Effect of feeding condensed tannins in high protein finishing diets containing corn distillers grains on ruminal fermentation, nutrient digestibility, and route of nitrogen excretion in beef cattle[1,2]

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ABSTRACT: Eight ruminally cannulated crossbred beef heifers (427 ± 41.2 kg, body weight) were used in a replicated 4 × 4 Latin square to determine the effects of feeding a condensed tannin (**CT**) extract with high protein diets containing corn dried distillers grains and solubles (**DG**) on ruminal fermentation, nutrient digestibility, and route of nitrogen (**N**) excretion. Dietary treatments included [dry matter (**DM**) basis]: 0 (**0DG**), 20 (**20DG**), and 40% DG (**40DG**), and 40% DG with 2.5% CT extract (1.33% CT) from *Acacia mearnsii* (**40DGCT**). The DG and CT extract were substituted for grain in a barley-based diet that contained 91% concentrate and 9% silage (DM basis) and was fed as a total mixed ration once daily. The crude protein concentrations of the diets were 12.9, 16.8, 20.4, and 20.5% for 0DG, 20DG, 40DG, and 40DGCT, respectively. Periods were 5 wk with 2 wk for transition to the DG level of the diets, 1 wk for adaptation to CT, and 2 wk for measurements. Feed offered and refused were measured daily. Total urine and fecal output were collected daily for 4 d consecutively. Data were analyzed using a mixed linear model with diet and period as fixed effects and square and animal within square as random effects. There was no effect ($P \geq 0.22$) of CT on DM intake, but 40% DG in the diet (40DG and 40DGCT) decreased ($P \leq 0.015$) DM intake compared with 20DG. As a result, nitrogen (**N**) intake was not different ($P > 0.15$) among heifers fed 20DG, 40DG, and 40DGCT (313 g N/d) and was less ($P \leq 0.001$) for heifers fed 0DG (220 ± 18 g N/d). Apparent total tract N digestibility was less ($P \leq 0.001$) in heifers fed 40DGCT (70.6 ± 1.07%) compared with to 0DG, 20DG, and 40DG (78.4%). There was no effect ($P = 0.84$) of CT (40DGCT vs. 40DG) on the total N output, however, feeding 40DGCT decreased ($P \leq 0.001$) the excretion of total urinary N and urea N in urine by 17 and 21%, respectively, compared with heifers fed 40DG and was equivalent ($P \geq 0.12$) to the amount excreted by heifers fed 20DG. The reduction of N digestibility reflected the protein binding effects of CT within the gastrointestinal tract and the shift in excess N excretion from labile urea N in urine to bound NDIN and ADIN in feces ($P \leq 0.001$) in heifers fed 40DGCT compared with 40DG. Supplementation of CT in high protein diets fed to feedlot cattle reduced urinary N and increased the capture of N in manure to potentially lesson the loss of N as ammonia and provide opportunities for improved nutrient management of beef production.

Key words: condensed tannins, corn distillers grains, feedlot cattle, nitrogen excretion

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INTRODUCTION

The expansion of the ethanol industry has increased the production of by-product feeds, primarily as corn distillers grains, making distillers grains an economical alternative to corn and other grains for beef cattle production (Klopfenstein et al., 2008). Dried distillers grains with solubles (**DG**) can be substituted as an energy source for up to 40% of corn and barley grain in the diets of finishing cattle with equal or better performance and carcass quality (Klopfenstein et al., 2008; Walter et al., 2010). However, feeding DG as an energy source at this level, particularly in barley-based diets with a higher basal crude protein (**CP**) concentration, can exceed requirements for CP by 50 to 80% (Walter et al., 2010; Hünerberg et al., 2013). Excess CP in the form of rumen degradable protein (**RDP**) or rumen undegradable protein (**RUP**) is metabolized and the excess nitrogen (**N**) excreted as urea in urine. Urinary urea is rapidly hydrolyzed to ammonium by microbial urease present in feces and the environment (Mobley and Hausinger, 1989) and then converted to ammonia which is readily volatilized and lost from the farm system to the environment.

Condensed tannins (**CT**) are secondary plant metabolites with complex and diverse chemical structures with a general affinity for binding to protein and to a lesser extent complex carbohydrates (Mueller-Harvey, 2006; Waghorn, 2008). The CT extract of *Acacia mearnsii* is an industrial tannin that has been investigated for improving N utilization (Ávila et al., 2015; Orlandi et al., 2015) and reducing methane emissions (Carulla et al., 2005; Grainger et al., 2009) in ruminant production. One of the most consistent effects of feeding the CT extract is a reduction in ruminal protein degradation and urinary N excretion. The objective of this study was to determine the effects of feeding the CT extract from *A. mearnsii* on ruminal fermentation, microbial protein synthesis, nutrient digestibility, and the route and chemical form of N excretion in beef cattle fed a high protein, barley-based concentrate diet with corn DG. The hypothesis was that the inclusion of the CT extract in a high protein finishing diet containing corn DG, and a greater proportion of RUP, would reduce total tract digestibility of protein and thereby reduce excretion of labile urinary urea N and increase stable N fractions in feces to improve the capture of excess N in manure (fertilizer value), lesson the potential loss of N to the environment, and improve whole farm nutrient balance of finishing beef feedlot operations.

MATERIALS AND METHODS

The study was conducted at Agriculture and Agri-Food Canada's Lethbridge Research and Development Centre (AB) in the Beef Metabolism Unit. Animals were cared for according to the guidelines of the Canadian Council on Animal Care (2009). Experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee at the Lethbridge Research and Development Centre.

Experimental Design, Animals, and Treatment Diets

The experiment was designed as a replicated 4 × 4 Latin square with 8 ruminally cannulated crossbred beef heifers [427 ± 41.2 kg, initial body weight (**BW**)], 4 dietary treatments, and 4 periods of 5 wk duration. Eight weeks prior to the start of the experiment, the heifers were ovariectomized and surgically fitted with permanent ruminal cannulas by a licensed veterinarian according to the procedures described by Bar Diamond (2011). Ruminal cannulas manufactured of flexible polyvinyl chloride with a 7.5 cm i.d. opening (number 4C; Bar Diamond, Inc., Parma, ID) were used for the first 6 to 8 wk after surgery and then replaced with a cannula with a 10 cm i.d. opening (number 9C; Bar Diamond, Inc.).

The heifers were housed in individual tie stalls on rubber mats bedded with wood shavings. The heifers were released to an outdoor pen for 1 to 2 h of exercise daily (1100 to 1300 h) as the measurement and sampling schedule permitted. Body weight was measured (without fasting but before fresh feed was offered) at 1300 h at the beginning of week 1 (d 1) and at the beginning and end of week 5 (d 29 and d 35) and averaged for the end BW of each period.

The dietary treatments [dry matter (**DM**) basis, Table 1] included: no corn dried distillers grains and solubles (**0DG**, control), 20% corn DG (**20DG**), 40% corn DG (**40DG**) and 40% corn DG with 2.5% CT extract powder from *A. mearnsii* (**40DGCT;** Weibull Black, Tanac SA, Montenegro, Brazil). The control diet contained 86% temper rolled barley grain, 9% whole crop barley silage, and 5% mineral and vitamin supplement. The DG and CT extract were substituted for the barley grain. The 20DG dietary treatment provided a CP concentration intermediate to the 0DG and 40DG and a mid-point reference for assessing the extent of relative effects of feeding 40DG with and without the CT extract. The dietary concentration of 2.5% CT extract was

Table 1. Ingredients and nutrient composition of the experimental diets and major feed ingredients

