4 with sour smell and green-yellow color. As reported by [15–17] who stated that good quality silage should contain pH between 3.5–4.5. These results have shown that FMS was a good alternative feed to improve the quality and for long dry season feeding. While the source of energy is the common limiting factor for anaerobic microbes growth, hence, the supply of urea and molasses have increased the microbial yield, leading to increased CP [18]. Providing good FMS with good protein, content can thus enhance the use of protein, particularly when fed with low quality roughage. However, the supplementation of FMS did not influence DM intake in all treatments. Nevertheless, it increased ($P < 0.05$) the digestibilities of DM, OM, CP, NFD and ADF. This finding agreed with the report of Phesatcha et al. [8] who supplemented *Flemingia macrophylla* hay which had increased the CP and NDF digestibilities. It has been reported that CT in the feeds combined with protein to protect protein degradability in the rumen [19]. Results suggest that the CT in FMS effectively impacted the nutrient digestibility and the microbial activity in the rumen, as well as increased the rumen fermentation efficiency.

Table 1
Feed ingredients and chemical composition of experimental diets

| Items | Concentrate | Rice straw | <i>Flemingia macrophylla</i> silage (FMS) |
|-------------------------------|-------------|------------|---|
| Ingredients (% air-day basis) | | | |
| Cassava chip | 60.00 | 0.00 | 0.00 |
| Brewery's grain dry | 12.00 | 0.00 | 0.00 |
| Rice bran | 9.00 | 0.00 | 0.00 |
| Palm kernel meal | 13.00 | 0.00 | 0.00 |
| Urea | 2.00 | 0.00 | 0.00 |
| Molasses | 2.00 | 0.00 | 0.00 |
| Sulfur | 0.50 | 0.00 | 0.00 |
| Salt | 1.00 | 0.00 | 0.00 |
| Mineral premix ^a | 0.50 | 0.00 | 0.00 |
| Chemical composition (% DM) | | | |
| Dry matter | 87.50 | 90.00 | 26.50 |
| Organic matter | 14.80 | 2.80 | 18.10 |
| Crude protein | 94.20 | 96.40 | 95.60 |
| Neutral detergent fiber | 28.90 | 71.70 | 47.50 |
| Acid detergent fiber | 17.20 | 47.70 | 37.20 |
| Condensed tannins | 0.00 | 0.00 | 10.20 |
| pH | 0.00 | 0.00 | 4.40 |
| Lactic acid (g/L) | 0.00 | 0.00 | 2.00 |
| Acetic acid (g/L) | 0.00 | 0.00 | 0.50 |

Rumen fermentation efficiency and blood urea nitrogen

Ruminal pH and BUN were not significantly shifted differently among treatments. This result agreed with Phesatcha et al. [8] who showed that pH and BUN did not change by *Flemingia* leaf supplementation. The normal rumen pH was reported to be 6.3–6.8 which can support cellulolytic bacteria's normal activity [20]. These values indicated that all levels of FMS feeding were in favorable condition. While, ruminal NH₃-N concentration was decreased as a result of the CT in FMS which could protect protein in the diet by forming tannin-protein complexes to by-pass to lower-gut [21, 22] reported that CT in the diets may also

reduce NH_3 concentration in the rumen by reducing protein degradation in the rumen. The optimal ruminal $\text{NH}_3\text{-N}$ concentration for rumen fermentation activities, ruminal microbial protein synthesis efficiency, voluntary dry matter intake, and digestibility of feeds were in the range of 15–30 mg/ml [23]. In this study, the ruminal $\text{NH}_3\text{-N}$ values (16.91 ± 0.08 to 19.31 ± 0.12 mg/ml) were found and could improve for rumen ecology in cattle crossbreds. With increased levels of FMS supplementation, the total VFA and C_3 were increased ($P < 0.05$), and the highest impact was found in the group fed with of 0.6 kg/day, while C_2 , C_2 to C_3 ratio were reduced. Phesatcha et al. [8] reported that, FM supplementation in dairy steers decreased C_2 whilst C_3 was increased. Makkar et al. [24] found that C_3 could be improved by supplementing CT and decreased concentration of C_2 . Furthermore, ruminal CH_4 production was decreased by CT and can inhibit methanogens growth in the rumen fermentation directly. CT inhibition of methanogenesis may also lead to a decrease in the ratio of C_2 to C_3 , as a result of increased hydrogen transfer to the synthesis of propionate [25]. Another possible influence of rumen CH_4 depression could be influenced by the suppression of protozoal population by FMS supplementation. This may be attributed to CT in FMS which affected the protozoa cell membrane, thus interfering with ion exchanges [26]. Pongchompu et al. [27] earlier reported that dairy heifer crossbreds supplemented with feeds containing phytonutrients had reduced protozoal count. The population of rumen protozoal and methane emission were significantly reduced [28].

