PLANT SECONDARY COMPOUNDS; THEIR IMPACT ON FORAGE NUTRITIVE VALUEAND UPON ANIMAL PRODUCTION

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Abstract

Both the anti-nutritional and beneficial effects of secondary compounds in a range of temperate and tropical forages have been reviewed. Major secondary compounds in temperate and tropical forage plants occur in the phenolic fraction and include condensed and hydrolysable tannins, phenolic monomers and lignin. Condensed tannins (CT) bind to plant protein by pH-reversible hydrogen bonding. In temperate legume forages this reduces rumen protein degradation and can increase the absorption of essential amino acids (EAA) from the small intestine, with reactivity depending on CT concentration, molecular weight and chemical structure. Low concentrations of CT in *Lotus corniculatus* (20-40g/kg DM) increased EAA absorption by 62% and increased wool growth (15%) and ovulation rate (25%) in grazing sheep and increased milk production in ewes and dairy cows, all without changing voluntary feed intake (VFI). High concentrations of CT in *Lotus pedunculatus* (80-100 g/kg DM) depressed VFI and depressed rates of body and wool growth in grazing sheep. Sulla, containing 80-120 g CT/kg DM, was particularly effective for counteracting the effects of parasitism and for promoting high rates of body growth in parasitised lambs. CT is present in tropical species such as Leucaena and Acacia at higher concentrations (60-200 g/kg DM) than in temperate species. Action of CT reduced rumen protein degradation in sheep fed tropical forages, but as yet there is no convincing evidence that this leads to increases in EAA absorption from the small intestine or that CT increases animal production. Further research is needed in these areas with tropical forages, particularly on the relationship between CT structure and its reactivity with proteins. Increasing CT concentration did not depress rumen microbial protein synthesis in sheep fed either temperate or tropical forages, until CT concentration exceeded 130 g/kg DM. Effect of CT upon undegraded, dietary protein release in the small intestine and upon endogenous protein secretion is defined as a future research area.

Flavonoids have been detected in tropical legume forages in the same concentrations as CT. They have anti-nutritional effects in terms of causing amino acid loss during their excretion as conjugates in the urine and by disturbing blood acid/base balance, leading to reduced VFI.

Research currently in progress with other secondary compounds in both temperate and tropical forages is reviewed. This includes sesquiterpene lactones in chicory, acubin in plantain, isoflavones in red clover and coumarin and dihydro-coumarin in glyricidia. The nutritional and anti-nutritional effects of these compounds for both ruminants and non-ruminants is discussed.

Introduction

Secondary compounds can exert both anti-nutritional and nutritionally beneficial effects upon forage feeding value. Recent advances in the field of secondary compounds that occur in both temperate and tropical forages. The major part of the review will concern phenolic compounds, including condensed tannins (CT) and monomeric phenolics. Their reactivity will be reviewed in relation to structure, followed by their effects upon voluntary food intake (VFI), digestive processes, animal production (including body and wool growth, lactation and reproduction) and animal health (bloat and parasite control). A feature of the paper will be to compare and contrast the effects produced by secondary compounds in animals grazing temperate and tropical forages.

The final part of the review will summarise research that is in progress involving other secondary compounds in both temperate and tropical forages. The objective here is to update readers on "research in progress" in these areas; firm conclusions are available in some cases but not in others at this stage.

Plant Phenolic Compounds

Condensed tannins (CT) occur in a number of temperate legumes such as *Lotus corniculatus*, *Lotus pedunculatus* and sulla (*Hedysarum coronarium*) and their effects upon forage nutritive value will be reviewed in this paper. Other temperate forages also contain low concentrations of CT (Table 1).

Condensed tannins

Temperate forage

Voluntary feed intake

High CT concentrations in *Lotus pedunculatus* (63 and 106 g/kg DM) substantially depressed VFI in sheep (-27%), in line with plant CT production being a defence against consumption by herbivores (Barry and Duncan 1984). Lower depressions in VFI (-12%) were produced by 55 g CT/kg DM in *Lotus pedunculatus* (Waghorn *et al*., 1994). However, medium CT concentrations in sulla (45 g/kg DM) and in *Lotus corniculatus* (34 and 44 g/kg DM) had no effect upon VFI (Terrill *et al*., 1992*a*; Wang *et al*., 1996*a*,*b*).

Digestive processes

When ruminants are fed fresh forages there is often an extensive fermentation of dietary protein to peptides, amino acids and ammonia in the rumen. Much of this nitrogenous substrate is reincorporated into microbial protein. However, the rapid release of ammonia often exceeds its incorporation into microbial protein, resulting in 20-35% of this N being lost as ammonia absorbed from the rumen (MacRae and Ulyatt 1974). A number of studies have shown that a low concentration of CT in the diet can increase the flow of non-ammonia-nitrogen (NAN) to the intestine, relative to N intake (for review see Barry and McNabb 1999). This results because CT can reduce protein (McNabb *et al.* 1996) and N (Waghorn *et al.* 1987*a*) degradation in the rumen.

When duodenal (abomasal) NAN flux per unit N eaten is plotted against CT concentration using data from a range of experiments where sheep were fed lotus species (Fig. 1), this clearly shows that total NAN flux increases with increasing CT concentration. This is in contrast to microbial N flux at the duodenum (abomasum) which shows no change as CT concentration increases (Fig. 1).

Waghorn *et al.* (1987*b*) reported that the CT in *L. corniculatus* (22 g kg DM^{-1}) fed to sheep increased abomasal flux of essential amino acids (EAA) by 50%. This change was associated with increased (63%) apparent absorption of EAA from the small intestine (Waghorn *et al.*, 1987*b*). Whilst the abomasal flux of nonessential amino acids (NEAA) was also increased (14%) by the CT, a significant reduction (20%) in the digestibility of NEAA in the small intestine resulted in the apparent absorption of NEAA being similar in control and polyethylene glycol (PEG)-supplemented sheep (Table 2). Strong complexes are formed between CT and the binding agents, PEG (MW 3350) and polyvinylpyrrolidone (PVP). Both these binding agents, and particularly PEG, have been used to study the interaction between CT and protein (Jones and Mangan 1977; Barry and Manly 1986). About 1.7-2.0 g of PEG/g CT is generally required to complex all the CT such that the CT from *Lotus pedunculatus* is unable to precipitate forage protein (Barry and Forss 1983). Therefore, the effects of CT can be deduced by comparing sheep receiving PEG, either orally or intraruminally (CT inactive) with sheep not receiving PEG supplementation (CT active).

The ratio of EAA:NEAA absorbed from the small intestine in the study of Waghorn *et al.* (1987*b*) was 0.87 for PEG-supplemented sheep. This same ratio in *L. corniculatus* and rumen bacteria was 1.14 and 1.08, respectively. Therefore, the value of 1.57 for the ratio of EAA:NEAA absorbed from the small intestine of sheep not receiving PEG could only arise from selective absorption of EAA. We have yet to fully understand how CT affects amino acid absorption from the small intestine.

