The effect of condensed tannins in sainfoin (*Onobrychis viciifolia*) and sulla (*Hedysarum coronarium*) on the digestion of amino acids in sheep

E.N. BERMINGHAM[1,2], K.J. HUTCHINSON[1,2], D.K. REVELL[3], I.M. BROOKES[2] AND W.C. MCNABB[1]

[1]Nutrition and Behaviour Group, AgResearch Limited, Palmerston North, New Zealand

ABSTRACT

Two experiments were conducted in order to compare the effect of condensed tannins (CT) in sainfoin (*Onobrychis viciifolia*) and sulla (*Hedysarum coronarium*) on the flux of amino acids (AA) through the gastrointestinal tract of sheep. In each experiment, one group of sheep (PEG group; CT inactive) received an intraruminal infusion of polyethylene glycol (PEG) to inactivate the CT present in the forages, while the other group received an intraruminal infusion of water (Tannin group; CT active). The CT in sainfoin (38.1 g/ kg DM) had no significant effect on apparent AA absorption from the small intestine. However, in the sulla-fed sheep, AA absorption in the small intestine was significantly increased by CT ($P<0.05$; CT = 64.4 g/ kg DM). In both forages a shift in AA digestion from the rumen to the small intestine was seen with increased abomasal fluxes of AA in the Tannin group of sainfoin- (92 versus 101 g/day in the PEG and Tannin group, respectively; $P<0.05$) and sulla-fed (65 versus 83 g/day; $P<0.001$) sheep. Differences in the digestion and absorption of AA in the two forages may be associated with differences in chemical structure of the CT.

Keywords: condensed tannins; amino acids; sheep; sulla; sainfoin

INTRODUCTION

Excessive production of ammonia indicates an inefficient use of dietary protein, which could otherwise be diverted to animal production (Lobley, 1992). Methods that can be used to decrease the amount of ammonia produced in the rumen of ruminants fed fresh forages, and thereby potentially increase animal production by increasing the amount of amino acids (AA) entering the small intestine (SI) for absorption include the feeding of condensed tannin-containing forages, such as *Lotus corniculatus* (Waghorn *et al*., 1987). Condensed tannins (CT) are naturally occurring secondary plant phenolic compounds located in plant vacuoles (Reid *et al*., 1974) that form stable complexes with protein at pH 3.5-7.0, reducing the digestion of dietary protein in the rumen. This increases the flow of dietary protein into the abomasum, where pH is less than 3.5, resulting in the dissociation of the CT-protein complex (Jones & Mangan, 1977) releasing AA for subsequent digestion and absorption in the SI (Waghorn *et al*., 1990).

Legumes containing CT currently used in New Zealand include *L. corniculatus* (*cv* Goldie) and *L. pedunculatus* (*cv* Maku). Due to differences in chemical structure and concentration of CT in *L. corniculatus* and *L. pedunculatus* these forages have different effects on animal production. *L. corniculatus* has a CT concentration in the range of 20-40 g/kg dry matter (DM; Barry, 1989) and when fed to sheep has many beneficial impacts on animal production including increasing overall production and efficiency of wool growth (Min *et al*., 1997). In contrast, *L. pedunculatus*, which has a higher CT concentration (75-90 g/kg DM) can have negative impacts on animal production (Barry & Duncan, 1984). The CT in *L. pedunculatus* has been shown to increase non-ammonia N (NAN) flow to the abomasum, however, the digestibility of AA in the SI is adversely affected. This has resulted in a small reduction in the apparent absorption of essential AA (EAA) from the SI of sheep fed *L. pedunculatus* (Waghorn *et al*., 1994).

Sainfoin (*Onobrychis viciifolia*) and sulla (*Hedysarum coronarium*) are both drought resistant legumes (Frame *et al*., 1998; Struffi *et al*., 1998) that contain CT. Feeding both sainfoin (Karnezos & Matches, 1991) and sulla (Terril *et al*., 1992a) has improved lamb production. However, very little is known about their effects on N digestion in the digestive tract. Therefore, it was the objective of this study to measure the flux of amino acids through the gastrointestinal tract (GIT) and the apparent absorption of AA from the SI in sheep fed fresh sainfoin and sulla.

