EFFECT OF OILS AND GRAPE SEED PROANTHOCYANIDIN EXTRACT ON RUMINAL FERMENTATION AND METHANE PRODUCTION IN DAIRY GOATS

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ABSTRACT

An *in vitro* experiment was conducted to investigate effects of supplementing oil or in combination with grape seed proanthocyanidin (PA) extract on true digestibility, ruminal fermentation and methane (CH_4) emission in dairy goats. For this purpose, four non lactating dairy goats were received an adapted diet containing concentrate and elephant grass (40:60, in DM) for 7 days. Animals were then collected rumen fluid before morning feeding to conduct batch *in vitro* fermentation. This study was carried out as a completely randomized design with 7 treatments and 4 replicates. A control diet consisted of concentrate and elephant grass at 40:60 while other 6 treatments were supplementation of 2.5% oil (either only soybean oil [S] or a mixture of soybean oil and tuna fish oil [SF]) combined without or with grape seed proanthocyanidin extract (contained 95% PA[P]) at 0, 0.4 and 0.8% DM, corresponding to Ctrl, SP_0, SFP_0, $SP_{0.4}$, $SP_{0.8}$, $SFP_{0.4}$ and $SFP_{0.8}$, respectively. Treatment diets had no effect on pH, NH_3-N concentration and *in vitro* true digestibility (P>0.05), however total VFA concentration at 48h incubation was greater in SFP_0 compared to that in Ctrl (P<0.05). Cumulative total gas production remained unchanged when feeding either oil alone or in combination withgrape seed PA extract (P>0.05). Methane (CH_4) concentration showed a strong drop in $SP_{0.8}$ and $SFP_{0.8}$ contrary to those in Ctrl and SP_0 (P<0.05). This resulted in decreased CH_4 production in both $SP_{0.8}$ and $SFP_{0.8}$ related to Ctrl (P<0.05), accounting for 33.57 and 34.90%, respectively. The present study demonstrates that feeding combination of grape seed PA extract at 0.8% DM with either soybean oil alone or a blend of soybean oil and tuna fish oil at 2.5% DM strongly suppresses CH_4 production without adverse effect on digestibility and ruminal fermentation in dairy goats.

Keywords: *digestibility, methane, oil, proanthocyanidin, ruminal fermentation*

INTRODUCTION

In recent years, dairy goat production rapidly develops in Viet Nam. Although average income per capita has been increasing, many health risks related to the modern lifestyle increase as well. Subsequently, customers are looking for natural and healthy food sources, including ruminant milk. In another thing, production of greenhouse gases (GHG) from livestock and their impact on climate change is a currentmajor concern (O'Mara, 2011). Enteric methane (CH_4) is one of the most important anthropogenic GHG emitted at the farm level in ruminant production systems, ranging from 48-65% in bovine milk production systems and from 56 to 65% in New Zealand dairy farms (Basset-Mens et al., 2009). The enteric CH_4 emission was projected to increase by over 30% from 2000 to 2020 (O'Mara, 2011). Moreover, energy loss from animals due to CH_4 production ranges from 2-12% of gross energy intake in mature cattle (Johnson and Johnson, 1995). An *in vitro* study showed that fish oil decreased CH_4 production by 30-50% (Castro-Montoya et al., 2012). Szczechowiak et al. (2016) reported that feeding oil blends had high potential to reduce CH_4 production in the ruminant. Thus, feeding oils and CT to dairy goats may bring not only environmentally friendly for the planet by mitigating CH_4 emission but also nutritional benefits for the animal. This study aimed to find out the proper level of CT in the diet which can reduce CH_4 production without or less affecting ruminal fermentation and digestibility.

MATERIALS AND METHODS

Materials

Feed: elephant grass, soybean oil, tuna fish oil, grape seed proanthocyanidin extract, ground corn, rice bran, soybean meal, copra meal, $CaCO_3$, DCP, NaCl, mineral and vitamin mix.

Animal: 4 non-lactating dairy goats (♂ Saanen × ♀ Bach Thao) used to collect rumen fluid.

Site and duration of study

This study was conducted from March to June 2020 at Laboratory of Ruminant Production Techniques, Department of Animal Sciences, College of Agriculture, Can Tho University.

