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Research Article

Dietary Hog Plum (*Spondias pinnata* (L.f) Kurz) Could Modulate Fermentation Process, and Feed Digestibility, As Well As and Reduce Protozoal Population: *In Vitro* Study"

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Abstract | The research was to elucidate the influences of dietary hog plum supplementation on nutrient digestibility, fermentation characteristics, protozoal population and kinetics of gas production. Completely randomized design (CRD) was used and the treatments were 6 levels of hog plum supplementation at 0, 0.5, 1.0, 1.5, 2.0 and 2.5 mg DM. Hog plum consisted of CP at 5.6% DM and saponins was found about 9.4%. Supplementation of hog plum did not alter gas production from the soluble fractions (*a*), gas production from the insoluble fraction (*b*) and the potential extent of gas production (*a+b*) and gas production rate constants for the insoluble fraction (*c*) (P>0.05). Furthermore, cumulative gas production from 96 h of incubation did not changed by hog plum complementation and were ranged from 154.7 to 158.8 ml/ 0.5 g DM. Ruminal pH of 2 h, 4 h after incubation and average pH were not significantly different among various doses of hog plum complementation (P>0.05). Average NH_3-N concentration were highest increased when hog plum supplementation at 2.0 to 2.5 mg and increased by 16.4 to 17.5%, respectively compared to no supplemented group. At 2 h after incubation, there were no changed on protozoal counts (P>0.05), whereas the reduction of protozoal counts were found when added hog plum at 4 h after incubation (P<0.05). In addition, mean concentration of protozoa was reduced when increasing doses of added hog plum (P<0.05). Supplementation of hog plum at 2.0 to 2.5 mg could reduce protozoal population by 71.2% when compared to no supplemented group. Supplementation of hog plum did not alter IVDMD and IVOMD and were average ranged from 57.1 to 58.5% DM and 62.4 to 64.1% DM, respectively (P>0.05). It could be summarized that supplementation hog plum did not adversary affect ruminal fermentation, kinetic of gas and *in vitro* digestibility, whereas population of protozoa was decreased when supplementation hog plum up to 2.0 mg.

Keywords | Tropical plant, Greenhouse gas, Saponin, Methane, Protozoa

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INTRODUCTION

The manipulation of ruminal fermentation was elucidate used to optimize rumen fermentation for enhancing feed use, animal production and reducing enteric methane (CH_4) emission (Hristov et al., 2013; Cherdthong et al., 2019). Johnson and Johnson (1995) noted that the CH_4 produced is not utilized by the ruminant itself, but instead represents an energy loss of 2–12% of gross energy. Accordingly, ruminal protozoa were removed by defaunation to reduce CH_4 emission in the rumen and enhance the protein outflow in the small intestine, indicating in the enhanced nutrient use and performance production of the ruminants (Santra and Karim, 2003). Some elimination options such as chemical inhibitors, and ionophores inhibit rumen protozoa.

However, consistent influences have not been verified for practical use (Hristov et al., 2013; Cieślak et al., 2016). Moreover, there is increasing interest in studying tropical plants as supplemental feed to reduce chemical inhibitors in animal nutrition.

Tropical plants consist of high contents of saponins which have become noticeable choices for prohibiting protozoa in the rumen (Gunun et al., 2019). These possibly be that these compounds may disturb the activity of ruminal protozoa, likely by attaching the proteins and enzymes of the protozoal membranes (Hristov et al., 2013). Numerous reports demonstrated that the adding condensed tannins sources such as *Terminalia chebula* Retz or the *Delonix regia* seed could reduce the ruminal concentration of protozoa by 60% to 70% (Anantasook et al., 2016; Supapong et al., 2017; Cherdtong et al., 2019).

Hog plum (*Spondias pinnata* (L.f) Kurz) is a tropical native plant to India, Myanmar, Indonesia, Southern China, and throughout Thailand (Florido and Cortiguerra, 2003). Fruits are borne in terminal clusters of 10-15 fruits (Campbell and Sauls, 1994). The fruit is ovoid, 45 cm in length, color is yellow in color, and fleshy, with the drupe having a finely edible pulp. It contains many chemical compounds which that are important for pharmacological activities. Chalise et al. (2010) reviewed that the concentration of saponins in hog plum was 8.14 mg/100 g and therefore, it was hypothesized that hog plum could modify ruminal fermentation and reduce the protozoal population.

Thus, the research was to elucidate the influences of dietary hog plum doses on nutrient digestibility, fermentation characteristics, the protozoal population and the kinetics of gas production.

