

Effects of Supplemental Virgin Coconut Oil and Condensed Tannin Extract from Pine Bark in Lactation Dairy Diets on Ruminal Fermentation in a Dual-Flow Continuous Culture System

Yang SY^{1*}, Ningrat RWS², Eun J-S¹ and Min BR³

¹Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT, USA

²Faculty of Animal Sciences, Andalas University, Padang, Indonesia

³Department of Agricultural and Environmental Sciences, Tuskegee University, Tuskegee, AL, USA

*Corresponding author: Si-Yong Yang, Department of Animal, Dairy and Veterinary Sciences, Utah State University, 4815 Old Main Hill, Logan, UT 84322-4851, USA, Tel: +1 435 757 1361; E-mail: sy.yang@usu.edu

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Abstract

The objective of our study was to investigate effects of virgin coconut oil (VCO) and condensed tannin extract from pine bark (PCT), either separately or in combination, as supplements to lactation dairy diets on *in vitro* ruminal fermentation profiles in a completely randomized design with four independent runs of continuous cultures. Four dietary treatments included: 1) control [CONT; total mixed ration (TMR) without supplement], 2) TMR with VCO (VCOT), 3) TMR with PCT (PCTT) and 4) TMR with VCO and PCT (VPT). Results showed that culture pH was maintained at least at 6.13 across dietary treatments, and supplementing VCO and/or PBE did not influence culture pH. Total volatile fatty acid concentration was similar in response to the supplements. Supplementing PCT decreased ammonia-nitrogen concentration both in PCTT and VPT ($P < 0.01$), while VCO supplementation resulted in no effect on ammonia-nitrogen concentration. Cultures offered VCO and PCT supplementation, either separately or in combination, showed no response on methane production. The decrease in ammonia-nitrogen concentration when PCT-containing diets (PCTT and VPT) were offered is likely attributed to condensed tannins in PCT, which indicates that PCT can affect dietary nitrogen utilization efficiency *in vivo* through condensed tannins on ruminal nitrogen metabolism. However, dietary concentration of VCO used in this study may have not been enough to manipulate ruminal fermentation.

Keywords: Continuous culture; Condensed tannin extract from pine bark; Ruminal fermentation; Virgin coconut oil

Introduction

Improving feed efficiency and reducing nutrient excretion into the environment are essential elements for sustainable dairy production worldwide. In high quality forage diets fed ruminants, majority of dietary proteins can be rapidly degraded, releasing between 56 and 65% of dietary nitrogen (N) in the rumen during microbial fermentation. Consequently, large losses of N as urea into urine (25-35%) occur after ammonia is absorbed through rumen wall [1], which is the primary source of volatile N to the environment [2]. Thus, losses of dietary N can be reduced by decreasing protein degradation in the rumen. Simultaneously, methane (CH₄) is produced in the rumen as a part of the normal process of ruminal feed digestion. Typically, about 6 to 10% of the total gross energy consumed by dairy cows is converted to CH₄ [3] which contributes to greenhouse gas emissions in the environment. A variety of strategies have been studied to improve ruminal N metabolism and mitigate CH₄ production, and feeding or supplementing specific substances as rumen modifiers that directly or indirectly inhibit ruminal N degradation as well as methanogenesis has been one of the most sought opportunities [4].

Natural plant compounds such as condensed tannins (CT) and coconut oil (CCO) have been recommended as potential ruminal fermentation modifiers to reduce dietary N degradation and methanogenesis in the rumen. Condensed tannins are prevalent in

