THE CONDENSED TANNIN CONTENT OF *LOTUS PEDUNCULATUS* AND ITS RELATIONSHIP TO AMINO ACID SUPPLY AND VOLUNTARY INTAKE

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SUMMARY

The condensed tannin content of *Lotus pedunculatus* (cv Grasslands Maku) was 20-40 g/kg DM when grown under high soil fertility conditions and 80-90 g/kg DM when grown in acid soils of low fertility. In the range 0-90 g/kg DM some 9% of total condensed tannin could not be bound by mascerates of the plant and was designated free tannin; increments in total condensed tannin above 90 g/kg DM all appeared as free tannin.

Nitrogen flow at the duodenum of sheep fed fresh low (46 g/kg DM) and high (106 g/kg DM) tannin lotus were greater than predicted for diets of non-tannin containing fresh forages, with post-ruminal digestibility of NAN being respectively 71 and 67%. Ruminal digestion of RFC and hemicellulose was depressed at both condensed tannin concentrations.

Voluntary intake was less for high-tannin than for low-tannin lotus and spraying lotus with PEG to bind the condensed tannins increased ME intake 44%. Daily drenching with PEG increased the growth of lambs grazing areas oversown with lotus by 40 g/d, but had no effect upon the growth of lambs grazing areas oversown with clovers.

It was concluded that presence of condensed tannins increased amino acid supply of sheep fed lotus (29% ME), compared with sheep fed ryegrass or white clover (20% ME). However, high contents of condensed tannins also depressed lotus voluntary intake. Methods of overcoming intake restriction, and application of the results to tannin - containing forages grown in Australia are discussed.

INTRODUCTION

Grazing ruminants in New Zealand (NZ) consume fresh forages of high metabolizable energy (ME) content (11 MJ/kg DM) and total N content (35 g/kg DM) for most of the annual production cycle. The proteins in grazed forages, predominately perennial ryegrass/white clover mixtures, are markedly degraded in the rumen (70%) and duodenal protein flow is considerably less than protein intake (MacRae and Ulyatt 1974; Ulyatt *et al.* 1975). Abomasal protein infusion experiments showed that amino acid absorption from the small intestine (17% ME intake) was insufficient for maximum rates of protein deposition in growing lambs (17 kg)' fed fresh ryegrass/white clover *ad libitum* (Barry 1982) .A subsequent review (Barry 1983) concluded that amino acid absorption was likely to be below the requirements of lactating ewes and dairy cattle, and recommended nutritional evaluation of legumes containing condensed tannins as a means of increasing amino acid supply in ruminants fed fresh forages.

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CONDENSED TANNINS AND PROTEIN SOLUBILITY

Condensed tannins are polymers of the phenolic compounds delphinidin and cyanidin (MW 300). In temperate species, condensed tannins occur in the leaves and stems of sainfoin *(Onobrychis viciifolia,* MW 17,000-28,000) and in the leaves and stems of *Lotus* species (MW 6,800-7,100) (Jones et *al.* 1976). They occur in the flower petals only of *Trifolium* species, and have not been detected in either lucerne *(Medicago sativa)* or the common temperate grasses.

During disintegration of the plant material, such as chewing by animals, condensed tannins react by hydrogen bonding with proteins to form a complex. The condensed tannin:protein complex is stable and insoluble in the pH range 3.5-7.0, but is unstable and releases protein at pH <3.0 and at pH 8.0 (Jones and Mangan 1977). Theoretically, condensed tannins should be able to increase amino acid supply in ruminants fed fresh forages, as the range where the tannin:protein complex is insoluble in dudes rumen pH (5.5-7.0), and the pH where protein is released by the complex corresponds with pH in the abomasum and small intestine. Because of the precipitation of forage proteins at rumen pH, legumes containing condensed tannins are also bloat resistant (Jones et *al.* 1973).