MATERIALS AND METHODS

Experimental design

Two similar experiments were conducted where sheep were fed the CT-containing legumes, sainfoin (*Onobrychis viciifolia*; Experiment 1) and sulla (*Hedysarum coronarium*; Experiment 2). The sites of N digestion and the apparent absorption of AA from the SI were calculated using digesta fluxes determined by measuring natural plant alkanes in the feed offered and in abomasal and ileal digesta, and a continuous intraruminal infusion of either Cr-EDTA (Experiment 1) or Co-EDTA (Experiment 2) according to the method described by Binnerts *et al*. (1968).

In both experiments, half the sheep (PEG group; CT inactive) received a continuous intraruminal infusion of polyethylene glycol (PEG; molecular weight 3500; Union Carbide, Danbury, CT, USA) to render the CT inactive. The remaining sheep (Tannin group; CT active) were infused with water.

Animals and feed

Experiment 1: Eight castrated male Romney sheep, approximately 15 months old, with mean live weight of 33.4 (SEM 0.2) kg, were fitted with rumen fistulae (63 mm ID) and abomasal cannulae (100 mm ID) approximately 30 days prior to commencing the experiment. Four sheep received an intraruminal infusion of Co-EDTA (equivalent to 50 mg Co/d) and PEG (160 g/d) in 480ml of water. The Tannin group received a continuous infusion of Co-EDTA (in 480 g of water) only, throughout the experimental period. The sheep were offered fresh sainfoin (*Onobrychis*

[2]Institute of Food, Nutrition and Human Health, Massey University, Palmerston North
[3]Department of Animal Science, University of Adelaide, Adelaide, Australia

viciifolia; 1000 g DM/d; g/kg DM; N 41.0, Total AA 159.0, Essential AA 99.3, Non-essential AA 59.7, CT 38.1)

Experiment 2: Nine castrated male Romney sheep, approximately 12 months old, with mean live weight of 30.3 (SEM 1.6) kg, were fitted with rumen fistulae (63 mm ID) and abomasal cannulae (100 mm ID) approximately 14 days prior to commencing the experiment. Five sheep received an intraruminal infusion of Cr-EDTA (50 mg Cr/day) and PEG (120 g/d)) in 340 g water while the remaining four sheep received an intraruminal infusion of Cr-EDTA only, throughout the experimental period. The sheep were offered fresh sulla (*Hedysarum coronarium*; 1000 g DM/d; g/kg DM; N 32.0, Total AA 90.0; Essential AA 55.8; Non-essential AA 34.2; CT 64.4).

In both experiments the forages were harvested thrice weekly by a sickle bar mower at 10.00 hours from a vegetative stand, (approximately 400 mm tall for sainfoin, and 1000 mm tall for sulla) and stored at 4°C until fed by overhead continuous feeders.

Dry matter and N digestibility of both forages were measured for seven days according to the method described by Waghorn *et al*. (1994) on a PEG free basis. The site of dry matter and N digestion was calculated by measuring digesta flow at the abomasum for three days following the digestibility period and at the ileum using a digesta sample collected at slaughter. After digesta sampling was completed the sheep were euthanased by an intravenous overdose of sodium pentobarbitone (300 mg/ml) and ileal digesta sampled according to the method described by Waghorn *et al*. (1994).

Abomasal digesta were sampled according to the method described by Ulyatt & Egan (1979). Natural plant alkanes present in the sainfoin and sulla were measured in the feed offered and refused and in abomasal and ileal digesta samples. Cobalt and Cr concentrations in the infusate and abomasal and ileal digesta samples were also determined. Digesta flow through the GIT was determined based on the method described by Faichney (1975).

Chemical and other analyses

The N in feed, feed refusals, faeces and digesta samples were determined by automated analysis of ammonia following Kjeldahl digestion (Williams & Twine, 1967). The CT concentration in feed samples was determined as extractable, protein-bound and fibre-bound fractions using the butanol-HCl procedure outlined by Terrill *et al.* (1992b).

The alkanes in feed and digesta samples and their supernatants were determined by gas chromatography according to the method described by Mayes *et al.* (1986). Freeze-dried and ground whole digesta samples (0.5g) were digested in concentrated nitric acid at 40°C for 96 hours according to the method described by Grace (1983) for the determination of Co and Cr concentrations. Cobalt and Cr concentrations in these samples, their supernatants and infusates were determined by inductively coupled argon plasma spectrometry (ICAPS; Lee, 1983).

