Digestion of ^{14}C-labelled condensed tannins from *Desmodium intortum* in sheep and goats

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An experiment was conducted to investigate the metabolism of condensed tannin (CT) in sheep and goats offered a mixture of *Digitaria decumbens* (700 g/kg) and *Desmodium intortum* (300 g/kg) hay. Radioactive $^{14}CO_2$ was used to label CT in young growing desmodium plants, [^{14}C]CT was extracted, purified and infused intraruminally, and the metabolism of [^{14}C]CT was followed in the rumen and lower digestive tract of both species. Digestion of DM, organic matter (OM), cell-wall constituents (CWC), N and the efficiency of rumen microbial synthesis were determined using a continuous intraruminal infusion of ^{51}Cr EDTA, $YbCl_3$ and $Na_2{}^{35}SO_4$. The measurements taken for sheep and goats respectively were: intake, 21 and 30 g/kg$^{0.9}$ per d; digestibilities (g/g) of DM, 0·566 and 0·505; OM 0·578 and 0·508; neutral-detergent fibre, 0·584 and 0·532; and acid-detergent fibre, 0·535 and 0·435. None of these measurements was significantly different ($P > 0·05$) between animal species. There was an apparent net gain in lignin across the rumen and whole intestinal tract for both animal species (19 and 29% for sheep and goats respectively). There were no significant differences between sheep and goats ($P > 0·05$) detected for any measurements of N excretion and utilization. The overall efficiency of N digestion and utilization was also similar between species. The routes of CT metabolism were compared for both colorimetric estimates (butanol–HCl) of dietary CT (DCT) and the specific radioactivity of [^{14}C]CT in digesta (abomasum) and excreta (urine and faeces) of both sheep and goats. [^{14}C]CT showed total losses of 57 and 56% in sheep and goats respectively whilst losses of DCT of 71 and 70% were detected with butanol–HCl in sheep and goats respectively. The apparent losses of DCT across the rumen of sheep and goats were 12 and 9% whilst higher losses (49 and 42% for sheep and goats respectively) were observed for [^{14}C]CT. Losses of DCT in the lower intestinal tract accounted for 69 and 71% of the total CT leaving the abomasum. By comparison, only 40 and 35% of [^{14}C]CT was lost during intestinal passage in sheep and goats respectively. It was concluded that the infused free [^{14}C]CT interacted with DCT and entered the protein and fibre-bound DCT pools. The loss of DCT during passage through the intestines was considered to be a consequence of either absorption of free CT or the degradation products of CT. It was assumed that free CT arose in the lower gastrointestinal tract from protein-CT and fibre-CT dissociation to be digested and/or absorbed. The higher recoveries of [^{14}C]CT in faeces (32 and 35%) compared with DCT (27 and 26%) for sheep and goats respectively) were associated with the excretion of [^{14}C] degradation products or conjugates which were not reactive to butanol–HCl. It was concluded that both methods (butanol–HCl and labelling CT with ^{14}C) detected a substantial disappearance of CT (free, protein, and fibre-bound) during metabolism in the gastrointestinal tract in sheep and goats.

Condensed tannin: Tannin metabolism: Sheep: Goats

Condensed tannins (CT) or proanthocyanidins are polymers of flavan-3-ols or flavan-3,4-diols (MW range 1000–30000) which are composed of catechin and epicatechin monomeric units (Porter, 1989). CT are naturally-occurring plant compounds which combine with proteins and other plant polymers such as cellulose, hemicellulose and pectin to form stable complexes (Mangan, 1988). Low levels of CT appear to be beneficial to ruminants by promotion of N retention and also prevention of bloat in cattle (Jones & Mangan, 1977;

* For reprints.

Barry & Manley, 1984; Waghorn *et al.* 1987). Although many studies have shown that CT will protect protein from digestion in the rumen, less is known about ruminal metabolism and intestinal absorption of the CT polymers. Drying is known to decrease the CT content of the leguminous tree species *Calliandra calothyrsus* (22–30%) and, apparently, to completely remove CT from *Gliricidia sepium* (Ahn, 1990). CT metabolism and/or absorption in the rumen varies with plant species, with the gliricidia CT being completely digested, whilst only 45–67% of calliandra CT appear to be metabolized in the rumen. Although CT have been observed leaving the abomasum, none appeared in the faeces in these experiments, suggesting that CT are either absorbed or excreted in faeces in a form not detected by CT assay (vanillin–HCl or butanol–HCl). It was suggested that CT was firmly bound to carbohydrates and could not be extracted either with solvents or detergents, or that the microflora in the intestinal tract facilitated CT digestion and absorption (Ahn, 1990).

