Journal of Animal and Feed Sciences, 26, 2017, 123–130 *https://doi.org/10.22358/jafs/73890/2017*
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In vitro fermentation, digestibility and methane production as influenced by *Delonix regia* seed meal containing tannins and saponins

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KEY WORDS: *Delonix regia*, seeds, meal, methane production, fermentation, glycosides, *in vitro*

Received: 13 January 2017
Revised: 16 March 2017
Accepted: 6 June 2017

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ABSTRACT. The aim of the study was to evaluate the effect of supplementation of *Delonix regia* (DR) seed meal containing tannins and saponins on gas kinetics, ammonia-nitrogen (NH_3-N) content, pH, methane (CH_4) production and dry matter (DM) digestibility using an *in vitro* gas production technique. The experimental design was completely randomized, and the dietary treatments included DR seed meal supplementation at levels of 0, 3.3, 5.0, 6.7, 8.3, 10, 11.7, 13.3, 15.0 and 16.7 mg DM added to 0.5 g of roughage and concentrate (70:30) mixture. The gas production was measured at several time points: 0, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 48, 72 and 96 h by a pressure transducer. The parameters of gas kinetics and cumulative gas production were not altered ($P > 0.05$), except for gas production rate constant for the insoluble fraction which was the highest at 11.7 mg DR seed meal inclusion. CH_4 production and total protozoa counts linearly decreased with increasing DR seed meal levels ($P < 0.05$). DR seed meal addition caused quadratic increase of *in vitro* DM digestibility with the highest value at 11.7 mg DR seed meal inclusion. No significant difference in volatile fatty acid profile ($P > 0.05$) was stated between treatments except for propionic acid. In conclusion, supplementation of DR seed meal resulted in improved *in vitro* gas kinetics and DM digestibility up to 11.7 mg level, while CH_4 production was reduced linearly. The further *in vivo* studies are necessary to examine practical of DR seed meal usage in animal production.

Introduction

Methane (CH_4) is a gas produced during fermentation in the rumen (Steinfeld et al., 2006). CH_4 is one of the main greenhouse gases (GHG), which concentration in the atmosphere has increased by approximately 40% from 1970 to 2004 (IPCC, 2007). For the CH_4 production is used approximately 5 to 7% of dietary gross energy (GE) (only 3% in cattle fed high-grain diets). This amount constitutes

approximately 16 to 26 g · kg^{-1} of dietary dry matter (DM) intake (this amount could be lower in diets containing high proportion of grain) (Hristov et al., 2013). Hence, CH_4 mitigation strategies in ruminants have focused on obtaining economic and anti-global warming benefits (Kara et al., 2015). Some mitigation options such as chemical inhibitors, defaunation and ionophores directly or indirectly inhibit methanogenesis in the rumen, but consistent effects for practical use have not been confirmed yet (Hristov et al., 2013). In addition, there is an increasing interest in exploiting tropical plant products as feed additives to solve the problems connected with animal nutrition and livestock production. A variety of nutritional amendments, such as more grain addition, inclusion of leguminous forages containing condensed tannins and saponins, supplementation of low-quality roughages, readily fermentable carbohydrates and fat, could be promising for CH_4 mitigation (Hristov et al., 2013; Cieslak et al., 2016).

Condensed tannins and saponins are secondary compounds found in many plants and are structurally diversed molecules divided into two groups: triterpene and steroid glycosides (Szumacher-Strabel and Cieślak, 2010). Plant secondary compounds have been reported to suppress CH_4 concentration, reduce rumen protozoa counts and modulate rumen fermentation patterns (Cieslak et al., 2013). Many studies have shown that forages containing condensed tannins reduce CH_4 emissions from ruminants (Guo et al., 2008; Cieslak et al., 2016).