Condensed tannins should not be confused with hydrolysable tannins, which are polymers of gallic or hexahydroxydiphenic acid with a carbohydrate (McLeod 1974). The pH v solubility of hydrolysable tannin:protein complexes differs from those of condensed tannins, and chemical methods that do not differentiate between the two forms of tannin will not yield reliable nutritional information. In N.Z., condensed tannins in forage plants are routinely determined by the vanillin-HCl procedure (Broadhurst and Jones 1978). Henceforth, the use of tannin in this paper refers to condensed tannins.

Polyethylene glycol (PEG; MW 4000) forms a soluble complex with condensed tannins (Jones and Mangan 1977), and this principle can be used to either prevent protein reacting with condensed tannin or to displace protein from a pre-formed tannin:protein complex.

LOTUS PEDUNCULATUS

Grasslands "Maku" lotus is a tetraploid selection of *Lotus pedunculatus* made to suit N.Z. conditions (Armstrong 1974). It can tolerate lower levels of soil fertility than white clover, and is being extensively used in South Island Hill Country development programmes on acid soils (pH 4.5-4.8) that are deficient in both available phosphate (P) and sulphur (S). Under conditions of minimal fertiliser input, Maku lotus has substantially out yielded white clover. Unless stated otherwise, the term lotus in this paper refers to Maku lotus.

Lotus has proved a poor competitor when grown at normal soil pH (5.8-6.5) with high levels of available soil P and S, and swards rapidly become infested with weed species, clover and grasses. In this paper reference is made to lotus grown in plots of high fertility soil only as an experimental tool. Such plots required extensive herbicide treatment to check competing species.

LOTUS CONDENSED TANNIN CONTENT

In high fertility soils, the condensed tannin content of lotus was 10-30 g/kg DM when grown in the North Island (John and Lancashire 1981) and 20-40 kg when grown in the South Island (Barry and Forss 1983). However, when grown in low fertility Hill Country soils under conditions of low fertiliser input (10 kg P + 10 kg S/ha/annum) we routinely record condensed tannin concentrations of 80-90 g/kg DM, with occasional values up to 120-140 g/kg DM when grown under very cold conditions. These levels can be reduced through fertiliser application (Table 1), with combinations of P and S being most effective. Condensed tannin content was negatively correlated with plant DM yield ($r = -0.94$; $P<0.001$), each 100 kg/ha increase in yield reducing the. tannin content by 6.7 g/kg DM (Barry & Forss 1983).

TABLE 1 Condensed tannin content of primary growth *Lotus pedunculatus* grown at a low soil fertility site as effected by fertiliser application

Fertiliser S (kg/annum)	0			50			SED
Fertiliser P (kg/annum)	0	10	40	0	10	40	
DM yield (kg/ha)	5	23	76	83	531	575	88.8
Condensed tannin (g/kg DM)	86	92	85	80	51	54	14.9
Total N (g/kg DM)	34.4	33.1	36.3	39.6	45.2	48.3	3.51
Total S (g/kg DM)	1.2	1.5	1.9	2.5	2.6	2.7	0.28
Total P (g/kg DM)	1.0	2.4	4.0	2.4	2.9	4.0	0.37

Barry and Forss (1983)

We have also developed an analytical method to measure the amount of tannin not bound by fine mascerates of lotus plants, and this has been designated "free" condensed tannin (Barry and Forss 1983). Relationships between free and total tannin contents in lotus (Fig. 1) have shown that over the range 0-90 g/kg DM of total tannin some 9% of this is in the free form. Increments in total tannin above 90 g/kg DM were all released in the free form, the tannin:protein complex apparently being saturated with condensed tannin. By definition, free tannin will not be bound by the plant after it is chewed by animals, and will therefore be released into the environment of the rumen. Free tannins are water soluble, have a high affinity for proteins, and are known to precipitate micro-organisms and digestive enzymes (McLeod 1974). A comprehensive evaluation programme is therefore being undertaken at Invermay to examine voluntary intake and digestion of lotus as affected by condensed tannin content. All experiments have been conducted using fresh lotus individually fed to sheep.

