Forages and Pastures: Antinutritive Compounds in Forages

M116 Fermentation and microbial protein synthesis from anthocyanidin accumulating Lc-alfalfa in rumen liquid. A. Jonker[1,2], M. Y. Gruber[2], Y. Wang[3], D. A. Christensen[1], J. J. McKinnon[1], and P. Yu*[1], *[1]Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, [2]Saskatoon Research Station, Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada, [3]Lethbridge Research Station, Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada.*

It is well documented that mono/polymeric anthocyanidin have the capacity to regulate ruminal fermentation and degradation characteristics of feed. Therefore, alfalfa was modified with a maize leaf color (Lc) gene to synthesize these metabolites. Three of these modified Lc-alfalfa progeny (BeavLc1, RambLc3, RangLc4) that expressed a purple-green phenotype were harvested at a vegetative pre-bud stage to determine fermentation and microbial protein synthesis. Ground freeze-dried samples were compared for their ruminal fermentation characteristics in rumen liquid with 15N as marker for microbial N using an in vitro gas production system. The 3 Lc-progeny had a similar anthocyanidin concentration with an average of 232 μg/g DM. Gas production rate was faster for RamLc3 ($P < 0.001$) than the other 2 Lc-progeny and faster for RangLc4 than BeavLc1. Methane production rate tended to be faster for RambLc3 ($P = 0.07$) compared with the other 2 Lc-progeny but total methane production was similar. Ammonia accumulation was faster ($P = 0.04$) but total ammonia accumulation and branch chain fatty concentration were lower ($P < 0.001$) for BeavLc1 compared with the other 2 Lc-progeny. Total volatile fatty acid accumulation was higher for RangLc4 ($P = 0.001$), propionate was higher and acetate lower for RambLc3 ($P < 0.001$) and butyrate was lower and acetate higher for BeavLc1 ($P < 0.001$). Microbial protein synthesis was higher for BeavLc1 ($P < 0.001$) compared with the other 2 Lc-progeny. In conclusion, all 3 Lc-alfalfa progeny accumulated anthocyanidin and fermentation profiles and end products differed between the 3 progeny.

Key words: anthocyanidin-accumulating alfalfa, in vitro ruminal fermentation and microbial-N, methane and ammonia

M117 How tannin deactivation can affect nutrient digestibility and metabolizable energy contents of sainfoin (*Onobrychis vicifolia*). H. Khalilvandi-Behroozyar*[1,2], M. Dehghan-Banadaky[1], and K. Rezayazdi[1], *[1]Department of Animal Science, University of Tehran, Karaj, Tehran, Iran, [2]Department of Animal Science, University of Urmia, Urmia, West Azerbaijan, Iran.*

Nutritive value of forages for ruminants is inherently variable and depends on many factors such as forage species, climate, degree of maturity, anti nutritional factors, etc. The objective of this study was to examine tannin deactivation effects on energy and nutrient availability from sainfoin. Second cut forage was shade dried and chopped (3 –5 cm length), and then exposed to nothing (Control) or 5% (w/v) solution of polyethylene glycol (PEG 6000 MW) that was sprayed on the forage (v/w ratio of 1:1). Water soaking was applied with tap water (v/w ratio of 4:1). Treatments were carried out at an ambient temperature of 25°C for 20 min with hand shaking for water, and overnight for PEG. Water was added to forage just before feeding in an in vivo trial. The extractable CT content was determined (Butanol–HCl reagent). Ruminally fistulated Holstein cows (3 multiparous, 680±20kg of BW) were used in 3×3 change over design. Each period consisted of 10 days for adaptation and 7 days for total fecal and forage sample collection. Forages were fed as sole diet (0800 and 1600) along with mineral/vitamins to meet 110% of maintenance requirements of dairy cows. Digestibility coefficients determined and ME estimated with ME= 0.0157 × DOMD. MIXED PROC of SAS 9.1 was used for statistical analysis at 0.05 probability level. Water and PEG deactivated 92.06 and 98.57% of CT, respectively. Digestibility coefficients of EE and ADF were not statistically different ($p \leq 0.05$). Differences of means for OM, NDF and CP digestibility and ME among treatments were statistically significant. Digestibility of NDF was 512.5^{b} for control and 610.1^{a} and 613.4^{a} g/kgDM for water and PEG groups respectively. Digestibility coefficients for CP were 649.8 ^{b}, 753.9 ^{a} and 770.4 ^{a} and for OM were 592.3 ^{c}, 731.4 ^{a} and 709.9 ^{b} g/kgDM for control, water and PEG groups respectively. ME content increased from 8.69 ^{c} to 10.72 ^{a} and 10.22 ^{b} for water and PEG treated forages. Tannin deactivation might be responsible for increasing ME.

Key words: sainfoin, polyethylene glycol, metabolizable energy

M118 Effects of sainfoin (*Onobrychis viciifolia*) processing for tannin deactivation on nitrogen content of cell wall and available nitrogen. H. Khalilvandi-Behroozyar*[1,2], K. Rezayazdi[1], and M. Dehghan-Banadaki[1], *[1]Department of Animal Science, University of Tehran, Karaj, Tehran, Iran, [2]Department of Animal Science, University of Urmia, Urmia, West Azerbaijan, Iran.*