Delonix regia (DR) is a well-studied species of the *Leguminosae* family originating from Africa (Madagascar) but as a wild or ornamental plant is present in various parts of the world, including Thailand. Bunches of scarlet red flowers appear from April to July when the tree loses its leaves, and its fruits ripen from August to October. DR seeds contain 20.50% crude protein (CP), 4.18–4.23% ether extract (EE), 4.45–6.84% crude fibre (CF) and secondary compounds of condensed tannins and saponins at 90–95 mg · 100 g^{-1} and 10–15%, respectively (Alemede et al., 2010; Kaga, 2011). Therefore, tannins and saponins in DR seed could affect the reduction of CH_4 in the rumen and improve ruminant efficiency. Recently, DR seed meal has been used as a protein source in diets, and no negative effect has been found for animal growth performance. It could lead to enhanced carcass weight in rabbit (Kaga, 2011) and tilapia (Bake et al., 2014). Furthermore, Alemede et al. (2010) showed that DR seed meal can be used as a 100% replacement of peanut meal in broilers without affecting blood parameters. However, the inclusion of DR seed meal containing condensed tannins and saponins to ruminant diets have not been studied yet. Therefore, the objective of this experiment was to investigate the effect of different levels of DR seed meal on kinetics, ammonia-nitrogen (NH_3-N) content, pH, CH_4 production and digestibility using an *in vitro* gas production technique. It was hypothesized that condensed tannins and saponins present in DR seed meal could influence *in vitro* rumen fermentation and so reduce CH_4 production.

Material and methods

The study was approved by the Animal Ethics Committee of Khon Kaen University (permission No. ACUC-KKU 34/2559), based on the Ethics of Animal Experimentation of National Research Council of Thailand.

Experimental treatments and design

The experimental design was completely randomized, and the dietary treatments included *Delonix regia* (DR) seed meal supplementation at doses of 0, 3.3, 5.0, 6.7, 8.3, 10, 11.7, 13.3, 15.0 and 16.7 mg DM added to 0.5 g of roughage and concentrate (70:30) mixture. DR seed pods were collected from the Khon Kaen province (Thailand) from August to October 2015. The pods were sun-dried for 2–3 weeks, and then were easily opened for seeds collection.

Animals and rumen fluid inoculum

Two male rumen-fistulated swamp buffaloes with a body weight (BW) of 400 ± 10 kg were used as rumen fluid donors. The rumen fluid was collected from animals fed concentrate (14% CP and 75% total digestible nutrient (TDN)) at 0.5% of BW in two equal portions at 08:00 and 16:00; rice straw was available *ad libitum*. The animals were kept in individual pens with free access to clean fresh water and mineral blocks. The rumen fluid was sampled before morning feeding, squeezed through 4-layer cheesecloth into pre-warmed thermo flasks with an O_2-free headspace, transported under anaerobic conditions to the laboratory at 39 °C and used as a source of inoculum. Before a batch culture preparation, rumen fluid from the two animals was pooled and mixed. Artificial saliva was prepared according to Menke and Steingass (1988), but the medium did not include a nitrogen source in the buffer. The artificial saliva and rumen fluid were mixed in 2:1 ratio to prepare a mixed rumen inoculum. One hour

before filling with 40 ml of the mixed rumen inoculum, the serum bottles with the respective substrates were pre-warmed in a water bath at 39 °C.

Fermentation substrates *in vitro*

Samples of 0.5 g of roughage and concentrate mixture (70:30) were weighed into 50 ml serum bottles and supplemented with the respective DR seed meal levels. For each treatment, three replications were prepared with five blanks. Bottles were sealed with rubber stoppers and aluminum caps, and incubated at 39 °C (96 h) for the *in vitro* gas production test. The bottles were gently shaken every 3 h. For each sampling time, five bottles containing only the rumen inoculum were included in each run, and the mean gas production values of these bottles were used as blanks. The blank values were subtracted from each measured value to give the net gas production. The 120 bottles (3 bottles per treatment × 10 treatments × 4 sampling times: 0, 2, 4 and 6 h incubation) were separately prepared for pH, NH_3-N, volatile fatty acids (VFAs) and CH_4 analyses. In total 10 ml of liquid samples was used for NH_3-N and VFAs analyses. A digestibility analysis was prepared with another set of 60 bottles (3 bottles per treatment × 10 treatments × 2 sampling times: 12 and 24 h incubation).

