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Influence of supplementing *Sesbania grandiflora* pod meal at two dietary crude protein levels on feed intake, fermentation characteristics, and methane mitigation in Thai purebred beef cattle

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Abstract

The aim of the study was to evaluate the effect of crude protein (CP) levels in concentrate and *Sesbania grandiflora* pod meal (SG) supplementations on feed intake, rumen fermentation, and methane (CH_4) mitigation in Thai purebred beef cattle. Four cattle with 100 ± 5.0 kg body weight were used in this study. A 2 × 2 factorial experiment in a 4 × 4 Latin square design were conducted, in which factor A was the CP contents in concentrate of 14, and 16% of dry matter (DM) and factor B was the supplement contents of SG at 0.4% and 0.6% DM intake, respectively. The results showed that the CP contents in concentrate and SG had no interaction effect on intake, digestibility, ruminal ecologies, ruminal fermentation products, and nitrogen utilization. Increasing CP contents in concentrate did not influence DM intake and nutrients' digestibility, and SG supplementation at 0.6% significantly ($P<0.05$) decreased CP digestibility. Increasing CP content to 16% increased significantly ($P<0.05$) the ruminal ammonia nitrogen (NH_3-N) concentration while decreased significantly ($P<0.05$) the NH_3-N concentration, protozoal number, and blood urea nitrogen (BUN) at 4 h post-feeding. The 0.6% supplementation of the SG increased significantly average total volatile fatty acids (VFAs) and propionate (C3) concentration while decreased significantly average acetate (C2), C2:C3 ratio, and CH_4 production, which was 2.71% for C2, 13.17% for C2:C3 ratio, and 4.37% for CH_4 production lower than 0.4% supplementation. Fecal nitrogen excretion was significantly decreased when supplemented with 0.6% of the SG. In conclusion, 0.6% of the SG supplementation showed a greater effect on intake, rumen manipulation, and CH_4 mitigation and would recommend supplementation to a concentrate-based diet containing either 14% or 16% CP content.

Keywords: *Sesbania grandiflora*, Tannins, Saponin, Methane, Fecal nitrogen, Ammonia, Propionate

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1. Introduction

The mission of methane (CH_4) is labeled as a greenhouse gas, which is one of the hot environmental problems [1]. Besides its influence on the environment, CH_4 is also responsible for the energy loss in ruminants from ingested feeds, which is up to 12 % of the gross energy intake [2]. Thus, mitigating CH_4 emission is not only a benefit to the environment but also enhances the energy utilization in ruminants. Various approaches including dietary manipulation, antibiotics, and plant secondary compounds (PSCs) for CH_4 mitigation have been tested, and using PSCs such as saponin and tannin shows the most effective approach recently [3-5] for CH_4 mitigation. Saponin affects CH_4 mitigation by lowering the protozoa population and change production patterns of volatile fatty acids (VFAs), and enhances, in addition, fiber degradation [6]. Tannins indirectly and directly affect methanogenesis resulting in lower CH_4 production. In addition, tannins can protect protein metabolism in the rumen by tannins-protein binding [7], which may enhance protein metabolism in the small intestine. However, the effect of saponin and tannins varies depend on sources, molecular weight, temperature, soil quality, nutrient stress, and topography [8,9]. *Sesbania grandiflora* (*S. grandiflora*) is a tree that is classed in *Fabaceae* family and *Sesbania* genus. The *S. grandiflora* pods contain 35% of crude protein (CP) and PSCs including tannin, flavonoids, steroids, and triterpenes [10,11]. Jayanegara et al. [9] reported that *Sesbania sesban* (*S. sesban*) leaves showed significantly mitigating *in vitro* CH_4 production when *S. sesban* leaves were used as a sole substrate and added to concentrate-based diets. However, the effect of *S. grandiflora* pod meal (SG) containing saponin and tannins with different dietary CP levels has not yet been evaluated. Regarding the saponin and tannins content, this could hypothesize that SG could greatly mitigate CH_4 emission and improve protein utilization via tannins-protein binding.

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Therefore, the aim of the study was to evaluate the effect of CP contents in concentrate and SG supplementation containing saponin and tannins on feed intake, rumen fermentation, and CH_4 mitigation in Thai purebred beef cattle.