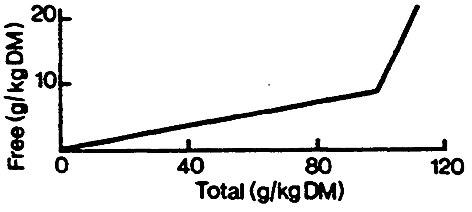

Fig. 1. Free condensed tannin concentration in *Lotus pedunculatus* as a function of total condensed tannin concentration.

VOLUNTARY INTAKE

Parry & Duncan (1983) generated low tannin (46 g/kg DM) and high tannin (106 g/kg DM) forms of lotus by growing the plant under high and low levels of soil fertility. Free tannin comprised respectively 3.0 and 14.2 g/kg DM. Voluntary ME intakes (MJ/kg liveweight (W) $^{0.75}$/d) were less for high tannin (0.77) than for low tannin (0.89) lotus.

As growing lotus under two extremes of soil fertility resulted in changes in concentration of a number of plant constituents in addition to tannin, an experiment was conducted where lotus was sprayed with PEG (MW 3350) before feeding to specifically bind condensed tannins. It was assumed that all the PEG ingested was quantitatively excreted in the faeces, and as condensed tannins bind to PEG in preference to protein (Jones & Mangan 1977) this effectively lowered the quantity of tannin available to react with plant constituents and enzymes in the rumen. Tannin which had not reacted with PEG, and could be measured by the vanillin-HCL procedure, was defined as total reactive condensed tannin.

When the total reactive condensed tannin content of lotus was reduced from 63 to 7 g/kg DM by spraying with PEG (Table 2), the apparent digestibility of structural carbohydrate (CHO) was increased 13% ($P<0.01$) and voluntary ME intake increased 44% ($P<0.05$) (Barry & Duncan 1983). This shows that condensed tannin content in the medium range was depressing voluntary energy intake, and could conceivably have depressed intake of the low as well as the high tannin lotus in the earlier comparison,

TABLE 2 Voluntary intake and apparent digestibility of lotus as affected by reducing the total reactive condensed tannin content through spraying with PEG (MW 3350)

	PEG addition		SED
	-	+	
Total condensed tannin (g/kg DM)	62.9	6.8	
Free condensed tannin (g/kg DM)	5.4	0.5	
Apparent) Cellulose (%)	52.7	57.9	2.62
Digestibility) Hemicellulose (%)	50.6	58.9	2.01
Voluntary) (MJ ME/kgW$^{0.75}$/d) intake)	0.48	0.69	0.097

Barry and Duncan (1983)

SITES OF DIGESTION

Sites of digestion of OM, CHO and nitrogen have also been determined in wether sheep fed the low tannin and high tannin lotus referred to earlier (Barry & Manley 1983). Dry matter fed was 750 g/d, given at hourly intervals. Faecal output was measured by direct collection, and duodenal flows estimated from intra-ruminal infusion of inert chromium EDTA and ruthenium phenanthroline markers (Faichney 1975).

Nett gains of N across the rumen (duodenal N flow - total N intake) were 1.8 and 10.5 g/d respectively for sheep fed low and high tannin lotus, versus predicted losses of 3.7 and 2.1 g/d for non-tannin containing fresh forage diets fed at the same level of N intake (Table 3). Despite dietary concentration and intake of total N being less for high tannin than for low tannin lotus, post-ruminal absorption of non-ammonia nitrogen (NAN) was similar for both diets. Apparent digestibility of NAN in the intestines was high for both diets, with the value for high tannin lotus (67%) being slightly but non-significantly lower than for low tannin lotus (71%) , and both values being only slightly less than predicted for non-tannin containing fresh forage diets (76%) MacRae & Ulyatt (1974). When expressed as NAN digested post-ruminally/g N eaten, the digestion of high tannin lotus was more efficient than that of low tannin lotus (P<0.01), but there was no difference between the two diets when calculated as g NAN digested post-ruminally/MJ ME consumed (2.7); Absorption of amino acids from the small intestine was calculated to be 29% of ME for both forms of lotus.

TART 3 Sites of nitrogen digestion in sheep fed lotus of low (46 g/kg DM) and high (106 g/kg DM) condensed tannin content.

