Effects of supplementing condensed tannin extract on intake, digestion, ruminal fermentation, and milk production of lactating dairy cows¹

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ABSTRACT

A lactation experiment was conducted to determine the influence of quebracho condensed tannin extract (CTE) on ruminal fermentation and lactational performance of dairy cows. The cows were fed a high forage (HF) or a low forage (LF) diet with a forage-to-concentrate ratio of 59:41 or 41:59 on a dry matter (DM) basis, respectively. Eight multiparous lactating Holstein cows (62 ± 8.8 d in milk) were used. The design of the experiment was a double 4×4 Latin square with a 2×2 factorial arrangement of treatments, and each period lasted 21 d (14 d of treatment adaptation and 7 d of data collection and sampling). Four dietary treatments were tested: HF without CTE, HF with CTE (HF+CTE), LF without CTE, and LF with CTE (LF+CTE). Commercial quebracho CTE was added to the HF+CTE and the LF+CTE at a rate of 3% of dietary DM. Intake of DM averaged 26.7 kg/d across treatments, and supplementing CTE decreased intakes of DM and nutrients regardless of forage level. Digestibilities of DM and nutrients were not affected by CTE supplementation. Milk yield averaged 35.3 kg/d across treatments, and yields of milk and milk component were not influenced by CTE supplementation. Negative effects of CTE supplementation on feed intake resulted in increased feed efficiency (milk yield/DM intake). Although concentration of milk urea N (MUN) decreased by supplementing CTE in the diets, efficiency of N use for milk N was not affected by CTE supplementation. Feeding the LF diet decreased ruminal pH (mean of 6.47 and 6.33 in HF and LF, respectively). However, supplementation of CTE in the diets did not influence ruminal pH. Supplementing CTE decreased total volatile fatty acid concentration regardless of level of forage. With CTE supplementation, molar proportions of acetate, propionate, and butyrate increased in the HF diet, but not in the LF diet, resulting in interac-

tions between forage level and CTE supplementation. Concentration of ammonia-N tended to decrease with supplementation of CTE. The most remarkable finding in this study was that cows fed CTE-supplemented diets had decreased ruminal ammonia-N and MUN concentrations, indicating that less ruminal N was lost as ammonia because of decreased degradation of crude protein by rumen microorganisms in response to CTE supplementation. Therefore, supplementation of CTE in lactation dairy diets may change the route of N excretion, having less excretion into urine but more into feces, as it had no effect on N utilization efficiency for milk production.

Key words: condensed tannin extract, lactating dairy cow, milk urea-N, ammonia-N

INTRODUCTION

In ruminants fed high-quality forage diets, most proteins are rapidly solubilized releasing between 56 and 65% of the N concentration in the rumen during fermentation; consequently, large losses of N occur (25–35%) as ammonia (NH_3) into urine (Min et al., 2000). Research is needed to improve N retention in animals. Natural plant compounds with a known ability to reduce proteolysis such as condensed tannin extract (CTE) offer a promising means of achieving this goal. Aerts et al. (1999a) found that condensed tannins (CT) in birdsfoot trefoil (*Lotus corniculatus*) and big trefoil (*Lotus pedunculatus*) markedly protected ribulose-1,5-bisphosphate carboxylase/oxygenase from degradation by mixed rumen microorganisms. Molan et al. (2001) demonstrated that CT concentrations of 400 $\mu\text{g}/\text{mL}$ or greater reduced the growth of a range of bacterial strains from the rumen. Furthermore, Min et al. (2002) reported that when the diet was changed from perennial ryegrass–white clover pasture (which does not contain CT) to birdsfoot trefoil (3.2% CT on DM basis) in sheep, populations of the proteolytic rumen bacteria decreased, confirming that the CT in the forages greatly reduces rumen proteolytic bacterial growth. These effects of CT on retarding forage N degradation supported greater milk production from cows fed birdsfoot trefoil over alfalfa silage (Hymes-

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Fecht et al., 2005). Alfalfa does not produce tannins except in the seed coats. Thus, feeding CT-containing forages in the diets containing alfalfa as a main forage source may increase N utilization and improve animal performance. However, tannin-rich forages are not agronomically suited in many areas and thus may not be readily available. Hence, a concentrated source of CT may be a possible alternative approach to feeding tannin-rich forages to enhance N utilization and improve lactational performance of dairy cows if similar dietary concentrations of tannins are provided from CTE as is seen in tannin-rich forage diets.

Although supplementation of CTE in lactating dairy diets has been extensively investigated, the literature lacks information on how ruminal fermentation characteristics are altered depending upon dietary composition, particularly forage-to-concentrate-ratio, which is considered one of the main driving forces directly affecting ruminal fermentation and production performance of lactating dairy cows. In addition, some studies (Jones et al., 1994; Molan et al., 2001) have shown that CT from different legume forages inhibit cell growth and division of ruminal microorganisms, including *Butyrivibrio fibrisolvens*, which is among those responsible for ruminal biohydrogenation (Jenkins et al., 2008). Grazing birdsfoot trefoil versus perennial ryegrass led to increased concentrations of C12:0, C14:0, C16:0, C18:2 n-6, and C18:3 n-3 fatty acids (FA), and reduced concentrations of *cis*-9 C18:1, *cis*-9, *trans*-11 conjugated linoleic acids (CLA), and *trans*-11 C18:1 FA in milk (Turner et al., 2005). The ruminal microbial population is an integral system with numerous interrelationships. Thus, it is likely that the inhibitory effects of CT influence ruminal biohydrogenation of unsaturated FA, leading to an altered biohydrogenation pathway (Vasta et al., 2008) and, consequently, changes in the FA composition of milk.

We hypothesized that supplementation of CTE would decrease ruminal NH_3 concentration and improve utilization of N for milk production, but its effects would differ between high (HF) and low forage (LF) diets. The objective of this study was to assess ruminal fermentation characteristics, digestibility, and lactational performance of early to mid lactating dairy cows fed an HF or LF diet without or with CTE supplementation. Additionally, we were interested in a possible link between supplementation of CTE and milk FA composition.

MATERIALS AND METHODS

The dairy cows used in this study were cared for according to the Live Animal Use in Research Guidelines

of the Institutional Animal Care and Use Committee at Utah State University (Logan).