Measurements and chemical analysis

The samples of DR seed meal, rice straw and concentrate were dried at 60 °C for 48 h, ground to pass through a 1-mm sieve (Cyclotech Mill, Tecator, Hoganas, Sweden) and used for chemical analysis and the *in vitro* gas production test. Dry matter (DM), ash, crude protein (CP), organic matter (OM) and acid detergent fibre (ADF) were determined according to the AOAC International (1998) method and expressed inclusive of residual ash. The neutral detergent fibre (NDF) in samples was estimated according to Van Soest et al. (1991). Content of condensed tannins in DR was analysed using the modified vanillin-HCl method based on Burns (1971). Saponins were analysed by using the modified vanillin-sulphuric acid method according to Wang and Fang (2004).

During the incubation, gas production was measured immediately after incubation at 0, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 48, 72 and 96 h using a pressure transducer and a calibrated syringe. A syringe fitted with an 18 gauge needle was used as a punch to a rubber stopper on the fermentation bottles, which were placed in the incubator. The metering system of the pressure transducer consisted of a three-way valve, a mechanical manometer fitted to a transducer, a gas-tight-syringe and a needle. The three-way valve was connected to the transducer to measure pressure in the serum bottles, and the gas-tight syringe measured the volume of produced gas. The third port was connected with a needle by a hose used to punch the rubber stopper on the serum bottle.

Fermentation liquor was sampled from 120 bottles at 0, 2, 4 and 6 h post inoculation (30 samples were obtained at each time) to measure the pH and then filtered through four layers of cheesecloth. Samples were centrifuged at 16 000 *g* for 15 min, and the supernatant was stored at −20 °C before NH_3-N analysis using the micro Kjeldahl methods (AOAC International, 1998) and VFAs analysis using high-performance liquid chromatography (Instruments by controller water model 600E, water model 484 UV detector, column novapak C18, column size 4×150 mm, mobile phase 10 mM H_2PO_4, pH 2.5; ETL Testing Laboratory, Inc., Cortland, NY, USA). After termination of incubation at 2 and 4 h, the content of the serum bottles was mixed properly, and a 1 ml sample was mixed with 6 ml of 4/100 formaldehyde for protozoa determination. The protozoal population was measured using the direct counting microscopic method (150×) based on the use of a haemocytometer (Boeco, Hamburg, Germany). *In vitro* digestibility was determined after termination of incubation at 12 and 24 h, when the content was filtered through pre-weighed Gooch crucibles (40 mm of porosity) and residual DM was estimated. The percentage of weight loss was determined and presented as *in vitro* DM digestibility (IVDMD) (Tilley and Terry, 1963). The methane in the total gas was measured at 24 h post incubation, and 10 ml of gas was withdrawn from the head space of the incubated serum bottle using a leak-proof syringe and analysed for methane using gas chromatography (Shimadzu Corporation, Kyoto, Japan).

Statistical analysis and calculations

Cumulative gas production data were presented accordingly to the equation of Ørskov and McDonald (1979) as:

$$Y = a + b\,(1 - e^{(-ct)})$$

where: a – gas production from immediately soluble fraction, b – gas production from insoluble fraction, c – gas production rate constant for insoluble fraction (b), t – incubation time, (a+b) – potential extent of gas production, Y – gas produced at time 't'.

Data were analysed as a completely randomized design by ANOVA using the General Linear Model (GLM) procedures of SAS (1989; Version 6.0; SAS Institute Inc., Cary, NC, USA). The model included the fixed effect of treatment. Treatment means were calculated using the Least Square Means (LSMEANS) option of SAS. When F-tests were significant, single degree of freedom orthogonal contrasts were used to determine linear and quadratic effects of increasing levels of DR seed meal supplementation. Since DR seed meal supplementation level increments was not equal in diet (i.e. 0, 3.3, 5.0, 6.7, 8.3, 10, 11.7, 13.3, 15.0 and 16.7 mg), coefficients for orthogonal polynomials were generated using IML (Interactive Matrix Language) procedures of SAS for unequal spacing (1989; Version 6.0; SAS Institute Inc., Cary, NC, USA). Moreover the Pearson correlation coefficient was calculated for number of total protozoa and digestibility of feed.

