Review

Unravelling the conundrum of tannins in animal nutrition and health†

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Abstract: This paper examines the nutritional and veterinary effects of tannins on ruminants and makes some comparisons with non-ruminants. Tannin chemistry per se is not covered and readers are referred to several excellent reviews instead: (a) Okuda T et al. Heterocycles 30:1195–1218 (1990); (b) Ferreira D and Slade D. Nat Prod Rep 19:517–541 (2002); (c) Yoshida T et al. In Studies in Natural Product Chemistry. Elsevier Science, Amsterdam, pp. 395–453 (2000); (d) Khanbabaee K and van Ree T. Nat Prod Rep 18:641–649 (2001); (e) Okuda et al. Phytochemistry 55:513–529 (2000). The effects of tannins on rumen micro-organisms are also not reviewed, as these have been addressed by others: (a) McSweeney CS et al. Anim Feed Sci Technol 91:83–93 (2001); (b) Smith AH and Mackie RI. Appl Environ Microbiol 70:1104–1115 (2004). This paper deals first with the nutritional effects of tannins in animal feeds, their qualitative and quantitative diversity, and the implications of tannin–protein complexation. It then summarises the known physiological and harmful effects and discusses the equivocal evidence of the bioavailability of tannins. Issues concerning tannin metabolism and systemic effects are also considered. Opportunities are presented on how to treat feeds with high tannin contents, and some lesser-known but successful feeding strategies are highlighted. Recent research has explored the use of tannins for preventing animal deaths from bloat, for reducing intestinal parasites and for lowering gaseous ammonia and methane emissions. Finally, several tannin assays and a hypothesis are discussed that merit further investigation in order to assess their suitability for predicting animal responses. The aim is to provoke discussion and spur readers into new approaches. An attempt is made to synthesise the emerging information for relating tannin structures with their activities. Although many plants with high levels of tannins produce negative effects and require treatments, others are very useful animal feeds. Our ability to predict whether tannin-containing feeds confer positive or negative effects will depend on interdisciplinary research between animal nutritionists and plant chemists. The elucidation of tannin structure–activity relationships presents exciting opportunities for future feeding strategies that will benefit ruminants and the environment within the contexts of extensive, semi-intensive and some intensive agricultural systems.

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NUTRITIONAL EFFECTS OF TANNINS

Lowry et al.1 have pointed out the surprising longevity of some commonly held perceptions – even if erroneous – of the harmful effects of tannins. They outlined how the first reviews in the 1960s and 1970s, which covered the effects of plant phenolics on mammals, had treated phenolics as toxic xenobiotics in general, despite reports demonstrating that many phenolics had very low toxicities. Nevertheless, generalisations persist that tannins are harmful or toxic to animals, which is at odds with the fact that humans have enjoyed tannins for thousands of years in drinks and foods.2–4

Beneficial nutritional effects

Tannins occur in many feeds such as fodder legumes, browse leaves and fruits.5–9 Some of these feeds (species of Acacia, Dichrostachys, Dorycnium, Hedysarum, Leucaena, Lotus, Onobrychis, Populus, Rumex and Salix) can produce useful benefits in ruminants, such as better utilisation of dietary protein, faster growth rates of liveweight or wool, higher milk yields, increased fertility, and improved animal welfare and health through prevention of bloat and lower worm burdens.1,10–15 Although tannin structures are chemically very diverse, they have one unifying property: tannins bind proteins. This characteristic is exploited in the production of leather, where tannins crosslink hide proteins. The main benefit of tannins in ruminant nutrition stems from their effect on protein digestion. Some, but not all, tannins can reduce the amount of protein that is digested in the rumen and enhance the amount of protein that is available for digestion in the small intestine.16 This shift in

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Figure 1. Tannins that bind to dietary protein increase the nitrogen flux from the rumen to the small intestine. This process has been referred to as 'ruminal escape protein'.

The extent to which protein digestion is affected depends on the source of the tannins. For example, *Lotus pedunculatus* Cav. tannins reduced the *in vitro* degradation of the major protein in green plants (Rubisco) much more than *L. corniculatus* tannins.21,22 It is likely that the binding strength in tannin–protein complexes is an important factor in ruminal protein digestion.23–25 However, the effect of tannins on bacteria, both in the rumen and in the intestine, may be another factor.26,27 In the presence of *L. corniculatus* tannins, lower concentrations of bacteria were found in the rumen and in the digesta flowing out of the rumen.28 Furthermore, the *L. corniculatus* tannins had a direct effect specifically on the proteolytic bacteria in the rumen.29 However, pre-incubation of *L. corniculatus* tannins with rumen bacteria resulted in more Rubisco degradation than pre-incubation with *L. pedunculatus* tannins.30 It is possible that *L. pedunculatus* tannins are not useful in ruminant nutrition because they inhibit the ruminal degradation of dietary protein too strongly.

**Detrimental nutritional effects**

Although it is timely to concentrate on the useful effects that can be derived from tannin-containing feeds, it must be emphasised that some tannins can also have harmful nutritional effects (N.B. 'Harmful and toxic effects' deals with the toxic effects separately). In fact, tannins were often described in the past as antinutritional factors because they can impact negatively on animal production.1.31–33 Typical responses by ruminants and non-ruminants are lower feed intake, protein and dry matter digestibilities, liveweight gains, milk yield and wool growth.32.34.35

Tannins can render feed constituents less digestible by binding to them. Protein digestibility tends to be reduced most, but carbohydrate, starch and cell wall digestibilities can also be affected.34.36 Typical examples of tannin-containing feeds with low protein digestibilities are *Acacia aneura* F. Muell., *Acacia angustisimia* (P. Mill.) Kuntze, *Acacia cyanophylla* Lindl., *Acacia karoo* Hayne Arzneyk, carob pulp (*Geranion siliqua* L.), fireweed flowers (*Epilobium angustifolium* L.), *L. pedunculatus,* *Robinia pseudacacia* L. and *Sebaniia sesban* L. Merr. (see also Table 1 in 'Appendix').1.2.12.34.35.37–41 Some tannin-containing feeds can result in rather high faecal N excretions, e.g. *A. karoo* and *Leucaena pallida* Britton & Rose.19,41 The origin of this faecal N has not yet been established and may stem from either dietary or endogenous protein sources. Inhibition of digestive enzymes or intestinal micro-organisms could be another cause of lower protein and dry matter digestibilities.37.42

The effects of tannins on lipid digestion were contradictory and may be due to altered ratios of lipolysis to lipogenesis.43 For example, carcass fatness in growing lambs (*Ovis aries* L.) was reduced with a low-tannin *Lotus* diet (20 g condensed tannins (CTs) kg⁻¹),44 while lipid digestibility in adult cockerels...
(Gallus gallus L.) was enhanced by bean hulls (160–210 g CTs kg\(^{-1}\)) but only in high-protein diets.\(^{45}\) Rats (Rattus rattus L.) consuming black locust (R. pseudoacacia) tannins had lower intestinal trypsin and \(\alpha\)-amylase activities but higher lipase activities.\(^{37}\) In contrast, chickens developed fatty livers after being fed sorghum (Sorghum bicolor (L.) Moench) grains.\(^{46}\) Therefore some of the above observations are perhaps explained by the effects of tannins on digestive enzymes, but effects on growth hormone levels have also been recorded.\(^{47}\)

### Structural diversity of tannins in feeds

The study of the nutritional effects of tannins is complicated by their great structural diversity, which has unfortunately been ignored or not sufficiently appreciated in many feeding trials and has led to considerable confusion in the literature.\(^{1,13,48}\) Attempts to attribute nutritional effects to generic tannin classes, e.g. condensed versus hydrolysable tannins (CTs vs HTs), have not proved useful (Table 1), but the perception is still widespread that HTs are toxic in comparison with CTs.\(^{12,49}\) The procyanidins and prodelphinidins (i.e. CTs; Fig. 2) in some species of Lotus, Onobrychis and Calliandra and the ellagitanins (i.e. HTs; Fig. 2) in chestnut wood extract (Castanea sativa Mill.) are beneficial, as demonstrated by higher liveweight gains or milk yields.\(^{12,48,50,51}\) However, the prosetinidins in S. lorentzii (i.e. CTs; Fig. 8(a)), the mixtures of CTs and phenolics in sorghum and the mixtures of CTs and HTs (gallo- plus ellagitanins) in some species of Acacia, Quercus and Terminalia can be harmful or even toxic to ruminants and result in liver and kidney lesions or death.\(^{1,43,52}\)

There is some evidence that slight changes in tannin structures can also produce measurable effects on intakes. For example, snowshoe hares (Lepus americanus Macfarlani) preferred bitterbrush (Purshia tridentata (Pursh) D.C.) or oatmeal treated with bitterbrush tannins (30 g kg\(^{-1}\)) rather than blackbrush (Coleogyne ramosissima Torr.) or oatmeal treated with blackbrush tannins.\(^{53}\) Bitterbrush tannins had higher catechin/epicatechin ratios (55:45) than blackbrush tannins (20:80). However, no other information was provided on the nutritional composition of these two feeds, such as total fibre, lignin or protein contents, which can also affect intakes. In another example a commercially available tannic acid preparation consisting mostly of tri- and tetragalloylglucoses did not decrease protein digestibility in deer (Cervus elaphus L.) or sheep, and no gallic acid was detected in the faeces.\(^{40}\) However, fireweed flowers containing mostly octa- and nonagalloylglycodies reduced protein digestibility, and 27% of the fireweed tannins were found as gallic acid in the faeces.\(^{40}\) We recently investigated two provenances of Calliandra calothyrsus Meissn. Patulul was nutritionally superior to San Ramón; its tannins also had a higher procyanidin/prodelphinidin (PC/PD) ratio (85:15) than San Ramón (30:70 to 37:63)\(^{48,51}\) and a lower degree of galloylation than San Ramón tannins (unpublished data). Similarly, L. coriunlatus has consistently produced better in vivo protein digestion than L. pedunculatus.\(^{12}\) Lotus coriunlatus tannins yield higher PC/PD ratios (67:33 to 79:21)\(^{9}\) than L. pedunculatus (18:81 to 23:77).\(^{54}\) However, before we conclude that PC/PD ratios are therefore likely to affect the nutritive value of tannin-containing feeds, let us consider sainfoin (Onobrychis vicifolia Scop.), which has a very high nutritive value.\(^{55–57}\) The PC/PD ratios in sainfoin tannins\(^{38}\) range from 7:93 to 38:62 and are comparable to the PC/PD ratios of L. pedunculatus, which has a poor nutritive value. This suggests either that the differences in the tannin structures from sainfoin and L. pedunculatus have not yet been identified or that other components in these plants modulate the tannin effects (see also ‘Colorimetric assays’ and ‘Isothermal titration calorimetry (ITC) and tannin solubilities’ (\(K_{ow}\) values')).

