COMPARISON OF THE IN VITRO FERMENTATION CHARACTERISTICS OF FRACTIONATED ALFALFA AND SAINFOIN

L.R. McMahon[1] , M. Goto[2] , T.A. McAllister[1], , G.A. Jones[3] and K.-J. Cheng[1]

[1] Agriculture and Agri-Food Canada Research Centre, PO Box 3000, Lethbridge, Alberta, Canada T1J 4B1

[2] Faculty of Bioresources, Mie University, Kamihama-cho, Tsu 514, Japan

[3] College of Agriculture, University of Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan, S7N 5A8

ABSTRACT

Alfalfa and sainfoin leaves were lyophilized and ground (A, S, respectively) or were fractionated into soluble (A_{SOL}, S_{SOL}) and insoluble (A_{INS}, S_{INS})components and lyophilized and incubated in vitro with diluted ruminal fluid for 24h. Whole leaf and soluble fractions were also incubated with polyethylene glycol 8000 (PEG). Gas production (GP), ammonia concentration [NH_3] and volatile fatty acid (VFA) production were lower ($P < 0.05$) from S and S_{SOL} than from A and A_{SOL} Insoluble fractions differed only in [NH_3] ($S_{INS} < A_{INS}$, $P < 0.05$). Inclusion of PEG increased ($P < 0.05$) GP, [NH_3] and VFA production from whole leaf and soluble fractions of sainfoin, but not alfalfa. Inactivating the condensed tannins in sainfoin with PEG overcame differences in degradability between these two forages.

KEYWORDS

Alfalfa, sainfoin, condensed tannin, rumen, fermentation

INTRODUCTION

Condensed tannins (CT) have been postulated (Kendall, 1966) to be responsible for the bloat-safety of some forage legumes (e.g., sainfoin (*Onobrychis viciifolia Scop.*) relative to alfalfa (Medicago sativa L.). These polymers bind soluble protein in the rumen and prevent formation of the stable foam associated with pasture bloat. However, these two forages differ in digestibility (55% vs 95% DMD from leaves of sainfoin and alfalfa, respectively, after 24 h ruminal incubation, Howarth et al., 1982) and in tissue strength and resistance to cell disruption (sainfoin > alfalfa, Lees et al., 1981). This study was conducted to determine the effect of CT on sainfoin degradation exclusive of the influences of leaf structure.

MATERIALS AND METHODS

Leaves from hand cut alfalfa (*Medicago sativa* cv Beaver, second cut, pre-bloom) and sainfoin (*Onobrychis viciifolia* cv Nova, first cut, early-bloom) were stripped from stems and frozen at -40°C.

Whole leaves: Frozen leaves were lyophilized (Freezemobile 6, Virtis Co. Inc., Gardiner, NY) and ground to pass a 1-mm sieve. These samples were designated A and S for alfalfa and sainfoin, respectively.

Fractionation of forage: Fractionation was conducted on ice with pre-chilled reagents. Frozen leaves were blended with 150 mL distilled water (Waring Blendor, high speed, 3 X 30 s (alfalfa) or 6 X 30 s (sainfoin) and filtered through 20-µm monofilament nylon mesh. Residues retained by the mesh were blended (both legumes 1 x 30 s) with 100 mL of methanol, re-filtered, then washed five times with acetone (5 min, 100 mL each). Insoluble residues of alfalfa and sainfoin were frozen at -40°C (AINS and SINS, respectively). SOLvents from methanol and acetone fractions were removed by rotary evaporation (Büchi Rotavator, Rinco Instruments Co. Inc., Greenville, IL) at 40°C, then the three extracts of each forage were combined and lyophilized. Soluble fractions of alfalfa and sainfoin were designated A_{SOL} and S_{SOL}, respectively.

In vitro incubations: Prior to the morning feeding, ruminal fluid was obtained from a cannulated Jersey steer fed a 70:30 (DM basis) concentrate:alfalfa silage diet, strained through four layers of cheesecloth into a CO_2-flushed vessel and incubated at 39°C for 30 min. Foam and floating particulates were removed and the liquid phase was used for incubations. Artificial medium (Scott and Dehority, 1965) was prepared without glucose or resazurin (for A_{INS} and S_{INS} incubations) and with casein also omitted (for A, S, A_{SOL} and S_{SOL} incubations).