Results and discussion

Chemical composition of diets. The concentrate and rice straw consisted of 14.2% and 4.3% CP, respectively (Table 1). The DR seed meal contained 21.5% CP, so it could be a beneficial alternative protein source for ruminants. This was probably influenced by different locations and the nutritional management of the planted DR, as previously stated by Egena et al. (2008) who determined that CP content in DR seed meal ranged from 18.0% to 25.0% DM. Condensed tannins and saponins content in DR seed meal was 93.1 mg · 100 g^{-1} DM and 12.3 g · kg^{-1} DM, respectively. The content of secondary compounds in DR was higher than in other tropical plants such as mangosteen peel (*Garcinia mangostana* L.), guava leaf (*Psidium guajava* L.) and Siam neem leaf (*Azadirachta indica*), which contained tannins and saponins at the level of 16.8, 15.8 and 11.4%, and 10.0, 2.8 and 2.8% DM, respectively (Ngamsaeng et al., 2006). Condensed tannins and saponins have been reported to possess anti-methanogenesis properties (Hristov et al., 2013; Cieslak et al., 2016). This information suggests that this plant may reduce CH_4, but no scientific work has been conducted to justify this hypothesis yet.

Gas kinetics and cumulative gas production. It was found that gas production from soluble fractions (*a*) ranged from −2.3 to −3.1 and was not significantly different among treatments ($P > 0.05$; Table 2). Furthermore, also the gas production from the insoluble fraction (*b*), the potential extent of gas production (*a*+*b*) and cumulative gas production

Table 1. Ingredient and chemical composition of concentrate, rice straw and *Delonix regia* seed meal

Indices	Concentrate	Rice straw	*Delonix regia* seed meal
Ingredients, g · kg^{-1} DM			
cassava chips	55.0		
rice bran	11.0		
coconut meal	12.9		
palm kernel meal	13.5		
urea	2.6		
pure sulphur	1.0		
mineral premix[1]	1.0		
molasses, liquid	2.0		
salt	1.0		
Chemical composition			
dry matter, g · kg^{-1}	89.8	92.1	89.5
organic matter, g · kg^{-1} DM	97.5	86.2	9.4
ash, g · kg^{-1} DM	9.3	13.2	4.8
crude protein, g · kg^{-1} DM	14.2	4.3	21.5
neutral detergent fibre, g · kg^{-1} DM	72.2	80.2	34.2
acid detergent fibre, g · kg^{-1} DM	12.1	56.4	24.5
tannins, mg · 100 g^{-1} DM	-	-	93.1
saponins, g · kg^{-1} DM	-	-	12.3

[1] mineral premix, per kg of premix: IU: vit. A 10 000 000, vit. E 70 000, vit. D 1 600 000; g: Fe 50, Zn 40, Mn 40, Co 0.1, Cu 10, Se 0.1, I 0.5

Table 2. Effect of *Delonix regia* seed meal on gas kinetics and cumulative gas production after 96 h of incubation

Indices	Kinetics of gas, ml · 0.5 g^{-1} DM				Cumulative gas production, ml gas · g^{-1} DM incubated
	a	*b*	*c*	*a*+*b*	
Delonix regia seed meal, mg DM					
0	−2.3	59.2	0.027[a]	56.7	117.6
3.3	−2.5	62.2	0.029[a]	59.8	124.2
5.0	−2.7	57.9	0.033[a]	54.6	116.8
6.7	−2.4	66.8	0.046[ab]	64.5	136.2
8.3	−3.0	66.2	0.045[ab]	63.4	133.2
10.0	−2.8	62.5	0.032[ab]	59.0	126.0
11.7	−3.1	65.3	0.056[b]	66.1	141.0
13.3	−2.9	58.8	0.034[a]	55.4	118.0
15.0	−3.0	65.9	0.029[a]	62.3	108.8
16.7	−3.1	62.3	0.033[a]	59.0	106.4
SEM	0.99	6.43	0.003	5.89	19.44
Contrasts					
linear	0.22	0.67	0.12	0.88	0.38
quadratic	0.98	0.11	0.04	0.09	0.08

a – gas production from immediately soluble fraction; *b* – gas production from insoluble fraction; *c* – gas production rate constant for insoluble fraction (b); *a*+*b* – potential extent (omit gas); SEM – standard error of the mean; [ab] – means with different superscripts within a column are significantly different at $P \leq 0.05$