Cows, Experimental Design, and Diets

Eight multiparous lactating Holstein cows were used; 4 cows were surgically fitted with ruminal cannulas. Days in milk ranged from 52 to 68 and from 49 to 73 for noncannulated and cannulated cows, respectively, at the start of the experiment. Average BW was 692 ± 69.7 kg at the beginning of the experiment and 710 ± 75.9 kg at the end of the experiment.

The design of the experiment was a double 4×4 Latin square with each period lasting 21 d (14 d of treatment adaptation and 7 d of data collection and sampling). The cows were allocated to squares by whether they were surgically cannulated, and the 2 squares were conducted simultaneously. Within square, cows were randomly assigned to a sequence of 4 diets. A 2×2 factorial arrangement was used; HF or LF diet with a forage-to-concentrate ratio of 59:41 or 41:59 (DM basis; Table 1), respectively, was combined with or without CTE to form 4 treatments: HF diet without CTE (HF-CTE), HF diet with CTE (HF+CTE), LF diet without CTE (LF-CTE), and LF diet with CTE (LF+CTE; Table 1). Water-soluble quebracho CTE in powder form (99% solubility; Chemtan Company Inc., Exeter, NH) was a crude extract of the bark of *Shinopsis* spp., and the appropriate quantity of quebracho CTE was applied into the mixer wagon to be mixed with other ingredients added to the HF+CTE and LF+CTE diets at a rate of 3% DM. The same quebracho CTE product was reported to contain 75% CT concentration with a small amount of simple phenolics (Min et al., 2006).

The forages used were alfalfa hay and corn silage; Table 1 shows diet composition. The concentrate contained steam-flaked barley and a pelleted supplement, and the formulation of the concentrate differed for the LF and HF diets. The diets are typical of high-producing cow diets in northern Utah with the inclusion of Rumensin (approximately 300 mg/head per day; Elanco Animal Health, Greenfield, IN), and were formulated based on NRC (2001) recommendations to provide sufficient NE_L , MP, vitamins, and minerals to produce 40 kg/d of milk with 3.5% fat and 3.0% true protein.

Cows were housed in individual tie stalls fitted with rubber mattresses and bedded with straw, and were fed a TMR for ad libitum intake with at least 10% of daily feed refusal. All cows were individually fed twice daily at 0830 and 1500 h with approximately 70 and 30% of total daily feed allocation at each feeding, respectively. Feed offered and refused was recorded daily, and daily

Table 1. Ingredients and chemical composition (means $\pm$ SD) of the TMR fed to lactating cows ($n = 4$)

Item	Diet ¹			
	HF		LF	
	– CTE	+ CTE	– CTE	+ CTE
Ingredient, % of DM				
Alfalfa hay	31.9	30.9	24.4	23.6
Corn silage	27.6	26.7	16.3	15.8
Barley, steam flaked	16.6	16.1	35.5	34.4
Beet pulp, pellets	4.00	3.89	3.98	3.89
Corn DDGS ²	9.30	9.01	9.26	9.00
Canola meal	3.71	3.61	3.70	3.61
Wheat middlings	2.28	2.22	2.28	2.21
Calcium carbonate	1.08	1.05	1.07	1.05
Sodium sesquicarbonate	1.03	1.01	1.03	1.01
Blood meal, flash dried	0.86	0.83	0.85	0.83
Salt	0.66	0.64	0.65	0.64
Urea	0.37	0.36	0.37	0.36
Magnesium oxide	0.04	0.04	0.04	0.04
Vitamins and minerals ³	0.57	0.55	0.57	0.55
Condensed tannin extract	—	3.0	—	3.0
Chemical composition, % of DM				
DM, %	54.1 $\pm$ 1.73	54.7 $\pm$ 1.23	60.1 $\pm$ 0.54	60.1 $\pm$ 0.53
OM	90.0 $\pm$ 0.15	90.6 $\pm$ 0.63	90.5 $\pm$ 0.71	90.5 $\pm$ 0.77
CP	18.5 $\pm$ 0.24	18.6 $\pm$ 0.27	18.4 $\pm$ 0.79	18.6 $\pm$ 0.68
RDP, ⁴ % of CP	69.1	69.4	69.6	69.8
RUP, ⁴ % of CP	30.9	30.6	30.4	30.2
NDF	37.3 $\pm$ 0.67	37.6 $\pm$ 1.35	34.1 $\pm$ 0.23	35.4 $\pm$ 0.38
ADF	21.9 $\pm$ 0.01	21.4 $\pm$ 1.09	18.3 $\pm$ 0.40	18.7 $\pm$ 0.33
Starch	23.0 $\pm$ 0.07	22.4 $\pm$ 0.71	29.3 $\pm$ 0.92	29.5 $\pm$ 0.92
Ether extract	3.64 $\pm$ 0.102	3.58 $\pm$ 0.013	3.59 $\pm$ 0.157	3.62 $\pm$ 0.262
NE _L , ⁴ Mcal/kg	1.64	1.62	1.73	1.70

¹Diets: high forage (HF) and low forage (LF) with (+ CTE) and without (– CTE) condensed tannin extract.

²Dried distillers grains with solubles.

³Formulated to contain (per kilogram of DM): 71.3 g of P, 68.9 g of K, 94.6 mg of Se (from sodium selenate), 6.56 g of Cu (from copper sulfate), 25.8 g of Zn (from zinc sulfate), 4,131.3 kIU of vitamin A, 515.4 kIU of vitamin D, 5,728.8 IU of vitamin E, and 19.6 mg of Rumensin (Elanco Animal Health, Greenfield, IN).

⁴Based on tabular value (NRC, 2001).

samples were collected to determine DMI. Cows had free access to water.

Cows were milked twice daily at 0400 and 1600 h. Milk production was recorded daily throughout the experiment. Cows were turned outside to a drylot for exercise for at least 1 h daily in the morning after being milked. Milk was sampled during the a.m. and p.m. milkings on 2 consecutive days (d 16 and 17) in each period. Individual milk samples were analyzed for fat, true protein, lactose, and MUN by the Rocky Mountain DHIA Laboratory (Logan, UT). Milk composition was expressed on weighted milk yield of a.m. and p.m. samples. Yields of milk fat, true protein, and lactose were calculated by multiplying milk yield from the respective day by fat, true protein, and lactose concentrations of the milk from an individual cow.