Whilst concentration in the diet is clearly important, other factors like chemical structure and source of the CT are equally important (for review, see Barry & McNabb, 1999). For example the CT in *L. pedunculatus* (55 g kg DM^{-1}) also increased (by 15%) the flux of EAA through the abomasum (Waghorn *et al.*, 1994). However, this CT reduced the apparent digestibility of EAA in the small intestine by 13 percentage units. The net effect of these changes in amino acid digestion was that apparent absorption of EAA from the small intestine was unaffected by this CT (Table 2). In that experiment, the CT in *L. pedunculatus* also reduced N digestibility by 12 percentage units and voluntary intake by 12%. In a similar experiment where sheep were fed a mixed diet consisting of *L. pedunculatus* and ryegrass (*Lolium perenne*) with a final CT concentration in the mixed diet of 18 g CT kg DM^{-1}, N digestibility was reduced by 13 percentage units (Waghorn & Shelton 1995). In that experiment the effects of CT were similar to *L. pedunculatus* fed as a sole diet even though the concentration of CT in the mixed diet more closely resembled *L. corniculatus*.

In this example the variation in nutritional responses to the two sources of CT was not solely mediated by the concentration of CT in the diet and it is likely that the chemical structure of the CT was important. The chemistry of CT is complex and a thorough discussion of this topic is beyond the scope of this review. However, key differences occur in the hydroxylation of the B-ring of the constitutive flavan-3-ol units. The stereochemistry of the heterocyclic C-rings takes the form of 2,3-*cis* or 2,3-*trans* and these dictate how flavan-3-ol subunits are attached relative to one another. Either C4/C8 or C4/C6 interflavanoid linkages link the constitutive flavan-3-ol units, and this affects the final shape of the polymer.

The number of constitutive flavan-3-ol units also varies. These differences produce an infinite number of chemical structures, which in turn affect the reactivity of the CT.

For example, the CT from *L. corniculatus* and *L. pedunculatus* differ considerably in their chemical structures (Foo *et al.*, 1996, 1997). The CT in *L. pedunculatus* predominantly consists of prodelphinidin subunits with epigallocatechin (64%) being prevalent. In contrast the CT from *L. corniculatus* is predominantly procyanidin with epicatechin (67%) dominating this CT. Finally, the average molecular weight (MW) of CT in *L. pedunculatus* is 2200, whilst in *L. corniculatus* it is 1900. In comparative experiments, CT from *L. pedunculatus* were more effective at reducing the degradation of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) protein by rumen micro-organisms than the CT from *L. corniculatus* (Aerts *et al.*, 1999). This suggests that the effect that CT has on protein degradation may be responsive to these differences in chemical structure.

Body growth, wool growth and reproduction

Lambs grazing *Lotus corniculatus* at very high DM allowances (i.e., *ad libitum* intakes) produced high rates of body and wool growth (Experiments 1 and 2; Table 2), but as there were no responses to PEG supplementation it seems that none of these effects were attributable to CT. However, when DM allowance was reduced in growing lambs (initial liveweight 22.4 kg) grazing *Lotus corniculatus* for four months during summer (Experiment 3), action of CT (i.e., unsupplemented sheep – PEG supplemented sheep) increased wool growth by 12% without affecting rate of body growth or VFI (Wang *et al.*, 1996a; Table 3). There was no response to PEG supplementation in comparable sheep grazing lucerne, containing only traces of CT (0.3 g/kg DM). Action of CT in dry ewes (initial liveweight 54 kg) restricted to maintenance feeding on *Lotus corniculatus* for four months during summer (Experiment 4) increased wool growth by 19% without affecting VFI or LWG (Table 3).

A review of many year's data implicated a role for protein nutrition in the ovulation rate of ewes (Smith 1991), and this was illustrated by an increase in ewes showing multiple ovulations in response to abomasal infusions of lactalbumin and soy protein isolate (73% vs. 55%; Cruickshank *et al*., 1998). Grazing trials were then carried out for 6 – 12 weeks, with ewes grazing perennial ryegrass/white clover pasture and *Lotus corniculatus*, containing 1 and 23 g CT/kg DM (Table 3), to study if the effect of improving protein supply on ovulation rate (OR) could be induced by CT. Grazing on *Lotus corniculatus* increased OR in all three years (Table 4), but the magnitude of the response differed between years. Where the ewes gained a small amount of weight during mating (Experiments 5 and 7), grazing on *Lotus corniculatus* increased OR by 32 and 21%, with a large part of the response due to the action of CT. Where the ewes lost a small amount of weight during mating (Experiment 6), grazing on *Lotus corniculatus* increased OR by 14%, with none of the effect attributable to action of CT. Averaged over all three experiments, grazing ewes on *Lotus corniculatus* during mating in autumn increased wool growth by 18%, but in contrast to results obtained during summer (Table 3) only a very small component of this was due to the action of CT.

In contrast to the increased productivity obtained from CT in *Lotus corniculatus*, the action of CT in *Lotus pedunculatus* containing 76-90 g CT/kg DM markedly depressed rates of both body growth and wool growth (Barry 1985) and high CT concentration in sulla (88 g/kg DM) restricted carcass gain in growing lambs (Douglas *et al*. 1999). This further illustrates the ecological role of high CT concentrations as a chemical defence.

Lactation

Feeding fresh forages imposes several limitations on both the yield and efficiency of milk and milk protein production. When lactating ruminants predominantly fed fresh forages have been given abomasal infusions of protein like casein, or fed supplementary protein protected from rumen degradation, production responses have included increased milk production in dairy cattle (Rogers *et al.*, 1980) and sheep (Penning *et al.*, 1988). This suggests that milk production is restricted when insufficient amino acids are absorbed from the small intestine relative to energy. Reduced availability of amino acids is due in part, to substantial losses of dietary protein from the rumen as a result of microbial fermentation to ammonia (MacRae and Ulyatt 1974) and the use of absorbed amino acids in the conversion of ammonia to urea in the liver (Lobley *et al.*, 1995).

Studies have shown that low concentrations of CT in the diet can increase milk yield and milk protein concentration. Wang *et al.* (1996b) conducted a grazing experiment to study the effects of CT in *L. corniculatus* on lactation performance of ewes rearing twin lambs. In that study, effects of CT were elucidated by comparing ewes orally supplemented with PEG, with ewes that had not received PEG. The milk yield and composition were similar for control (CT acting) and PEG-supplemented (CT not acting) ewes at peak lactation. However, as the lactation progressed, control ewes experienced a slower decline in milk production. In mid and late lactation, control ewes were producing more milk (21%) and more milk protein (14%) than the comparable PEG-supplemented ewes.

