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Effect of purple prairie clover (*Dalea purpurea* Vent.) hay and its condensed tannins on growth performance, wool growth, nutrient digestibility, blood metabolites and ruminal fermentation in lambs fed total mixed rations

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ABSTRACT

This study evaluated the effects of purple prairie clover (PPC, *Dalea purpurea* Vent.) hay and its condensed tannins (CT) on feed intake, growth performance, wool growth, nutrient digestibility, blood metabolites and rumen fermentation in lambs fed diets containing PPC and alfalfa hay. Alfalfa and PPC were harvested at similar growth stage, sun-cured to <12% moisture, and stored in a shed for 120 d. Thirty six individually fed lambs were randomly allocated into three groups and fed total mixed ration containing 40% (dry matter (DM) basis) of a pelleted barley grain based concentrate and 60% of alfalfa hay (AH), or 60% PPC hay (PH) or PH supplemented with polyethylene glycol (PH-p) for 77 d. Lambs were fed once daily and weighed bi-weekly. Faeces samples were collected in the 5th wk for 5 d to determine nutrients digestibility using acid insoluble ash as a marker. Lambs were slaughtered at the end of experiment to evaluate the carcass characteristics. Wool yield and quality were measured using a 10 cm dye band applied on d 0 and harvested on d 75. Blood samples were collected to analyze serum metabolites and antioxidant enzymes, and rumen fluid was sampled to analyze rumen fermentation products. Lambs fed PH had lower ($P < 0.01$) DM intake than AH or PH-p. Growth performance, wool growth parameters and carcass characteristics did not differ among diets. Lambs fed PH-p had greater ($P < 0.05$–0.001) DM, organic matter (OM), crude protein (CP), neutral detergent fibre (aNDF) and acid detergent fibre (ADF) digestibility than those fed AH, and greater ($P < 0.05$–0.001) CP, aNDF and ADF digestibility than those fed PH diet. Lambs fed

Abbreviations: ADF, acid detergent fibre; ADG, average daily gain; ADL, acid detergent lignin; AH, alfalfa hay; AIA, acid insoluble ash; ALB, albumin; ALKP, alkaline phosphatase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; BW, body weight; Ca, calcium; CAT, catalase; CHOL, cholesterol; CP, crude protein; CREA, creatinine; CT, condensed tannins; DM, dry matter; DMI, dry matter intake; FE, feed efficiency; GLOB, globulin; GLU, glucose; GSH-Px, glutathione peroxidase; LRDC, Lethbridge Research and Development Centre; MDA, malondialdehyde; N, nitrogen; aNDF, neutral detergent fibre; OM, organic matter; P, phosphorus; PEG, polyethylene glycol; PPC, purple prairie clover; PH, purple prairie clover hay; PH-p, PPC hay along with polyethylene glycol; SOD, superoxide dismutase; TAC, total antioxidant capacity; TBIL, total bilirubin; TMR, total mixed rations; VFA, volatile fatty acid.

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PH exhibited greater apparent total tract digestibility of DM ($P < 0.05$) and OM ($P < 0.05$) and tended ($P = 0.059$) to have greater CP digestibility compared to those fed AH. Lambs consuming PH diet had lower ($P < 0.05$) blood urea nitrogen and creatinine than AH and lower ($P < 0.05$) blood glucose and urea than PH-p, but greater ($P < 0.01$) total antioxidant capacity and catalase activity than AH diet. Lambs fed PPC CT had lower ($P < 0.05$) concentrations of ammonia, total VFA, propionate, *iso*-butyrate, *iso*-valerate and protozoa. It was concluded that PPC hay had greater nutritive value to alfalfa hay owing to its greater DM, OM and protein digestibility, but did not improve lamb growth.

1. Introduction

The low cost of grain and the high feed efficiencies of grain diet have resulted cattle production in North America concentrating in large industrial scale production systems. However, the confined feeding of large-scale and high-energy grains to beef cattle is faced with increasing challenges in terms of animal health and welfare, environmental pollution and food safety (Owens et al., 1998; Vasconcelos and Galyean, 2008). Consequently, there is a need to increase the use of forages in cattle production systems that maintain cattle health, are economically sustainable and reduce negative environmental impacts.

Alfalfa is by far the most important forage in North America because of its high yield, adaptability, high nutritive value and ability to sustain rates of gain that are comparable to those obtained in feedlots (Popp et al., 2000). However, feeding alfalfa to cattle has limitations as it frequently causes bloat. In contrast, forages that contain condensed tannins (CT) are considered bloat-safe as the CT bind to proteins upon chewing and ruminating, preventing the formation of the proteinaceous, gas-trapping foam associated with legume bloat (Waghorn and Jones, 1989; McMahon et al., 1999). In addition, CT at medium concentrations (40–50 g extractable CT/kg dry matter (DM)) have been found to improve protein utilization by reducing protein degradability (Wang et al., 1994b; Waghorn et al., 1999), resulting in increased productivity of animal feeding temperate forages with high protein content (Wang et al., 1996a,b; McMahon et al., 1999).

There are a variety of forages grown in North America that contain CT with vastly different chemical properties (McAllister et al., 2005; Berard et al., 2011). Purple prairie clover (PPC; *Dalea purpurea* Vent.) is a native legume widely distributed in prairie region of North America. It is considered to be an important palatable forage with high nutritive value for ruminants (Posler et al., 1993; Schellenberg and Banerjee, 2002). The CT in PPC possess strong anti-*Escherichia coli* and anti-*E coli* O157:H7 activity as demonstrated by numerous *in vitro* and *in vivo* studies (Liu et al., 2013; Wang et al., 2013; Jin et al., 2015; Huang et al., 2015).