	Diet[1]						
Item	0DG	20DG	40DG	40DGCT	Barley grain	Barley silage	Corn dried distillers grains and solubles
Ingredient, % DM							
Barley silage[2]	9.0	9.0	9.0	9.0			
Barley grain, temper rolled[3]	86.0	66.0	46.0	43.5			
Corn dried distillers grains and solubles	0	20.0	40.0	40.0			
Condensed tannin extract (*Acacia mearnsii*)[4]	0	0	0	2.50			
Supplement[5]							
Barley grain, ground	3.30	3.30	3.30	3.30			
Calcium carbonate	1.35	1.35	1.35	1.35			
Salt	0.162	0.162	0.162	0.162			
Trace mineral and vitamin premix[6]	0.054	0.054	0.054	0.054			
Vitamin E (500,000 IU/kg)	0.006	0.006	0.006	0.006			
Rumensin premix[7]	0.017	0.017	0.017	0.017			
Molasses	0.105	0.105	0.105	0.105			
Flavoring agent[8]	0.003	0.003	0.003	0.003			
Nutrient composition, % DM unless noted otherwise[9]							
DM, % as fed	77.1 ± 1.29	78.1 ± 1.30	78.6 ± 0.95	79.1 ± 1.39	36.3 ± 4.74	85.7 ± 1.07	90.7 ± 0.54
OM	95.6 ± 0.60	95.7 ± 0.81	95.3 ± 0.32	95.3 ± 0.15	92.1 ± 0.44	97.5 ± 0.32	96.7 ± 0.14
CP	13.0 ± 1.04	16.8 ± 0.98	20.4 ± 0.90	20.5 ± 1.04	11.2 ± 0.74	12.9 ± 1.24	30.9 ± 0.88
NDICP, % CP	24.0 ± 7.37	43.9 ± 8.86	46.6 ± 1.87	49.3 ± 3.80	11.9 ± 1.20	25.3 ± 2.15	55.1 ± 1.94
ADICP, % CP	1.60 ± 0.38	7.90 ± 0.60	11.4 ± 0.96	11.5 ± 0.67	5.56 ± 0.60	1.18 ± 0.29	17.0 ± 2.25
Starch	52.4 ± 2.31	40.9 ± 3.43	31.6 ± 1.32	29.6 ± 1.57	23.2 ± 2.74	56.4 ± 4.91	4.57 ± 0.35
NDF	20.2 ± 1.96	27.1 ± 4.60	32.0 ± 4.16	29.4 ± 1.85	46.9 ± 1.28	18.5 ± 1.59	42.2 ± 0.82
ADF	7.41 ± 0.93	10.5 ± 0.84	13.3 ± 1.37	13.1 ± 0.63	28.1 ± 1.55	5.40 ± 0.61	20.6 ± 0.91
Crude fat	2.43 ± 0.11	4.00 ± 0.16	5.57 ± 0.37	5.47 ± 0.24	2.95 ± 0.23	2.10 ± 0.19	10.2 ± 0.26
GE, Mcal/kg DM	4.25 ± 0.04	4.43 ± 0.04	4.71 ± 0.08	4.65 ± 0.06	4.24 ± 0.05	4.33 ± 0.06	5.23 ± 0.03

ADF = acid detergent fiber; ADICP = acid detergent insoluble crude protein; CP = crude protein; DM = dry matter; GE = gross energy; NDF = neutral detergent fiber; NDICP = neutral detergent insoluble crude protein; OM = organic matter.

[1]0DG = no corn dried distillers grains and solubles (DG), 20DG = 20% of diet DM as DG, 40DG = 40% of diet DM as DG, 40DGCT = 40% of diet DM as DG and 2.5% of diet DM as condensed tannin extract of *Acacia mearnsii*.

[2]Particle size distribution of the silage using the Penn State Particle Separator = 8.02 ± 3.00% > 19.0 mm, 54.4 ± 4.10% 19.0 to 8.0 mm, 35.5 ± 5.60% 8.0 to 1.18 mm, and 2.04 ± 1.48% < 1.18 mm.

[3]Bushel weight of whole barley was 65.0 ± 1.80 kg/hL (50.4 ± 1.39 lb/Winchester bu) and the processing index of the temper-rolled barley was 77.4 ± 4.07%.

[4]Weibull Black, Tanac SA, Montenegro, Brazil.

[5]Ingredients of the supplement were pelleted [0.34 cm (1/4 in) diameter].

[6]Lethbridge Research and Development Centre Feedlot Premix provided per kg of diet DM: 55 mg/kg of Zn, 14 mg/kg of Cu, 25 mg/kg of Mn, 0.6 mg/kg of I, 0.27 mg/kg of Se, 0.2 mg/kg of Co, 9200 IU/kg of vitamin A, 460 IU/kg of vitamin D, and 13 IU/kg of vitamin E.

[7]Provided monensin at 33 mg/kg diet. Premix contained monensin (as monensin sodium) at 200 g/kg of premix (DM basis); Elanco Animal Health, Guelph, ON.

[8]Anise 422 powder; Canadian Bio-Systems Inc., Calgary, AB.

[9]Nutrient composition of diets and major ingredients (barley silage, barley grain, and corn dried distillers grains and solubles) determined by chemical analysis, mean ± SD, n = 4 periods.

selected as this was highest dietary concentration of CT with no adverse effects on DM intake (**DMI**) when included in a high protein diet containing corn DG (Koenig et al., 2018). The extractable CT concentration of the *A. mearnsii* extract was 53.0% (DM basis). Diets were formulated to meet the mineral and vitamin requirements for beef cattle (NRC, 2000). The diets were prepared daily as total mixed rations (**TMR**) in a Data Ranger (American Calan, Northwood, NH) and were offered once daily (1400 h) in an amount to permit ad libitum consumption (minimum refusals of 10% of the daily feed offered). Water was freely available from individual automatic bowls throughout the experiment. The heifers were assigned to their dietary treatments such that each treatment followed every other treatment at one time during the experiment to balance for any residual effects. The 5-wk periods consisted

of 2 wk for transition to the DG levels of the diets, 1 wk for adaptation to CT, and 2 wk for measurements and sample collection.

Feed Intake and Sampling (day 1 to 35)

Feed offered and refused (1330 h) were recorded daily. Samples (~1 L volume) of the TMR and orts were collected and frozen for 5 d of each week and composited by week for each of the 5 wk. The weekly composites of the TMR and orts were thawed, split using a riffle splitter (to ~1 L), and DM was determined at 55 °C in a forced-draft oven for 48 h. The DMI was calculated as the difference in the DM offered and refused. An additional subsample of the composite for the TMR and orts for week 4 and 5 were combined for each period and dried at 55 °C. Samples of the barley grain, corn DG, and supplement were also collected daily for 5 d and composited for each of week 4 and 5 which were then combined for each period. Barley silage (10 L volume from the face of the pit) was collected once a wk, split, frozen, composited for the period, and then dried. Dried feeds and orts were ground through a 4-mm diameter screen and then through a 1-mm diameter screen (model 4 Wiley mill; Thomas Scientific, Swedesboro, NJ). Dried and ground feed and ort samples were stored at room temperature until analyzed for analytical DM, organic matter (**OM**), starch, N, neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), neutral detergent insoluble N (**NDIN**), acid detergent insoluble N (**ADIN**), crude fat, and gross energy (**GE**). Particle size distribution was determined using the Penn State Particle Separator with 3 sieves with apertures of 19.0, 8.0, and 1.18 mm [Lammers et al., (1996) as modified by Kononoff et al., (2003)] on subsamples of the barley silage. Samples of whole and temper rolled barley grain were collected from the Lethbridge Research and Development Centre Feed Mill as each 1-t batch was processed (approximately weekly) and averaged to determine the processing index. The processing index was defined as the volume weight of the grain after processing divided by the volume weight of whole barley grain before processing × 100% (on a DM basis; 0.5 L cup, Seedburo, Chicago, IL). Samples (~250 g) of the CT were collected from each 25-kg bag and were combined for the experiment.

Ruminal pH and Fermentation Characteristics (day 22 to 29)

Ruminal pH was monitored for 7 consecutive days using a continuous pH measurement system (LRCpH Data Logger System, Dascor, Inc., Escondido, CA; Penner et al., 2006) positioned in the ventral sac. The pH sensors were calibrated with standard buffers of pH 7.0 and 4.0 at 39 °C immediately before placement in the rumen on day 22 and after removal on day 29. Any drift in pH (mV) between the pre and postcalibration curves was assumed to be linear and interpolated to estimate calibration curve parameters for each reading based on the time of measurement to convert millivolts to pH units. Ruminal pH (in mV) was recorded at 1 min intervals. Data for ruminal pH were summarized for each heifer in each period as daily mean, minimum and maximum pH, and duration and area under the curve below pH thresholds of 5.8 (indicative of reduced fiber digestibility) and 5.6 (indicative of subacute ruminal acidosis). Ruminal pH at 30-min intervals over the feeding cycle is also reported.

Ruminal digesta (~1 L) was collected from 4 sites within the rumen (250 mL from the cranial, ventral, central, and caudal areas) at 0, 1, 3, 6, 9, 12, 18, and 21 h after feeding on day 25 and 28 and was squeezed through polyester monofilament fabric (355 µm mesh opening, PECAP, B. & S. H. Thompson, Ville Mont-Royal, QC) to separate into filtrate and particles. At each time point, 5 mL of the ruminal filtrate were combined with 1 mL of 25% (wt/vol) meta-phosphoric acid and another 5 mL of the filtrate were combined with 1 ml of 0.2 *M* H_2SO_4 and stored frozen (−20 °C) until volatile fatty acids (VFA) and ammonia N analysis, respectively.