The metabolic fate of CT in the digestive tract may be best studied by tracing the routes of excretion of radioactively labelled CT. ^{14}C-labelled epicatechin and procyanidins have been used to study the metabolism of polyphenols during beer malting and brewing (Eastmond & Gardner, 1974; McGuinness *et al.* 1975; Laws *et al.* 1976). Radioactive [U-^{14}C]-(+)-catechin has been used also to study the routes of metabolism of the flavanol (+)-catechin in animals and humans (Shaw & Griffiths, 1980; Shaw *et al.* 1982). Recently, Terrill *et al.* (1994) reported an experiment in which the fate of CT during digestion was measured after infusion of [^{14}C]CT from *Lotus pedunculatus* into the abomasum of sheep. In the present study $^{14}CO_2$ was used to label the tannins of *Desmodium intortum*, which after extraction and purification as free tannins were infused into the rumen of sheep and goats to trace their metabolism in the intestinal tract.

MATERIALS AND METHODS

Labelling and isolation of desmodium condensed tannins

D. intortum cv. green leaf was grown in pots under controlled environmental conditions for approximately 9 weeks before being transferred to a plant growth chamber. All plants were then exposed to $^{14}CO_2$ for 48 h. After this period unfixed $^{14}CO_2$ was expelled to atmosphere, and all plants were harvested, oven dried at 65–70° (48 h) and ground to pass a 3·0 mm sieve. Some plants were freeze-dried (FD). Duplicate samples (approximately 200 mg) were extracted with 12 ml acetone (700 ml/l) and, after removal of acetone, this crude water-soluble tannin (CWST) fraction was further purified by extraction with diethyl ether and ethyl acetate which removed contaminating small-molecular-weight phenolic compounds, and this clean tannin fraction (CTF) was assayed for [^{14}C] activity. Although relatively high activity was found in CTF, further purification by gel-filtration chromatography was undertaken using Sephadex LH-20 (Pharmacia, Uppsala, Sweden) pre-swollen with methanol (500 ml/l). CTF samples (2 ml) were added to the column and washed with four bed volumes (64 ml, flow rate 1 ml/min) of 500 ml/l methanol. Acetone was used to elute the tannin fraction from the column, and the eluate was dried by rotary evaporation (40°) to remove acetone. After confirming that pure free CT had been successfully labelled with ^{14}C, a larger-scale extraction and purification was carried out using the same procedures. This technique has been described in more detail by Perez-Maldonado (1994).

Animals and their diets

Two 3-year-old castrated Border Leicester × Merino sheep, mean live weight 40·0 (SE 0·1) kg, and two 8-month-old castrated male cashmere goats, mean live weight 22·0 (SE 3·53) kg, were used as experimental animals. Each animal was fitted with permanent rumen and

abomasal cannulas and then adapted to a pangola grass (*Digitaria decumbens*) hay diet. All animals were drenched against internal parasites before being transferred to an air-conditioned animal house at the University of Queensland. All animals were held individually in metabolism cages and fed from automatic overhead feeders that dispensed feed hourly. A mixed legume and grass hay diet (300 g legume hay (*D. intortum*) + 700 g pangola grass hay/kg) was offered at a fixed rate (585 g DM) daily to each animal. Free access to water was provided to all animals which were weighed at the beginning and at the end of the experimental period.

Experimental plan and measurements taken

The experiment was conducted over 28 d. The first 11 d was an adaptation period followed by 7 d for N balance and feed digestibility determinations (days 12–19). The next 5 d (19–24) were allowed for the continuous infusion of desmodium [^{14}C]CT. The final 5 d (24–28) were allocated for digesta-flow studies during which ^{51}Cr-EDTA, $YbCl_3$ (solid phase marker) and ^{35}S were continuously infused intraruminally. Metabolism cages were equipped with sieve collectors that separated urine from faeces. Urine samples were collected in a plastic bucket (4 litres) which contained 30 ml glacial acetic acid. A funnel containing a wad of glasswool was placed through the bucket's lid to aid the collection of urine free from contamination. Faeces were collected in plastic bags attached to the sieve collector. Urine and faeces collected from each animal were weighed every 2 d and sub-sampled (10%), pooled and stored at −20° for later analysis.