However, the nutritive value and feeding value of PPC for ruminant livestock has rarely been determined. Jin et al. (2012) reported CT concentrations in PPC up to 94 g/kg DM had minimal impact on *in vitro* DM degradability. Huang et al. (2015) showed that CT at 35–36 g/kg DM in green-chop PPC decreased protein digestibility, but did not affect organic matter (OM) or fibre digestion in lambs fed PPC-alfalfa mixture. Because legume forage is usually preserved as hay to be used in intensive feedlot industry, it is important to know the nutritive and feeding value of PPC hay to evaluate the feasibility of PPC as a potential forage source.

The objective of this study was to assess the effects of PPC hay and its CT on growth performance, wool growth, nutrient digestibility, blood metabolites and rumen fermentation in lambs fed diets containing PPC or alfalfa hay.

2. Materials and methods

This experiment was conducted between December 2015 and February 2016 at the Lethbridge Research and Development Centre (LRDC), Lethbridge, Canada. All lambs used in this study were cared for according to guidelines set by the Canadian Council on Animal Care (CCAC, 2009) and the protocol was approved by the LRDC Animal Care Committee.

2.1. Forage preparation

Pure stands of alfalfa (cv. AC Longview; mid- to full-bloom stage) and PPC (common seed; full-bloom stage) were harvested from irrigated plots of the Swinton silt loam soil at the LRDC. The plots had been established for two years and were cut using a forage harvester (John Deere 6610; Moline, IL, USA), sun cured in the field to <12% moisture, baled in approximately 20 kg square bales ($90 \times 50 \times 30$ cm) and stored in an enclosed shed at ambient temperature for 120 d. Prior to feeding, both PPC and alfalfa hay were mechanically chopped to ≈10 cm lengths using an Agri-Chopper (Valmetal, QC, Canada) and stored in a closed shed. Thereafter, hays were sampled weekly starting from the 1st week of lambs being fed chopped hays for chemical analysis (Table 1). The samples were taken from top, middle and bottom of haystack which were then composited as a single sample at each sampling time.

Table 1
Chemical composition of Alfalfa and purple prairie clover (PPC) hay.

Composition[1]	Alfalfa	PPC	SEM	P value
Dry matter (g/kg hay)	924	921	1.0	0.124
Organic matter	917	923	9.2	0.687
Total N	28	25	1.2	0.136
Neutral detergent fibre	493	518	19.1	0.408
Acid detergent fibre	413[b]	429[a]	3.3	0.025
Acid detergent lignin	85[b]	98[a]	0.9	<0.001
Extractable condensed tannins	ND[2]	64.1 ± 1.0[3]	–	–
Protein condensed tannins	ND	12.4 ± 0.1	–	–
Fibre condensed tannins	ND	5.6 ± 0.3	–	–
Total condensed tannins	–	82.2 ± 1.2	–	–

[a, b] Means with different letters differ (P < 0.05).
[1] All g/kg dry matter basis otherwise indicated.
[2] ND: not detectable.
[3] Values = mean ± standard error.

Table 2
Ingredient and chemical compositions (g/kg dry matter) of experimental diets containing alfalfa hay (AH), purple prairie clover hay (PH) or PH supplemented with polyethylene glycol (PH-p).

Item	Diets		PH-p
	AH	PH	
PH-p Ingredient			
Alfalfa hay	600	0	0
Purple prairie clover hay	0	600	600
Barley	368	363	363
Dried molasses	15	15	15
Urea	0	5	5
Ammonium chloride	3	3	3
Dicalcium phosphate	4	4	4
Calcium carbonate	3.2	3.2	3.2
Sheep mineral[a]	6.5	6.5	6.5
Vitamin mix[b]	0.2	0.2	0.2
Deccox premix[c]	0.1	0.1	0.1
Chemical composition			
Dry matter	920	919	919
Organic matter	928	932	932
Total nitrogen	24	25	25
Neutral detergent fibre	382	382	382
Acid detergent fibre	278	287	287
Starch	208	202	202
Acid insoluble ash	8.6	10.9	10.9
Extractable condensed tannins	ND[d]	38.2 ± 0.6[e]	–
Protein condensed tannins	ND	7.6 ± 0.1	–
Fibre condensed tannins	ND	3.4 ± 0.2	–
Total condensed tannins	–	49.3 ± 0.7	–

[a] Sheep mineral: including 93.1% NaCl, 1.25% Mg, 0.9% Zn, 0.94% Mn, 0.13% Cu, 0.003% Se, 1.25% S, 1.25% K and 1.25% Fe.
[b] Vitamin mix: containing vitamin A, 10,000 IU/g; vitamin D, 1250 IU/g; vitamin E, 10 IU/g.
[c] Deccox premix: including 60 g/kg decoquinate (Rhone-Poulenc Canada, Mississauga, ON).
[d] ND: not detectable.
[e] Values = mean ± standard error.

2.2. Animals, diets and experiment design

Thirty-six Canadian Arcott ram lambs (39.5 ± 7.4 kg initial body weight (BW); 4.1 ± 0.3 month) were blocked by BW and randomly allocated to the three diets and housed in 36 indoor pens (2.82 × 1.0 m). The pens were bedded with sawdust and each was equipped with an individual feeder and watering bowl. Diets contained 40% (DM basis) of a barley grain based concentrate and either 60% alfalfa hay (AH), 60% PPC hay (PH) or PH diet supplemented with polyethylene glycol (PH-p). Polyethylene glycol (PEG) specifically binds with CT, neutralizing CT activity. Therefore, comparison between diets with and without PEG supplementation (PH vs. PH-p) would define the effects of CT. Urea was added to the PPC diets to ensure that all diets were iso-nitrogenous (Table 2). Diets were formulated to meet or exceed the nutrient requirement for 4–7 month old sheep with modest growth (NRC, 2007). The ingredients of the concentrates were first pelleted, and then mixed with chopped hay in Data Ranger (American Calan, Northwood, NH, USA) as total mixed ration (TMR) upon feeding. The PEG (MW 3350; Sigma) was added to the PH-p diet by spraying 300 g/L of PEG solution onto the diet during mixing so as to achieve 1 CT: 2 PEG ratio in the diet. The same amount of deionized water was also applied to AH and PH diets.