	Low tannin lotus	High tannin lotus	SED
Diet N concentration (g/kg DM)	41.3	31.6	
Intake Total N (g/d)	28.7	24.8	0.19
Duodenum) Total N (g/d)	30.5 (25.0)†	35.3 (22.7)†	1.45
) NAN (g/d)	28.7	33.4	1.34
Faeces NAN (g/d)	8.3	11.0	0.72
Apparent digestibility (%)	71	56	2.70
NAN digested) (g/d)	20.4	22.5	1.52
Post-ruminally) (g/g N eaten)	0.71	0.91	0.053

Barry and Manley (1983)

† Calculated from equation of Ulyatt and Egan (1979) for non-tannin containing fresh forage diets fed at same level of N intake used by Barry and Manley (1983).

The proportion of cellulose totally digested that was digested in the rumen was simlar for both high tannin and low tannin lotus (87%), this value being as predicted from the equations of Ulyatt & Egan (1979) for non-tannin containing diets. However, less hemicellulose was digested in the rumen of sheep fed both the low tannin (61% total) and high tannin (38% total) farms of lotus than predicted (77% total). Likewise, ruminal digestion of readily fermentable CHO (RFC; water soluble CHO + pectin; 83% total for both diets) was less than calculated for fresh ryegrasses and white clover diets (99% total; MacRae & Ulyatt 1974). These findings imply that lotus condensed tannins were impairing ruminal CHO digestion, with hemicellulose digestion being most depressed.

GRAZING TRIALS

Liveweight gain can be considered as a function of both voluntary intake and nutritive value/DM eaten (Ulyatt 1973). As the presence of condensed tannins in lotus depressed both voluntary intake and ruminal digestion of RFC and hemicellulose, it seemed probable that high concentrations of condensed tannins could be restricting liveweight gain. To test this hypothesis, growing sheep grazed areas of tussock oversown with either lotus or a mixture of white clover and red clover in two experiments each of 6 weeks duration (T.N. Barry and W.L. Lowther, unpublished data). Experiment 1 was conducted over late spring/early summer using lotus primary growth, whilst Experiment 2 was conducted during autumn with secondary growth lotus and clover. Intitial weight of the sheep was respectively 36.0 and 25.5 kg in Experiments 1 and 2. Half of the sheep grazing each forage were drenched daily with PEG (100 g/d Expt 1; 75 g/d Expt 2) made up to 200 ml in water, whilst control sheep were drenched at the same times with 200 ml water.

Liveweight gains (Table 4) were understandably greater in sheep grazing primary than secondary growth lotus, and were increased by PEG supplementation in both experiments ($P<0.05$). Administration of PEG to lambs grazing areas oversown with clover produced no change in liveweight gain, the beneficial effects of PEG thus being specific to lambs grazing a legume of high condensed tannin content.

TABLE 4 Responses to daily drenches of PEG (MW 3350) in growing sheep grazing Hill Country tussock areas oversown with either Maku lotus or a mixture of white and red clover.

Experiment No.	Legume	Condensed tannin (g/kg DM)		Liveweight gain (g/d)		SED
		Total	Free	Water	PEG	
1	lotus	76	8	125	166	16.7
2	(lotus	89	7	27	70	9.2
	(clover	<1	ND†	28	26	9.2

† non-detectable

DISCUSSION AND CONCLUSIONS

Amino acid supply

These investigations have shown that condensed tannins in *Lotus pedunculatus* markedly increased duodenal N flows compared with predicted values for non-tannin containing fresh forages, and also allowed a high release of NAN in the intestines. In laboratory studies Barry and Forss (1983) found that high concentrations of lotus condensed tannins (40-80 g/kg DM) were necessary to prevent protein deamination when mixtures of fresh lotus and white clover were mascerated and incubated with rumen fluid, and this is confirmed by the *in vivo* finding of Barry and Manley

(1983). The high release of NAN in the intestines contrasts with effects produced by protecting protein from ruminal degradation using high rates of formaldehyde, which can include a reduction in post-ruminal digestibility (Beever et *al.* 1977). The difference may be that association between condensed tannins and protein is by means of hydrogen bonding (McLeod 1974), versus a fixed chemical bonding in the reaction of formaldehyde with proteins, Secondly, the formaldehyde:protein bond is only reversible in the low pH of the abomasum, whereas protein can be released from condensed tannin:protein complexes at pH values corresponding to those in both the abomasum and small intestine (Jones and Mangan 1977).