[^{14}C]*condensed tannin and marker infusion studies*

Desmodium [^{14}C]CT (specific radioactivity 2·978 MBq/g tannin) was continuously infused (100 ml/d) into the rumen of each animal for 5 d (days 19–24) at a rate of 0·8–0·9 g CT/animal per d. During this period, total urine and faeces, blood (10 ml), rumen contents (200 g) and abomasal contents (200 g) were collected every 8 h and stored (−20°) for assay of radioactivity and CT content. From day 24 until day 28, digesta flow at the abomasum and microbial protein synthesis were determined from an intraruminal infusion (300 ml/d) of ^{51}Cr-EDTA (14·8 MBq/d per animal), $YbCl_3$ (100 mg Yb/kg DM intake) and $Na_2{}^{35}SO_4$ (5·55 MBq/d per animal). Samples of strained rumen fluid (3 ml) and abomasal digesta (300 g/animal) were collected at 8, 16, 24, 32, 40 and 48 h commencing on day 3 of the 5 d period. After the termination of the intraruminal infusion, samples of rumen contents were collected at 4, 8, 12, 20, 28, 36, 48, 60 and 72 h. Samples of rumen contents were withdrawn with syringes through a semi-rigid intraruminal probe covered with nylon mesh to exclude the larger feed particles. Individual abomasal samples from each animal were mixed and bulked over each 24 h collection, thereby providing two samples from each animal for analysis. The bulked samples of abomasal digesta were strained through double cheesecloth to obtain a filtrate, and both whole digesta (WD) and filtrate samples were assayed for radioactivity (^{51}Cr) and Yb from which digesta-flow rates were determined by the double marker technique of Faichney (1980). Before freeze-drying, a representative sample (80 ml) of WD was taken and mixed with 40 g/l Cab-osil (SiO_2; Fluka Chemie AG, Buchs, Switzerland) and assayed for ^{51}Cr-EDTA activity. At the same time, a sample of 100 ml WD was also separated for determination of Yb, and fractions used for the determinations of the specific activity of cysteine-^{35}S and for the analysis of all constituents (DM, organic matter (OM), N, neutral-detergent fibre (NDF), acid-detergent fibre (ADF), lignin, and free, protein-bound, and fibre-bound CT). The second portion of filtrate was subdivided into two parts (A and B). Filtrate A was used for ^{51}Cr and Yb determinations and the remaining fraction was freeze-dried and analysed as described for WD. Filtrate B was lightly centrifuged (500 ***g*** for 10 min) and the supernatant fraction was re-centrifuged at

20000 *g* for 20 min. This supernatant fraction was used for NH_3 determination and the microbial residue was freeze-dried and subsequently analysed for cysteine-^{35}S.

Analytical methods

The DM content of feed, feed refusals, faeces, and digesta was determined by drying the material at 70° for 48 h, followed by ash determination (4 h at 600°). Total N in feed, feed refusals, faecal samples, rumen, abomasal filtrate, WD and urine samples was analysed by the Kjeldahl method (Association of Official Agricultural Chemists 1960). ADF, NDF, and lignin fractions were determined in feed, feed refusal, faeces, and WD by the method of Goering & Van Soest (1970). Hemicellulose and cellulose contents were calculated from ash-free detergent-fibre fractions (hemicellulose = NDF OM − ADF OM, cellulose = ADF OM − lignin) (Minson, 1971). NH_3 concentrations in abomasal contents were determined by steam distillation followed by titration with 0·01 M-HCl. ^{51}Cr-EDTA activity was determined in a Hewlett Packard Gamma counter (model 5212, Packard Instrument Co., Downers Grove, IL, USA). Rumen fluid volume and outflow rate from the rumen were obtained from the disappearance of ^{51}Cr after the continuous infusion of ^{51}Cr-EDTA solution was stopped (Faichney 1975). The proportion of cysteine of microbial origin in WD, supernatant, and abomasum microbial fractions was determined by the method of Elliott & Armstrong (1982). ^{35}S activity was determined in a Hewlett Packard scintillation counter (Tri-carb, model 4430 Hewlett Packard, Palo Alto, CA, USA). $YbCl_3$ concentrations in abomasal samples (WD and supernatant fractions) were determined in an inductively coupled plasma emission spectrometer (ICP) after HNO_3–$HClO_4$ digestion.

Condensed tannin analysis

Free, protein-bound and fibre-bound CT fractions were determined in feed, feed refusals, abomasal digesta and faecal samples using a modification of the method of Terrill *et al.* (1992) as described by Perez-Maldonado & Norton (1996). The ^{14}C-labelled CT infusion was also analysed, and contained only free tannins. Urine samples were also analysed for total phenolics by the method of Singleton & Rossi (1965).

Measurement of [^{14}C]condensed tannin radioactivity

Samples (0·5 and 1·0 ml) of the [^{14}C]CT infusion solution were mixed with 9·0 ml emulsifier-safe LSC-cocktail (scintillation liquid) in a 20 ml vial and counted in a Hewlett Packard scintillation counter (Tri-Carb, model 4430). Abomasal and faecal samples were oven dried (60°), ground with a mortar and pestle and 300–400 mg pelletized in ashless tissue paper. Pellets were then oxidized (0·9–1·1 min) in a Packard Tricarb sample oxidizer (Model 306). Carbosorb II (9 ml) and Permafluor V (12 ml) (Packard Instruments Co.) were used as a $^{14}CO_2$ absorbant and scintillator respectively. The absorbing column was washed with deionized water between samples to remove any residual effects. A ^{14}C-standard (paper disc 5000 disintegrations/min (dpm), Amersham Radiochemicals, Amersham, Bucks.) was oxidized after every twenty samples. Specific activity in each sample was obtained by dividing dpm by dry weight (mg). Portions of urine (3 ml) from each animal at each time of collection were mixed with 10 ml scintillation solution (high safe III, Pharmacia, Uppsala, Sweden) and counted for 60 min (Hewlett Packard scintillation counter, Tri-Carb, model 4430).

