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Effects of hydrolyzable tannin with or without condensed tannin on methane emissions, nitrogen use, and performance of beef cattle fed a high-forage diet[1,2]

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ABSTRACT: Sustainability of animal agriculture requires efficient use of energy and nitrogen (**N**) by ruminants fed high-forage diets. Thus, there is a need to decrease methane (CH_4) emissions and prevent excessive N release into the environment. Therefore, this experiment examined the long-term effects of feeding hydrolyzable tannin (**HT**) with or without condensed tannin (**CT**) on animal performance, rumen fermentation, N use, and CH_4 production in beef cattle fed a high-forage diet. A total of 75 weaned crossbred steers (292 ± 4.1 kg) were grouped by body weight (BW), housed in individual pens, and randomly assigned to 1 of 5 dietary treatments (15 animals/treatment) in a completely random design. The animals were fed a basal diet of alfalfa:barley silages (50:50; dry matter [DM] basis) with a crude protein content of 17.1% and supplemented with HT extract (chestnut, **CN**) or a combination (50:50) of HT and CT extracts (quebracho, **Q**) in a powdered form at different levels of dietary DM. The treatments for determining animal performance and N use were control (no tannin), 0.25% CN, 1.5% CN, combination of CN and Q at 0.125% each (0.25% CNQ), and CN and Q at 0.75% each (1.5% CNQ) of dietary DM. The treatments for the CH_4 measurement were control, 1.5% CN, and 1.5% CNQ of dietary DM. The first 84 d of the study were used to measure animal performance, rumen fermentation, and N use, and the next 30 d were used to measure CH_4 emissions with the tracer gas technique. There were no effects of treatment on DM intake (DMI), BW, average daily gain, and gain: feed ($P \geq 0.10$). The plasma urea N concentration was greater ($P < 0.05$) for 1.5% CN and 1.5% CNQ than those fed 0.25% CNQ (120.9 and 120.4 vs. 111.7 mg/L, respectively), but not different ($P > 0.05$) from animals fed control or 0.25% CN (117.2 and 117.5 mg/L, respectively). Tannin inclusion did not affect rumen pH, total volatile fatty acid concentration, proportions of acetate and propionate, and total protozoa populations ($P \geq 0.16$). Tannin, irrespective of type or dose, decreased ($P < 0.01$) ruminal ammonia concentration. Tannin type and dose did not affect ($P = 0.54$) daily CH_4 production (154 ± 5.9 g/d) but 1.5% CNQ tended to decrease CH_4 yield compared with control (20.6 vs. 22.0 g/kg DMI; $P = 0.094$). HT from CN alone or in combination with CT from Q can be added at a low (0.25% DM) or high (1.5% DM) level to a forage-based diet to decrease ruminal ammonia concentration in growing beef cattle fed a high-protein diet without adverse effects on animal performance. A combination of HT and CT at a concentration of 1.5% dietary DM also tended to decrease CH_4 emissions without negatively affecting performance.

Key words: beef cattle performance, enteric methane, high-forage diet, nitrogen use, tannin

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INTRODUCTION

There is inefficiency in dietary nitrogen (**N**) utilization by cattle that are fed forage diets high in soluble protein, because greater intake of soluble N increases N excretion in feces and urine (Dijkstra et al., 2013). In addition, growing concerns of elevated methane ($\mathbf{CH_4}$) emissions from ruminant production have prompted efforts to develop mitigation strategies. Beef cattle fed high-forage diets lose about 6% to 12% of their energy intake into the atmosphere as enteric CH_4 (Johnson and Johnson, 1995) representing a potential source of inefficiency. Tannins are plant secondary compounds with the ability to form complexes with proteins and carbohydrates that can potentially improve N use and decrease CH_4 production (Patra and Saxena, 2011). However, the effects of these polyphenolic polymers may depend on the type and quantity consumed.

Tannins can be categorized as condensed tannin (**CT**) or hydrolyzable tannin (**HT**) based on their structure and reactivity. CTs have high binding capacity for dietary protein and can decrease degradability of protein in the rumen, which can be beneficial for animals fed diets with high concentrations of rumen degradable protein. For instance, feeding the CT-containing forage birdsfoot trefoil as a sole diet improved weight gain of sheep due to an increase in amino acid absorption from the small intestine (Wang et al., 1996; Waghorn, 2008). However, net flow of metabolizable protein (MP) to the small intestine is not always increased when feeding tannins because increased flow of undegraded feed protein may be offset by decreased microbial crude protein (CP) synthesis (Wang et al., 1996; MacAdam and Villalba, 2015). Consequently, the effects of tannins on N use and animal performance can be highly variable due to factors such as origin, concentration, molecular structure, and dosage of tannin.

CTs can also decrease enteric CH_4 production (Carulla et al., 2005; Grainger et al., 2009), but the binding capacity of CT may also decrease fiber digestibility (Reed, 1995). HTs have smaller molecular weight (500 to 3,000) than CT (1,900 to 28,000). Compared with CT, HT have a weaker affinity for proteins and thus are more easily absorbed from the digestive tract increasing potential toxicity to the animal (McLeod, 1974). For this reason, previous research on use of tannin in ruminant diets focused on CT rather than HT. However, an in vitro study indicated that there was no difference between tannin sources in preventing protein degradation (Getachew et al., 2008). Furthermore, supplementing HT at 3% of dry matter (DM) showed no evidence of toxicity to sheep; yet, CH_4 emissions were decreased by 24% compared with control (Liu et al., 2011). HTs can permeate rumen protozoa and exert toxic effects, thereby disrupting their association with methanogens (Patra and Saxena, 2011).