many plants and can reduce ruminal protein degradation, which can increase intestinal protein flow when provided at moderate doses of 20 to 40 g·kg⁻¹ CT in dry matter (DM) [5]. Pine (*Pinus taeda* L.) tree bark (PB) is one of the abundant timber industry by-products and contains up to 110 g·kg⁻¹ CT on a DM basis [6]. Condensed tannins in PB are mostly procyanidins; total CT consisted of 876 g·kg⁻¹ procyanidins and 124 g·kg⁻¹ prodelphinidins [7]. Min et al. [6] reported that feeding PB increased average daily gain and feed efficiency without adverse effects on animal health when added to meat goat diets up to 300 g·kg⁻¹ DM, and the positive effects were ascribed to various functional effects of CT by altering ruminal fermentation: reduction of ammonia-N (NH₃-N) concentration and beneficial shifts in volatile fatty acid (VFA) composition. On the other hand, certain plant oils and dietary lipids have been identified as potential CH₄-suppressing feed ingredients for ruminants [3]. Coconut oil as a source of medium-chain fatty acids (MCFAs) proved to sizable reduction in CH₄ emissions *in vivo* [8]. The CCO produced through the wet method is known as virgin coconut oil (VCO). The VCO is rich in MCFAs [60-63 g·100 g⁻¹ fatty acid (FA) methyl esters; 7.19-8.81% C8:0, 5.65-6.59% C10:0, 46.9-48.0% C12:0 and 16.2-18.9% C14:0] [9], and consequently it has a potential to be used as a rumen modifier to lessen ruminal CH₄ production.

We hypothesized that supplementation of CT extract from pine bark (PCT) and VCO in lactation dairy diets would alter the ruminal fermentation pattern in a desirable manner by reducing NH₃-N concentration and CH₄ production as a result of inhibiting microbial proteolytic activity and methanogenesis. Hence, the purpose of our study was to evaluate the effect of PCT and VCO supplementation and

their combined treatment on *in vitro* fermentation characteristics with focus on NH₃-N and CH₄.

Materials and Methods

Experimental design, diets and continuous culture operation

Experimental design: Four dietary treatments were tested in a completely randomized design with four independent runs of continuous cultures (n = 4). The four dietary treatments included: 1) control (CONT; TMR without supplement), 2) TMR with VCO (VCOT), 3) TMR with PCT (PCTT) and 4) TMR with VCO and PCT (VPT; Table 1). Before use in fermentors, the diets were dried at 55°C for 48 h and ground through a 4.0 mm screen (standard model 4, Arthur Thomas Co., Philadelphia, PA, USA).

Item	Experimental diet ^a			
	CONT	VCOT	PCTT	VPT
Ingredient (g·kg ⁻¹)				
Alfalfa hay	480	480	480	479
Corn silage	166	167	143	141
Corn grain, steam-flaked	166	130	148	114
Beet pulp, shreds	53.6	55.5	61	57.1
SBMCM ^b	116	120	120	133
VCO	0	29.8	0	29.6
PCT	0	0	29.8	29.5
Vitamins and minerals ^c	18.1	17.5	17.4	17.4
Chemical composition (g·kg ⁻¹ dry matter)				
Dry matter (g·kg ⁻¹)	944 ± 0.3	946 ± 0.1	942 ± 0.1	944 ± 0.2
Organic matter	894 ± 1.8	895 ± 0.7	893 ± 2.7	897 ± 1.9
Crude protein	204 ± 2.0	198 ± 2.4	205 ± 2.2	204 ± 2.2
Neutral detergent fiber	282 ± 6.4	270 ± 42.0	282 ± 11.2	249 ± 40.7
Acid detergent fiber	182 ± 5.9	177 ± 25.8	182 ± 7.1	157 ± 31.2
Ether extract	9.3 ± 1.12	43.1 ± 2.51	10.2 ± 3.02	48.5 ± 2.33
NFC ^d	399	359	407	373
NEL ^e (MJ·kg ⁻¹ DM)	6.74	7.2	6.82	7.28
Condensed tannins	0.5	1.3	21.2	24.6