2. Materials and methods

Approval no. ACUC-KKU 10/62 was issued by the Animal Ethics Committees of Khon Kaen University.

2.1. *S. grandiflora* pods meal

The fresh *S. grandiflora* pods were collected from Udon-Thani and Khon Kaen provinces (Thailand) from September to November 2020. The pods were sun-dried for 2 to 3 weeks and ground through a 1-mm sieve (Cyclotech Mill, Tecator, Höganäs, Sweden). The SG sample was analyzed for saponin and tannins content. The analysis procedure was modified from Kwon et al. [12] and Edeoga et al. [13]. In brief, 5 g SG were put into Erlenmeyer flask and added with 80% methanol, then the flask was evaporated using a microwave for 30 min and transferred through Whatman No. 41 into a new flask. The same process was repeated four times. After filtered, the sample was evaporated using a rotary vacuum evaporator to obtain the final volume of approximately 25 ml. The obtained solution was separated with 99.9% ether using a separatory funnel, then the residue at the bottom-funnel was reseparated with Butanol-N and washed two times using 5% NaCl. After washed, the residue was heated at 80°C for 30 min in a water-bath. The crude saponin content of SG was obtained after the residue was oven-dried overnight at 60°C. The tannins content was analyzed by modifying the method of Burns [14]. Chemical composition of SG was analyzed according to AOAC [15] including dry matter (DM, ID 967.03), organic

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matter (OM, ID 942.05), CP (ID 984.13), ether extract (EE, ID 920.39), and acid detergent fiber (ADF) and Van Soest et al. [16] for neutral detergent fiber (NDF). The crude saponin and tannins content and chemical composition of the SG were shown in Table 1.

2.2. Cattle, design, and feeding

Four Thai purebred beef cattle of 100 ± 5.0 kg initial body weight were used in this study. A 2 × 2 factorial experiment in a 4 × 4 Latin square design were conducted, in which factor A was the CP contents in concentrate of 14, and 16% of DM and factor B was the supplement contents of SG at 0.4% and 0.6% dry matter intake, respectively. Cattle were placed in an individual pen with free access to mineral block and clean water and fed dietary treatments at 7:00 am and 4:00 pm. Four periods, 14-days were used as treatment adaptation and 7-days were used for sample collection and digestibility study. The concentrate was fed at 1.0% BW, and rice straw (RS) was used as an exclusive roughage source and provided *ad libitum*. The chemical composition of concentrate and RS was provided in Table 1.

2.3. Sample collection and sampling procedures

The offered feeds including concentrate and RS and they are remaining were daily recorded during the experiment. The cattle were transferred to metabolism crates and stayed there for 7-days. The samples of concentrate and RS and their remaining portions were collected and separated (two parts); the first part of samples was analyzed for DM content and the remaining part of samples was pooled and frozen by cattle and period for chemical composition analysis. Fecal and urine samples were collected for 7-days using the total collection method to study digestibility and nitrogen balance. 5% feces of total fresh weight were withdrawn and separated (two parts); the

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first part of fecal samples was analyzed for DM content and the remaining part of fecal samples was pooled and frozen by cattle and period for chemical composition analysis. The frozen samples including feeds (concentrate and RS), refusals (concentrate and RS), and feces were thawed, oven-dried at 60°C, and ground through a 1-mm sieve for chemical composition analysis including DM, ash, CP, and ADF following the AOAC [15] method and NDF according to Van Soest et al. [16]. The frozen urine samples were thawed and analyzed for urinary nitrogen using the Kjeldahl method according to AOAC [15].