Sampling, Data Collection, and Chemical Analyses

Chopped alfalfa hay, corn silage, and concentrates were sampled weekly to determine DM concentration.

Diets were adjusted weekly to account for changes in DM concentration. Diets of TMR samples were collected on d 20 and 21 for particle size analysis by using the Penn State Particle Separator as described by Kononoff et al. (2003) equipped with 3 sieves (19, 8, and 1.18 mm) and a pan. The recommended proportions for TMR are 2 to 8% on the 19-mm sieve, 30 to 50% on the 8-mm sieve, and 30 to 50% on the 1.18-mm sieve (Kononoff and Heinrichs, 2007). All diets were within the recommended range, except for the proportion on the top screen for the HF diet, which was greater than recommend.

Samples of the TMR fed and orts for individual cows were collected daily during the data collection period, dried at 60°C for 48 h, ground to pass a 1-mm screen (standard model 4, Arthur H. Thomas Co., Philadelphia, PA), and stored for subsequent analyses. Contents of DM of the samples were used to calculate intakes and digestibilities of DM and nutrients.

Analytical DM concentration of samples was determined by oven drying at 135°C for 3 h; OM was deter-

mined by ashing, and N content was determined using an elemental analyzer (Leco TruSpec N, St. Joseph, MI; AOAC, 2000). The NDF and ADF concentrations were sequentially determined using an Ankom^{200/220} Fiber Analyzer (Ankom Technology, Macedon, NY) according to the methodology supplied by the company, which is based on the methods described by Van Soest et al. (1991). Sodium sulfite was used in the procedure for NDF determination and pre-treatment with heat-stable amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation, St. Louis, MO). Starch concentration of feed was determined by using a 2-step enzymatic method (Rode et al., 1999) with a microtiter plate reader (Dynatech Laboratories, Chantilly, VA) to read glucose release colorimetrically at 490 nm.

Weekly samples of dietary ingredients were analyzed for total FA concentration and FA profile according to the procedure of Sukhija and Palmquist (1988) and Kleinschmit et al. (2007) using a GLC (model 6890 series II, Hewlett Packard Co., Avondale, PA) fitted with a flame-ionization detector. The injector port temperature was 230°C with a split ratio of 20:1. The column was 100 m, with an inside diameter of 0.25 mm (CP-Sil 88, Varian, Lake Forest, CA). The carrier gas was helium at a rate of 2.0 mL/min. Initial oven temperature was 50°C held for 1 min, and then increased to 145°C at a rate of 5°C per min and held for 30 min. The temperature was then increased at 10°C/min to 190°C and held for 30 min. Finally, the temperature was increased at 5°C/min to 210°C and held for 35 min. The total run per sample lasted 123.5 min.

Weighted composite milk samples from individual cows were analyzed for FA composition. Milk fat was extracted by boiling milk in a detergent solution (Hurley et al., 1987). Extracted fat was derivatized to methyl esters using an alkaline methylation procedure by mixing 40 mg of fat with a sodium methoxide methylation reagent (NaOCH₃/MeOH) as described by Chouinard et al. (1999). After FA methyl esters were formed, anhydrous calcium chloride pellets were added and allowed to stand for 1 h to remove water in the sample. Samples were then centrifuged at $1,016 \times g$ at 4°C for 20 min.

Separation of FA was achieved by using a GLC (model 6890 series II) fitted with a flame-ionization detector. Samples containing methyl esters in hexane (1 µL) were injected through the split injection port (100:1) onto the column (CP-Sil 88). Oven temperature was set at 80°C and held for 10 min, and then increased to 190°C at 12°C/min for 39 min. The temperature was then increased again to 218°C at 20°C/min and held for 21 min. Injector and detector were set at 250°C. Total run time was 71 min. Heptadecadenoic acid was used as a qualitative internal standard. Individual FA

concentrations were obtained by taking the specific FA area as a percentage of total FA, and were reported as grams per hundred grams of FA methyl esters.

Feed DM and nutrient digestibility was measured during the last week in each period using acid-insoluble ash (**AIA**) as an internal marker (Van Keulen and Young, 1977). Fecal samples (approximately 200 g wet weight) were collected for each cow from the rectum twice daily (a.m. and p.m.) every 12 h, moving ahead 2 h each day for the 6 d of fecal sampling beginning on d 15. This schedule provided 12 representative samples of feces for each cow. Samples were immediately subsampled (about 50 g), composited across sampling times for each cow and each period, dried at 55°C for 72 h, ground to pass a 1-mm screen (standard model 4), and stored for chemical analysis. Apparent total-tract nutrient digestibilities were calculated from concentrations of AIA and nutrients in diets fed, Orts, and feces using the following equation: apparent digestibility = $100 - [100 \times (AIA_d/AIA_f) \times (N_f/N_d)]$, where AIA_d = AIA concentration in the diet actually consumed, AIA_f = AIA concentration in the feces, N_f = concentration of the nutrient in the feces, and N_d = concentration of the nutrient in the diet actually consumed (Eun and Beauchemin, 2005).

Ruminal Fermentation Characteristics

Ruminal pH was continuously measured for 2 consecutive days starting on d 18 using the Lethbridge Research Centre Ruminal pH Measurement System (**LRCpH**; Dascor, Escondido, CA) as described by Penner et al. (2006). Readings in pH buffers 4 and 7 were recorded before placing the LRCpH system in the rumen. Ruminal pH readings were taken every 30 s and stored by the data logger. After about 48 h of continuous pH measurement, the LRCpH was removed from the rumen and washed in 39°C water; millivolt readings were recorded in pH buffers 4 and 7. The daily ruminal pH data were averaged for each minute and summarized as minimum pH, mean pH, and maximum pH. In addition, daily episodes, duration (h/d), and area (pH × min) when ruminal pH was <5.5 were calculated. The threshold 5.5 was assigned because it has been defined by others (Beauchemin and Yang, 2005) to cause ruminal acidosis.