Woodward *et al.* (1999) reported that CT in *L. corniculatus* fed to dairy cows in late lactation also increased milk and milk protein yield, and milk protein concentration. In that study, CT was responsible for 57% of the increase in the milk protein concentration because cows fed *L. corniculatus* had a higher milk protein concentration (3.61%) than comparable cows fed *L. corniculatus* supplemented with PEG (3.44%), ryegrass (3.31%) or ryegrass supplemented with PEG (3.30%). Changes in lactation performance were not a consequence of changes in intake because CT did not affect this parameter in either study.

Animal health

Ruminants grazing forage diets are subject to a number of diseases, some of which have a nutritional component. Two such conditions are rumen frothy bloat in cattle and internal parasite infections in young grazing sheep, cattle, deer and goats.

Bloat is caused by very high solubility of forage proteins leading to the development of a stable foam in the rumen, and is very prevalent in cattle fed on legumes, especially in spring (Mangan, 1959). Because of their protein-precipitating properties, grazing CT-containing legumes has long been known to eliminate bloat (Jones *et al*. 1973). However, the minimum plant CT concentration needed to make forage bloat-safe was not known; this has recently been proposed to be 5 g CT/kg DM or greater (Li *et al*. 1996).

Effects of CT on parasitism can be assessed by grazing animals on legumes that contain differing levels of CT but have similar morphology and are similar in other aspects of chemical composition. Anthelmintic treated (i.e., parasite free) lambs grew at similar rates when grazing CT-containing legumes (sulla and *Lotus pedunculatus*) or non-CT-containing lucerne (Table 5). However, non-drenched (i.e., parasitised) lambs grew much better on the CT-containing legumes, indicating that they could better tolerate the parasites. Parasite burdens at slaughter were similar for lambs grazing *Lotus pedunculatus* and sulla, but were considerably lower for lambs grazing sulla.

Two possible mechanisms could be involved. Firstly, improved EAA supply from the action of the CT may counteract the protein loss caused by gut parasitism and may stimulate the immume system, enabling the animals to better resist a parasite burden. Secondly, the CT may directly react with and inactivate parasite larvae during passage through the gut. Using *in vitro* studies, Molan *et al*. (2000*a,b*) have shown that CT extracted from sainfoin, sulla, *Lotus pedunculatus* and *Lotus corniculatus* can inhibit infective gut worm larvae of sheep and both gut worm and lungworm in farmed deer, with the effect influenced by both CT concentration and structure. Therefore, it seems that CT may counteract parasites by one or both of the above mechanisms, and that the mechanism involved may differ for sulla and *Lotus pedunculatus* CT.

Tropical forages

The majority of available data on tropical CT point to a negative influence, not always on VFI but often on the availability of nitrogen, per unit of forage. Few data are available on the impact of tropical CT on animal performance over the longer term (months). Available data suggest a negative impact. The potential for low concentrations of tropical CT to enhance animal productivity and health over the longer term remains largely unexplored and should be a future research priority. *In vivo* studies that have incorporated the use of PEG supplementation to determine the effect of CT, unconfounded by other plant factors, are the focus of this section.

Voluntary feed intake

Tropical CT's can depress intake. Depressions may not be immediately evident in short-term preference trials, but can emerge over the longer term. Comprehensive preference trials by both Kaitho *et al.* (1998) involving 40 "cut and carried" browse *spp.* (fed to sheep, CT range 2–81 g/kg DM), and Faint *et al.* (1998) with 21 directly grazed leucaena accessions (fed to cattle, CT range 0–277 g/kg DM), showed no significant negative correlation between preference rating and CT content. Nor was preference improved when the accessions were sprayed with PEG (Faint *et al.* 1998). However, in a related but only preliminary trial, foliar applications of PEG to harvested leucaena improved the preference of cattle for the high CT *L. pallida* (CT = 147 g/kg DM), while the preference of the already well accepted lower CT *L. leucocephala* (CT = 75 g/kg DM) remained unchanged (Hannigan and McNeill 1998). Consistent with this we have recently shown in a digestibility trial that the CT fraction in *L. pallida* depresses VFI in sheep by as much as 45%. In similar protocols, no depression was noted for the lower CT *L. leucocephala* or the hybrid of the 2 species (Table 6). CT's in *A. aneura* and *A. saligna* (= *A. cyanophylla*) have depressed the VFI of sheep in digestibility trials, run over the course of 1-2 weeks (Degen *et al.* 1998, 15%, Ben Salem 1999*b*, 19%). However, there are instances where intakes depressions have not been detected (Miller *et al.* 1997, Ben Salem 1999a), or depressions were evident but minimal (< 10%, Barahona *et al.* 1997, for *Desmodium ovalifolium* and *Flemingia macrophylla*).

The above data are consistent with the reasonable expectation that the higher the CT content the greater the depression of VFI and provide a note of caution with regard to a reliance on preference trials as a means of predicting longer term intakes of CT-rich forages.

Animal Productivity

There are few data from longer term feeding trials for VFI or animal productivity responses. Available data appear largely confined to low quality forages such as *Acacia aneura* (Mulga) and *A. saligna* (= *A. cyanophylla*); (Table 7). Effects on VFI appear variable, while effects on liveweight and wool production tend to be negative. Similarly, in grass-

based diets supplemented with *Calliandra calothyrsus* (30% of the diet), Palmer and McSweeney (2000) have preliminary data for a positive response to PEG in wool production in the order of 20-30%. Surprisingly, no similar information is available on PEG responses in the high quality legumes such as *Leucaena leucocephala*, Gliricidia, and the Desmodiums. We are not aware of any published data on the impact of tropical CT's on milk production, or measures of reproductive performance such as ovulation rate.
[Insert Table 7 near here]

Animal Health

Hypotheses relating to the potential of tropical CT's to reduce worm burdens are being actively pursued, although to date there have been few in vivo studies and their results are inconclusive (Kahn and Diaz-Hernandez 2000). Supplementing weaner sheep with Calliandra calothyrsus for one week did not reduce naturally acquired worm burdens (Parker and Palmer 1991). It may be that CT has to be fed over a longer period for benefits to be detected. For example, by supplementing lambs with quebracho CT for 10 weeks, artificially induced worm burdens were reduced (Butter et al, 1998). Although even then, liveweight gains remained depressed.

No data appear to be available on the ability of tropical CTs to protect against bloat. However, the consistent ability of CT in tropical forages to protect protein in the rumen (see later) indicates a protective effect against bloat is highly likely.