MATERIALS AND METHODS

Hog Plum Preparation

Hog plum fruits were collected during August, 2017 to January 2018, in Khon Kaen province, Thailand. The fruit were moved out of seeds and collected the peel for the test. The peel were oven dried at 60 °C for 24 hour, then was grounded to pass a 1-mm sieve and kept until test.

Experimental Design And Treatments

Completely randomized design (CRD) was used and the treatments were 6 levels of hog plum supplementation at 0, 0.5, 1.0, 1.5, 2.0 and 2.5 mg DM with 0.5 g of substrates (60% roughage and 40% concentrate) (see Table 1).

Ruminal Inocula

All processes between the ruminal fluid moving and inoculum periods were anaerobic techniques and sterile. Two male dairy steer with an initial body weight of 400±20 kg were used as rumen fluid donors. Ruminal fluid samples were obtained from animals before feeding in the morning, then was filtered through four layers of cheesecloth into prewarmed thermos flasks before moved to the *in vitro* laboratory.

Table 1: Feed formulation and chemical composition of diets.

Item	Concentrate	Rice straw	Hog plum
Ingredients, kg DM			
Cassava chip	55.0		
Rice bran	11.0		
Coconut meal	12.9		
Palm kernel meal	13.5		
Urea	2.6		
Pure sulfur	1.0		
Mineral premix*	1.0		
Molasses, liquid	2.0		
Salt	1.0		
Chemical composition			
Dry matter (%)	91.3	93.3	87.6
% of DM			
Organic matter	87.1	85.3	88.7
Ash	12.9	14.7	11.3
Crude protein	13.6	2.6	5.6
Neutral detergent fiber	10.8	19.7	56.8
Acid detergent fiber	6.9	11.3	36.3
Saponins, mg/ 100 g DM	-	-	9.4

*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.

The 0.5 g of substrate was obtained from 60% of roughage and 40% of concentrate. Substrates were milled to a 1- mm screen and weighed to 0.5 g of DM into 50 mL bottles for 96 h. Media solution was performed as following to the method of Menke and Steingass (1988). All bottles with the mixture of substrate treatments were prepared in a water bath at 39 °C for 1 h before filling with 40 mL of the artificial saliva solution.

Fermentation Characteristics Determination

The production of gas was collected at 0, 1, 2, 4, 6, 8, 10, 12, 18, 24, 48, 72 and 96 h and was calculated to the equation of Ørskov and McDonald (1979). The rumen solution mixture was sampled at 0, 4, 8, 12 and 24 h after inoculation. Rumen fluid inocula were then filtered through four layers of cheesecloth. Samples were used for ruminal pH determination using Hanna Instruments' HI 8424 microcomputer (Kallang Way, Singapore) and nitrogen (NH_3-N) (AOAC, 1998). The protozoal count was determined at 2 and 4 h of incubation using the direct counting microscopic (150×) method based on the use of a haemocytometer (Boeco, Hamburg, Germany). *In vitro* DM digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) were elucidated after dissolving at 12 and 24 h after inoculum according to the method of Tilley and Terry (1963).

Chemical Composition Analysis

Hog plum peel, roughage 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 nutritional determination and the gas analysis. The diets were chemically evaluated for dry matter (DM), ash and crude protein (CP), organic matter (OM) and acid detergent fiber (ADF) (AOAC, 1998). The content of neutral detergent fiber (NDF) was determined by the method of Van Soest et al. (1991). Saponins were evaluated by method based on Wang and Fang (2004) using the modified vanillin-sulfuric acid. Ingredients and nutritional content of diets are demonstrated in Table 1.

Statistical Assay

All data from the experiment were statistically analyzed as a CRD using the GLM procedure of SAS (1998) (Version 6.0; SAS Inst. Inc. Cary, NC). Results are presented as mean values with the standard error of the means. Differences between dietary means were evaluated by Duncan's New Multiple Range Test with $P<0.05$ level of significance. Orthogonal polynomial contrast was performed to determine their responses.

RESULTS AND DISCUSSION

Nutritional Value Of Feed And Hog Plum

Table 1 shows the ingredient and nutritional composition of feed and hog plum. The concentrate diet contains 13.6% DM of CP, which could be because of the additional urea (2.6% DM) in the ration. A high level of NPN in the ratio might be synchronized with a fermentable carbohydrate source from the cassava chip which include makes up 55% of the concentrate diet. It might be support ruminal microbial protein production and improve ruminal ecosystem proficiency. Hog plum consisted of CP at 5.6% of DM, which could be an additional protein source when incorporated into the rumen. Furthermore, a secondary compound in hog plum, namely saponins, was found to make up about 9.4% of DM, which was greater than those reported by Chalise et al. (2010; 8.14%). It might possibly be that due the differences in numerous factors, such as age, soil fertility, and preparation process. Saponins content in hog plum was closely to that of other tropical plants, such as *Terminalia chebula* Retz (9.9%; Anantasook et al., 2016) and the *Delonix regia* seed (12.3%; Supapong et al., 2017). Thus, based on the content of saponins in hog plum, it might be potential possible to modulate rumen fermentation, with more elucidation being required.