(after 96 h of incubation) were not altered by increasing concentrations of DR seed meal ($P > 0.05$). In comparison to control group gas production rate constant for the insoluble fraction (*c*) was significantly higher ($P < 0.05$) only in treatment in which DR seed meal was added at a dose of 11.7 mg (0.056 ml · h^{-1}). This enhanced performance of kinetics gas '*c*' could be attributed to higher nutrients levels in DR seed meal, such as CP, starch and vitamins, resulting in effective ruminal fermentation. However, the further increase of DR seed meal level above 11.7 mg did not result in further growth of parameter '*c*' but there was observed the decrease to values which did not differ from the control one. This could be due to the higher amount of secondary plant compounds contained in DR seed meal at 13.3 to 16.7 mg levels. Makkar (2003) showed that intake of high levels of tannins and saponins by animals can produce toxic rumen microorganisms, which could result in the inhibition of feed digestion and rumen fermentation.

***In vitro* ruminal fermentation and CH_4 concentration.** Ruminal pH and NH_3-N concentration in bottle serum was not altered by inclusion of various levels of DR seed meal (Table 3). The pH was in a stable range from 6.5 to 6.6, which is a suitable value for rumen ecology, similar to that reported by Van Soest et al. (1991). NH_3-N concentration ranged from 103.0 to 124.1 mg · l^{-1} for all treatments. No differences in NH_3-N concentration were observed due to low levels of DR supplementation (0 to 16.7 mg) which did significantly change the level of CP content in the substrate. However, these values were above the optimum for rumen microorganism growth (30 to 80 mg · l^{-1}), as stated by Satter and Slyter (1974), and the range was similar to the one (107–157 mg · l^{-1}) determined by Cherdthong and Wanapat (2014).

DR seed meal supplementation caused linear decrease of CH_4 concentration measured at 24 h post-incubation from 72.4 to 114.4 ml · l^{-1} and in all these groups CH_4 concentration was lower than in control group without DR seed meal addition ($P < 0.05$). Supplementation of DR seed meal at 11.7 mg reduced CH_4 content by 19.9%, whereas 16.7 mg DR seed meal supplementation by 42.4%. Furthermore, with the increasing level of DR seed meal to 16.7 mg, the methane concentration expressed per unit of total gas production or gram of *in vitro* dry matter digestibility also decreased. Reduction of CH_4 concentration by DR seed meal supplementation could be due to the presence of condensed tannins and saponins. Guo et al. (2008) demonstrated that saponins inhibited the expression of the methyl-coenzyme M reductase (*mcrA*) gene which takes part in the final step of methanogenesis. In addition, the CH_4-suppressing effects of saponins were presumably a direct action against the rumen microbes involved in CH_4 formation, including methanogens and protozoa (Cieslak et al., 2013, 2016; Anantasook et al., 2016). Similar findings were found in this study: the protozoal population was also linearly reduced with increased DR seed meal levels

Table 3. Influence of *Delonix regia* seed meal supplementation on pH, methane concentration, ammonia-nitrogen content and *in vitro* dry matter (DM) digestibility (IVDMD) using *in vitro* gas production technique

Indices	pH	NH_3-N content, mg · l^{-1}	IVDMD, g · kg^{-1} DM	Methane concentration			Protozoal population counts, ×10^6 cell · ml^{-1}
				ml CH_4 · l^{-1}	ml · g^{-1} IVDMD	ml per unit of total gas production	
Delonix regia seed meal, mg DM							
0	6.6	113.3	507.2^{a}	125.8^{e}	496.3^{e}	2.1^{b}	7.2^{c}
3.3	6.5	109.1	538.1^{a}	114.4^{d}	425.3^{d}	1.8^{ab}	7.0^{c}
5.0	6.6	103.0	543.3^{b}	110.3^{cd}	406.3^{d}	1.9^{ab}	6.8^{c}
6.7	6.6	113.2	613.5^{bc}	107.6^{c}	351.1^{c}	1.6^{a}	6.5^{bc}
8.3	6.5	121.1	621.4^{bc}	105.8^{c}	340.7^{c}	1.6^{a}	5.3^{b}
10.0	6.6	108.0	602.7^{bc}	105.4^{c}	350.2^{c}	1.7^{a}	5.3^{b}
11.7	6.6	106.4	656.5^{c}	100.8^{bc}	307.3^{ab}	1.4^{a}	5.0^{b}
13.3	6.5	112.3	552.2^{b}	95.9^{b}	347.5^{b}	1.6^{a}	4.4^{ab}
15.0	6.6	119.4	545.5^{b}	94.1^{b}	345.3^{b}	1.7^{a}	4.4^{ab}
16.7	6.6	124.1	520.4^{a}	72.4^{a}	278.5^{a}	1.4^{a}	3.0^{a}
SEM	0.18	9.6	13.6	0.59	15.77	0.03	0.13
Contrasts							
linear	0.44	0.29	0.45	0.04	0.05	0.01	0.03
quadratic	0.89	0.09	0.03	0.23	0.01	0.22	0.12