Feeding HT or CT at a dose of 0.6% dietary DM to feedlot Holstein steers had no effect on growth or DM intake (DMI), but a combination of the 2 sources (0.3% each) increased average daily gain (ADG) and DMI compared with the control treatment (Rivera-Méndez et al., 2016). The relatively low dose of tannin used in the study of Rivera-Mendez et al. (2016) may have avoided potential toxicity of HT to steers. Aguerre et al. (2015) supplemented a combination of HT and CT at 0%, 0.45%, and 1.8% of dietary DM in the diet of lactating dairy cattle and reported a linear increase in DMI with no adverse effect on milk production but a decrease in milk urea N and urine N. Thus, there may be an advantage of combining CT and HT as a supplement for beef cattle. Both sources of tannins may improve N use, but CT may decrease hydrogen available for methanogens (Reed 1995), whereas HT may exert toxic effects on methanogens (Jayanegara et al., 2015). However, there is limited information available on their combined effects and optimum dose.

Therefore, we hypothesized that a combination of HT and CT at an optimum dose compared with the control may lower rumen ammonia concentration (an indicator for N excretion) and decrease CH_4 emissions without adverse effects on animal performance. The objective of the study was to determine the effects of HT alone and in combination with CT at low and high doses on growth performance, N use, ruminal fermentation, protozoa populations, and CH_4 emissions in growing beef steers.

MATERIALS AND METHODS

The experimental protocol (ACC 1630) was reviewed and approved by the Animal Care

Committee of the Lethbridge Research and Development Centre. The animals were cared for and the experiment was conducted according to the guidelines of the Canadian Council on Animal Care (2009).

Animals, Diet, and Experimental Design

A total of 75 weaned crossbred steers with an initial body weight (BW) of 292 ± 4.1 kg (mean ± SEM) were grouped by BW (3 groups; 25 steers per group) and randomly assigned to 1 of 5 dietary treatments (15 animals per treatment) within group. Before the study, the steers were processed using standard management procedures for ear tagging and vaccination. Over a 2-wk period, they were gradually introduced to the control diet and adapted to their individual pens (2.5 × 3 m) bedded with wood shavings. Each pen had a separate feeder, and animals in adjacent pens shared a water bowl. The steers were fed a basal diet of alfalfa and barley silages (50:50; DM basis) and a supplement containing minerals and vitamins to meet or exceed the nutrients requirement of growing beef cattle (NASEM, 2016; Table 1). The basal diet was supplemented with HT extract (chestnut, **CN**; 74% tannin) or a combination (50:50) of HT and CT extract (quebracho, **Q**; 91% tannin) in powdered forms (SilvaTeam, León, Gto., Mexico) at different levels of dietary DM (Table 1). The treatments for determining animal performance were control (no tannin), 0.25% CN, 1.5% CN, combination of CN and Q at 0.125% each (0.25% **CNQ**), and CN and Q at 0.75% each (1.5% CNQ) of dietary DM.

After the performance study, 45 steers from 3 of the treatment groups were used for CH_4 measurements. Treatments for the CH_4 measurement were control, 1.5% CN, and 1.5% CNQ of dietary DM. The lower levels of tannins were not considered because an effect on CH_4 emissions was not expected at lower application rates (Jayanegara et al., 2012). Individual feed ingredients including

Table 1. Feed ingredients of dietary treatments and chemical composition of the basal diet

	Dietary treatment[1]				
		CN		CNQ	
Item	Control	0.25%	1.5%	0.25%	1.5%
Ingredients, % DM					
Alfalfa silage[2]	47.50	47.38	46.75	47.38	46.75
Barley silage[3]	47.50	47.38	46.75	47.38	46.75
Supplement[4]	5.00	5.00	5.00	5.00	5.00
Barley ground	4.90	4.90	4.90	4.90	4.90
Salt (sodium chloride)	0.05	0.05	0.05	0.05	0.05
Vitamin and mineral premix[5]	0.05	0.05	0.05	0.05	0.05
Chestnut extract[6]	—	0.25	1.50	0.125	0.75
Quebracho extract[6]	—	—	—	0.125	0.75
Chemical composition, % DM					
DM (as is)	35.8				
CP	17.1				
NDF	43.8				
Starch	12.7				
NEg (Mcal/kg DM)[7]	0.82				

[1]CN = Chestnut; CNQ = chestnut and quebracho mix.

[2]Contained 31.9% ± 1.48% DM and 79.6% ± 1.55% OM, 21.7% ± 0.74% CP, 43.7% ± 1.35 % NDF, and 35.0% ± 1.24% ADF on a DM basis using pooled samples for each period and from the gas measurement phases (mean ± SD; n = 7).

[3]Contained 38.0% ± 0.70% DM and 91.9% ± 0.53% OM, 13.1% ± 0.51% CP, 46.4% ± 1.45% NDF, 25.5% ± 1.07% ADF, and 18.7% ± 4.44% starch on a DM basis using pooled samples for each period and from the gas measurement phases (mean ± SD; n = 7).

[4]Contained 89.8% ± 0.23% DM and 94.7% ± 0.81% OM, 10.8% ± 0.32% CP, 19.7% ± 3.39% NDF, 6.13% ± 1.19% ADF, and 60.1% ± 3.83% starch on a DM basis using pooled samples for each period and from the gas measurement phases (mean ± SD; n = 7), provided as mash.

