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Difference in the nature of tannins on in vitro ruminal methane and volatile fatty acid production and on methanogenic archaea and protozoal populations

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ABSTRACT

Six plant sources of hydrolyzable tannins (HT) or HT and condensed tannins (CT; designated as HT1, HT2, HT3, HT + CT1, HT + CT2, and HT + CT3) were evaluated to determine their effects in vitro on CH_4 production and on ruminal archaeal and protozoa populations, and to assess potential differences in biological activities between sources containing HT only or HT and CT. Samples HT1, HT2, and HT3 contained only HT, whereas samples HT + CT1, HT + CT2, and HT + CT3 contained HT and CT. In experiment 1, in vitro incubations with samples containing HT or HT + CT resulted in a decrease in CH_4 production of 0.6 and 5.5%, respectively, compared with that produced by incubations containing the added tannin binder polyethylene glycol-6000. Tannin also suppressed the population of methanogenic archaea in all incubations except those with HT2, with an average decrease of 11.6% in HT incubations (15.8, 7.09, and 12.0 in HT1, HT2, and HT3) and 28.6% in incubations containing HT + CT (35.0, 40.1, and 10.8 in HT + CT1, HT + CT2, and HT + CT3) when compared with incubations containing added polyethylene glycol-6000. The mean decrease in protozoal counts was 12.3% in HT and 36.2% in HT + CT incubations. Tannins increased in vitro pH, reduced total VFA concentrations, increased propionate concentrations, and decreased concentrations of iso-acids. In experiment 2, when a basal diet was incubated with graded levels of HT + CT1, HT + CT2, and HT + CT3, the total gas and CH_4 production and archaeal and protozoal populations decreased as the concentration of tannins increased. Our results confirm that tannins suppress methanogenesis by reducing methanogenic populations in the rumen either directly or by reducing the protozoal population, thereby reducing methanogens symbiotically associated with the protozoal population. In addition, tannin sources containing both HT and CT were more potent in suppressing methanogenesis than those containing only HT.
Key words: tannin, methane, methanogenic archaea, protozoon

INTRODUCTION

Ruminal methanogenic organisms use hydrogen produced during carbohydrate fermentation to reduce CO_2 to CH_4, thereby maintaining low partial pressures of hydrogen, which allows the oxidation of reduced NAD (Schonhusen et al., 2003). Despite this beneficial role in the rumen microbial ecosystem, the production of CH_4 is an energetically wasteful process to ruminants (Anderson et al., 2003). Methane emission by ruminants has received considerable attention because of its contribution to global warming (Lassey, 2007). Therefore, CH_4 reduction strategies should improve ruminant production efficiency and mitigate global warming.

Direct ruminal intervention is a means to control ruminant CH_4 emissions (Joblin, 1999), because CH_4-producing archaea, known as methanogens, are a distinct group of organisms that form a normal component of the rumen microbial ecosystem (Tavendale et al., 2005). Hydrogen and CO_2 are the major substrates for ruminal methanogens, and compounds that inhibit the activity of methanogens are likely to reduce or eliminate CH_4 production. Based on their structure and chemical properties, tannins are divided into hydrolyzable tannins [**HT**, which have a central carbohydrate core to which number of phenolic carboxylic acids are bound by esters of gallic acid (gallotannin) or ellagic acid (ellagitannins)] and condensed tannins (**CT**, or proanthocyanidines, which have no carbohydrate core and are derived by condensation of flavonoid precursors or polymers of flavonoids; Baker, 1999). Although tannins are generally regarded as antinutritional, certain tannins at low concentrations alter ruminal fermentation (Bhatta et al., 2002) and microbial protein synthesis (Bhatta et al., 2001). Tannins also reduce ruminal CH_4 production when included either as temperate legumes (Waghorn et al., 2002) or as purified tannin extracts

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Table 1. Nutrient composition of the TMR, hydrolyzable tannins (HT), or hydrolyzable and condensed tannins (HT + CT)[1]