SEM – standard error of the mean; $^{a-e}$ – means with different superscripts within a column are significantly different at $P \leq 0.05$

($P < 0.05$). Increasing DR seed meal level up to 16.7 mg decreased protozoa by 58.3% when compared to the control group. Moreover, tannins may also inhibit, through bactericidal or bacteriostatic activities, the growth or activity of rumen methanogen and protozoa, likely by binding proteins and enzymes of the microbial cells. The current study is in agreement with the data of Anantasook et al. (2016), who found that supplementing secondary plant compounds from *Terminalia chebula* Retz (containing 8.4% condensed tannins and 9.9% saponins) at a dose of 12 mg reduced CH_4 production by 60.9% in an *in vitro* experiment. Furthermore, 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 (Foiklang et al., 2016). Therefore, DR seed meal is an interesting tropical plant to use as a feed additive to reduce environmental pollution from CH_4 and could be applied for commercial natural products for ruminants. However, the potential methanogenic properties of feed containing tannins and saponins may be related not only to the condensed tannin and saponin content but also to other factors (Cieslak et al., 2013). The lack of the effect of plants containing tannins and saponins on the number of methanogens in rumen liquid under *in vitro* conditions may be the use of too low dose of tannins and saponins in the supplement (Szumacher-Strabel and Cieślak, 2010).

In vitro DM digestibility (Table 3) was significantly different among DR level supplemented ($P < 0.05$). Because the *in vitro* gas technique has been used as a measurement of feed degradation, high gas production rate (*c*) indicated the high digestibility of substrates (Cherdthong and Wanapat, 2014). In the present study, it was found that treatments in which a higher gas production rate was observed (such as 11.7 mg DR seed meal) also showed higher *in vitro* digestibility of DM. Increasing the DR seed meal concentration had quadratic effects on DM digestibility and was the highest with 11.7 mg inclusion (656.5 g · kg^{-1} DM). However, supplementation of DR seed meal in doses higher than 11.7 mg (from 13.3 to 16.7 mg) reduced *in vitro* DM digestibility by 10.3–13.6%. So as 16.7 mg treatment did not differ from group without DR seed meal supplementation. The improvement of DM digestibility in *in vitro* study with an optimal sugar concentration from DR seed meal supplementation indicated the availability of more potentially fermentable sugar with a N source for the proliferation of rumen microbes as reported by Udén (2006). However, *in vitro* DM digestibility decreased when DR seed meal levels were higher than 11.7 mg. Cieslak et al. (2016) stated that soluble sugar can interact with phytochemicals, and consequently, the soluble sugar can be physically less available for rumen microorganisms. This interaction results in decreased activity of the microorganism and reduced DM digestibility. Furthermore, this may be due to the high content of tannins and saponins in DR seed meal and may reduce cell wall digestibility by binding bacterial enzymes and (or) forming indigestible complexes with cell wall carbohydrates. Scalbert (1991) identified three mechanisms of tannin and saponin toxicity in microorganisms: 1. enzyme inhibition and substrate deprivation, 2. action on membranes and 3. metal ion deprivation. Jones et al. (1994) found that tannins and saponins inhibited the cell-associated protease activity of *Butyrivibrio fibrosolvens*, which is predominant cellulolytic bacteria. The distinction between the inhibition of cellulolytic enzymes and the formation of complexes with cellulose that are resistant to hydrolysis by cellulases may be difficult. Bae et al. (1993) indicated that the extracellular endoglucanase from the ruminal bacterium *Fibrobacter succinogenes* was more susceptible to inhibition by the tannins and saponins than cell-associated enzymes. These results suggest that bacterial enzymes may be more susceptible to high level of tannins and saponins in DR seed meal and could decrease DM digestibility.