[5]Contained 35.01% $CaCO_3$, 10.37% $CuSO_4$, 28.23% $ZnSO_4$, 0.15% ethylenediamine dihydriodide (80% concentration), 5.01% Se 1% (10,000 mg Se/kg), 0.1% $CoSO_4$, 14.54% $MnSO_4$, 1.71% vitamin A (500,000,000 IU/kg), 0.17% vitamin D (500,000,000 IU/kg), and 4.7% vitamin E (500,000 IU/kg).

[6]Chestnut extract contained 74% tannin and quebracho extract contained 91% tannin in powdered forms (SilvaTeam, León, Gto., Mexico); thus, the 0.25% CN, 1.5% CN, 0.25% CNQ, and 1.5% CNQ treatments contained 0.19, 1.11, 0.20 (0.09% HT and 0.11% CT), and 1.24% (0.56% HT and 0.68% CT) tannin, respectively.

[7]Estimated using the NASEM (2016), Empirical Level of Solution, with the measured nutrient compositions of the alfalfa silage, barley silage, and supplement with animal, management, and environmental inputs.

tannins were mixed as a total mixed ration (**TMR**) using a Calan Data Ranger (American Calan, Northwood, NH) and offered once a day (0930 h) to the steers for ad libitum (5% ort) intake.

Sampling Procedures

Steers were weighed before feeding on 2 consecutive days at the beginning (November 8 and 9, 2016) and end (January 31 and February 1, 2017) of the performance study. In addition, animals were weighed before feeding every 3 wk so that there were 4 experimental periods (period 1, weeks 1 to 3; period 2, weeks 4 to 6; period 3, weeks 7 to 9; and period 4, weeks 10 to 12). The amount of TMR offered was recorded daily and sampled weekly; orts were collected daily, weighed, composited, and subsampled weekly. The DM of the weekly sample of TMR was determined and used to calculate weekly DMI. The silages, supplements, and tannins were sampled weekly to monitor DM and adjust diet composition whenever necessary. A subsample of a weekly composite of the silages and supplements in each period was used for chemical analysis. ADG and feed efficiency (ADG/DMI; gain:feed [G:F]) were calculated by period and averaged over the entire study.

Blood samples were collected from all 15 animals per treatment at the beginning of the study and at the end of every period at the time of weighing animals. Blood samples were taken from the jugular vein into sterile-evacuated tubes containing an anticoagulant (10 mL, lithium heparin, Vacutainer, Becton Dickinson, Oakville, ON). The blood was centrifuged at 3,000 × *g* and 4 °C for 20 min to obtain plasma and stored at −20 °C until analysis of plasma urea N (**PUN**) concentration as an indicator of protein status of the animal (Kohn et al., 2005).

Rumen fluid samples were collected orally 3 h postfeeding near the end of period 4 from 7 animals per treatment using the method described by Paz et al. (2016). A 3-m tube was passed through a speculum that was inserted via the mouth into the ventral sac. The other end of the tube was connected to a 1,000-mL Nalgene polypropylene vacuum flask (Thermo Scientific Inc., Waltham, MA). A suction pump (model DD195, Precision Scientific, Niagara Falls, NY) connected to the vacuum flask was applied for a few seconds to free any blockage and facilitate the collection of rumen samples. To avoid cross contamination between animals, the speculum and tubing were thoroughly washed with warm water and the first 200-mL sample was discarded to avoid saliva contamination. The following 200 mL collected was strained through a PECAP polyester screen (355-µm pore size; B & S H Thompson, Ville Mont-Royal, QC, Canada) and retained for analysis of volatile fatty acids (VFA), ammonia concentration, and protozoa enumeration. The pH of the unfiltered rumen fluid was immediately measured using a pH meter (Orion Model 260A, Fisher Scientific, Toronto, ON, Canada). For VFA determination, 5 mL of the filtered ruminal fluid was added to 1 mL of 25% meta-phosphoric acid (wt/vol). Another 5 mL of filtered ruminal fluid was added to 1 mL of 1% sulfuric acid (vol/vol) for ammonia determination. Also, for protozoa enumeration, 5 mL of the filtrate was mixed with 5 mL of methyl green–formalin–saline solution and stored in the dark at room temperature until analyzed.

Enteric CH_4 production from all control, 1.5% CN, and 1.5% CNQ animals was measured using the sulphur hexafluoride (**SF_6**) tracer gas technique as described by McGinn et al. (2006). To facilitate the measurements, the animals were first assigned to 3 groups with 5 animals per treatment in each group (15 animals per group) with each group measured sequentially. At least 7 d before CH_4 measurement, a permeation tube was introduced into the rumen of each animal using a tube within a speculum that was introduced via the mouth into the ventral sac. The permeation tubes were stored for at least 1 mo at 39 °C to determine the release rate of SF_6 (mean ± SD release rate; 4.79 ± 0.559 mg/d). Before CH_4 measurement, animals were adapted to wearing the halters and yokes. Background levels of SF_6 and CH_4 were measured daily by suspending 3 yokes in the barn. The yokes were replaced every 24 h for 6 d for each animal. Three 20-mL gas subsamples were taken from each yoke with a syringe and injected into 6.8-mL exetainer vials (Labco Ltd, Wycombe, Bucks, UK) for further analysis. During the CH_4 measurement, TMR and orts were collected daily to determine DM content and calculate daily DMI. Ingredients were sampled once for each group and used for chemical analysis.