2.3. Animal feeding, sampling and measurements

Lambs were adapted to the diets for 7 d, followed by a 77-d period of data collection. Lambs were individually fed once daily (08:00 h), ensuring at least 5% orts during the adaptation and majority of the experimental period by adjusting the amount of offered diet based on the intake in previous day. The exception was on d 28–32 when lambs were fed at 90% of *ad libitum* intake to estimate nutrient digestibility using acid insoluble ash (AIA) as an internal digestibility marker. Orts were weighted weekly to estimate dry matter intake (DMI). Samples of the diet and orts were collected weekly, stored at −20 °C and composited at the end of study for chemical analysis. Lambs were weighed after an overnight fast, twice at the beginning and at the end of the experiment respectively, and once bi-weekly between. Lambs had free access to water during the entire experimental period.

Faeces samples were collected twice daily (09:00 and 15:00 h) for 5 d (d 28–32 of the experimental period) from each lamb via digital rectal retrieval. Samples for the collection period were combined for each lamb and stored immediately at −20 °C until they were freeze-dried for chemical analyses. Diets were also sampled daily and composited over the 5-d period for chemical analysis.

A 10 cm dye band, using L'oreal Paris ("NF02 noir fatal") hair dye, was applied to the right mid-side of each lamb on d 0. The hair dye cream was mixed with the activator (Oxigenta 6%) immediately before application and applied in a thin line at skin level using the applicator provided. At the completion of the experiment (d 75), the fleece containing the dye bands was shorn using clippers (Oster clippers, No. 40 cutting head) at skin level and stored in plastic bags before being sent to Riverina Wool Testers Pty Ltd (Wagga Wagga, NSW, Australia) for wool test analysis.

Rumen fluid was sampled from the same 6 randomly selected lambs by stomach tube just prior to feeding on d 50, 71 and 77. The pH of rumen fluid was immediately measured using a potable pH meter (VWR, Mississauga, ON, Canada). Rumen fluid was filtered through four layers of cheesecloth and subsequently sampled for analyses of volatile fatty acid (VFA), ammonia and protozoa using the procedures described by Wang et al. (1994a).

Blood samples were collected using a 20 gage needle into acutainer tubes (BD Franklin Lakes, NJ, USA) via the jugular vein of each lamb on d 0, 29 and 77 of the experimental period. Blood samples were kept at room temperature for 20 min and centrifuged at 8000 × *g* for 10 min. The resultant serum was divided into two portions and immediately stored at −80 °C for subsequent analysis of the serum metabolites and antioxidant enzyme activity.

At the end of the experiment (d 77), 10 lambs from each diet were randomly selected and slaughtered after being fasted for 18 h at a commercial abattoir (SunGold Specialty Meats LTD., Innisfail, AB, Canada). Trained personnel recorded carcass traits according to the procedures described by Stanford et al. (2000). Carcass characteristics included hot carcass weight, fat thickness between the 12th and 13th rib on longissimus dorsi muscle and dressing percentage were determined using the procedures described by He et al. (2013).

2.4. Laboratory analyses

All forage, diets, orts and faeces samples were analyzed for DM and OM (AOAC, 1991, # 943.01), N by flash combustion analysis using a NA1500 nitrogen analyzer (Carlo Erba Instruments, MI, Italy), and aNDF (with addition of sodium sulfite and α-amylase for analysis) and ADF using an Ankom 200 system (Ankom Technology Corp., Fairport, NY, USA) as described by McGinn et al. (2004). Acid detergent lignin (ADL) was determined according to method of AOAC (1999, # 973.18). The AIA content of diets, orts and faeces samples collected for the estimation of digestibility were determined using the procedure described by Van Keulen and Young, (1977). Starch was quantified using the enzymatic method described by Beauchemin et al. (1997). Diet and ort samples were analyzed for CT (extractable, protein bound and fibre bound components) by the butanol-HCl method of Terrill et al. (1992) with purified CT from whole plant PPC used as the standard. Rumen fluid samples were analyzed for VFA using a 5890A gas liquid chromatograph (Phenomenex, Torrance, CA, USA) as described by Wang et al. (2006), ammonia by the phenol-hypochlorite method (Weatherburn, 1967) and protozoa using light microscopy (Wang et al., 1994a). A pre-packed panel (General Health Profile, IDEXX Laboratories, ME, USA) for measuring serum metabolites of albumin (ALB), alkaline phosphatase (ALKP), alanine aminotransferase (ALT), blood urea nitrogen (BUN), calcium (Ca), cholesterol (CHOL), creatinine (CREA), glucose(GLU), globulin (GLOB), phosphorus (P), total bilirubin(TBIL) and total protein (TP) was conducted with an IDEXX VetTest® Chemistry Analyzer (IDEXX Laboratories, Westbrook, ME, USA). Commercial kits (Cayman Chemical Company, Ann Arbour, MI, USA) were used to determine serum anti-oxidant enzyme activity including catalase (CAT, # 707002), glutathione peroxidase (GSH-Px, # 703102), malondialdehyde (MDA, # 700870), superoxide dismutase (SOD, # 706002) and total antioxidant capacity (TAC, # 709001), following the corresponding manufacturer's instructions. Yield, strength, length, fibre diameter, fibres >30.5 mm, comfort factor, curvature and spinning fineness of wool which were used to assess the effects of three dietary treatments on wool parameters were analyzed accordingly to test methods described in the "Red Book" (International Wool Textile Organisation (IWTO) 2015).