The objective of this study was to examine the effectiveness of tannin deactivation on potential nitrogen availability of sainfoin. DM was determined by drying at 105°C overnight. Extractable condensed tannin content of second cut sainfoin was determined using the butanol-HCl reagent. Forages were chopped, and then exposed to nothing (Control) or 5%(w/v) solution of polyethylene glycol (PEG 6000 MW) that was sprayed on the forage (v/w ratio of 1:1). Soaking with water was applied using tap water (v/w ratio of 4:1). Treatments were carried out at an ambient temperature of 25°C for 20 min with hand shaking for water, and overnight for PEG. Treated forages (3 replicates) were then exposed to 40°C temperature in a forced air oven, for 48 h. Neutral detergent fiber and ADF analyzed using Fibertech system. Sodium sulfite not included in NDS solution. Nitrogen content of entire feed and ADF and NDF residues were measured by the Kjeldahl method. Crude protein was calculated as N×6.25 and available nitrogen was considered TN-ADIN. Comparison of means was done using GLM procedure of SAS 9.1(SAS, 2002). CRD and Duncan's multiple range test was conducted to comparison of means between treatments were used ($p \leq 0.05$). Condensed tannin content declined from 21.3^{a} (control) to 1.7 ^{b} and 0.3 ^{c} g/kg DM respectively for water and PEG treated forages. On the other hand total nitrogen content (g/kgDM) was increased for treated forages due to DM losses of 19.4^{c} to 20.7^{a} and 19.9^{b} for water and PEG treatments, respectively. Acid detergent insoluble nitrogen decreased from 6^{a} (unprocessed forage) to 2.1^{b} and 2.2^{b} g/kgDM for water and PEG treatments, respectively. Also, NDIN decreased from 6.3^{a} in untreated forage to 5.7^{b} and 5.1^{c} g/kgDM for water and PEG treatments, respectively and this contributed to increased available nitrogen content (38.8 and 32.1%). Efficient deactivation of tannins can is likely responsible for the results.

Key words: sainfoin, available nitrogen, tannin

M119 Effects of tannin deactivation with different chemicals on protein fractions of sainfoin (*Onobrychis viciifolia* Scop.) in Cor-

nell Net Carbohydrate and Protein System (CNCPS). H. Khalilvandi-Behroozyar*[1,2], M. Dehghan-Banadaky[1], and K. Rezayazdi[1], *[1]Department of Animal Science, University of Tehran, Karaj, Tehran, Iran, [2]Department of Animal Science, University of Urmia, Urmia, West Azerbaijan, Iran.*

Sainfoin (*Onobrychis viciifolia* Scop.) is tanniferous legume forage. Reports about sainfoin crude protein and condensed tannin contents have a wide range from 102 to 285 and 25.2 to 100 (g/kg of DM), respectively. Cornell Net Carbohydrate and Protein System (CNCPS) is a growing feed evaluation and ration balancing system in the world and create data about feed protein fractions is essential for accurate ration balancing for dairy cattle to improve cattle performance and reduction of environmental pollution due to nitrogen excretion from dairy industry. To our knowledge this is the first report on CNCPS protein fractions of sainfoin and effects of tannin deactivation on this measure. Sainfoin hay was treated with different chemicals to deactivate tannin. Treatment procedure and data about phenolic compounds content after treatment were presented in a companion abstract (M124). The CNCPS protein fractions of sainfoin, determined according to standardized procedure where the A fraction (non-protein N including ammonia, peptides and amino acids and considered to be completely soluble) was determined using trichloroacetic acid solution. Neutral detergent insoluble nitrogen and ADIN were determined as N content of residuals after neutral and acid detergent procedures, respectively. The B_2 fraction was calculated by difference, and results are presented as percentage of CP. All chemical analysis and forage treatments were done in triplicate. Data were analyzed by SAS 9.1, using GLM procedure for a completely randomized design ($P \leq 0.05$). Total phenolics, total tannins and condensed tannins content of sainfoin were 39.4 ± 0.6, 38.5 ± 1 and 21.3 ± 0.4 g/kg of DM respectively. Crude protein content was 121.3 ± 1.7g/kg of DM. Results (Table 1) showed that in untreated forage a large portion of crude protein was in the C fraction, which is unavailable the for animal. This can be due to condensed tannin- protein complexes. Tannin deactivation treatments improved nutritional availability of protein by decreasing the C fraction and increasing the A and B3 fractions.

Table 1. Effects of tannin deactivation on CNCPS protein fractions of sainfoin. (CP percentage)

	A	B_1	B_2	B_3	C
Control	16.8 ^{c}	9.7 ^{b}	28.6 ^{c}	13.9 ^{d}	30.9 ^{a}
Water	34.2 ^{ab}	1.3 ^{e}	37.2 ^{b}	17.1 ^{b}	10.2 ^{d}
Urea	17.1 ^{c}	13.4 ^{a}	40.9 ^{d}	7.5 ^{c}	21.1 ^{b}
$KMnO_4$	30.1 ^{b}	9.7 ^{b}	27.7 ^{c}	17.3 ^{b}	15.2 ^{c}
PEG	16.6 ^{c}	6.7 ^{c}	52.4 ^{a}	13.6 ^{d}	10.6 ^{d}
Wood ash	36.4 ^{a}	1.3 ^{e}	34.1 ^{bc}	16.6 ^{b}	11.6 ^{d}
NaoH	11.1 ^{d}	7.5 ^{c}	36.5 ^{b}	24.1 ^{a}	20.9 ^{b}
$NaHCo_3$	35.5 ^{a}	4.4 ^{d}	28.1 ^{c}	16.8 ^{bc}	15.2 ^{c}
S.E.M	1.53	0.45	3.11	0.95	1.05

$^{a–e}$Means within each column with different superscript letters are statistically different ($P \leq 0.05$).