Nitrogen Excretion and Nutrient Digestibility (day 29 to 35)

Urine and feces were collected separately and quantitatively and for 4 consecutive 24-h periods at 1100 h from day 31 to 35. No bedding (shavings) was used during total collections. Urine was collected from the bladder using indwelling catheters (Foley catheter, Bardex Lubricath, 2-way, 75 cm^3 balloon size, 8.7 mm catheter diameter; Bard Canada, Inc., Oakville, ON) and directed through tubing into 20 L plastic buckets containing 500 mL of 2 *M* H_2SO_4 to reduce the pH of the urine to less than 2.5 to prevent microbial activity and volatilization of ammonia. The volume of urine was recorded daily. A 20-mL sample of the acidified urine for each day was diluted to 100 mL with deionized water (18 mΩ) to avoid precipitation of uric acid (Chen and Gomes, 1992) and was stored frozen (−20 °C) until analysis. Feces were collected in pans positioned behind the heifers. The daily

output of feces was weighed and mixed, and a sample (~1 kg wet weight) was dried at 55 °C in an oven for 48 to 72 h to determine DM and then stored at ambient temperature until further processing and analysis. The dry fecal samples were ground and stored as described for the feed and orts samples. Fecal samples for each day were analyzed for DM, OM, starch, N, NDF, ADF, NDIN, ADIN, crude fat, and GE. Urine samples for each day were analyzed for total N, urea N, ammonia N, and purine derivatives (allantoin and uric acid).

Dry matter intake was calculated for each heifer during total collection as the difference between the amount of diet DM offered and refused from day 30 to 34 of each period (beginning 1 d before the total collection of feces and urine). Nutrient intake was calculated by multiplying the daily DM offered and refused by the nutrient content of the TMR and orts composited for week 4 and 5, respectively, and computing the difference between the daily nutrients offered and refused. Apparent total tract nutrient digestibility was calculated as the difference between the daily nutrient intake and the amount of the corresponding nutrient appearing in the feces.

Blood was collected on day 29 and 35 at 1300 h via puncture of the jugular vein into sterile evacuated tubes containing an anticoagulant (10 mL, lithium heparin, Vacutainer, Becton Dickinson, Oakville, ON) and centrifuged at 3,000 × *g* and 4 °C for 20 min. The plasma was transferred to individual vials and stored at −20 °C until analysis of plasma urea N (**PUN**).

In Situ Ruminal Organic Matter and Crude Protein Degradability

Ruminal digestion kinetics (rate and extent of digestion) of the major feed ingredients (barley grain, barley silage, and corn DG) and 4 TMR treatment diets were determined using the in situ technique. After completion of the replicated Latin square experiment, the 8 heifers were transitioned and adapted to the diets that they were assigned during the first period (2 heifers per treatment diet). Feed was offered in an amount to permit ad libitum intake and was offered once per day (1400 h).

The feed ingredients and TMR samples collected for each of the 4 periods were composited for the in situ experiment and dried at 55 °C in a forced-draft oven. Barley grain, corn DG, and the 4 TMR were ground through a 2-mm screen and barley silage was ground through a 4-mm diameter screen (model 4, Wiley mill). Samples of each feed (6 ± 0.05 g) were weighed into bags (8.5 × 16 cm) made from monofilament polyester fabric (pore size, 51 μm; PECAP, B. & S. H. Thompson) and heat sealed (22 mg/cm^2 surface area). The bags (12 maximum) were placed in large (20 × 30 cm) mesh sacs with 3 × 5-mm pores that permitted ruminal fluid to enter freely and contained a weight to control the position of the sac within the ventral rumen. The bags were soaked for 10 min in warm water (39 °C) before being incubated in the rumen in duplicate for 2, 4, 8, 12, and 16 h and in triplicate for 24, 36, 48, and 72 h over 2 wk. The series of bags for each of the TMR were incubated in the 2 heifers fed the corresponding treatment diets. The bags for barley grain, barley silage and corn DG were incubated in the 2 heifers fed the 40DG and 40DGCT diets. Upon removal from the rumen, the bags were immersed in cold water to stop fermentation and remove excess rumen contents. The bags were transferred to clean mesh bags and then machine washed in cold water using a delicate cycle for 6 min. The mesh bags were removed from the machine before the spin cycle. Once drained the bags were returned to the machine for a second rinse cycle for 6 min. Bags were dried at 55 °C for 72 h to determine DM disappearance. Quadruplicate 0 h bags were incubated in rumen buffer solution (Goering and Van Soest, 1970) at 39 °C for 30 min and washed as described for the bags incubated in the rumen. The residues were combined for each time point within each animal and analyzed for analytical DM, OM, and N.

Ruminal disappearance of the nutrients at each individual incubation time were calculated as the difference between the nutrient content of the initial sample and the residue remaining after incubation in the rumen and expressed as a percentage of the nutrient content of the initial sample. Kinetic parameters of nutrient disappearance in the rumen were estimated using the Marquardt iterative method of the nonlinear regression procedure (Release 9.3, SAS Inst., Inc., Cary, NC; McDonald, 1981) to fit the first-order equation: $y = A + B (1 - e^{-kd(t - L)})$ for t > L, where: y is the percentage of OM or CP disappearance at time t (%), A is the soluble rapidly degraded fraction (%), B is the insoluble potentially degradable fraction (%), k_d is the fractional rate constant for the degradation of fraction B (/h), L is the lag time (h), and t is the time of incubation (h). The undegradable fraction C was calculated as 100 − (A + B). The RDP was equated with the effective ruminal degradability (**ERD**) and was calculated from the equation: $ERD = A + (B \times k_d)/(k_d + k_p)$, where: k_p is the fractional passage rate

from the rumen (0.06/h) and constants A, B, and k_d are as defined previously. The RUP content was calculated as 100 – ERD of CP for the feeds.

Chemical Analysis

All chemical analyses were performed on each sample in duplicate and reported on a DM basis. Dry matter for correction of chemical results to a DM basis was determined by weighing 0.5 g of sample into a porcelain crucible and then placing in a force-air oven at 135 °C for 2 h (AOAC, 2016, method no 930.15) followed by hot weighing. The OM content was determined on the same sample as the difference between 100 and the percentage ash (AOAC, 2016, method no. 942.05). Samples previously ground through a 1-mm sieve were further ground using a ball mill (Mixer Mill MM2000, Retsch, Haan, Germany) to a fine powder for determination of starch and N. Urine was freeze dried for total N analysis. Starch was determined by enzymatic hydrolysis of α-linked glucose polymers as described by Koenig et al. (2013). Nitrogen was quantified by flash combustion with gas chromatography and thermal conductivity detection (AOAC, 2016, method no. 990.03; Carlo Erba Instruments, Milan, Italy). The CP was calculated as N × 6.25. The NDF was determined based on the procedure of AOAC (2016, method 2002.04) using heat-stable α-amylase (Termamyl 120 L, Type L, Novozymes A/S) and sodium sulfite, and ADF was determined based on AOAC (2016, method 973.18) with modifications to each procedure for use in an ANKOM Fiber Analyzer (ANKOM Technology Corp., Fairport, NY). The NDIN and ADIN was determined by analyzing the NDF (obtained using the above NDF procedure without the use of sodium sulfite) and ADF residues, respectively, for N. Samples of the major feed ingredients and the TMR for the 4 periods were combined to make one representative sample each for the experiment for crude fat analysis. Crude fat was determined by ether extraction for 6 h (AOAC, 2016, method 920.39; E-816 Hot Extraction Unit, Buchi Labortechnik AG, Flawil Switzerland). Gross energy was determined using a bomb calorimeter (E2K Isothermal Bomb Calorimeter, Digital Data Systems, Pty Ltd., Gauteng, South Africa). The CT concentration was determined in the *A. mearnsii* extract using the butanol-HCl assay with reference standards prepared from the purified extract (Hagerman, 2002).

Volatile fatty acid concentrations in ruminal fluid were quantified using crotonic acid as an internal standard by gas chromatography (Model 6890N; Agilent Technologies Santa Clara, CA) with a capillary column (30 m × 0.32 mm i.d. × 1.0 µm film thickness, nitroterephthalic acid modified polyethylene glycol; Zebron ZB-FFAP, Phenomenex, Torrence, CA) and flame ionization detection. Ammonia N concentration in ruminal fluid and urine was determined using the salicylate-nitroferricyanide-hypochlorite procedure and a continuous flow colorimetric analyzer (Astoria 2 Analyzer; Astoria-Pacific, Inc., Clackamas, OR).

Urea N was determined in plasma and urine by reaction with diacetyl monoxime and colorimetric detection using a flow analyzer (method A332, Astoria 2 Analyzer). Uric acid in urine was determined using a colorimetric procedure (Uric Acid; Pointe Scientific, Inc., Canton, MI) with volumes adjusted accordingly for a microplate reader (Appliskan; Thermo Electron Corporation). Allantoin was measured in urine according to the procedure of Chen and Gomes (1992) adjusted for a microplate reader. The intestinal flow of microbial N was calculated using the relationship for cattle with the purine derivatives measured in urine (Chen and Gomes, 1992).