Ruminal contents were sampled from cannulated cows 0, 3, and 6 h after the a.m. feeding on d 20 and 21. Approximately 1 L of ruminal contents was obtained from the anterior dorsal, anterior ventral, medial ventral, posterior dorsal, and posterior ventral locations within the rumen, composited by cow, and strained through a polyester screen (pore size 355 µm; B & S H Thompson, Ville Mont-Royal, QC, Canada). Five milli-

liters of the filtered ruminal fluid was added to 1 mL of 1% sulfuric acid, and samples were retained for $\text{NH}_3\text{-N}$ determination. Concentration of $\text{NH}_3\text{-N}$ in the ruminal contents was determined as described by Rhine et al. (1998). Another 5 mL of the filtered ruminal fluid was taken at 3 h after the a.m. feeding and added to 1 mL of 25% of meta-phosphoric acid, and the samples were retained for VFA determination. The VFA were quantified using a GLC (model 6890 series II) with a capillary column (30 m $\times$ 0.32 mm i.d., 1- μm phase thickness, Zebtron ZB-FAAP, Phenomenex, Torrance, CA), and flame-ionization detection. Crotonic acid was used as an internal standard. The oven temperature was 170°C held for 4 min, which was then increased by 5°C/min to 185°C, and then by 3°C/min to 220°C, and held at this temperature for 1 min. The injector temperature was 225°C, the detector temperature was 250°C, and the carrier gas was helium.

Statistical Analyses

Data were summarized for each cow by measurement period. All data were statistically analyzed using the mixed model procedure in SAS (SAS Institute, 2001). Data for intake, BW, digestibility, energy balance, and milk production were analyzed by using a model that included the effects of forage level in the diet (HF vs. LF), square (noncannulated vs. cannulated cows), CTE supplementation (without vs. with CTE), and the interaction between forage level in the diet and CTE. Cow, period, and cow by period by square were the terms of the random statement.

Data for VFA profiles and $\text{NH}_3\text{-N}$ concentration were analyzed with a model that included the effects of forage level (HF vs. LF), CTE supplementation (without vs. with CTE), and the interaction between forage level and CTE. In addition, the fixed effect of time after feeding was included using the repeated option. Cow and period were the terms of the random statement. The covariance structure that resulted in the lowest values for the Akaike's information criteria and Schwarz's Bayesian criterion was used (Littell et al., 1998). Data for DMI were reported using the heterogeneous compound symmetry structure, whereas milk yield data were analyzed by the autoregressive structure. Data for milk components and efficiency of feed and N utilization were analyzed using the unstructured covariance structure. In addition, data for VFA and $\text{NH}_3\text{-N}$ were analyzed by the unstructured and unstructured variance-covariance structure.

Residual errors were used to test main effects and interactions. Differences were considered significant at $P < 0.05$ and trends were discussed at $P < 0.10$. When the interaction between forage level in the diet

and CTE was $P < 0.10$, contrasts were used to examine the effects of CTE within forage level using single degree of freedom contrasts. Contrasts were considered significant at $P < 0.05$. Results are reported as least squares means.

RESULTS AND DISCUSSION

Characteristics of Experimental Diets

Ingredients and chemical composition of experimental diets are listed in Table 1. Alfalfa hay was used as the main forage in the HF and LF diets, and the alfalfa hay was high quality, being clean, bright green, and fine stemmed with a chemical composition of 21.7 ± 1.68 , 40.6 ± 0.09 , and $30.7 \pm 0.27\%$ DM for CP, NDF, and ADF, respectively. Therefore, digestive and nutritive contributions by the alfalfa hay in the current study would be relatively high due to its large dietary proportion (31.9 and 24.4% DM for the HF and LF diets, respectively) and its high nutritive quality. Corn silage used in this study contained 7.2 ± 0.70 , 41.7 ± 1.67 , and $23.4 \pm 1.12\%$ DM for CP, NDF, and ADF, respectively. Concentrations of CP were similar between the HF and the LF diet, and the RDP to RUP ratio was maintained at 70:30, on average, across diets. Concentrations of NDF and ADF were higher in the HF diet compared with the LF diet, whereas starch concentration was higher in the LF than in the HF diet (29.4 vs. 22.9% on average).

Fatty acid profiles of experimental diets are presented in Table 2. The concentration of *cis*-9 C18:1 was higher in the LF diet, whereas C18:3 n-3 was present at a higher concentration in the HF diet. Diets were similar in other FA and total saturated and unsaturated FA.

Intake and Digestibility

The effects of increasing the forage proportion of the diet observed in this study were as expected based on previous literature (Yang et al., 2001; Eun and Beauchemin, 2005). Yang et al. (2001) reported no difference on DM intake in response to forage-to-concentrate ratios of 35:65 or 55:45 (DM basis) in lactating cows, whereas Eun and Beauchemin (2005) reported that total-tract digestibilities of DM, OM, and NDF were not affected by a forage-to-concentrate ratio of 60:40 vs. 34:66 (DM basis) in lactating cows. The discussion of these effects is limited because the focus of this study was to understand the interaction between level of forage in the diet and CTE supplementation, rather than the effects of forage-to-concentrate ratio per se.

Supplementing CTE in the diet decreased intake of DM, OM, CP, NDF, and ADF, regardless of forage

Table 2. Fatty acid composition (means $\pm$ SD) of the TMR fed to lactating cows ($n = 4$)