Nutrient utilisation

The data in Table 6 are representative of the trends seen in the wider literature for the effect of CT on nutrient availability *in vivo*. While tropical CT may not always depress VFI, OMD, or flows of microbial N, they consistently depress the apparent whole-tract digestibility of N (Table 6, and Pritchard *et al*. 1992, Carrulla 1994, Barahona 1997, Miller *et al*. 1997, Norton and Ahn 1997, Ben Salem *et al*. 1999*ab*). As CT content rises, depressions in N digestibility lead to depressions in N retention. The higher the CT content, the greater the depression.

The reduction in the digestibility of N starts in the rumen. This is evidenced by the remarkably consistent observation that tropical CT increase the proportion of bypass protein in the diet, and often increase the total flow of protein to the abomasum (e.g. for *Leucaena leucocephala*, Table 6, *Desmodium ovalifolium*, Carrulla 1994 and Barahona *et al.* 1997). However, evidence that this extra protein advantages the animal remains elusive. The important question is whether or not this extra protein is available for absorption across the small intestine. Few have attempted to measure this for tropical CT. Barahona *et al.* (1997) have, and they detected no beneficial effect. To date there is no clear evidence that tropical CT can improve the delivery of metabolisable protein per unit of forage ingested.

For tropical CT, post-ruminal impacts may prove to be more important than ruminal. In monogastrics it is well recognised that CT exacerbate the obligatory losses of endogenous protein (see review by McNeill *et al.* 1998). If the same occurs in ruminants fed CT-containing tropical forages it may explain why improvements in post-ruminal flows of protein may not be complemented by improvements in N retention. Increased flows of bypass protein may be offset by an increased loss in endogenous protein. Such a response would be dependent on ingested CT remaining active following passage through the rumen. Whether the result of overprotection of feed protein or an exacerbation of endogenous loss, evidence for postruminal activity is mounting. Abomasal infusions of PEG induced a greater wool growth response than did ruminal infusions, in sheep fed hay plus 30% of the diet as

Calliandra calothyrsus (Palmer and McSweeney 2000). Komolong and McNeill (unpublished) have shown that as the intake of a crude extract containing quebracho CT was increased across the range 0 to 6% of DMI (actual CT levels 0, 13, 26, 38 g/kg DM in a lucerne chaff based diet fed at 1000 gDM/lamb/day), bypass protein flows remained unaltered whilst apparent absorption of NAN from the small intestine declined dramatically, and linearly, from 12.6 to 7.2 g/day ($P<0.05$). Further investigation is required to quantify the extent to which the extra protein reaching the end of the small intestine is of endogenous as compared to feed origin in response to a dietary load of CT.

Comparing and contrasting the effects of CT in temperate and tropical forages

From the information available to date, it is clear that animal responses to CT differ between temperate and tropical forages. Whereas increases in EAA absorption and in animal production have been shown from the action of CT in some temperate forages (e.g., *Lotus corniculatus*), no such increases have been demonstrated yet for tropical forages. One reason is that there are many more CT-containing tropical forages than temperate forages and less nutritional work has been done on tropical than on temperate CT; it may be that beneficial effects of one or more tropical CT remain to be discovered.

A further reason is that CT concentration is generally higher in tropical than in temperate forages and that there may well be differences in molecular weight and structure between tropical and temperate CT. Work is needed on the structure of CT from different tropical forages and their relationship to reactivity. Current information with tropical CT points to adequate protection of dietary protein from rumen digestion, but no net benefit in protein absorption from the small intestine (as is also the case for CT in the temperate legume *Lotus pedunculatus*). Possible reasons include incomplete release of CT from protein in the small intestine and/or increases in endogenous protein excretion, leading to increases in faecal N excretion. This could well be a function of CT structure. It highlights the small intestine as an area for nutritional investigation in ruminants fed CT-containing forages.

Whilst there may be differences between tropical and temperate CT in action in the small intestine, there appears to be minimal effect of either upon rumen microbial protein synthesis, unless CT concentration is extremely high (for instance over 130 g/kg DM; Table 6). Rumen micro-organisms may have evolved methods of protecting themselves from high concentrations of dietary CT (McSweeney *et al.* 2000).

Monomeric phenolics

Monomeric phenolics (simple phenolics, low molecular weight phenolics, phenolic acids) are, or are closely related to, the building blocks of tannins and lignin. The variety of monomeric phenolics and their relevance to animal nutrition has been reviewed by Harborne (1988), Lowry *et al.* (1996), and Foley *et al.* (1999). Their significance to animal performance derives from their absorption from the digestive system whereupon they interfere with metabolism. Despite their common occurrence in tropical forages and likely impact on metabolism, effects on animal performance remain to be properly defined.

Examples include the cinnamates (e.g. p-coumaric acid and ferulic acid in tropical grasses) and the flavanoid monomers, grouped as the flavones (luteolin and apigenin in the shrub legume Tagasaste; *Chamaecytiscus proliferens*), flavonols (e.g. quercetin and myricetin in leucaena), and the flavanols or catechins. Flavanoid monomers are commonly within the soluble fraction of forage, and often in glycosidic form (i.e. with a sugar attached), whilst the cinnamates play a structural role in the cell wall. Concentrations of cinnamates in tropical grasses can be in the order of 10-50 g/kg DM, flavanol glycosides in *Leucaena leucocephal*

60 g/kg DM, and flavones in tagasaste fluctuate from approximately 50-150 g/kg DM (Lowry *et al*. 1984, Lowry *et al*. 1993b, Lowry *et al.* 1996, Edwards 2000). Hydrolysable tannins and related phenolics such as the catechin gallates occur at high concentrations in *Acacia nilotica* (up to 450 g/kg DM) and should be capable of supplying large quantities of phenolic monomers upon hydrolysis by microbes in the rumen environment (Lowry *et al.* 1993a, and pers comm.).

Monomeric phenolics can impede digestion *in vitro*, but effects *in vivo* appear to be of minimal importance (Jung and Fahey 1983, Jung 1985, Lowry *et al.* 1996, Edwards 2000). If the microbial population is appropriately adapted it appears that significant parts of the molecules can be converted to metabolisable energy, e.g. any attached sugars, plus parts of the carbon rings, other than the difficult to degrade "B" ring, may be degraded to acetates (Lowry *et al.* 1996). Instead, it is likely that effects on post-absorption on animal metabolism is of greater importance.

Potential effects on animal performance may be mediated by a change in the acid-base balance of the animal. This in turn may impede appetite and also the efficiency of utilisation of absorbed N. Once ingested, rumen microbes degrade the 3-ringed flavanoids and the single-ringed cinnamates to leave mainly single-ringed structures such as phenylpropionic and phenylacetic acids. These are quantitatively absorbed into the blood stream (80% or more, Pagella *et al.* 1997). Following conjugation in the liver, the absorbed phenolics are excreted in the urine as benzoic acid derivatives or "phenolic conjugates". The majority is as hippuric acid (benzene conjugated with glycine), but include benzoic acid, phenylacetic, and p-cresol variously conjugated with either glycine, sulphur, or glucuronic acid (Martin 1969*ab*, 1982, 1983, Pagella *et al.* 1997, Foley *et al*. 1999).