Laboratory Analyses

Samples of TMR, orts, and ingredients were dried in a forced air oven at 55 °C for 72 h to determine DM content. The dried ingredients were ground through a 1-mm screen (Wiley mill; A.H. Thomas, Philadelphia, PA). The ground ingredients were used to determine analytical DM (method 930.15; AOAC, 2005), which was used to correct the chemical analysis to a DM basis. Ash (method 942.05; AOAC, 2005), neutral detergent fiber (NDF;

heat-stable amylase and sodium sulfite were used; Ankom A200, Ankom Technology, Fairport, NY), and acid detergent fiber (ADF; Ankom Technology) were analyzed. The ingredients were further ground using a ball grinder (Mixer Mill MM 2000; Retsch, Haan, Germany) and analyzed for starch (Koenig et al., 2013) and total N concentration using flash combustion and thermal conductivity detection (Carlo Erba Instruments, Milan, Italy). The CP content of the ingredients was calculated by multiplying N content by 6.25.

Concentrations of VFA in ruminal fluid were analyzed as described by Romero-Perez et al. (2015) using gas chromatography (model 5890; Hewlett Parkard, Wilmington, DE) with crotonic acid as an internal standard. Ruminal ammonia concentration was determined by the salicylate–nitroprusside–hypochlorite method using segmented flow analyzer (Rhine et al., 1998). Ruminal protozoa were enumerated under a light microscope using a counting chamber (Neubauer Improved Bright-Line counting cell, 0.1 mm depth; Hausser Scientific, Horsham, PA) as described by Veira et al. (1983).

The PUN concentrations were determined using microsegmented flow analysis (model Astoria2; Astoria Pacific Inc., Clackamas, OR). The PUN was used to estimate the quantity of urine N excreted (**UNE**) using the equation of Kohn et al. (2005), UNE = CR × BUN × BW, where CR is N clearance rate of kidney in beef cows (1.3 L • d^{-1} • kg^{-1}), BW is the mean BW for the period, and BUN is the blood urea N. The PUN was presumed to be equivalent to BUN because urea diffuses freely into and out of blood cells.

The CH_4 and SF_6 concentrations were analyzed using gas chromatography as described by McGinn et al. (2006). Standard curves were generated throughout the study using 8 gas standards (1.64 to 248 μmol/mol for CH_4 and 18.3 to 1540 nmol/mol for SF_6 with correlation coefficient exceeding 99.9% for all curves).

Statistical Analyses

All data were analyzed using a mixed procedure of SAS (SAS Inst., Inc., Cary, NC). Animal was the experimental unit for all variables. Normality of distribution and homogeneity of variance were determined using the Univariate procedure of SAS. For the DMI, weekly data were averaged for each animal in each period. A $\log_{10}$ transformation was applied to the protozoa data before analysis because the data were not normally distributed and inverse $\log_{10}$ of the least square means was reported. The initial and final BW, VFA, rumen pH, ruminal ammonia concentration, and protozoa data were analyzed using a model that included treatment as fixed effect and animal as random effect. The DMI, ADG, G:F, PUN, and UNE data were analyzed using a statistical model that included treatment, period, and their interaction as fixed effects and the random effect of animal within treatment with period as a repeated measure. For PUN and UNE, the data included their baseline measurements (day 0) as covariates.

The model used to analyze CH_4 production and DMI during gas measurements included fixed effect of treatment with group as random effect and day of sampling (days 1 to 6) within each group as a repeated measure. For all data, covariance structure (autoregressive) that yielded the smallest Akaike and Bayesian information criteria value was used and means were separated at $P < 0.05$ while tendencies were indicated at $0.05 \leq P < 0.10$. The Tukey–Kramer was used to determine significant differences among means.

RESULTS

The DMI during the growth performance phase averaged 7.51 ± 0.198 kg/d and did not differ ($P = 0.10$) among treatments (Table 2). However, there was a treatment × period interaction for DMI ($P < 0.01$); during weeks 10 to 12, the DMI of steers fed 1.5% CNQ was greater ($P < 0.05$) than that of steers fed 0.25% CN and 0.25% CNQ (8.66 vs. 7.49 and 7.45 kg/d, respectively), although it did not differ ($P > 0.05$) from that of steers fed control and 1.5% CN diets (8.04 and 8.36 kg/d, respectively). There was a treatment × period interaction ($P = 0.04$) for ADG, whereby during weeks 10 to 12, the ADG of steers fed 1.5% CN and 1.5% CNQ was greater ($P < 0.05$) than that of steers fed 0.25% CN (1.075 and 1.085 vs. 0.778 kg/d, respectively). The G:F was greater ($P < 0.05$) for 0.25% CN compared with 0.25% CNQ in weeks 4 to 6, whereas the opposite occurred during weeks 10 to 12. Regardless of these treatment differences, the ADG and G:F of animals fed the tannin treatments did not differ ($P > 0.05$) from those of the control in any of the periods and there was no effect ($P \geq 0.80$) of treatment on ADG and G:F when examined over the entire study.