Fatty acid ¹	Diet ²			
	HF		LF	
	– CTE	+ CTE	– CTE	+ CTE
C14:0	0.38 $\pm$ 0.035	0.36 $\pm$ 0.029	0.35 $\pm$ 0.027	0.38 $\pm$ 0.029
C16:0	20.9 $\pm$ 0.28	21.3 $\pm$ 0.66	20.8 $\pm$ 0.65	21.8 $\pm$ 0.24
C17:0	0.17 $\pm$ 0.023	0.17 $\pm$ 0.009	0.17 $\pm$ 0.025	0.17 $\pm$ 0.007
C18:0	2.60 $\pm$ 0.189	2.60 $\pm$ 0.141	2.60 $\pm$ 0.109	2.80 $\pm$ 0.087
C18:1 <i>trans</i> -9	0.08 $\pm$ 0.004	0.07 $\pm$ 0.008	0.07 $\pm$ 0.019	0.07 $\pm$ 0.022
C18:1 <i>cis</i> -9	20.0 $\pm$ 0.29	19.6 $\pm$ 0.17	21.2 $\pm$ 0.60	21.2 $\pm$ 0.52
C18:2	45.0 $\pm$ 0.56	44.9 $\pm$ 0.26	45.5 $\pm$ 0.44	44.8 $\pm$ 0.75
C18:3 n-6	0.55 $\pm$ 0.159	0.50 $\pm$ 0.137	0.49 $\pm$ 0.128	0.45 $\pm$ 0.141
C18:3 n-3	8.31 $\pm$ 0.299	8.16 $\pm$ 0.212	6.51 $\pm$ 0.438	6.14 $\pm$ 0.387
C20:0	0.53 $\pm$ 0.043	0.52 $\pm$ 0.109	0.48 $\pm$ 0.055	0.49 $\pm$ 0.011
C20:2	0.04 $\pm$ 0.006	0.03 $\pm$ 0.013	0.03 $\pm$ 0.015	0.05 $\pm$ 0.033
C20:4	0.18 $\pm$ 0.015	0.20 $\pm$ 0.017	0.20 $\pm$ 0.038	0.19 $\pm$ 0.009
C22:0	0.41 $\pm$ 0.042	0.48 $\pm$ 0.040	0.43 $\pm$ 0.012	0.49 $\pm$ 0.012
C24:0	0.73 $\pm$ 0.073	0.72 $\pm$ 0.021	0.59 $\pm$ 0.053	0.61 $\pm$ 0.087
Saturated fatty acids	26.1 $\pm$ 0.59	26.7 $\pm$ 0.37	26.2 $\pm$ 0.71	27.2 $\pm$ 0.27
Unsaturated fatty acids	73.9 $\pm$ 0.59	73.3 $\pm$ 0.37	73.8 $\pm$ 0.71	72.8 $\pm$ 0.27

¹Fatty acid composition expressed as g/100 g of fatty acid methyl esters.

²Diets: high forage (HF) and low forage (LF) with (+ CTE) and without (– CTE) condensed tannin extract.

level (Table 3). Previous studies on the effects of CT on feed intake in ruminants have yielded inconsistent results. Decreases of DMI with CT have already been reported either in cows (McNabb et al., 1996 with 5.5% of CT; Priolo et al., 2000 with 2.5% of CT) or in sheep (Barry and McNabb, 1999 with 7.5 to 10.0% of CT). This depressing effect would be attributed to degraded palatability (Cooper and Owen-Smith, 1985) or to a short-term effect of astringency (Landau et al., 2000). Others found no effect of CT on DMI in either Jersey heifers (0.60% of quebracho CTE; Baah et al., 2007) or lactating dairy cows (0.45% of CT; Benchaar et al., 2008). Conversely, DMI increases were also observed with CT as in Woodward et al. (2001) and Carulla et al. (2005) with 2.59 and 2.50% of CT, respectively. These results suggest that CT fed at relatively high concentrations has negative effects on feed intake in ruminants, and the effects may vary with the source of CT.

Although supplementing CTE in the diet decreased feed intake, total-tract digestibilities of DM and nutrients were not affected, regardless of the level of forage in the diet. Similar to our result, Benchaar et al. (2008) observed no effects on digestibilities of DM and nutrients (OM, CP, and ADF) with the addition of quebracho CTE at 0.64% of DM. In contrast, Beauchemin et al. (2007) reported that the digestibility of CP decreased in beef cattle with the addition of 1 to 2% quebracho CTE. Carulla et al. (2005) observed that supplementing the diet of sheep with 2.5% DM of CTE from black wattle tree decreased digestibilities of OM, CP, NDF, and ADF. Although binding of tannins to protein im-

proves efficiency of N utilization in ruminants (Aerts et al., 1999b), it may influence CP digestibility depending upon concentration and source of tannins. In fact, a diet containing 1.8% CT from big trefoil resulted in a reduced CP digestibility, whereas the same concentration of CT from birdsfoot trefoil had less effect on CP digestibility (Waghorn and Shelton, 1997). Similar to our findings, Baah et al. (2007) reported that digestibilities of DM, OM, and NDF were not altered in LF diets supplemented with quebracho CTE at 0.6% DM when fed to Jersey heifers. Based on CTE supplementation having no effect on digestibility in this study, it is likely that the quebracho CTE supplementation at 3% DM in the diets tested in our study simply depressed feed intake due to palatability without affecting feed digestion.

Milk Production and Efficiency

Milk yield averaged 34.6 and 36.1 kg/d for the HF and LF diets, respectively, and was not influenced by CTE supplementation (Table 4), which agreed with Benchaar et al. (2008) and Aguerre et al. (2010) with lower dietary inclusions of CTE (0.64 and 1.8%, DM basis, respectively). Concentrations of milk fat, true protein, and lactose were not affected by CTE supplementation. Yield of milk components was not altered by CTE supplementation. Milk fat yield and composition were not affected when supplementing CTE in lactation dairy diets up to 1.8% DM (Aguerre et al., 2010). Benchaar et al. (2008) reported that milk composition was not changed when supplementing quebracho CTE

Table 3. Nutrient intake and total-tract digestibility of lactating cows fed high (HF) or low forage (LF) diets without or with condensed tannin extract (CTE) supplementation

Item	Diet				SEM	Significance of effect ¹		
	HF		LF			FL	CTE	FL × CTE
	– CTE	+ CTE	– CTE	+ CTE				
Intake, kg/d								
DM	27.7	25.4	27.5	26.3	1.25	0.53	<0.01	0.32
OM	24.0	22.8	24.6	23.8	1.17	0.10	0.04	0.63
CP	4.94	4.68	5.08	4.96	0.224	0.02	0.03	0.41
NDF	10.0	9.37	9.67	9.19	0.495	0.14	<0.01	0.59
ADF	5.75	5.39	5.12	4.89	0.287	<0.01	<0.01	0.54
Digestibility, %								
DM	68.8	65.9	71.1	70.0	2.28	0.17	0.39	0.70
OM	70.2	67.6	72.4	71.2	2.30	0.23	0.42	0.76
CP	70.8	67.1	73.4	70.5	3.01	0.29	0.24	0.87
NDF	53.1	51.1	55.0	54.9	3.55	0.43	0.76	0.79
ADF	49.8	47.5	48.1	48.7	3.89	0.95	0.82	0.72

¹FL = forage level in the diet (HF vs. LF); CTE = without versus with CTE supplementation; and FL × CTE = interaction between FL and CTE.

to lactating dairy cows. When supplementing CTE at different levels, Aguerre et al. (2010) reported that milk true protein concentration increased at 0.45% CTE supplementation, whereas supplementing CTE at 1.8% DM decreased milk true protein concentration. It seems that milk composition depends on the concentration of CT in the diet tested, and in our case, CTE supplementation at 3% DM would have minor effects on milk composition. However, it is not clear how the smaller dose of CTE at 1.8% DM tested by Aguerre et al. (2010) decreased milk true protein concentration.

