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PLANT TANNINS, BLOAT AND NUTRITIVE VALUE

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SUMMARY

Results are presented which add support to the view that the presence of tannins in the feed of ruminants can have beneficial effects. These include a reduction of bloat and, by partial protection of protein from degradation in the rumen, improvement of nitrogen utilization. Problems arising when attempting to exploit these actions of tannins are discussed. The importance is stressed of correct methodolgy in working with tannin-containing forages.

INTRODUCTION

Great interest has arisen in plant tannins because of their ability to combine with plant proteins in the rumen and the implications this has in reducing bloat and improving protein utilization.

The purpose of the present paper is to discuss those implications, and, particularly, to draw attention to the problems encountered when trying to turn them to practical advantage.

THE CHEMISTRY OF TANNINS

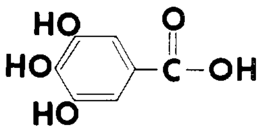
Tannins are special types of phenolic compounds of plant origin, traditionally distinguished by their ability to tan animal hides. Tanning involves the formation of insoluble tannin-protein complexes, a characteristic property of tannins which can readily be demonstrated *in vitro* with protein solutions.

There are two chemically distinct types of tannins (Fig. 1). They are:

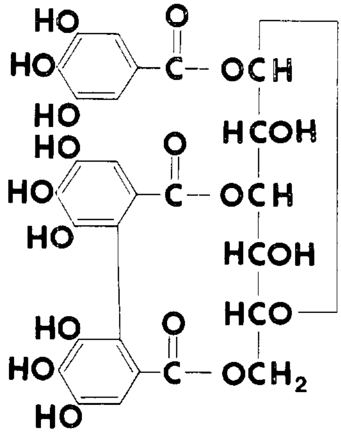
(1) *Hydrolysable tannins* (gallotannins and ellagitannins). These are polyesters of gallic acid, and other phenolic acids derived from it, with a sugar, normally glucose, which are readily hydrolysed by acid.

(2) *Condensed tannins* (flavolans). These are polymers (M.W. ~ 1 000 to > 20 000) of catechins, which are flavonoid phenols. The linkage between the monomers — typically a carbon-carbon condensation — is relatively stable under the conditions which cleave ester linkages in hydrolysable tannins.

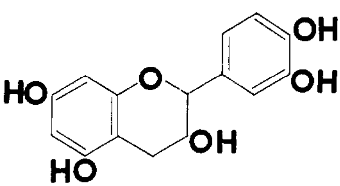
Tannins are found in many plants. In a particular genus, some species may be found to contain high levels, others to



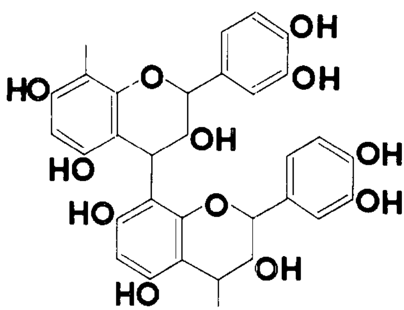
Gallic acid



A hydrolysable tannin



Catechin



Linkage in condensed tannin

FIG. 1: *Structures of hydrolysable and condensed tannins and constituent units.*

contain little or none. Most commonly, tannins are found in the bark of trees, in unripe fruit, and in galls, but they can also occur in other tissues. Their concentration in the plant can be influenced by environmental factors. Their functions in the plant are obscure.

The existence of the two types of tannin has frequently been ignored in past research. Yet they differ in their properties, particularly in the detailed characteristics of their reactions with proteins; and they require different methods of chemical analysis for their specific identification. As if to confuse this situation further, tannin preparations from a given source will often contain a range of compounds of either or both types.

Tannin-protein Bonding

Tannins bind to proteins primarily by hydrogen bonding (Loomis and Battaile, 1966). Quantitatively, binding is strongly influenced by such conditions of this reaction as pH and the relative and absolute concentrations of the reactants (Calderon *et al.*, 1968). The two types of tannins show differences in their behaviour. Thus hydrolysable tannins bind strongly at pH 3-4, but to a continuously decreasing extent above pH 5. Condensed tannins, on the other hand, have been found to bind to protein almost independently of pH below neutrality, and to a sharply decreasing extent above pH 8 (Loomis and Battaile, 1966).

The tannin-protein bonds can be split under certain conditions, *i.e.*, proteins can be de-tanned. However, even under conditions that favour disruption of hydrogen bonding, detanning is often incomplete; this is especially true of condensed tannin-protein complexes (Goldstein and Swain, 1965). Apparently other, more stable, probably covalent bonds can occur through partial oxidation of the phenolic groups.