2.5. Calculation and statistical analysis

Feed intake was determined as the gravimetric difference between the feed offered and orts. Average daily gain (ADG) was estimated as the linear regression coefficient of BW vs. time of 77 d of the study and feed efficiency (FE) was expressed

Table 3
Dry matter intake, growth performance and carcass characteristics of Canadian Arcott ram lambs fed diet containing alfalfa hay (AH), purple prairie clover hay (PH) or PH supplemented with polyethylene glycol (PH-p).

Item	Diets[1]			SEM	P value
	AH	PH	PH-p		
Dry matter intake (g/d)	1160[a]	1067[b]	1124[ab]	20.7	0.009
Initial live weight (kg)	39.0	38.0	39.0	2.14	0.942
Final live weight (kg)	52.5	50.8	52.1	1.98	0.822
Weight gain (kg)	13.5	12.8	13.2	0.48	0.554
Average daily gain (g/d)	187.1	181.1	186.0	6.69	0.778
Feed efficiency (g/g)[2]	0.16	0.17	0.17	0.01	0.762
Carcass characteristics					
Hot carcass weight (kg)	25.38	24.71	25.18	0.70	0.789
Dressing percentage (%)[3]	46.01	46.34	46.63	0.59	0.761
Grade rule (mm)[4]	11.8	11.4	11.8	0.98	0.946

[a, b] Means with different letters differ ($P < 0.05$).
[1] AH: total mixed diet containing 60% chopped alfalfa hay and 40% barley grain based concentrate; PH: total mixed diet containing 60% chopped purple prairie clover (PPC) hay and 40% barley grain based concentrate; PH-p: total mixed diet containing 60% chopped PPC hay, 40% barley grain based concentrate and supplemented with polyethylene glycol (3350 MW) to neutralize the biological activity of PPC condensed tannins.
[2] Calculated as: average daily gain/dry matter intake.
[3] Calculated as the hot carcass weight proportion of the fasting live weight.
[4] Body wall thickness between 12th and 13th rib, 11 cm from the carcass midline (Kirton, 1989).

as ADG/DMI. Indigestible AIA in diet ingested and in faeces samples was used as marker to estimate nutrient digestibility using the equation described by Huang et al. (2015).

$$\text{Digestibility}(\%) = [1 - (a \times b)/(c \times d)] \times 100\%$$

where, a and c = AIA concentration in diet ingested and faeces respectively; b and d = nutrient concentration in faeces and diets ingested respectively. a and d were calculated as difference between the diet offered and orts.

Rumen fluid and serum data obtained during data collection period were averaged across different sampling times for each lamb prior to statistical analysis.

All data were statistically analyzed as completely randomized design by ANOVA of mixed procedure of SAS (2012) with treatment as fixed effect and lamb as experimental unit using model of $Y = \mu + Bi + Dj + Eij$, where μ = the overall mean; Bi = the fixed effect of diet ($i = 1–3$); Dj = the random effect of animal ($j = 1–12$); and Eij = the residual error. Serum data obtained at the beginning of the experiment (d 0) was initially used as covariate in analyzing serum biochemical parameters and antioxidant indices using equal slope model because regression slopes of each measurements were tested to be equal among treatments. The covariance analysis showed that these initial data had no effect, and consequently was excluded in the final analysis. Significant differences among treatments were tested using LSMEANS with the PDIFF option with significance declared at $P \leq 0.05$, and a tendency was reported if $0.05 < P \leq 0.10$.

3. Results

3.1. Composition of forage

Alfalfa and PPC hay had similar ($P > 0.05$) OM, N and aNDF content, but PPC hay had greater ($P < 0.05$) ADF and ADL than alfalfa hay (Table 1). Of the total CT in PPC hay (82.2 g/kg DM) the majority (78%) were extractable with the remaining being protein-bound (15%) and fibre-bound (7%). No CT were detected in alfalfa hay. All analyzed nutrient concentrations were similar among three diets with extractable CT about 38.2 g/kg DM in the total mixed PH diet (Table 2).

3.2. Lamb growth performance, wool yield and quality and nutrients apparent digestibility

All lambs appeared healthy throughout the entire experimental and no morbidity or mortality occurred. Dry matter intake of lambs fed PH diet was less ($P < 0.01$) than those of lambs fed AH diet, and tended to be lower ($P = 0.075$) than lambs fed PH-p (Table 3). There was no difference in DMI between AH and PH-p fed lambs. All lambs had similar ($P > 0.05$) initial BW, final BW, ADG, FE and carcass traits.

Wool yield, strength, length, fibre diameter, comfort factor, curvature and spinning fineness were all not affected ($P > 0.05$) by dietary treatments (Table 4).

Lambs consuming PH exhibited greater apparent total tract digestibility of DM ($P < 0.05$) and OM ($P < 0.05$) and tended ($P = 0.059$) to have greater CP digestibility compared to those fed AH, but lower digestibility of CP ($P < 0.001$), aNDF ($P < 0.01$) and ADF ($P < 0.05$) than those fed PH-p (Table 5). Digestibilities of DM, OM, CP, aNDF and ADF were all greater ($P < 0.01$) for PH-p than for AH. Addition of PEG to PH increased ($P < 0.05$) CP, aNDF and ADF digestibility, tended ($P = 0.094$) to increase

Table 4
Effects of feeding lambs alfalfa hay (AH), purple prairie clover hay (PH) and purple prairie clover hay supplemented with polyethylene glycol (PH-p) diets on wool yield and quality.