Concentration of MUN decreased by 16.9 and 16.2% in the HF and LF diets, respectively, in this study. Milk urea N decreased by 7.2% in cows supplemented with CTE at 1.8% DM, but not at lower concentrations of 0.45 to 0.9% DM (Aguerre et al., 2010). Likewise, Benchaar et al. (2008) observed no effects of CTE supplemented at 0.64% of DM on MUN. Although the CP concentration in our diets was relatively high compared with typical lactation diets, the CP concentration and RDP to RUP ratio were similar across diets. Therefore, the effect of CTE supplementation at 3% of DM used

Table 4. Milk production and composition and efficiencies of DM and N use for milk production of lactating cows fed high (HF) or low forage (LF) diets without or with condensed tannin extract (CTE) supplementation

Item	Diet				SEM	Significance of effect ¹		
	HF		LF			FL	CTE	FL × CTE
	− CTE	+ CTE	− CTE	+ CTE				
Milk yield, kg/d	34.1	35.0	36.2	36.0	1.82	<0.01	0.42	0.21
Milk composition, %								
Fat	3.75	3.69	3.59	3.61	0.111	0.02	0.75	0.42
True protein	3.07	3.05	3.11	3.09	0.059	0.05	0.28	0.76
Lactose	4.83	4.81	4.89	4.87	0.049	<0.01	0.15	0.95
MUN, mg/dL	14.8	12.3	14.8	12.4	0.80	0.91	<0.01	0.96
Milk component yield, kg/d								
Fat	1.29	1.29	1.30	1.30	0.091	0.84	0.92	0.93
True protein	1.05	1.05	1.12	1.10	0.050	<0.01	0.55	0.43
Lactose	1.66	1.69	1.76	1.75	0.099	0.04	0.84	0.61
Efficiency								
Milk yield/DMI	1.23 ^b	1.37 ^a	1.33	1.37	0.071	0.05	<0.01	0.07
Milk N/N intake ²	0.229	0.231	0.236	0.236	0.0116	0.13	0.75	0.80

^{a,b}Means in the same row within HF and LF subgroups with different superscripts differ based on single degree of freedom contrasts ($P < 0.05$).

¹FL = forage level in the diet (HF vs. LF); CTE = without versus with CTE supplementation; and FL × CTE = interaction between FL and CTE.

²Efficiency of use of feed N to milk N = [(milk true protein, kg/d ÷ 0.93) ÷ 6.38] ÷ N intake, kg/d (Dschaaka et al., 2010).

in this study would not be influenced by diets to elicit a positive effect on MUN concentration. Milk urea N is used as a management tool to improve dairy herd nutrition and monitor the nutritional status of lactating dairy cows. Urinary N excretion has been shown to have a positive linear relationship with MUN (Ciszuk and Gebregziabher, 1994; Jonker et al., 1998). Elevated MUN indicates that excess CP has been fed to the dairy cow for her level of production (Broderick and Clayton, 1997; Jonker et al., 1998). Broderick (1995b) reported that MUN more clearly reflected dietary CP intake than did ruminal ammonia concentration. In our case, however, it is clear that forming CT-protein complexes decreased protein degradation and $\text{NH}_3\text{-N}$ production in the rumen as discussed later, resulting in decreased MUN concentration. However, CTE supplementation did not affect milk protein concentration and yield in spite of the decreased MUN in this study. Nelson (1996) suggested that when total milk protein was 3.0 to 3.2% and MUN concentration was 12 to 16 mg/dL, protein degradability fractions and NE_L were likely in balance. The MUN concentration and the milk protein concentration in our study were within this range, and CT supplementation clearly decreased MUN concentration. Lower MUN by CTE supplementation suggests that supplementing CTE in lactating dairy diets may reduce N excretion into urine because urinary N excretion has been shown to have a positive linear relationship with MUN (Jonker et al., 1998).

Contrary to the effect of CTE on MUN, efficiency of N use for milk N was not affected by CTE supplementation (Table 4), probably because CTE did not affect milk protein yield. Feed efficiency (milk yield/DMI) increased by CTE supplementation in the HF diet, but not in the LF diet, leading to a tendency for an interaction between forage level and CTE supplementation ($P = 0.07$). Aguerre et al. (2010) reported that feed efficiency was not influenced by supplementing CTE up to 1.8% DM. Makkar (2003) indicated that the greater animal performance observed in some studies when animals were fed CT has been related to the protection of dietary protein from rumen microbial degradation, resulting in increased supply of AA to the intestine and greater absorption into blood. However, in our case it is likely that the negative effects of CTE supplementation on feed intake resulted in increased feed efficiency in the HF diet.

Ruminal Fermentation Characteristics

Feeding the LF diet decreased ruminal pH with mean ruminal pH of 6.47 and 6.33 in the HF and the LF diet, respectively (Table 5). Because of the higher starch concentration in the LF diet (Table 1), we expected

that the LF diet would decrease ruminal pH. However, no notable depression in ruminal pH was shown in minimum pH and $\text{pH} < 5.5$. Yang and Beauchemin (2007) fed LF diets (35:65 of forage-to-concentrate ratio) to lactating dairy cows and observed mean ruminal pH of 6.02 with area under $\text{pH} 5.5 \times \text{min}$ of 75, whereas feeding the LF diet in this study resulted in area under $\text{pH} 5.5 \times \text{min}$ of 1.73. Although we used barley grain as a main ingredient of the concentrate mixture, dietary concentration of the barley grain was 35.5% DM in this study, which is notably lower than that of LF diet (56.1% DM) tested by Yang and Beauchemin (2007). Thus, the relatively low dietary concentration of barley grain in the LF diet and proper particle size distribution of the diet prevented the decrease in ruminal pH.