Experimental design and treatments

This study was divided into two experiments including: 1) syringe gas production technique to measure gas and CH_4 production, and 2) bottle incubation to determine ruminal fermentation and *in vitro* digestibility. The experiments were conducted as a completely randomized design with the treatments included: 1) basal diet (60% elephant grass + 40% concentrate) without supplementing oil or PA (Ctrl), 2) 2.5% soybean oil (SP_0), 2.5% soybean oil and tuna oil (3:2) (SFP_0), 2.5% soybean oil + 0.4% grape seed proanthocyanidin extract ($SP_{0.4}$), 2.5% soybean oil + 0.8% grape seed proanthocyanidin extract ($SP_{0.8}$), 2.5% soybean oil and tuna oil (3:2) + 0.4% grape seed proanthocyanidin extract ($SFP_{0.4}$), and 2.5% soybean oil and tuna oil (3:2) + 0.8% grape seed proanthocyanidin extract ($SFP_{0.8}$). Soybean oil was supplemented as a pure product while tuna oil was added as crude oil. A commercial grape seed proanthocyanidin extract was used in this study as a source of CT, in form of PA.

Substrates, rumen fluid, and medium preparation

Elephant grass was ground in a Retsch mill (Cutting Mill SM 100 model, Retsch, Haan, Germany) to pass a 1-mm mesh prior to *in vitro* incubation. The incubation substrate consisted of elephant grass, concentrate and grape seed proanthocyanidin extract were mixed as calculated ratio in Table 1 and stored until incubation. Oils were prepared and added into incubation syringes and bottles as an oil-ethanol solution (1:15, w/v). Chemical characteristics of feeds used in this study are presented in Table 2.

Table 1. Feed ingredients used in the study

Item	Treatments						
	Ctrl	***SP_0***	***SFP_0***	***$SP_{0.4}$***	***$SP_{0.8}$***	***$SFP_{0.4}$***	***$SFP_{0.8}$***
Ingredient (%DM)							
Elephant grass	60.00	58.50	58.50	58.26	58.02	58.26	58.02
Ground corn	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Rice bran	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Soybean meal	23.60	24.69	24.69	24.86	25.04	24.86	25.04
Copra meal	4.20	2.11	2.11	1.78	1.44	1.78	1.44
$CaCO_3$	0.90	0.90	0.90	0.90	0.90	0.90	0.90
DCP	0.60	0.60	0.60	0.60	0.60	0.60	0.60
NaCl	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Mineral and vitamin mix	1.40	1.40	1.40	1.40	1.40	1.40	1.40
Soybean oil	-	2.50	-	2.50	2.50	-	-
Soybean oil + Tuna oil (3:2)	-	-	2.50	-	-	2.50	2.50
Grape seed proanthocyanidin extract	-	-	-	0.4	0.8	0.4	0.8
Total	*100*	*100*	*100*	*100*	*100*	*100*	*100*
Roughage (%)	60.00	58.50	58.50	58.26	58.02	58.26	58.02
Concentrate (%)	40.00	39.00	39.00	38.84	38.68	38.84	38.68
Dietary CP (%)	16.60	16.60	16.60	16.60	16.60	16.60	16.60
Dietary DM (%)	45.41	44.31	44.31	44.13	43.96	44.13	43.96

Note: CP: crude protein, DM: dry matter.

Rumen fluid were collected before the morning feeding from four non-lactating dairy goats (♂ Saanen×♀ Bach Thao) fed a diet based on elephant grass and 20% CP concentrate (R:C 60:40, w/w on DM basis). The animals were fed twice daily at 07:30 and 17:00 for 1-week period priorto taking the rumen fluid. Rumen fluid was collected (300 ml/goat), immediately kept in thermos flasks, and then filtered through a metal sieve into pre-warmed thermos flasks to retain small particles.

Medium solution was prepared according to Menke and Steingass (1988) with some minor modifications. Briefly, KH_2PO_4 content in 1 L macromineral solution was reduced from 6.2 g to 6.0 g while $FeCl_2.6H_2O$ concentration in 1 L micromineral solution was increased from 0.8 g to 8.0 g. Medium solution was also added tryptone, L-cysteine hydrochloride and sodium sulfite.

Table 2. Chemical composition (%) of feeds used in the study

Ingredient	DM	OM	Ash	CP	NDF	PA
Elephant grass	15.19	91.43	8.57	7.27	67.35	-
Ground corn	87.90	99.01	0.99	8.11	27.71	-
Rice bran	91.58	88.38	11.62	7.45	48.86	-
Soybean meal	90.32	93.08	6.92	45.58	11.03	-
Copra meal	88.97	95.47	4.53	18.50	41.68	-
Grape seed proanthocyanidin extract	100	-	-	-	-	95

Note: DM: dry matter, OM: organic matter, Ash: total mineral, CP: crude protein, NDF: neutral detergent fiber, PA: proanthocyanidin.