	Diets[a]			SEM	P value
	AH	PH	PH-p		
Yield (%)	65.6	66.5	65.9	1.29	0.884
Strength	27.6	23.8	26.7	3.19	0.669
Length (cm)	58.6	59.9	57.4	2.42	0.883
FD[b] (μm)	27.6	26.8	27.2	0.60	0.596
FDSD[c] (μm)	6.4	5.9	6.3	0.20	0.132
FDCV[d] (μm)	23.4	22.0	23.0	0.49	0.172
Fibres >30.5 mm (%)	30.2	24.6	27.9	3.56	0.541
Comfort factor (%)	69.8	75.4	72.1	3.56	0.541
Curvature (deg/mm)	87.8	92.8	89.4	2.86	0.454
Spinning fineness (μm)	27.5	26.3	27.0	0.62	0.395

[a] AH: total mixed diet containing 60% chopped alfalfa hay and 40% barley grain based concentrate; PH: total mixed diet containing 60% chopped purple prairie clover (PPC) hay and 40% barley grain based concentrate; PH-p: total mixed diet containing 60% chopped PPC hay, 40% barley grain based concentrate and supplemented with polyethylene glycol (3350 MW) to neutralize the biological activity of PPC condensed tannins.
[b] FD: fibre diameter.
[c] FDSD: fibre diameter standard deviation.
[d] FDCV: fibre diameter coefficient of variation.

Table 5
Nutrient apparent digestibility (%) of Canadian Arcott ram lambs fed diet containing alfalfa hay (AH), purple prairie clover hay (PH) or PH supplemented with polyethylene glycol (PH-p).

Item[2]	Diets[1]			SEM	P value
	AH	PH	PH-p		
Dry matter	59.8[b]	67.6[a]	68.8[a]	1.88	0.003
Organic matter	60.8[b]	66.9[a]	71.2[a]	1.76	<0.001
Crude protein (N × 6.25)	61.0[b]	65.5[b]	76.5[a]	1.75	<0.001
Neutral detergent fibre	43.0[b]	47.9[b]	59.2[a]	2.68	<0.001
Acid detergent fibre	43.3[b]	44.8[ab]	51.0[a]	1.94	0.022

[a, b] Means with different letters differ ($P < 0.05$).
[1] AH: total mixed diet containing 60% chopped alfalfa hay and 40% barley grain based concentrate; PH: total mixed diet containing 60% chopped purple prairie clover (PPC) hay and 40% barley grain based concentrate; PH-p: total mixed diet containing 60% chopped PPC hay, 40% barley grain based concentrate and supplemented with polyethylene glycol (3350 MW) to neutralize the biological activity of PPC condensed tannins.
[2] Dgestibility was determined by acid insoluble ash (AIA) method using equation: digestibility (%) = $[1 - (a \times b)/(c \times d)] \times 100\%$ where, a and c = AIA concentration in diet ingested and faeces respectively; b and d = nutrient concentration in faeces and diets ingested respectively. a and d were calculated as difference between the diet offered and orts.

OM digestibility but did not affect DM digestibility. Digestion of starch was nearly complete (99.9%) for all lambs and there was no difference in starch digestion among lambs fed the three diets (data no shown).

3.3. Serum metabolites and antioxidant enzymes

All the serum metabolites measured in this study were in the normal range or very close to this range of a healthy sheep (Table 6). Lambs fed PH-p had greater GLU ($P < 0.05$) and BUN ($P < 0.001$) than those fed PH. Concentrations of CREA and BUN were lower ($P < 0.05$) for lambs fed PH than lambs fed AH. In contrast, lambs fed PH had greater serum TAC ($P < 0.01$) and CAT ($P < 0.001$) than those fed AH with TAC also tending ($P = 0.077$) to be higher in lambs fed PH-p. Similarly, lambs fed PH-p had greater ($P < 0.001$) serum CAT activity than lambs fed AH.

3.4. Rumen metabolites

Treatment did not affect ($P > 0.05$) rumen fluid pH (Table 7). However, rumen fluid collected from lambs fed PH had lower ($P < 0.05$) concentrations of ammonia, VFA and protozoa as compared to lambs fed AH or PH-p. Lambs fed the PH diet also produced VFA with lower ($P < 0.05$) molar proportions of propionate and *iso*-valerate, but greater ($P < 0.01$) acetate: propionate ratio than those fed AH or PH-p. All these measured rumen metabolites were similar ($P > 0.05$) between AH and PH-p fed lambs, except for the molar proportion *iso*-butyrate which was greater ($P < 0.01$) for AH fed than for PH-p fed lambs.

Table 6
Serum biochemical parameters and serum antioxidant indices of Canadian Arcott ram lambs fed diet containing alfalfa hay (AH), purple prairie clover hay (PH) or PH supplemented with polyethylene glycol (PH-p).

Item	Diets[1]			SEM	P value
	AH	PH	PH-p		
Serum metabolites (Reference range[2])					
Albumin (24–34 g/L)	32.54	31.14	32.25	0.83	0.360
Alkaline phosphatase (50–228 U/L)	213.67	220.50	222.17	11.83	0.870
Alanine aminotransferase (35–80 U/L)	45.12	45.60	46.80	1.89	0.809
Calcium (91–108 mg/L)	110.16	105.90	108.82	1.61	0.148
Cholesterol (0.44–0.82 g/L)	0.35	0.37	0.35	0.03	0.740
Creatinine (6.0–15.0 mg/L)	10.11^{a}	9.08^{b}	9.46^{ab}	0.28	0.037
Glucose (0.50–0.80 g/L)	0.79^{ab}	0.74^{b}	0.84^{a}	0.02	0.014
Phosphorus (40–89 mg/L)	83.65	87.61	87.37	3.15	0.401
Total bilirubin (1.0–4.0 mg/L)	3.58	2.67	2.89	0.40	0.220
Total protein (56–78 g/L)	64.63	62.55	63.07	1.15	0.389
Blood urea nitrogen (0.105–0.42 g/L)	0.50^{a}	0.39^{b}	0.50^{a}	0.01	<0.001
Globin (32–41 g/L)	31.42	32.06	31.97	0.51	0.609
Serum antioxidant indices					
Total antioxidant capacity (mM)	1.95^{b}	2.05^{a}	1.97^{ab}	0.04	0.021
Superoxide dismutase (U/ml)	5.44	5.31	5.12	0.29	0.727
Glutathione Peroxidase (nmol/min/ml)	29.72	32.84	31.68	1.99	0.508
Catalase (nmol/min/ml)	83.11^{b}	108.64^{a}	101.34^{a}	3.65	<0.001
Malondialdehyde (μM)	5.86	6.98	6.52	0.49	0.267

$^{a, b}$ Means with different letters differ ($P < 0.05$).