Supplementation of CTE did not influence ruminal pH in the HF or LF diet (Table 5). Similar to our results, Benchaar et al. (2008) and Aguerre et al. (2010) reported no effects on ruminal pH by feeding CTE to dairy cows. We could not test effects of CTE supplementation on its potential to prevent ruminal acidosis by reducing rapid starch hydrolysis, because feeding the LF diet did not create a severely acidotic fermentative environment in the rumen.

Supplementation of CTE decreased total VFA concentration regardless of the level of forage in the diet (Table 5). Decreased VFA production corresponds to the decreased DMI in this study. Molar proportions of acetate, propionate, and butyrate increased in the HF diet, but not in the LF diet due to CTE supplementation, resulting in interactions between forage level and CT supplementation. Supplementing CTE in the HF diet decreased the acetate-to-propionate (A:P) ratio, but in the LF diet, CT supplementation increased the A:P ratio, resulting in an interaction between forage level and CT supplementation. Effects of CTE on total VFA concentration and VFA pattern have been variable among studies depending on the dosage rate and the source of CT. For example, Beauchemin et al. (2007) reported that despite a lack of effect of quebracho CTE supplementation on digestibility of DM, increasing supplementation levels of quebracho CTE (up to 2% of DMI) tended ($P = 0.08$) to decrease total VFA concentration, and decreased acetate molar proportion and A:P ratio. In contrast, despite a decrease in digestibility of OM, Carulla et al. (2005) observed no change in total VFA concentration in sheep supplemented with black wattle tree CTE, but the molar proportion of acetate decreased, and that of propionate increased in sheep fed diets supplemented with CTE. Benchaar et al. (2008) and Aguerre et al. (2010) reported that total concentrations of VFA and molar proportions of individual VFA were not affected by feeding quebracho CTE.

Table 5. Ruminal fermentation characteristics of lactating cows fed high (HF) or low forage (LF) diets without or with condensed tannin extract (CTE) supplementation

Item	Diet				SEM	Significance of effect ¹		
	HF		LF			FL	CT	FL × CTE
	− CTE	+ CTE	− CTE	+ CTE				
Minimum pH	5.63	5.45	5.39	5.30	0.152	0.02	0.73	0.71
Mean pH	6.43	6.50	6.33	6.33	0.080	<0.01	0.38	0.35
Maximum pH	7.01	7.07	6.93	6.89	0.074	0.02	0.84	0.27
pH <5.5								
Daily episodes	0.00	0.25	1.00	0.50	0.401	0.04	0.62	0.17
Duration, h/d	0.00	0.06	0.19	0.29	0.161	0.12	0.54	0.85
Area, pH × min	0.00	0.17	1.17	2.28	1.193	0.14	0.53	0.64
Total VFA, mM	129.5	121.8	137.0	131.9	3.44	<0.01	0.03	0.64
Individual VFA ²								
Acetate (A)	64.3 ^b	65.9 ^a	57.9	58.9	0.96	<0.01	0.09	<0.01
Propionate (P)	18.7 ^b	20.3 ^a	25.7	24.4	0.69	<0.01	0.65	0.01
Butyrate	10.5 ^b	11.5 ^a	13.0	13.6	0.49	<0.01	0.16	<0.01
A:P	3.48 ^a	3.29 ^b	2.29 ^b	2.47 ^a	0.114	<0.01	0.83	<0.01
NH ₃ -N, mg/dL	10.4	8.49	8.52	7.60	0.902	0.10	0.09	0.52

^{a,b}Means in the same row within HF and LF subgroups with different superscripts differ based on single degree of freedom contrasts ($P < 0.05$).

¹FL = forage level in the diet (HF vs. LF); CTE = without versus with CTE supplementation; and FL × CTE = interaction between FL and CTE.

²Expressed as mol/100 mol.

An interesting finding of this experiment was that molar proportions of VFA were affected by CTE in the HF diet, but not in the LF diet. In addition, CTE supplementation decreased the A:P ratio in the HF diet, whereas CTE supplementation increased A:P ratio in the LF diet. The mechanism whereby CTE supplementation resulted in an opposite effect on A:P ratio between the HF and LF diets is difficult to explain. Supplementing CTE would beneficially manipulate ruminal fermentation in the HF diet that contained a higher dietary proportion of alfalfa hay compared with the LF diet that contained a higher level of steam-flaked barley. A favorable change in A:P ratio in the HF diet due to CTE supplementation may contribute to the increasing feed efficiency discussed earlier. The differences in the A:P ratio between the HF and the LF diets detected in this study may be due to several factors, such as ruminal bacterial growth rates due to different starch concentrations between the HF and the LF diet, substrate affinities with CT (alfalfa hay protein vs. barley protein), or microbial inhibition. Further research needs to elucidate how CTE supplementation would beneficially affect ruminal fermentation in HF diets, particularly diets containing high dietary proportions of alfalfa hay.

The concentration of NH₃-N tended to decrease ($P = 0.09$) with supplementation of CTE (Table 5). A decrease in NH₃-N concentration was reported when CTE was supplemented at 1.8% DM, whereas no effects were

observed at a lower concentration (0.45 and 0.9% DM; Aguerre et al., 2010). Moreover, a diet containing 1.8% of CT from big trefoil resulted in a decreased ruminal NH₃-N concentration, whereas the same concentration of CT from birdsfoot trefoil had lesser effects (Waghorn and Shelton, 1997). The N-binding effects of CT have been well documented (Waghorn et al., 1987; Barry and McNabb, 1999; Beauchemin et al., 2007). The CT present in birdsfoot trefoil have been found to inhibit the growth of proteolytic bacteria and may also precipitate plant protein, making it less available for proteolysis (Min et al., 2000; Molan et al., 2001), thereby inhibiting NH₃ production. When protein is rapidly degraded in the rumen, NH₃ is produced more quickly than the microbes can utilize it for protein synthesis, resulting in more protein being degraded than synthesized (Broderrick, 1995a). Powell et al. (2009) recently reported a shift of N excretion routes by feeding CT-containing forages, because the ratio of N excreted in feces and urine was greatest for low-tannin and high-tannin birdsfoot treatments and lowest for alfalfa treatment. Reduced excretion of urinary N would result in reduced environmental losses through nitrate leaching, NH₃ volatilization, and nitrous oxide emissions (Place and Mitloehner, 2010). Because supplementation of CTE had no effect on N utilization efficiency for milk production in this study, supplementation of CTE in lactation dairy diets may change the route of N excretion, having less excretion into urine but more into feces.

