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Effect of *Delonix regia* seed meal supplementation in Thai native beef cattle on feed intake, rumen fermentation characteristics and methane production

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ABSTRACT

The aim of this research was to investigate the effect of supplementing *Delonix regia* (DR) seed meal on feed intake, digestibility, rumen fermentation, nitrogen balance and CH_4 production in Thai native beef cattle fed on rice straw. Four Thai native beef cattle with the initial body weight (BW) of 100 ± 5.0 kg were randomly assigned according to a 4 × 4 Latin square design to receive DR seed meal supplementation at 0, 90, 180 and 270 g/d. The present results revealed that the total intake (g/kg $BW^{0.75}$) was significantly increased with the inclusion of dry matter (DM) seed meal at 270 g ($P < 0.05$). DM and OM digestibility were decreased when increasing DR seed meal levels ($P < 0.05$). Ruminal NH_3-N concentration increased in beef cattle receiving DR seed meal. Supplementation of DR seed meal did not alter fungal zoospores' concentration ($P > 0.05$), whereas the protozoal population was at 0, 4 h post feeding, and the mean values reduced when increasing the levels of DR seed meal supplemented ($P < 0.05$). The concentration of propionic acid at 4 h post feeding and its average concentration were significantly highest when 270 g DR seed meal was supplemented ($P < 0.05$). Estimation of CH_4 concentrations and CH_4 per dry matter intake were found reduced when increasing its DR seed meal concentration. In addition, N absorption, N retention and proportion of N retention to N intake were enhanced when 270 g DR seed meal was supplemented ($P < 0.05$). Thus, the inclusion of DR seed meal at 270 g/d resulted in improving total feed intake, rumen fermentation and N balance whereas there was reduced DM digestibility, protozoal population and CH_4 production in beef cattle fed rice straw base.

1. Introduction

Beef cattle are economically important domestic animals and have a long tradition in Thai agriculture. However, beef production

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in Thailand is often suboptimum, characterized by slow growth performance and low reproductive efficiency (Cherdthong et al., 2014). This could be due to insufficient quantities of energy and protein during the dry season, especially when the cattle feed on rice straw. Thus, the new feed resources that are rich in nutrients and available in each area may benefit from further research.

Livestock contributes to about 18% to the global anthropogenic greenhouse gas (GHG) emissions, accounting for about 37% of the total anthropogenic methane (CH_4) and 65% of global anthropogenic nitrous oxide. CH_4 is produced normally during the fermentation of feed by methanogenic bacteria (Hristov et al., 2013). The removal of ruminal protozoa can also reduce CH_4 production as some population of methanogens remains attached to protozoa (Cieślak et al., 2016). Tannin and saponin-containing tropical plants have been demonstrated to decrease CH_4 production both *in vitro* and *in vivo* experiments. The inhibitory effect of these compounds on rumen methanogenesis have been ascribed due to their direct effect on methanogenesis archaea, protozoal associated CH_4 production and indirectly through a depression of fiber digestion in the rumen. Anantasook et al. (2016) demonstrated that supplementing *Terminalia chebula* Retz. containing tannins and saponins could improve rumen fermentation by reducing CH_4 production and protozoa populations' *in vitro* gas technique. They also investigated the inhibitory effects of tannins and saponins from *Samanea saman* could depress CH_4 production, methanogens and protozoal populations in rice straw-fed dairy cows (Anantasook et al., 2015). Similarly, Gunun et al. (2016) showed that the concentration of CH_4 was reduced at 8% when goats supplemented with condensed tannins in Mao (*Antidesma thwaitesianum* Muell. Arg.) seed meal. Thus, directly inhibitory to methanogens, tannins and saponins have been shown to lower protozoal numbers, which may also decrease protozoal-associated methanogenesis.