Metabolic acid-base balance is perturbed toward to acidic since the phenolic conjugates formed by the liver are relatively strong organic acids. As outlined by Foley *et al*. (1999) and Foley *et al.* (1995), absorbed phenolics could influence N metabolism in two ways, as a urinary loss of N in the glycine of conjugated phenolics (e.g. hippuric acid), or indirectly via a reduction in acid-base balance. In order to maintain pH homeostasis, HCO_3^- is required to neutralise excess H^+ (Stewart 1983). One source is via the degradation of amino acids by the kidney resulting in the simultaneously release of $NH3^+$ into the urine and HCO_3^- into the blood stream (Halperin *et al.* 1992). There is also speculation that processes such as ureagenesis and gluconeogenesis may be impeded by absorbed phenolics, possibly due to the requirement for HCO_3^- in these pathways competing with that required for pH homestasis (Foley *et al.* 1995, Foley *et al.* 1999). Hence absorption of phenolic monomers are hypothesised to stimulate a wastage of circulating amino acids and therefore a reduction in the efficiency of conversion of absorbed N into tissue N. Illius and Jessop (1995) have modeled such an effect, in sheep, and defined a decline in rate of gain in tissue protein from +2 g/d to –12 g/d as the absorption of allelochemical (e.g. phenolics) increases from 0 to 0.5 mol/d. However, as far as we are aware, *in vivo* data for ruminants, necessary to validate such predictions, have yet to be published.

Evidence in support of the above hypotheses is as follows. In terms of direct loss of N, Lowry *et al.* (1993b) noted N losses as hippuric acid in sheep fed tropical grasses in the order of 0.5 to 1.0 g N/day, and these comprised 5 to 17% of total N intake. Other evidence is more circumstantial. With regard to appetite depression, as concentration of phenolic monomers in Tagasaste rise, in hot dry summer and autumn months, liveweight gains of cattle decline, largely due a reduction in VFI, even though there is plenty of feed on offer (Edwards *et al*, 1997ab, Edwards 2000; Figure 2). In the Mediterranean climate of Western Australia the temperate shrub legume Tagasaste (*Chhomaecytisus proliferus*) produces high LWG in young cattle during winter (1.2 kg/day) when the concentration of total phenolics in leaf plus edible stem is low (35 g/kg DM), but low LWG during summer/autumn (0-0.75 kg/day) when the concentration of total phenolics is high (80 g/kg DM); Edwards 2000). The principal phenolic compounds in Tagasaste are the flavones apigenin and luteolin; extractable CT concentrations are very low but measurable CT levels have been found in the protein-bound and fibre-bound fractions (Edwards 2000; Table 8). Moreover, in line with a hypothesised effect of phenolic monomers on acid-base balance, the urine pH of cattle grazing Tagasaste also declines to unusually low levels (pH 5.8 to 6.0), the lowest pH's occurring when the concentration of phenolics in the Tagasaste have previously been shown to peak. In cattle grazing temperate grass-based pastures, urine pH is commonly well above 8 units (Roche *et al*. 2000). We have also found the urine pH's of sheep fed leucaena to be less than those fed lucerne chaff (pH 6.68 v. 8.34, $P<0.05$); (McNeill *et al.* 2000*b*). These declines in urine pH should be indicative of a decline in the acid-base balance of the whole animal (Halperin *et al.* 1992, Horst *et al.* 1997).

Others compounds present in temperate and tropical forages

A range of these other compounds and their potential affects upon animals is summarised in Table 8. Selections of the herb chicory (*Chichorium intybus*) for vegetative growth have considereable potential in temperate agriculture. The cultivar 'Grasslands Puna' is of high OMD (82%), breaks down rapidly in the rumen and has a very rapid fractional outflow rate from the rumen; consequently VFI is high under grazing and it promotes high rates of animal growth, especially during late summer/autumn when ryegrass-based pastures are of low feeding value (Barry 1998; Kusmartono *et al*. 1996). However, early studies with dairy cows fed diets of chicory alone identified a bitter taint in the milk, and for this reason chicory feeding to dairy cows is limited to 2 h/day, generally following the morning milking, to restrict chicory intake to *c*. 25% of the total daily DM intake. Degradation products of the sesquiterpene lactones present in chicory, namely dihydrolactucin, tetrahydrolactucin and hydroxyphenylacetic acid (HPAA) have been identified as the taint compounds in the milk of chicory-fed cows (Visser 1992). Tetrahydrolactucin and dihydrolactucin are probably formed by hydrogenation in the rumen, whilst HPAA is a degradation product of lactupicrin. A selection programme has now been carried out to produce low sesquiterpene lactone chicory, with a view to feeding this to dairy cows as a greater proportion of the diet. However, whilst this may solve the milk taint problem, extra care will be needed with management of this plant, as the reduced concentration of sesquiterpene lactones may have lowered its chemical defence against fungal diseases.

The herb plantain (*Plantago lanceolata*) contains both condensed tannins and the iridoid glycosides acubin and catalpol and is being investigated for its potential anthelmintic properties for grazing ruminants (Rumball *et al*. 1997). Despite its high OMD when in the vegetative state (80%), growth of lambs was much lower than found for chicory and was similar to that for lambs grazing perennial ryegrass (Frazer and Rowarth 1996). Niezen *et al.* (1998) found very low growth in non-parasitised lambs grazing plantain (52 g/d) and no evidence that it sustained growth in parasitised lambs.

Red clover (*Trifolium pratense*) contains the iso-flavones formononetin, biochanin, daidzein and genistein. High concentrations of formonontin present in the cultivars Pawera, Hamera and Turoa (7-14 g/kg DM) cause problems of depressed ovulation rate, returns to service and barrenness if fed to sheep before and during mating (Barry and Reid 1984). However, these problems have now been overcome with the successful selection of red clover cultivars for low formononetin content (<3 g/kg DM; McDonald *et al*. 1994). Whilst high concentrations of iso-flavones in red clover cause reproductive problems in sheep, they are desirable for use in a developing human pharmaceutical industry in Australia and New Zealand, that is being driven by the Novogen Company (Novogen 1999). The iso-flavones are extracted from vegetative red clover and consumed in tablet form as dietary supplements to Western-type diets, which are normally low in iso-flavones, with the objective of reducing pre- and post-menopause problems in women and of reducing prostate enlargement in men. Current research is also focussed on modifying the iso-flavones to produce a range of prescription drugs for the early treatment of cancer, athrosclerosis, hypertension and as anti-flammatory drugs.