Table 1: Ingredients and chemical composition of experimental diets offered to continuous cultures with virgin coconut oil (VCO) and/or condensed tannins extract from pine bark (PCT). ^aCONT = TMR without supplement, VCOT = TMR with VCO, PCTT = TMR with PCT, and VPT = TMR with VCO and PCT. ^bSBMCM, mixture of soybean meal and canola meal at 50:50 on a DM basis. ^cFormulated to contain (per kg DM): 226.7 mg of Se (from sodium selenite), 9278.7 mg of Cu (from copper amino acid complex), 40,537.4 mg of Zn (from zinc amino acid complex), 38,653.4 mg of Mn (from manganese amino acid complex), 552.6 mg of Co (from cobalt carbonate), 1,234,585.2 IU of vitamin A, 152,808.1 IU of vitamin D, 3,815.1 IU of vitamin E, and

295 mg of Rumensin (Elanco Animal Health, Greenfield, IN, USA). ^dNon-fiber carbohydrates = 1000 - CP - NDF - ether extract - ash (g·kg⁻¹ DM). ^eNet energy for lactation based on tabular value [10,11].

Diets: Condensed tannin extract from pine bark was prepared by an extraction procedure using a mixture of 70% acetone containing 0.1% ascorbic acid, and plant pigments and waxes were removed by three rounds of methylene chloride extraction. The remaining CT fractions were freeze-dried, re-dissolved in 50% methanol (v/v) and purified by gel filtration using a Sephadex LH-20 column (Pharmacia, Uppsala, Sweden) washing with 50% methanol (v/v) and elucidating with 70% acetone (v/v) [10]. Then, purified CT were freeze-dried and stored in the dark at 4°C for the future application.

Virgin coconut oil obtained from University of Andalas, Indonesia, contained 8.9% C8:0, 7.0% C10:0, and 51.0% C12:0 on a g·100 g⁻¹ FA methyl esters basis. Both PCT and VCO were added at 29.8 g·kg⁻¹ DM to lactation dairy TMR with a forage-to-concentrate ratio of 63:37 on average (Table 1). As a source of forage, alfalfa hay was included in the diets at a same dietary proportion (480 g·kg⁻¹ DM) across the diets. In addition, a mixture of soybean meal and canola meal (50:50 on a DM basis) was added to the diets at a similar dietary concentration (122 g·kg⁻¹ DM) so that crude protein (CP) concentration was similar across the diets (203 g·kg⁻¹ DM on average). The diets were formulated to meet NRC [11] recommendations for rumen degradable and undegradable proteins, minerals and vitamins of a mid-lactation dairy cow weighing 780 kg (body condition score = 3.0) and producing 36.3 kg of milk·d⁻¹ containing 35 g·kg⁻¹ fat and 30 g·kg⁻¹ true protein.

Continuous culture operation: Ruminal fluid (average pH of 6.9) was collected before the morning feeding (i.e. 07:00 h) from two ruminally cannulated lactating Holstein dairy cows fed a TMR composed of 432 g·kg⁻¹ chopped alfalfa hay, 224 g·kg⁻¹ corn silage, 226 g·kg⁻¹ rolled corn grain and 118 g·kg⁻¹ concentrate (DM basis). Care, handling and sampling of the donor animals used in this study were approved by the Utah State University Institutional Animal Care and Use Committee. Ruminal fluid was collected from various locations within the rumen, placed into preheated insulated containers, and transported to the laboratory where rumen contents were strained through a polyester screen (PeCAP, pore size 355 µm; B and SH Thompson, Ville Mont-Royal, QC, Canada). The filtered ruminal inoculum (700 mL) was added to each continuous culture fermentor. A dual-flow continuous culture system with airtight glass culture vessels (1 L total capacity) was used, and fermentor design and operating conditions were reported elsewhere [12].