Key words: sainfoin, condensed tannin, CNCPS

M120 Effects of chemical treatments for tannin deactivation on in situ organic matter degradability of sainfoin (*Onobrychis vicifolia*). H. Khalilvandi-Behroozyar*[1,2], K. Rezayazdi[1], and M. Dehghan-Banadaky[1], *[1]Department of Animal Science, University of Tehran, Karaj, Tehran, Iran, [2]Department of Animal Science, University of Urmia, Urmia, West Azerbaijan, Iran.*

Sainfoin is a temperate legume forage, with medium to high concentrations of condensed tannins (CT). The objective of present study was to examine the effectiveness of tannin deactivation on ruminal organic matter (OM) degradability of sainfoin. Forages (second cut) were chopped and treated without (control) or with solutions of NaOH (0.05 *M*) and wood ash (180g/l) with forage to solution ratio of 1:4. Also, 5% solution of PEG (6000 MW) was sprayed on the forages (1:1 w/v). The pH of solutions were determined (12.28 and 12.13 for NaOH and wood ash, respectively). condensed tannin contents were determined using butanol-HCl reagent. Organic matter degradability was determined using three ruminally fistulated Holstein cows, fed balanced rations with forage:concentrate ratio of 60:40. samples were ground to pass 2 mm screen and 5 g was weighed into nylon bags with 50 micron pore size (sample size:surface area was 12.5 mg/cm^2). Duplicates were incubated for 4,8,12,24,48,72 and 96 in the ventral rumen. Effective degradability (ED) was calculated with NEWAY computer package. Complete randomized block design (animals as block), GLM PROC of SAS 9.1 and Duncan's Test were used for data analysis ($P \leq 0.05$). The CT concentration of the control forage was 21.3+0.4 g/kg dry matter. PEG caused a marked increase in the soluble fraction, but not in the "b" fraction. NaoH slightly increased the "a" fraction but wood ash decreased it compared with control. Only wood ash increased the rate of degradation of the "b" fraction, although this was not statistically significant. Effective degradability (K=0.05) were 54.83^{b} + 1.91, 54.33^{b} + 3.40, 50.30^{c} + 0.56 and 62.70^{a} +0.61 for control, NaoH, wood ash and PEG treatments, respectively.

Key words: sainfoin, organic matter, tannin

M121 Chemical compositions and anti-nutritive factors of *Acacia mangium*. T. Clavero* and R. Razz, *Centro de Transferencia de Tecnologia en Pastos y Forrajes, Universidad del Zulia, Maracaibo, Estado Zulia, Venezuela.*

A field experiment was carried out under tropical dry forest conditions in northwest Venezuela to determine the chemical compositions and anti-nutritive factors of *Acacia mangium*. The study included 3 defoliation frequencies (6, 9, 12 weeks). Treatments were replicated 3 times in a randomized block design. Measurements included total nitrogen (TN), in vitro dry matter digestibility (IVDMD), non-structural carbohydrates (TNC), Ca, Mg, P and anti-nutritional compounds (condensed tannins-CT-, saponins-S-, phenols, alkaloids-ALK-, steroids-ST-). Data were subjected to ANOVA, using general linear models procedures of SAS statistical package. Treatments means were contrasted using Tukey test. Several chemical changes of *A. mangium* were noted from 6 to 12 weeks of growth. At each interval TN content declined significantly ($P < 0.05$). The highest value of IVDMD (56.4% ± 2.1) was obtained on 6 weeks of growth and declined on 12.9 digestible units from 6 to 12 weeks. TNC did not differ ($P > 0.05$) among treatments. Concentrations of Ca, Mg, P were affected significantly ($P < 0.05$) by maturity. Mineral concentrations decrease due to translocation from leaves to stems with increasing maturity. The highest concentrations of CT, phenols and ALK (0.48, 2.91 and 0.80%) respectively, were observed at 6 weeks of growth and significantly affected ($P < 0.05$) by the stage of growth. In contrast, S and ST were not affected by stage of growth. Young leaves had higher content of secondary metabolites than older leaves. Because the levels of CT and phenols were under 4% in all treatments, no adverse effects in ruminants would be expected. The chemical compositions and anti-nutri-

tional values of *Acacia mangium* indicate that this specie has potential as feed source in tropical ruminant diets.

Key words: *Acacia mangium*, chemical compositions, anti-nutritional factors

M122 Nutrient composition, polyphenolic compound content, in situ degradation and in vitro rumen fermentation characteristics of leaves from three mulberry species. H. J. Yang* and W. X. Wang, *State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing, China.*

Mulberry leaves of 14 cultivars of *M. atropurpurea* Roxb. (MAR), 7 cultivars of *M. alba* Linn (MAL) and 8 cultivars of *M. multicaulis* Perr (MMP) were harvested in May and June in Guangzhou city of China and assessed for differences in nutritive value and potential for use as an alternative forage by chemical analysis, in situ ruminal degradation and in vitro batch culture. CP (N × 6.25), NDF, ADF and ether extract (EE) in the leaves ranged from 243 to 262, 260–289, 162–177 and 32–42 g/kg DM, respectively. Means and standard errors (s.e) for CP, NDF, ADF and EE in mulberry leaves were 253 ± 4.5, 275 ± 7.1, 169 ± 3.5 and 37 ± 2.5, respectively. No differences were found for CP or NDF. EE content was ranked: MMP > MAL > MAR ($P < 0.05$), with the highest mean (60 g/kg DM) of cultivar Tongxiangqing in MMP. Total phenol, total tannin and condensed tannin contents were lower than those reported for other tree leaves in literature and did not differ among mulberry species. In situ degradation rate (c) of either DM or CP in 3 cannulated lactating dairy cows, fitted to an exponential curve of the type: $Y = a + b \times (1- e^{-c \times time})$, was similar for mulberry species. However, differences were found in the effective degradation of DM (0.48 in MAR, 0.45 in MAL and 0.41 in MMP with a pooled s.e of 0.008; $P < 0.01$), CP (0.40 in MAR, 0.40 in MAL and 0.36 in MMP with a pooled s.e. of 0.012; $P = 0.05$) and in vitro DM disappearance ($P < 0.01$), and all of these parameters consistently were ranked: MAR > MAL > MMP. In vitro cumulative gas production (also fitted to the same exponential curve), ammonia N, volatile fatty acid characteristics showed no differences among mulberry species. Total phenolic and tannin content were positively correlated with the degradation rate of DM and CP ($P < 0.05$). MAR had the lowest total tannin content and was more degradable in the rumen than MAL and MMP. In general, mulberry leaves had a higher protein and lower fiber content. Consequently, mulberry leaves could be an acceptable and highly digestible rumen-by-pass protein feed supplement for use by ruminant animals on the occasions when silkworm production is not always profitable.