Statistical methods and calculations

Calculations of liquid and solid digesta flow were performed using the double-marker method of Faichney (1980). The significance of differences between animal species was tested using a pooled variance *t* test (Steel & Torrie, 1980).

Table 1. *Chemical composition (g/kg DM) of pangola* (Digitaria decumbens) *and desmodium* (Desmodium intortum) *hay offered to sheep and goats as a diet*

Component	Pangola hay	Desmodium hay
Organic matter	932	924
Nitrogen	9·7	20·8
Neutral-detergent fibre	732	719
Acid-detergent fibre	442	630
Hemicellulose	297	90
Cellulose	386	430
Lignin	58	209
Condensed tannins*		
Free	ND	8·5
Protein-bound	ND	12·6
Fibre-bound	ND	12·7
Total condensed tannin	ND	33·8

ND, not detected.
* *Desmodium intortum* condensed tannin equivalent.

RESULTS

Chemical composition, intake and digestibility of the components of dietary dry matter

The chemical compositions of pangola grass and desmodium hay fed to sheep and goats are presented in Table 1. Mean values for intake and digestibilities of DM, OM, and cell-wall constituents of the diet given to sheep and goats are shown in Table 2. Sheep tended to have higher digestibilities of all dietary constituents than goats but none of these differences was significant. For both sheep and goats there was an apparent net gain of lignin during passage through the digestive tract.

Digestion of nitrogen and efficiency of microbial protein synthesis in the rumen

Table 3 presents mean values for N intake and N excretion of sheep and goats given the mixed pangola–desmodium-hay diet. There were no significant differences between species for any measurements of N excretion and utilization.

The daily flow, sites of digestion and fate of non-NH_3 nitrogen (NAN) in the digestive tract of sheep and goats fed on pangola grass–desmodium hay are shown in Table 4. Although there were significant ($P < 0{\cdot}05$) differences between sheep and goats for N intake and NAN flow to the small intestines, the amount (g) of NAN leaving the abomasum per g N intake was not different ($P > 0{\cdot}05$) between animal species. The efficiency of microbial N synthesis, expressed as g microbial N/kg OM apparently digested in the rumen (OMADR) and g microbial N/kg OM truly digested in the rumen (OMTDR), was similar for both animal species. Similar trends were observed between species for the efficiency with which N was absorbed from the small intestine and for the apparent degradabilities of feed NAN in the rumen.

Recovery of infused free [^{14}C]condensed tannin in the digestive tract of sheep and goats offered a mixture of pangola grass and desmodium hay as a diet

The recovery of [^{14}C] after continuous infusion of labelled [^{14}C]CT to sheep and goats fed on a mixed diet of pangola grass and desmodium hay is shown in Table 5. The amounts (kBq/d) of [^{14}C]CT infused into the rumen of sheep and goats were not significantly

Table 2. *Comparison of intake and digestibility of pangola grass* (Digitaria decumbens) *and desmodium* (Desmodium intortum) *hay offered to sheep and goats as a diet*†

(Mean values for two animals per group)

Measurement (g/d)	Sheep	Goats	SED	Significance of difference
DM				
Offered	585	585	—	—
Intake	585	473	16·8	*
Intake (g/kg$^{0·9}$ per d)	21	30	2·0	NS
Digestibility (g/g)	0·566	0·505	0·0747	NS
Organic matter				
Digestibility (g/g)	0·578	0·508	0·0841	NS
Proportion digested in rumen	0·715	0·825	0·1498	NS
Neutral-detergent fibre				
Digestibility (g/g)	0·584	0·532	0·0734	NS
Proportion digested in rumen	0·930	1·002	0·1198	NS
Acid-detergent fibre				
Digestibility (g/g)	0·535	0·434	0·1110	NS
Proportion digested in rumen	1·034	1·065	0·1327	NS
Lignin				
Digestibility (g/g)	−0·192	−0·290	0·1250	NS
Intake	60·5	46·9	0·83	*
Leaving abomasum	64·6	40·2	13·24	NS
In faeces	72·2	60·6	7·06	NS

* $P < 0·05$.