Kinetics Of Gas And Cumulative Gas

Table 2 elucidates the effect of hog plum on gas kinetics and gas production after incubation at 96 h. The supplementation of hog plum did not alter gas production from the soluble fractions (*a*), gas production from the insoluble fraction (*b*), and the potential extent of gas production (*a*+*b*) and gas production rate constants for the insoluble fraction (*c*) ($P>0.05$). Kinetic gas "c" were ranged from 0.044- to 0.048 ml/ 0.5 g of DM, while whereas the value of "b" were ranged from 151.8- to 155.3 ml/ 0.5 g of DM. Furthermore, cumulative gas production from 96 h of incubation did not changed due to hog plum complementation and were ranged from 154.7 to 158.8 ml/ 0.5 g of DM. Thus, increasing doses of hog plum did not adversary affect the kinetics of gas and could be maintain a normal condition of ruminal fermentation, as well as the and digestion of feed. In contrast, some previous research work indicated that the inclusion of a high level of saponins could prohibit ruminal bacteria, resulting in the suppression of *in vitro* gas production (Makkar, 2003; Jouany and Morgavi, 2007; Cieślak et al., 2016).

Table 2: Effect of hog plum on gas kinetics and cumulative gas at 96 h after incubation.

Hog plum supplementa-tion, mg DM	Kinetic of gas*, ml/ 0.5 g DM				
	a	b	c	a+b	Cumulative gas production, ml DM incubated
0	-1.6	155.6	0.048	154.0	157.8
0.5	-1.0	155.1	0.047	154.0	158.0
1.0	-1.6	152.2	0.045	152.9	156.1
1.5	-1.9	155.4	0.045	154.4	157.6
2.0	-1.2	153.0	0.045	155.3	158.8
2.5	-1.1	152.0	0.044	151.8	154.7
SEM	1.1	1.53	0.020	1.7	1.72
P-value	0.66	0.93	0.10	0.99	0.98
Contrasts†					
Linear	0.11	0.15	0.33	0.52	0.44
Quadratic	0.97	0.88	0.55	0.12	0.25

**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); †L: Linear; Q: Quadratic.

Ruminal Fermentation And Protozoal Population

The influence of hog plum inclusion on rumen pH and NH_3-N concentrations at 2 h and 4 h after incubation is shown in Table 3. The ruminal pH of 2 h and, 4 h after incubation and average pH were not significantly different among various doses of hog plum supplementation (P>0.05). Average pH were ranged from 6.8 to 6.9 which was suitable rage for rumen microbial activity in the rumen (Calabrò et al., 2005). Concentration of NH_3-N at 2 h after incubation did not alter by hog plum level (P>0.05), whereas increasing hog plum levels were significantly increased NH_3-N concentration at 4 h after incubation. Average NH_3-N concentration were highest increased when hog plum supplementation at 2.0 to 2.5 mg and increased by 16.4 to 17.5%, respectively compared to no supplemented group. This could be due to additional of CP content from hog plum (5.6% CP) may provide protein supply for NH_3-N synthesis. However, optimum range of NH_3-N concentration were found in present study which could be indicate suitable rumen ecology and potential breakdown of feed when hog plum supplementation (11-20 mg%; Cherdthong et al., 2019).

Table 3: Influence of hog plum supplementation on ruminal pH, ammonia-nitrogen (NH_3-N) concentration and ruminal protozoal population at 2 h and 4 h after incubation.

Item	Hog plum supplementation, mg DM						P-value	Contrasts*	
	0	0.5	1.0	1.5	2.0	2.5		L	Q
Rumen parameters									
pH									
2 h	7.1	6.9	7.1	6.9	7.1	7.1	0.12	0.85	0.96
4 h	6.7	6.7	6.9	6.5	6.9	6.9	0.22	0.25	0.85
Mean	6.9	6.8	6.9	6.8	6.8	6.8	0.45	0.87	0.18
NH_3-N, mg%									
2 h	11.2	12.1	11.5	12.6	12.1	12.5	0.35	0.05	0.87
4 h	14.1^a	15.4^a	14.9^a	16.8^{ab}	18.7^b	17.8^b	0.04	0.02	0.76
Mean	12.7^a	13.8^a	13.2^a	14.7^{ab}	15.4^b	15.2^b	0.02	0.04	0.23
Ruminal protozoal population, x 10^6 cell/ml									
2 h	8.9	9.5	8.6	8.9	8.1	7.8	0.22	0.52	0.74
4 h	8.9^a	7.8^a	6.5^{ab}	5.3^b	2.3^c	2.5^c	0.03	0.04	0.88
Mean	8.9^a	8.7^a	7.6^a	7.1^{ab}	5.2^b	5.2^b	0.04	0.02	0.55