Table 6. Fatty acid composition in the milk of lactating cows fed high (HF) or low forage (LF) diets without or with condensed tannin extract (CTE) supplementation

Fatty acid ¹	Diet				SEM	Significance of effect ²		
	HF		LF			FL	CTE	FL × CTE
	– CTE	+ CTE	– CTE	+ CTE				
C16:0	34.4	33.7	34.6	34.2	0.94	0.57	0.38	0.71
C16:1 <i>trans</i> -9	0.46	0.55	0.52	0.50	0.034	0.81	0.08	0.01
C16:1 <i>cis</i> -9	1.28	1.31	1.52	1.34	0.124	0.05	0.31	0.13
C17:0	0.57	0.62	0.60	0.61	0.039	0.85	0.16	0.43
C17:1 <i>cis</i> -10	0.16	0.17	0.18	0.16	0.014	0.71	0.81	0.43
C18:0	8.82	9.03	8.25	8.36	0.508	0.03	0.57	0.85
C18:1 <i>cis</i> -9	17.8	18.4	17.2	17.5	0.76	0.21	0.49	0.83
C18:1 <i>trans</i> -10	0.27	0.29	0.28	0.28	0.020	0.94	0.61	0.37
C18:1 <i>cis</i> -11	0.87	0.91	0.87	0.95	0.077	0.77	0.32	0.68
C18:1 <i>trans</i> -11	1.23	1.38	1.23	1.32	0.085	0.70	0.12	0.71
C18:1 <i>trans</i> , total	2.50	2.76	2.50	2.73	0.146	0.91	0.04	0.87
<i>cis</i> -9, <i>trans</i> -11 CLA	0.32	0.37	0.38	0.42	0.032	0.09	0.15	0.82
<i>trans</i> -10, <i>cis</i> -12 CLA	0.06	0.08	0.07	0.08	0.008	0.77	0.06	0.81
C18:2 n-6	2.68	2.65	2.74	2.89	0.119	0.21	0.59	0.41
C18:3 n-3	0.35	0.39	0.34	0.39	0.024	0.74	0.02	0.79
C18:3 n-6	0.10	0.11	0.11	0.11	0.009	1.00	0.64	0.60
C20:0	0.12	0.14	0.13	0.12	0.007	0.45	0.12	0.03
C20:1	0.11	0.13	0.13	0.17	0.015	0.12	0.07	0.57
C20:2	0.10	0.12	0.10	0.11	0.011	0.56	0.11	0.76

¹Fatty acid composition was expressed as g/100 g of fatty acid methyl esters. C18:1 *trans*, total = *trans*-4,5 C18:1 + *trans*-6,8 C18:1 + *trans*-9 C18:1 + *trans*-10 C18:1 + *trans*-11 C18:1 + *trans*-12 C18:1 + *trans*-13,14 C18:1; CLA = conjugated linoleic acid.

²FL = forage level in the diet (HF vs. LF); CTE = without versus with CTE supplementation; and FL × CTE = interaction between FL and CTE.

It is also possible that the tendency on lower ruminal NH₃-N with CTE supplementation was due in part to the lower CP intake as reported in Table 3.

Milk FA Composition

In general, supplementation of CTE had minor effects on milk FA profiles regardless of forage level in the diet (Table 6). Concentration of total C18:1 *trans* FA increased with CTE supplementation. Cows fed CTE-supplemented diets tended to increase ($P = 0.06$) *trans*-10, *cis*-12 CLA. Supplementing CTE increased the proportion of C18:3 n-3 FA regardless of forage level. In addition, supplementing CT tended to increase ($P = 0.07$) C20:1 FA. Limited information exists on the use of CTE to alter the ruminal biohydrogenation process and manipulate the FA profile of bovine milk and meat. Benchaar and Chouinard (2009) reported that milk FA composition was not affected by supplementing quebracho CTE. Jones et al. (1994) showed inhibition of the growth of *B. fibrisolvens*, which is involved in ruminal biohydrogenation of FA. Using 24-h in vitro batch culture, Kronberg et al. (2007) reported that quebracho CTE reduced biohydrogenation of C18:3 FA. Using continuous cultures, Khiaosa-Ard et al. (2009) reported an inhibition of the last step of biohydrogenation by CTE supplied at a rate of 7.89% DM, leading to the

accumulation of *trans*-11 C18:1 FA. It is likely that the CTE supplementation at 3% DM tested in this study would not interfere in the biohydrogenation process in the rumen and consequently have no major effect on FA profiles in the milk.

CONCLUSIONS

Experimental cows used in this study maintained overall productive performance without any negative response on digestibility, milk production, and ruminal fermentation when cows were fed CTE-supplemented diets. Condensed tannins decreased intakes of DM and nutrients, but CT improved feed efficiency in the HF diet. The greater response to CTE supplementation in the HF diet may have resulted from a higher dietary proportion of alfalfa hay in the HF diet, which highlights that CTE supplementation needs to be focused on diets containing high forage N degradability in the rumen. The most significant findings in this study were that cows receiving CTE-supplemented diets decreased ruminal NH₃-N and MUN concentrations without loss of milk protein yield. This indicates that less ruminal N was lost as NH₃ because of decreased CP degradation by rumen microorganisms in response to CTE supplementation. Therefore, dietary manipulation with the use of CTE in dairy diets may alter ruminal metabo-

lism and N excretion into urine. Because of a lack of effect on N utilization efficiency, the benefit may be an increase of N excretion into feces, a more stable form of N, influencing the ratio of fecal N to urinary N, but not total N excretion.

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