### What are optimum tannin concentrations in feeds?

Several authors have advised that dietary concentrations of \(<50\) g CTs kg\(^{-1}\) are beneficial.\(^{1,11,17,59,60}\) These recommendations originated mainly from feeding trials with Lotus species and may not be applicable to other feeds. For example, sulla (Hedysarum coronarium L.) with 72 g CTs kg\(^{-1}\) and sainfoin (O. vicifolia) with 80 g CTs kg\(^{-1}\) had high nutritive values for sheep,\(^{61,62}\) but carob pulp with only 25 g CTs kg\(^{-1}\) depressed growth rates.\(^{39}\) The relatively low concentrations (\(<30\) g CTs kg\(^{-1}\)) in L. leucocephala and S. sesban had no beneficial effects on N balances in Brazilian sheep, because Brachiarica decumbens Stapf has apparently supplied insufficient energy.\(^{63}\) Diets need to balance the energy and protein requirements of ruminants before tannins can exert beneficial effects through ruminal escape protein.\(^{10}\) However, the tropical forage Lespedeza cuneata (Dum. Curs.) G. Don with 5–12 g CTs kg\(^{-1}\) had higher nutritive values than similar forages without CTs.\(^{64}\) Many browses in the tropics and other zones can contain very high levels of tannins or total phenolics (100–500 g kg\(^{-1}\)),\(^{1,5,6,65}\) and Acacia nilotica L. pods are a particularly rich source of flavanol gallate tannins (contents up to 500 g kg\(^{-1}\) have been measured).\(^{66}\) In some parts of the world, ruminants may spend most of their time on such tannin-rich browses,\(^{67}\) which can be useful feeds especially during the dry season.\(^{1}\) Even tannin-rich browses can yield positive N balances and growth rates, which on occasion are comparable to those achieved with legume hay or commercial protein meals.\(^{15,38,51,68–74}\)

When ruminants such as sheep consume the tannin-containing L. corniunlatus, amino acids are absorbed along the entire length of the small intestine.\(^{16}\) However, if these tannins are neutralised by polyethylene glycol (PEG), the majority of amino acids are absorbed in the proximal third. Lotus coriunlatus tends to have
Figure 2. Examples of tannin structures: condensed tannins from fodder legumes (procyanidins, prodelphinidins); hydrolysable tannins (ellagitannins) from chestnut wood (castalagin, vescalagin, castalin, vescalin); taratannins; procyanidin A2 from peanut skins; procyanidins B2, B3 and B5; casuarictin; geraniin; a gallo-tannin from tannic acid, (pentagalloylglucose). Continued on next page.

lower tannin levels than *L. pedunculatus*; but these concentration differences are not sufficient to explain their different nutritional effects. *Lotus pedunculatus* tannins are ‘more potent’ even if diluted below the levels of *L. corniculatus* tannins. Recently, fundamental differences between these *Lotus* tannins were reported that may account for their contrasting nutritional effects. *Lotus pedunculatus* had a tannin fraction of very high

\[ \text{R} = H: \text{procyanidins} \]
\[ \text{R} = \text{OH}: \text{prodelphinidins} \]

\[ R_1 = \text{OH}, R_2 = \text{H}: \text{castalagin} \]
\[ R_1 = \text{H}, R_2 = \text{OH}: \text{vescalagin} \]

\[ R_1 = \text{OH}, R_2 = \text{H}: \text{castalin} \]
\[ R_1 = \text{H}, R_2 = \text{OH}: \text{vescalin} \]

\[ n = 1 - 7 \]

\[ \text{Taratannins} \]

\[ \text{Procyanidin A2} \]

\[ \text{Casuarictin} \]
molecular weight (ca 12 300 Da), but \textit{L. corniculatus} tannins were less than 5300 Da.

Limited evidence indicates that some tannins may disappear in the digestive tract and could even function as nutrients\textsuperscript{70,77,78} because studies by Lowry and Kennedy\textsuperscript{79} suggest that some phenolics can act as mammalian nutrients: quercetin and rutin, but not catechin, were degraded into acetate by a mixed rumen inoculum within the time span of rumen passage.

**Effects of added tannins or tannin-rich supplements**

Attempts have been made to determine the nutritional effects of tannins by adding them to animal diets. Tannins such as quebracho (2’Sprofisetinidins; see Fig. 8(a) below) or tannic acid (Fig. 2) are extracted from wood and are commercially available but are not necessarily representative of the tannins that occur in most animal feeds. Generally speaking, these particular tannins have not produced nutritional benefits\textsuperscript{20,40,80} However, a small number of feed additives such as taratannins (\textit{Caesalpinia spinosa} (Mol.) Kuntze), chestnut tannins, tannin-rich peanut (\textit{Arachis hypogea} L.) skins and tamarind (\textit{Tamarindus indica} L.) seed husks have yielded positive nutritional effects (Table 1). Treatment of soybean (\textit{Glycine max} (L.) Merr.) meal with 100 g taratannins kg\textsuperscript{-1} (Fig. 2) resulted in reduced proteolysis in the rumen and improved daily weight gains, feed efficiencies and N balances in lambs\textsuperscript{81}. Low doses of chestnut tannins significantly reduced ammonia in an in vitro Rusitec fermentation system\textsuperscript{59} they also enhanced (by 5\%) non-ammonia N flow to the duodenum in growing bulls (\textit{Bos taurus} L.) and resulted in higher apparent intestinal protein digestion\textsuperscript{82}. However, 21 g chestnut tannins kg\textsuperscript{-1} added to soybean meal produced no significant effect on voluntary intake, feed conversion, daily gain or length of fattening period when lambs were finished from 15 to 25 kg.\textsuperscript{83} Although there were no signs of histopathological toxicity, raised \(\gamma\)-glutamyl-transferase and aspartate amino-transferase activities will require further investigation. In contrast, a higher level of chestnut tannins (80 g kg\textsuperscript{-1}) sprayed onto a hay diet significantly decreased dry matter (DM) digestibility in sheep (from 63.3 to 57.7\%) and goats (\textit{Capra hircus} L.) (from 63.7 to 60.7\%), but animal liveweight and body condition were not affected over a 10 day collection period\textsuperscript{84}.

Lactating dairy cows fed diets containing 80–160 g peanut skins kg\textsuperscript{-1} (180 g CTs kg\textsuperscript{-1}; Fig. 2) showed higher DM intakes, milk yield and milk fat content and had lower ruminal NH\textsubscript{3} levels and milk protein content. It was concluded that diets containing 160 g peanut skins kg\textsuperscript{-1} were optimal.\textsuperscript{85} Lactating dairy cows also benefited from diets containing 75 g tamarind seed husks kg\textsuperscript{-1} (140 g CTs kg\textsuperscript{-1}). Husk additions significantly increased liveweight gains (by 46\%). They also increased faecal N and decreased urinary N, but the increase in N balance was not significant.\textsuperscript{86}

**Nutritional implications of tannin–protein complexes**

Some of these seemingly discrepant results involving added tannins may well be due to the fact that the binding strengths in different tannin–protein complexes can vary over several orders of magnitude\textsuperscript{87–90} (see also ‘Isothermal titration calorimetry (ITC) and tannin solubilities (K\textsubscript{ow} values)’). An interesting comparison showed that tannins from three \textit{Leucaena} accessions, i.e. \textit{L. leucocephala}, \textit{Leucaena trichandra} (Zucch.) Urban and \textit{L. pallida}, differed markedly in their affinity for protein and that this was related to their effects on protein digestion and N retention in ruminants.\textsuperscript{24} Different binding strengths may also account for the observation that apparently lower CT levels in \textit{A. angustissima} seemed to interfere more with protein use than the higher CT levels in \textit{L. leucocephala}.\textsuperscript{38}
The tannins in *Rumex obtusifolius* L. seem to be particularly ‘active’ – which presumably means strongly protein-binding – because a concentration as low as 2 g tannins kg\(^{-1}\) diet was sufficient to precipitate 50% of the soluble dietary protein and to prevent bloat\(^{91}\) (see also ‘Bloat’). In contrast, the interaction between quebracho tannins and feed protein was so weak that the protein flux to the abomasum was not increased,\(^{20}\) which means that no ruminal escape protein was generated by these tannins (Fig. 1). Current evidence therefore suggests that tannin–protein interactions are optimal only in some feeds, e.g. *Dichrostachys cineria* L., *L. leucocephala*, *L. corniculatus* and sainfoin.\(^{12,15,34}\)

**Salivary tannin-binding proteins**

Tannin-binding salivary proteins comprise proline-rich proteins (PRPs) and histatins (Hsts), which have a strong affinity for tannins.\(^{92,93}\) These proteins are probably the first line of defence by some animal species against dietary tannins; moreover, these proteins tend to precipitate only those tannins that usually occur in their diets, which suggests an evolutionary link between the specificity of these salivary proteins and the dietary tannin structures.\(^{94}\) For example, salivary proteins from moose (*Alces alces* L.) and beaver (*Castor canadensis* Kuhl) only bound linear CTs, which are common in their diets (*Salix* spp., *Betula* spp., *Populus tremula* L.); mule deer (*Odocoileus hemionus* Rafinesque) salivary proteins bound linear and branched CTs and gallotannins, which is consistent with their more generalised diets; and salivary proteins from the omnivorous black bear (*Ursus americanus* Pallas) bound linear and branched CTs and also gallo- and ellagittannins.\(^{94}\) When the authors added, to beaver, deer and sheep diets, only those tannins which were not bound by their salivary proteins, they measured significant reductions in protein digestibilities.