In vitro incubation

To measure ruminal fermentation products and *in vitro* true digestibility, substrates were weighed to 625 mg of DM into pre-vacuum 100-mL glass bottle. However, to measure total gas and CH_4 production, substrates were weighed to 625 mg of DM into 100-mL glass syringes. Incubated bottles and syringes were then supplemented with 250 µL of oil-ethanol solution which providing 15.63 mg of added oil. Four blank syringes for gas production were added 250 µL of absolute ethanol (99.99%) without any substrate and supplementation. Under continuous CO_2 flushing, 50 mL mixture (1:4, v/v) of filtrated rumen fluid andpre-warmed (39°C) medium were introduced into gas tight glass syringes and bottles. The syringes were incubated in a shaking water bath (WNB 45, Memmert, Germany) at 39°C for 48 h. All bottles were incubated in a shaking incubator (ISS-4075R, Jeiotech, Korea) at 39°C for 48 h.

Sampling, measurements, and chemical analysis

Rumen fluid was collected at 24 and 48 h post incubation. Incubation was stopped by placing the bottles into ice-cold water, and the pH of bottle contents was immediately measured by pH meter (HI5222, Hana Instruments, US). The incubated contents were then filtrated through four layers of cheesecloth. The samples for NH_3-N and VFA analyses were acidified with 1 M H_2SO_4 (10/1, v/v), centrifuged and supernatant was then stored at – 20°C. At 24 and 48 h post inoculation, some bottle samples were stopped to determine *in vitro* true digestibility (IVTD) following the method described by Van Soest and Robertson (1985). Gas volume produced was recorded at 2, 4, 6, 8, 10, 12, 18, 24, 36, and 48 h incubation. Gas production was collected into gas bags (22952, Restek, US) and stored until analysis of CH_4 concentration. Dry matter (DM) and crude protein (CP) were determined by methods of AOAC (1990). Neutral detergent fiber (NDF) was determined using the methods described by Van Soest et al. (1991), adapted for Fiber Analyzer. The rumen NH_3-N concentration was determined by a micro Kjeldahl method

(AOAC, 1990). The samples (without acid digestion) were distillated throught a distillation system in boric acid (2%) and then titrated with diluted sulfuric acid (0.1 N). Concentration of total volatile fatty acids (VFA) was determined following the method of Barnett and Reid (1957). Methane concentration in gas samples was measured by gas analyzer (GeoTech GA5000, Queensway, UK).

Statistical analysis

Data were analyzed by ANOVA procedure of Minitab 16.2 for a completely randomized design. Overall differences between treatment means were considered to be significant as $P<0.05$, mean while a tendency toward significance was declared at $0.10>P\geq0.05$. Significant differences among treatment means were assessed by Tukey's multiple comparison tests after a significant F-test. Data are expressed as mean ± SEM, which represents the pooled SEM for the model.

RESULTS AND DISCUSSION

Ruminal fermentation patterns and digestibility

The treatment diet had no effect on ruminal pH and NH_3-N concentration at 24 and 48 h post incubation (Table 3). That NH_3-N concentration ranged from 36.05 to 60.90 mg/dL in this study was in the agreement with Khang et al. (2016), who reported that's rumimal NH_3-N concentration was between 39.89 and 70.55 mg/dL after 48 h incubation. VFA content in SFP_0 (139.9 mM) was higher ($P<0.05$) than that in the Ctrl (99.6 mM). Kook et al. (2002) also found the greater VFA concentration when animals were fed fish oil at 5% in the diet. However, Roy et al. (2017) reported no response on total VFA concentration as diet was added soybean oil. Ferreira et al. (2015) found that increasing amount of soybean oil and fish oil blend in the diet had caused no shift of total VFA concentration. That treatment diet had no any influence on IVTD in this study was in agreement with the finding of Roy et al. (2017), who reported that supplementing different oil sources at 3 and 4% didn't show any effect on *in vitro* DM digestibility.