[1] AH: total mixed diet containing 60% chopped alfalfa hay and 40% barley grain based concentrate; PH: total mixed diet containing 60% chopped purple prairie clover (PPC) hay and 40% barley grain based concentrate; PH-p: total mixed diet containing 60% chopped PPC hay, 40% barley grain based concentrate and supplemented with polyethylene glycol (3350 MW) to neutralize the biological activity of PPC condensed tannins.

[2] The range value was provided by the IDEXX VetTest Chemical Analyzer for healthy sheep (General Health Profile, IDEXX Laboratories, Westbrook, ME, USA).

Table 7
Rumen metabolite and protozoa concentrations of Canadian Arcott ram lambs fed diet containing alfalfa hay (AH), purple prairie clover hay (PH) or PH supplemented with polyethylene glycol (PH-p).

Item	Diets[1]			SEM	P value
	AH	PH	PH-p		
pH	5.93	5.97	5.99	0.09	0.874
Ammonia (mmol/L)	10.78^{a}	6.84^{b}	13.78^{a}	1.00	<0.001
Total VFA (mmol/L)	105.72^{a}	88.94^{b}	107.69^{a}	5.69	0.026
Molar percentage (%)					
Acetate	64.57	66.63	64.90	1.34	0.512
Propionate	24.67^{a}	19.04^{b}	22.74^{a}	1.30	0.013
Butyrate	9.35	11.00	9.95	0.92	0.337
Iso-butyrate	0.49^{a}	0.25^{b}	0.29^{b}	0.05	0.005
Valerate	1.79	2.06	1.65	0.17	0.147
Iso-valerate	0.21^{a}	0.02^{b}	0.15^{a}	0.03	<0.001
Caproic acid	0.50	0.52	0.32	0.10	0.187
Acetate: propionate	2.74^{b}	3.52^{a}	2.91^{b}	0.19	0.008
Protozoa ($\times 10^5$/mL)	1.48^{a}	0.08^{b}	1.49^{a}	0.25	<0.001

$^{a, b}$ Means with different letters differ ($P < 0.05$).

[1] AH: total mixed diet containing 60% chopped alfalfa hay and 40% barley grain based concentrate; PH: total mixed diet containing 60% chopped purple prairie clover (PPC) hay and 40% barley grain based concentrate; PH-p: total mixed diet containing 60% chopped PPC hay, 40% barley grain based concentrate and supplemented with polyethylene glycol (3350 MW) to neutralize the biological activity of PPC condensed tannins.

4. Discussion

4.1. Feed intake and lamb growth performance

Although PPC is considered an important palatable component of prairie forage (Schellenberg and Banerjee, 2002), little information is available regarding its nutritive and feeding value as livestock feed. To our knowledge, this study is the first to determine feed intake, growth performance and wool yield and quality of ruminants fed PPC hay as part of TMR. Feeding value is a function of intake and nutritive value and nutritive value is a measure of available nutrients (Waghorn and Clark, 2004). This study demonstrated that PPC hay had greater nutritive value owing to its greater DM, OM and protein digestibility, but similar feeding value to that of alfalfa hay due to its reduced feed intake, as judged by the similar growth performance, carcass traits and wool yield of lambs fed diet containing 60% respective forages. The 8% lowered feed intake of lambs fed PH