PRPs and Hsts also appear to mediate the effects of tannins on carbohydrate digestion by sparing the enzymes involved in their degradation. PRPs and Hsts protected α-amylase from inhibition by tannic acid.\(^{95}\) However, the effects of tannins on digestive enzymes are not uniform or predictable.\(^{96}\) In an *in vitro* enzyme mixture containing pepsin, trypsin, chymotrypsin and peptidase, tannic acid reduced the hydrolysis of bovine serum albumin (BSA) but increased the hydrolysis of casein, pea (*Pisum sativum* L.) meal, soybean meal and haemoglobin.\(^{96}\)

If tannins are not administered orally, tissue necrosis can result. For example, barium tannic acid enemas produced fatal liver damage in humans.\(^{97}\) Liver and kidney necrosis also occurred after intra-abomasal dosing of sheep and appeared to be caused by free tannic acid in the plasma.\(^{98,99}\) However, if given orally to sheep, tannic acid led to several urinary metabolites (gallic acid, pyrogallol, 4-O-methyl gallic acid) and no detectable levels of tannic acid in the rumen, plasma or urine (N.B. ruminal pyrogallol was significantly correlated with blood methaemoglobin concentrations\(^{98}\)). It is likely that rumen micro-organisms protected sheep from any negative effects of tannic acid, because sheep do not produce salivary proteins.

PRPs are either constitutive or inducible by tannins, depending on the animal species, and occur for example in the saliva of humans, pigs (*Sus scrofa* L.), rats, rabbits (*Oryctolagus cuniculus* L.), goats, deer and bears (*Ursus spp.*),\(^{77,100}\) but the saliva of hamsters (*Mesocricetus auratus* Waterhouse) shows few or no PRPs. As a result, rats are more resistant to tannins than hamsters, and mule deer and goats are more resistant than sheep and cows.\(^{77,101}\) It therefore follows that much less *L. corniculatus* tannin could be extracted from plant material that had been chewed by deer (27%) than by sheep (90%),\(^{11}\) which suggests that salivary proteins from deer bound very strongly to these dietary tannins. Robbins *et al.*\(^{77}\) hypothesised that the presence of PRPs maximised the amount of quebracho tannin that was bound per unit of protein and thus minimised N losses.

A little-known practice that involves the successful use of a tannin-rich feed comes from the Mediterranean region. Iberian pigs (*Sus scrofa meridionalis* De Beaux & Festa) relish acorns and have traditionally been fed extensively during the fattening phase on acorns from several oak species,\(^{102}\) despite the fact that acorns have high levels of galloyl glucoses (see ‘Harmful and toxic effects’).\(^{103}\) The resulting meat is evenly infused with fat, and products fetch very high prices. It is possible that pigs will be protected by their PRPs against these tannins (however, ‘Browse mixtures’ explores an alternative explanation). It may therefore be worthwhile to investigate the potential for other tannin-rich feeds in pig nutrition.

However, not all reports concur with the above hypothesis that salivary proteins are responsible for resistance to tannins. For example, there were no significant differences in protein and carbohydrate digestion between sheep and goats that had been fed tannin-containing *Desmodium intortum* (Mill.) Urb. or *C. calothyrsus*.\(^{104}\)

Another interesting experiment compared the interactions of CTs from *D. intortum* and *L. pedunculatus* and tannic acid with BSA, plant leaf proteins and salivary mucoproteins from sheep and goats.\(^{105}\) Insoluble tannin–protein complexes resulted with BSA and leaf proteins, but soluble complexes with the salivary proteins. This study raised several questions: what effect do other compounds in the digestion matrix have on tannin–protein complexation; is protein digestibility affected by the solubility of the tannin–protein complexes; are tannins in the soluble complexes more readily washed out of the rumen than those in insoluble complexes and how does this affect their post-ruminal digestion?
PHYSIOLOGICAL EFFECTS OF TANNINS

Harmful and toxic effects

The harmful effects of tannins in ruminant and monogastric animals can range from producing chronic or systemic effects (subacute intoxication), e.g. A. angustissima and Quercus coccifera L. leaves, to causing occasional deaths, e.g. A. nilotica fruits, Terminalia oblongata F. Muell., Cedrela toona (L.) D. Don, Quercus spp. leaves and green acorns.102

Many monogastric animal species appear to be more sensitive to tannins than ruminants, but caution is needed when making such generalisations, because the presence or absence of salivary tannin-binding proteins cuts across ruminants and non-ruminants (see ‘Nutritional implications of tannin–protein complexes’).1,34,106 For example, the Iberian pigs in Mediterranean countries ingest per day up to 20 g of gallic and ellagic acid esters and preferentially eat Quercus rotundifolia Lam. acorns, which have higher tannin concentrations than Quercus suber L. acorns, without suffering any organ damage.102,103

Responses to tannic acid were generally negative and resulted in gastric ulceration and hypersecretion of mucus.112 It is, however, worth bearing in mind that feeding trials have been carried out with only a limited number of animal species or diets and may also have been too short-term for adaptation to take place.1,34,45,113 Furthermore, in some experiments the effects of tannins could have been confounded with the effects of other compounds; for example, high-tannin sorghums contain many co-occurring phenolics, such as the relatively rare 3-deoxyanthocyanidins and flavan-4-ols,114 and faba beans (Vicia faba L.) contain lectins and trypsin inhibitors. This might explain why lactating pigs fed sorghum/soybean meal had a poor N balance,115 whereas Iberian pigs thrive on acorns.102,103

Bioavailability of tannins

The evidence for absorption of CTs is still equivocal and is based on findings of higher plasma antioxidant capacity in rabbits which had been fed grapeseed (Vitis vinifera L.) tannins116 and on higher radioactivity in gastric faeces.77 The authors presumed that the quebracho tannins could be absorbed, perhaps as the relatively rare 3-deoxyanthocyanidins and flavan-4-ols,114 and faba beans (Vicia faba L.) contain lectins and trypsin inhibitors. This might explain why lactating pigs fed sorghum/soybean meal had a poor N balance,115 whereas Iberian pigs thrive on acorns.102,103

(ȝ) and that the higher oligomers (Fig. 2) and grapeseed oligomers were neither cleaved into monomers nor absorbed in rats.123 However, small quantities (<1%) of two purified procyanidin dimers, B2 and B5 (Fig. 2), were transferred to the serosal side of an isolated small intestine.124 It is also worth noting that dimeric to hexameric procyanidins from cocoa were cleaved at pH 2 in sodium phosphate buffer solution and also in simulated gastric juice (pH 2) over 3.5 h at 37°C and that the higher oligomers degraded much faster than the lower oligomers.121 These results may help to dispel the view that only ‘hydrolysable’ tannins are readily degraded. It remains to be seen whether these reactions are nutritionally important, as gastric pH values are rarely that low after a meal.

After intragastric administration of oligomeric CTs from apple (Malus domestica Borkh.), dimers to tetramers could be detected within 30 min in rat plasma by liquid chromatography/tandem mass spectrometry (LC/MS/MS).125 It is likely that PRPs would have bound to these tannins had they been fed orally, but not when given intragastrically, and this could have affected their bioavailability. As mentioned above (‘Nutritional implications of tannin–protein complexes’), the fatal liver damage that resulted from barium tannic acid enemas97 could have been caused by direct absorption from the colon of either tannic acid or its metabolites, as they would not have been bound by PRPs or Hsts. To date, there is no evidence for the direct absorption of higher-MW condensed tannins across the intestinal membrane in either ruminants or monogastrics, but evidence for the absorption of punicalagin, an HT, is contradictory (see ‘Metabolism of tannins and phenolics’). It is worth remembering though that only a few studies have so far investigated the bioavailability of a limited range of tannins.

It is interesting that all quebracho tannins could be recovered from the faeces of deer and bears (probably these tannins were complexed by salivary proteins; see ‘Salivary tannin-binding proteins’) but only 75% from sheep faeces.27 The authors presumed that the quebracho tannins had been metabolised in sheep, possibly by the rumen micro-organisms. Indeed, there is considerable evidence that points to tannin degradation by intestinal micro-organisms, and the resulting metabolites might be more bioavailable than the original tannin compounds.126–128 Tannin degradation does not only depend on the presence of micro-organisms. Some tannins are also degraded at physiologically relevant pH values: the procyanidins (CTs) from cocoa (Theobroma cacao L.) and casuarictin, an ellagitannin (ET) (Fig. 2), from strawberries (Fragaria ananassa Duch.) degraded at pH 2 and pH 7–8 respectively into their monomeric constituents,121,129

Metabolism of tannins and phenolics

Several studies have shown that tannins can disappear in the intestinal tract of ruminants and non-ruminants:
(1) Perez Maldonado and Norton detected substantial losses of 14C-labelled CTs from D. intortum in the gastrointestinal tract of sheep and goats; (2) Dépré et al. found that 14C-labelled polymeric procyanidins which had been isolated from the leaves of Salix caprea L. and were free of catechin monomers or proanthocyanidin dimers and trimers were catabolised by human intestinal micro-organisms into phenolic acids; and (3) Abia and Fry concluded that 14C-labelled carob CTs were not inert in the gut of rats – approximately 18% of the CTs had undergone degradation such as depolymerisation and/or metabolism. In contrast, Nakamura and Tonogai concluded that the oligomers of grape seed extract were not absorbed or metabolised by rats.

It is well known that micro-organisms can degrade gallic acid and tannic acid into 4-O-methyl gallic acid, pyrogallol, phloroglucinol and resorcinol (Fig. 3) and that catechin and epicatechin are methylated or conjugated to sulfate esters or glucuronides. Ring fission of catechins produces valerolactones and phenylpropionic acids. Similar degradation products have been reported from Pycnogenol, which is a commercial procyanidin-rich medicinal pine bark extract. This product yielded two urinary metabolites after oral administration to humans: glucuronic acid or sulfate conjugates of δ-(3,4-dihydroxyphenyl)-γ-valerolactone and δ-(3-methoxy-4-hydroxyphenyl)-γ-valerolactone. Similarly, 3H-labelled epigallocatechin gallate fed to rats resulted in 5-(5′-hydroxyphenyl)-γ-valerolactone 3′-O-β-glucuronide as the main urinary metabolite and 5-(3′, 5′-dihydroxyphenyl)-γ-valerolactone as a faecal metabolite (Fig. 4). Ellagitannins from strawberries, raspberries and walnuts were also apparently degraded by human intestinal micro-organisms and excreted as urolithin B-glucuronide (Fig. 5).