Delonix regia (DR) is one kind of the plants containing secondary compound. It is abundant during the dry season and contains a large amount of underutilized condensed tannins and saponins. DR seeds contains 20.50% and secondary compounds of condensed tannins and saponins at 90–95 mg/100 g and 10–15%, respectively (Alemede et al., 2010; Kaga, 2013). The incorporation of DR seed into animal diets potentially may enable manipulation of rumen ecosystems. DR seed meal has been used as a protein source in animal diets and could be enhancing carcass weight in tilapia (Bake et al., 2014), rabbits (Kaga, 2013), broilers (Egena et al., 2008) and Savanna Brown does (Alemede et al., 2010). Recently, Supapong et al. (2017) demonstrated that supplementation of DR seed meal at 11.7 mg resulted in improved *in vitro* kinetics gas and DM digestibility while reducing CH_4. However, utilization of condensed tannins and saponins in DR seed meal for beef cattle has not been studied. The aim of this research was to investigate the effect of supplementation of DR seed meal on feed intake, digestibility, rumen fermentation, nitrogen balance and CH_4 production in Thai native beef cattle fed on rice straw.

2. Materials and methods

Animals involved in this study were approved by the Animal Ethics Committee of Khon Kaen University (record no. ACUC-KKU 34/2559), based on the Ethic of Animal Experimentation of National Research Council of Thailand.

2.1. Dietary preparation

Rice straw and concentrate diet were obtained from the Ruminant Metabolism Center, Tropical Feed Resources Research and Development Center (TROFREC). DR seed pods were collected from the Khon Kaen province in Thailand from August to October 2015. The pods were sun-dried for 2–3 weeks, and then the pods were easily opened for seed collection. DR seed pods were dried at 60 °C for 48 h, ground to pass through a 1-mm sieve (Cyclotech Mill, Tecator, Hoganas, Sweden) and used for experimental test.

2.2. Animals, experimental design and feeding

Four, Thai native beef cattle with initial body weight (BW) of 100 ± 5.0 kg were randomly assigned according to a 4 × 4 Latin square design to receive DR seed meal supplementation at 0, 90, 180 and 270 g/d. A concentrate mixture (Table 1) was fed to animals at 0.5% BW daily. DR seed meal and concentrate were offered in two equal meals per day at 0700 and 1600. Cattle were fed rice straw ad libitum basis and clean fresh water all times. All animals were kept in individual pens. The experiment was conducted for 4 periods with 21 days per each. The first 14 days were for adaptation period and last 7 days were for samples collection as animals were moved to metabolism crates and fed the rice straw at 90% of the previous voluntary feed intake of straw. Concentrate was still offered at 0.5% BW daily.

2.3. Sample collection and sampling procedures

The samples of DR seed meal, concentrate, and rice straw offered and refusals samples were collected during the last 7 days of each period. Fecal and urine samples were collected during the last 7 days of each period by using total collection method as animal were on the metabolism crates to study on the nutrient digestibility and nitrogen balance. The fecal samples were collected about 5% of total fresh weight and divided into two parts; first part for DM analysis every day and second part was kept in refrigerator and pooled by cattle at the end of each period for chemical analysis. Feeds, refusals and fecal samples were chemically analyzed DM (ID 967.03), N (ID 984.13), EE (ID 954.02), ash (ID 942.05), and ADF (ID 973.18) according to the AOAC (1998) method. The neutral detergent fiber (NDF) in samples was estimated according to Van Soest et al. (1991). Content of condensed tannins in DR was analyzed by using the modified vanillin-HCl method based on Burns (1971). Saponins were analyzed by using the modified vanillin-sulfuric acid method based on Wang and Fang (2004). Table 1 present the ingredient and chemical composition of concentrates, DR and rice straw used in the experiment. Metabolizable energy (ME) was calculated according to the equation described by Robinson

Table 1
Ingredient and chemical composition of concentrate, rice straw and *Delonix regia* (DR) seed meal used in the experiment.

Item	Concentrate	Rice straw	DR seed meal
Ingredients, kg DM			
Cassava chips	55.0		
Rice bran	11.0		
Coconut meal	12.9		
Palm kernel meal	13.5		
Urea	2.6		
Pure sulfur	1.0		
Mineral premix[a]	1.0		
Molasses, liquid	2.0		
Salt	1.0		
Chemical composition			
Dry matter, g/kg	898	921	895
Organic matter, g/kg DM	907	868	842
Ash, g/kg DM	93	132	158
Crude protein, g/kg DM	142	43	215
Neutral detergent fiber, g/kg DM	121	722	342
Acid detergent fiber, g/kg DM	74	564	245
Metabolizable energy (ME), MJ/kg DM	120	70	140
Tannins, mg/100 g DM	–	–	93
Saponins, g/kg DM	–	–	12

[a] 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.

et al. (2004). Digestible organic matter fermented in the rumen (DOMR) was calculated according to the equation described by ARC (1984) as:

$$\text{DOMR (kg/d)} = \text{digestible organic matter intake (DOMI, kg/d)} \times 0.65$$

where: DOMI = [digestibility of organic matter (kg/kg DM) × organic matter intake (kg/d)]/100, 1 kg DOMI = 15.9 MJ ME/kg (Kearl, 1982).