Ruminal volatile fatty acid concentration. There were observed no significant differences ($P > 0.05$) in total volatile fatty acids (TVFA), acetic acid (C2) or butyric acid (C4) content between

Table 4. Influence of *Delonix regia* seed meal supplementation on volatile fatty acids (VFAs) content using *in vitro* gas production technique

	Acetic acid	Propionic acid	Butyric acid	Total VFAs, mmol · l^{-1}
Delonix regia seeds meal, mg DM				
0	65.0	21.6^{a}	12.0	98.6
3.3	64.1	23.0^{ab}	12.5	99.6
5.0	63.4	20.5^{a}	15.0	99.3
6.7	62.5	20.0^{a}	13.0	97.5
8.3	64.8	23.0^{ab}	13.0	99.8
10.0	61.4	20.5^{a}	12.5	97.9
11.7	61.5	26.0^{b}	11.5	104.0
13.3	64.0	24.5^{ab}	12.0	103.5
15.0	65.9	23.1^{ab}	13.5	101.5
16.7	64.6	22.9^{ab}	15.0	103.5
SEM	3.03	1.15	1.00	4.31
Contrasts				
linear	0.29	0.12	0.77	0.87
quadratic	0.87	0.04	0.34	0.22

DM – dry matter; SEM – standard error of the mean; ^{ab} – means with different superscripts within a column are significantly different at $P \leq 0.05$

treatments (Table 4). However, propionic acid (C3) content was altered among DR seed meal levels and was the highest with 11.7 mg DR seed meal inclusion. Anantasook et al. (2016) explained that condensed tannins present in plants may be suppressing CH_4 production by shifting hydrogen from the CH_4 pathway to produce C3. Moreover, saponins may also induce rechanneling of metabolic hydrogen from CH_4 to C3 production (Kara et al., 2015). Furthermore, Waghorn (2008) revealed that plant secondary compounds may adversely affect cellulolytic bacteria and consequently anaerobic fermentation of carbohydrates to short-chain fatty acids, particularly C3, thereby reducing CO_2 and H_2 formation, which is required for methanogenesis, such as the succinate pathway. Therefore, an increase in propionate production would be expected if propionate formation has become an alternative pathway for H_2 extirpation in rumen. These results are in agreement with the findings of Anantasook et al. (2016), who reported that C3 concentration could increase at 4.1% with the supplementation of tannins and saponins from *T. chebula* at a dose of 12 mg. Similarly, Foiklang et al. (2016) also found that the supplementation of grape pomace powder as a source of tannins and saponins in dairy steer increased C3 concentration by 6.9% when compared to control. In addition, Beauchemin et al. (2007) reported a linear decline in C2 concentration and the acetate to propionate ratio when 10 and 20 g · kg^{-1} DM of *Schinopsis quebracho-colorado* (red quebracho) tannins were supplemented.

Conclusions

Supplementation of *Delonix regia* (DR) seed meal may result in the improved gas production rate constant for the insoluble fraction and *in vitro* dry matter digestibility up to 11.7 mg, whereas the reduced CH_4 production can be obtained with the linear increase supplementation level of DR seed meal. However, these findings should be further investigated in *in vivo* experiments to elucidate the actual effect of DR seed meal addition on animal production.

Acknowledgments

The authors would like to express their sincere thanks to the Increase Production Efficiency and Meat Quality of Native Beef and Buffalo Research Group, Khon Kaen University (KKU), and Research Fund for Supporting Lecturer to Admit High Potential Student to Study and Research on His Expert Program Year 2015 for providing financial support for the research and the use of the research facilities. This work was also supported by Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, KKU and the Thailand Research Fund (TRF) contract grant IRG5980010.

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