The PUN concentration averaged over the entire study for animals receiving tannin treatments was greater ($P < 0.05$) for 1.5% CN and 1.5% CNQ than those fed 0.25% CNQ (120.9 and 120.4 vs. 111.7 mg/L, respectively), but not different ($P > 0.05$) from animals fed control and 0.25% CN (117.2

Table 2. Effects of hydrolyzable tannin with or without condensed tannin on growth performance of beef steers (n = 15) fed an alfalfa:barley silage (50:50) diet

	Treatment[2]						
		CN		CNQ			
Item[1]	Control	0.25%	1.5%	0.25%	1.5%	SEM	P value
BW, kg							
Initial	292	290	293	293	293	4.1	0.98
Final	352	345	353	353	357	4.1	0.78
DMI, kg/d							
Weeks 1 to 3	6.98	6.44	6.96	6.90	6.88	0.220[3]	<0.01[3]
Weeks 4 to 6	7.65	7.06	7.54	7.55	7.69		
Weeks 7 to 9	7.68	7.33	7.62	7.77	8.12		
Weeks 10 to 12	8.04[ab]	7.49[b]	8.36[ab]	7.45[b]	8.66[a]		
Overall	7.59	7.08	7.62	7.41	7.84	0.198[4]	0.10[4]
ADG, kg/d							
Weeks 1 to 3	0.551	0.454	0.620	0.635	0.635	0.0822[3]	0.04[3]
Weeks 4 to 6	0.875	0.913	0.901	0.755	0.847		
Weeks 7 to 9	0.526	0.516	0.329	0.512	0.437		
Weeks 10 to 12	0.913[ab]	0.778[b]	1.075[a]	0.959[ab]	1.085[a]		
Overall	0.716	0.665	0.733	0.715	0.751	0.0501[4]	0.80[4]
G:F							
Weeks 1 to 3	0.077	0.069	0.089	0.089	0.090	0.0099[3]	0.04[3]
Weeks 4 to 6	0.114[ab]	0.127[a]	0.120[ab]	0.099[b]	0.107[ab]		
Weeks 7 to 9	0.067	0.070	0.043	0.065	0.055		
Weeks 10 to 12	0.111[ab]	0.099[b]	0.125[a]	0.126[a]	0.125[a]		
Overall	0.092	0.092	0.094	0.095	0.094	0.0058[4]	0.99[4]

[a,b]Within a row, means without a common superscript letter differ at $P < 0.05$.

[1]BW = Body weight; DMI =dry matter intake; ADG = average daily gain; G:F = gain:feed.

[2]CN = chestnut; CNQ = chestnut and quebracho mix.

[3]The SEM and P-values of treatment × period interaction for DMI, ADG, and G:F.

[4]The SEM and P values of the overall effect of treatment for DMI, ADG, and G:F.

and 117.5 mg/L, respectively; Table 3). However, the treatment × period interaction ($P < 0.01$) indicated that the differences among treatments differed among periods, with differences occurring later in the study. From weeks 7 to 9, the PUN of animals fed 1.5% CN was greater ($P < 0.05$) than that of animals fed control, 0.25% CN, and 0.25% CNQ (128.9 vs. 110.3 mg/L) but not different ($P > 0.05$) from that of animals fed 1.5% CNQ (119.9 mg/L). These differences decreased over time such that from weeks 10 to 12, the PUN of animals fed 1.5% CN and 0.25% CNQ was actually less ($P < 0.05$) than that of animals fed control (95.2 and 97.4 vs. 110.6 mg/L, respectively) but not different ($P > 0.05$) from those fed 0.25% CN and 1.5% CNQ (101.1 and 105.9 mg/L, respectively). The overall estimated UNE was less ($P < 0.05$) for 1.5% CN, 0.25% CNQ, and 1.5% CNQ compared with the control (51.1, 49.1, 43.0, vs. 54.8 g/d) with 0.25% CN being intermediate (52.1 g/d). The treatment × period interaction ($P = 0.01$) occurred because 1.5% CNQ differed ($P < 0.05$) from control in all periods, 0.25% CNQ differed ($P < 0.05$) from control in all periods except the first, whereas 1.5% CN differed ($P < 0.05$) from control in weeks 7 to 9 only.

The ruminal fluid pH averaged 7.30 ± 0.890 and was not affected by tannin addition ($P = 0.35$; Table 4). Total ruminal VFA concentration averaged 81.18 ± 7.783 mM was also not affected ($P = 0.16$) by adding tannins to the diet. Also, tannin inclusion did not affect the molar proportions of acetate, propionate, and isobutyrate ($P \geq 0.19$), and thus, acetate:propionate ratio was not affected ($P = 0.62$). However, butyrate proportion was greater ($P < 0.05$) for 1.5% CNQ compared with control, 0.25% CN, and 0.25% CNQ (11.29 vs. 10.31, 9.18, and 9.57 mol/100 mol, respectively). Tannin supplementation at 1.5% CN and 1.5% CNQ decreased ($P < 0.05$) valerate and isovalerate proportions compared with control. Ammonia concentration was decreased ($P < 0.01$) with the addition of tannin, irrespective of tannin source or dose compared with the control (mean; 6.64 vs. 11.96 mM). Total protozoa numbers in rumen fluid were not affected by tannin source or dose (mean, 4.20×10^5/mL; $P = 0.14$).