Fraction samples (200 mg of A, S, A_{SOL} and S_{SOL}; 100 mg of A_{INS} and S_{INS}) were weighed into fifteen replicate 100-mL serum vials. Second sets of whole leaf and soluble fraction vials were prepared, to which 40 mg of polyethylene glycol 8000 (PEG) were added (yielding samples A_{PEG}, S_{PEG}, $A_{SOL-PEG}$ and $S_{SOL-PEG}$, respectively). After addition of ruminal fluid (2 mL) and artificial medium (18 mL), all vials were incubated (39°C, 70 rpm) in an orbital shaker (Lab-Line Instruments, Inc., Melrose Park, IL). Gas production was determined by water displacement (Fedorak and Hrwdey, 1983). After 8, 12 and 16 h (for soluble fraction samples) and 12, 16 and 24 h (for all others) of incubation, the contents of quintuplicate vials were centrifuged (200 x *g*, 7 min) and the supernatants stored at -40°C until analyzed for ammonia (Weatherburn, 1967) and volatile fatty acids (VFA, ZoBell et al., 1997). Duplicate vials of each sample type were processed without incubation, to provide background values. Total N in all substrates was determined in duplicate using a mass spectrometer nitrogen analyzer (NA1500, Carlo Erba Instruments, Rodano, MI, Italy) and used to calculate crude protein and to normalize ammonia accumulation values for substrate N. Data were analyzed using the General Linear Models procedure and differences among least squares means were determined using the PDIFF option of SAS (1989).

RESULTS

Crude protein contents of 41.9%, 43.1% and 29.7% and 36.5%, 29.1% and 32.8% were determined for A, A_{SOL}, A_{INS} and S, S_{SOL} and S_{INS}, respectively.

For whole leaves, gas production (GP) from sainfoin was consistently lower ($P < 0.05$) than from alfalfa (Table 1), and at 24 h GP from S was only 82% of that from A. Polyethylene glycol increased ($P < 0.05$) GP only from S, and at all time points, GP from S_{PEG} was similar ($P > 0.05$) to that from A and A_{PEG}. With and without PEG (A_{SOL} *vs* S_{SOL}; $A_{SOL-PEG}$ *vs* $S_{SOL-PEG}$), GP was consistently lower ($P < 0.05$) from sainfoin than from alfalfa soluble fraction. Beyond 8 h of incubation, PEG increased ($P < 0.05$) GP from A_{SOL} and S_{SOL}. At 16 h, GP from $S_{SOL-PEG}$ was similar ($P > 0.05$) to that from A_{SOL}. In contrast to whole leaf and soluble fractions, GP from A_{INS} and S_{INS} was generally similar ($P > 0.05$), although there was a transient lag (at 12 and 16 h) in production from A_{INS} relative to S_{INS}.

Ammonia concentration ([NH_3], normalized for substrate N) was lower ($P < 0.05$) with S than with A, and with S_{INS} than with A_{INS}, throughout the incubation. PEG increased ($P < 0.05$) [NH_3] with sainfoin whole leaves, but this effect was not observed with alfalfa. By 24 h, [NH_3] in S_{PEG} vials had exceeded the amounts measured in A vials. In S vials, [NH_3] decreased between 12 and 24 h, indicating that microbial uptake of NH_3 exceeded production. With the soluble fractions, [NH_3] was lower in S_{SOL} than in A_{SOL} only after 8 h. Including PEG increased ($P < 0.05$) [NH_3] in S_{SOL} vials but not in A_{SOL} vials. Total VFA production was lower (84%, $P < 0.05$), in S vials than in A vials (Table 2) and VFA production profiles from the two forage types differed. Millimolar percentages of acetate and butyrate were higher ($P < 0.05$) and propionate and isobutyrate lower ($P < 0.05$) with S than with A (data not shown). Production of VFA from A was unaffected ($P > 0.05$) by PEG, but propionate and isobutyrate production from S were increased ($P < 0.05$). Total VFA was also lower (78%, $P < 0.05$) from S_{SOL} than from A_{SOL} but the forage effect was not observed in the presence of PEG. Branched-chain fatty acid (BCFA, isobutyrate and isovalerate) and valerate production were lower ($P < 0.05$) from $S_{SOL-PEG}$ than from A_{SOL} or $A_{SOL-PEG}$. Forage type did not affect ($P > 0.05$) VFA production from insoluble fractions.