Nitrogen balance, excretion of purine derivatives and microbial protein synthesis

FMS supplementation affected N-balance as shown in Table 4. The N-absorbed and retention were linearly increased with FMS supplementation due to N-intake and CP digestibility, however N-excretion of fecal and urine were similar among treatments. Agreed with, Viennasay et al. [12] who reported that increased digestibility of the CP, consequently affected on the increase in retained N. The high intake of CP and CT increases the flow of protein from the rumen to the small intestine [29]. The urine PD excretion is considered to be an important determinant to predict the productivity of microbial proteins in the rumen. The synthesis of ruminal microbial proteins plays an important role in ruminants because it mainly provides high-quality protein resources for the host animals. Essentially, microbial protein synthesis and efficiency depend on many factors such as DM intake, nitrogen and carbohydrate ruminal degradability rate, rumen dilution rate, mineral and other factors [30]. The ammonia is the main N source of the rumen microbes, but the diet availability of amino acids, peptides, and both together increases the growth of cellulolytic and amylolytic bacteria [31]. In adequate doses, the efficiency in microbial synthesis and the microbial yield were increased by including saponins [32] or CT [33]. Under this study, supplementation of FMS containing both CT and SPN, could provide additional protein available at the lower-gut for the host ruminant.

Conclusion

Under this experiment, the results revealed the potential use of FMS as a good-quality feed to improve nutrients digestibility, rumen fermentation, microbial protein synthesis, and to mitigate methane production. FMS supplementation at 0.6 kg DM/day exhibited the best result. Making FMS should be encouraged to prepare for on-farm use especially during the long dry season. Furthermore, *in vivo* feeding trials should be conducted in both beef cattle and dairy cattle in order to obtain more relevant data.

Materials And Methods

This study was approved by the Institutional Animal Care and Use Committee of Khon Kaen University, Thailand. Record No. IACUC-KKU-94/61 and Reference No. 0201.2.11/73.

Preparation of *Flemingia macrophylla* Silage

Flemingia macrophylla was planted by stems on the experimental plots of Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Thailand with close supervision of the advisory Professor. All plant parts were kept and stored at TROFREC. *Flemingia macrophylla* (FM) whole top plant was harvested from the shrub after three months of regrowth. Young stem, leaf and branch were chopped with a machine to about 3 cm in length. Molasses: urea: water at 2:1:10 were mixed well with 100 kg chopped FM and stored in the plastic barrel after pressing well (approximately 60 kg/barrel), and tightly covered for 21 days. *Flemingia macrophylla* silage (FMS) was sampled from various barrels, oven-dried at 60 °C for 48 hours and then ground, stored in bottles for chemical analysis. Standard methods were employed to analyze for chemical compositions including dry matter (DM), ash, crude protein (CP), neutral-detergent fiber (NDF), acid-detergent fiber (ADF) [34, 35]. While condensed tannins (CT) was by the vanillin-HCl [36], method modified by [37] FMS sample was collected weighing 40 g soaked in 200 ml of cool distilled water for 12 hours. The mixture was then filtered and supernatant divided into 4 aliquots for determination of pH using a portable pH meter (HANNA Instruments HI 8424 microcomputer, Singapore). Apart from that, a FMS sample was washed with deionizing water for analyzed lactic acid and acetic acid analysis [38].

Animals and Design

These experimental beef crossbreds belonged to TROFREC, Khon Kaen University and were provided for use to support Ph.D. students for research. After the termination the experiment, they have been maintained in good health and were used for another experiment by a student. Four, beef cattle about two year old with 172 ± 43 kg liveweight, were randomly assigned to in a 4×4 Latin square design. Concentrate was offered at 0.5 kg of body weight (BW)/day and rice straw offered *ad libitum* with supplementation of FMS at 0, 0.2, 0.4 and 0.6 kg DM/head/day. The trial was conducted for four periods each was consisted of 21 days, during the first 14 days was for matter feed intake measurement, while during the last 7 days was for sample collection using total collection method. All animals were in individual pens, clean water and mineral-salt blocks were available at all times. The diet was offered to