Table 3. Effects of hydrolyzable tannin with or without condensed tannin on plasma urea N and urine N excretion of beef steers (n = 15) fed an alfalfa:barley silage (50:50) diet

	Treatment[2]					SEM	P value
		CN		CNQ			
Item[1]	Control	0.25%	1.5%	0.25%	1.5%		
PUN, mg/L							
Weeks 1 to 3	128.6	130.8	132.4	122.9	131.8	4.59[3]	<0.01[3]
Weeks 4 to 6	118.8	119.2	126.9	125.3	123.8		
Weeks 7 to 9	110.7^{bc}	118.8^{b}	128.9^{a}	101.5^{c}	119.9^{ab}		
Weeks 10 to 12	110.6^{a}	101.1^{ab}	95.2^{b}	97.4^{b}	105.9^{ab}		
Overall	117.2^{ab}	117.5^{ab}	120.9^{a}	111.7^{b}	120.4^{a}	2.78[4]	0.047[4]
UNE, g/d[5]							
Weeks 1 to 3	50.8^{a}	51.1^{a}	49.8^{a}	51.2^{a}	40.4^{b}	2.09[3]	0.01[3]
Weeks 4 to 6	55.0^{a}	49.2^{b}	52.0^{ab}	42.3^{c}	40.1^{c}		
Weeks 7 to 9	57.2^{a}	51.3^{b}	47.1^{bc}	51.9^{b}	42.1^{c}		
Weeks 10 to 12	56.0^{a}	56.7^{a}	55.6^{a}	51.0a^{b}	49.2^{b}		
Overall	54.8^{a}	52.1^{ab}	51.1^{b}	49.1^{b}	43.0^{c}	1.17[4]	<0.01[4]

$^{a-c}$Within a row, means without a common superscript letter differ at $P < 0.05$.

[1]PUN = Plasma urea nitrogen; UNE = urine nitrogen excretion.

[2]CN = chestnut; CNQ = chestnut and quebracho mix.

[3]The SEM and P values of treatment × period interaction for PUN and UNE.

[4]The SEM and P values of the overall effect of treatment for PUN and UNE.

[5]Estimated using the BW and PUN measured and the N clearance rate of kidney in beef cattle (1.3 L • d^{-1} • kg^{-1}), using the equation by Kohn et al. (2005).

During CH_4 measurement, the DMI averaged 7.27 ± 0.322 kg/d and was not affected (P = 0.54) by treatment (Table 5). The daily CH_4 produced did not differ (P = 0.84) among treatments (154.3 ± 5.94 g/d). However, adding tannin at 1.5% CNQ tended (P = 0.094) to decrease CH_4 yield by 6.4% compared with the control (20.6 vs. 22.0 g/kg DMI).

DISCUSSION

This study examined the effects of supplementing HT alone or in combination with CT as a means of improving N use status and reducing CH_4 emission of growing beef cattle fed a high-forage diet consisting of blend of alfalfa and barley silages. Animal performance was measured to determine whether any changes in N use or CH_4 emissions resulted in a change in ADG or G:F. The basal diet was high in CP (17.1%) to maximize animal performance; however, silage diets high in soluble protein increase the amount of N excreted in feces and urine (Dijkstra et al., 2013). As tannins have the ability to bind to proteins, they may decrease soluble protein degradation in the rumen and increase protein flow to the small intestine leading to improved animal performance, if MP supply is limiting. An improvement in animal performance is generally attributed to an increase in MP supply to the small intestine increasing absorption of amino acids to the blood. Using the Empirical Level of Solution of the Beef Cattle Nutrient Requirements Model (NASEM, 2016), MP required was estimated at 447.5 g/d for cattle gaining 1.0 kg/d while it was estimated that the control diet supplied 553.9 g/d of MP. Thus, the lack of increase in ADG for cattle fed the tannin treatments is consistent with MP supply not being limiting for growth.

The effect of added tannin sources was examined at low (0.25% DM) and high (1.5% DM) concentrations of HT alone or in combination with CT, with CN used as the source of HT and Q as the source of CT. The low level of tannin used in the present study was chosen based on the study of Rivera-Méndez et al. (2016), where 0.2% to 0.6% DM of supplemental CT improved G:F of Holstein steers, compared with a control diet. The high level of tannins used in the study was chosen to maximize the potential effects of tannins, without reducing DMI and potentially animal performance. Accounting for the tannin concentrations of the CN and Q, the 0.25% CN, 0.25% CNQ, 1.5% CN, and 1.5% CNQ treatments contained 0.19%, 0.20% (0.09% HT and 0.11% CT), 1.11%, and 1.24% (0.56% HT and 0.68% CT) tannin, respectively. These tannin concentrations are below the concentration of tannin (i.e., <2% of dietary DM) reported by Jayanegara et al. (2012) that may depress DMI and animal performance. Supplementing ruminant diets with tannins at high concentration may decrease DMI

due to a reduction in palatability and digestibility, which negatively affect ADG of the animal (Waghorn, and Shelton, 1997; Mueller-Harvey, 2006; Waghorn, 2008). In the present study, the overall lack of effect of tannin supplementation on DMI and ADG was consistent with feeding <2% of dietary DM as tannin. Beauchemin et al. (2007) also reported no effects of Q extract at 0%, 1%, and 2% dietary DM on DMI or ADG in growing cattle (initial BW, 223 kg) fed a diet of 70% forage DM. Also, Krueger et al. (2010) showed no difference in DMI, ADG, or G:F when 1.5% of HT extract was added to a 64% corn grain diet (DM basis) compared with a control diet fed to finishing beef steers (initial BW; 414 kg). In contrast, adding a combination of HT and CT at 0.6% CNQ (50:50) dietary DM increased DMI and ADG of feedlot Holstein steers (initial BW, 392 kg) compared with the control, whereas no effect occurred when the tannins were applied separately (Rivera-Méndez et al., 2016). In the present study, the greater DMI intake in period 4 for the high concentration of CNQ compared with the low dosage of CN coincided with greater ADG and improved G:F of cattle fed CNQ during this period. The effect of tannin supplementation of diets on animal performance may depend on the dosage and type of tannin, nutrient composition of the diet, and animal nutrient requirements.