† For details of diets and procedures, see Table 1 and pp. 502–504.

Table 3. *The digestion and utilization of nitrogen by sheep and goats offered a mixed diet of pangola grass* (Digitaria decumbens) *and desmodium* (Desmodium intortum) *hay*†

(Mean values for two animals per group)

Measurement	Sheep	Goats	SED	Significance of difference
N intake (NI):				
g/d	7·62	6·46	0·153	*
g/kg$^{0·9}$ per d	0·28	0·40	0·046	NS
N in urine:				
g/d	1·41	1·05	0·461	NS
As a proportion of NI	0·184	0·162	0·0141	NS
Apparent digestibility N (g/g)	0·452	0·412	0·0920	NS
N retained:				
g/d	2·04	1·61	0·432	NS
mg/kg$^{0·9}$ per d	73·7	96·7	17·64	NS
As a proportion of NI	0·267	0·250	0·0723	NS
As a proportion of digested N	0·602	0·595	0·1162	NS

* $P < 0·05$.

† For details of diets and procedures, see Table 1 and pp. 502–504.

Table 4. *The sites of digestion and fate of non-ammonia nitrogen (NAN) in sheep and goats offered pangola grass* (Digitaria decumbens) *and desmodium* (Desmodium intortum) *hay as a diet*†

(Mean values for two animals per group)

Measurement	Sheep	Goats	SED	Significance of difference
Leaving abomasum (g/d)				
Total N	11·4	9·1	0·25	*
NAN	10·9	8·6	0·32	*
Microbial-N	7·1	5·0	0·68	NS
NAN leaving abomasum/N intake (g/g)	1·4	1·3	0·04	NS
Efficiency of microbial N synthesis				
g microbial-N/kg OMADR	32·1	27·7	1·38	NS
g microbial-N/kg OMTDR	23·9	21·4	0·78	NS
Post-rumen N digestion (g/g)	0·63	0·58	0·062	NS
Apparent degradability of feed-N in rumen‡	0·50	0·45	0·054	NS

OMADR, organic matter apparently digested in the rumen; OMTDR, organic matter truly digested in the rumen, assuming 1 g microbial-N = 10·714 g organic matter (Faichney & White, 1977).

* $P < 0·05$.

† For details of diets and procedures, See Table 1 and pp. 502–504.

‡ Calculated as (N intake − feed NAN entering small intestine)/N intake.

Table 5. *The recovery of ^{14}C from free [^{14}C]condensed tannin ([^{14}C]CT) infused into the rumen of sheep and goats offered a mixture of pangola grass* (Digitaria decumbens) *and desmodium* (Desmodium intortum) *hay as a diet*†

(Mean values for two animals per group)

Measurement	Sheep	Goats	SED	Significance of difference
[^{14}C]CT (kBq/d):				
Infused	26·0	23·7	0·71	NS
Leaving abomasum	13·4	13·8	2·12	NS
In urine	2·7	2·2	0·35	NS
In faeces	8·5	8·3	1·17	NS
Proportion of [^{14}C]CT infused:				
In urine	0·105	0·091	0·0141	NS
In faeces	0·328	0·351	0·0483	NS
Apparently absorbed	0·672	0·649	0·0140	NS
Proportion of [^{14}C]CT absorbed:				
Lost from rumen	0·723	0·633	0·1235	NS
Lost post-abomasally	0·277	0·367	0·1251	NS
[^{14}C] apparently retained:				
As a proportion of [^{14}C]CT infused	0·567	0·555	0·0631	NS
As a proportion of apparently absorbed	0·844	0·853	0·0340	NS

† For details of diets and procedures, see Table 1 and pp. 502–504.

Table 6. *The digestion of dietary condensed tannin (CT) and infused (free) [^{14}C]CT in sheep and goats offered a mixture of pangola grass* (Digitaria decumbens) *and desmodium* (Desmodium intortum) *hay as a diet*†

(Mean values for two animals per group)

Measurement (g/d)	Sheep	Goats	SED	Significance of difference
CT intake:				
Free infused [^{14}C]CT	0·85	0·8	0·02	NS
Free (dietary)	1·5	1·2	0·04	*
Total free	2·3	2·0	—	—
Protein-bound	2·2	1·8	0·06	*
Fibre-bound	2·2	1·8	0·01	*
Total CT intake	6·8	5·6	0·18	*
DM intake (%)	1·2	1·2	0·01	NS
CT in urine‡	0·15	0·23	0·045	NS
CT intake (%)	2·2	4·2	0·93	NS
CT in faeces:				
Free	0·01	0·03	0·012	NS
Protein-bound	1·2	0·87	0·050	*
Fibre-bound	0·61	0·58	0·124	NS
Total CT	1·8	1·5	0·093	NS
CT intake (%)	26·9	26·2	2·51	NS
CT (%) apparently digested:				
Free	99·6	98·8	0·01	NS
Protein-bound	45·6	51·8	0·03	*
Fibre-bound	72·5	67·5	0·08	NS
Total	73·1	73·8	1·41	NS
CT apparently retained:				
g/d	4·8	3·9	0·32	NS
CT intake (%)	70·9	69·6	3·43	NS
CT digested (%)	97·1	94·3	1·46	NS