Urine samples were analyzed for urinary N using the Kjeldahl procedure described by the AOAC (1998) and were calculated for N utilization. At the 21st day of each period, jugular vein blood samples (10 ml) were collected at 0 h (before feeding) and 4 h after feeding for analyze blood urea-nitrogen (BUN). All samples were taken using a 21-ga needle, and the tubes containing 12 mg of EDTA as anticoagulant and plasma was separated by centrifugation at 500 × *g* for 10 min at 4 °C and stored at −20 °C until used.

Approximately, 45 ml of rumen fluid was taken from the rumen by a stomach tube connected to a vacuum pump at the same time of blood sampling. Ruminal pH and temperature were determined using a portable pH and temperature meter (HANNA Instruments HI 8424 microcomputer, Singapore). Rumen fluid samples were filtered through four layers of cheesecloth. The samples were divided into two portions. One portion was used for ammonia nitrogen (NH_3-N) determination using 5 ml of sulphuric acid (H_2SO_4) added to 45 ml of rumen fluid. The mixture was centrifuged at 16,000 × *g* for 15 min, and the supernatant was stored at −20 °C before NH_3-N analysis (Kjeltech Auto 1030 analyzer, Tecator, Hoganiis, Sweden). Total volatile fatty acid (VFA), acetic, propionic and butyric acid was done using HPLC. Calculation of ruminal methane (CH_4) production using VFA proportions according to Moss et al. (2000) as follows: CH_4 production = 0.45(acetate) −0.275(propionate) +0.40 (butyrate).

For determination of protozoa and fungal population, the second portion was fixed using a solution of 10% formalin in sterilized 0.9% saline, and were measured using the direct counting microscopic method based on the use of a haemocytometer (Boeco, Hamburg, Germany).

2.4. Statistical analysis

Statistical analysis accounted for the 4 × 4 Latin square design using the GLM procedure of SAS (Version 6.0; SAS Inst. Inc. Cary, NC, 1989). Data were analyzed using the model:

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

where: Y_{ijk}, observation from animal j, receiving diet i, in period k; μ, the overall mean, M_i, effect of the different level of DR seed meal level (i = 1, 2, 3, 4), A_j, the effect of animal (*j* = 1, 2, 3, 4), P_k, the effect of period (k = 1, 2, 3, 4), and ε_{ijk} the residual effect. Results are presented as mean values with the standard error of the means. Differences between treatment means were determined by Duncan's New Multiple Range Test, and differences among means with $p < 0.05$ were represented as statistically significant differences. Orthogonal polynomials for diet responses were determined by linear and quadratic effects.

Table 2
Influence of different levels of *Delonix regia* seed meal on feed intake, nutrient intake and apparent digestibility in Thai native beef cattle.