Statistical Analysis

The data for nutrient digestibility, route of N excretion and ruminal pH were analyzed as a replicated 4 × 4 Latin square design using a mixed linear model (Release 9.3; SAS Inst., Inc., Cary, NC) with dietary treatment and period as fixed effects and square and animal within square as random effects. Data for ruminal fermentation characteristics were analyzed using a similar model with time (hour relative to feeding) included as a repeated measure. Data for in situ ruminal digestion kinetics of each feed were analyzed using a mixed linear model with diet as a fixed effect and animal as a random effect. Heifer was the experimental unit for all models. The REML method was used for estimating the variance components and the Kenward-Roger's option was used to adjust the degrees of freedom. Differences among the dietary treatments were compared using Fisher's protected LSD test (i.e., when $P \leq 0.05$ for the main effect of treatment). The covariance structure used for the model with repeated measures was compound symmetry or heterogeneous compound symmetry. The most appropriate covariance structure was selected based on the lowest Akaike's information criterion. When the treatment × time interaction was significant, tests of the simple effects (i.e., treatment and hour) were determined using the SLICE option in

the LSMEANS statement. Differences among time points for each treatments and treatments at each time points where determined when the test for the effect of the corresponding slice was significant. Differences were declared significant at $P \leq 0.05$ and tendencies were discussed at $0.05 < P \leq 0.10$.

RESULTS

Dry matter intake of heifers was less (kg/d, $P \leq 0.015$ and % of BW, $P \leq 0.027$; Table 2) when fed 40% DG (40DG and 40DGCT) compared with 20DG, but was not different ($P \geq 0.13$) compared with when fed 0DG when expressed as kg/d. When expressed as a % of BW, DMI was not different ($P = 0.38$) between heifers fed 40DGCT and 0DG, but was less ($P = 0.050$) when fed 40DG compared with 0DG. There was no negative effect ($P \geq 0.22$) of CT on DMI (kg/d and % of BW) of the 40% DG diet (40DGCT vs. 40DG). Fecal output was greater ($P \leq 0.009$) in heifers fed 20DG and 40% DG (40DG and 40DGCT) compared with 0DG. There was a tendency ($P = 0.08$) towards greater fecal output for heifers fed the 40DGCT compared with 40DG. Urine output was less ($P = 0.007$) in heifers fed 40DGCT than 40DG, and was not different ($P \geq 0.32$) from heifers fed the lower CP diets (0DG and 20DG).

As was observed for DMI, there was no effect of the CT extract (40DGCT vs. 40DG, $P \geq 0.16$; Table 2) on OM, starch, NDF, ADF, and GE intake. Organic matter intake was less ($P \leq 0.014$) in heifers fed 40% DG (40DG and 40DGCT) compared with heifers fed 20DG, but was not different ($P = 0.28$) for heifers 40DGCT compared with those fed 0DG. Starch intake decreased as the proportion of DG that replaced barley grain in the diets increased from 0 to 20 and 40% ($P < 0.001$). Intake of fiber (NDF and ADF) did not differ ($P \geq 0.12$) among heifers fed the 20DG and 40% DG (40DG and 40DGCT) diets, but was greater ($P \leq 0.002$) than in heifers fed 0DG. Gross energy intake by heifers was not different ($P = 0.13$) among dietary treatments.

Apparent total tract digestibility of OM, starch, and GE was reduced ($P \leq 0.05$; Table 2) with increasing DG. Digestibility of NDF was greater ($P = 0.016$) in heifers fed 20DG compared with 0DG, and was intermediate for 40DG. Digestibility of ADF did not differ ($P = 0.35$) between heifers fed 20DG and 40DG and was greater ($P \leq 0.002$) than those fed 0DG. Although there was no effect ($P \geq 0.16$) of feeding the CT extract on nutrient intake, apparent total tract digestibility of OM, NDF, ADF, and GE was less ($P \leq 0.049$) in heifers fed 40DGCT compared with 40DG. There was, however, no effect ($P = 0.92$) of the CT extract on

Table 2. Nutrient intake and apparent total tract digestibility in beef heifers fed high protein finishing diets containing corn dried distillers grains and solubles and a condensed tannin extract

	Diet[1]					
Item[2]	0DG	20DG	40DG	40DGCT	SEM	*P*-value
DMI, kg/d	10.6^{a,b}	11.5^{a}	9.75^{b}	10.1^{b}	0.541	0.021
DMI, % of BW	1.97^{a,b}	2.10^{a}	1.79^{c}	1.89^{b,c}	0.056	0.013
Feces, kg/d	2.07^{b}	2.66^{a}	2.54^{a}	2.84^{a}	0.204	0.001
Urine, L/d	8.54^{b}	8.69^{b}	11.2^{a}	9.20^{b}	0.618	0.003
Nutrient intake, kg/d						
OM	10.2^{a,b}	11.0^{a}	9.28^{b}	9.61^{b}	0.517	0.018
Starch	5.65^{a}	4.65^{b}	3.34^{c}	3.04^{c}	0.181	<0.001
NDF	2.07^{b}	3.21^{a}	3.11^{a}	2.86^{a}	0.196	<0.001
ADF	0.76^{b}	1.19^{a}	1.26^{a}	1.28^{a}	0.079	<0.001
GE, Mcal/d	45.1	50.6	45.1	46.8	2.51	0.13
Apparent total tract digestibility, % intake						
OM	82.0^{a}	78.2^{b}	75.3^{c}	73.1^{d}	0.99	<0.001
Starch	95.2^{a}	93.3^{a,b}	92.6^{b}	92.7^{b}	0.72	0.049
NDF	52.8^{b,c}	60.5^{a}	57.8^{a,b}	51.7^{c}	1.96	0.021
ADF	33.5^{b}	49.1^{a}	53.3^{a}	38.1^{b}	2.83	<0.001
GE	80.4^{a}	76.8^{b}	74.5^{c}	72.1^{d}	0.98	<0.001

ADF = acid detergent fiber; BW = body weight; DMI = dry matter intake; GE = gross energy; NDF = neutral detergent fiber; OM = organic matter.

$^{a-d}$Means within a row with different superscript letters differ ($P \leq 0.05$, $n = 8$).

[1]0DG = no corn dried distillers grains and solubles (DG), 20DG = 20% of diet DM as DG, 40DG = 40% of diet DM as DG, 40DGCT = 40% of diet DM as DG and 2.5% of diet DM as condensed tannin extract of *Acacia mearnsii*.

[2]Dry matter intake determined during week 5 (d 29 to 35) of each period.

apparent total tract digestibility of starch (40DGCT vs. 40DG).

Nitrogen intake was not different ($P \geq 0.22$; Table 3) for heifers fed 20DG and 40% DG (40DG and 40DGCT) and was greater ($P \leq 0.001$) than for 0DG. The CP concentration of the diet increased with increasing DG, but DMI was less ($P \leq 0.015$) in heifers fed 40% DG (40DG and 40DGCT) compared with 20DG. In addition, there was a tendency ($P \leq 0.10$) for increased N concentration in refusals as the proportion of DG increased (data not presented). Therefore, N intake did not differ ($P \geq 0.22$) between heifers fed 20 and 40% DG. The intestinal flow of microbial N was greater ($P \leq 0.007$) in heifers fed 20DG than 0DG and 40DG. Microbial N flow was reduced ($P = 0.012$) for the 40DGCT diet compared with the 40DG diet.

Total N and fecal N output (g/d) were greater ($P \leq 0.01$; Table 3) from heifers fed 20DG and 40DG compared with 0DG. There was a tendency ($P = 0.06$) towards greater apparent total tract N digestibility for 20DG compared with 0DG, but there was no difference between 40DG compared with 0DG ($P = 0.42$) and 20DG ($P = 0.29$) and when averaged for the 3 treatments was 78.4 ± 1.07%. There was no effect ($P = 0.84$) of feeding the CT extract on total N output (40DGCT vs. 40DG). There was, however, a shift in the route of N excretion resulting in an increase ($P < 0.001$) in fecal N output and a reduction ($P < 0.001$) in apparent total tract N digestibility (40DGCT vs. 40DG). Apparent total tract N digestibility in heifers fed 40DGCT was 70.6%. The amount of urinary N output increased ($P \leq 0.008$) with increasing DG,

Table 3. Nitrogen intake, microbial flow, apparent total tract digestibility, and route and chemical form of excretion in beef heifers fed high protein finishing diets containing corn dried distillers grains and solubles and a condensed tannin extract