VFI of Tagasaste grown in Spain was low and negatively correlated to its concentration of alkaloids ($P < 0.01$; Ventura *et al*. 2000; Table 8). Further study is needed of the secondary compounds in Tagasaste, to establish the direct causes of the summer/autumn depression in VFI.

The shrub legume *Glyricidia sepium* grows widely throughout the tropics and is known to be of high nutritive value, if not for the common observation that stock will not eat it as high proportion of the diet thought to be due to its pungent odour. It is frequently fed as a supplement to low quality roughage diets (Preston and Leng 1987). Leaves of Glyricidia are high in N content (43 g/kg DM) and contain approximately 40 g CT/kg DM; an unusual feature is that most of this CT is protein-bound with the extractable CT level being close to zero (Jackson *et al*. 1996). Therefore analytical methods that measure extractable CT only, will incorrectly classify Glyricidia as not containing CT. Recent investigations (Karda 2000; Norton *et al*. 2000) suggest high concentrations of dihydro-coumarin, rather than coumarin, are associated with low levels of acceptability of Glyricidia leaves by animals but supplementation studies involving adding this compound to the diet are required; binding CT with additions of PEG did not change acceptability.

VFI of immature leaves of the tropical shrub legume *Cratylia argentia* is low and is increased following wilting (Raaflaub and Lascano 1995); possible contributing factors are its contents of hydroxy-coumarins, terpenes and condensed tannins and further research is required.

The tropical grass *Brachiaria decumbens* (signalgrass) contains saponins which have been correlated with a photosensitisation reaction in young grazing cattle in Colombia (Lascano, personal communication), and have been detected in rumen contents of sheep intoxicated through feeding on signalgrass in Malaysia (Lajis *et al*. 1993; Salam Abdullah *et al*. 1992). Relative concentration of saponins in signalgrass is approximately 3.5 times higher than found in *Brachiaria humidicala* and *Brachiaria brizantha* (Lascano, personal communication). A major plant-breeding programme is underway with *Brachiaria decumbens* in Colombia including identification of genotypes that do not contain saponin. Further research is clearly required in this area.

Conclusions

It is concluded that the presence of secondary compounds can have a profound effect upon both the nutritive value and the feeding value of both temperature and tropical forages and that these effects can be beneficial in some instances as well as being detrimental in others. In order to fully understand the effects of these compounds upon grazing animals, it is necessary to develop a knowledge of their chemical structure and reactivity, particularly with proteins, so that an understanding of their mode of action can be determined. From a knowledge of this, together with their effects upon nutrient supply and upon VFI and growth of the grazing animal, it will be possible to define if the concentration of specific secondary compounds in forages should be either decreased or increased in concentration.

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Table 1 - The extractable and bound condensed tannin content of legumes, grasses and herbs fed to ruminants in temperate grazing systems, measured by the butanol- HCI method*.

Forage	**Condensed tannin (g/kg DM)**			
	Extractable	**Protein-bound**	**Fibre-bound**	**Total**
Legumes:				
Big trefoil (*Lotus pedunculatus*)	61	14	1	77
Birdsfoot trefoil (*Lotus corniculatus*)	36	9	2	47
Sulla (*Hedysarum coronarium*)				
Autumn	35	14	2	51
Spring	73	9	2	84
Sainfoin (*Onobrychis vicifolia*)	29			
Red clover (*Trifolium pratense*)	0.4	0.6	0.7	1.7
Lucerne (*Medicago sativa*)	0.0	0.5	0.0	0.5
Grasses:				
Perennial ryegrass (*Lolium perenne*)	0.8	0.5	0.5	1.8
Herbs:				
Chicory (*Chicorium intybus*)	1.4	2.6	0.2	4.2
Sheeps burnet (*Sanguisorba minor*)	1.0	1.4	1.0	3.4

* From Terrill *et al*. (1992*b*); Jackson *et al*. (1996); Hoskin *et al*. (1999).

Table 2 - The effect of condensed tannins (CT) on amino acid digestion in the small intestine of sheep fed *Lotus corniculatus* (22 g CT/kg DM) or *Lotus pedunculatus* (55 g CT/kg DM) with (-CT) or without (+ CT) a continuous intra-ruminal infusion of polyethylene glycol (PEG; MW 3500).

	Lotus corniculatus[1]		***Lotus pedunculatus***[2]	
	+CT	**-CT**	**+CT**	**-CT**
N intake (g N/d)	37.80	37.80	42.40	47.60
N digestibility	0.70	0.78	0.67	0.81
Abomasal NAN flux (g/d)	29.50	25.80	34.00	31.30
Abomasal EAA flux (g/d)	95.60	63.90	121.00	105.60
Apparent EAA absorption (g/d)	58.80	36.10	81.40	83.50
EAA digestibility in SI	0.69	0.65	0.66	0.79
Abomasal NEAA flux (g/d)	68.50	60.00	84.30	77.70
Apparent NEAA flux (g/d)	37.40	41.30	50.80	57.20
NEAA digestibility in SI	0.55	0.69	0.59	0.73

[1] From Waghorn *et al*. (1997)
[2] From Waghorn *et al*. (1994)
N, nitrogen; NAN, non-ammonia-nitrogen; EAA, essential amino acids; SI, small intestine, NEAA, nonessential aminoacids

Table 3 - Voluntary feed intake, liveweight gain, carcass gain and wool growth in lambs (Experiment 1) and dry ewes (Experiment 2) grazing the forage legumes *Lotus corniculatus* (27-34 g CT/kg DM) and lucerne (0.3 g total CT/kg/DM) during summer.

	Lotus		**Lucerne**		**SE**
	CT acting	PEG supplemented	CT acting	PEG supplemented	
	Experiment 1 (1991/92; 27.9 kg LW[1]; 4.5 kg DM/lamb/day[2])				
VFI (kg OM/day)	1.76	ND	1.65	ND	0.04
LWG (g/day)	228	ND	183	ND	8.2
Carcass weight (kg)	20.4	ND	17.8	ND	0.82
Fleece weight (kg)	2.78	ND	2.25	ND	0.091
	Experiment 2 (1994/95; 19.3 kg LW; 5.3 kg DM/lamb/day)				
LWG (g/d)	271	250	ND	ND	8.0
Carcass weight (kg)	21.1	19.8	ND	ND	0.57
Fleece weight (kg)	1.75	1.78	ND	ND	0.067
	Experiment 3 (1992/93); 22.4 kg LW; 2.5 kg DM/lamb/day)				
Rumen ammonia (mg N/1)	255	370	555	535	
VFI (kg OM/day)	1.19	1.20	1.32	1.34	0.056
LWG (g/d)	203	188	185	178	5.8
Carcass gain (g/d)	79	75	68	63	2.9
Wool growth (g/d)	12.1	10.9	10.8	10.2	0.39
	Experiment 4 (1995/96; 54.0 kg LW; 1.3 kg DM/ewe/day)				
Rumen ammonia (mg N/1)	221	278	ND	ND	8.5
VFI (kg OM/day)	1.23	1.20	ND	ND	0.051
LWG (g/d)	54	67	ND	ND	9.3
Wool growth (g/d)	13.2	11.1	ND	ND	0.66

From Wang *et al*. (1996a); Min *et al*. (1998); Douglas *et al*. (1995; 1999)
ND = not determined
[1] Initial liveweight.
[2] Daily green forage allowance.