* $P < 0·05$.
† For details of diets and procedures, see Table 1 and pp. 502–504.
‡ Determined as total phenolics.

different ($P > 0·05$), but goats had higher infusions in relation to body size. There were no significant differences between sheep and goats for the amounts of radioactive C recovered in urine and faeces or for the apparent absorption and retention of ^{14}C.

Digestion and apparent absorption or disappearance of free [^{14}C]condensed tannin and dietary condensed tannins in the digestive tract of sheep and goats

The intake and excretion of dietary CT (DCT) from sheep and goats infused with labelled free tannins and fed on the mixed pangola–desmodium hay diet is shown in Table 6. Sheep had significantly higher ($P < 0·05$) intakes of CT than goats, and also had significantly higher faecal excretions of protein-bound CT than goats. However the amounts of total DCT excreted in urine and faeces and the apparent absorption and digestion of the feed CT were not significantly different between animal species.

The amounts of DCT entering the small intestines of sheep and goats infused with [^{14}C]CT and fed with a CT-containing diet of pangola grass and desmodium hay are shown in Table 7. Both the proportion of CT intake lost from the rumen and the amount lost in the small intestine after leaving the abomasum were not statistically different among animal species ($P > 0·05$).

Table 7. *Dietary condensed tannin (CT) entering the small intestines of sheep and goats infused with [^{14}C]CT and offered pangola grass* (Digitaria decumbens) *and desmodium* (Desmodium intortum) *hay as a diet*†

(Mean values for two animals per group)

Leaving abomasum (g/d)	Sheep	Goats	SED	Significance of difference
Free	ND	ND	—	—
Protein-bound	3·93	3·43	0·421	NS
Fibre-bound	2·13	1·62	0·052	*
Total CT	5·98	5·05	0·357	NS
CT intake (%)	88·4	90·5	1·41	NS
CT intake lost in rumen (%):				
Free	100	100	—	NS
Protein-bound	−78·1	−92·0	23·60	NS
Fibre-bound	7·82	9·94	2·287	NS
Total	11·7	9·5	7·17	NS
Abomasum flow lost post-abomasally (%):				
Free	ND	ND	—	—
Protein-bound	69·3	74·8	2·38	NS
Fibre-bound	70·2	64·2	8·48	NS
Total CT	69·4	71·1	1·95	NS

ND, not detected.
* $P < 0·05$.
† For details of diets and procedures, see Table 1 and pp. 502–504.

DISCUSSION

Intake and digestion of organic matter and cell wall fractions

The diet used was formulated to represent the type of feed which might be selected by animals grazing tropical grass and legume pastures in which *D. intortum* raised the protein content of the diet to meet the minimum requirements (80 g crude protein/kg) for sheep and goats (Agricultural Research Council, 1984; National Research Council, 1985). The site and extent of digestion of OM and cell-wall components were similar to those recorded for sheep given similar forage-based diets (Standing Committee on Agriculture, 1990), with 72–83% of the OM digested being apparently digested in the rumen. In the present experiment, fibre digestion in the rumen appeared to be overestimated for NDF (93–100% of total) and for ADF (103–107% of total) fractions. An apparent net gain of lignin (Table 2) with passage through the digestive tract was also detected in sheep and goats (19 and 29% respectively) but the difference between species was not significant ($P > 0·05$). The net balance in lignin across the rumen (sheep +7%, goats −8%) was within the limits of accuracy of the techniques used to measure digesta flow. However, a substantial apparent increase in lignin flow occurred in the lower gut for both species (sheep 19%, goats 51%), representing net 'artifact' lignin gains of 12 and 14 g/d for sheep and goats respectively. Similar observations of lignin gain across the gut have been reported in experiments in which feeds of high CT content have been fed (Goodchild, 1989; Ahn, 1990). CT–protein complexes have been identified as crude lignin in faeces thereby producing this artifact lignin in faeces (Carre & Brillouet, 1986; Van Soest *et al.* 1987). Since direct colorimetric detection of CT in faeces by butanol–HCl was not possible, there is a need to develop methodologies to determine CT not only in faecal material but also in other biological matrices such as urine, blood, abomasum and rumen contents.