Sheep fed tannic acid had glucuronides of resorcinol and 2-carboxy-2′,4′,4,6-tetrahydroxy diphenyl 2,2′-lactone as urinary metabolites; leaves of T. oblongata (containing ETs) also produced these glucuronides plus punicalagin (Fig. 5), which was considered to be a toxic ET degradation product. However, the acute hepatotoxicity and nephrotoxicity in sheep that were ascribed to punicalagin were surprising, because (1) it had been thought that this large compound (1083 Da) would not be absorbed from the gut, (2) relatively large dose rates were required to produce toxic effects in mice (1 mg g⁻¹ liveweight) and (3) a diet containing 60 g punicalagin kg⁻¹ was also not toxic when fed to rats for 37 days, despite the fact that punicalagin and related metabolites such as gallagic acid (Fig. 5) were found in plasma, liver and kidney. Moreover, Cerdá et al. pointed out that pomegranate (Punica granatum L.) juice contains ≥2 g punicalagin L⁻¹ and is consumed by humans.

Figure 3. Microbial degradation products of gallic acid.

Figure 4. Urinary and faecal metabolites of epigallocatechin gallate.
Systemic effects

The toxic effects observed in chickens that had been fed high-tannin sorghum grains were caused by an inhibition of the post-digestive metabolism. The authors concluded that absorption of low-MW polyphenols, which are associated with the tannins in sorghum grain, rather than tannins themselves was responsible for the effects on body tissues and should be viewed as a systemic effect. This caused leg abnormalities in chickens, induction of liver enzymes, higher concentrations of urinary glucuronides, and animal deaths that were too rapid to be caused by reduced nutrient digestibility. A recent review on the health benefits of plant phenolics also suggested that the epidemiology of flavonols and catechins points to a systemic effect, which results from direct absorption of these compounds or their metabolites.

It has been hypothesised that animals can reduce their intakes in order to allow time to detoxify such compounds. Iason and Murray conducted two interesting experiments with compounds that occur naturally in ericaceous plants. They injected orcinol (3,5-dihydroxytoluene; Fig. 6) intravenously at likely physiological concentrations into sheep and found that energy expenditure increased significantly by 5%. When orcinol and quinol (p-hydroxybenzene) were infused together into the rumen at likely dietary concentrations, they observed an increase in urinary energy excretions and a reduction in digestible energy intake, which they attributed to chronic toxic effects in the tissues. Herbivores browsing plants with high concentrations of secondary plant compounds tend to have well-developed detoxification mechanisms, e.g. a large induction response of liver enzymes. Foley et al. suggested the use of detoxification indices such as glucuronic acid or hippuric acid excretions in order to assess the costs of detoxifying different types of phenolic-rich feeds. Although these urinary excretion indices would need to be calibrated against dietary intakes of known plant phenolics and their excretion rates, it would be worth pursuing such a line of enquiry, especially for unconventional feed resources.

Further studies will be needed to elucidate the effects of tannins and their associated low-MW phenolics in a range of feeds, as there are many more
compounds than have been investigated so far and the various consumers possess different digestive systems. It is worth bearing in mind also that not all phenolics produced systemic effects, as some phenolics can act as nutrients.1

TREATMENTS AND FEEDING METHODS TO OVERCOME NEGATIVE EFFECTS OF TANNINS

Addition of supplements

The liveweight losses associated with feeding livestock CT-rich mulga (A. aneura) during drought periods in Australia have traditionally been overcome with mineral supplements containing N, P and S.146 These may assist in the detoxification process and also provide additional nutrients to rumen micro-organisms, as mulga tannins reduced protein digestibility.1

Additions of methionine and choline to chick diets alleviated the toxicity of tannic acid and also the toxicity of CTs or possibly the low-MW phenolics in sorghum (see ‘Systemic effects’),100 presumably because they functioned as methyl donors in the detoxification process and facilitated the excretion of compounds such as 4-O-methyl gallic acid.46 However, methionine additions did not overcome the toxic effects of faba bean tannins in chick diets.147 These discrepancies between sorghum and faba bean diets have not yet been explored.

Addition of tannin-binding polymers

Several polymers such as PEG and polyvinylpoly-pyrrolidone (PVPP) (Fig. 7) bind strongly to tannins and can reduce their antinutritional or toxic effects in vitro and in vivo in rats, rabbits and sheep.1,106,148,149 However, given the fact that some tannins have positive nutritional effects, it is not surprising that there are instances where PEG effects were species-dependent and removed the beneficial tannin effects.15,62,107,150–152 Furthermore, whilst PEG was effective against quebracho tannins, it failed to reverse the negative effects of tannic acid in an in vitro fermentation system (rate and extent of fermentation).153 PEG also failed to correct the negative N balances in sheep fed C. calothyrsus leaves.150

It is therefore not justified to promote the widespread use of a relatively expensive feed additive, as has happened during a drought in the 1990s in southern Africa (personal observation), without prior feeding trials.

Alkaline treatments

Alkaline treatments such as Ca(OH)2, NaOH or wood ash can be very effective in preventing the toxic or antinutritional effects of tannins and/or associated phenolics in the leaves of Quercus stellata Wangenh., Quercus incana Bartr. or C. hirta or in sorghum grain.40,66,108,154,155 The fact that ammonia is just as effective as these metal hydroxides demonstrated that high OH− concentrations, rather than chelating metal ions, were responsible for improving the nutritive value.156 Interestingly, NaOH treatment was more effective with whole than with ground sorghum grain,157 which could be due to the fact that most of the sorghum phenolics are located in the outer pericarp. The nutritive value of L. leucocephala, however, was not improved by urea or wood ash treatments,158 which is not surprising, as these tannins appear to be particularly suited for generating ruminal escape protein.10 It may be concluded that alkaline treatments are probably most useful for overcoming the acute toxic effects of certain – as yet unknown – tannin or phenolic compounds.

Browse mixtures

Lowry159 observed that farmers usually minimise antinutritional problems by feeding leaf mixtures, which dilute or reduce toxic effects. Recent research by just a few authors has indeed shown that mixtures produced less deleterious effects than tanniniferous browses fed as sole feeds.11,160–163 This supports the hypothesis derived from ecological studies78 that the best strategy for herbivores would be to mix diets in order to minimise the energy costs of detoxification. The benefit that may be derived from such an approach to utilising tannin-rich feeds has hardly been explored in animal nutrition.

Silages

Silages prepared from tannin-containing plants tend to have reduced soluble N contents, which improves their feeding value.164–166 Lotus corniculatus silages compared favourably with alfalfa (Medicago sativa L.) and red clover (Trifolium pratense L.) silages for feeding dairy cows.167 Dry matter intakes were similar among these diets, but milk and protein yields were higher for the Lotus silages. Lotus corniculatus silage also achieved a significantly higher N balance in lambs than red clover or alfalfa silage.168

Up to now, few studies have investigated the merits of conserving tannin-rich fodder for dry season feeding in tropical countries. In one study, mixtures of tropical grasses and tree leaves from L. leucocephala or Gliricidia sepium (Jacq.) Walp. were ensiled, resulting in high-quality silages which promoted weight gain in sheep.169 It is worth noting that a significant proportion of the ‘ruminal escape protein’ apparently

Figure 7. Two tannin-binding polymers.
survived the silage fermentation process.\textsuperscript{169} Similarly, Titterton\textsuperscript{170} found that silages containing maize (\textit{Zea mays} L.) stover mixed with tree leaves from species of \textit{Calliandra}, \textit{Acacia}, \textit{Gliciridia} or \textit{Lespedeza} could be used to replace commercial feed supplements for dry season feeding and, in a good year, into lactation without loss of milk yield from dairy cows. Ensiling green tea (\textit{Camellia sinensis} (L.) O. Kuntze) waste with whole-crop oat (\textit{Avena sativa} L.) increased N retention in goats, and the authors also concluded that feed proteins survived the ensiling process and were digested post-ruminally, as the added N did not increase ruminal ammonia, urinary or faecal N.\textsuperscript{171,172}

\textbf{Contribution of sugars or energy}

As mentioned in ‘Systemic effects’, the detoxification of intravenously injected phenolics carried an energy cost.\textsuperscript{144} It is therefore interesting that high levels of sugars can alleviate the antinutritional effects of some tannins. The following tannin-containing feeds have high levels of soluble sugars and/or starch and also a high nutritive value for both ruminants and non-ruminants: fruits from \textit{Gleditsia triacanthos} L. and \textit{Piliostigma thonningii} (Schum.) Milne-Redh., acorns from \textit{Quercus} spp., \textit{Prosopis cineraria} (L.) Druce leaves and sainfoin.\textsuperscript{173,174,175,176}

Although even low concentrations of carob tannins (25 g kg\textsuperscript{-1}) produced growth-depressing effects, the high concentrations of sugars (470 g kg\textsuperscript{-1}) in carob pulp may be responsible for the low but positive lamb growth rates of 48 g day\textsuperscript{-1}.\textsuperscript{39} A good source of energy is likely to assist in the detoxification of some tannins, co-occurring lower-MW phenolics or their metabolites. This could also explain why sugar-rich cactus (\textit{Opuntia ficus-indica} (L.) P. Mill.) fruits, cactus pads and molasses removed the toxic effects of kermes oak (\textit{Q. cocifera}) and increased the nutritive value of several browses.\textsuperscript{177,178} The addition of maize grain to browse leaves may have increased the weight gain of sheep either by balancing energy and protein requirements or by supplying energy for the detoxification process.\textsuperscript{180} This hypothesis is also supported by observations that European roe deer (\textit{Capreolus capreolus} L.) selected diets containing high concentrations of both phenolics (including BSA protein-binding tannins) and soluble sugars.\textsuperscript{181}