	Supplementation of *Delonix regia* seed meal, g DM					Contrast	
	0	90	180	270	SEM	Linear	Quadratic
DM intake							
Rice straw							
kg/day	2.0	2.0	2.0	2.1	1.00	0.44	0.34
g/kg $BW^{0.75}$	64.7	65.2	63.2	63.9	1.47	0.56	0.78
Concentrate							
kg/day	0.5	0.5	0.5	0.5	0.56	0.43	0.87
g/kg $BW^{0.75}$	16.7	16.4	15.3	15.3	1.03	0.77	0.44
Delonix regia seed meal							
g/day	0.00	0.09	0.18	0.27	–	–	–
g/kg $BW^{0.75}$	0.00	2.9	5.6	8.4	–	–	–
Total intake							
kg/day	2.5	2.6	2.7	2.8	0.89	0.45	0.89
g/kg $BW^{0.75}$	81.3[a]	84.4[b]	84.1[b]	87.5[c]	1.55	0.04	0.05
Nutrient intake, kg/d							
Dry matter	2.5	2.6	2.7	2.8	0.89	0.53	0.97
Organic matter	2.2	2.3	2.4	2.5	1.02	0.78	0.34
Crude protein	0.2	0.2	0.2	0.2	0.45	0.61	0.33
aNeutral detergent fiber	1.5	1.6	1.6	1.6	0.77	0.55	0.98
Acid detergent fiber	1.2	1.1	1.1	1.1	0.56	0.17	0.12
Estimated energy intake							
DOMI[d], kg/d	1.5	1.6	1.6	1.6	0.10	0.89	0.33
DOMR[e], kg/d	1.0	1.0	1.0	1.0	0.98	0.76	0.23
ME, MJ/d	23.6	24.6	24.9	25.1	0.82	0.67	0.87
ME, MJ/kg DM	9.4	9.4	9.2	9.0	0.41	0.55	0.99
Digestibility coefficients							
Dry matter	0.63[a]	0.60[b]	0.60[b]	0.58[c]	0.01	0.02	0.03
Organic matter	0.67[a]	0.67[a]	0.66[a]	0.64[b]	0.01	0.03	0.04
Crude protein	0.62	0.63	0.63	0.63	0.04	0.16	0.32
aNeutral detergent fiber	0.54	0.55	0.53	0.53	0.05	0.38	0.98
Acid detergent fiber	0.45	0.45	0.44	0.43	0.05	0.19	0.33

[a,b,c]Means in the same row with different superscripts differ ($P < 0.05$). [d]DOMI = Digestible organic matter intake. [e]DOMR = Digestible organic matter fermented in the rumen.

3. Results

3.1. Feed intakes and digestibility coefficients

The influence of different levels of DR seed meal on feed intake, nutrient intake and apparent digestibility in Thai native beef cattle are presented in Table 2. The intake of rice straw and concentrate were similar among DR seed meal fed groups ($P > 0.05$), whereas the total intake (g/kg $BW^{0.75}$) was linearly increased when DM seed meal was included at 270 g ($P < 0.05$). The total intake ranged from 81.31 to 87.52 g/kg $BW^{0.75}$. The supplementation of DR seed meal did not change the intakes of nutrient and energy ($P > 0.05$). The digestibility of CP, NDF and ADF were similar among various DR seed meal levels ($P > 0.05$). However, DM and OM digestibility were significantly different with DR seed meal supplementation and decreased when increasing DR seed meal levels ($P < 0.05$).

3.2. Rumen ecology, microorganisms and blood urea-nitrogen

Rumen pH and temperature were not changed by DR seed meal levels supplemented (Table 3; $P > 0.05$). Ruminal NH_3-N at 0, 4 h post feeding, and mean values linearly increased in beef cattle receiving DR seed meal at 90, 180 and 270 g DM ($P < 0.05$), respectively. Similarly, blood urea-N at 4 h post feeding was enhanced with the inclusion of DR seed meal when compared to the control fed group ($P < 0.05$). The supplementation of DR seed meal did not alter the fungal zoospores concentration ($P > 0.05$), whereas protozoal population was at 0, 4 h post feeding, and the mean values reduced when increasing the levels of DR seed meal supplemented ($P < 0.05$).

3.3. Ruminal volatile fatty acid (VFA) profiles and methane (CH_4) estimation

Feeding of DR seed meal to cattle did not influence the concentration of total VFA, acetic acid and butyric acid in the rumen

Table 3
Rumen ecology, microorganism and blood urea-nitrogen (BUN) in Thai native beef cattle fed different levels of *Delonix regia* seed meal.