Ruminal fluid pH was similar to expectations for cattle fed forage diets (Kiro, 2017). The lack of effect of tannin treatments on pH supports the findings of other studies with tannins fed to cattle or sheep (Krueger et al., 2010; Liu et al., 2011; Aguerre et al., 2016).The lack of treatment effect on total VFA and the main VFA proportions (acetate and propionate) indicated that there were likely no effects of tannins on rumen digestibility of the diet. Similarly, regardless of CP concentration of the diet (15.3% vs. 16.6%), tannin supplemented as CNQ (0%, 0.45%, 0.9%, and 1.8 % dietary DM) did not affect total VFA or proportions of acetate and propionate in dairy cattle (Aguerre et al., 2016). Beauchemin et al. (2007) reported a linear

Table 4. Effects of hydrolyzable tannin with or without condensed tannin on ruminal fermentation of beef steers (n = 7) fed an alfalfa:barley silage (50:50) diet

	Treatment[1]						
		CN		CNQ			
Item	Control	0.25%	1.5%	0.25%	1.5%	SEM	*P*-value
pH	7.19	7.26	7.43	7.36	7.24	0.890	0.35
Total VFA, m*M*	94.70	86.34	69.42	72.53	82.90	7.783	0.16
VFA, mol/100 mol							
Actate (A)	68.49	70.10	69.37	69.46	68.55	0.601	0.31
Propionate (P)	14.06	14.60	14.02	14.70	14.23	0.242	0.19
Butyrate	10.31^{bc}	9.18^{d}	10.69^{ab}	9.57^{cd}	11.29^{a}	0.298	<0.01
Valerate	1.81^{a}	1.54^{ab}	1.36^{b}	1.44^{b}	1.41^{b}	0.109	0.049
Isobutyrate	1.76	1.64	1.64	1.69	1.57	0.065	0.34
Isovalerate	2.64^{a}	2.24^{b}	2.19^{b}	2.48^{ab}	2.18^{b}	0.124	0.049
A:P	4.88	4.82	4.96	4.73	4.83	0.104	0.62
Ammonia, m*M*	11.96^{a}	8.07^{b}	5.56^{b}	7.16^{b}	5.76^{b}	1.287	<0.01
Protozoa, cells × 10^{5}/mL[2]	3.17	3.79	4.10	3.18	6.74	0.093	0.14

$^{a–d}$Within a row, means without a common superscript letter differ or tend to differ at $P < 0.05$ or $0.05 \leq P < 0.10$, respectively.

Samples were taken on week 12 in period 4.

[1]CN = chestnut; CNQ= chestnut and quebracho mix.

[2]Data were $\log_{10}$ transformed before statistical analysis and inverse $\log_{10}$ least squares mean reported herein.

Table 5. Effects of hydrolyzable tannin with or without condensed tannin on intake and methane (CH_4) emission of beef steers (n = 15) fed an alfalfa:barley silage (50:50) diet

	Treatment[1]				
Item	Control	1.5% CN	1.5% CNQ	SEM	*P* value
DMI, kg/d	7.01	7.27	7.52	0.322	0.54
CH_4, g/d	151.5	156.4	155.0	5.94	0.84
CH_4, g/kg DMI	22.0^{a}	21.7^{ab}	20.6^{b}	0.47	0.094

^{a,b}Within a row, means without a common superscript letter tend to differ at $0.05 \leq P < 0.10$.

[1]CN = Chestnut; CNQ = chestnut and quebracho mix.

reduction in total VFA concentration and acetate:propionate ratio with increasing levels of CT tannin from Q extract (0%, 1%, or 2% dietary DM) added to a high-forage diet fed to growing beef cattle. The present study sampled ruminal fluid 3 h after feeding only in week 12 of the experiment while Beauchemin et al. (2007) sampled 2 h after feeding every 21 d. In the present study, the rumen microbiota may have been changed to favor microbes that hydrolyze tannin but the changes were likely minor as there were no effects on total VFA concentration and proportions of acetate and propionate. Similarly, the overall population structure of rumen microbiota was not significantly altered in Holstein steers fed a mix of CT and HT compared with control cattle, although tannins decreased bacterial richness (Carrasco et al., 2017). At the greater dose of 1.5% CNQ in the present study, the proportions of butyrate (4-carbon) increased and valerate (5-carbon) decreased relative to the control. Greater hydrolysis of tannins by rumen microbes in animals fed the 1.5% CNQ may have increased the butyrate proportion. Similarly, the butyrate proportion increased on day 42 in the study of Krueger et al. (2010) who fed 1.5% CN or Q to feedlot cattle. Both HT and CT can be hydrolyzed to butyrate through a series of enzymatic action using 3-hydroxy-5-oxohexanoate pathways (Krumholz and Bryant, 1986; Bhat et al., 1998).

Tannins bind to proteins and as such decrease ruminal degradation of protein (Reed, 1995) and consequently decrease urinary N excretion (Carulla et al., 2005; Grainger et al., 2009). In the present study, irrespective of dosage or type, tannin decreased ruminal ammonia concentration relative to the control with a 53% decrease for the greater dosage and 36% for the lower dosage. Similarly, other studies with tannin supplementation in vitro (Getachew et al., 2008) and in vivo (Beauchemin et al., 2007) have shown a reduction in ammonia concentration. Isovalerate is a branched-chain VFA from the deamination of leucine, and in the present study its proportion decreased with a greater dose of tannin. The decrease in isovalerate with a greater dose of tannin may indicate a reduction in ruminal protein degradation, which could partly explain the observed decrease in ruminal ammonia concentration for the greater dose tannin.