In Fig. 2 the quantities of NAN absorbed post-ruminally/N intake has been compared for a number of fresh N.Z. forages fed to sheep. The data of Barry & Manley (1983) with *Lotus pedunculatus* has been combined with that of John and Lancashire (1981) for Lotus *corniculatus* and plotted as a function of lotus condensed tannin concentration, NAN absorbed post-ruminally/N intake was linearly related to tannin concentration (r = 0.998, P<0. OS), with the intercept value corresponding to zero condensed tannin concentration (0.57 ± 0.008) being similar to that obtained for diets of the non-tannin containing species ryegrasses and white clover (MacRae and Ulyatt 1974). Whilst there are differences in amino acid supply between the ryegrasses and white clover, these effects are small compared to those produced by concentrations of lotus tannins in the range 40-80 g/kg DM. Condensed tannins in the sainfoin used by Ulyatt and Egan (1979) appeared to be slightly less effective on a w/w basis than lotus condensed tannins for increasing amino acid supply.

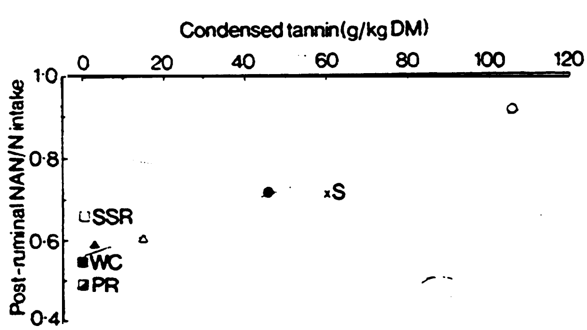

Fig. 2. Ratio NAN digested post-ruminally/total N intake in sheep fed lotus sp compared with perennial and short rotation ryegrass (PR; SSR; ◪), white clover (WC; ■) and sainfoin (S; X) fed at same N intake. Line refers to lotus sp. only. low and high tannin ● ○ *Lotus pedunculatus* (Barry and Manley 1983). low and high tannin ▲ △ *Lotus corniculatus* (John and Lancashire 1981).

When expressed as amino acids absorbed from the small intestine/ MJ ME intake, values for lotus (29% ME) were higher than for white clover or perennial ryegrass (20% ME), with sainfoin being intermediate (25% ME) .

Rumen carbohydrate digestion

The PEG supplementation experiment of Barry and Duncan (1983) showed hemicellulose to be the structural CHO most depressed by high concentrations of condensed tannins, and this appears to be confined to a depression of digestion in the rumen (Barry and Manley 1983). When feeding kale *(Brassica oleracea)* diets, Barry et *al.* (1983) likewise found ruminal hemicellulose digestion to be the only aspect of CHO digestion depressed by the protein-binding substance dimethyl disulphide, produced from microbial fermentation of dietary S-methyl-L-cysteine sulphoxide (SMCO). It seems that ruminal hemicellulose digestion is particularly inhibited by the presence of water soluble protein binding substances in the rumen. Ruminal digestion of all CHO constituents in sheep fed sainfoin was similar to that of sheep fed other fresh forages (Ulyatt and Egan 1979), showing that condensed tannins (60 g/kg DM) of larger MW than in lotus do not depress hemicellulose digestion in the rumen.

Voluntary intake and liveweight gain

In contrast to effects upon amino acid supply, it is clear that high concentrations of condensed tannin depress the voluntary intake of Lotus *pedunculatus* by sheep, and that this is probably the principle way that the tannin restricts the growth of grazing sheep. We are currently undertaking experiments at Invermay to construct dose/response relationships for both voluntary intake and amino acid supply, to determine the concentration of condensed tannin in lotus sp. that will prevent sane deamination of protein without depressing voluntary intake.