	Supplementation of *Delonix regia* seed meal, g DM					Contrast	
	0	90	180	270	SEM	Linear	Quadratic
Rumen ecology							
Ruminal pH							
0 h post feeding	6.9	6.8	7.0	7.1	0.45	0.66	0.34
4 h post feeding	6.9	6.7	6.9	7.0	0.55	0.45	0.67
Mean	6.9	6.7	6.9	7.0	0.53	0.54	0.32
Ruminal temperature, °C							
0 h post feeding	38.9	38.3	38.8	39.0	2.59	0.67	0.89
4 h post feeding	39.7	39.5	39.6	39.8	3.34	0.77	0.34
Mean	39.3	38.9	39.2	39.4	3.00	0.55	0.98
NH_3-N concentration, mg/dl							
0 h post feeding	13.8^{a}	14.8^{a}	16.0^{b}	19.8^{c}	0.77	0.02	0.06
4 h post feeding	17.5^{a}	18.9a^{b}	20.4^{b}	23.2^{c}	0.98	0.03	0.07
Mean	15.7^{a}	16.9^{ab}	18.2^{b}	21.5^{c}	0.87	0.04	0.05
Blood urea-N concentration, mg/dl							
0 h post feeding	9.8	10.5	11.8	12.5	0.98	0.07	0.33
4 h post feeding	10.3^{a}	12.0^{b}	12.8^{b}	14.0^{c}	1.22	0.02	0.09
Mean	10.0	11.3	12.3	13.3	1.20	0.06	0.67
Ruminal microbes, cell/ml							
Protozoa, x10^{6}							
0 h post feeding	5.5^{a}	4.3^{ab}	2.5^{b}	2.6^{b}	0.54	0.03	0.98
4 h post feeding	8.3^{a}	6.5^{b}	5.0^{bc}	4.1^{c}	1.01	0.02	0.23
Mean	6.9^{a}	5.4^{b}	3.8^{b}	3.4^{b}	0.78	0.03	0.07
Fungal zoospores, x10^{4}							
0 h post feeding	1.2	1.2	1.2	1.2	1.67	0.15	0.88
4 h post feeding	1.5	1.6	1.6	1.6	1.86	0.98	0.99
Mean	1.4	1.4	1.4	1.4	1.79	0.34	0.11

^{a,b,c}Means in the same row with different superscripts differ ($P < 0.05$).

($P > 0.05$). However, the concentration of propionic acid at 4 h post feeding and the average concentration were significantly different among treatments, which were highest when 270 g DR seed meal was supplemented ($P < 0.05$). The estimation of CH_4 concentrations at 4 h post feeding, mean value and CH_4 per dry matter intake (DMI) were found significantly different with various levels of DR seed meal supplemented and was linearly reduced when increasing DR seed meal concentration (Table 4; $P < 0.05$).

3.4. N utilization

There were no differences in total N excretion, fecal N excretion or urinary N excretion in cattle fed different DR seed meal ($P > 0.05$). Increasing DR seed meal supplementation in cattle led to increased N intake from 22.5 to 34.5 g/d. In addition, N absorption, N retention and proportion of N retention to N intake were enhanced when 270 g DR seed meal was supplemented ($P < 0.05$) (Table 5).

4. Discussion

4.1. Feed intakes and digestibility coefficients

Increasing the total intake was expressed as g/kg $BW^{0.75}$ in beef cattle fed with DR seed meal due to increasing the intake of DR seed meal level supplementation. Intake of DR seed meal was increased at 8.4 g/kg $BW^{0.75}$ when 270 g of DR seed meal was fed to the cattle. It might be due to the aspect that DR seed meal is favored due to it containing soluble sugar, which is highly attractive to animals. However, there were no adversary effects on other feed intake and nutrient intake, thus feeding DR seed meal could be an alternative feed animal will be implied without a negative response toward its palatability. Similarly, Alemede et al. (2010) revealed that DR seed meal can be used to substitute groundnut cake up to 100% without any deleterious effects on the feed intake of Savanna Brown does. Gunun et al. (2016) showed that feed and nutrient intakes were not affected by the *Antidesma thwaitesianum* Muell. Arg. seed meal's containing of condensed tannins supplementation. Conversely, the addition of plants containing condensed tannins and saponins to ruminant diets at a high concentration usually suppresses feed intake because of the reduced palatability, decreased rate of digestion and development of conditioned aversion. Beauchemin et al. (2008) demonstrated that feed intake was reduced when raising condensed tannins uptake to doses higher than 50 g/kg DM. The negative effect of condensed tannins on feed intake was caused by the astringency of condensed tannins and short-term post digestional malaise (Landau et al., 2000).

DM intake of feed is the amount of DM consumed by the animal and is a central concept to any discussion of animal nutrition.

Table 4
Concentrations of ruminal volatile fatty acid (VFA) profiles and methane (CH_4) estimation in Thai native beef cattle fed with various levels of *Delonix regia* seed meal.