DISCUSSION

Including PEG in incubations of sainfoin resulted in degradability characteristics similar to alfalfa, both with ground leaves and with sainfoin fractionated to overcome leaf structure. These observations indicate that CT were largely responsible for depressing digestibility

in the sainfoin in this study. The VFA production profiles from whole leaves were consistent with inactivation of CT by PEG (Wang et al., 1994), in that BCFA concentrations with S_{PEG} were increased relative to S. The BCFA arise from deamination of branched-chain amino acids, which is inhibited by CT binding to the substrate protein. This effect was less obvious in the soluble fraction, which may indicate that the PEG included was insufficient to inactivate all of the CT concentrated in the soluble fraction. This study confirms a role of CT in determining overall digestibility of sainfoin.

REFERENCES

Fedorak, P.M. and S.E. Hrwdey. 1983. A simple apparatus for measuring gas production by methanogenic cultures in serum bottles. Environ. Technol. Lett. **4:** 425.

Howarth, R.E., B.P. Goplen, S.A. Brandt and K.-J. Cheng. 1982. Disruption of leaf tissues by rumen microorganisms: An approach to breeding bloat-safe forage legumes. Crop Sci. **22**: 564-568.

Kendall, W.A. 1966. Factors affecting foams with forage legumes. Crop Sci. **6**: 487-489.

Lees, G.L., R.E. Howarth, B.P. Goplen and A.C. Fesser. Mechanical disruption of leaf tissues and cells in some bloat-causing and bloat-safe forage legumes. Crop Sci. **21**: 444-448.

SAS Institute, Inc. 1989. SAS User's Guide: Statistics, Version 6 ed. SAS Institute, Inc., Cary, NC.

Scott, H.W. and B.A. Dehority. 1965. Vitamin requirements of several cellulolytic rumen bacteria. J. Bact. **89**: 1169-1175.

Wang, Y., G.C. Waghorn, T.N. Barry and I.D. Shelton. 1994. The effect of condensed tannins in Lotus corniculatus on plasma metabolism of methionine, cystine and inorganic sulphate by sheep. Br. J. Nutr. **72**: 923-935.

Weatherburn, M.W. 1967. Phenol-hypochlorite reaction for the determination of ammonia. Anal. Chem. **39**: 971-974.

ZoBell, D.R., R.D. Wiedmeier, T.A. McAllister, T. Entz, M. Goto and K.J. Cheng. 1997. In vitro fermentation and digestion characteristics of six cereal grains. Proc. West. Sect. Amer. Soc. Anim. Sci. **48**: (In press).

Table 1
Gas production and ammonia accumulations during in vitro incubations of alfalfa and sainfoin leaf materials with diluted ruminal fluid.