	Diet[1]					
Item	0DG	20DG	40DG	40DGCT	SEM	*P*-value
Intake, g N/d	220^{b}	307^{a}	303^{a}	329^{a}	17.7	<0.001
Microbial N flow[2], g N/d	145^{b}	166^{a}	137^{b}	117^{c}	4.53	<0.001
Apparent total tract digestibility, %	77.1^{a}	79.9^{a}	78.3^{a}	70.6^{b}	1.07	<0.001
Total N output, g N/d	145^{c}	210^{b}	231^{a}	233^{a}	11.6	<0.001
Fecal N, g N/d	50.0^{c}	67.6^{b}	64.9^{b}	96.0^{a}	5.34	<0.001
Fecal N, % of N intake	22.9^{b}	22.0^{b}	21.9^{b}	29.5^{a}	0.98	<0.001
Fecal N, % total N output	34.9^{b}	32.4^{b}	28.7^{c}	41.4^{a}	1.37	<0.001
Fecal NDIN, g N/d	9.58^{c}	15.04^{b}	16.58^{b}	36.43^{a}	2.14	<0.001
Fecal NDIN, % fecal N	19.0^{d}	21.8^{c}	25.3^{b}	38.3^{a}	0.73	<0.001
Fecal NDIN, % total N output	6.61^{b}	7.01^{b}	7.37^{b}	15.7^{a}	0.44	<0.001
Fecal ADIN, g N/d	5.45^{c}	9.47^{bc}	10.3^{b}	26.0^{a}	1.71	<0.001
Fecal ADIN, % fecal N	11.2^{b}	13.8^{b}	16.0^{b}	27.9^{a}	1.87	<0.001
Fecal ADIN, % total N output	3.88^{b}	4.44^{b}	4.65^{b}	11.6^{a}	0.67	<0.001
Urine N, g N/d	95.2^{c}	144^{b}	166^{a}	137^{b}	7.53	<0.001
Urine N, % N intake	43.7^{b}	53.7^{a}	55.3^{a}	42.1^{b}	2.77	0.009
Urine N, % total N output	65.1^{b}	69.9^{a}	71.6^{a}	58.7^{c}	0.99	<0.001
Urea N, g N/d	69.3^{c}	111^{b}	128^{a}	101^{b}	7.68	<0.001
Urea N, % total N output	30.0^{c}	47.9^{b}	55.4^{a}	43.9^{b}	3.33	<0.001
Urea N, % urine N	71.9	75.6	78.1	73.9	2.25	0.21
Ammonia N, g N/d	0.89^{c}	1.70^{b}	2.27^{a}	1.78^{a,b}	0.169	<0.001
Ammonia N, % urine N	0.95^{f}	1.12^{e,f}	1.38^{e}	1.41^{e}	0.12	0.062
Allantoin N, g N/d	10.3^{b}	11.9^{a}	9.59^{b}	8.29^{c}	0.46	<0.001
Allantoin N, % urine N	10.9^{a}	8.15^{b}	5.91^{c}	6.28^{c}	0.39	<0.001
Uric acid N, g N/d	0.83^{a,b}	0.89^{a}	0.92^{a}	0.70^{b}	0.058	0.016
Uric acid N, % urine N	0.87^{a}	0.61^{b}	0.57^{b}	0.54^{b}	0.03	<0.001
Plasma Urea N, mg N/L	115^{c}	168^{b}	202^{a}	172^{b}	13.1	<0.001

ADIN = acid detergent insoluble N; NDIN = neutral detergent insoluble N.

$^{a-d}$Means within a row with different superscript letters differ ($P \leq 0.05$, $n = 8$).

^{e,f}Means within a row with different superscript letters differ ($0.05 < P \leq 0.10$, $n = 8$).

[1]0DG = no corn dried distillers grains and solubles (DG), 20DG = 20% of diet DM as DG, 40DG = 40% of diet DM as DG, 40DGCT = 40% of diet DM as DG and 2.5% of diet DM as condensed tannin extract of *Acacia mearnsii*.

[2]Microbial N flow was determined from purine derivative excretion in urine.

and when expressed as a percentage of N intake and output, were greater ($P \leq 0.025$) in heifers fed 20DG and 40DG compared with the 0DG diet. Urinary N output was decreased ($P = 0.001$) in heifers fed 40DGCT compared with 40DG.

Fecal NDIN and ADIN output were also increased ($P \leq 0.001$; Table 3) in heifers fed 40DGCT compared with 40DG and accounted for 38.3% and 27.9% of fecal N output, respectively, from heifers fed 40DGCT which was 13 and 12 percentage units greater, respectively, than from heifers fed 40DG. Output of urine N and urea N from heifers fed 40DGCT was less ($P \leq 0.001$) than from heifers fed 40DG, equal ($P \geq 0.12$) to those fed 20DG, and greater ($P < 0.001$) than those fed 0DG. When expressed as a percentage of urine N output, urea N was not different ($P = 0.21$) among the dietary treatments and averaged 74.9 ± 2.25%. The amount ($P \leq 0.05$) and percentage (tendency, $P \leq 0.10$) of ammonia N output increased with increasing DG with no effect ($P = 0.87$) of the CT extract (40DGCT vs. 40DG) on the percentage of ammonia N, although output in urine was low (≤1.41% urine N). As urine N output increased with DG, the proportion of allantoin N and uric acid N decreased ($P \leq 0.05$). There was no effect ($P \geq 0.23$) of the CT extract on the proportions of each of the purines derivatives excreted (40DGCT vs. 40DG), but the CT extract reduced ($P \leq 0.021$) the daily amounts of purine derivative N excreted.

Plasma urea N concentration increased ($P \leq 0.05$; Table 3) with increasing DG. Supplementation of the CT extract reduced PUN in heifers fed 40% DG (40DGCT vs. 40DG, $P = 0.004$) to the level observed in heifers fed 20DG ($P = 0.71$) reflecting the effect that the CT extract had on reducing total tract CP digestibility.

Ruminal ammonia N concentration tended ($P \leq 0.10$; Table 4) to increase with increasing DG. Supplementation of the CT extract tended to

Table 4. Ruminal fermentation characteristics and pH in beef heifers fed high protein finishing diets containing corn dried distillers grains and solubles and a condensed tannin extract

	Diet[1]					*P*- value		
Item	0DG	20DG	40DG	40DGCT	SEM	Diet	Time	Diet × Time
Ammonia, mg N/L	70.9^{e}	84.8^{d,e}	106^{d}	70.3^{e}	19.2	0.078	<0.001	0.050
VFA, m*M*	166^{a}	166^{a}	158^{a}	137^{b}	9.2	<0.001	<0.001	0.75
Individual VFA, mol/100 mol								
Acetic acid	46.4	46.5	46.8	46.8	0.95	0.98	<0.001	0.20
Propionic acid	37.2^{a}	39.2^{a}	36.8^{a}	32.0^{b}	1.42	0.006	<0.001	0.66
Butyric acid	10.6^{b}	8.56^{b}	10.0^{b}	15.2^{a}	1.49	0.006	<0.001	0.013
Branched chain acids[2]	2.75^{b}	2.67^{b}	2.89^{a,b}	3.55^{a}	0.31	0.049	<0.001	0.70
Long chain acids[3]	2.99	3.09	3.47	2.44	0.363	0.24	0.049	0.93
Acetic:propionic acid ratio	1.31^{b}	1.25^{b}	1.34^{b}	1.68^{a}	0.099	0.011	<0.001	0.015
Ruminal pH								
Mean	5.70^{b}	5.56^{c}	5.81^{a,b}	5.89^{a}	0.057	< 0.001		
Minimum	5.09^{b}	5.03^{b}	5.16^{b}	5.32^{a}	0.054	0.002		
Maximum	6.45^{a}	6.30^{b}	6.58^{a}	6.51^{a}	0.047	0.007		
Range	1.36^{d}	1.27^{d,e}	1.42^{d}	1.19^{e}	0.067	0.080		
pH < 5.8								
Duration, h/d	13.8^{b}	17.1^{a}	11.7^{b,c}	9.80^{c}	1.30	<0.001		
Area, pH units × min/d	360^{b}	457^{a}	263^{b}	161^{c}	50.2	<0.001		
Bouts, no/d	13.0	11.9	11.7	13.6	1.34	0.61		
Bout duration, min/bout	139	212	120	74	38.4	0.14		
pH < 5.6								
Duration, h/d	10.6^{a,b}	13.4^{a}	8.23^{b,c}	5.79^{c}	1.38	<0.001		
Area, pH units × min/d	212^{a,b}	272^{a}	144^{b}	68.3^{c}	34.7	<0.001		
Bouts, no/d	12.7^{a}	13.8^{a}	9.91^{b}	9.34^{b}	0.74	0.003		
Bout duration, min/bout	94.0	102	74.9	40.9	22.2	0.21		

VFA = volatile fatty acids.

$^{a-c}$Means within a row with different superscript letters differ ($P \leq 0.05$, $n = 8$).

^{d,e}Means within a row with different superscript letters differ ($0.05 < P \leq 0.10$, $n = 8$).

[1]0DG = no corn dried distillers grains and solubles (DG), 20DG = 20% of diet DM as DG, 40DG = 40% of diet DM as DG, 40DGCT = 40% of diet DM as DG and 2.5% of diet DM as condensed tannin extract of *Acacia mearnsii*.

[2]Isobutyric acid and isovaleric acid.