	Supplementation of *Delonix regia* seed meal, g DM				SEM	Contrast	
	0	90	180	270		Linear	Quadratic
Total VFA, mmol/l							
0 h post feeding	106.0	105.0	104.3	105.5	3.33	0.32	0.44
4 h post feeding	109.5	109.5	114.0	113.5	5.89	0.93	0.33
Mean	107.8	107.3	109.2	109.5	5.04	0.85	0.42
VFA profiles, mol/100 mol							
Acetic acid							
0 h post feeding	66.5	65.2	65.1	64.2	2.98	0.32	0.45
4 h post feeding	67.7	66.6	66.5	65.2	4.01	0.55	0.99
Mean	67.1	65.9	65.8	64.7	3.33	0.12	0.87
Propionic acid							
0 h post feeding	21.2	22.9	23.7	24.4	1.54	0.22	0.95
4 h post feeding	22.5[a]	25.8[b]	26.9[b]	30.3[c]	1.89	0.02	0.12
Mean	21.9[a]	24.4[b]	25.3[b]	27.4[c]	1.01	0.03	0.23
Butyric acid							
0 h post feeding	12.3	11.9	11.2	11.4	2.11	0.84	0.34
4 h post feeding	9.8	7.6	6.6	4.5	2.01	0.43	0.44
Mean	11.1	9.8	8.9	8.0	1.89	0.33	0.65
Acetic/propionic acid ratio	3.1[a]	2.7[b]	2.6[b]	2.4[c]	0.12	0.04	0.05
Acetic plus butyric/propionic acid ratio	3.6[a]	3.1[b]	3.0[b]	2.7[c]	0.14	0.02	0.03
CH_4 estimation (mM/l)							
0 h post feeding	29.0	27.8	27.3	26.7	1.13	0.06	0.34
4 h post feeding	28.2[a]	25.9[b]	25.2[b]	22.8[c]	1.04	0.01	0.03
Mean	28.6[a]	26.9[b]	26.2[b]	24.8[c]	0.98	0.03	0.04
CH_4 (mM/l)/DMI (kg)	11.4[a]	10.2[ab]	9.7[b]	8.8[c]	0.09	0.02	0.05

[a,b,c]Means in the same row with different superscripts differ ($P < 0.05$).
CH4 = (0.45x acetic acid) − (0.275x propionic acid) + (0.40x butyric acid) (Moss et al., 2000).

Table 5
Effects of different levels of *Delonix regia* seed meal on N utilization of Thai native beef cattle.

	Supplementation of *Delonix regia* seed meal, g DM					Contrast	
	0	90	180	270	SEM	Linear	Quadratic
N intake, g/d	25.5[a]	24.8[a]	29.4[b]	34.5[c]	2.34	0.01	0.12
N excretion	16.5	16.7	17.4	16.9	2.03	0.53	0.44
Fecal excretion, g/d							
Output, kg/d	1.0	1.0	1.2	1.0	0.45	0.67	0.43
Total N, g/d	5.4	5.4	6.2	6.3	1.66	0.16	0.32
Total N/N excretion	33.0	34.3	35.8	36.9	1.89	0.07	0.97
Urinary excretion							
Output, L/d	3.3	3.3	3.4	3.5	0.65	0.37	0.98
Total N, g/d	11.0	10.9	11.1	10.7	0.98	0.44	0.22
Total N/N excretion	67.0	65.7	64.2	63.1	6.78	0.13	0.17
N absorption, g/d	20.0[a]	19.0[a]	23.2[b]	28.3[c]	2.01	0.03	0.02
N retention, g/d	8.9[a]	8.0[a]	12.0[b]	17.8[c]	1.58	0.03	0.03
N retention/N intake	35.3[a]	32.5[a]	40.9[b]	50.9[c]	3.05	0.02	0.04

[a,b,c]Means in the same row with different superscripts differ ($P < 0.05$).