	Gas production (mL/vial)[z]			Ammonia accumulation (mg NH_3-N mg^{-1} N fed)					
Incubation time (h	4	8	12	16	24	8	12	16	24
Forage fractio[y]									
Whole leaf									
A	4.51*a*	12.56*a*	19.40*a*	23.48*a*	29.94*a*	na[x]	0.348*a*	0.409*ab*	0.526*b*
A_{PEG}	5.62*a*	13.91*a*	20.97*a*	23.83*a*	27.43*a*	na	0.307*bc*	0.369*b*	0.550*b*
S	2.41*b*	8.63*b*	15.88*b*	20.53*b*	24.42*b*	na	0.280*c*	0.237*c*	0.345*c*
S_{PEG}	4.76*a*	13.52*a*	20.29*a*	25.21*a*	28.83*a*	na	0.347*ab*	0.423*a*	0.604*a*
SEM[w]	0.58	0.58	0.58	0.68	0.97	-	0.014	0.014	0.014
Soluble									
A_{SOL}	3.40*a*	8.17*a*	12.50*b*	14.33*b*	na	0.293*b*	0.337*b*	0.393*a*	na
$A_{SOL-PEG}$	3.51*a*	8.73*a*	14.07*a*	17.75*a*	na	0.276*b*	0.315*bc*	0.387*a*	na
S_{SOL}	2.36*b*	5.73*b*	9.00*d*	11.55*c*	na	0.302*b*	0.297*c*	0.316*b*	na
$S_{SOL-PEG}$	2.46*b*	6.49*b*	10.92*c*	14.56*b*	na	0.345*a*	0.384*a*	0.415*a*	na
SEM	0.31	0.31	0.38	0.54	-	0.010	0.010	0.010	-
Insoluble									
A_{INS}	1.60	6.09	9.96*b*	11.96*b*	12.26	na	0.863*a*	0.907*a*	1.146*a*
S_{INS}	2.04	6.82	10.85*a*	13.07*a*	13.28	na	0.700*b*	0.821*b*	1.033*b*
SEM	0.29	0.29	0.29	0.35	0.50	-	0.026	0.026	0.026

[z]Whole leaf and soluble fractions contained 200 mg/vial; insoluble fraction contained 100 mg/vial.
[y]A, S, A_{SOL}, S_{SOL}, A_{INS} and S_{INS} indicate alfalfa and sainfoin; whole leaf, soluble fraction and insoluble fraction, respectively. PEG indicates inclusion of 40 mg polyethylene glycol 8000 per vial.
[x]na: not analyzed.
[w]SEM: standard error of the mean.
a-d: Values for a given fraction and time followed by different letters differ ($P < 0.05$).

Table 1
Volatile fatty acid production during in vitro incubations of alfalfa and sainfoin leaf materials with diluted runinal fluid.

		VF_Az (mM)						
Forage fraction[y]	Incubation (h)	A	P	B	IB	V	IV	Total
Whole leaf	24							
A		19.69	6.65_a	0.89	1.66_a	1.03	1.61	31.52_a
A_{PEG}		18.08	5.99_{ab}	0.87	1.55_{ab}	1.02	1.50	29.02_{ab}
S		18.17	4.80_c	0.99	0.58_c	0.50	1.53	26.56_b
S_{PEG}		18.49	5.81_b	0.99	1.33_b	0.94	1.66	29.22_{ab}
SEM[x]		0.59	0.24	0.07	0.10	0.56	0.29	1.11
Soluble	16							
A		17.20_a	5.39_a	0.63	1.00_a	0.44_b	1.07_a	25.73_a
A_{PEG}		15.98_b	5.44_a	0.65	1.12_a	0.52_a	1.13_a	24.85_{ab}
S		13.75_c	4.13_b	0.69	0.41_c	0.32_c	0.75_b	20.05_c
S_{PEG}		15.83_b	5.48_a	0.60	0.73_b	0.32_c	0.76_b	23.73_b
SEM		0.27	0.11	0.05	0.06	0.01	0.09	0.38
Insoluble	24							
A		14.20	3.00	0.67	1.25	0.99	1.11	21.22
S		14.79	3.10	0.80	1.31	0.96	1.30	22.26
SEM		0.48	0.13	0.05	0.06	0.04	0.08	0.58

[z] A:acetate; P:propionate; B:butyrate; IB:isobutyrate; V:valerate; IV:isovalerate.
yA, S, A_{SOL}, S_{SOL}, AINS and S_{INS} indicate alfalfa and sainfoin; whole leaf, soluble fraction and insoluble fraction, respectively. Whole leaf and soluble fractions contained 200 mg/vial; insoluble fraction contained 100 mg/vial. $_{PEG}$ indicates inclusion of 40 mg polyethylene glycol 8000 per vial.
[x]SEM: standard error of the mean.
a,b,c,: Values in a column for a given forage fraction followed by different letters differ ($P < 0.05$).