การปรับเปลี่ยนกระบวนการหมักในรูเมนด้วยอาหารอัดเม็ดที่มีสารประกอบเชิงซ้อนจากพืชเป็นองค์ประกอบในโคเนื้อ

Rumen manipulation by pellets containing plant secondary compound for beef cattle diet

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บทคัดย่อ: วัตถุประสงค์ของการทดลองครั้งนี้ เพื่อศึกษาผลของอาหารอัดเม็ดที่มีเมล็ดฝักหางนกยูงเป็นองค์ประกอบ (pellets containing Delonix regia seed meal; PEDEM) ต่อการกินได้ กระบวนการหมักในรูเมนและประชากรจุลินทรีย์ในโคเนื้อพื้นเมืองไทย โดยใช้โคจำนวน 4 ตัว น้ำหนักตัวเริ่มต้น 125 ± 5.0 kg ใช้แผนการทดลองแบบ 4 x 4 Latin Square เพื่อให้สัตว์ทุกตัวได้รับอาหารทั้ง 4 ปัจจัย ซึ่งประกอบไปด้วยระดับการเสริม PEDEM ที่ระดับ 0, 50, 100 และ 150 g/d ตามลำดับ PEDEM มีองค์ประกอบของโปรตีนหยาบ 25.42% ในขณะที่มีแทนนินส์และซาโปนินส์ที่ระดับ 84.75 mg/ 100 g DM และ 11.04 g/kg ตามลำดับ การกินได้ของอาหารและโภชนะ ไม่มีการเปลี่ยนแปลงตามระดับการเสริม PEDEM (P>0.05) โดยปริมาณการกินได้รวมอยู่ในช่วง 2.59-2.76 kg/d ซึ่งถือเป็นช่วงปกติของสัตว์ที่ได้รับฟางข้าวเป็นอาหารหยาบหลัก การเสริม PEDEM ที่ระดับ 50-150 g/d จะทำให้โคเนื้อได้รับแทนนินส์และซาโปนินส์ที่ระดับ 0.42-0.127 mg/ 100 g DM และ 0.006-0.017 g/kg ตามลำดับ การเสริม PEDEM ไม่ส่งผลต่อค่าความเป็นกรด-ด่างและอุณหภูมิในรูเมน (P>0.05) แต่มีผลทำให้ค่าความเข้มข้นของแอมโมเนีย-ไนโตรเจน มีค่าเพิ่มขึ้นเมื่อเปรียบเทียบกับกลุ่มควบคุม (P<0.05) ระดับการเสริม PEDEM ที่เพิ่มขึ้น ส่งผลทำให้ประชากรโปรโตซัวมีค่าลดลงแบบเป็นเส้นตรง (P<0.05) และมีค่าต่ำที่สุดเมื่อมีการเสริม PEDEM ที่ระดับ 150 g/d เมื่อเปรียบเทียบกับกลุ่มควบคุมพบว่า การเสริม PEDEM ที่ระดับ 150 g/d ทำให้ประชากรโปรโตซัวลดลง 76.64% หลังจากที่รับอาหารแล้ว 4 ชั่วโมง อย่างไรก็ตาม การเสริม PEDEM ไม่ส่งผลกระทบในทางลบต่อประชากรของแบคทีเรีย (P>0.05) ดังนั้น การเสริม PEDEM ที่ระดับ 150 g/d ในโคเนื้อพื้นเมืองไทย สามารถคงสภาพของการหมักในรูเมนให้ปกติ และลดประชากรของโปรโตซัวได้

คำสำคัญ: สารประกอบเชิงซ้อนจากพืช, กระบวนการหมักในรูเมน, แก๊สโลกร้อน, โปรโตซัว, การแปรรูปอาหาร

ABSTRACT: The aim of this experiment was to determine the pellets containing Delonix regia seed meal (PEDEM) on feed intake, rumen fermentation and microbial population in Thai native beef cattle. Four, Thai native beef cattle with initial body weight (BW) of 125 ± 5.0 kg were randomly assigned according to a 4 ×4 Latin square design to receive PEDEM supplementation at 0, 50, 100 and 150 g/d. PEDEM contain high concentration of CP at 25.42% while, tannins and saponins were 84.75 mg/ 100 g DM and 11.04 g/kg DM, respectively. The intake of feeds and nutrients were no significantly among treatments (P>0.05). The total intake ranged from 2.59 to 2.76 kg/d which was the normal range when animal fed on low quality roughage such as rice straw. Supplementation of PEDEM at 50