diet as compared to that of AH diet is likely due to the differences in chemical composition and/or palatability between the two forages. Feed intake is usually regulated by both digestibility and palatability of the diet (Greenhalgh and Reid, 1967), and both are closely associated with the physical property and chemical composition of the feed (Van Soest, 1965). In this study, the two forages contained similar nutrient content except for greater ADF and ADL content in PPC hay than in alfalfa hay, and all diets were formulated to similar nutrient concentrations. This, coupled with the fact that digestibilities of DM and OM was greater and CP digestibility tended to be greater for the PH than for AH diet and aNDF and ADF digestibility were similar between the two diets, suggests that the lower feed intake of PH fed lambs as compared to that of AH fed ones was unlikely caused by their nutrient composition or digestibility. Wool production is strongly correlated with feed intake (Rangel and Gardiner, 2009). Therefore, the similar feeding value of the current diets potentially explains the similar wool yield and quality of lambs among the three dietary treatments. Sheaffer et al. (2009) reported that PPC and alfalfa had similar palatability. However, their result was based on the percentage of total leaf mass consumed by lambs during grazing, and did not account for the plant stem. In the current study, both forages were fed as chopped whole-plant hay including stems. In general, stems contain twice as much lignin as that in leaves and native grasses were found to contain more lignin than cultivated grasses likely due to greater proportion of stems in native than in cultivated grasses (Ramírez-Lozano, 2015). The same situation might also be true for native vs. cultivated legume forage. It has been demonstrated that animal prefer leaves than stems (Heady and Torell, 1959). Another factor that might have lowered feed intake for lambs fed PPC diet is the presence of CT in PPC hay. The astringent nature of CT during chewing can reduce the palatability of CT containing forages and thereby decrease feed intake (Mueller-Harvey, 2006). Although, some recent studies indicated that CT concentrations up to 97 g/kg DM have no effect on feed intake of sheep (Heckendorn et al., 2006; Theodoridou et al., 2012), the majority of the literature indicates more than 50 g of CT/kg DM generally reduces feed palatability and depresses feed intake (Kumar and Singh, 1984; Wang et al., 1996b; Mueller-Harvey, 2006; Krueger et al., 2010) although there were variations among plant sources of CT (i.e. different CT structures). In this study, levels of extractable CT were 64.1 g/kg DM in PPC hay and 38.2 g/kg DM in PH diet. The tendency for lower feed intake of PH diet fed lambs than PH-p diet fed lambs suggests that PPC CT in the present study might have decreased feed intake. However, the complete consumption of the entire PH diet indicated that 64.1 g/kg DM of CT in PPC hay did not cause lambs to sort PPC out of the total mixed diet. This suggests that concentration of PPC CT up to 64.1 g/kg had minimal effect on palatability of PPC hay. Huang et al. (2015) also showed that extractable CT in fresh PPC (47 g/kg DM) did not affect the palatability or feed intake of lambs. Therefore, despite its strong protein-binding capacity (Liu et al., 2013) and anti-*E. coli* activity (Wang et al., 2013; Liu et al., 2013; Jin et al., 2015), its seems that CT at the concentration of 64.1 g/kg DM in PPC hay in the current study and 47 g/kg DM in fresh PPC of Huang et al. (2015) did not prevent lambs from consuming PPC in either a sun-dried or fresh form. The observation that CT in PH diet decreased digestibilities of aNDF, ADF and CP and tended to decrease OM digestibility compared with PH-p diet might have reduced feed intake of PH diet fed lambs. Because feed intake of the animal is negatively related to the nutrient digestibility of the diet (Andersen et al., 1959; Van Soest, 1983). Reduced feed intake and nutrient digestibility by dietary CT have been reported in the literature (Wang et al., 1996b; Min et al., 2003). Overall, these results demonstrate that feeding value of PPC hay prepared at the full-bloom stage is comparable to that of alfalfa hay harvested at the mid-bloom stage. Furthermore, PPC CT at the concentration of 38.2 g/kg DM had no effect on lamb growth performance although it slightly reduced feed intake.

4.2. Nutrient digestibility and rumen fermentation metabolites

It is interesting to note that the DM and OM digestibility of PPC diet was greater and CP digestibility tended to be greater than that of alfalfa diet. Given the fact that both diets contained the same level of a barley grain-based concentrate, the greater DM and OM digestibility of PPC diet suggests that PPC hay was more digestible than alfalfa hay. On the contrary, Huang et al. (2015) reported that digestibilities of OM and CP of PPC were lower than that of alfalfa. The discrepancy between the two studies is likely due to the different diets forms and CT concentrations in the two studies. Huang et al. (2015) used green chopped alfalfa and alfalfa-PPC mixtures as diets, whereas this study used alfalfa and PPC hay mixed with barley grain in a TMR. In addition, the alfalfa used in Huang et al. (2015) was harvested at early bloom stage and contained less fibre but more protein than PPC. In contrast, the alfalfa hay in this study was made from alfalfa harvested at mid- to full-bloom stage and had a fibre and protein content that was similar to PPC.

Generally, biological activity of CT depends on both their concentration and chemical structure (Mueller-Harvey, 2006; Waghorn, 2008). In this study, protein and fibre digestibilities were decreased by the presence of CT in PPC hay. Decreased protein digestibility is a common observation with CT-containing forages (Waghorn et al., 1990; Perez-Maldonado and Norton, 1996; Mezzomo et al., 2011) and antimicrobial activity against rumen fibrolytic and proteolytic bacteria of other sources of CT has been well documented (Bae et al., 1993; Jones et al., 1994; Min et al., 2005; Wang et al., 2009). Huang et al. (2015) also reported that PPC CT at a concentration of 35–36 g/kg DM in a fresh forage diet decreased protein digestibility. The current study and Huang et al. (2015) showed that PPC CT at the dietary concentrations between 35 and 38 g/kg DM had a negative impact on protein and fibre digestibility and this effect became greater as the CT concentration increased because CT concentration at 35 g/kg DM decreased protein digestibility and tended to decreased fibre digestibility in Huang et al. (2015) whereas CT concentration at 38.2 g/kg DM decreased both protein and fibre digestibility and tended to decreased OM digestibility in this study. However, the negative effect of PPC CT on protein and fibre digestion did not negatively affect animal productive performance in this study as indicated by a similar growth rate, feed efficiency, carcass traits and wool