Key words: mulberry leaves, in situ degradation, cumulative gas production

M123 Fluoride content of leaves and stems of alfalfa hay at different stages of maturity. C. Arzola*[1], M. R. Murphy[2], J. Salinas[3], R. Copado[1], A. Corral[1], O. Ruiz[1], C. Rodriguez[1], E. Santellano[1], and H. Gaytan[1], *[1]Universidad Autonoma de Chihuahua, Chihuahua, Chihuahua, Mexico, [2]University of Illinois, Urbana-Champaign, [3]Universidad Autonoma de Tamaulipas, Cd. Victoria, Tamaulipas, Mexico.*

Fluoride (F) accumulation by forages can be of great significance for cattle nutritionists because high-contents of this element can cause dental and osseus lesions on cattle. Besides ingestion of fluoride by drinking water, alfalfa which is a very common forage for dairy cattle can be a source of fluoride. Because there is some concern about the F content of water in some areas of the central part of the state of Chihuahua in Mexico, it is believed that crops can accumulate F. To investigate the pattern and amount of fluoride deposition of 2 varieties of alfalfa ('Cuff-101' and 'Excellent multileaf') harvested on 2 seasons (summer and fall), F content was analyzed in leaves and stems of hay harvested over a range of maturity following an initial phenologic stage characterized by an average stem length of about 0.3 m, (but not visible buds, flowers, or seedpods) within the 2 seasons. Within each season, plots were clipped initially (d 0) and then additional sampling dates were scheduled at 5-d intervals for the next 20 d, resulting in a total of 5 clipping dates (0, 5, 10, 15, and 20 d). Data were analyzed as a split-plot experiment, the plots arranged factorially in a randomized complete block design, being the alfalfa varieties and season the main effects, and maturity the subplot term. The overall mean content of fluoride in the alfalfa hay (94.24 ppm as fed basis) was in general high as compared with typical fluoride concentration reported in tables. When comparing F values in hay samples of the whole plant, no differences ($P > 0.05$) were detected between varieties, season, or stage of maturity. In leaves there was a greater ($P < 0.05$) concentration of F in Cuff-101 variety (104.53 ± 9.97 ppm) than in Excellent multileaf (82.99 ± 10.28). In stems, Cut-101 also had a greater ($P < 0.05$) of F as compared with Excellent multileaf (97.66 ± 9.76 and 86.75 ± 9.60, respectively). A general conclusion was that F content was high regardless of the stage of maturity and harvesting season of hay. A more detailed study should be conducted to determine the levels of F in ingesta to provide information regarding the F status of cattle consuming alfalfa hay.

Key words: fluoride, alfalfa, *Medicago sativa*

M124 Distribution of antiherbivory compounds in *Flourensia cernua*. R. E. Estell*, E. L. Fredrickson, D. K. James, and D. M. Anderson, *USDA-ARS, Jornada Experimental Range, Las Cruces, NM.*

Flourensia cernua is used as a shrub model to study the influence of terpenes on intake by livestock at the Jornada. Two studies (20 plants per study) were conducted using a completely randomized design to examine within plant distribution of volatile compounds to improve sampling protocol. Leaves from 3 positions (outer canopy, subcanopy, and basal) were collected from 4 quadrants (northeast, southeast, northwest, southwest) in Exp. 1. Leaves were removed from 2 leaders of current year's growth for each position in each quadrant. In Exp. 2, 10 leaders of current year's growth were collected from the outer canopy in each quadrant and 3 leaf age categories were formed by separating leaders into thirds and removing leaves. Terpenes were extracted from 5 leaves in duplicate with ethanol and analyzed with gas chromatography/mass spectrometry. Data were log-transformed and analyzed by univariate ANOVA and means separated using Tukey's honestly significant difference. Ninety-three compounds (including 15 unknowns) were present on the leaf surface of *F. cernua*. Only 7 compounds in Exp. 1 and 9 in Exp. 2 differed ($P < 0.05$) among quadrants, and no consistent effect of quadrant was observed in either study. In Exp. 1, 31 compounds differed ($P < 0.05$) among leaf positions, but outer canopy and subcanopy leaves did not differ for any compound. Basal leaves contained greater concentrations than outer canopy and subcanopy leaves for 10 compounds, and less than the other 2 positions for 10 compounds. Thirty-two compounds differed for leaf age in Exp. 2, with immature leaves containing greater concentrations than mature leaves for 30 compounds and greater than the middle age category for 26 compounds ($P < 0.05$). Total concentration (cumulative concentration of all compounds) did not differ among leaf positions but differed for leaf age (greater in immature than mature leaves; $P <$

0.05). Terpenes that differed in Exp. 1 were represented about equally by mono- and sesquiterpenes, whereas compounds affected by leaf age were predominantly sesquiterpenes (3 of 32 were monoterpenes). Both leaf position and age affect terpene concentrations and sampling variability in this shrub.