Plasma urea N increases in cattle when they are fed high-protein diets (Ruiz et al., 2002; Kohn et al., 2005; Wickersham, 2008) and therefore can indicate the protein status of the animal. The mean range in PUN between 95 and 132 mg/L was below the value of 150 mg/L reported by Byers and Moxon (1980) for growing beef cattle fed a corn silage diet with dietary CP content of 16.5% DM. Johnson and Preston (1995) suggested that optimal PUN concentration for protein deposition by beef steers is between 60 and 80 mg/L. The greater average PUN reported in the present study compared with 80 mg/L in the study of Johnson and Preston (1995) is an indication of excess dietary protein relative to the protein and energy requirements of the animal. Furthermore, the increase in PUN with the greater dose of CN and CNQ compared with the lower dose of CNQ was unexpected, given that all tannin treatments decreased rumen ammonia concentrations compared with the control. Usually as ruminal ammonia concentration decreases, PUN concentration decreases as reviewed by Kennedy and Milligan (1980). However, it is possible that the lower ruminal ammonia concentration for the animals fed the tannin treatments corresponded to greater postruminal flow of MP, and greater intestinal absorption of amino acids, leading to increased PUN, especially at the greater dose of CNQ. Also, the decrease in ruminal ammonia concentration coupled with an increase in PUN for the greater dose of CN and CNQ relative to the lower dose of CNQ may partly be due to an increase in the rate of urea transfer through the blood to the rumen (Kennedy and Milligan, 1978). However, UNE is likely a better indication of N use status than PUN alone, because it accounts for both PUN and BW of animals. The decrease in UNE for 1.5% CN, 0.25% CNQ, and 1.5% CNQ by 6.8%, 10.4%, and 21.5%, respectively, is consistent with the decrease in rumen ammonia and suggests that the tannin treatments decreased protein degradation in the rumen leading to decreased absorption of ammonia from the rumen, and a shift in route of N excretion with proportionately less excreted in urine and more excreted in feces. Similarly, CNQ at 1.8% or 0.9% vs. 0% DM has been shown to shift the partitioning of N from urine to feces, thereby decreasing UNE (Aguerre et al., 2016). Hence, for diets containing excess N, feeding a high dose of HT and CT in combination may be beneficial for the environment, because decreasing urinary excretion of N would be expected to decrease the volatilization of N in the form of ammonia.

A meta-analysis from 30 in vivo experiments showed that a decrease in CH_4 production is consistent with tannin concentration greater than 2% but tannin at this dietary concentration can negatively affect DMI (Jayanegara et al., 2012). Thus, CH_4 was not measured at the lower doses of tannins in the present study as a decrease in CH_4 was not expected. Although daily CH_4 produced was not affected by tannin supplementation, CH_4 yield (g/kg DMI) tended to decrease by 6% with supplementation of 1.5% CNQ relative to the control. Other studies have reported a 13% decrease in CH_4 yield for sheep (Carulla et al., 2005) and a 22% decrease for dairy

cattle (Grainger et al., 2009) with CT applied at 2.5% and 1.8% of dietary DM, respectively. On the contrary, other studies in beef cattle supplemented with CT at 1.8% (Beauchemin et al., 2007) and 1% (Ebert et al., 2017) dietary DM reported no effect of tannin on CH_4 yield. It has been proposed that tannins decrease CH_4 emissions by directly inhibiting methanogens (Field et al. 1989) and ruminal microbes that produce hydrogen and are associated with methanogens such as protozoa (Bhatta et al., 2009; Cieslak et al., 2012), or indirectly by decreasing fiber degradation (Carulla et al., 2005) in the rumen. The lack of effect of tannins on DMI, total VFA concentration, and the major VFA proportions in the present study suggests that fiber degradation may not have been affected. Although the methanogens were not measured, the lack of effect of tannin on the protozoa population indicates that the decrease in CH_4 production for 1.5% CNQ relative to the control was not directly related to protozoa. However, a decrease in CH_4 production is not always concomitant with a decrease in protozoa (Bhatta et al., 2013), as some tannins may decrease methanogens that are not associated with protozoa. The discrepancies between the present study and that of Beauchemin et al. (2007) who did not observe any effect of tannin on CH_4 when beef cattle were fed a high-forage diet supplemented with 1.8% CT may be due to the differences in the type of tannin used. In vitro, HT combined with CT was more potent in terms of reducing CH_4 compared with CT applied alone with a decrease in the methanogen population when 6 commercial sources of tannins containing HT or CT were compared with control without tannin (Bhatta et al., 2009). Also, Carrasco et al. (2017) reported a decrease in methanogenic archaea in Holstein steers fed a diet supplemented with HT and CT blend from CN and Q extracts, respectively. It is also possible that the decrease in CH_4 production for the 1.5% CNQ combination was due to the slightly greater tannin concentration in this treatment relative to 1.5% CN.

In conclusion, adding CN to supply HT, alone or in combination with Q to supply CT, to a high-forage diet (95% DM) at a low (0.25% DM) or greater dose (1.5% DM) decreased ruminal ammonia concentration without adversely affecting animal performance of growing beef cattle. Adding HT from CN alone to a high-forage diet at a concentration of 1.5% (50:50) dietary DM did not affect CH yield but a combination of HT and CT from CN and Q, respectively, at a concentration of 1.5% (50:50) dietary DM (1.24% DM total tannin) tended to decrease CH_4 yield from growing beef cattle, without negative effects on animal performance.

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