Table 3. Ruminal fermentation patterns and digestibility

Item[1]	Treatment[2]							SEM	P
	Ctrl	***SP_0***	***SFP_0***	***$SP_{0.4}$***	***$SP_{0.8}$***	***$SFP_{0.4}$***	***$SFP_{0.8}$***		
pH									
24 h	6.66	6.63	6.72	6.75	6.76	6.77	6.77	0.05	0.209
48 h	6.46	6.61	6.67	6.76	6.73	6.76	6.80	0.08	0.069
NH_3-N, mg/dL									
24 h	53.55	53.67	60.90	56.00	54.25	53.55	49.70	6.36	0.938
48 h	46.20	50.40	50.40	44.80	36.05	44.80	42.70	4.35	0.310
VFA, mM									
24 h	108.0	105.8	137.6	134.7	100.4	130.4	127.0	15.99	0.499
48 h	99.6^{b}	109.9^{ab}	139.9^{a}	122.4^{ab}	116.9^{ab}	130.8^{ab}	131.7^{ab}	7.42	0.014
IVTD, %									
24 h	73.46	75.11	73.77	74.78	74.98	73.87	75.06	0.74	0.503
48 h	79.04	79.03	78.12	79.37	79.57	80.76	79.86	1.27	0.863

Note: [1]VFA: volatile fatty acid, IVTD: in vitro true digestibility.

[2]Ctrl: only basal diet, SP_0: 2.5% soybean oil, SFP_0: 2.5% soybean oil + tuna oil (3:2), $SP_{0.4}$: 2.5% soybean oil + 0.4% grape seed proanthocyanidin extract, $SP_{0.8}$: 2.5% soybean oil + 0.8% grape seed proanthocyanidin extract, $SFP_{0.4}$: 2.5% soybean oil + tuna oil (3:2) + 0.4% grape seed proanthocyanidin extract, and $SFP_{0.8}$: 2.5% soybean oil + tuna oil (3:2) + 0.8% grape seed proanthocyanidin extract.

[a,b]Means within a row with different superscripts are significantly different at $P<0.05$.

Total gas and CH_4 production

Total gas production remained unchanged among the treatments (Table 4). Szczechowiak et al. (2016) reported that oil blend and CT had no effect on total gas production in dairy cows. Treatment diet didn't have any effect on total gas production during the early (0-6 h) and late periods (24-48 h) of incubation (Figure 1). However, at the middle stage of incubation (8-12 h), Ctrl diet always showed the higher value of total gas production ($P<0.05$) compared to other diets, and the similar trend was observed at 18 h incubation($P<0.1$).Combination of single oil or oil blend with PA at a high dose caused a remarkable drop ($P<0.05$) of CH_4 concentration relative to Ctrl. As a result of reducing CH_4 concentration without changing total gas production, CH_4 production (mL) significantly decreased ($P<0.05$) in $SP_{0.8}$ and $SFP_{0.8}$ compared to Ctrl, accounting for 33.57 and 34.90%, respectively.This was in agreement with Szczechowiak et al. (2016), who indicated that supplementing a mixture of oil and CT decreased CH_4 production in dairy cows.

Table 4. Total gas and CH_4 production

Item[1]	Treatment[2]							SEM	P
	Ctrl	***SP_0***	***SFP_0***	***$SP_{0.4}$***	***$SP_{0.8}$***	***$SFP_{0.4}$***	***$SFP_{0.8}$***		
Total gas (48 h)									
mL	141.5	133.2	128.4	125.1	121.2	125.9	125.1	7.01	0.490
mL/gDM	226.5	213.1	205.5	200.1	193.9	201.5	200.1	11.21	0.490
mmol	6.32	5.95	5.73	5.58	5.41	5.62	5.58	0.31	0.490
mmol/gDM	10.11	9.51	9.17	8.93	8.65	8.99	8.93	0.50	0.490
Methane (48 h)									
%	17.73^{a}	17.51^{a}	16.66^{ab}	15.11^{ab}	13.51^{b}	14.71^{ab}	13.01^{b}	1.56	0.044
mL	24.96^{a}	23.28^{ab}	21.47^{ab}	19.22^{ab}	16.58^{b}	18.56^{ab}	16.25^{b}	2.04	0.045
mL/gDM	39.93^{a}	37.24^{ab}	34.36^{ab}	30.76^{ab}	26.52^{b}	29.70^{ab}	26.00^{b}	3.26	0.045
mmol	1.11^{a}	1.04^{ab}	0.96^{ab}	0.86^{ab}	0.74^{b}	0.83^{ab}	0.73^{b}	0.09	0.045
mmol/gDM	1.78^{a}	1.66^{ab}	1.53^{ab}	1.37^{ab}	1.18^{b}	1.33^{ab}	1.16^{b}	0.15	0.045

Note: [1]DM: dry matter.