[3]Valeric acid and caproic acid.

reduce ruminal ammonia N in heifers fed 40% DG (40DGCT vs. 40DG, $P \leq 0.10$) to the concentration ($P \geq 0.33$) in heifers fed 20DG and 0DG. There was diet × time interaction ($P = 0.05$) where the CT extract (40DGCT vs. 40DG) reduced ($P \leq 0.050$) ruminal ammonia N at 0, 1, and 12 h and tended ($P \leq 0.10$) to reduce it at 9 and 21 h relative to feeding (Fig. 1). There was no effect ($P \geq 0.13$, Table 4) of increasing DG on ruminal total VFA concentration and the molar proportions of individual VFA. However, ruminal total VFA concentration was reduced ($P \leq 0.001$) in heifers fed 40DGCT compared with all other dietary treatments. There was a diet × time interaction for the molar proportion of butyric acid ($P = 0.013$) and the acetic to propionic acid ratio ($P = 0.015$). The proportion of propionic acid was reduced ($P \leq 0.05$) and, therefore, the acetic to propionic acid ratio was greater ($P \leq 0.05$) in heifers fed 40DGCT than the 0DG, 20DG, and 40DG diets. Butyric acid was greater ($P \leq 0.05$) in heifers fed 40DGCT compared with the other dietary treatments except at 0, 12, and 15 h when the molar proportion for 40DGCT only tended ($P \leq 0.09$) to be greater than for 0DG. Branched chain acids (isobutyric acid and isovaleric acid) were also greater ($P \leq 0.05$) for 40DGCT than 0DG and 20DG, and tended to be greater than ($P = 0.06$) heifers fed 40DG.

Ruminal mean pH and maximum pH were less ($P \leq 0.05$; Table 4) and conversely the duration of time and area that pH was < 5.8 were greater ($P \leq 0.05$) in heifers fed 20DG than the other dietary treatments. Supplementation of the CT extract increased (40DGCT vs. 40DG, $P = 0.024$) the minimum pH and tended to reduce ($P \leq 0.10$) the pH range. There was no effect ($P = 0.20$) of CT (40DGCT vs. 40DG) on the duration of time that pH was < 5.8, although there was a tendency ($P = 0.09$) for a reduced duration of time that the pH was < 5.6. In addition, the areas for pH < 5.8 and < 5.6 were less ($P \leq 0.049$) for heifers fed 40DGCT vs. 40DG. Ruminal pH differed (diet × time interaction, $P \leq 0.05$; Fig. 2) among the dietary treatments for the 30-min time points except from 10.5 to 12.5 and 13.5 h. The decline in ruminal pH from 5 to 8.5 h after feeding was less ($P \leq 0.05$) severe for heifers fed 40DGCT vs. 40DG.

The in situ ERD of OM in the TMR was reduced ($P \leq 0.05$; Table 5 and Fig. 3) from 77.3 to 61.7% with increasing DG due to a decrease ($P \leq 0.05$) in the proportion of the degradable B fraction and its rate of degradation and an increase ($P \leq 0.05$) in the proportion of the undegradable C fraction. The effect of increasing the proportion of DG in the TMR had a greater effect on the ERD of CP as it was reduced ($P \leq 0.05$) from 76.2 to 47.1% due to a reduction ($P \leq 0.05$) in the percentage of the soluble A fraction and rate of degradation of the B fraction. There was no effect of the CT extract (40DGCT vs. 40DG, $P \geq 0.13$) on the digestion kinetics of OM in the TMR, but ERD of CP was

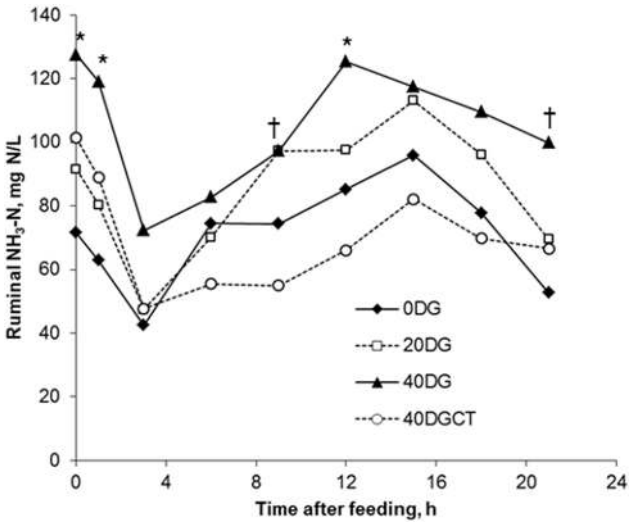

Figure 1. Ruminal ammonia N concentration in beef heifers fed high protein finishing diets containing corn dried distillers grains and solubles (DG) and a condensed tannin (CT) extract. 0DG = no corn DG, 20DG = 20% of diet DM as DG, 40DG = 40% of diet DM as DG, and 40DGCT = 40% of diet DM as DG and 2.5% of diet DM as CT extract of *Acacia mearnsii*. (diet × time interaction, SEM 19.2, $P = 0.05$; *$P \leq 0.05$, †$0.05 < P \leq 0.10$).

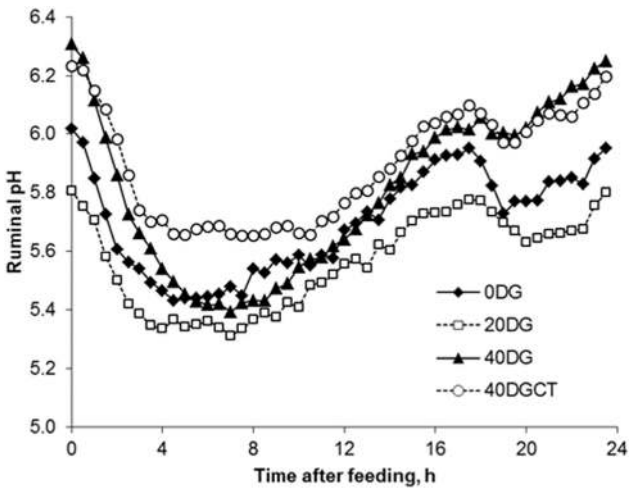

Figure 2. Ruminal pH in beef heifers fed high protein finishing diets containing corn dried distillers grains and solubles (DG) and a condensed tannin (CT) extract. 0DG = no corn DG, 20DG = 20% of diet DM as DG, 40DG = 40% of diet DM as DG, and 40DGCT = 40% of diet DM as DG and 2.5% of diet DM as CT extract of *Acacia mearnsii*. Ruminal pH differed among the dietary treatments at the 30-min time points except from 10.5 to 12.5 and 13.5 h (diet × time interaction, SEM 0.069, $P \leq 0.05$). Ruminal pH was greater (40DGCT vs. 40DG; $P \leq 0.05$) in heifers fed the tannin extract from 5 to 8.5 h and tended ($P \leq 0.10$) to be greater at 4, 4.5, 9, and 9.5 h.

Table 5. In situ ruminal organic matter and crude protein digestion kinetics of feed in beef heifers fed high protein finishing diets containing corn dried distillers grains and solubles and a condensed tannin extract

	Diet[1,2]					
Parameters[3]	0DG	20DG	40DG	40DGCT	SEM	*P*-value
Organic matter						
Total mixed rations						
A, %	38.5	38.9	38.6	38.6	0.94	0.99
B, %	47.8[a]	45.5[a]	39.3[b]	35.2[b]	1.42	0.011
C, %	13.7[c]	15.7[b,c]	22.1[a,b]	26.0[a]	1.91	0.030
k_d, /h	0.259[a]	0.131[b]	0.086[b]	0.096[b]	0.013	0.002
ERD, %	77.3[a]	70.0[b]	61.7[c]	60.1[c]	0.60	<0.001
Crude protein						
Total mixed rations						
A, %	40.1[a]	21.2[c]	23.0[b,c]	25.1[b]	0.65	<0.001
B, %	54.3[e,f]	65.2[e]	66.3[e]	43.0[f]	5.02	0.084
C, %	5.6[f]	13.7[f]	10.6[f]	31.9[e]	5.28	0.084
k_d, /h	0.120[a]	0.074[b]	0.034[c]	0.041[c]	0.006	0.002
ERD, %	76.2[a]	57.0[b]	47.1[c]	41.6[d]	1.01	<0.001
Barley grain						
A, %	-	-	39.4	44.1	1.58	0.17
B, %	-	-	56.2	48.7	1.83	0.10
C, %			4.4	7.2	2.22	0.46
k_d, /h	-	-	0.132	0.101	0.026	0.50
ERD, %	-	-	77.9	73.9	1.73	0.25
Barley silage						
A, %	-	-	74.3	74.6	0.40	0.66
B, %	-	-	12.5	2.75	0.62	0.008
C, %			13.2	22.7	1.00	0.022
k_d, /h	-	-	0.008	0.050	0.026	0.37
ERD, %	-	-	75.8	75.6	0.20	0.44
Corn distillers grains and solubles						
A, %	-	-	15.0	14.3	0.12	0.050
B, %	-	-	68.7	46.6	3.78	0.054
C, %			16.2	39.2	3.78	0.050
Lag, h	-	-	0.44	1.53	0.21	0.068
k_d, /h	-	-	0.031	0.029	0.002	0.44
ERD, %	-	-	38.6	29.2	0.69	0.011

[a–d]Means within a row with different superscript letters differ ($P \leq 0.05$, $n = 2$).