*L: Linear; Q: Quadratic; ^{a,b,c}: means with different superscripts within a column are significantly different at P ≤ 0.05.

Ruminal protozoal population at 2 h and 4 h after incubation with various doses of hog plum supplementation are shown in Table 3. Numerous tropical plants consisting great contents of saponins have become interesting choices for interrupting protozoa in the rumen (Gunun et al., 2019). At 2 h after incubation, there were no changed on protozoal counts (P>0.05), whereas the reduction of protozoal counts were found when added hog plum at 4 h after incubation (P<0.05). In addition, mean concentration of protozoa was reduced when increasing doses of added hog plum (P<0.05). Supplementation of hog plum at 2.0 to 2.5 mg could reduce protozoal population by 71.2% when compared to no supplemented group. This could possibly be because saponins may disturb the activity of ruminal protozoa, likely by attaching the proteins and enzymes of the protozoal cells (Cieślak et al., 2016). Thus, lowering in rumen protozoa could be relate to reduce CH_4 production. Previous report demonstrates that supplementation of saponins source from *Delonix regia* seed reduced protozoal counts by 58% to 68.3% (Supapong et al., 2017; Cherdtong et al., 2019). The results of this trial clearly demonstrate the possibility to use the tropical plant to manipulate rumen fermentation, which is very important for animal performances as well as for the environment (Hristov et al., 2013).

Table 4: Influence of hog plum supplementation on *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) at 12 h and 24 h after incubation.

Item	Hog plum supplementation, mg DM						P-value	Contrasts*	
	0	0.5	1.0	1.5	2.0	2.5		L	Q
IVDMD, %DM									
12 h	55.2	54.6	54.8	56.4	55.2	55.4	0.22	0.26	0.39
24 h	58.9	59.5	59.8	60.6	60.5	59.7	0.25	0.35	0.11
Mean	57.1	57.1	57.3	58.5	57.9	57.6	0.11	0.33	0.88
IVOMD, %DM									
12 h	60.1	61.2	60.6	61.3	61.1	62.3	0.77	0.45	0.36
24 h	64.6	65.1	65.8	66.8	65.5	64.1	0.85	0.87	0.78
Mean	62.4	63.2	63.2	64.1	63.3	63.2	0.24	0.98	0.18

*L: Linear; Q: Quadratic.

In Vitro Digestibility

Table 4 are shown the influence of hog plum supplementation on *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) at 12 h and 24 h after incubation. Supplementation of hog plum did not alter IVDMD and IVOMD and were average ranged from 57.1 to 58.5% DM and 62.4 to 64.1% DM, respectively (P>0.05). Thus, increasing doses of hog plum did not adversary effect on reduction of nutrient digestibility and maintain normal feed utilization. In contrast, Cherdtong et al. (2019) indicated that increasing the saponins source (*Delonix regia* seed) at 14 mg could be depressed *in vitro* fiber digestibilities by 10.5% *in vitro* digestibilities when compare to 12 mg. This might possibly be because high saponins in *Delonix regia* seed might inhibitory ruminal fibrolytic bacterial numbers via interfere enzymes activity and action on cell membranes and metal ion deprivation (Patra et al., 2010). However, no negatively influences of hog plum on feed digestion in present study might be due to the lower content of saponin (9.4 *vs* 12.3%) and lower dose supplementation (2.5 *vs* 16.7 mg) compared with the *Delonix regia* seed.

CONCLUSION

Based on the current study, it could be concluded that adding of hog plum did not adversary affect ruminal fermentation, the kinetics of gas, and *in vitro* digestibility. Moreover, the protozoa count was reduced hog plum was added up to 2.0 mg. However, the concerning the necessity of further researches to confirm the influence of hog plum supplementation directly measuring the methane production is needed to confirm the influence of hog plum supplementation.

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CONFLICT OF INTEREST

Authors declare no conflicts of interest of the manuscript.

AUTHORS' CONTRIBUTION

All authors contributed substantially to this study and are in full agreement with the content of the manuscript.

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