Key words: terpenes, chemical distribution, leaf age

M125 Degradation kinetics of calcium caseinate incubated in vitro with increasing levels of tannin extract from *Acacia mearnsii* with or without polyethylene glycol addition. D. Zeni*, A. C. Fluck, G. V. Kozloski, A. A. Martins, F. Zanferari, and S. Stefanello, *Universidade Federal de Santa Maria, Santa Maria, RS, Brazil.*

The effects of tannin extract from *Acacia mearnsii* and polyethylene glycol (PEG) on in vitro gas production from calcium caseinate was evaluated in a study with a 3 × 2 factorial treatment arrangement. Approximately 1 g of calcium caseinate was incubated in triplicate for 48 h with 100 mL of buffered-ruminal fluid in 160 mL glass jars. Treatments were 0 (no tannin) or 20, 40 or 60 g tannin extract/kg of calcium caseinate with presence (+PEG) or absence (-PEG) of 2 g of PEG. Inoculum was obtained from a rumen fistulated steer receiving ad libitum fresh ryegrass (*Lolium multiflorum*). Pressure in the jars, which was converted into gas volume, was measured at 2, 3, 4, 5, 6, 9, 12, 18, 24, 36, and 48 h after incubation. Gas production data were fitted to an exponential unicompartmental model to estimate degradation kinetic parameters. ANOVA analysis was performed and when the treatment effect was significant ($P < 0.05$), linear and quadratic effects were tested by regression analysis. Gas volume increased at increased levels of tannin with addition of PEG ($P = 0.040$) and was not affected without PEG addition (Table 1). Degradation) rate linearly decreased at increasing levels of tannin addition without PEG ($P = 0.047$), but remained constant when PEG was added. Lag time was not affected by tannins without addition of PEG whereas it decreased linearly (P= 0.078) when PEG was added to the incubation media. In conclusion, the tannin extract did not negatively affect calcium caseinate fermentation in vitro.

Table 1.

Tannin	Gas volume (mL)		Degradation rate (%/h)		Lag time (h)	
(g/kg DM)	-PEG	+PEG	-PEG	+PEG	-PEG	+PEG
0	80.7*	57.0	2.83	3.71*	10.3	9.80
20	80.7*	63.0	3.87	3.75	6.73	12.5*
40	92.7*	75.3	2.59	3.90*	8.0	8.10
60	95.7*	63.7	2.82	3.12*	5.26	10.2*
SD	9.34	8.30	0.52	0.25	2.61	2.27
P-value[1]	0.040	0.186	0.486	0.047	0.078	0.650

[1]Linear tannin effect. *Effect of PEG by ANOVA ($P \leq 0.05$).

Key words: tannins, PEG, rumen degradable protein

M126 Degradation kinetics of cellulose incubated in vitro with increasing levels of tannin extract from *Acacia mearnsii* with or without polyethylene glycol addition. D. Zeni*, A. C. Fluck, G. V. Kozloski, A. A. Martins, F. Zanferari, and T. R. Longo, *Universidade Federal de Santa Maria, Santa Maria, RS, Brazil.*

The effects of tannin extract from *Acacia mearnsii* and polyethylene glycol (PEG) on in vitro gas production from cellulose was evaluated in a study with a 3 × 2 factorial treatment arrangement. Approximately 1 g of crystalline cellulose (AVICEL) was incubated in triplicate for 48 h with 100 mL of buffered-ruminal fluid in 160 mL glass jars. Treatments were 0 (no tannin) or 20, 40 or 60 g tannin extract/kg of cellulose DM in the presence (+PEG) or absence (-PEG) of 2 g of PEG. Inoculum was obtained from a rumen fistulated steer fed ad libitum fresh ryegrass (*Lolium multiflorum*). Pressure in the jars, which was converted into gas volume, was measured at 2, 3, 4, 5, 6, 9, 12, 18, 24, 36, and 48 h after incubation. Gas production data was fitted to an exponential unicompartmental model to estimate degradation kinetics parameters. ANOVA analysis was performed and when the treatment effect was significant ($P < 0.05$), linear and quadratic effects were tested by regression analysis. Gas volume was not affected by tannin extract with or without PEG addition (Table 1). However, PEG increased gas volume ($P < 0.05$) independently of tannin level. Gas production rate linearly decreased at increased levels of tannin extract, independently of PEG addition ($P < 0.05$). Lag time, in turn, increased linearly with tannin levels without PEG ($P < 0.05$) whereas it was not affected by tannins when PEG was added to the incubation media. Tannin extract negatively affected cellulose fermentation in vitro. However, this effect was not clearly overcome by PEG inclusion. This implies that the contribution of PEG alone to gas production should be accounted for when it is used as a tannin inhibitor in in vitro assays.

Table 1. Degradation kinetics from cellulose incubated in vitro with 20, 40, or 60 g/kg DM of tannin extract with or without PEG

Tannin	Gas volume (mL)		Degradation rate (%/h)		Lag time (h)	
(g/kg DM)	-PEG	+PEG	-PEG	+PEG	-PEG	+ PEG
0	221	229.3*	3.86*	3.54	5.97	5.45
20	225*	205.3	3.74*	3.49	5.43	6.76*
40	219*	210.6	3.32	4.01*	6.16*	5.31
60	222*	212.3	3.71*	3.65	5.2	5.6
SD	7.93	10.3	0.25	0.34	0.54	0.38
P-value[1]	0.821	0.332	0.036	0.024	0.849	0.016

[1]Linear tannin effect. *Effect of PEG by ANOVA ($P \leq 0.05$).