[e–f]Means within a row with different superscript letters differ ($0.05 < P \leq 0.10$, $n = 2$).

[1]0DG = no corn dried distillers grains and solubles (DG), 20DG = 20% of diet DM as DG, 40DG = 40% of diet DM as DG, 40DGCT = 40% of diet DM as DG and 2.5% of diet DM as condensed tannin extract of *Acacia mearnsii*.

[2]Each of the total mixed rations were incubated in situ in two heifers fed the corresponding treatment diet. The major feed ingredients were incubated in two heifers fed the 40DG and 40DGCT diets.

[3]Parameters were determined by fitting the equation: $y = A + B(1 - e^{-kd(t - L)})$ for $t > L$, where: y is the percentage of the OM or CP disappearance at time t (%), A is the soluble fraction (%), B is the slowly degradable fraction (%), k_d is the fractional rate constant for the degradation of fraction B (/h), L is the lag time (h), and t is the time of incubation (h). The undegradable fraction C was calculated as 100 - (A + B). Effective ruminal degradability (ERD, %) was calculated from the equation: $ERD = A + (B \times k_d)/(k_d + k_p)$, where: k_p is the fractional passage rate from the rumen (0.06/h).

reduced ($P = 0.021$) due to tendencies ($P \leq 0.10$) towards a reduced percentage of the degradable B fraction and a greater percentage of the undegradable C fraction. For the major feed ingredients, there was no effect of the CT extract (40DGCT vs. 40DG, $P > 0.10$) on the digestion kinetics of OM in barley grain and barley silage which averaged 76.8 ± 0.95% and 49.2 ± 0.56% ERD, respectively, and of NDF in barley silage which averaged 21.01 ± 1.76% ERD (data not shown). The ERD of OM in DG was, however, less for heifers fed 40DGCT vs. 40DG (42.1 vs. 46.0 ± 0.64, $P = 0.049$, data not shown). The B fraction of CP was reduced for barley silage ($P = 0.008$) and tended to be reduced for barley grain ($P = 0.10$),

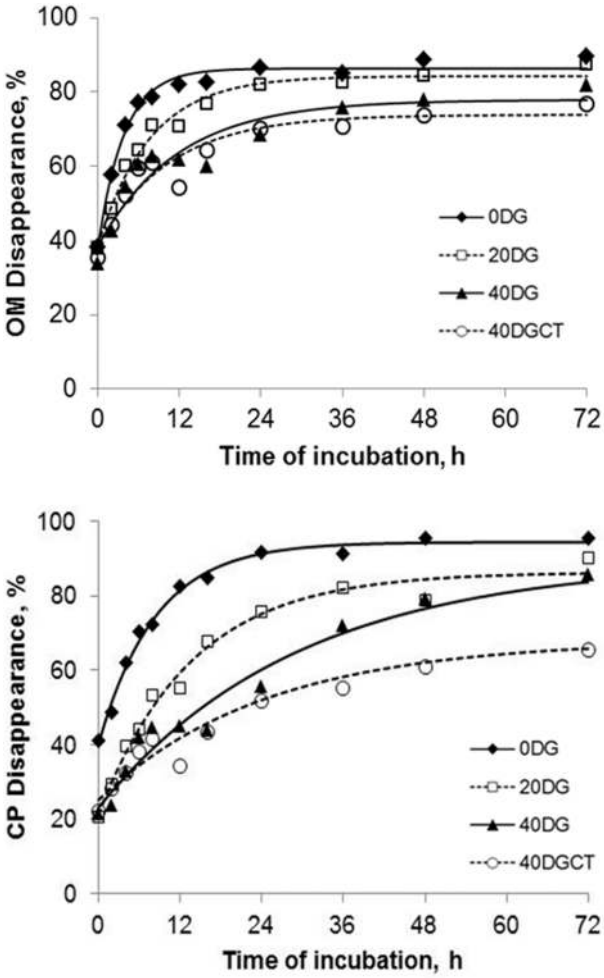

Figure 3. In situ ruminal organic matter and crude protein disappearance of high protein finishing diets containing corn dried distillers grains and solubles (DG) and a condensed tannin (CT) extract incubated in the rumen of two beef heifers. 0DG = no corn DG, 20DG = 20% of diet DM as DG, 40DG = 40% of diet DM as DG, and 40DGCT = 40% of diet DM as DG and 2.5% of diet DM as CT extract of *Acacia mearnsii*.

although there was no effect on ERD of CP ($P \geq 0.25$) when incubated in the heifers fed the 40DGCT vs. 40DG diets. In contrast, the soluble fraction was reduced ($P = 0.050$), the degradable fraction tended to be reduced ($P = 0.054$), the undegradable fraction was increased ($P = 0.050$), and as a result the ERD of CP in corn DG was reduced ($P = 0.011$) from 38.6 to 29.2% in heifers fed the 40DGCT compared with the 40DG diet.

DISCUSSION

Dietary supplementation of 2.5% of the CT extract (1.33% CT from *A. mearnsii*) in a barley grain-based high protein finishing diet containing 40% corn DG reduced apparent total tract CP digestibility and shifted the route and chemical form of excess N excretion from soluble urea N in urine to insoluble bound forms (NDIN and ADIN) in feces. There was a minor 3.0 and 3.2% reduction in apparent total tract OM and GE digestibility, respectively, due to the reduction in the degradability of the CP component of the diet with CT. The shift in the route and chemical form of N excretion by feeding CT in a high protein diet indicates a potential increase in the capture and stability of N in manure. In a companion feedlot finishing study of 83-d duration, feeding 2.5% CT extract in similar diets with 40% corn DG had no adverse effects on cattle performance (DMI, average daily gain, gain:feed) and carcass traits (Koenig et al., 2018). In addition, feeding 2.5% CT extract reduced volatilization of ammonia by 23% from the pen of feedlot cattle in the companion study.

The CT extract from *A. mearnsii* (black wattle tree) is produced commercially from the tree bark following harvest, aqueous autoclave extraction, evaporative concentration, and is available in a liquid or spray-dried powder (Chan et al., 2015). The extractable CT concentration of the *A. mearnsii* extract powder used in this study was 53.0% (DM basis). The concentration for similar products assayed with the butanol-HCl procedure was approximately 60.6% CT (Carulla et al., 2005; Grainger et al., 2009; Griffiths et al., 2013), although concentrations as low as 20.3% have also been reported (Gerlach et al., 2018).

Feed Intake, Ruminal Fermentation and Nutrient Digestibility

Condensed tannins are the most common form of tannin found in forages, shrubs and legumes and are well-recognized for their affinity to bind protein and to a lesser extent fiber, starch, and minerals and depending on the source and amount consumed can have nutritionally detrimental or beneficial effects on livestock production (Makkar, 2003; Mueller-Harvey, 2006; Waghorn, 2008). Generally, intake of herbage containing naturally high levels of CT (>5%) is reduced with negative effects on digestibility and more importantly on productivity (Min et al., 2003). The upper limit for the dietary concentration of the commercial CT extract (*A. mearnsii*) appears to be lower than the limit for CT occurring naturally in herbage to avoid the associated negative effects on intake and performance. The CT extract was fed at 2.5% of the diet DM (1.33% CT of diet DM) and at this concentration when included in a high-protein TMR based on barley grain with 40% corn DG had no negative effect on DMI and nutrient intake. This is in agreement with

a companion feedlot study where up to 2.5% CT extract in a high protein TMR consisting of barley grain with 20 and 40% corn DG had no effect on DMI of growing and finishing beef cattle (Koenig et al., 2018). Feed intake was, however, reduced in the cattle when the CT extract was increased to 3.5% (1.86% CT) of the diet. Likewise, there was no effect of up to 2.7% CT extract (*A. mearnsii*) on DMI of forage-based diets fed to beef steers (Ávila et al, 2015; Orlandi et al., 2015). Carulla et al. (2005) fed CT extract (*A. mearnsii*) at a higher concentration of 4.1% (2.5% CT) of a forage-based diet to sheep and found a small but significant increase in DMI. Grainger et al. (2009), however, found that a CT extract (*A. mearnsii*) when administered in a liquid drench twice daily at estimated concentrations ranging from 1.43 to 3.15% of DMI (0.86 to 1.9% CT) lowered feed intake and milk production in dairy cattle grazing pasture which appeared to be exacerbated when dietary CP was low (16% CP).