On day 21st of each period, 10 ml of blood samples at jugular vein position were collected from each cattle at 0 and 4 h after feeds were offered. The samples of blood were stored in EDTA as an anticoagulant, and plasma was obtained using a centrifuge (500 × g rpm, 10 min, 4°C), then was analyzed for blood urea nitrogen (BUN) [17]. The fluid (100 ml) from the rumen was withdrawn from each cattle via a stomach tube attached to a vacuum pump. A portable pH (HANNA, HI 8424, Singapore) was used for the fluid-pH measurement. The fluid samples were gone through cheesecloth (4-layers) and separated (two parts); the first part (45-ml fluid) was mixed with H_2SO_4 (5-ml) at the ratio of 1:9. The clear samples solution were obtained via centrifuge (16,000 × g, 15-min), then were analyzed for NH_3-N (Kjeltech Auto 1030 analyzer, Sweden) and volatile fatty acids (VFA) proportions (acetate-C2, propionate-C3, butyrate-C4) using high-performance liquid chromatography [18]. The VFA proportions concentration were calculated for the CH_4 concentration. The stoichiometrical model used for estimating CH_4 from VFA composition was as following Moss et al. [19]. Even though the determination of CH_4 production is usually counted by using a respiratory chamber or by using the gas chromatography technique, unfortunately very costly, and such facilities may not be available especially in developing countries. Thus, the calculation of CH_4 production from VFA profiles is expected to be a solution to the problem. The

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remaining part was mixed in 10% formalin for protozoal population study using a direct count technique under microscopic (Boeco, Hamburg, Germany).

2.4. Calculations and statistical analysis

All data were analyzed according to a 2 × 2 factorial in a 4 × 4 Latin square design using a Generalized Linear Model (GLM) procedure. The model is as follows:

$Y_{ijk} = \mu + M_i + A_j + P_k + \varepsilon_{ijk}$

where Y_{ijk} is the observation, μ is the overall mean, M_i is the treatments' effect, A_j is the effect of the animal, P_k is the effect of the period, and ε_{ijk} is the residual effect. Differences between treatment means were tested by Duncan's new multiple range test [20] and $p<0.05$ was considered the statistical difference.

3. Results

3.1. Chemical composition of diets

The concentrate was formulated to contain 14.10 and 16.06% DM intake to test the effect of SG containing saponin and tannins on nitrogen utilization efficiency. SG composes of 162 g/kg DM of saponin and 108.7 g/kg DM of tannins.

3.2. Feed intakes and digestibility coefficients

The influence of CP contents in concentrate and SG supplementation on feed intake and apparent digestibility was presented in Table 2. The CP contents in concentrate and supplementation of SG had no interaction effects on DM intake and nutrients' digestibility. Increasing CP contents in concentrate did not influence DM intake and nutrients' digestibility; in contrast, SG

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supplementation significantly (P<0.05) affect the CP digestibility, although others did not differ. Increasing SG supplementation to 0.6% significantly decreased CP digestibility, which was 5.7% lower than 0.4% supplementation.

3.3. pH, ammonia nitrogen, protozoa, and blood urea-nitrogen

The influence of CP contents in concentrate and SG supplementation on ruminal pH, NH_3-N, protozoal count, and BUN was shown in Table 3. The CP contents in concentrate and SG supplementation had no interaction effects on pH, NH_3-N, protozoal number, and BUN. Increasing CP content in concentrate to 16% increased significantly (P<0.05) for the ruminal NH_3-N concentration at 4 h post-feeding, which was 5.05% higher than 14% CP in concentrate. The average concentration of ruminal NH_3-N was 19.06 mg/dl for 14% CP and 19.88 mg/dl for 16% CP in concentrate (Table 3). Increasing SG supplementation to 0.6% decreased significantly (P<0.05) for ruminal NH_3-N concentration, protozoal number, and BUN at 4 h post-feeding, which decreased by 12% for NH_3-N, 45% for protozoal number, and 9% for BUN.

3.4. Ruminal volatile fatty acids and methane estimation

The influence of CP contents in concentrate and SG supplementation on total VFAs, C2, C3, C4, C2:C3 ratio, and CH_4 estimation was shown in Table 4. The CP contents in concentrate and SG supplementation had no interaction effects on ruminal total VFAs and their portions and CH_4 production. The CP contents in concentrate did not affect the ruminal fermentation products and CH_4 estimation. Increasing SG supplementation affected significantly total VFAs, C2, C3, C2:C3 ratio, and CH_4 production, although C4 was not affected. A 0.6% supplementation of SG increased significantly average total VFAs and C3 concentration, which was 0.84% for total VFAs and

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7.13% for C3 higher than 0.4% supplementation. In contrast, 0.6% supplementation of SG decreased significantly average C2, C2:C3 ratio, and CH_4 production, which was 2.71% for C2, 13.17% for C2:C3 ratio, and 4.37% for CH_4 production lower than 0.4% supplementation.