Key words: tannin, PEG, in vitro

M127 Nutrient and tannin contents of purple prairie clover (*Petalostemon purpureum*) harvested at different growth stages. L. Jin*[1,2], Z. Xu[1], A. D. Iwaasa[3], Y. G. Zhang[2], M. P. Schellenberg[3], T. A. McAllister[1], and Y. Wang[1], *[1]Agriculture and Agri-Food Canada, Lethbridge Reserach Centre, Lethbridge, AB, Canada, [2]Department of Animal Science, Northeast Agricultural University, China, [3]SPARC-AAFC, Swift Current, SK, Canada.*

The native legume purple prairie clover (PPC; *Petalostemon purpureum*) is well adapted to the prairie region of Canada and is considered an important palatable component of prairie hay. However, there is no information available on its nutritive value for ruminants. Whole plants of PPC were harvested from 5 regions of native pastures at Swift Current, SK, Canada at vegetative (VEG) and full-flowering/early seeding (FL) stages (4 samples for each maturity stage in each region). Proportions of leaf, stem and flower were determined. Whole plants were analyzed for organic matter (OM), N, neutral detergent fiber (NDF) and acid detergent fiber (ADF). Leaf, stem, flower and whole plant were also analyzed for total phenolics, total tannins and condensed tannin (CT). Plant had higher ($P < 0.001$) proportion of leaf, but lower ($P < 0.001$) proportion of flower at VEG than at FL stage. Contents of OM, NDF, ADF and their associated N were higher ($P < 0.01$), but

N content was lower (21.4 vs. 26.7 g/kg DM; $P < 0.01$) for FL than for VEG stage. Condensed tannin was detected in all tissues (i.e. leaf, stem and flower) of the plant; with flower containing the highest (198-213 g/kg DM) and stem containing the lowest (17-18 g/kg DM) levels. Contents of total phenolics, total tannins and total CT in leaf were all higher ($P < 0.01$) at the VEG than at the FL stage, whereas they were present at similar concentrations in stem and flowers at both growth stages. Whole plant harvested at the VEG contained lower (59 vs. 94 g/kg DM; $P < 0.001$) level of CT than that harvested at the FL stage, due to the increased proportion of flower at the FL stage. Overall, PPC is characterized by its high level of N content and a unique array of bioactive compounds and therefore may have considerable potential as a high-quality forage for beef cattle. Its yield and persistence need to be further evaluated.

Key words: purple prairie clover, nutrient, tannins

M128 Evaluation of tannins in indigenous forage plants of the Brazilian semi-arid. M. L. Chizzotti*[1,2], F. R. B. Oliveira[2], R. T. S. Rodrigues[2], K. C. Busato[2], T. S. Silva[2], J. A. Siqueira[2], and F. H. M. Chizzotti[1], *[1]Universidade Federal de Lavras, Lavras, MG, Brazil, [2]Universidade Federal do Vale do São Francisco, Petrolina, PE, Brazil.*

This experiment was conducted to evaluate the presence of tannins in indigenous plants of the Brazilian semiarid region Caatinga. Samples of seedling and adult leaves of fourteen species were used: *Albizia inundata*, *Amburana cearensis*, *Anadenanthera colubrina*, *Caesalpinia ferrea*, *Erythrina velutina*, *Handroanthus impetiginosus*, *Handroanthus spongiosus*, *Hymenaea martiana*, *Myracrodruon urundeuva*, *Pseudobombax marginatum*, *Spondias tuberose*, *Syagrus coronata*, *Tabebuia aurea* and *Ziziphus joazeiro*. The condensed tannin complexing agent, Polyethylene glycol (PEG), was used to evaluate the presence of tannins by semi- automated in vitro gas production and in vitro digestibility after 96 h. Five replicates were dried at 55° C and ground (1 mm) and 100 mg of samples were incubated with or without 200 mg of PEG. The fermentation kinetic parameters were fitted to the following exponential model: $Y = b \times (1 - e^{(-c \times (t - L))})$, where Y is the accumulated gas, mL/100g of incubated DM; b is the asymptote of gas production, mL/100g of incubated DM; c is the fractional rate of gas production, %/h ; t is the time, h; and L is the lag time, h, for t>L. The 95% confidence interval was used to compare coefficients. There was an effect of PEG ($P < 0.05$) on asymptote of gas production for the leaves of adult plants *A. colubrina*, *H. martiana*, and *M. urundeuva*, indicating the presence of tannins in these species. For the rate of gas production, there was an effect of PEG ($P < 0.05$) for seedling leaves of *A. colubrina*, *H. martiana*, *M. urundeuva*, and *P. marginatum*. The DM digestibility after 96 h of incubation was higher ($P < 0.05$) in the presence of PEG for the adult and seedlings leaves of *A. colubrina* (56.5% vs. 39.4% and 74.2% vs. 61.0%, respectively), for seedling leaves of *M. urundeuva* (72.4% vs. 59.0%), and for adult leaves of *C. ferrea* (51.5% vs. 47.4%) and *H. martiana* (47.7% vs. 29.3%). The leaves of species *Albizia inundata*, *Amburana cearensis*, *Erythrina velutina*, *Handroanthus impetiginosus*, *Handroanthus spongiosus*, *Spondias tuberose*, *Syagrus coronata*, *Tabebuia aurea* and *Ziziphus joazeiro* did not present a significant content of tannins. Funded by FACEPE, CRAD and CNPq.