Table 4 - The effect of grazing ewes on *Lotus corniculatus* or perennial ryegrass/white clover (pasture), and of supplementation with polyethylene glycol (PEG) on maximum ovulation rate and on wool production.

	Experiment[1]	**Pasture**	**Lotus**		Legume effect (%)[2]	CT effect (%)[3]
		+/- PEG	+ PEG	CT-acting		
			(Ovulation rate)			
Min *et al*. (1999)	5	1.33	1.46	1.76	9.8	22.6
Luque *et al*. (2000)	6	1.45	1.66	1.64	14.5	0
Min *et al*. (2001)	7	1.48	1.58	1.79	6.6	14.2
Mean		**1.42**	**1.57**	**1.73**	**10.3**	**12.3**
			(Clean fleece weight; kg)			
Min *et al*. (1999)	5	1.12	1.31	1.35	17.0	3.6
Luque *et al*. (2000)	6	1.54	1.69	1.73	9.7	2.6
Min *et al*. (2001)	7	1.41	1.61	1.71	14.2	7.1
Mean		**1.36**	**1.54**	**1.60**	**13.6**	**4.4**
			(Liveweight gain; g/day)			
Min *et al*. (1999)	5	12	34	40		
Luque *et al*. (2000)	6	-12	-20	-25		
Min *et al*. (2001)	7	43	16	22		

[1] Initial liveweight was respectively 54, 60 and 53 kg in Experiments 5, 6 and 7.

[2] Calculated as $\frac{\text{(Lotus PEG – Pasture) x 100}}{\text{Pasture}}$

[3] Calculated as $\frac{\text{(Lotus CT acting – Lotus PEG) x 100}}{\text{Pasture}}$

Table 5 - The effect of grazing condensed tannin-containing legumes (sulla and *Lotus pedunculatus*) upon the growth and parasite status of anthelmintic drenched (parasite free) and non-drenched (parasitised) lambs. Lucerne was also grazed as a CT-free control legume.

	Lucerne	**Sulla**	**Lotus pedunculatus**
		Experiment 1 (28 days)	
Total condensed tannin (g/kg DM)	1	120	
Liveweight gain (g/day):			
Anthelmintic drenched	263	316	
Non-drenched	28	231	
Faeces egg count (eggs/g):			
Non-drenched	2,220	1,320	
		Experiment 2 (42 days)	
Total condensed tannin (g/kg DM)	2	99	
Liveweight gain (g/day):			
Anthelmintic drenched	184	200	
Non-drenched	-39	129	
Total worm burden:			
Non-drenched	19,268	8,016	
		Experiment 3 (42 days)	
Liveweight gain (g/d):			
Anthelmintic drenched	243	226	232
Non-drenched	121	175	160
Total worm burden:			
Non-drenched	18,084	13,090	23,665

From Niezen *et al*. (1995; 1998).

Table 6 - Impact of dietary condensed tannin (CT) from *Leucaena spp*. on nitrogen and organic matter utilisation in sheep, with or without CT neutralised by polyethylene glycol (PEG).

	L. leucocephala (CT = 73 g/kg DM, fed fresh)		Leucaena KX2 hybrid (CT = 129 g/kg DM, fed as dried leaf)		L. pallida CT = 201 g/kg DM, fed as dried leaf)	
	+ PEG	**CT acting**	**+ PEG**	**CT acting**	**+ PEG**	**CT acting**
Intake, DM or OM (g/d)	984 a	934 a	921 a	896 a	1080 b	595 a
OM digestibility (%)	64.4 a	65.0 a	0.61 b	0.54 a	48.8 b	37.5 a
Nitrogen intake (g/d)	33.5 a	31.7 a	28.3 a	27.9 a	27.7 a	19.5 b
Non-bacterial NAN (g/d)	2.6 a	14.2 a[1]	ND	ND	ND	ND
Microbial N (g/d)	18.5 a	17.6 a	10.5 a	9.7a	5.4a	2.0a
Faecal N (g/d)	7.5 b	11.3 a	8.1 b	15.9 a	11.9 b	19.4 a
N digestibility (%)	77.5 b	64.2 a	71.4 b	43.2 a	57.2 b	0.9 a
N retention (g/d)	10.37 a	7.21 a	4.96 b	3.01 a	-4.5 b	-9.9 a

From McNeill *et al.* (1998), McNeill *et al.* (2000*a*), and Gobius (unpublished)
Across rows and within each forage, means lacking a common superscript differ (P<0.05)
KX2 = *L.leucocephala* x *L. pallida* hybrid
ND = not determined, NAN = non-ammonia N
CT assayed by the rapid Butanol-HCl methodology as modified by Dalzell and Kerven (1998)
[1]P<0.10

Table 7 - Voluntary feed intake (g DM/day), liveweight gain (g/day), and wool growth (mg/cm^2.day) in sheep fed *Acacia spp*. foliage for extended periods.

	CT acting	**PEG supplemented**	**CT effect (%)[1]**
A. aneura, Pritchard et al. (1992), 10 weeks pen fed, CT = 96 g/kg of total DMI			
VFI	368 [a]	655 [b]	-44
LWG	-64 [a]	35 [b]	-283
Wool growth, Period 1	0.30 [a]	0.40 [a]	ns
Wool growth, Period 2	0.18 [a]	0.50 [b]	-64
A. aneura, Miller et al. (1997), 25 weeks grazing, CT intake unknown			
LWG, Period 1	22 [a]	44 [b]	-50
LWG, Period 2	70 [a]	46 [b]	52
Wool growth, Period 1	0.745 [a]	0.809 [b]	-8
Wool growth, Period 2	1.061 [a]	1.075 [a]	ns
A. cyanophylla (=A. saligna), Ben Salem et al. (1999a), 10 weeks pen fed, CT = 25 g/kg of total DMI			
VFI (kg OM/day)[2]	337 [a]	278 [a]	ns
LWG (g/d)	69 [a]	95 [b]	-27

[a,b] Within rows, means with a similar superscript do not differ ($P<0.05$)
ns not significant at (($P<0.05$)
[1] Calculated as (CT acting – PEG supplemented)/PEG supplemented x 100
[2] Intakes are of foliage only; all sheep were also offered 400g barley grain per day

Table 8 - Other secondary compounds present in a range of temperate and tropical forages and their effects upon animals.