3.5. Nitrogen utilization

The influence of CP contents in concentrate and SG supplementation on nitrogen (N) intake, N excretion, N absorption, and N retention was shown in Table 5. The CP contents in concentrate and SG supplementations had no interaction effects on N utilization. Increasing CP content in concentrate did not influence N utilization (Table 5). SG supplementations influenced significantly total N excretion and fecal N excretion; others did not differ. Increasing SG supplementation decreased significantly for total N excretion and fecal N excretion, which decreased by 13% for total N excretion and 14% for fecal N excretion when supplemented at 0.6% of the SG.

4. Discussion

4.1. Feed intakes and digestibility coefficients

The CP contents in concentrate and SG supplementations had no interaction effects on feed intake and digestibility of nutrients. This result agreed with the previous report [21-24]. The CP contents in concentrate did not affect total DM intake and nutrients' digestibility, this was similar to the finding of Norrapoke et al. [23]. In addition, Norrapoke et al. [23] found that mangosteen peel pellets did not affect the nutrients' digestibility. In this study, SG supplementations significantly decreased the CP digestibility. This could be due to the tannins content in SG that could prevent ingested protein metabolism in the rumen via tannins-protein complexes formation [7]. Cherdthong et al. [4] found that increased *Delonix regia* seed meal levels decreased CP

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digestibility, but it failed to meet the statistical difference. This discrepancy for CP digestibility could relate to many factors including supplement sources, sources form, dose study, the composition of diet [25,26]. SG supplementations did not affect DM intake and digestibility of DM, OM, NDF, and ADF. This was similar to the finding of Cherdthong et al. [4] who, found a non-significant difference for DM intake and digestibility of DM, OM, and fibrous portions but supplemented *Delonix regia* seed meal (50, 100, and 150 g DM) showed lower digestibility of DM, OM, NDF, and ADF compared to the control treatment. In this study, supplemented 0.6% of the SG showed higher digestibility of DM, OM, NDF, and ADF compared to the 0.4% of the SG supplementation. The improvement of fiber degradation could be due to the synergistic effect of saponin-tannins binding in the rumen which, is beneficial to fiber degrading microbes. Gutierrez et al. [27] stated that saponin-tannins binding could relieve saponin suppression of bacterial activity and ruminal degradation of saponin. Rira et al. [6] suggested that in addition to CH_4 mitigation, saponin may enhance fiber degrading bacteria and fungi in the rumen. Saponin may act as a substrate for tannins chelator [28]. Consuming chemical chelators like tannins and other toxic compounds like saponin could reduce the toxicity produced by tannins [29], thus fiber degradation in the rumen was enhanced.

4.2. pH, ammonia nitrogen, protozoa, and blood urea-nitrogen

The CP contents in concentrate and SG supplementations did not show any interaction effects on pH, NH_3-N, protozoal number, and BUN (Table 3). This was in agreement with Norrapoke et al. [23] who found a non-significant interaction effect between protein levels and mangosteen peel pellets on pH, NH_3-N, protozoal number, and BUN. The ruminal pH was slightly dropped in hour 4 post-feeding. Similarly, Ampapon and Wanapat [24] revealed that protein levels and rambutan