Amino acid hydrolysates were prepared from feeds and digesta samples by hydrolysing 50 mg of freeze-dried material in 7.5M HCl at 110°C for 22h as described by Waghorn *et al*. (1994).

Statistical analysis

Analysis of variance to test the differences between treatment means was carried out using GLM (general linear models) procedures (SAS, 1989) for both experiments.

RESULTS

The CT concentration in both Experiment 1 and 2 did not affect DM intake, N intake, nor total AA, EAA or NEAA intakes (Table 1).

Dry matter flow through the abomasum and ileum was not significantly different between the PEG and Tannin sheep in both experiments (Table 1). However, N flow through both the abomasum and ileum was significantly higher in the Tannin sheep in Experiment 1 and Experiment 2 (Table 1).

The major effect of the CT in both Experiments was to significantly decrease both rumen and total N digestibility (Table 1). Total DM digestibility was decreased by 8-11% in Experiment 1 and Experiment 2 ($P<0.05$) during the digestibility period.

The presence of CT in both experiments resulted in a

TABLE 1: Dry matter (DM), and nitrogen (N) intakes, flux and sites of digestion in sheep fed sainfoin (*Onobrychis viciifolia*) and sulla (*Hedysarum coronarium*) with or without an intraruminal infusion of polyethylene glycol (PEG).

	Sainfoin				Sulla			
	PEG Mean	Tannin Mean	SE	P	PEG Mean	Tannin Mean	SE	P
n	**4**	**4**		**5**	**4**			
Intake (g/day)								
DM	796	822	12.8	NS[1]	832	845	28.2	NS
N	32	32	0.2	NS	28	29	0.6	NS
Digestibility (proportion of intake)								
DM								
Rumen	0.36	0.37	0.031	NS	0.45	0.42	0.010	†
Total	0.76	0.68	0.017	**	0.69	0.58	0.015	**
N								
Rumen	0.37	0.14	0.032	**	0.24	0.10	0.032	*
Total	0.76	0.65	0.011	***	0.81	0.70	0.008	***
DM Flow (g DM/day)								
Abomasum	506	517	25.9	NS	458	490	22.4	NS
Ileum	198	305	31.3	*	321	282	22.9	NS
N Flow (g N/day)								
Abomasum	15	28	3.3	*	21	26	0.9	**
Ileum	8	12	1.2	†	6	8	0.5	*

[1.] NS= not significant; * P<0.05; ** P<0.01; *** P<0.001; † P<0.10%

TABLE 2: AA Intakes, flux and site of digestion in sheep fed sainfoin *(Onobrychis viciifolia)* and sulla *(Hedysarum coronarium*) with or without an intraruminal infusion of polyethylene glycol (PEG).

	Sainfoin				Sulla			
	PEG Mean	Tannin Mean	SE	P	PEG Mean	Tannin Mean	SE	P
n	4	4			5	4		
Intake (g/day)								
Total AA[1]	127	131	1.6	NS[2]	75	75	2.9	NS
EAA	80	82	1.0	NS	46	47	1.8	NS
NEAA	48	49	0.6	NS	29	29	1.1	NS
Abomasal AA Flux (g/day)								
Total AA	92	101	2.1	*	65	83	2.4	***
EAA	54	60	1.1	**	40	51	1.4	***
NEAA	39	41	1.1	NS	26	32	1.0	**
Ileal AA Flux (g/day)								
Total AA	37	41	6.0	NS	20	31	1.5	***
EAA	22	25	3.6	NS	12	19	1.0	***
NEAA	15	16	2.5	NS	8	12	0.5	***
Digestibility of AA in the small intestine								
Total AA	0.68	0.60	0.040	NS	0.69	0.63	0.020	*
EAA	0.66	0.59	0.045	NS	0.69	0.63	0.020	***
NEAA	0.70	0.62	0.035	NS	0.69	0.63	0.018	*
Apparent Absorption of AA from the small intestine (g/day)								
Total AA	63	60	3.8	NS	45	52	2.1	*
EAA	36	35	2.5	NS	27	32	1.3	*
NEAA	27	26	1.4	NS	18	20	0.9	†

[1] The total AA reported here did not include aspartic acid, proline, methionine, cysteine, or tryptophan
[2] NS= not significant; * P<0.05; ** P<0.01; *** P<0.001; † P<0.10%

significantly higher total AA and EAA flux to the abomasum (Table 2). However, only in Experiment 2 did CT increase the total AA and EAA flow at the ileum (P<0.001). The digestibility of AA in the small intestine did not differ between treatments in Experiment 1. This is in contrast to Experiment 2, which had significantly lower AA digestibility in the small intestine (Table 2).