Digestibility of OM and GE was reduced by 3.0 and 3.2%, respectively, due largely to a reduction in ruminal and total tract digestibility of the protein component of the diet when cattle were fed the CT extract. In situ ruminal degradability of OM in the TMR and barley grain, and OM and NDF in barley silage were not affected by feeding the CT extract with 40% DG. There was, however, a reduction in ruminal total VFA concentration and higher ruminal pH (indicated by greater pH throughout the feeding cycle and lesser area under the pH thresholds of 5.8 and 5.6) suggesting lower ruminal OM fermentation in cattle fed the CT extract. In addition to the reduction in total VFA concentration, there was also a shift in the molar proportions of the individual VFA in cattle fed the CT extract. Acetic acid was not affected by the CT extract, but the molar proportion of propionic acid was reduced and the acetic to propionic acid ratio was increased. This decrease in the molar proportion of propionic acid may indicate lower starch digestion in the rumen (Bannink et al., 2008). There was no effect of feeding the CT extract on total tract digestibility of starch (averaged 92.7% of intake), suggesting that a reduction in ruminal starch digestion was compensated by a shift in the site of digestion to the lower tract. Ebert et al. (2017) reported a linear decrease in total tract digestibility of starch in cattle fed steam-flaked corn-based diets with 25% corn distillers by-products and 0, 0.5, and 1% CT extract from quebracho after 34 d on feed, but there was no effect of the CT extract at 95 d on feed. The CT of *A. mearnsii* and quebracho differ in their chemistry and response in various tannin assays (Mueller-Harvey, 2006; Venter et al., 2012). In an in vivo study where CT extracts of *A. mearnsii* and quebracho were fed to beef cattle at 2.5% of a barley-based diet with 20% wheat distillers grain (Koenig and Beauchemin, 2017), there was no effect of either of the CT extracts on apparent total tract starch digestibility (K. Koenig, unpublished data).

Nitrogen Metabolism and Excretion of Excess Nitrogen in Feces and Urine

The inclusion of corn DG at 20 and 40% of diet DM increased the CP concentration by 29.2 and 56.9% to 16.8 and 20.4% CP compared with the 13% CP barley grain-based finishing diet that is typical of the standard feedlot diet in Western Canada and adequate in metabolizable protein (Koenig and Beauchemin, 2013). Increasing the DG from 20 to 40% of DM, however, decreased feed intake such that N intake was not different for the 2 levels of DG (305 g N/d) and was 38.6% greater than the diet without DG (220 g N/d). With increasing DG, RDP of the TMR was reduced from 76.2 to 47.1% and conversely RUP was increased from 23.8 to 52.9% of CP. Excess dietary CP whether in the form of RDP or RUP is metabolized and the excess N excreted in the form of urea N in urine. In the metabolizable protein system, RDP is required to meet the N requirements of rumen microorganisms. The RDP consists of a variable portion of feed protein and NPN (e.g., urea, amino acids, and nucleic acids) that is degraded by ruminal microorganisms to ammonia, amino acids, and peptides (Walker et al., 2005). Ammonia is the primary end product and major N source for ruminal microbial protein synthesis. The ammonia that is not incorporated into bacterial protein is absorbed across the ruminal epithelium and lower sections of the gastro-intestinal tract, enters the portal vein and is transported to the liver where it is removed and converted to urea for excretion in urine (Reynolds and Kristensen, 2008). The RUP consisting of feed protein that escapes microbial degradation in the rumen, microbial protein, and endogenous protein passes to the small intestine for host enzyme hydrolysis to constituent amino acids, gut tissue oxidation, and absorption into the portal blood (Lapierre et al., 2006). Amino acids absorbed in excess or with an imbalanced profile in relation to maintenance and production requirements are extracted and deaminated in the liver and the N is also excreted in the form of urea N in urine.

Condensed tannins complex with protein through covalent H-bonding and hydrophobic

interactions of which the latter can be reversed depending on their chemical structure and pH (Hagerman et al., 1998). Ruminal protein digestion may be reduced directly by CT binding to dietary protein and reducing availability for microbial degradation and indirectly by binding to extracellular enzymes (Makkar et al., 1988), metalloenzymes (Smith et al., 2005) and cell membranes to inhibit activity, transport of nutrients, and growth of the microorganism (McSweeney et al., 2001). From in vitro studies with the CT of sainfoin, Jones and Mangan (1977) proposed that stable tannin—feed protein complexes are formed at the pH of the rumen between 5.0 and 7.0 preventing microbial degradation which then dissociate at the pH of abomasum (at pH < 3) and small intestine (at pH > 7) allowing subsequent digestion and release of amino acids for absorption. In the present study, the complexation of CT (*A. mearnsii*) and feed protein persisted throughout the gastrointestinal tract reducing apparent total tract digestion of dietary protein. In addition to the reducing microbial degradation of dietary protein, host enzyme digestion and intestinal absorption of amino acids may have also been inhibited. In addition, PUN concentration was also reduced in heifers fed the CT extract suggesting reduced ruminal ammonia N absorption and amino acid absorption. Dietary protein was fed in excess of requirements and, therefore, if the CT-protein complexes disassociated post-ruminally and amino acids were absorbed in excess, PUN would be expected to be similar to cattle fed the high protein diet without CT.

The effect of CT on ruminal protein degradation resulted in a decrease in ruminal ammonia N concentration and in situ CP degradability. Ruminal ammonia N concentration was reduced from 106 to 70.3 mg N/L when the CT extract was fed with 40% DG and was equal to the concentration observed in cattle fed the barley-based diet without DG (70.9 mg N/L). Ruminal ammonia N concentrations in cattle fed the barley-based diets with and without DG were in agreement with the range of values (60 to 118 mg N/L; Walter et al., 2012; Hünerberg et al. 2013; Koenig and Beauchemin, 2013) observed for cattle fed similar diets. The decrease in ruminal ammonia N concentration with the CT extract (*A. mearnsii*) is consistent with the results of other studies (Carulla et al., 2005; Orlandi et al., 2015). In situ ruminal degradability of CP in the TMR was reduced by 13% from 47.6 to 41.6% when the CT extract was included in the barley-based finishing diet with 40% DG. Feeding CT had the largest effect on the ruminal CP degradability of corn DG which was reduced by 32% from 38.6 to 29.2% of CP (or alternatively RUP increased from 61.4 to 70.8% of CP) as a result of a lower proportion of the soluble and slowly degradable fraction and a doubling of the rumen undegradable fraction. The RUP in corn DG was considered within the normal range of 61.7 to 74.2% (based on the mean ± SD; NASEM, 2016). In situ ruminal degradability of CP in the barley grain was not affected by the CT, although there was tendency towards a smaller slowly degradable fraction. The slowly degradable fraction was reduced in the barley silage, although it only comprised ≤12.5% of the CP; 74.5% of the CP was soluble. With the in situ technique, the soluble CP fraction (e.g., soluble protein, peptides, and amino acids) is assumed to be instantaneously and completely degraded in the rumen (0% escape). However, the ruminal degradation rate and escape of soluble protein can vary from 21 to 56% for a variety of concentrate feeds and fresh forages (Hedqvist and Uden, 2006). The formation of insoluble CT-protein complexes reduce the rate and extent of protein digestibility but soluble CT-protein complexes may also form (Hagerman and Robbins, 1987) and if unaccounted for, degradability would be overestimated when determined with the in situ technique.

Microbial N flow in the cattle was reduced by feeding the CT extract. The effects of CT extract (from *A. mearnsii*) on microbial populations and N flow reported in the literature are variable. In forage-based diets with 4.1% CT extract, ruminal bacteria and ciliate protozoal numbers in sheep were unaffected, although holotrich protozoal numbers were reduced (Carulla et al., 2005). Likewise, Kozlozki et al. (2012) reported no negative effect on microbial N flow when the CT the extract was infused into the rumen of sheep in an amount equivalent to up to 2.7% of the diet DM. In contrast, Orlandi et al. (2015) reported a tendency towards reduced microbial N flow in cattle fed the CT extract at a concentration at 2.7% of diet DM.

Total N output was not different between the heifers fed the high protein diets with 40% DG without and with the CT extract. Feeding the 2.5% CT extract (1.33% CT) reduced dietary CP degradability in the rumen and total tract and consequently shifted the route of excess N excretion from urine to feces. Inclusion of the CT extract in the diet increased the fecal N output by 48% compared with the heifers fed the 40% DG diet and of this increase 63.8% of the N was recovered in the fecal NDIN fraction and 50.5% in the fecal ADIN fraction. Urinary N and urea N output was reduced by

the CT extract, although there was no effect of the CT extract on N composition of urine which averaged 73.9% urea N, 6.8% purine derivative N, and 1.4% ammonia N. The shift in the route of N excretion is consistent and in agreement with other studies where the CT extract (*A. mearnsii*) was fed to sheep, dairy cattle, and steers fed a variety of diets (Carulla et al., 2005, Grainger et al., 2009, Orlandi et al., 2015).

Supplementation of a CT extract (*A. mearnsii*) in high protein finishing diets containing DG shifted the route of excess N excretion from labile urea-N in urine to stable bound forms in feces. Development of CT extracts as natural feed additives to increase the capture of N in manure and lesson the loss of N as ammonia will enable beef cattle producers to take advantage of the economics of high protein by-product or other feeds and allow for improved nutrient management in environmentally sustainable beef cattle production systems.

Conflict of interest statement. None declared.

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