Digestion and utilization of nitrogenous constituents

In the present study there were no differences between sheep and goats in overall efficiency of N use. Sheep and goats appeared to utilize dietary N in the rumen similarly, with similar apparent degradabilities of dietary N (0·50 and 0·45) and similar efficiencies of microbial protein synthesis (142 and 141 g microbial protein/kg digestible OM intake). These values were comparable with those reported for sheep (130–146 g/kg digestible OM intake) given similar diets (Agricultural Research Council, 1984; Standing Committee on Agriculture, 1990).

The routes of condensed tannin loss in sheep and goats

Studies of the fate of CT from sorghum hay, *Leucaena leucocephala* (leucaena) and *Acacia aneura* (mulga) in the gastrointestinal tract of sheep have suggested substantial losses of CT (89, 92 and 88% for sorghum, leucaena and mulga respectively), of which 75–80% occurred in the post-rumen tract (Goodchild, 1989). Ahn (1990) calculated that between 68 and 60% of total CT (estimated by the vanillin–HCl method) disappeared in the rumen, but could find no tannin–protein complexes in the abomasum or CT in the faeces of sheep fed on *C. calothyrsus*. In this study it was concluded that CT was completely digested and/or absorbed in the post-rumen tract. Experiments with sheep fed on fresh and dried *C. calothyrsus* with and without PEG have been undertaken by Ahn (1990) to investigate further the fate of dietary CT. In this study free and bound (protein+fibre) CT were assayed, but again no CT were detected in abomasal and faecal samples. It was suggested from these studies that CT lost from the rumen (45 and 85% fresh and dried respectively) was possibly bound to plant carbohydrates during the transit through the digestive tract. Distel & Provenza (1991) have also reported difficulties in CT analysis in studies with goats. Despite the use of powerful solvents such acetic acid, SDS, acetone and ether, only 6% of ingested CT was recovered in faeces, and they recognized that the extraction of CT is a major problem for workers in this field.

Recently Terrill *et al.* (1994) have compared the absorption and/or metabolism by sheep of CT estimated colorimetrically (butanol–HCl) with values obtained from concurrent studies with ^{14}C-labelled CT. They found little [^{14}C]CT-carbon was absorbed from small intestine, whereas the colorimetric method showed a significant disappearance of CT from both the rumen and the small intestine.

The preceding discussion suggests that CT may be lost from the digestive tract by absorption as free CT, absorption as breakdown products or conversion to conjugates which lack the reactive CT 'ring' structure and are simply not chemically detected by butanol–HCl. In fact, estimation of bound CT in digesta by Terrill *et al.* (1992) used a SDS solution with a pH adjusted to 8·0 with HCl which may not be sufficiently alkaline to break tannin–protein complexes. Results from *in vitro* experiments in our laboratory have shown that solution pH must be greater than 10 for dissociation of tannin–protein complexes (Perez-Maldonado *et al.* 1995).

In the present study, CT from *D. intortum* were radioactively labelled with ^{14}C and infused into the rumen of sheep and goats in an attempt to trace the route of CT loss in the digestive tract of ruminants. The recovery of CT was then calculated by two methods, first the balance of DCT as estimated by colorimetric assay and second the balance of radioactivity following the infusion of [^{14}C]CT.

It was assumed that the fate of labelled [^{14}C]CT in the digestive tract of sheep and goats would be similar to that of DCT, and that the infused free [^{14}C]CT would undergo similar reactions to dietary free tannins. Radioactive [^{14}C]CT showed total losses (not recovered in urine or faeces) of 57 and 56% in sheep and goats respectively (Table 5). Comparable values for DCT loss were 73 and 74% for sheep and goats respectively (Table 6). Urinary

losses of radioactivity predicted that 9–11% of infused [^{14}C]CT was excreted in urine, whereas no DCT was found in urine. This suggests that [^{14}C]CT had been extensively degraded into radioactive fragments either during passage through the digestive tract or by metabolism in the body tissues. It was also found that only 33–35% radioactivity of infused [^{14}C]CT appeared in faeces, compared with some 26–27% of DCT intake which was found in faeces. This observation may be explained if the radioactivity appearing in faeces represents both intact radioactive CT and some degradation products which are completely or partially non-reactive with butanol–HCl. Alternatively the CT excreted in faeces may be more highly polymerized than those ingested, and may be less reactive per g CT (by butanol–HCl method), thus underestimating the amount of DCT excreted.

Metabolism of radioactive and dietary condensed tannins in the rumen

In the present study, only 12 and 10% of DCT ingested was apparently lost during passage through the rumen of sheep and goats respectively (Table 8), these values being similar to that found for sheep given desmodium diets (17%) in an earlier experiment (Perez-Maldonado & Norton, 1996). Much higher losses of radioactively labelled CT were observed in the same animals (49 and 42% for sheep and goats respectively; Table 7) and the reasons for this discrepancy are not clear.