Key words: anti-nutritional factors, Caatinga, polyethylene glycol

M129 Effect of grazing toxic tall fescue prior to or immediately following insemination on beef cattle reproductive performance. M. G. Burns*[1], J. G. Andrae[1], S. L. Pratt[1], W. C. Bridges[1], and F. N. Schrick[2], *[1]Clemson University, Clemson, SC, [2]University of Tennessee, Knoxville.*

The impact of toxic tall fescue (F) on beef cattle reproductive performance has been sporadically addressed, and few studies examine the effect that exposure timing has on conception rates. The objective of this study was to determine if F affects cattle reproduction differently when cattle are exposed before or immediately following insemination, to assess effects during gamete development or in altered uterine environment, respectively. Two and 3 yr old beef cows (99 hd total) were blocked by breed, BCS and age and allotted to groups (n = 50) grazing F-(>92% wild-type infected) or alternate forages (O; common bermudagrass and annual ryegrass) for 210 d before timed insemination. On d −8 all cows were subjected to a standard 5-d CIDR estrous synchronization program and were artificially inseminated on d0. Immediately following insemination, 25 cows from each group were switched to the alternate grazing treatment for the remainder of the trial, consistent with a 2 × 2 factorial arrangement. This resulted in 4 total forage treatment combinations when grazed pre and post breeding: fescue-fescue (FF n = 25), fescue-other (FO n = 25), other-fescue (OF n = 24), and other-other (OO, n = 25). Cows were visually checked for estrus behavior from d0 to d10 after which bulls were placed with cows for 60d. Blood was collected on d-18 and d-8 for P4 analysis to assess cyclicity. Blood was also collected on d-8 and d10 for prolactin concentrations. Pregnancy was determined using ultrasonography at d 130 and verified with calving records. Data were analyzed using PROC GLM of SAS. Cattle grazing tall fescue on d-8 had lower ($P < 0.05$) serum prolactin levels than cattle grazing O. There was a F x O interaction ($P < 0.05$) for serum prolactin on d10. Prolactin concentrations of FF and OF did not differ and were lower ($P < 0.01$) than both OO and FO groups. Prolactin levels of FO were higher ($P < 0.05$) than OO at d10. Pre AI treatment had no effect ($P > 0.05$) on final pregnancy rate. Cattle grazing F post AI had lower ($P < 0.01$) final pregnancy rates compared with O.

Key words: fescue, beef cattle, reproduction

M130 Endophyte-infected tall fescue seed extract induces constriction of bovine vasculature. A. P. Foote*[1], D. L. Harmon[1], K. R. Brown[2], J. R. Strickland[2], K. R. McLeod[1], L. P. Bush[1], and J. L. Klotz[2], *[1]University of Kentucky, Lexington, [2]USDA-ARS, FAPRU, Lexington, KY.*

Ergovaline (ERV) has been extensively used to study vasoactive effects of endophyte (*Neotyphodium coenophialum*) infected tall fescue (*Lolium arundinaceum*). However preliminary in vitro tests show that an extract of toxic tall fescue seed (E+EXT) is more potent than ERV alone indicating other compounds contribute to vasoconstriction. Thus, experiments were conducted to determine if vasoactivity of an E+EXT is different than a mixture of ergot alkaloids (ALK) of equal concentration and to determine if an endophyte-free extract (E-EXT) is vasoactive. Segments of lateral saphenous vein and right ruminal artery and vein were collected from steers (n = 6) shortly after slaughter. Vessels were cleaned of excess connective tissue and fat and sliced into segments that were suspended in a multi-myograph chamber with 5 mL of continually oxygenated Krebs-Henseleit buffer, equilibrated for 90 min, and exposed to a reference compound, 120 m*M* KCl for ruminal vessels and 0.1 m*M* norepinephrine for saphenous vein. Increasing concentrations of each treatment (E+EXT,

E-EXT, ALK, and ERV) were added to the respective chamber every 15 min following buffer replacement. Data were normalized as a % of maximal contractile response of the reference compound and analyzed as a CRD. For saphenous vein, ALK and E+EXT induced similar responses ($P = 0.19$ for $10^{-6}M$; $P = 0.28$ for $10^{-7}M$) that were greater than $10^{-6}M$ ERV ($P < 0.01$ and $P = 0.09$ respectively) and $10^{-7}M$ ERV ($P < 0.01$ and $P < 0.01$ respectively). The potency of ALK and E+EXT was greater than ERV ($P < 0.01$) in saphenous vein. For ruminal artery, ALK and E+EXT induced similar responses ($P = 0.31$ for 10^{-6}; $P = 0.06$ for 10^{-7}) that were greater than ERV at $10^{-6}M$ ($P < 0.01$ and $P < 0.01$ respectively) and $10^{-7}M$ ($P = 0.04$ and $P < 0.01$ respectively). For ruminal vein, ALK and E+EXT induced similar responses ($P = 0.13$) but E+EXT did not differ from ERV ($P = 0.61$). The E-EXT did not induce a contractile response in any vessel tested ($P > 0.1$). Although low in concentration, non-ergovaline alkaloids in E+EXT contribute to the observed contractile response and should be considered when studying fescue toxicosis.