Effects of Dehydration

From analogy with the behaviour of simpler phenols (Forsyth, 1964), drying or curing, and especially drying by heating, of tannin-containing plant material would be expected to promote stable tannin-protein bonding through partial oxidation. Partially oxidized tannins may also bind strongly to other plant constituents besides protein, *e.g.*, cellulose (Roux, 1955). Such effects would lead to reduced extractability of tannins from plant materials, as was noted by Lyford and Smart (1967) and Bate-Smith (1973) with condensed tannins. As with the distinction between the types of tannins, the consequences of dehydration appear to have been largely ignored in research into tannin-containing forages. It is a common practice to air or oven dry and then grind samples of plant material for tannin analysis.

Analytical Methods

Reliable analytical methods depend on specific, structurally-dependent reactions with the tannins. Specific methods are available for condensed tannins (Jones *et al.*, 1973) and for ellagitannins (Bate-Smith, 1972), the more common of the hydrolysable tannins. Unfortunately, non-specific methods have been widely used, detracting from the value of much of the work to date on tannin-containing plants. Methods which depend on measuring total plant phenols are quite unsatisfactory: red clover, for example, has a high phenolic content but no tannins. These analytical uncertainties compound those introduced by sample preparation, referred to earlier.

TANNINS AND BLOAT

Bloat is a familiar hazard when cattle are fed certain common legumes — white, red, subterranean clovers, and lucerne. Satisfactory methods of control, based on the regular administration of bloat-preventing substances, have been evolved for dairy cattle under intensive management conditions (Reid, 1972). However, these methods are time-consuming and relatively expensive, and they are not well adapted to other forms of management, particularly extensive grazing systems. With the expansion of beef production in New Zealand agriculture and improvement of hill country pastures, largely by the introduction of improved legumes, there is thus an increasing risk of bloat in a situation where it cannot easily be controlled.

The primary cause of bloat is the formation of stable foam in the reticulo-rumen (Reid, 1960). Although a number of factors contribute to formation of this foam (Clarke and Reid, 1974) soluble plant protein appears to be a major foaming agent.

From *in vitro* studies with leaf extracts, Kendall (1966) proposed that when legume species that do not cause bloat are fed to cattle, foaming is limited by the presence of tannins in the feed and the pH of the rumen fluid. He did not identify the tannins involved and gave no details of their action. Work at this laboratory has shown that the presence of condensed tannins in the leaves of legumes (Jones *et al.*, 1973) correlates with the reported absence of bloating (Jones and Lyttleton, 1971) and the reduced liberation of soluble protein on the disruption of the leaves (Jones *et al.*, 1970). This work has shown tannins to be acting as protein precipitants.

To study the effects of a tannin-containing legume on rumen function, feeding experiments have been started at this laboratory with sainfoin (*Onobrychis viciifolia*, Scop.). This species is not normally grown in New Zealand, but has long been known overseas as a valuable feed plant. The variety used here is "Fakir"; it has been found to contain 16 to 23 DM % protein and 1 to 1.5% of condensed tannins.

Sainfoin is reputed not to cause bloat (Piper, 1914, quoted by Eslick, 1968). In 4 small trials here, 2 with stall-fed and 2 with grazing cows, no case of bloat has occurred in any animal feeding on sainfoin (Reid and Gurnsey, unpubl. results). Pooled data from these trials are shown in Table 1. The feed in both kinds of trial was fresh, usually in early bloom, and was offered in 2 × 2 h feeds a day, the animals being kept at other times in bare yards with access to water. The total number of animal/feeds (*i.e.*, opportunities to bloat) is so far 302, over 45 days. In control groups of animals fed fresh red clover (*Trifolium pratense*) bloat occurred on 15 of 46 days.

TABLE 1: INCIDENCE OF BLOAT IN NON-LACTATING DAIRY CATTLE FED SAINFOIN AND RED CLOVER

(Pooled data from 2 stall-feeding and 2 grazing trials, during period April 1972 to January 1974)

	Feed	
	Sainfoin	*Red Clover*
Total No. cows used[1]	12	12
Total No. days fed[2, 3]	45	46
Total No. days bloating[4]	0	15

[1] Maximum number cows on either feed in any trial was 6. Several sets of identical twins were included.

[2] The duration of sainfoin feeding in individual trials was 6, 8, 15 and 16 days.

[3] In 3 trials sainfoin and red clover were fed simultaneously, identical twins being split between feeds. In the other trial, red clover was fed before and after the period on sainfoin.

[4] Bloat varied in both incidence and severity in all trials; 11+ cases of moderate or severe bloating occurred.