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to 150 g/d, the animals received tannins and saponins for 0.042 to 0.127 mg/ 100 g DM and 0.006 to 0.017 g/kg DM, respectively. Adding various doses of PEDEM did not alter ruminal pH and ruminal temperature (P>0.05). Concentration of NH3-N concentration was significantly different among various doses PEDEM supplementation (P<0.01). Increasing doses of PEDEM supplementation were linearly increased NH3-N concentration. Increasing doses of PEDEM were linearly reduced protozoal numbers (P<0.01) which was lowest concentration when PEDEM added at 150 g. Compared to no PEDEM fed group, supplementation PEDEM at 150 g reduced population of protozoa for 76.64% when rumen fluid was sampled at 4 h after feeding. However, population of total bacterial counts did not adversary affect by PEDEM supplementation (P>0.05). Supplementation of PEDEM at 150 g/d in Thai native beef cattle could maintain rumen fermentation, whereas reduce protozoal numbers.
Keywords: Plant secondary compound, Rumen fermentation, Greenhouse gas, Protozoa, Feed processing

Introduction

Manipulation of the rumen is more interesting particularly improve rumen efficiency, feed utilization and reduce environmental pollution (Kang et al., 2016). Strategic feeding of pellet containing phytochemical compounds to ruminant is one approach could enhance rumen fermentation and decrease greenhouse gas. Delonix regia (DR) a wild plant otherwise called flame of the forest originated from America (Madagascar) but now distributed in several countries of the tropical regions including Thailand. It is produces tones of pods containing seed which contain rich of tannins and saponins at 93.1 mg/ 100 g DM and 12.3 g/kg DM, respectively (Supapong et al., 2017). Previous study by Supapong et al. (2017) elucidated that DR seed meal successfully manipulate rumen fermentation, reduce protozoal population and CH4 production in beef cattle. However, the development of DR as pellet feed to improve the value added and to enhance rumen efficiency in beef cattle needs to be developed.

Therefore, the aim of this experiment was to determine the pellets containing Delonix regia seed meal (PEDEM) on feed intake, rumen fermentation and microbial population in Thai native beef cattle.

Materials and methods

Delonix regia (DR) seed pods were sun-dried for three weeks, and then the pods were easily opened for seed collection and grouKnd to pass through a 0.1 cm sieve (Cyclotech Mill, Tecator, Sweden). Then, the DR seed meal was mixed other ingredients (Table 1). The mixture was put into a pelleting machine and left to sun- dry for 2–3 d to reduce moisture before being fed to the animals. Four, Thai native beef cattle with initial body weight (BW) of 125 ± 5.0 kg were randomly assigned according to a 4 ×4 Latin square design to receive PEDEM supplementation at 0, 50, 100 and 150 g/h/d. PEDEM and 0.5% BW of concentrate were offered in two equal meals per day at 0700 and 1600. Rice straw was fed ad libitum. All steers were kept in individual pens, and clean fresh water and mineral blocks were available at all times. The experiment was 4 periods, and each lasted 21 d. During the first 14 d, all animals were fed their respective diets in the pens, while during the last 7 d they were moved

to metabolism crates for fecal and urine collection .Table 1 shows the chemical composition of the concentrates, PEDEM and rice straw. Feeds, refusals and fecal samples were chemically analyzed according to the standard method. Content of condensed tannins in PEDEM was analyzed by using the modified vanillin-HCl method while, saponins were analyzed by using the modified vanillin sulfuric acid method. At the end of each period, rumen fluid was collected at 0 h and 4 h after feeding. Approximately 100 ml of rumen fluid was taken from middle part of the rumen by a stomach tube connected to a vacuum pump. Rumen fluid was immediately measured for pH and temperature using (Hanna Instruments HI 8424 microcomputer, Singapore) after withdrawal. Rumen fluid samples were then filtered through 4 layers of cheesecloth. Samples were divided into 2 portions; first portion was used for NH3-N analysis with 5 ml of 1 mol H2SO4 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 NH3-N analysis using the Kjeltech Auto 1030 Analyzer. A second portion was fixed with 10% formalin solution in sterilized 0.9% saline solution. The total direct count of bacteria and protozoa were made based on the use of a hemocytometer (Boeco, Hamburg, Germany).

All 4×4 Latin square design data from the experiment were analyzed using the SAS GLM procedure. Significance was declared at $P < 0.05$ as representing statistically significant differences.