Each independent run lasted 8 d (5 d of treatment adaptation and 3 d of data and sample collection). The first day of each run allowed for microbial adaptation by offering CONT to all fermentors, and experimental diets were offered to pre-determined, corresponding fermentors from d 2. Anaerobic condition in the fermentors was maintained by infusion of CO₂ at a rate of 20 mL·min⁻¹. Artificial saliva prepared according to Slyter et al. [13] was continuously infused into fermentors at a rate of 1.2 mL·min⁻¹ using a peristaltic pump (Model 323, Watson-Marlow Inc., Wilmington, MA, USA) to maintain a fractional dilution rate of 10.0 %·h⁻¹. To mimic rumen motility, cultures were continuously stirred by a central paddle attached to an electric motor. Each fermentor received a total of 20 g of DM·d⁻¹ that was fed in two equal portions at 08:00 and 20:00 h. Diets were manually fed to the fermentors through a feed port on the fermentor vessel.

Sample collection

On d 6 and 7 of each run, culture pH was measured hourly through a pH electrode connected to a pH meter (sympHony™ B10P benchtop meters, VWR International, Inc., Radnor, PA, USA). At 08:00, 12:00 and 16:00 h, CH₄ samples were collected from the headspace gas of each fermentor using a 10 µL gastight syringe (Hamilton Co., Reno, NV, USA) and analyzed for CH₄ with a GLC (Model CP-3900, Varian, Walnut Creek, CA, USA). Daily CH₄ production (mM·d⁻¹) was calculated as reported by Williams et al. [14] using the equation: CH₄ proportion in fermentor headspace (mM·mL) × CO₂ gas flow through the fermentor headspace (20 mL·min⁻¹) × 60 min × 24 h. Two sets of 5 mL culture fluid samples were collected for VFA and NH₃-N analysis.

Chemical analysis

Analytical DM content of samples was determined by oven drying at 105°C for 3 h ([15]; method 930.15), and organic matter was determined by ashing at 550°C for 5 h ([15]; method 942.05). Content of N was determined using an organic elemental analyzer (Flash 2000; CE Elantech Inc., Lakewood, NJ, USA) ([15]; method 990.03). Neutral detergent fiber (aNDF) and acid detergent fiber, both expressed inclusive of residual ash, were sequentially determined using an ANKOM^{200/220} Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) according to the methodology supplied by the company, which is based on the methods described by Van Soest et al. [16]. Heat stable α-amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation, St. Louis, MO, USA) and sodium sulfite were used in the aNDF analysis. Ether extract was measured ([15]; method 2003.05) using a fat analyzer (ANKOM Technology). Total extractable CT concentration in experimental diets was determined using a butanol-HCl colorimetric procedure [17,18].

Culture VFA were separated and quantified using a GLC (Model 6890 series II, Hewlett Packard Co., Avondale, PA, USA) with a capillary column (30 m × 0.32 mm i.d., 1 µm phase thickness, Zebron ZB-FAAP, Phenomenex, Torrance, CA, USA) and flame ionization detection. The oven temperature was held at 170°C for 4 min, increased to 185°C at a rate of 5°C·min⁻¹, then increased by 3°C·min⁻¹ to 220°C and held at this temperature for 1 min. The injector and the detector temperatures were 225 and 250°C, respectively, and the carrier gas was helium [19]. Concentration of NH₃-N was determined as described by Rhine et al. [20] using a plate reader (MRX^c, Dynex Technologies, Chantilly, VA, USA).

Statistical analysis

Data were analyzed using the MIXED procedure of SAS [21]. Dietary treatment was included as a fixed effect, with independent run and fermentor as random effects in the model. Denominator degrees of freedom were estimated using the Kenward-Roger option. The same mixed model was used for variables that were repeated in time (i.e. culture pH, NH₃-N and CH₄), but sampling time and a repeated statement were added to the model. One of three model structures was used depending on the finite sample corrected Akaike's information criterion value for data that best fit the model. The structures were unstructured and compound symmetry, unstructured and first-order autoregressive, and unstructured and unstructured variance-covariance structure. Data for culture pH, VFA concentration and

profile [except acetate - to - propionate (A:P) ratio] and total CH₄ production were reported using the unstructured and compound symmetry structure, whereas data for A:P ratio, CH₄ production and NH₃-N concentration were reported using the unstructured and unstructured variance-covariance structure. Effects were declared significant if P<0.05, and trends were discussed at 0.05 ≤ P<0.10.