	HT[2]			HT + CT[3]			
Item	HT1	HT2	HT3	HT + CT1	HT + CT2	HT + CT3	TMR[4]
DM	88.6	90.9	90.9	88.7	93.4	90.5	87.5
OM (g/kg of DM)	98.6	98.7	95.7	92.2	98.2	91.0	95.9
Ash (g/kg of DM)	1.40	1.29	4.35	7.80	1.80	9.00	4.07
CP (g/kg of DM)	1.02	2.39	1.42	0.710	2.13	1.10	13.1
NDF (g/kg of DM)	4.03	3.98	4.07	4.06	3.86	5.06	41.6
ADF (g/kg of DM)	2.01	1.96	2.14	1.78	1.85	2.67	24.2
Gross energy (Mcal/kg of DM)	3.70	3.78	3.56	4.45	4.78	4.37	4.04
Tannin concentration (% of DM)							
HT	13.0	13.2	18.5	3.94	7.62	7.78	—
CT	—	—	—	1.33	3.67	1.50	—

[1]HT = hydrolyzable tannin as gallotannin; CT = condensed tannin as leucocyanidin equivalent.

[2]HT1, HT2, and HT3 = samples containing only HT (HT1 from myrabolam; HT2 and HT3 from chestnut).

[3]HT + CT1, HT + CT2, and HT + CT3 = samples containing HT plus CT (HT + CT1 and HT + CT2 from quebracho; HT + CT3 from mimosa).

[4]TMR contained 65% timothy hay, 20% crushed corn, and 15% soybean meal.

(Roth et al., 2002). However, there are no reports on potential differences in the activities of HT and CT on CH_4 production and on methanogenic archaeal and ciliated protozoal populations. The present study was conducted to determine the effects of plant materials containing different tannins (HT or HT + CT) on in vitro CH_4 production and on ruminal archaeal and ciliated protozoal populations, and to determine the difference, if any, in tannin sources containing HT only or both HT and CT.

MATERIALS AND METHODS

Source of Tannins

Six commercially available natural sources of tannins (designated as HT1, HT2, HT3, HT + CT1, HT + CT2, and HT + CT3) were used in this study. According to information obtained from the supplier (Kawamura and Co. Ltd., Asakusabashi, Taito-ku, Tokyo, Japan), HT1 (130 g of HT/kg of DM) was an extract from myrabolam, HT2 (132 g of HT/kg of DM) and HT3 (185 g of HT/kg of DM) were from chestnut, HT + CT1 (39.4 g of HT/kg of DM + 13.3 g of CT/kg of DM) and HT + CT2 (76.2 g of HT/kg of DM + 36.7 g of CT/kg of DM) were from quebracho, and HT + CT3 (77.8 g of HT/kg of DM + 15.0 g of CT/kg of DM) was from mimosa (Table 1). The products were supplied in the form of a fine dry powder.

Tannin Estimation

A 0.1-g sample of each tannin source was extracted with 10 mL of 70% (vol/vol) aqueous acetone in a 50-mL stoppered Erlenmeyer flask for 20 h at room temperature. After centrifugation at 2,795 × *g* for 15 min, the supernatant was made up to 10 mL with butanol HCl in the presence of iron, using rhodanine reagent (Makkar, 2003). Condensed tannin was expressed as leucocyanidin equivalent and hydrolyzable tannin as gallotannin (Makkar, 2003).

In Vitro Gas Production

In vitro gas production was determined by the procedure of Menke and Steingass (1988). In experiment 1 (with tannin-containing samples only), the effects of the different tannin-containing samples on in vitro methanogenesis was assessed by incubating samples with and without addition of a tannin binder, polyethylene glycol (**PEG**; PEG-6000, Wako Pure Chemical Industries Ltd., Osaka, Japan; 400 mg). Tannin-containing samples (200 mg) were weighed into 100-mL calibrated glass syringes (Häberle Labortechnik, Ettlenschieß, Germany) with pistons lubricated with Vaseline. Buffered mineral solution (Menke and Steingass, 1988) was prepared and placed in a water bath at 39°C under continuous flushing with CO_2. Rumen fluid was collected before the morning feeding from 3 ruminally cannulated, nonlactating Holstein cows (466 kg of mean BW) fed 5.8 kg of timothy hay, 1.6 kg of crushed corn, and 0.80 kg of soybean meal. Ruminal contents were collected into a prewarmed insulated flask, transported to the laboratory, homogenized, and filtered through 3 layers of cheesecloth. An anaerobic condition was maintained by continuous flushing with CO_2. The well-mixed and CO_2-flushed, strained ruminal fluid was added to the buffered mineral solution (1:2). The mixture was kept stirred with a magnetic stirrer under CO_2 in a water bath at 39°C. A mixture of ruminal fluid and buffer