Results and discussion

Table 1 shown the chemical composition of PEDEM product and feed used in present study. PEDEM contain high concentration of CP at 25.42% which could be related to addition 1% urea in the ration. A feeding strategy when an ingredient containing a high level of urea included in the pellets is to provide the cattle with a readily available source of rumen-fermentable carbohydrates. D. regia and molasses are the ingredients which is rich in water-soluble carbohydrates, and is an inexpensive source of energy in some regions compared to starchy feeds. This would show a more synchronized supply of nitrogen and energy to rumen microbes, which might have led to an improved microbial protein synthesis in the rumen. Furthermore, PEDEM product consisted concentration of 84.75 mg/ 100 g DM of tannins and 11.04 g/kg DM of saponins, which could be beneficial on rumen manipulation particularly CH4 mitigation in the rumen. Concentration of tannins in PEDEM product was slightly lower than those previous pellet studies such as banana flower power pellet (96 mg/ 100 g DM; Kang et al., 2016) or mangosteen (Garcinia mangostana) peel pellet (99.5 mg/100 g DM; Norrapoke et al., 2012). This might be due to tannins concentration of the raw plants was differ among sources. Table 2 shows the intake of feeds and nutrients as affect by different doses of PEDEM supplement to animals. The intake of feeds and nutrients were no significantly among treatments ($P>0.05$). The total intake ranged from 2.59 to 2.76 kg/d which was the normal range when animal fed on low quality roughage such as rice straw. However, addition of PEDEM may provide more nutrients supply to rumen microorganisms and support animal host. Supplementation of PEDEM at 50 to 150 g/d, the animals received tannins and

saponins for 0.042 to 0.127 mg/ 100 g DM and 0.006 to 0.017 g/kg DM, respectively. These concentration of plant metabolites could be beneficial effect on ruminal fermentation and reduction of CH_4.

Table 1. Ingredient and chemical composition of concentrate, pellets containing *D. regia* seed meal (PEDEM) and rice straw.

Items	Concentrate	PEDEM	Rice straw
Ingredients, %DM			
Cassava chips	55.50	-	
Rice bran	11.00	-	
Coconut meal	13.00	-	
Palm kernel meal	13.40	-	
D. regia seeds meal	-	90.00	
Cassava starch	-	5.00	
Minerals and vitamins*	-	1.00	
Sulfur	1.00	1.00	
Urea	2.60	1.00	
Salt	1.00	1.00	
Molasses	2.00	1.00	
Chemical composition			
Dry matter, %	93.53	96.73	94.54
Organic matter, %DM	92.75	93.61	93.12
Crude protein, %DM	14.04	25.42	2.83
Neutral detergent fiber, %DM	11.85	18.95	66.87
Acid detergent fiber, %DM	7.97	12.31	43.37
Tannins, mg/100 g DM	-	84.75	-
Saponins, g/kg DM	-	11.04	-

Table 2 shows the rumen ecology, and ruminal microbes in Thai native beef cattle fed different levels of PEDEM. Adding various doses of PEDEM did not alter ruminal pH and ruminal temperature and which were suitable ranged for microbial enzyme activity of feed digestion. Ruminal pH and temperature were ranged from 6.84 to 6.92 and 38.71 °C to 39.20 °C which was similar reported by Supapong et al. (2017) who investigated in the same animal condition. Concentration of NH3-N concentration was significantly different among various doses PEDEM supplementation ($P<0.01$). Increasing doses of PEDEM supplementation were linearly increased NH3-N concentration. Supplementation of PEDEM at 150 g/d increased mean NH3-N concentration at 6.70 mg/dl when compared to no-PEDEM supplemented group. This may be due to high CP intake when PEDEM added, thus, lead to high CP available for microbial breakdown to NH3-N in the rumen. Rumen NH3 is the major end-product of protein CP diet 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 NH3 pool in the rumen. Thus, the high-CP content in the rumen could increase ruminal NH3-N concentration. These results were in agreement with Hung et al. (2013) indicated that concentration of ruminal NH3–N was increased with Leucaena leaf pellet supplementation at 450 g/d compared to with no-pellet fed group.

Table 2 Influence of different levels of pellets containing DR seed meal (PEDEM) on feed intake, nutrient intake, rumen fermentation and rumen microorganisms in Thai native beef cattle.