the animals twice in the morning (07:00a.m.) and afternoon (04.00p.m.). The liveweight of each cattle was weighed at the beginning and the end of each period to calculate feed intake. Feed provided and refusals were measured daily throughout the experimental period. Feed samples were collected twice a week for DM analysis [34]. Samples of feeds including concentrate, FMS, rice straw, feces were collected randomly daily during the last 7 days of each period. A daily sample of feces of each animal (about 100 g) was collected to be analysed [34]. Urine samples were acidified immediately after collection by diluting one volume of urine with four volumes of 0.1 N H₂SO₄ and stored at - 20 °C urine samples were analyzed for total Nitrogen content [34] total purine derivatives (PD) excretion per day was calculated as the sum of allantoin and uric acid the daily absorbed exogenous purines estimated, and microbial N supply (MNS) predicted [39, 40], respectively. In the last day of each period, rumen fluid samples were collected at 0 and 4 hour post-feeding. Details of sampling procedures of rumen fluid for animal of volatile fatty acids (VFA) [38], protozoal population count [41], and estimated methane (CH₄) production [42] are presented in details in Wanapat et al. [43]. Blood samples (about 10 ml) were collected from the jugular vein at each rumen sampling time and kept into the tubes to which 0.1 g EDTA was added for analysis of blood urea-nitrogen (BUN) [44].

Data Management and Statistical Analysis

All data were subjected to ANOVA according to a 4 × 4 Latin square design using the General Linear Models (GLM) procedures [45] (24). The results were presented as mean values with the standard error of the means. Difference among means with $P < 0.05$ was accepted as statistical differences while $0.05 < P < 0.10$ was accepted as a tendency. Treatment means were statistically compared by Duncan's New Multiple Range Test [46] (25).

Abbreviations

ADF: acid detergent fiber; BUN: blood urea nitrogen; BW: body weight; C: concentrate; CP: crude protein; C₂: acetate; C₃: propionate; C₄: butyrate; CP: condensed tannins; DM: dry matter; EMNS: efficiency of microbial nitrogen supply; FMS: *Flemingia macrophylla* silage; NDF: neutral detergent fiber; NH₃-N: ammonia-nitrogen; N: nitrogen; MNS: microbial nitrogen supply; OM: organic matter; PD: purine derivatives; SP: saponins; VFA: volatile fatty acid

Declarations

Ethics approval and consent to participate

The experiment was officially agreed and approved by the Khon Kaen University Committee of Animal Care and Use for Research. The experimental cattle were provided by our research farm (TROFREC, KKU).

Consent for publication

Not applicable.

Availability of data and materials

All experimental data are responsible and available under the holding of the corresponding author.

Competing interests

The authors declare that they have no competing interests.

Funding

This research was supported by Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University. Thailand Research Fund and International Research Network (TRF-IRN) TRF-IRN57W0002. KKU Scholarship for ASEAN and GMS Countries' Personnel. The Funding donors did not have roles in the design of the study; research conduct, samples collection, analysis, and interpretation of data; and in writing the manuscript.

Ethics approval

The experiment was officially agreed and approved by the Khon Kaen University Committee of Animal Care and Use for Research. Record No. IACUC-KKU-94/61 and Reference No. 0201.2.11/73.

Author contributions

MW: BV designed the experiments. BV: Conducted the animal experiments. BV: performed the analyses. MW, BV: Wrote the manuscript. All authors reviewed and contributed to the manuscript. MW revised the final draft of manuscript. All authors read and approved the final manuscript.

Acknowledgments

The technical support rendered by Animal Nutrition Research Institute, Department of Livestock Development-Thapra, Dairy Production Organization of Thailand, Northeast (DPO-NE), are greatly acknowledged.