Typically, DM intake increases as the digestibility of the diet increases. However, anti-quality components such as tannins and saponins in feeds may decrease digestibility. The supplementation of DR seed meal containing condensed tannins and saponins at 270 g when compared to the nom-supplemented group showed a reduction of the digestibility of DM and OM at 4.2 and 3.1%, respectively. This probably is due to the inhibitory effects of the plant's secondary compounds in feed digestibility which was caused by the reduction of rumen bacterial populations and the inhibition of enzymes activity (Patra et al., 2006). Tannins have been implicated for their inhibitory effect on feed digestion in many experiments (Hristov et al., 2013). Patra et al. (2006) reported that DM and OM digestibility were significantly suppressed by about 6% with the addition of tannins sources from *T. chebula*. Similarly, Cieslak et al. (2016) reported that including *Sanguisorba officinalis* tannin extracts at 100 mg was reduced *in vitro* dry matter digestibility at 25.6%. Previous experiments confirmed that supplementing of DR seed meal higher than 11.7 mg (13.3–16.7 mg) reduced *in vitro* DM digestibility by 16–21% (Supapong et al., 2017). On the other hand, feeding plants containing condensed tannins

and saponins from *Antidesma thwaitesianum* Muell. Arg. seed meal to goats did not affect the apparent nutrient digestibility (Gunun et al., 2016), whereas *in vitro* true digestibility was increased when *T. chebula* included (Anantasook et al., 2016).

4.2. Rumen ecology, microorganisms and blood urea-nitrogen

The ruminal pH and temperature values for all the DR seed meal levels were optimal for normal rumen fermentation, microbial growth and microbial activity to digest fiber and feed (Cherdthong et al., 2015). The feeding of protein supplements effects on ruminants is based on the knowledge that NH_3 is the major end-product of protein degradation in the rumen and on the belief, which appears to have been generally accepted, that most of the N utilized by rumen microbes comes from the NH_3 pool in the rumen. The average ruminal NH_3-N increased from 15.7 to 21.5 mg/dl when increasing DR seed meal to its highest level. This could be due to the cattle consuming a higher level of crude protein from DR seed meal than those who did not receive the supplement. In this experiment, the cattle received crude protein at 58.1 g from 270 g DR seed meal supplementation. Thus, the high-crude protein content in the rumen could increase ruminal NH_3-N concentration. In the study by Cieslak et al. (2016) the concentration of NH_3-N slightly increased with the increasing level of *Sanguisorba officinalis* tannin extract at 4 mg. However, Gunun et al. (2016) demonstrated that the concentration of ruminal NH_3-N did not change when supplementing *Antidesma thwaitesianum* Muell. Arg. seed meal to goats. High concentrations of ruminal NH_3 increased the flux of NH_3 into the blood. A diffusion of NH_3 across the rumen wall has been demonstrated in cattle (Cherdthong et al., 2014). Blood urea levels could indicate whether or not adequate N is available to the rumen microbial population. At 4 h post feeding, blood urea-N concentration had increased to 14.0 mg/dl when increasing ruminal NH_3-N concentration in the cattle fed 270 g DR seed meal. However, the blood urea-N concentrations were in the normal range between 10 and 15 mg/dl (Byers and Moxon, 1980). This finding agrees with Alemede et al. (2010), who reported that the inclusion of DR seed meal at 100% in the diet of Savanna Brown does increase blood urea-N concentration at 0.75 m/mol.

The protozoal population reduced when increasing the level of DR seed meal containing condensed tannins and saponins. Liu et al. (2011) explained that tannins may inhibit the growth or activity of ruminal protozoa. This is probably a result of binding the proteins and enzymes of a protozoal cell. Furthermore, saponins have a potent antiprotozoal activity by forming complexes with sterols in protozoal cell membranes (Jouany and Morgavi, 2007). Supapong et al. (2017) demonstrated that inclusion of DR seed meal at 16.7 mg resulted in reduced protozoal counts at 41.7% in *in vitro* experiment. Similarly, the supplementation of *T. chebula* slightly decreased protozoal numbers (Anantasook et al., 2016).