\section*{ANIMAL HEALTH AND WELFARE}

\textbf{Parasites}

Although farmers have traditionally used plants for de-worming animals,\textsuperscript{182,183} the evidence that animals use plants for self-medicating purposes is still equivocal.\textsuperscript{184} Much work remains to be done so that traditional knowledge can aid the development of plant-based anthelmintic products that yield consistent results. Samples from different geographical regions have produced variable results,\textsuperscript{183} as the synthesis of secondary plant products can be affected by environmental growing conditions.\textsuperscript{185} \textit{Moringa oleifera}

Lam K. represents an excellent example where the combination of traditional knowledge and scientific study resulted in an improved product for human nutrition.\textsuperscript{186}

Several tropical legumes have also shown some promising results. Grazing of \textit{L. cuneata} forage (50 g CTs kg\textsuperscript{-1}) achieved remarkably high reductions (57–100\%) in faecal egg counts (FECs), total faecal egg output and the numbers of parasitic nematodes (species of \textit{Haemonchus}, \textit{Teladorsagia} and \textit{Trichostrongylus}) in goats.\textsuperscript{64} High levels of dried \textit{A. karoo} leaves, which contain ca 240 g CTs kg\textsuperscript{-1},\textsuperscript{187} also significantly reduced FECs and \textit{Haemonchus contortus} Rud. worm burdens in goats.\textsuperscript{187} In comparison, \textit{A. nilotica} leaves had hardly any effect on FECs despite very high concentrations (ca 400 g kg\textsuperscript{-1}) of catechin gallate tannins.\textsuperscript{5,6,187} However, weight gains on both browse diets were comparable to a commercial goat meal diet after 7 weeks, which illustrates just some of the complexities of this type of research.\textsuperscript{187} Feeding of \textit{Acacia polyacantha} Willd. reduced the FECs of a mixed nematode population by approximately 30\% and the \textit{Oesophagostomum columbianum} Curtice worm burden by 13\% in goats.\textsuperscript{189} The authors also noted that quebracho extracts appeared to be much more anthelmintic than wattle (\textit{Acacia mearnsii} De Wild) extracts \textit{in vivo}. This could stem from either concentration or slight structural differences between the tannins in these commercial extracts (see ‘Methane emissions from ruminants’; Fig. 8). Pregnant goats given free access to tannin-rich browse in Ugandan rangelands over a 6 month period had significantly lower FECs and worm burdens and gained more weight than goats given daily PEG drenches that neutralised the tannins.\textsuperscript{190} Unfortunately, no details were given of the browse plants consumed. Mixtures of browse diets might therefore be useful both to control intestinal parasites and to reduce the antinutritional effects of high-tannin diets (see ‘Browse mixtures’).

Recent research demonstrated that quebracho tannins had a direct anthelmintic effect on the economically important nematode \textit{Trichostrongylus colubriformis} (Giles) Loos in sheep.\textsuperscript{191} Apparently, the direct inhibitory effect of CTs is dependent on their species of origin. The \textit{in vivo} migration of \textit{T. colubriformis} decreased in the following order when exposed to CTs: \textit{Dorycnium pentaphyllum} Scop. > \textit{O. viciifolia} > \textit{L. pedunculatus} > \textit{Dorycnium rectum} (L.) Ser. > \textit{R. obtusifolius} > \textit{H. coronarium} > \textit{L. corniculatus}.\textsuperscript{192} This may explain why \textit{L. pedunculatus} and \textit{H. coronarium} were able to minimise the effects of heavy parasite burdens on animal performance and/or reduce the numbers of parasites in sheep and deer more effectively than \textit{L. corniculatus}.\textsuperscript{11,12}

The diet of lambs had a significant impact on egg hatching and on the development of \textit{T. colubriformis} larvae both in the laboratory and in the field.\textsuperscript{193} Larval recoveries from faeces were significantly lower from lambs fed \textit{D. rectum} and \textit{L. corniculatus} rather than white clover (\textit{Trifolium repens} L.), alfalfa or \textit{H.}}
Parasitic nematodes go through several developmental stages in their life cycles, and an in vitro migration assay, whilst useful as a screening tool, may not predict the more complex outcomes in vivo and amongst different animal species. Ramirez-Restrepo et al. evaluated whether L. corniculatus could be used in an organic sheep-farming system to control intestinal parasites. Lotus corniculatus swards resulted in significantly lower FECs in ewes and lambs, together with higher liveweight gains and wool production, than perennial ryegrass (Lolium perenne L.)/white clover pastures. They concluded that L. corniculatus eliminated the need for pre-lambing anthelmintic treatments. However, FECs increased in lambs after weaning, and the authors therefore suggested further research on integrating other CT-containing forages that are highly active against parasites, e.g. senna, into the grazing system.

These are important findings, because the sheep and goat industries are encountering serious nematode resistance to synthetic anthelmintics, which already goats are currently not widely used by farmers, as their agronomic performance tends to be inferior to that of alfalfa or clover. Nevertheless, modern North American sainfoin varieties have shown good yields, and current research is aimed at improving their agronomic performance in order to develop an economically viable forage legume that benefits animal health.17,55,204

The discovery that BAN genes encode a new enzyme, anthocyanidin reductase, has led to a significant breakthrough in our understanding of CT synthesis in plants. This enzyme converts cyanidin to (−)-epicatechin, which is an important chain extension unit in many CTs. Transgenic expression of BAN in tobacco resulted in CT accumulation in the petals. CT biosynthesis occurred also in alfalfa forage after transformation with a maize anthocyanin regulatory gene. Therefore it should now be possible to develop new CT-containing plants that benefit animal health and welfare by preventing bloat and reducing the worm burdens in ruminants. Although it is not yet known which particular tannin molecules or what molecular properties are responsible for preventing bloat, reducing nematode mobility or lowering parasitic worm burdens, plants with defined tannin compounds will be most useful for elucidating the structure–activity relationships. Molecular biology could play a useful role by creating designer plants to probe these relationships. Such information will be of great use in future transformation studies of tannin-containing plants with improved nutritional and veterinary properties.

**ENVIRONMENTAL EFFECTS**

Ruminants are important producers of greenhouse gases such as ammonia and methane. The proposed imposition of tariffs to mitigate greenhouse gas emissions will affect the agricultural industry, and therefore dietary options to reduce such emissions are currently being investigated.

**Nitrogen emissions from ruminants**

The digestion of high-quality forages is relatively inefficient in ruminants and results in large ammonia losses from the rumen (20–35% of dietary N), most of which is excreted as urea in urine (Fig. 1). Similarly, a grass/alfalfa pasture with 10% dock (R. obtusifolius), or 2 g tannins kg⁻¹ in the diet, also prevented bloat. It is therefore suggested that the contributions by tannin-containing ‘weeds’ to ruminant welfare and bloat prevention should be considered.

However, some tannin-containing fodder legumes such as sainfoin are currently not widely used by
Methane emissions from ruminants

It is well known that low-quality feeds account for ca 90% of the world’s methane production by ruminants. Van Soest\textsuperscript{210} therefore pointed out that manipulation of cattle diets with high nutritive value in intensive farming systems would have little effect on worldwide methane outputs. Recent research, however, has provided some interesting data on how tannin-containing diets can reduce methane emissions from ruminants. Freshly fed \textit{L. cuneata} (180 g CTs kg\textsuperscript{-1}) resulted in lower methane emissions, expressed as either quantity per day (7.4 vs 10.6 g day\textsuperscript{-1}) or relative to dry matter intake (6.9 vs 16.2 g kg\textsuperscript{-1} DM intake), compared with a diet containing \textit{Digitaria ischaemum} (Schreb. ex Muhl) and \textit{Festuca arundinacea} (Schreb.) Schreb. \textit{ex} Muhl and \textit{Festuca arundinacea} Schreb.\textsuperscript{216} Similar results were obtained with fresh tannin-containing \textit{L. pedunculatus} and goat willow (\textit{S. caprea}), which reduced methane emissions from the rumen by 16–20% per unit intake.\textsuperscript{217,218} Silage from \textit{L. corniculatus} also reduced methane emissions per unit intake (g CH\textsubscript{4} kg\textsuperscript{-1} DM intake) by 23% and per unit production (g CH\textsubscript{4} kg\textsuperscript{-1} milk solids) by 13%.\textsuperscript{209} The question remains whether lower methane losses also result in lower energy losses from ruminants.\textsuperscript{12}

Roth \textit{et al.}\textsuperscript{219} compared quebracho (CTs), mimosa (\textit{A. mearnsii}; CTs) and chestnut (ETs) tannins for their effects on \textit{in vitro} gas production, methane production and digestibility. They chose relatively high concentrations of tannins (90–440 g tannins kg\textsuperscript{-1}), and their results suggested that appropriate doses of tannins would reduce methane production but not digestibility. It remains to be seen whether the small effect of the quebracho tannins was due to a low tannin concentration in the extract or to the structural differences that exist between the proflavetinidins in quebracho and those in mimosa extracts. Mimosa proflavetinidins are characterised by 2\textit{R} stereochemistry, a preponderance of 4 \textit{→} 8 links to the terminal catechin and a 3,4-\textit{trans} configuration (Fig. 8); quebracho proflavetinidins are characterised by 2\textit{S} stereochemistry, a higher proportion of 4 \textit{→} 6 links to the terminal catechin and a 3,4-\textit{cis} configuration (Fig. 8).\textsuperscript{220,221} Furthermore, mimosa tannins have a higher degree of hydroxylation and quebracho tannins have a lower water solubility.\textsuperscript{222}

A greater use of traditional tannin-containing feeds such as species of \textit{Lotus}, \textit{Onobrychis}, \textit{Salix} and \textit{Gleditsia}, \textit{Quercus} spp. acorns, etc. could therefore assist in the move from intensive to more extensive feeding systems in temperate countries, not only by improving the efficiency of nutrient utilisation but also by reducing the need for anthelmintics.\textsuperscript{12,102,223}