Results and Discussion

Experimental diets

Ingredients and nutrient composition of diets are presented in Table 1. The diets ranged in CP content from 198-205 g·kg⁻¹ DM. Supplementing VCO sizably increased ether extract contents in VCOT and VPT, whereas it decreased contents of non-fiber carbohydrates in both of the diets. Contents of CT in PCT-containing PCTT and VPT were 21.2 and 24.6 g·kg⁻¹ DM, respectively.

Culture pH and VFA profiles

Supplementation of VCO and/or PCT did not affect culture pH and concentration of total VFA which averaged 6.19 and 37.2 mM, respectively (Table 2). The cultures in this study were offered high-forage diets, and consequently it was expected that dietary treatments would not have a negative effect on culture pH. Total VFA concentration did not differ in response to supplementing VCO and/or PCT. Therefore, supplementation of VCO and PCT at 29.8 g·kg⁻¹ DM in lactation dairy TMR with a forage-to-concentrate ratio of 63:37 on average would not interfere with ruminal physiological conditions.

Supplementation of VCO decreased acetate proportion in VCOT, but not in VPT (Table 2). Cultures offered VCO-containing diets (VCOT and VPT) increased butyrate proportion. Dohme et al. [22] observed that supplementing CCO (53 g·kg⁻¹ DM) decreased propionate and increased butyrate proportions by suppression of ciliates in continuous cultures. Van Nevel and Demeyer [23] reported that supplementing C10:0 and C12:0 at concentrations of 0.01-0.1 g·L⁻¹ stimulated butyrate-producing bacteria (*Butyrivibrio* sp.).

Supplementation of PCT increased acetate proportion, while it did not influence propionate proportion, leading to a tendency for an increase in A:P ratio (P = 0.10; Table 2). Valerate proportion decreased by PCTT, whereas isobutyrate proportion decreased by VPT. According to meta-analysis of in vitro studies (15 experiments and 130 treatments) by Jayanegara et al. [24], acetate proportion tended to increase (P = 0.08), while propionate tended to decrease (P = 0.08) with increasing contents of dietary tannins, resulting in an increase in ratio of Dschaak et al. [25] reported that molar proportions of acetate, propionate, and butyrate increased in a high-forage lactation dairy diet (a forage-to-concentrate ratio of 59:41), but not in a low-forage lactation dairy diet (a forage-to-concentrate ratio of 41:59) due to quebracho (*Schinopsis* sp.) CT extract supplementation at 30 g·kg⁻¹ DM to dairy cows, resulting in interactions between forage level and CT supplementation. Additionally, supplementing quebracho CT extract in the high-forage diet decreased A:P ratio, but in the low-forage diet, leading to an interaction between forage level and CT supplementation. Thus, dosage rate, source of CT, dietary composition, and their interactions will clearly contribute to inconsistent effects of CT on VFA patterns.

Item	Dietary treatment ^a				SEM	P
	CONT	VCOT	PCTT	VPT		
Mean culture pH	6.18	6.21	6.13	6.23	0.07	0.27
Total VFA (mM)	37.8	38.6	38.4	34.1	1.81	0.19
Individual VFA (mol·100 mol ⁻¹)						
Acetate (A)	52.7 ^b	48.1 ^c	55.1 ^a	52.8 ^{ab}	1.95	<0.01
Propionate (P)	31.4 ^a	29.2 ^{ab}	28.2 ^{abc}	26.0 ^c	1.55	0.02
Butyrate	9.81 ^c	14.6 ^a	11.6 ^{abc}	14.5 ^{ab}	1.38	0.01
Valerate	3.33 ^b	4.78 ^a	2.31 ^c	3.72 ^b	0.89	<0.01
Isobutyrate	0.63 ^a	0.63 ^{ab}	0.52 ^{abc}	0.47 ^c	0.06	<0.01
Isovalerate	1.44	1.63	2.08	1.49	0.13	0.08
A:P	1.71	1.68	1.97	2.03	0.13	0.1