Perez-Maldonado & Norton (1996) observed a net gain of 46% protein-bound CT across the rumen of sheep given a pangola grass and desmodium hay diet, confirming the hypothesis that tannin–protein complexes increase through the formation of complexes between free tannin and plant and endogenous protein in the rumen. In the present experiment the free dietary and [^{14}C]CT interacted with dietary protein and fibre, increasing the net protein-bound by 79 and 91%. At the same time there was a small reduction in fibre-bound CT across the rumen (3 and 10% for sheep and goats respectively). These results also indicate that intraruminal infusion of free [^{14}C]CT may have altered the proportions of free CT available for complex formation in the rumen, and for this reason much higher amounts of protein-bound CT were found than observed in a previous trial (Perez-Maldonado & Norton, 1996).

The [^{14}C]CT losses from the rumen (49–42%) may be explained by bacterial degradation which led to appearances of 9 and 11% in the urine of sheep and goats respectively (Table 5). The remaining losses of ^{14}C may be accounted by incorporation into methane, excretion as $^{14}CO_2$ and by accumulation of ^{14}C in microbial and animal tissues. There is evidence that simple phenolic compounds related to flavanoids ((+)-catechin) are extensively degraded by rat-caecal microflora (Shaw & Griffiths, 1980; Groenewoud & Hunt, 1986). However the mechanisms of higher-CT depolymerization and the enzymes involved in microbial metabolism are still unknown (Groenewoud & Hunt, 1986; Deschamps, 1989). In studies with rats and monkeys, Hackett (1986) found a large accumulation of these metabolites in the liver, kidney and other tissues before they were finally excreted. Another possible explanation for this apparent loss of [^{14}C]CT may be associated with analytical errors which resulted in either an overestimation of radioactivity loss or an underestimation of DCT loss.

Metabolism of condensed tannins in the lower digestive tract

The intestinal loss (absorption) of DCT in sheep and goats accounted for 69 and 71% of the total CT leaving the abomasum (Table 7), these values being similar to those found for sheep and goats fed on desmodium (700 g/kg) in a previous trial (Perez-Maldonado & Norton, 1996). Goodchild (1989) found that 75 and 88% of DCT of leucaena and mulga were apparently absorbed post-ruminally by sheep, and Ahn (1990) reported complete absorption of CT by sheep fed on *C. calothyrsus*. Recently, Terrill *et al.* (1994) found that

76% of DCT (*L. pedunculatus*) leaving the abomasum disappeared in the small intestine. CT loss during passage through the intestines may be due to absorption of free CT or the products of CT degradation. Since no free CT were found to enter the intestines from the abomasum, it is possible that free CT was released from tannin–protein and tannin–fibre complexes in the duodenum by the detergent action of bile salts. Conformational changes of the ring structure of CT may also occur during passage through the small intestine rendering CT products unreactive to butanol–HCl and therefore undetectable in intestinal contents; these products would therefore appear to have been absorbed. Plant substances and bile pigments may also produce high background values in digesta, which may interfere with the colorimetric (butanol–HCl) determination and lead to underestimation of DCT in faeces.

Terrill *et al.* (1994) found that little, if any, of the radioactive [^{14}C]CT was absorbed from the small intestine of sheep abomasally infused with [^{14}C]CT, but it may be seen from their data that a substantial amount of [^{14}C]CT was apparently lost between the caecum and faeces. In contrast, these workers estimated that 76% of DCT was lost during passage from abomasum to faeces, and explained this loss as a failure of the analytical technique (butanol–HCl) to estimate DCT which had changed its conformation during passage through the intestines. These workers could find no ^{14}C in blood and negligible amounts in urine and faeces, although the experimental period (400 min) may not have been long enough to detect the products of CT metabolism in these pools. In the present experiment, 40 and 35% of [^{14}C]CT was lost between the abomasum and faeces of sheep and goats respectively, and losses of DCT (69 and 71%) were similar to those found by Terrill *et al.* (1994). However ^{14}C from CT was found in urine, indicating that tannins were being absorbed and metabolized by both sheep and goats.

The results from both experiments suggest the following mechanisms of post-abomasal CT metabolism and absorption. CT enters the duodenum mainly in a bound form, and dissociation of these complexes is facilitated immediately by the detergent action of bile salts and later by pH (> 8) as digesta moves further down the intestines. The free CT released undergoes conformational change during intestinal passage, rendering the CT residues unreactive to butanol–HCl, and these residues of high molecular weight pass unabsorbed to the caecum. CT residues are then metabolized by microbial action in the caecum, with a reduction in molecular size and degradation of the flavanoid ring structure. The degradation products from this activity may then be absorbed from the caecum–large intestine, or be excreted directly in faeces.

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