**ANALYSIS OF TANNIN MIXTURES AND PREDICTION OF ANIMAL RESPONSES**

**Colorimetric assays**

Tannins can be detected after producing a colour with several different reagents. The Prussian blue, Folin–Ciocalteu, HCl/butanol and vanillin methods are the most widely employed, but the use of 4-dimethylaminocinnamaldehyde and rhodanine has also been advocated.\textsuperscript{224–226} The Folin–Ciocalteu reagent reacts with phenolic groups in general to produce a blue colour, but is therefore not specific for tannins. The HCl/butanol assay cleaves condensed tannins oxidatively and yields brightly coloured anthocyanidins. However, this reaction is not quantitative and colour yields are dependent on inter-flavanoid linkages (4 \textit{→} 8 vs 6 \textit{→} 8 bonds; \textit{A- vs B-type} tannins) and the presence or absence of 5-OH groups.\textsuperscript{227} This explains for example why quebracho tannins (no 5-OH groups) yield less colour than \textit{Lotus} tannins (with 5-OH groups). Schofield \textit{et al.}\textsuperscript{225} and Mueller-Harvey\textsuperscript{226} reviewed in detail several methods for quantifying condensed and hyrolysable tannins and suggested that, given the limitations of current tannin assays, more than one assay be employed concurrently. Moreover, feeds can contain several different types of tannins (CTs, GTs, ETs), which cannot be measured adequately by a single assay. The statement by Lowry \textit{et al.}\textsuperscript{1} that ‘the simplicity of absorbance measurements masks the problems of extracting meaningful data’ cannot be emphasised strongly enough. Not surprisingly, results from simple colorimetric assays have not proved particularly useful for predicting the nutritional responses to tannin-containing feeds.

One of the most important steps in quantifying tannins depends on the use of appropriate standards.\textsuperscript{228} Stewart \textit{et al.}\textsuperscript{48} showed that the best approach is to prepare a separate crude tannin standard for each plant accession. Even the use of a standard prepared from a different accession of the same species can be misleading: a standard based on tannins from \textit{C. calothyrsus} of San Ramón provenance would have significantly underestimated the tannins in \textit{C. calothyrsus} of Patulul provenance. Simple solvent extractions are one possible approach for isolating crude tannin mixtures from plants.\textsuperscript{229} Chromatography on Sephadex LH20 is another widely used method for preparing crude tannin mixtures, which can then be used as standards in colorimetric assays.\textsuperscript{48} However, two recent papers\textsuperscript{76,230} demonstrated that the usual procedure of eluting tannins from Sephadex LH20 with acetone/water (7:3) missed the higher-MW fractions of \textit{L. pedunculatus} and \textit{Dorycnium} spp. tannins, which could be eluted instead with water or methanol/water (1:1). It would appear that the ability of Sephadex...
LH20 to operate partially in a size exclusion mode has been overlooked for many years.\textsuperscript{230,231}

The composition of these tannin mixtures can be characterised by MALDI-TOF-MS\textsuperscript{9,119,120,232,233} or electrospray ionisation mass spectrometry (ESI-MS).\textsuperscript{76,230,234,235} Normal phase, reverse phase and gel permeation high-performance liquid chromatography (HPLC) are suitable for separating and quantifying
individual compounds. These techniques are invaluable for identifying the main features of different tannin mixtures and are a necessary complement to colorimetric assays when attempting to relate the effects of tannins to in vivo responses.

### Protein precipitation assays

Several techniques have been devised to determine tannin concentrations by measuring the amount of protein they precipitate. Another assay, the so-called radial diffusion assay, is also based on protein precipitation and is suitable for screening large numbers of samples. Tannins are placed in the centre of an agar plate which contains BSA as the protein. As tannins diffuse outwards, they precipitate BSA, and the diameter of the resulting ring is measured and compared with diameters obtained with known tannin standards.

Much work remains to be done in order to unravel which particular factors influence most the digestion of proteins in tannin-containing plants. Relevant factors could be (1) the total quantity of protein that is precipitated by tannins during digestion, (2) the astringency of a tannin, i.e. unit of protein bound per unit of tannin, or (3) the binding strength in protein–tannin complexes. Tannins and proteins can bind via two distinctly different mechanisms, i.e. hydrophobic interactions and hydrogen bonding. Both types of interactions are likely to occur in dietary tannin–protein complexes, but their relative importance has not yet been elucidated. Hagerman et al. discovered two model tannins, each of which bound predominantly via one of these two mechanisms to BSA: the non-polar pentagalloylglucose (PGG; Fig. 2) formed a hydrophobic coat around the protein, whereas a polar epicatechin polymer, epicatechin16 (4 → 8) catechin, formed hydrogen-bonded crosslinks between protein molecules. The epicatechin polymer was more efficient than PGG at precipitating BSA on a molar or mass basis, and the epicatechin polymer–BSA complex was also much more stable than the PGG–BSA complex. Similarly, the profisetinidin CTs from quebracho interacted with salivary histatins by a different mode than PGG.

Although it has been known for some time that the binding strengths in different tannin–protein complexes can vary over orders of magnitude, surprisingly few studies have related the protein-binding abilities of tannins to in vivo protein digestibilities. Robbins et al. concluded that the reduction in protein digestibility in wild ruminants was proportional to the BSA protein-precipitating capacity of plant tannins. Osborne and McNeil came to a similar conclusion, as L. leucocephala tannins have a weaker BSA-binding capacity than L. pallida tannins and are nutritionally superior. An interesting recent study by Andarbi et al. compared two techniques which used either 15N- or 125I-labelled protein–CT complexes for in vivo assessments of post-ruminal protein digestibilities. The 125I technique proved more precise and was capable of detecting differences in protein digestibilities. These digestibilities were correlated with CT astringency values, which measure the amount of protein precipitated by CTs on a g g⁻¹ basis.

It remains to be seen (1) whether such distinctly different interaction mechanisms also occur between naturally occurring mixtures of tannins and nutritionally relevant proteins and (2) whether these two types of binding interactions (hydrophobic versus hydrogen bonds) and the binding strengths in the resulting complexes have any bearing on protein digestion, tannin toxicity or bioavailability in ruminant and non-ruminant feeds. An interesting proposition was made by Wróblewski et al., who suggested that the much weaker interaction of epigallocatechin gallate (compared with pentagalloyl glucose) with salivary Hsts may allow its uptake into the body and be related to the reported health benefits of tea.

It is questionable whether the widely used BSA is a suitable model for the fraction I protein (Rubisco), which accounts for 32–40% of protein in forage leaves. A protein precipitation technique that used Rubisco instead of BSA was unable to differentiate between the nutritional properties of Lotus, sainfoin, sulla and dock tannins. The authors concluded that measurements of Rubisco degradation rather than precipitation may be more relevant to ruminant nutrition. Rubisco consists of large and small subunits (LSUs, SSUs) and, in the absence of CTs, rumen micro-organisms degraded the LSUs much more rapidly than the SSUs. CTs inhibited LSU degradation much more than SSU degradation. Interestingly, Tanner et al. also found that there were only small differences between the inhibitory effects of CTs from species of Dorycnium, Hedysarum, Lotus and Onobrychis. However, if the CTs were first pre-incubated with rumen fluid, LSU degradation was affected much more by the L. pedunculatus than the L. corniculatus CTs, which suggested that the L. pedunculatus tannins were more inhibitory to proteolytic bacteria. Differential binding of tannins to rumen micro-organisms could well be another important factor in protein digestion.

Tannin-resistant bacteria tend to secrete an extracellular polysaccharide and form a thick glycoprotein, which has a high affinity for tannins. Tannins from several tropical forages (Arachis pintoi Krapov & W.C. Greg., Desmodium ovalifolium (Schum.) Walp., G. sepium and Manihot esculenta Crantz) precipitated different amounts of BSA compared with alfalfa leaf protein in a radial diffusion assay which had been modified to mimic rumen conditions (pH 6.8, 39 °C). In the same assay, quebracho tannins and tannic acid precipitated BSA but not alfalfa leaf protein. The authors concluded that measures of ‘biological tannin activity’ based on this particular BSA precipitation assay might not reflect the ability of tannins to precipitate plant proteins from herbivore diets.
Another point to remember is that complexation between tannins and proteins can lead to both soluble and insoluble complexes. Soluble complexes are likely to be washed out of the rumen more quickly than insoluble complexes, and therefore both types of complex formation will be relevant to animal nutrition, but only insoluble complexes will be estimated in an assay based on protein precipitation.

**Isothermal titration calorimetry (ITC) and tannin solubilities (K\text{ow} values)**

We recently probed tannin–protein interactions by isothermal titration calorimetry (ITC). ITC measures the amount of energy that is released when tannins are slowly titrated into a protein solution. The ITC protein-binding curves exhibited higher affinities for myrabolan (*Terminalia chebula* Willd.) and *A. nilotica* tannins. The binding curve of *D. cinerea* tannins with gelatin was sigmoidal, suggesting a highly specific interaction. In contrast, the *A. nilotica* tannins bound via a two-stage process: the first stage was specific interaction. In contrast, the *D. cinerea* tannins bind non-specifically and relatively weakly, whereas tannins with lower *K\text{ow}* values bind specifically and more strongly to proteins and improve the efficiency of N utilisation in ruminants (Table 2). *K\text{ow}* values in the literature appear to support this hypothesis. PGG (Fig. 2) has higher *K\text{ow}* values (ranging from 32 to 129 depending on the experimental conditions) and is an important constituent of tannic acid, which can be toxic. However, castalagin has a lower *K\text{ow}* value (0.1) and is the major constituent of chestnut tannins, which are approved as feed additives in Switzerland. It is of note that the ranges of known *K\text{ow}* values for HTs and CTs overlap: values for HTs range from 0.0001 to 100 and values for CTs from 0.002 to 0.13. This fact may indicate why classifying tannins into CTs and HTs is not helpful for predicting animal responses.

Techniques based on protein precipitation, ITC and *K\text{ow}* values allow the study of real-life, complex tannin mixtures. The author would like to propose that these techniques be evaluated in animal-feeding trials in order to identify the characteristics of desirable and safe tannins.