Another possibility is that ruminants may be able to adapt to lotus containing high concentrations of condensed tannins. In investigations of low growth rates in lambs grazing forage kale, plots were produced containing normal (65 g/kg DM) and low (30 g/kg DM) concentrations of SMCO. Growth of lambs grazing these respective forages were 75 and 150 g/d during the first six weeks, with both groups recording 150 g/d during weeks 7-12. Thus lambs grazing normal kale adapted to its high SMCO content after a grazing period of six weeks, and a similar phenomenon may account for the high growth rates (200 g/d) obtained where lambs have grazed high tannin lotus for long periods (W.L. Lowther unpublished data).

TABLE 5 Growth of young sheep grazing pure species plots grown under high soil fertility conditions.

Species	RFC structural CHO	Lignin (g/kg DM)	Condensed tannin (g/kg DM)	Liveweight gain (relative)	Liveweight gain (g/d)
Huia white clover	1.3	25	T	100	190-354
Fakir sainfoin	1.0	48	60	97	182-230
Maku lotus	0.8	116	20	87	153-315
Wairau lucerne	ND	ND	0	78	123-267
Hamau red clover	ND	ND	T	78	127-234
Ruanui ryegrass	0.3	20	0	52	88-198

John and Lancashire (1981)

ND - not determined; T - trace amounts

As growth, N retention and lactation yield of ruminants consuming fresh forages are likely to be restricted by amino acid supply (see Introduction), the increase in amino acid supply produced by lotus condensed tannins will be beneficial, once the problems of depressed intake are resolved. This is illustrated in Table 5 for young sheep grazing pure species plots for periods up to 6 and 12 weeks. Growth rates of lambs grazing sainfoin and lotus exceeded those of lambs grazing lucerne, red clover and perennial ryegrass, but were less than those of lambs grazing white clover. The two tannin containing legumes had lower ratios of RFC: structural CHO and higher lignin contents than white clover, and this may account for lamb growth being slightly lower on these forages than on white clover.

Extrapolation to other tannin - containing legumes

The high levels of condensed tannin encountered in the work discussed here could be specific to *Lotus pedunculatus* or perhaps to the cultivar Grasslands Maku. In an experiment conducted at a semi-arid site of soil pH 5.5, condensed tannin content of the Lotus *corniculatus* cultivars Granger, Maitland and El Boyero (15-22 g/kg DM) was considerably lower than that of Maku lotus (53 g/kg DM), whilst the Lotus *corniculatus* cultivars Winnar and Empire contained only trace amounts (2 g/kg DM) of condensed tannins (W.L. Lowther, T.R. Manley and T.N. Barry, unpublished data). Thus lotus condensed tannin content can be lowered through choice of cultivar. As the condensed tannin content of Lotus sp. has a heritability of 53% (Dalrymple 1982), it may be possible to select for *Lotus pedunculatus* plants of lower tannin content when grown in acid soils under minimum levels of fertiliser input.

Condensed tannins are also present in a range of tropical legumes grown in Australia (McLeod 1974). Whilst it seems probable that growing these legumes under minimum or zero fertiliser inputs will produce plants containing high concentrations of condensed tannins, their effects upon the animal cannot be predicted without a knowledge of condensed tannin MW and reactivity. Based upon N.Z. data with temperature species it seems that high MW condensed tannins (17,000-28,000) present in sainfoin do not inhibit aspects of ruminal CHO digestion as do the lower MW tannins present in lotus sp. (6,800-7,100), but on a w/w basis are slightly less effective at increasing amino acid supply than are lotus tannins,

Unlike lotus and sainfoin that contain condensed tannins evenly distributed throughout their leaf and stem tissue, plants such as *Trifolium* species that only contain condensed tannins in flower petals are not bloat resistant. To prevent bloat, and probably to increase amino acid supply, it therefore seems that condensed tannins must be uniformly distributed throughout all tissues of the grazed plant.

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