Apparent absorption of total AA from the small intestine was changed by the presence of CT only in Experiment 2 (Table 2).

DISCUSSION

The principal aim of these experiments was to examine the effects of CT on the sites of AA digestion in the GIT of sheep fed fresh sainfoin (Experiment 1) and sulla (Experiment 2). The CT concentration in sainfoin was within the range that is usually considered beneficial for animal production (30-40 g/kg DM: Barry *et al*., 1986) while the sulla CT-concentration was in the CT range that is usually considered anti-nutritional (Barry & Duncan, 1984).

Polyethylene glycol preferentially binds with CT, preventing it from reacting with protein (Jones and Mangan, 1977). Therefore, comparing the Tannin group with the PEG group provides a means of elucidating the effects of the CT present in the forages. Active CT (i.e., not bound to PEG) in sainfoin and sulla decreased N digestibility in the rumen and whole GIT, which is consistent with other studies (Waghorn *et al*., 1987; Waghorn *et al*., 1994).

Abomasal flux of all AA was increased by the presence of active CT in sulla. However, only total and EAA flux increased in the sainfoin-fed sheep. These results are similar to studies with other forages. For example, sheep fed *L. corniculatus* had higher NEAA and EAA fluxes (96 and 69 g/day, respectively; Waghorn *et al*. 1987) than those receiving PEG, as with sheep fed *L. pedunculatus* (84 and 121 g/d in NEAA and EAA, respectively; Waghorn *et al*., 1994). Increased flux of AA from the abomasum indicates a decrease in rumen proteolysis (Waghorn *et al*., 1987).

Despite the increased flux of AA to the SI associated with the presence of active CT in the diet, there was a decrease in AA digestibility in the SI in both sainfoin- and sulla-fed sheep. A reduction in AA digestibility was also noted in *L. pedunculatus* (Waghorn *et al*., 1994), with both EAA and NEAA having a lower digestibility in the presence of active CT (0.66 and 0.59, respectively). The causes of the decrease in intestinal AA digestibility are not clear. However, two mechanisms may exist (Waghorn, 1996). Firstly, the tannin-protein complex may not be completed dissociated in the intestine, thus reducing the effectiveness of proteolytic enzymes. Secondly, CT may still be active in the small intestine and interfere with proteins involved in digestion. If CT remain biologically active in the intestinal environment, it is possible that they interfere with proteins involved in AA transport. However, it appears that such interactions, if they occur, are actually positive because the apparent absorption of AA from the SI was significantly increased in the sulla-fed sheep in the current study from 45 g/day to 52 g/day (PEG and Tannin sheep, respectively), and sheep fed *L. corniculatus* (Waghorn *et al*., 1987).

Structural differences have been observed in *L. corniculatus* (Foo *et al*., 1996) and *L. pedunculatus* (Foo *et al*., 1997), with these being the likely cause of the differences in AA digestibility in different CT-containing forages (McNabb *et al*., 1998; Aerts *et al*., 1999). From the current results it appears that the CT in sainfoin acts in a similar manner to the CT in *L. pedunculatus* despite the lower concentration of CT in this forage. In contrast, the CT in sulla acts in a similar manner to those in *L. corniculatus*, despite the higher concentrations in sulla.

In conclusion, the active CT in sainfoin resulted in a

small increase in appearance of AA in the SI. However, this was negated by a slight reduction in digestibility in the SI such that apparent absorption of AA was not affected by the presence of active CT. In sulla, the CT improved apparent absorption of AA in the SI, despite a reduction in the intestinal digestibility of AA because of a large increase in flux of AA to the SI. The differences in AA digestion and absorption in sainfoin- and sulla-fed sheep appear to be associated with different chemical characteristics of the CT in each forage. It is important that the structures of the CT in both these forages be determined in order to fully understand how they affect nutritional parameters.

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