Plant	Type of Plant	Secondary compound(s)	Approx. concentration (g/kg DM)	Nutritional effects	Author	Country
			TEMPERATE FORAGES			
Chicory	Herb	Sequiterpene lactones	3.5	Detrimental milk flavour	Barry (1998)	New Zealand
Plantain	Herb	Acubin Catalpol Condensed tannin	22 8 14	Possible anthelmintic properties	Rumball et al. (1997)	New Zealand
Red clover	Forage Legume	Isoflavones	7-14	Adverse effects on reproduction in sheep. Beneficial effects in human nutrition.	Barry & Reid (1984) Novogen (1999)	Australia & New Zealand
Tagastaste	Shrub Legume	Flavones Condensed tannin Alkaloids	50-110 25-50 2-11	Restrict VFI	Edwards (2000) Ventura et al. (2000)	Australia Spain
			TROPICAL FORAGES			
Glyricidia	Legume Tree	Coumarin Dihydro-coumarin Condensed tannin	0.1[1] 7.2[2] 1.2 0.3 70 26	Possible volatile intake repellants	Karda (1999)	Australia
Cratylia argentia	Shrub Legume	Hydroxy-coumarins Terpenes Condensed tannin		Possible intake repellants	Raaflaub & Lascano (1995)	Colombia
Brachiaria decumbens	Grass	Saponins		Photosensitisation in young cattle	Lajis et al. (1993) Salam Abdullah et al. (1992)	Malaysia

[1] Very low acceptability.
[2] Better acceptability.

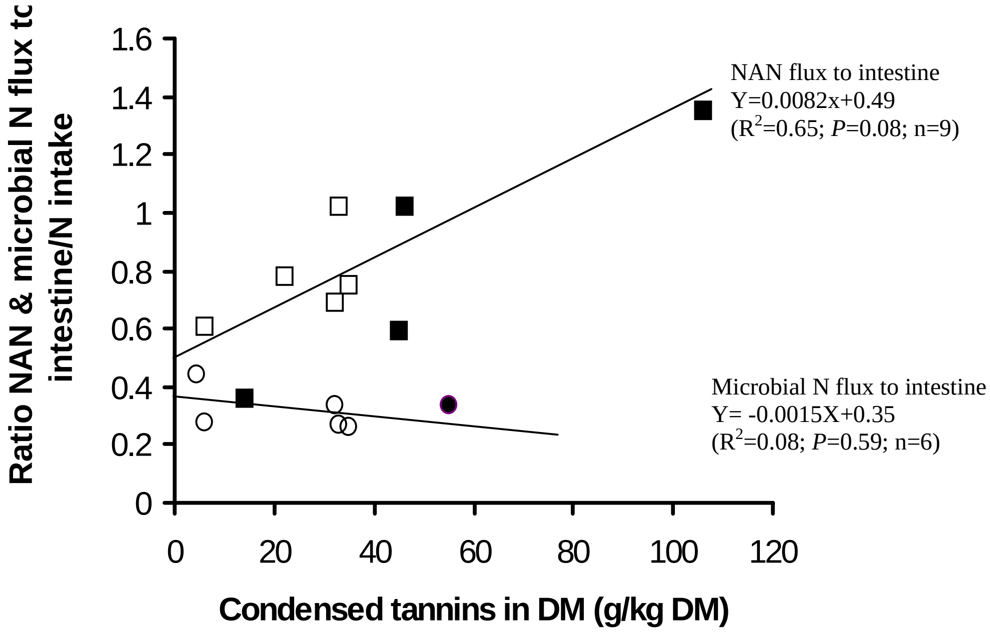

Figure 1 The relationship between condensed tannins concentration in lotus species dry matter (X), and the ratio of non-ammonia-nitrogen (NAN) flowing at the abomasum or duodenum (□, *L. corniculatus*; ■, *L. pedunculatus*) and microbial N (O, *L. corniculatus*; ●, *L. pedunculatus*) per unit of N eaten by sheep. Sources: Barry & Manley 1984; Barry *et al*. 1986; Waghorn *et al*. 1987*a & b,* 1994; McNabb *et al*. 1993; Wang *et al*. 1996*b*.

From Min (1999).

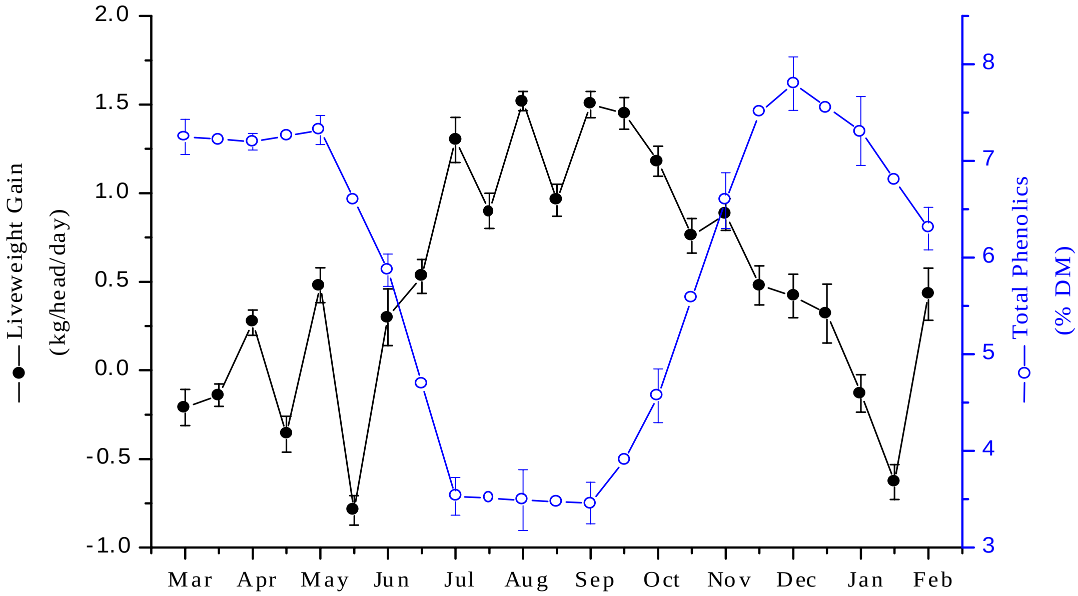

Figure 2 Seasonal fluctuations in the concentration of phenolic compounds in hand-picked portions of the edible leaf and stem material of tagasaste (○) and liveweight performance of cattle grazing that material (●) in 1994/95. Values are means +/- s.e.m.

From Edwards (2000).