**CONCLUSIONS AND OUTLOOK**

Plants tend to produce complex mixtures of tannins, and not all tannins have the same effects. Digestion of tannin-rich feeds appears to be facilitated by co-feeding with a ready source of energy to support the detoxification of any associated phenolic compounds, or by feeding mixtures rather than single species of high-tannin materials. The focus of this review has been on tannin–protein interactions and their effects on protein digestion and animal health. Caution is needed when attributing physiological effects to tannins: in the case of sorghum grain, other compounds rather than the tannins are probably responsible for the observed toxic effects. A similar situation may exist in *T. oblongata*, but this has not yet been proven beyond doubt. Although not covered in this review, one also needs to bear in mind that the digestive tract is a complex system which contains micro-organisms and many different matrices that may interact with tannins and thus modulate their effects.

Tannins in *L. corniculatus*, *L. leucocephala*, *O. viciifolia* and *D. cinerea* are particularly effective at improving protein digestion in ruminants and appear to interact in an optimal fashion with feed proteins. The underlying mechanisms for their superior effects require further study. To summarise, there is some evidence that the following structural features of tannins affect the nutritive value of feeds: procyanidin/prodelphinidin ratios; degree of galloylation; molecular weights; and binding strengths in tannin–protein complexes. It is also of note that tannins interact with proteins via either hydrophobic or hydrogen bonds or via a combination of these two distinctly different binding mechanisms. It remains to be shown which of these mechanisms is more important in terms of animal nutrition or health.

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**K\text{ow}-value**

\[ K\text{ow-value} = \frac{\text{Concentration in octanol}}{\text{Concentration in water}} \]

Higher *K\text{ow}* values indicate that a molecule is more ‘fat-soluble’ than ‘water-soluble’ and that it may be absorbed into body tissues. Tannins with higher *K\text{ow}* values are therefore more likely to be absorbed and to exert physiological, i.e. toxic or medicinal, effects. Indeed, this seems to be the case with the partially galloylated CTs from Chinese rhubarb (*Rheum palmatum* L.), which are substantially insoluble in water but highly soluble in organic solvents. Tanaka *et al.* also found that hydrophobic interaction of these galloylated CTs with rhein 8-O-glucoside, the major anthraquinone glycoside in rhubarb, significantly increased their water solubility and may assist in the transfer of these bioactive CTs from the food matrix to the absorbing body tissues. These results also demonstrate that co-occurring matrix compounds will influence the nutritional or medicinal effects of tannins.

It is conceivable that toxic tannins have higher *K\text{ow}* values and bind non-specifically and relatively weakly, whereas tannins with lower *K\text{ow}* values...
It could be argued that tannins that interact predominantly via hydrogen bonds form stronger complexes with proteins (e.g. dietary or salivary proteins) and are therefore more likely to generate ruminal escape protein. Circumstantial evidence suggests that there is an optimum interaction between certain tannins and feed proteins, i.e. neither too strong nor too weak an interaction. Conversely, tannins that interact via hydrophobic bonds form weaker complexes with proteins and may therefore be more easily dissociated from such complexes, resulting in their metabolism in the digestive tract or in medicinal, veterinary or toxic physiological effects. This and alternative hypotheses need to be scrutinised, e.g. the differential effects of tannins on proteolytic rumen micro-organisms. One of the keys to elucidating the biological effects of tannins depends on understanding which diets produce high levels of urinary phenolics or increased faecal N. Does faecal N originate from dietary protein, bacterial cells or secreted proteins, animal tissue protein or salivary proteins? How important is the fact that some tannins shift the site of amino acid absorption along the entire length of the small intestine? Feeds with well-defined compositions of tannins, proteins and fermentable carbohydrates would allow us to evaluate the structure–activity relationships of the tannin–protein hypothesis and also to test alternative hypotheses.

Preliminary evidence suggests that the strength of interactions between tannins and proteins is related to their solubilities in solvents such as water and octanol and that the resulting $K_{ow}$ values may be of use in predicting these interactions. It is also of note that the $K_{ow}$ values do not distinguish between CTs and HTs, which could explain why the classification into CTs and HTs has not been helpful for predicting animal responses. If true, this could explain why quebracho tannins, which have lower water solubilities than the closely related mimosa tannins (or higher $K_{ow}$ values), have only very weak interactions with dietary proteins but have better anthelmintic properties. Similarly, it has been suggested that the weak interaction between epigallocatechin gallate (compared with pentagalloylglucose) and salivary histatins may account for the health benefits of green tea. The reader will notice from Table 2 that some contradictions or unresolved issues still exist with these arguments. This review has attempted to unravel several facets of the tannin conundrum, but further research is needed, especially at the interface between tannin chemistry and in vivo effects.

Tannins form a highly diverse group of natural products with promising nutritional, veterinary and environmental effects. Current research focuses in particular on their anthelmintic properties and on their ability to improve the efficiency with which nutrients are used by ruminants in order to reduce environmental losses of methane and, more importantly, nitrogen. However, progress is hampered by a lack of suitable analytical techniques that are capable of predicting these biological effects. The establishment of structure–activity relationships and the development of meaningful tannin assays would be aided by experimental plant models with altered tannin compositions. Any newly developed tannin assays will assist plant breeders and molecular biologists with the screening of plant germplasm for the benefit of animals and the environment.

ACKNOWLEDGEMENTS
I am grateful to Professor PJ Van Soest and an anonymous reviewer for their very helpful comments.

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APPENDIX

Table 1. Summary of the available information on feeds with tannins and their nutritional or veterinary effects

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Tannins</th>
<th>Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia brevispica</td>
<td>17% Ph; PD</td>
<td>Good N retention by sheep; good growth rates by calves</td>
<td>6,71</td>
</tr>
<tr>
<td>Acacia karoo</td>
<td>24% CT; sulfate esters of 4-O-β-D-glucopyranosyl gallic acid</td>
<td>High faecal N, low N retention (goats); anthelmintic</td>
<td>41,187,259</td>
</tr>
<tr>
<td>Acacia nilotica</td>
<td>30–49% Ph (mostly catechin gallates)</td>
<td>Valuable browse, but low intakes (sheep, goats, cattle)</td>
<td>1,5,6,41,188,260</td>
</tr>
<tr>
<td>Acacia nilotica</td>
<td>76% Ph; epigallocatechin-7-gallate; epigallocatechin-5,7-digallate</td>
<td>Low intakes: weight loss; can be toxic at high intakes (goats)</td>
<td>25,109,261–265</td>
</tr>
<tr>
<td>Acacia tortilis</td>
<td>15–41% Ph; epigallocatechin-7-gallate; epigallocatechin-5,7-digallate</td>
<td>Sheep and calves: good growth rates, better N retention than on alfalfa</td>
<td>71,265</td>
</tr>
<tr>
<td>Arachis hypogaea</td>
<td>18–24% PC: A- and B-type bonds; proanthocyanidins A-1, A-2;</td>
<td>&lt;16% peanut skins in diet is useful for dairy cows: increased dry matter intake, milk yield and % milk fat, but lower % milk protein</td>
<td></td>
</tr>
<tr>
<td>Calliandra calothyrsus</td>
<td>12–36% CT; PC/PD ratios</td>
<td>Contradictory results: negative or low N balance; did not increase weight gains, but adding PEG increased digestibility and wool growth; useful feed (lambs, goats, cattle); increasing milk production in dairy cows and goats; increased growth rates of goat kids; anthelmintic in lambs</td>
<td></td>
</tr>
<tr>
<td>Camellia sinensis</td>
<td>CE; GE; epicate gallate; epigallocate galate</td>
<td>Stops diarrhoea in calves; improves intestinal microflora balance; prevents digestive diseases</td>
<td>198,273,274</td>
</tr>
<tr>
<td>Castanea sativa</td>
<td>7% castalin/vescalin; 30% castalagin/vescalagin; 3% PGG</td>
<td>2% tannins: no toxic effects (lambs); 8% tannin sprayed onto hay: no hepatic damage; improved N supply to duodenum (bulls); treatment against diarrhea (pigs, cattle); reduced ammonia emission from urine and from stored manure from lactating cows</td>
<td></td>
</tr>
<tr>
<td>Ceratonia siliqua</td>
<td>PC, PD dimers, trimers; highly galloylated GE; hexose and pentose gallates</td>
<td>CT were modified in rat gut; 2.5% carob pulp tannins produced low growth rates (lambs), negative effects on digestibility despite high sugar and pectin contents (47%); 0.9% tannin diet had no negative effect on weight gain</td>
<td></td>
</tr>
<tr>
<td>Ceratonia siliqua</td>
<td>7–29% CT</td>
<td>Goats: no toxic effects</td>
<td>39,130,174,277</td>
</tr>
<tr>
<td>Desmodium ovalifolium</td>
<td>18% CT; A- and B-type bonds</td>
<td>Ruminal escape protein</td>
<td>279–281</td>
</tr>
<tr>
<td>Dichrostachys cinerea</td>
<td>15–20% CT; PC/PD ratios</td>
<td>Ruminal escape protein (goats)</td>
<td>15,232,263</td>
</tr>
<tr>
<td>Dorycnium spp.: leaves</td>
<td>13:87; MW 3300–35 600</td>
<td>Anthelmintic (in vitro and in vivo)</td>
<td>192,193,230,282</td>
</tr>
</tbody>
</table>