4.3. Ruminal volatile fatty acid (VFA) profiles and methane (CH_4) estimation

The current experiment has shown that propionic acid increased while the acetic- to-propionic acid ratio and the acetic plus butyric-to-propionic acid ratio decreased in the highest level supplement group. The expected shift in the VFA profile from acetic to propionic acid ratio was associated with a shift of hydrogen from the CH_4 pathway making, propionic acid producing available (Anantasook et al., 2016). Therefore an alternative electron sink metabolic pathway to dispose of the reducing power has to occur. The succinate-propionic acid pathway that leads to propionic acid production could be a possibility (Newbold et al., 2005). In addition, soluble sugar contained in DR seed meal might be also served as carbohydrate substrate to produce propionic acid. Beauchemin et al. (2007) who demonstrated that acetic acid to propionic acid ratio was reduced when 20 g/kg DM of Quebracho tannin was included. This agrees with Anantasook et al. (2016), who found that inclusion of *T. chebula* was significantly increased propionic acid, while the acetic acid and acetate-to-propionic acid ratio decreased when compared to non-supplemented group.

The fermentation and VFA production by the rumen microbes in the rumen of cattle influence the production of CH_4. Increasing the DR seed meal concentration linearly reduced estimations of CH_4 concentrations at 4 h post feeding, mean value and CH_4 per dry matter intake (DMI). A reduction of CH_4 from DR seed meal supplementation could be due to the effect of the condensed tannins and saponins. Condensed tannins and saponins have been reported to suppress CH_4 production, reduce rumen protozoal number and modulate rumen volatile fatty acid (Hristov et al., 2013). A decrease in CH_4 production by DR seed meal could be mediated through: (i) a reduction in protozoa and/or methanogen populations associated with the protozoa surface; (ii) a direct inhibition of methanogen population; (iii) changes in volatile fatty acids profile and the acetate to propionate ratio and (iv) inhibition of fibrolytic enzymes activity and thus the feed digestibility (Cieslak et al., 2016). In the previous *in vitro* study, the supplementation of DR seed meal at 11.7 mg g could reduce CH_4 at 19.9%, whereas 42.4% reduced with 16.7 mg DR supplementations (Supapong et al., 2017). Gunun et al. (2016) found that the concentration of CH_4 reduced by 8% when goats were supplemented with condensed tannins in the Mao (*Antidesma thwaitesianum* Muell. Arg.) seed meal. Similarly, inclusion of *T. chebula* (8.4% condense tannins and 9.9% saponins) at 12 mg could reduce CH_4 production by 60.9% in an *in vitro* experiment (Anantasook et al., 2016). Furthermore, Foiklang et al. (2016) demonstrated that the CH_4 concentration was reduced by 88.3% when dairy steer were fed with grape pomace powder as a source of tannins and saponins. However, the potential methanogenic properties of feeds containing tannins and saponins may be related not only to the condensed tannin and saponin content but also to other factors (Cieslak et al., 2016).

4.4. N utilization

Major consequences of reducing the N utilization are the increasing cost of supplemental proteins to compensate for the high ruminal losses of feed proteins and the excessive N excretion in urine that potentially contributes to the N contamination of surface water and groundwater. A shift in N excretion from urine to feces in the cattle that are fed DR seed meal can exert beneficial effects environmentally as urinary N is mainly in the form of urea, which is rapidly converted into NH_3 and then to volatile nitrous oxides

(green house gases) (Patra and Saxena, 2011). Moreover, nitrate produced by the oxidation of NH_3 causes water pollution (Eckard et al., 2010). As a result, there was less nitrogen excreted in the urine and hence, less impact on the environment. The inclusion of DR seed meal had shifted the partitioning of N from urine to feces, which is consistent with the results of most studies on tannin effects (Ahnert et al., 2015). In this study, increasing the condensed tannins and saponins levels from DR seed meal there was a slight decrease in urinary N at 3.9% of N excretion, whereas a trend to increase in fecal N excretion for 3.9% of N excretion when comparing the control and 270 g DR seed meal. Condensed tannins and saponins increase availability of high quality protein for absorption in the lower gut of the ruminants. The supplementation of DR seed meal at 270 g improved N absorption and N retention. N retention is considered the most common index of the protein nutrition status of ruminants. Condensed tannins at lower levels have been reported to have a positive influence or increased N retention in ruminants (Ahnert et al., 2015).

5. Conclusions

The supplementation of DR seed meal at 270 g/d resulted in improving total feed intake, rumen fermentation and N balance while there was reduced DM digestibility, protozoal population and CH_4 production in beef cattle. However, additional studies on the utilization of DR seed meal should be conducted under the field conditions for ruminant production.

Conflict of interest

We declare that no conflict of interest exists among the authors.

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