References

1. Wanapat M. The role of cassava hay as animal feed. DOA CIAT 504.
2. Binh DV, Tien NP, Mui NT. Study on biomass yield and quality of *Flemingia macrophylla* and on soil fertility. In Proc. Workshop Anim Nutr Sci. 1998;137.
3. Andersson MS, Lascano CE, Schultze-Kraft R, Peters M. Forage quality and tannin concentration and composition of a collection of the tropical shrub legume *Flemingia macrophylla*. J Sci Food Agric. 2006;86:1023–1031.
4. Tiemann TT, Lascano CE, Wettstein HR, Mayer AC, Kreuzer M, Hess HD. Effect of the tropical tannin-rich shrub legumes *Calliandra calothyrsus* and *Flemingia macrophylla* on methane emission and nitrogen and energy balance in growing lambs. Anim. 2008;2:790–799.
5. Kang S, Wanapat M, Phesatcha K, Norrapoke T, Foiklang S, Ampapon T, Phesatcha B. Using krabok (*Irvingia malayana*) seed oil and *Flemingia macrophylla* leaf meal as a rumen enhancer in an *in vitro* gas production system. Anim Prod Sci. 2017;57:327–333.
6. Williams CM, Eun JS, MacAdam JW, Young AJ, Fellner V, Min BR. Effects of forage legumes containing condensed tannins on methane and ammonia production in continuous cultures of mixed ruminal microorganisms. Anim Feed Sci Technol. 2011;166:364–372.
7. Fagundes GM, Modesto EC, Fonseca CEM, Lima HRP, Muir JP. Intake, digestibility and milk yield in goats fed *Flemingia macrophylla* with or without polyethylene glycol. Small Ruminant Res. 2014;116:88–93.
8. Phesatcha B, Wanapat M, Phesatcha K, Ampapon T, Kang S. Supplementation of *Flemingia macrophylla* and cassava foliage as a rumen enhancer on fermentation efficiency and estimated methane production in dairy steers. Trop Anim Health Prod. 2016;48:1449–1454.
9. Cheli F, Campagnoli A, Dell’Orto V. Fungal populations and mycotoxins in silages: From occurrence to analysis. Anim Feed Sci Technol. 2013;183:1–16.
10. Wanapat M, Phesatcha K, Viennasay B, Phesatcha B, Ampapon T, Kang S. Strategic supplementation of cassava top silage to enhance rumen fermentation and milk production in lactating dairy cows in the tropics. Trop Anim Health Prod. 2018;50:1539–1546.
11. Giang NTT, Wanapat M, Phesatcha K, Kang S. Level of *Leucaena leucocephala* silage feeding on intake, rumen fermentation, and nutrient digestibility in dairy steers. Trop Anim Health Prod. 2016;48:1057–1064.
12. Viennasay B, Wanapat M, Phesatcha K, Phesatcha B, Ampapon T. Replacement of rice straw with cassava-top silage on rumen ecology, fermentation and nutrient digestibilities in dairy steers. Anim Prod Sci. 2019;59:906–913.
13. Wanapat M, Polyorach S, Boonnop K, Mapato C, Cherdthong A. Effects of treating rice straw with urea or urea and calcium hydroxide upon intake, digestibility, rumen fermentation and milk yield of dairy cows. Livest Sci. 2009;125:238–243
14. Wanapat M, Sundstøl F, Garmo TH. A comparison of alkali treatment methods to improve the nutritive value of straw. I. Digestibility and metabolizability. Anim Feed Sci Technol. 1985;12:295–309.

15. Carpintero CM, Henderson AR, McDonald P. The effect of some pre-treatments on proteolysis during the ensiling of herbage. *Grass Forage Sci.* 1979;34:311–315.
16. Man NV, Wiktorsson H, Effect of molasses on nutritional quality of cassava and gliricidia tops silage. *Asian-Australas J Anim Sci.* 2002;15:1294–1299.
17. Ali M, Cone JW, Van Duinkerken G, Klop A, Blok MC, Bruinenberg M, Khan NA, Hendriks WH. Variation between individual cows in in situ rumen degradation characteristics of maize and grass silages. *NJAS-NJAS-Wagen J Life Sci.* 2016;78:167–173.
18. Staples CR, Fahey Jr GC, Rindsig RB, Berger LL. Evaluation of dairy waste fiber as a roughage source for ruminants. *J Dairy Sci.* 1981;64:662–671.
19. Wanapat M, Pilajun R, Polyorach S, Cherdthong A, Khejornsart P, Rowlinson P. Effect of carbohydrate source and cottonseed meal level in the concentrate on feed intake, nutrient digestibility, rumen fermentation and microbial protein synthesis in swamp buffaloes. *Asian-Australas J Anim Sci.* 2013;26:952.
20. Calabrò S, Cutrignelli MI, Piccolo G, Bovera F, Zicarelli F, Gazaneo MP, Infascelli F. *In vitro* fermentation kinetics of fresh and dried silage. *Anim Feed Sci Technol.* 2005;123:129–137.
21. Hess HD, Mera ML, Tiemann TT, Lascano CE, Kreuzer M. *In vitro* assessment of the suitability of replacing the low-tannin legume *Vigna unguiculata* with the tanniniferous legumes *Leucaena leucocephala*, *Flemingia macrophylla* or *Calliandra calothyrsus* in a tropical grass diet. *Anim Feed Sci Technol.* 2008;147:105–115.
22. Hassanat F, Benchaar C. Assessment of the effect of condensed (acacia and quebracho) and hydrolysable (chestnut and valonea) tannins on rumen fermentation and methane production *in vitro*. *J Sci Food Agric.* 2013;93:332–339.
23. Wanapat M, Pimpa O. Effect of ruminal NH₃-N levels on ruminal fermentation, purine derivatives, digestibility and rice straw intake in swamp buffaloes. *Asian-Australas J Anim Sci.* 1999;12: 904–907.
24. Makkar HP, Becker K, Abel HJ, Szegletti C. Degradation of condensed tannins by rumen microbes exposed to quebracho tannins (QT) in rumen simulation technique (RUSITEC) and effects of QT on fermentative processes in the RUSITEC. *J Sci Food Agric.* 1995;69:495–500.
25. Dschaak CM, Williams CM, Holt MS, Eun JS, Young AJ, Min BR. Effects of supplementing condensed tannin extract on intake, digestion, ruminal fermentation, and milk production of lactating dairy cows. *J Dairy Sci.* 2011;94:2508–2519.
26. Klita PT, Mathison GW, Fenton TW, Hardin RT. Effects of alfalfa root saponins on digestive function in sheep. *J Anim Sci.* 1996;74:1144–1156.
27. Pongchompu O, Wanapat M, Wachirapakorn C, Wanapat S, Cherdthong A. Manipulation of ruminal fermentation and methane production by dietary saponins and tannins from mangosteen peel and soapberry fruit. *Arch Anim Nutr.* 2009;63:389–400.
28. Patra AK, Saxena J. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *J Sci Food Agric.* 2011;91:24–37.