Table 2: Ruminal pH and volatile fatty acid (VFA) profiles in continuous cultures offered lactation dairy diets with virgin coconut oil (VCO) and/or condensed tannins extract from pine bark (PCT). ^aCONT = TMR without supplement, VCOT = TMR with VCO, PCTT = TMR with PCT, and VPT = TMR with VCO and PCT. ^{a-c}Means within a row that do not have a common superscript differ at P<0.05.

Concentration of NH₃-N

Compared with CONT, supplementing PCT decreased NH₃-N concentration in both PCTT and VPT by 37 and 24%, respectively (Figure 1A). However, VCO supplementation resulted in no effect on NH₃-N concentration. The decrease in NH₃-N concentration when PCT-containing diets (PCTT and VPT) were offered is likely attributed to CT in PCT. Min et al. [6] reported a linear decrease in ruminal NH₃-N concentration when meat goats were fed with pine bark at 150 and 300 g·kg⁻¹ DM. The N-binding effects of CT have been well documented [3,26]. Tannin-protein binding activity in the PCT-containing diets would be expected to decrease NH₃-N concentrations in two ways: 1) reducing dietary protein degradation via formation of insoluble tannin-protein complexes or decreasing the solubility of protein [1,27]; and 2) inhibiting proteolytic bacteria and/or proteolytic enzyme activity [28]. *In vivo*, this in turn would shift protein to digestion in the small intestine and be expected to increase N utilization efficiency. Under typical cattle feeding conditions, manipulation of ruminal protein degradation or the efficiency of N utilization in the rumen is the most effective strategy to reduce N losses [29]. The presence of optimum CT contents (20-40 g·kg⁻¹ DM) in the diet can reduce protein degradation in the rumen and improve bypass protein flow to the small intestine [5], thus enabling more enzymatic hydrolysis of dietary protein in the lower tract. Hence, the reduction in the NH₃-N concentration through CT in PCT-containing diets can contribute to improving utilization of dietary N in ruminal fermentation and reducing N excretion *in vivo*. Although binding of tannins to protein improves efficiency of N utilization in ruminants [30], it may influence CP digestibility depending upon their content and source. In fact, a diet containing 18 g·kg⁻¹ CT from big trefoil resulted in a reduced CP digestibility, whereas the same content of CT from birdsfoot trefoil had less effect on CP digestibility [31]. In the current study, the decrease in NH₃-N concentration when PCT-containing diets (PCTT and VPT) were offered indicates that PCT has a potential to affect dietary N utilization efficiency *in vivo* through CT on ruminal N metabolism.