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peel powder had no interaction effects on *in vitro* ruminal pH, NH_3-N, and protozoal number. Increasing CP contents in concentrate increased significantly the ruminal NH_3-N concentration. The NH_3-N concentration was 19.06 mg/dl for 14% CP and 19.88 mg/dl for 16% CP in concentrate. The greater NH_3-N concentration with 16% CP in concentrate suggested that more protein come into the rumen that allowed microbes to convert it into NH_3-N when compared with 14% CP in concentrate. Similarly, Ampapon and Wanapat [24] found that increasing CP levels (14%, 16%, and 18%) in concentrate affected the *in vitro* ruminal NH_3-N concentration. Norrapoke et al. [23] showed that protein levels (16 *vs* 19%) did not affect the ruminal NH_3-N concentration of lactating dairy cows but affected significantly the BUN concentration. SG supplementations significantly decreased NH_3-N, BUN, and protozoal numbers. The decrease of NH_3-N concentration when supplemented at 0.6% of the SG could be due to the protected protein metabolism in the rumen by tannins via tannins-protein complexes formation while the lower BUN concentration caused by the decrease of NH_3-N concentration. A similar finding had been reported by Bhatta et al. [29] who found a significant decrease of *in vitro* NH_3-N concentration when increased levels of trees (*Autocarpus integrifolis*, *Azardirachta indica*, and *Ficus bengalensis*) containing PSCs. Also, Holtshausen et al. [30] revealed that feeding saponin-containing *Yucca schidigera* and *Quillaja Saponaria* decreased quadratically on *in vitro* NH_3-N concentration compared to the control. SG supplementations decreased significantly the protozoal number at 4 h post-feeding. This result was in agreement with the previous studies, Norrapoke et al. [23] used mangosteen peel pellets in lactating dairy cows, Ampapon and Wanapat [24] used rambutan peel powder in the *in vitro* study, and Cherdthong et al. [4] used *Delonix regia* seed meal (*Delonix regia*) in Thai native beef cattle. The reduction of protozoal number could be due to the presence of saponin and tannins in the SG. Saponin has been reported to be toxic to protozoa [31].

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Mechanism of saponin on protozoa has been proposed by Makkar et al. [32] that saponin may form complexes with a lipid membrane, which increased permeability, caused an imbalance, and consequently occur cell lysis, and Wallace et al. [33] proposed that saponin formed complexes with sterols on the surface of the protozoal membrane, which caused impairing and disintegrating. Tannins can directly affect methanogen bacteria, but not for protozoa [34]. It may indirectly affect protozoal number [35], and a possible mechanism has been proposed by Tavendale et al. [36], tannins may bind to proteinaceous adhesin or part of the cell envelope of methanogenic archaea, which impaired the methanogen-protozoa complex formation and decreased interspecies hydrogen transfer and inhibited methanogen growth. Methanogenic archaea and protozoa had a symbiotic association [35], thus tannins indirectly affected the protozoal number.

4.3. Ruminal volatile fatty acid profiles and methane estimation

The interaction effect between CP contents in concentrate and SG supplementations was not found for total VFAs, C2, C3, C4, C2:C3 ratio, and CH_4 estimation. The result was in agreement with Aguerre et al. [21], Norrapoke et al. [23], and Ampapon, Wanapat et al. [24]. The CP contents in concentrate and SG supplementations independently affected the ruminal fermentation and the CH_4 estimation. An increase of the SG supplementations significantly increased the total VFAs and C3 concentration while decreased significantly the C2:C3 ratio and CH_4 estimation. The increase of the total VFAs concentration when SG supplementation was increased could be due to the greater DM intake and digestibility of the DM, OM, NDF, and ADF as benefit from saponin-tannins binding resulting in less toxicity on ruminal microbe activity. Also, total VFAs production is closely related to the production of the VFA proportions including C2, C3, and C4. A reduction of the total VFAs occurred with the change of the VFAs proportion such as, increase C2 and

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decrease C3 [37,38]. In this study, the increase in the SG supplementations significantly decreased C2 concentration. Similarly, Beauchemin et al. [39] found a decrease of the C2 when fed quebracho containing tannins, and Castro-Montoya et al. [40] fed mimosa, sumach, and chestnut resulting decrease of the C2 concentration. Saponin effects on VFAs products vary depending on the studied dose. An increase the SG supplementation significantly increased C3 concentration. This could be related to the decrease of the protozoal number and the CH_4 production providing more available hydrogen. Similar results have been reported [31,40]. Saponin may inhibit acetate producer and protozoa and may favor propionate producing bacteria resulting in greater C3 concentration in the rumen [33]. The CH_4 emission was mitigated by the SG supplementations. A reduction of the CH_4 production by saponin and tannins had been reported widely [3,4,8,23]. Saponin has been revealed to mitigate CH_4 production and change ruminal fermentation [8]. The reduction of CH_4 production could link to the reduction of the protozoal number and C2:C3 ratio. Reduction of the C2:C3 ratio provided more hydrogen for the C3 production resulting in less hydrogen for the methanogenesis and subsequently reduced CH_4 production. Protozoa can provide hydrogen for the methanogen bacteria and act as the host for methanogen [25], thus lowering protozoal number with a reduction of CH_4 production. Tannins can directly affect methanogenesis by suppressing ruminal archaea, but not removing protozoa [34]. As methanogens attached to protozoa, tannin's effect on protozoa could decrease CH_4 production because protozoa can synergistically offer hydrogen for the methanogenesis process [25].