[2]Ctrl: only basal diet, SP_0: 2.5% soybean oil, SFP_0: 2.5% soybean oil + tuna oil (3:2), $SP_{0.4}$: 2.5% soybean oil + 0.4% grape seed proanthocyanidin extract, $SP_{0.8}$: 2.5% soybean oil + 0.8% grape seed proanthocyanidin extract, $SFP_{0.4}$: 2.5% soybean oil + tuna oil (3:2) + 0.4% grape seed proanthocyanidin extract, and $SFP_{0.8}$: 2.5% soybean oil + tuna oil (3:2) + 0.8% grape seed proanthocyanidin extract.

[a,b]Means within a row with different superscripts are significantly different at $P<0.05$.

A decrease in CH_4 production in the diet containing oil and PA may result from hydrogen reduction, a substrate for rumen microbiota (Wencelová et al., 2015). Our findings suggest a synergistic effect of feeding both oil and PA on CH_4 reduction, we did not observe any effect on CH_4 production when diet was added only oils. The decreased CH_4 production in the $SP_{0.8}$ and $SFP_{0.8}$ diets probably happened due to the unsaturated fatty acids (UFA), which escaped from ruminal biohydrogenation as supplementing both oils and condensed tannins in the diet (Carreño et al., 2015). UFA may reduce protozoa counts, hence protozoa-associated methanogens, and may be also direct inhibitory effect on the membrane transport of methanogens (Beauchemin et al., 2008). Earlier studies (Patra and Yu, 2013, 2015) concluded that binary and ternary combination of anti-methanogenic inhibitors with complementary mechanisms of actions on methanogenesis may alter the archaeal communities and may decrease CH_4 production additively without negatively impacting upon rumen fermentation

and nutrient degradability. We also found an additive effect in decreasing CH_4 production as oil and PA were combined in the diet. The decrease of rumen CH_4 concentration, concurrently with a constant ruminal fermentation products and true digestibility in diets containing both oils and PA at high dose suggested that the hydrogen was recast to end-products capturing more digestible energy from fermented organic matter, resulting in a more efficient usage of feed energy (McGuffey et al., 2001).

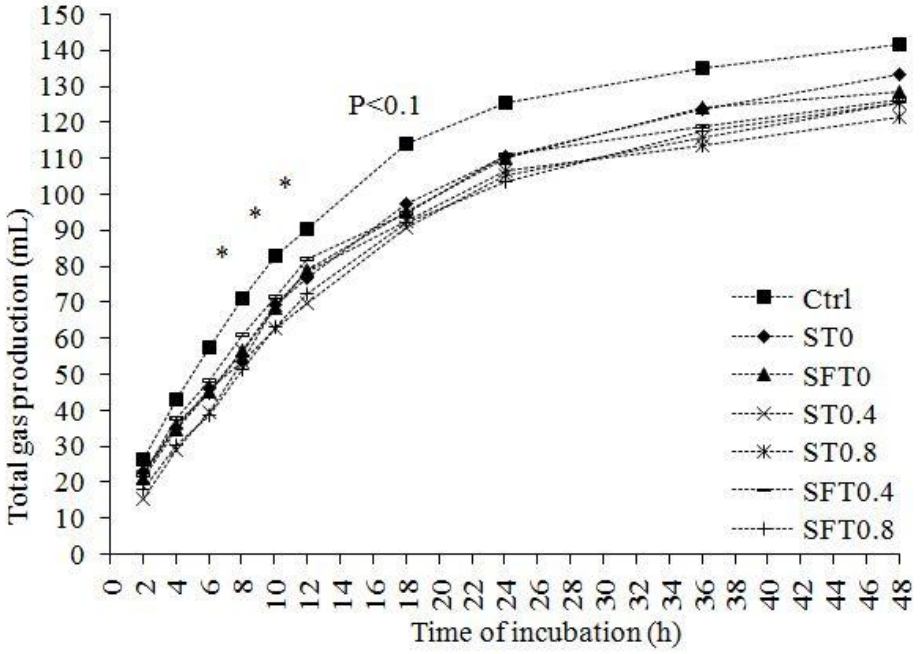

Figure1. Cumulative gas production changes during the incubation. *: P<0.05

CONCLUSIONS

Supplementing combination of grape seed proanthocyanidin extract at 0.8% DM with either soybean oil with or without tuna fish oil at 2.5% DM strongly suppresses CH_4 production without adverse effect on digestibility and ruminal fermentation in dairy goats.

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