Methane production

Cultures offered VCO and PCT supplementation, either separately or in combination, showed no response on CH₄ production, although feeding VCOT numerically decreased CH₄ production by 13.5% compared with CONT (Figure 1B). Medium-chain FA, which is present in high contents in CCO, is known to modify ruminal fermentation and to mitigate greenhouse gas emissions [32]. *In vitro* ruminal protozoal populations were completely eradicated by MCFA (particularly C10:0 and C12:0) within 6 h and decreased by long-chain unsaturated FA (C18:3, C18:2, C18:1) [33]. Odongo et al. [34] observed a 36% CH₄ reduction in dairy cattle fed a TMR with 50 g·kg⁻¹ (DM) C14:0 supplementation. Dong et al. [35] reported that the supplementation of 100 mL·L⁻¹ of CCO at a whole orchardgrass (*Dactylis glomerata*) hay diet and a mixture of 900 g·kg⁻¹ wheat and 100 g·kg⁻¹ hay diet decreased the CH₄ production compared with control diets at 0.51 vs. 1.84 mmol·d⁻¹ and 0.49 vs. 3.94 mmol·d⁻¹, respectively, in continuous cultures. On the other hand, the effectiveness of adding lipids to the diet to reduce CH₄ emissions depends on many factors including level of supplementation, fat source, FA profiles, form in which the fat is administered (i.e. either as refined oil or as full-fat oilseeds) and the type of diet [36]. Refined oils that are high in MCFA, such as CCO, palm kernel oil, canola oil or pure C14:0 are particularly effective in reducing CH₄, especially toward high-concentrate diets and low Ca diets [37]. The primary mechanism whereby MCFA reduce CH₄ is through the toxicity they exhibit on rumen methanogens [37]. The authors indicated that with a forage-based diet, probably more C14:0 was attached to the feed particles and less to the methanogens than with a concentrate-based diet [37]. Similarly, Cosgrove et al. [38] observed that oil infusion (3:1 mixture of linseed oil and sunflower oil) up to 50 g·kg⁻¹ DM intake to the rumen of sheep fed ryegrass pasture did not affect CH₄ production. Consequently, a potential effect of the VCO tested in the current study may have been inhibited on ruminal fermentation possibly due to the binding between VCO and forages (630 g·kg⁻¹ DM) in the diets. Another possibility for the absence of VCO supplementation would be

a relatively low dietary content of VCO (29.8 g·kg⁻¹ DM) used in the present study.