4.4. Nitrogen utilization

Interaction between CP contents in concentrate and SG supplementations did not affect intake, excretion, absorption, and retention of the N. The CP contents in concentrate did not affect the

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intake, excretion, absorption, and retention of the N (Table 5). Increasing SG supplementation significantly decreased total N excretion and N excretion in feces. However, SG supplementation did not affect either N absorption or retention. A decrease of fecal N excretion when increased SG supplementation suggested that post-ruminal protein metabolism was enhanced by SG-containing tannins and saponin. The lower fecal N excretion could also be due to the lower NH_3-N concentration in the rumen (Table 3) when increased SG supplementation. Tannins have been widely reported for their effect on slowing down the degradation rate of protein in the rumen [31] via tannins-protein complexes formation at the ruminal pH and subsequently reduced NH_3-N concentration in the rumen. The tannins-protein complexes disassociation at the abomasum post escaping ruminal fermentation affected the N excretion [31]. Lowering fecal N excretion when increased SG supplementation may suggest that tannins-protein complexes were well-disassociated at the abomasum and subsequently absorbed in the small intestines resulting in low N excretion. However, some studies have been reported for higher fecal N excretion when fed diets containing tannins [34,41]. The higher fecal N excretion could explain by that tannins-protein complexes were not completely disassociated at the abomasum and subsequent digestive tracts resulting in higher fecal N excretion [31]. The ability of tannins binding to protein varied depend on its sources and chemical properties such as molecular weight, and the post-ruminal disassociation process between tannins and protein [42,43], thus, would be different that may cause the contrast finding in term of fecal N excretion compared to the previous studies.

5. Conclusions

From this study, the CP contents in concentrate and SG containing saponin and tannins supplementation did not have an interaction effect on feed utilization, ruminal ecology, ruminal

fermentation products, and N utilization. Increasing CP contents in concentrate did not affect intake, digestibility, ruminal ecologies, ruminal fermentation products except C3 and NH_3-N concentration, and N utilization. The 0.6% of SG supplementation enhanced intake, digestibility of nutrients (except CP), ruminal fermentation products mainly total VFAs and C3 concentration while reduced NH_3-N, protozoal number, C2:C3 ratio, and CH_4 production and increased fecal N excretion when compared to the 0.4% supplementation of SG. The authors would recommend supplementing SG at 0.6% to the concentrate-based diet containing either 14% or 16% CP.

Authors' contribution: Planning and design of the study, N.U., A.C.; Conducting and sampling, N.U., S.S.; Samples analysis, N.U.; Statistical analysis, N.U.; Manuscript drafting, N.U.; Manuscript editing and finalizing, N.U., S.S., A.C.

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Table 1 Feed ingredients and chemical composition of concentrate diet containing various crude protein (CP), rice straw and *S. grandiflora* pods meals (SG).

Items	Concentrate		Rice straw	SG
	14% CP	16% CP		
Ingredient, % Dry matter				
Cassava chip	53.0	52.5	-	-
Soybean meal	12.5	16.5	-	-
Rice bran	15.0	12.0	-	-
Palm kernel meal	14.0	13.5	-	-
Urea	1.6	1.6	-	-
Premix*	1.0	1.0	-	-
Molasses	1.4	1.4	-	-
Sulfur	0.5	0.5	-	-
Salt	1.0	1.0	-	-
Chemical composition, %				
Dry matter, %	91.06	91.43	92.20	94.19
Organic matter, %DM	84.97	86.10	90.61	93.86
Crude protein, %DM	14.10	16.06	3.27	22.48
Ether extract, %DM	2.05	2.17	1.46	4.42
Neutral detergent fiber, %DM	30.87	28.76	71.74	56.67
Acid detergent fiber, %DM	13.57	12.68	47.73	43.12
Gross energy (GE), MJ/kg DM	4.06	4.14	3.83	-
Condensed tannin, (g /kg DM)	-	-	-	108.7