(continued overleaf)
### Table 1. Continued

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Tannins</th>
<th>Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gleditsia triacanthos</em> (honey locust): fruits</td>
<td>5.4% CT; sulfate ester of 4-O-β-D-glucopyranosyl gallic acid</td>
<td>High, avid intakes by sheep; not toxic; sugar-rich</td>
<td>102,259,283</td>
</tr>
<tr>
<td><em>Hedysarum coronarium</em> (sulla)</td>
<td>7% CT</td>
<td>Anthelmintic</td>
<td>62,64,284</td>
</tr>
<tr>
<td><em>Leucaena leucocephala</em></td>
<td>1–5% CT</td>
<td>Ruminal escape protein; good liveweight gains</td>
<td>10,19,24,270,285,286</td>
</tr>
<tr>
<td><em>Lespedeza cuneata</em> (Sericea lespedeza)</td>
<td>5–18% CT; mostly PD; MW 14 000–20 000</td>
<td>High-tannin variety: lower methane emissions; anthelmintic</td>
<td>64,216,284,287</td>
</tr>
<tr>
<td><em>Lotus corniculatus</em></td>
<td>PC/PD ratios 67:33 to 84:16; CE as terminal units; GE as extender units; mostly 4 → 8, some 4 → 6 interflavan linkages; small amounts of epicatechin monomers and glucose in CT; DP 2–44 (MW &lt; 12 300)</td>
<td>Ruminal escape pass protein, better in vivo protein digestion than <em>L. pedunculatus</em>; anthelmintic</td>
<td>7,9,12,64,76</td>
</tr>
<tr>
<td><em>Lotus pedunculatus</em></td>
<td>PC/PD ratios 19:81 to 23:77; CE and GE occur in terminal and extender units; small amounts of glucose in CT; 4 → 8 interflavan linkages; DP 2–44 (MW &lt; 12 300)</td>
<td>Ruminal escape protein, but less amino acids absorbed in lower intestines compared with <em>L. corniculatus</em>; anthelmintic</td>
<td>8,12,54,64,76,284</td>
</tr>
<tr>
<td><em>Onobrychis viciifolia</em> (sainfoin)</td>
<td>PC/PD ratios 7:93 to 38:62; mostly epicatechin; cis/trans ratios 67:33 to 86:14</td>
<td>Ruminal escape protein; high growth rates; high N retention; anthelmintic</td>
<td>58,64,288,289</td>
</tr>
<tr>
<td><em>Prosopis cineraria</em>: leaves</td>
<td>6–12% CT</td>
<td>Some weight gain, but addition of PEG increases weight gain</td>
<td>176</td>
</tr>
<tr>
<td><em>Quercus robur</em> L. (pedunculate oak): leaves</td>
<td>Pedunculagin, vescalagin, castalagin; CT (but no PGG)</td>
<td>Excellent for growing and finishing Mediterranean pigs and ruminants</td>
<td>102,103,290</td>
</tr>
<tr>
<td><em>Quercus semecarpifolia Smith</em> (Himalayan evergreen)</td>
<td>4% tannins</td>
<td>Positive N retention, weight gain (goat kids)</td>
<td>291</td>
</tr>
<tr>
<td><em>Quercus gambelii</em> Nutt. (gambel oak)</td>
<td>80% oak diet with 9% tannins</td>
<td>Intake depressed, but no toxic effects (goats)</td>
<td>68</td>
</tr>
<tr>
<td><em>Rumex obtusifolius</em> (dock)</td>
<td>PC: 2,3-cis-flavan units; epicat as extender units; some galloyl groups</td>
<td>Prevents bloat</td>
<td>249</td>
</tr>
<tr>
<td><em>Salix caprea</em> (goat willow)</td>
<td>Adult tree: PC/PD ratio 10:1; PC consist only of cat units. Young tree: PC/PD ratio 6:23; cat and gallocat units</td>
<td>Reduces ruminal methane emissions</td>
<td>218,295,296</td>
</tr>
<tr>
<td><em>Salix viminalis</em> L. (<em>osier willow</em>), <em>S. matsudana</em> Koidz × <em>alba</em> L. (<em>tree willow</em>)</td>
<td>8–7% CT in leaves</td>
<td>Useful during droughts to lessen weight loss of cattle; improved reproductive performance of sheep</td>
<td>14,223,297</td>
</tr>
<tr>
<td><em>Schinopsis lorentzii</em> (quebracho): wood</td>
<td>Profisetindins; DP 6.5</td>
<td>4% added CT – lower feed intake, N retention, body fat deposition (rats); 5% added CT – ulceration, increase in mucosal histiocytes, reduced apparent digestibilities (sheep); up to 8% added CT – sheep healthy; 17% added CT – toxic (no rumen fermentation); anthelmintic effects</td>
<td>59,80,191,298,299</td>
</tr>
</tbody>
</table>

(continued overleaf)
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Table 1. Continued

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Tannins</th>
<th>Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sorghum bicolor</em> (L.) Moench. (sorghum): seeds</td>
<td>Up to 7% CT; mixture of CT depends on sorghum variety: PC with A- and B-type bonds, glucosylated proapigeninidins and proluteolinidins with terminal eriodictyol</td>
<td>Systemic effects in rats and chickens attributed to low-MW phenolics; 14C-CT not absorbed by chickens</td>
<td>114,120,142</td>
</tr>
<tr>
<td><em>Tamarindus indica:</em> seed husk</td>
<td>15% tannins</td>
<td>Lactating dairy cows: more N retained; improved weight gains; no effect on intake or milk yield; higher faecal N, lower urinary N</td>
<td>86</td>
</tr>
<tr>
<td><em>Terminalia oblongata:</em> leaves</td>
<td>11–29% tannins; HT, ET</td>
<td>Hepatotoxic and nephrotoxic effects; poisoning and deaths of cattle and sheep; resorcinol glucuronide, 2-carboxy-2′,4′,4,6-tetrahydroxy diphenyl 2,2′-lactone glucuronide in urine</td>
<td>138–140</td>
</tr>
<tr>
<td><em>Ventilago viminalis</em> Hook</td>
<td>HT</td>
<td>Toxic</td>
<td>300</td>
</tr>
<tr>
<td><em>Vitis vinifera</em> (grapeseed)</td>
<td>Highly galloylated procyanidins; CE, GE, epicatechin gallate</td>
<td>Not toxic (rats)</td>
<td>119,301,302</td>
</tr>
</tbody>
</table>

Abbreviations: cat = catechin; CE = catechin or epicatechin; CT = condensed tannins; DP = degree of polymerisation; epicat = epicatechin; ET = elagitannins; GE = galallocatechin or epigallocatechin; GT = gallotannins; HT = hydrolysable tannins; MW = molecular weight (Da); PC = prodelphinidins; PD = prodelphinidins; PEG = polyethylene glycol; PGG = pentagalloylglucose; Ph = total phenolics; % = g tannin or phenolic per 100 g (dry matter basis).

Table 2. *K* <sub>ow</sub> values of tannins and, where available, information on their interactions with proteins and their biological effects (see Table 1 for details of nutritional or veterinary effects)

<table>
<thead>
<tr>
<th>Tannins or tannin-containing feeds</th>
<th><em>K</em> &lt;sub&gt;ow&lt;/sub&gt; values</th>
<th>Information on protein binding</th>
<th>Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ellagitannins</td>
<td>0.0001–100</td>
<td></td>
<td>Not toxic (rats)</td>
<td>258,303,304</td>
</tr>
<tr>
<td>Punicalagin</td>
<td>Low <em>K</em> &lt;sub&gt;ow&lt;/sub&gt;: water solubility 2 g L&lt;sup&gt;−1&lt;/sup&gt;</td>
<td></td>
<td>141</td>
<td></td>
</tr>
<tr>
<td>Epicatechin&lt;sub&gt;16&lt;/sub&gt; catechin</td>
<td>0.002</td>
<td>Hydrogen bonding</td>
<td>Positive nutritional effects</td>
<td>244</td>
</tr>
<tr>
<td>Castalagin/vescalagin (in chestnut tannins)</td>
<td>0.10</td>
<td>Water-soluble</td>
<td>Positive nutritional effects</td>
<td>85,266,267</td>
</tr>
<tr>
<td>Dichrostachys cinerea: fruits</td>
<td>0.17</td>
<td>Specific binding</td>
<td>Positive nutritional effects</td>
<td>15,25</td>
</tr>
<tr>
<td>Procyanidins: dimer up to 15-mer (Malus spp., apple)</td>
<td>0.2–0.3</td>
<td></td>
<td>304–306</td>
<td></td>
</tr>
<tr>
<td>Epigallocatechin (in tea)</td>
<td>0.28–0.5</td>
<td>Non-specific binding</td>
<td>Can be toxic (goats)</td>
<td>90,109</td>
</tr>
<tr>
<td>Digenyogal glucose (in hamamelitannin)</td>
<td>0.5</td>
<td>Soluble complexes with salivary histatins</td>
<td>Medicinal effects</td>
<td>93,244,304</td>
</tr>
<tr>
<td>Heptagalloyl glucose</td>
<td>1.0</td>
<td></td>
<td>244</td>
<td></td>
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<tr>
<td>Trigalloyl glucose</td>
<td>1.5–4</td>
<td></td>
<td>258,304</td>
<td></td>
</tr>
<tr>
<td>Hexagalloyl glucose</td>
<td>1.5</td>
<td></td>
<td>244</td>
<td></td>
</tr>
<tr>
<td>Epicatechin</td>
<td>1.6–2.4</td>
<td></td>
<td>244,304,306</td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>1.74–8.1</td>
<td></td>
<td>307–309</td>
<td></td>
</tr>
<tr>
<td><em>Acacia nilotica:</em> fruits</td>
<td>3.3</td>
<td></td>
<td>244</td>
<td></td>
</tr>
<tr>
<td>Epigallocatechin gallate (in tea)</td>
<td>5.2–12.1</td>
<td></td>
<td>93,244,304</td>
<td></td>
</tr>
<tr>
<td>Tetragalloyl glucose</td>
<td>10.1–39.8</td>
<td></td>
<td>258,304</td>
<td></td>
</tr>
<tr>
<td>Procyanidin B-2 gallate (<em>Rheum palmatum</em>)</td>
<td>27</td>
<td>Medical effects</td>
<td>258,310</td>
<td></td>
</tr>
<tr>
<td>Epicatechin gallate</td>
<td>48</td>
<td>Hydrophobic bonding</td>
<td>Can be toxic (ruminants, monogastrics, humans)</td>
<td>244,258,304</td>
</tr>
<tr>
<td>Pentagalloyl glucose (a component of tannin acid)</td>
<td>32–129</td>
<td></td>
<td>303,311</td>
<td></td>
</tr>
<tr>
<td>Geraniin (Fig. 2)</td>
<td>&gt;100</td>
<td></td>
<td>2037</td>
<td></td>
</tr>
</tbody>
</table>

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