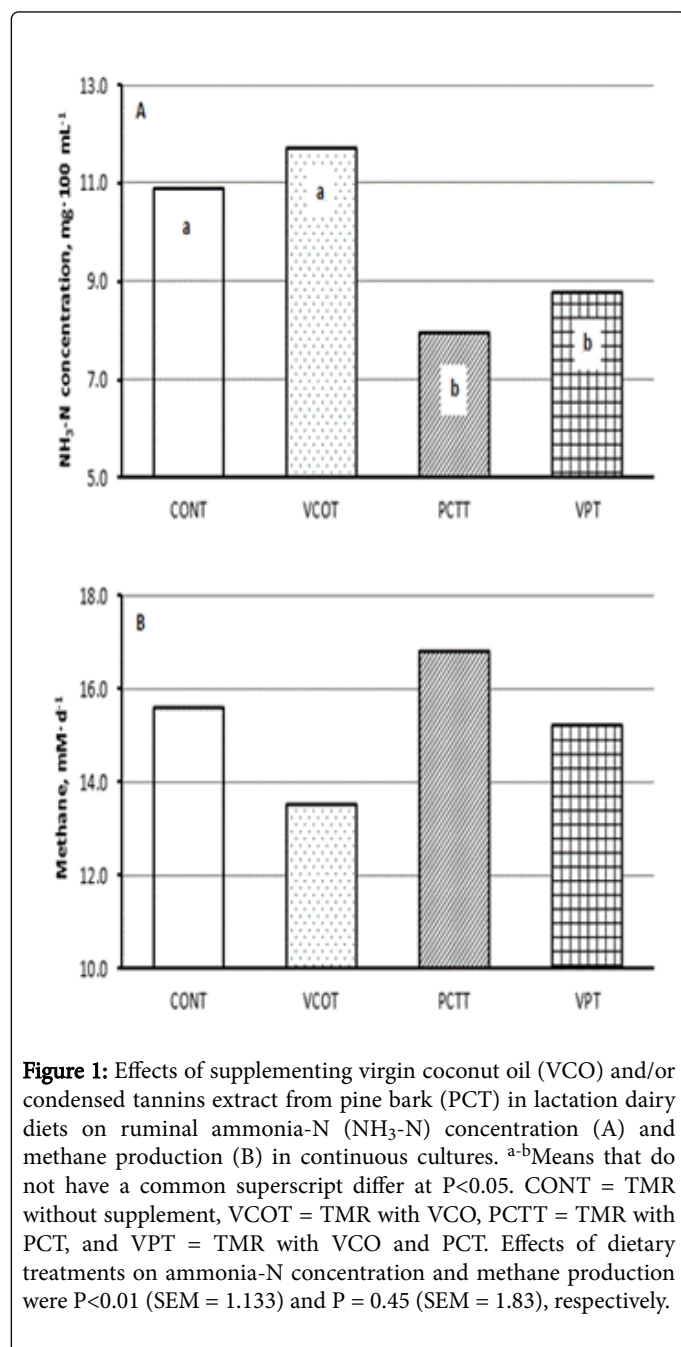


Figure 1: Effects of supplementing virgin coconut oil (VCO) and/or condensed tannins extract from pine bark (PCT) in lactation dairy diets on ruminal ammonia-N (NH₃-N) concentration (A) and methane production (B) in continuous cultures. ^{a-b}Means that do not have a common superscript differ at P<0.05. CONT = TMR without supplement, VCOT = TMR with VCO, PCTT = TMR with PCT, and VPT = TMR with VCO and PCT. Effects of dietary treatments on ammonia-N concentration and methane production were P<0.01 (SEM = 1.133) and P = 0.45 (SEM = 1.83), respectively.

Studies using CT extract or CT-containing forages reported reductions in CH₄ emissions [14,39]. The CT modify growth of rumen microflora, reduce feed protein degradation, and lower feed energy losses to CH₄ [40]. However, it seems that findings on whether CT are able to genuinely suppress ruminal CH₄ formation per unit of digestible nutrient and the extent to which this effect occurs, appear to be inconsistent [24]. For example, Beauchemin et al. [3] found that feeding quebracho CT extract at up to 20 g·kg⁻¹ of DM failed to reduce CH₄ emissions from growing beef cattle. The authors indicated that the reason for this discrepancy may be due to the different CT, and/or that the level of supplementation of quebracho tannin at 20 g·kg⁻¹ DM

would be below the threshold required to cause a reduction in CH₄ production [3]. Condensed tannins from various plant species have shown not only dose-dependent, but also plant-specific effects on ruminal fermentation [41], which could be related to their different chemical structures [30] and molecular weights [39,42]. Meta-analysis by Jayanegara et al. [24] indicated that the variation in CH₄ production per digestible organic matter *in vivo* was very high at low concentrations of dietary tannins of <20 g·kg⁻¹ DM, whereas variability clearly decreased with increasing tannin concentrations. The authors suggested that the influence of other dietary components such as proteins and carbohydrates may mask that of tannins at low levels [24]. McAllister et al. [43] reported that CT extracted from different plants vary greatly in their capacity to bind carbohydrates and proteins. In the current study, CT concentration (21.2 and 24.6 g·kg⁻¹ DM for PCTT and VPT, respectively) in PCT-containing diets would be insufficient to lessen ruminal methanogenesis.

Conclusion

Overall results on the present study partially supported our hypothesis by reducing NH₃-N concentration due to PCT supplementation in lactation dairy diets. We have yet to explore the effects of supplementing PCT on microbial community structure to address N transaction, microbial growth and energetic efficiency. Supplementation of PCT and/or VCO showed no response on CH₄ production. Therefore, the concentration of PCT and VCO used in this study would not be enough to affect ruminal protozoal population and methanogenic archaea. Our study demonstrated that PCT, although not effective in mitigating CH₄ production, has a potential to improve N utilization efficiency in lactation dairy diets through the decreased NH₃-N production in ruminal fermentation. However, caution should be exercised due to the fact that CT fed at relatively high concentrations may have negative effects on feed intake in ruminants, and the effects may also vary with the source of CT. Thus, further research needs to be carried out to confirm the *in vitro* result on N metabolism in lactating dairy cows fed with PCT and its long-term effects on lactational performance as a feed additive to improve N utilization efficiency by dairy cows.

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