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Saponins, g /kg DM	-	-	-	162.0

* Minerals and vitamins (each kg contains): Vitamin A: 10,000,000 IU; Vitamin E: 70,000 IU; Vitamin D: 1,600,000 IU; Fe: 50 g; Zn: 40 g; Mn: 40 g; Co: 0.1 g; Cu: 10 g; Se: 0.1 g; I: 0.5 g.

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Table 2 Effects of crude protein (CP) levels (14 *vs* 16%) in concentrate with *S. graniflora* pods meal (SG, 0.4% *vs* 0.6% dry matter intake) on feed intake and digestibility in Thai native beef cattle.

Item	14% CP		16% CP		SEM	P-value		
	0.4% SG	0.6% SG	0.4% SG	0.6% SG		CP	SG	CP*SG
Dry matter intake								
Roughage intake, kg/d	2.29	2.32	2.31	2.33	0.18	0.949	0.992	0.820
%BW*	1.71	1.71	1.75	1.70	0.09	0.886	0.721	0.721
g/kg $BW^{0.75}$	54.88	57.06	54.68	54.53	3.31	0.571	0.673	0.629
Concentrate intake, kg/d	1.58	1.58	1.58	1.58	0.06	0.440	0.640	0.917
%BW	1.03	1.03	1.04	1.05	0.009	0.479	1.000	0.721
g/kg $BW^{0.75}$	36.63	35.89	36.21	36.38	1.56	0.976	0.800	0.687
Total intake kg/d	3.97	4.01	3.99	4.02	0.29	0.971	0.927	0.860
Total intake, %BW	2.74	2.75	2.79	2.74	0.57	0.530	0.600	0.539
g/kg $BW^{0.75}$	94.91	96.82	94.29	94.85	4.40	0.685	0.698	0.831
Nutrient digestibility, %								

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Dry matter	65.82	65.52	67.56	69.27	4.40	0.395	0.825	0.752
Organic matter	69.32	69.15	70.84	71.60	2.48	0.281	0.866	0.796
Crude protein	70.01	67.82	73.16	67.62	1.90	0.296	0.014	0.239
Neutral detergent fiber	68.46	69.48	68.52	67.39	2.01	0.490	0.969	0.468
Acid detergent fiber	55.44	59.20	55.40	56.50	3.44	0.585	0.338	0.595

*CP = Crude protein, CP*SG= interaction between CP and *S. graniflora* pods meal, BW = body weight, SEM= standard error of the mean.

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Table 3 Effects of crude protein (CP) levels (14% *vs* 16%) in concentrate with *S. graniflora* pod meal (SG, 0.4% *vs* 0.6% dry matter intake) on ruminal pH, ammonia nitrogen, ruminal protozoal population, and blood urea-nitrogen concentration in Thai native beef cattle.

Item	14% CP		16% CP		SEM	P-Value		
	0.4% SG	0.6% SG	0.4% SG	0.6% SG		CP	SG	CP*SG
pH								
0 h post-feeding	6.76	6.80	6.77	6.81	0.16	0.933	0.738	0.983
4 h post-feeding	6.59	6.71	6.62	6.70	0.09	0.850	0.148	0.762
Mean	6.67	6.76	6.70	6.76	0.13	0.860	0.582	0.910
Ammonia nitrogen, mg/dl								
0 h post-feeding	15.96	16.32	16.26	16.95	0.56	0.266	0.208	0.687
4 h post-feeding	23.43	20.54	24.42	21.89	0.69	0.013	<0.001	0.394
Mean	19.70	18.43	20.34	19.42	0.42	0.008	0.003	0.334
Protozoa, $\times 10^5$ cell/mL								
0 h post-feeding	7.01	6.70	7.25	7.02	0.47	0.288	0.309	0.866
4 h post-feeding	10.69	7.15	10.60	7.49	0.62	0.783	<0.001	0.637

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Mean	9.13	6.49	9.31	6.96	0.27	0.251	<0.001	0.905
Blood urea-nitrogen concentration, mg/dl								
0 h post-feeding	10.28	10.39	10.76	11.05	0.40	0.087	0.492	0.760
4 h post-feeding	12.10	11.33	12.44	11.15	0.55	0.838	0.021	0.522
Mean	11.19	10.86	11.60	11.10	0.32	0.069	0.224	0.713

*CP = Crude protein, CP*SG = interaction between crude protein and *S. graniflora* pod meal, BW = body weight, SEM= standard error of the mean.