29. Giang NTT, Wanapat M, Phesatcha K, Kang S. Effect of inclusion of different levels of Leucaena silage on rumen microbial population and microbial protein synthesis in dairy steers fed on rice straw. *Asian-Australas J Anim Sci.* 2017;30:181.
30. Stern MD, Bach A, Calsamiglia S. Alternative techniques for measuring nutrient digestion in ruminants. *J Anim Sci.* 1997;75:2256–2276.
31. Kernick BL. The effect of form of nitrogen on the efficiency of protein synthesis by rumen bacteria in continuous culture.
32. Hess HD, Monsalve LM, Lascano CE, Carulla JE, Diaz TE, Kreuzer M. Supplementation of a tropical grass diet with forage legumes and *Sapindus saponaria* fruits: effects on *in vitro* ruminal nitrogen turnover and methanogenesis. *Austral J Agric Res.* 2003;54:703–713.
33. Puchala R, Min BR, Goetsch AL, Sahlu T. The effect of a condensed tannin-containing forage on methane emission by goats. *J Anim Sci.* 2005;83:182–186.
34. AOAC, Official Methods of Analysis, 19th Association of Official Analytical Chemists, Gaithersburg MD. 2012.
35. Van Soest PV, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci.* 1991;74:3583–3597.
36. Burns RE. Method for estimation of tannin in grain sorghum 1. *Agron J.* 1971;63:511–512.
37. Wanapat M, Pongchompu O. Method for estimation of tannin by vanillin-HCl method (A modified method of Burns, 1971). Department of Animal Science, Khon Kaen University, Khon Kaen, 4002. 2001.
38. Samuel M, Sagathevan S, Thomas J, Mathen G. An Hplc Method for Estimation Of volatile Fatty Acids in Ruminal Fluid. *Indian J Anim Sci.* 1997;67:805–807.
39. Chen XB, Gomes MJ. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives-An over of the technical details. International Feed Resources Unit, Rowett Reseach Institute, Bucksburn Aberdeen AB2 9SB, UK (1995).
40. Valadares RFD, Broderick GA, Valadares Filho SC, Clayton MK. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. *J Dairy Sci.* 1999;82:2686–2696.
41. Galyean M. Laboratory procedure in animal nutrition research. *Department of Animal and Life Science.* New Mexico State University, USA 188. 1989.
42. Moss AR, Jouany JP, Newbold J. Methane production by ruminants: its contribution to global warming. *Annal Zootech.* 2000;49:231–253.
43. Wanapat M, Gunun P, Anantasook N, Kang S. Changes of rumen pH, fermentation and microbial population as influenced by different ratios of roughage (rice straw) to concentrate in dairy steers. *J Agric Sci.* 2014;152:675–685.
44. Crocker CL, Rapid determination of urea nitrogen in serum or plasma without deproteinization. *Amer J Med Technol.* 1967;33:361.

45. SAS (Statistical Analysis System). User's Guide: Statistic, Version 9.4th SAS Inst. Inc., Cary, NC (2013).
46. Steel RG and Torrie JH. Principles and procedures of statistics, a biometrical approach (No. Ed. 2). McGraw-Hill Kogakusha, Ltd (1980).