yield and quality between PH and PH-p fed lambs. It has been shown that CT can shift protein digestion site from rumen to the small intestine, resulting in improved protein utilization (Wang et al., 1996b,c; Waghorn and Shelton, 1997; Patra and Saxena, 2011). Therefore, it is possible that the negative effect of CT on total tract protein and fibre digestibility was offset by the positive effect of CT on site of protein digestion within the gastrointestinal tract. Condensed tannin mediated reductions in ruminal protein degradation are usually associated with a decline in the concentration of ruminal ammonia (Waghorn et al., 1994) and total VFA (Wang et al., 1996b). The lower rumen ammonia concentrations in PH fed lambs than in PH-p fed lambs is another indicative of reduction in ruminal protein degradation. Previous reports also showed that PPC CT at various concentrations significantly reduced ammonia concentration *in vitro* (Jin et al., 2012, 2013, 2015). Amino acid deamination is mostly attributed to rumen Gram-positive bacteria with a high specific activity of NH_3 production (Bento et al., 2015) and CT from other sources have well been recognized to inhibit rumen Gram-positive bacteria (Jones et al., 1994). The lower total VFA concentration might have partially been caused by reduced feed intake and fibre digestion in the rumen because rumen is the main site for fibre digestion. The lower molar proportion of ruminal propionate and the subsequent higher acetate: propionate ratio in lambs fed PH fed than those fed AH or PH-p, suggest that it was PPC CT that caused this difference between AH and PH diets. The reduction of the molar proportion of propionate by PPC CT in this study is in agreement with the *in vitro* observations of Jin et al. (2012, 2013). However, the reason why PPC CT decreased propionate molar proportion is unknown. Narvaez et al. (2013) documented that rumen fermentation into propionate mainly involved *Prevotella bryantii* and *Ruminobacter amylophilus*. Moreover, the presence of CT has been observed to reduce growth of both *P. bryantii* (Molan et al., 2001; Min et al., 2005) and *R. amylophilus* (Jones et al., 1994). Therefore, the negative effect of PPC CT on propionate production in this study might be through inhibiting rumen levels of these bacteria, but this was not determined. With respect to branched-chain VFA, such as *iso*-butyrate and *iso*-valerate, they are produced from the deamination of valine and leucine during rumen fermentation (El-Shazly, 1952). Therefore, the reduced concentrations of *iso*-butyrate and *iso*-valerate for lambs fed PH than other two diets in this study also indicated that PPC CT decreased deamination of these essential amino acids in the rumen. Decreased rumen concentration of branched-chain VFA has also been reported for other sources of CT (Wang et al., 1994a, 1996b). Similarly, the lower protozoa number in the rumen fluid in lambs fed PH as compared to the other diets suggests that PPC CT had anti-protozoa activity, which is also not reported for PPC CT. This observation was consistent with the reports for CT in *Lotus corniculatus* (Wang et al., 1994a, 1996b). The anti-protozoa activity of PPC CT may also contribute to the decreased rumen ammonia concentration in PH group because protozoa is one of the major ammonia producers within the rumen (Warner, 1956; Hino and Russell, 1987). Further research is needed to determine the effect of PPC CT on rumen microbial community and on protein metabolism and utilization.

4.3. Blood metabolites and antioxidant capacity

The observation that measured serum metabolites were within or close to the normal range for healthy sheep indicated that diets used in this study did not adversely impact these metabolite profiles. The lower BUN concentration for lambs fed PH than those fed AH and PH-p is consistent with the lower CP digestibility and lower rumen ammonia observed with this treatment. The lower serum GLU concentration of PH than PH-p fed lambs is consistent with Huang et al. (2015). The blood sugar-decreasing effect of CT was also reported for other CT-containing forages fed to sheep (Wang et al., 1996a; Mahgoub et al., 2008). The mechanism by which CT decrease blood glucose concentration is not clear. However, this study showed PPC CT decreased molar proportion of propionate which is the only gluconeogenic VFA, which may in part account for the lower blood glucose concentration. Decreased molar proportion of propionate in total VFA by PPC CT was also reported by Jin et al. (2012) in an *in vitro* study. Condensed tannins have been shown to induce β-cell regeneration and directly act on adipose cells, possibly enhancing insulin receptors of fat cells (Anderson and Polansky, 2002; Kim et al., 2003; Klein et al., 2007), but this requires that CT molecules contact directly with the cells. Whether this mechanism was contributed to the lowered serum glucose concentration in this study is not known because digestion of PPC CT was not determined.

The greater serum TAC in lambs fed PH than for those fed AH suggests that PPC hay may improve the antioxidant status of lambs. However, the similar serum TAC between the PH and PH-p fed lambs suggests that the improved antioxidant status of PH was not solely due to CT. Superoxide dismutase, GSH-Px and CAT are all antioxidant enzymes that assist in maintaining a healthy cellular anti-oxidant status. In particular, CAT plays a key role in maintaining H_2O_2 homeostasis by catalyzing the decomposition of H_2O_2, a major reactive oxygen species in living organisms, thereby is a very important enzyme in protecting the cell from oxidative damage. The similar difference in TAC and CAT between lambs fed PH diet and AH diet suggests the greater TAC in PH fed lambs is mainly due to its greater CAT activity. This result indicated that lambs fed PH diet had improved antioxidant status than lambs fed AH diet. However, the similar CAT activity and TAC between PH and PH-p fed lambs indicated that the improved antioxidant status in PH fed lambs could not be attributable solely to PPC CT even though there is an abundant evidences showing that tannins possess strong anti-oxidant activity (Hagerman et al., 1999; Dutta et al., 2012; Dey and De, 2014). Antioxidants are widely believed to be important line of defense against cellular oxidative stress (Regoli and Principato, 1995). Improved animal health and productive performance have been shown to be closely associated with enhanced antioxidant capacity of animals fed diets containing antioxidants (Sahin et al., 2003; Kumar et al., 2009; Wang et al., 2011). In this study, the negative effect of reduced feed intake on animal growth performance in PH fed lambs may have partially been neutralized by the effect of phenolic compounds in improving anti-oxidant status of the lambs.

5. Conclusion

Purple prairie clover hay containing 64.1 g/kg DM of extractable CT had greater nutritive value to alfalfa hay in terms of its greater DM, OM and protein digestibility, but did not improve lamb growth performance. Altogether, this study demonstrated that PPC hay prepared at full-bloom stage had similar feeding value as alfalfa hay prepared at mid- to full-bloom stage, but CT in PPC had negative effect on protein, fibre and OM digestion at the concentration of 38.2 g/kg dietary DM when PPC hay was used as an ingredient in formulated TMR. Further study is needed to determine the optimum amount of PPC hay in the formulated TMR in order to obtain the most benefit of its high nutritive value whilst to minimize its CT's negative effect on nutrient digestion.

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