	Supplementation of PEDEM, g DM					Contrast	
	0	50	100	150	SEM	Linear	Quadratic
Total intake							
kg/day	2.59	2.67	2.76	2.75	0.18	0.50	0.83
g/kg $BW^{0.75}$	63.23	65.14	67.34	67.73	2.83	0.26	0.80
Nutrient intake, kg/d							
Dry matter	2.59	2.67	2.76	2.75	0.18	0.50	0.83
Organic matter	2.50	2.58	2.67	2.67	0.17	0.48	0.83
Crude protein	0.16^{a}	0.17^{a}	0.19^{a}	0.20^{b}	0.01	0.02	0.88
Neutral detergent fiber	1.65	1.69	1.74	1.72	0.13	0.70	0.83
Acid detergent fiber	1.04	1.07	1.10	1.09	0.08	0.64	0.81
Rumen ecology							
pH	6.84	6.90	6.92	6.90	0.06	0.42	0.56
Temperature, °C	39.01	38.71	38.91	39.20	0.18	0.29	0.15
NH_3-N, mg/dl	14.80^{a}	16.33^{b}	18.21^{c}	21.50^{d}	0.11	<0.01	<0.05
Ruminal microbes, cell/ml							
Protozoa, $x10^{6}$	11.51^{a}	6.01^{b}	4.93^{b}	2.73^{c}	0.6	<0.01	<0.05
Bacteria × 10^{8}	16.69	15.92	16.90	16.32	1.51	0.35	0.83

Effect of PEDEM supplementation doses on rumen protozoal population is show in Table 3. Increasing doses of PEDEM were linearly reduced protozoal numbers (P<0.01) which was lowest concentration when PEDEM added at 150 g. Compared to no PEDEM fed group, supplementation PEDEM at 150 g reduced population of protozoa for 76.64% when rumen fluid was sampled at 4 h after feeding. Reduction of protozoal population could be due to tannins and saponis contain in PEDEM which these compounds may inhibit the activity of rumen protozoa, likely by binding the proteins and enzymes of the protozoal membranes as noted by Cieślak et al. (2016). Similar results by Kang et al. (2016) who revealed that supplementation of banana flower powder pellet at 60 g/kg of substrate in in vitro study shows reduce protozoal numbers for 58.01% compared to no pellet added. In addition, the dairy cows receiving 300 g/d of mangosteen peel pellet could decreased

27.62% of protozoal counts, while Hung et al. (2013) found protozoa reduced 36.11% when cattle receiving Leucaena leaf pellet 450 g/d. Moreover, population of total bacterial counts did not adversary affect by PEDEM supplementation (P>0.05). Thus, supplementing of pellets containing plant secondary compounds could be beneficial on reduction of protozoal concentration and maintain bacterial population in the rumen.

Conclusions

Supplementation of PEDEM at 150 g/d in Thai native beef cattle could maintain feed intake and rumen fermentation, whereas reduce protozoal numbers. Thus, feeding of PEDEM might be an alternative feed product which is a potential for improve rumen fermentation efficiency and may reduce environmental effect for ruminant production. However, further research on diversity of methanogenic bacteria as affected by PEDEM are require to elucidate.

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References

Cieslak, A., P. Zmora, A. Matkowski, I. Nawrot-Hadzik, E. Pers-Kamczyc, M. El-Sherbiny and M. Szumacher-Strabel. 2016. Tannins from Sanguisorba officinalis affect in vitro rumen methane production and fermentation. J. Anim. Plant Sci. 26:54-62.

Hung, L.V., M. Wanapat, and A. Cherdthong. 2013. Effects of Leucaena leaf pellet on bacterial diversity and microbial protein synthesis in swamp buffalo fed on rice straw. Livest. Sci. 151:188–197.

Kang, S., M. Wanapat and V. Bounnaxay. 2016. Supplementation of banana flower powder pellet and plant oil sources on in vitro ruminal fermentation, digestibility, and methane production. Trop. Anim. Health Prod. 48:1673–1678.

Norrapoke, T., M. Wanapat and S. Wanapat. 2012. Effects of protein level and mangosteen peel pellets (mago-pel) in concentrate diets on rumen fermentation and milk production in lactating dairy crossbreds. Asian-Australas. J. Anim. Sci. 25:971–979.

Supapong, C., A. Cherdthong, A. Seankamsorn, B. Khonkhaeng, M. Wanapat, S. Uriyapongson, N. Gunun, P. Gunun, P. Chanjula and S. Polyorach. 2017. In vitro fermentation, digestibility and methane production as influenced by Delonix regia seed meal containing tannins and saponins. J. Anim. Feed Sci. 26:123-130.