Key words: tall fescue, vasoconstriction

M131 Contractile response of bovine lateral saphenous vein to ergovaline, serotonin$_{2A}$, α_{2A}-, and α_{2C}-adrenergic receptor agonists relative to time off endophyte-infected tall fescue. J. L. Klotz[1], G. E. Aiken[1], A. P. Foote*[2], L. P. Bush[2], K. R. Brown[1], B. M. Goff[2], and J. R. Strickland[1], *[1]USDA-ARS-FAPRU, Lexington, KY, [2]University of Kentucky, Lexington.*

Previous research has demonstrated differences in contractile responses to ergot alkaloids, serotonin (5HT), and adrenergic agonists by lateral saphenous veins collected from cattle that grazed either endophyte (*Neotyphodium coenophialum*)-infected or endophyte-free tall fescue (*Lolium arundinaceum*), possibly influenced by an altered vascular biogenic amine receptor profile. To aid in understanding of how cattle recover after exposure to toxic tall fescue, lateral saphenous vein biopsies were conducted on 21 predominantly Angus steers (357 ± 3 kg) at 0 (n = 6), 7 (n = 6), 14 (n = 5), and 28 d (n = 4) after removal from grazing KY31 tall fescue pasture (3.0 ha) for 126 d. Off-pasture, animals were housed in a dry lot and fed a corn silage and soybean hull mixed diet. Biopsied segments of vein were sliced into 2–3 mm cross-sections and suspended in myograph chambers containing 5 mL of oxygenated Krebs-Henseleit buffer (95% O_2/5% CO_2; pH = 7.4; 37°C). Veins were exposed to increasing concentrations (1×10^{-11} to $10^{-4}M$) of ergovaline (ERV), TCB2 ($5HT_{2A}$ agonist), guanfacine HCl (GF; α_{2A}-adrenergic agonist), and (R)-(+)-m-nitrobiphenyline oxalate (NBP; α_{2C}-adrenergic agonist). Data were normalized to a reference addition of $1 \times 10^{-4}M$ norepinephrine and analyzed as a CRD for main effects of days off pasture, agonist concentration, and d off pasture × concentration with steer as experimental unit using mixed models of SAS. Agonist concentration and day (d 7 and 14 were greater than d 0 and 28) were significant ($P < 0.01$), but days off pasture × concentration was not significant ($P > 0.1$) for all 4 compounds tested. Ergovaline and TCB2 had the greatest maximal responses and 2 adrenergic agonists elicited relatively smaller responses. Response to increasing concentrations GF was the least and varied most across days. The α_{2C} agonist NBP, was the more vasoactive adrenergic agonist. The effect of animal recovery during the 28-d off of tall fescue did not affect the relative response through changes in the biogenic amine receptor profile as evaluated pharmacologically in the bovine saphenous vein.

Key words: Bovine, ergovaline, tall fescue

M132 Differences in chemical composition of crown rust resistant and susceptible oat cultivars in Northern Mexico. H. Bernal-Barragán*[1,4], M. A. Cerrillo-Soto[2,4], A. S. Juárez-Reyes[2,4], F. G. Ríos-Rincón[3,4], E. Gutiérrez-Ornelas[1,4], M. Guerrero-Cervantes[2,4], N. C. Vásquez-Aguilar[1], and J. E. Treviño-Ramírez[1], *[1]Facultad de Agronomía UANL, Escobedo, N.L., México, [2]Facultad de Medicina Veterinaria y Zootecnia UJED, Durango, Dgo., México, [3]Facultad de Medicina Veterinaria y Zootecnia UAS, Culiacán, Sin., México, [4]Red Internacional de Nutrición y Alimentación en Rumiantes, México.*

Forage nutritive value of 2 groups of oat (*Avena sativa* L.) cultivars (about 120 days growing season) bred in Mexico were determined and related to their differences in the susceptibility to crown rust disease caused by *Puccinia coronata*. Three crown rust resistant oat cultivars (CRR= code cultivars L112, L124 and L164), released in 2007 from the Agronomy Department at the University of Nuevo Leon (UANL) in Mexico, and two commercial crown rust susceptible oat cultivars (CRS=Guelatao and Chihuahua), were sown in December 2007. No crown rust disease was present in any of the plots during this experiment. Three random whole plant samples were collected from small plots at 101, 110 and 117 days of growth. Samples were oven dried at 55° C, and ground to pass through 1-mm screen. dry matter, ash, CP, fat, NDF, ADF, and ADL, were determined and expressed on a DM basis and contents of cellulose, and hemicellulose were calculated. A 2 × 3 factorial arrangement with two groups of oat cultivars (resistant and susceptible), and three cutting stages (101, 110, and 117 days), was used in a complete randomized design, with three replicates per cultivar. There were no significant interactions between the main factors. Ash content was lower ($P < 0.05$) in CRS (12.1%) than in CRR (13.1%). Differences in CP ($P < 0.05$) were found between 101 (11.1%) and 117 days (8.5%). Fat content was similar ($P > 0.05$) between groups of oat cultivars; however fat content at 117 days (3.5%) was higher than at 101 and 110 days (2.9% in average). Crown rust resistant cultivars had higher ($P < 0.05$) NDF (56.6%) than CRS (54.6%). There were no differences ($P > 0.05$) in ADF (31.0%), hemicellulose (24.6%) and cellulose (26.4%) between CRR and CRS; however cellulose content at 101 days (27.7%) was higher ($P < 0.05$) than at 117 days (24.8%). There were small ($P = 0.051$) differences in lignin content between CRR (4.8%) and CRS (4.3%) oat cultivars, and between cutting at 101 (4.2%) and 117 days (4.9%). In conclusion, differences in ash, NDF and lignin contents were detected between crown rust resistant and susceptible oat varieties cultivated in northeastern Mexico.

Key words: *Avena sativa*, nutritive value, crown rust resistance