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Table 4 Effects of crude protein (CP) levels (14% *vs* 16%) in concentrate with *S. graniflora* pod meal (SG, 0.4% *vs* 0.6% dry matter intake) on volatile fatty acid profile, and methane estimation in Thai native beef cattle.

Item	14% CP		16% CP		SEM	P-Value		
	0.4% SG	0.6% SG	0.4% SG	0.6% SG		CP	SG	CP*SG
Total volatile fatty acid, mmol/l								
0 h post-feeding	100.60	101.34	100.70	101.32	0.54	0.913	0.105	0.873
4 h post-feeding	105.66	106.63	106.66	107.87	0.68	0.278	0.288	0.902
Mean	103.13	103.98	103.68	104.59	0.69	0.456	0.013	0.830
Volatile fatty acid, profiles, %								
Acetic acid								
0 h post-feeding	65.14	63.92	65.10	63.68	0.55	0.779	0.061	0.384
4 h post-feeding	67.43	65.40	67.08	64.76	0.51	0.065	<0.001	0.961
Mean	66.28	64.66	66.09	64.22	0.41	0.307	<0.001	0.224
Propionic acid								
0 h post-feeding	21.40	22.31	21.08	22.63	0.60	0.393	0.182	0.659
4 h post-feeding	22.61	24.42	23.01	25.50	0.84	0.420	<0.001	0.853

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Mean	22.00	23.37	22.05	24.06	0.55	0.890	<0.001	0.975
Butyric acid								
0 h post-feeding	13.45	13.76	13.80	13.68	0.53	0.504	0.567	0.671
4 h post-feeding	10.22	10.17	9.90	9.73	0.62	0.574	0.595	0.893
Mean	11.84	11.96	11.85	11.70	0.43	0.329	0.311	0.522
Acetic acid to propionic acid	3.01	2.71	3.09	2.68	0.06	0.760	<0.001	0.419
Methane estimation, mM/l								
0 h post-feeding	28.81	28.13	29.02	27.91	0.55	0.984	0.413	0.588
4 h post-feeding	27.77	26.62	27.60	25.80	0.43	0.209	<0.001	0.208
Mean	28.29	27.38	28.31	26.85	0.35	0.409	<0.001	0.223

*CP = Crude protein, CP*SG = interaction between crude protein and *S. graniflora* pod meal, BW = body weight, SEM= standard error of the mean.

CH_4 estimation= (0.45 ×acetic acid) − (0.275 × propionic acid) + (0.40 × butyric acid) [19].

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Table 5 Effects of crude protein (CP) levels (14% *vs* 16%) in concentrate with *S. graniflora* pod meal (SG, 0.4% *vs* 0.6% dry matter intake) on nitrogen (N) balance in Thai native beef cattle.

Item	14% CP		16% CP		SEM	P-Value		
	0.4% SG	0.6% SG	0.4% SG	0.6% SG		CP	SG	CP*SG
N intake, g/d	36.41	36.16	36.45	36.02	2.01	0.971	0.817	0.949
N excretion, g/d	16.03	13.78	15.95	14.39	1.02	0.716	0.021	0.641
Fecal N excretion, g/d	13.36	11.42	13.09	11.77	0.82	0.723	0.036	0.929
Urinary N excretion, g/d	2.67	2.36	2.86	2.62	0.30	0.474	0.076	0.997
N absorption, g/d	23.05	24.75	23.36	24.25	1.19	0.945	0.357	0.771
N retention, g/d	20.38	22.39	20.50	21.63	2.09	0.867	0.271	0.792

* CP = Crude protein, CP*SG = interaction between crude protein and *S. graniflora* pod meal, SEM, standard error of the mean.