The state of the rumen contents of the cows feeding on sainfoin was observed through rumen cannulae on a number of occasions. No foam was present.

In sheep feeding experiments, no froth was seen in either ruminal or duodenal contents of animals fed sainfoin. This contrasts markedly with the situation in sheep fed white clover (*Trifolium repens*), when the ruminal contents are characteristically very frothy, and froth commonly occurs in the duodenal contents. Other features of the ruminal contents of sheep fed sainfoin were a distinctive yellowish appearance as compared with the green colour of the digesta of the clover-fed animals, and a tendency for "rafting" of fibre over free liquid.

These observations are consistent with the hypothesis that the inclusion of tannin in the ruminant diet can be beneficial in reducing the likelihood of bloat.

TANNINS AND NUTRITIVE VALUE

Serious wastage of feed protein can result from its microbial degradation in the rumen and subsequent absorption as ammonia. For this reason, methods have been evolved to prevent protein degradation in the rumen yet allow it to be available for digestion in the small intestine. Thus heat treatment (Chalmers *et al.*, 1954), coating protein with inert compounds (Sibbald *et al.*, 1968), treatment with aldehydes (Ferguson *et al.*, 1967) and plant tannins (Zelter and Leroy, 1966) have been tried with varying success.

The main requirements of tanning as a system of protein protection have been set out by Zelter *et al.* (1970). These are first, that the tannin-protein complexes be stable in the rumen but do not interfere with the later enzymic digestion of the protein in the lower gut; secondly, that the metabolism of the micro-organisms is not adversely affected; and, thirdly, that the tannins are harmless to the animal.

Initial work with tannins has demonstrated the viability of the system. Treatment of peanut, soybean, linseed, rapeseed, sunflower seed meals, dried skim milk and casein with aqueous solutions of chestnut tannin (mainly hydrolysable tannins) prevented degradation by rumen micro-organisms *in vitro* (Zelter *et al.*, 1970). Similarly, Driedger and Hatfield (1972) found that treatment of soybean meal with various aqueous concentrates of both hydrolysable and condensed tannins resulted in up to a 90% decrease of deamination *in vitro* in rumen fluid.

There are conflicting reports on the availability of tanned proteins to enzymatic digestion beyond the rumen. Important factors would appear to be the proportion and kind of tannin in the complex. The above authors all found that tanning of protein did not affect proteolysis with pepsin *in vitro*. However, although Delort-Laval and Viroben (1969) found tanning to cause only slight inhibition of pepsin and trypsin activity, Driedger and Hatfield (1972) found that pancreatic digestion decreased significantly with increasing tannin concentration, and that the source of the tannin had an effect. Feeny (1969) also demonstrated inhibition of protein digestion by trypsin increasing with the proportion of tannin present, and found condensed tannins to be more inhibitory than hydrolysable tannins.

There are several reports on the effects of tanning on digestion and utilization of protein *in vivo* by sheep which suggest that tannin treatment improves nitrogen utilization in ruminants. Thus Zelter and Leroy (1966) reported reduced rumen ammonia concentrations and plasma urea concentrations, Delort-Lavel *et al.* (1972) and Driedger and Hatfield

(1972) reported increased N retention and the latter authors also found improved liveweight gains and feed efficiency.

Several legumes used as herbages in agriculture naturally contain tannins. Two which contain condensed tannin (Jones *et al.*, 1973), *Lotus pedunculatus* Cav, and sainfoin, have been used to study nutritive value in this laboratory. The effectiveness of lotus tannin in slowing deamination in the rumen was tested in an *in vitro* experiment (Lyttleton and Ulyatt, unpubl.). Tubes containing 4.0 g of ground fresh plant material were made up to 40 ml with phosphate buffer (pH 7.5), gassed with CO_2 and then inoculated with 10 ml of strained rumen liquor from a cow fed red clover. The tubes were incubated for 5 h at 40° C then the contents were strained through glass wool and samples of filtrate analysed for ammonia by the Conway method. The results are shown in Table 2. After

TABLE 2: AMMONIA PRODUCTION *IN VITRO* FROM WHITE CLOVER AND LOTUS LEAF HOMOGENATES INCUBATED WITH RUMEN MICRO-ORGANISMS

	NH_3-N produced (mg/50 ml)
White clover	0.49
Lotus	— 0.02
White clover + lotus:	
(a) mixed *before* homogenizing	0.24
(b) mixed *after* homogenizing	0.31

5 h much less ammonia was present in lotus tubes as compared with white clover. Mixing white clover and lotus led to a reduction in ammonia formation. There was a small difference according to whether the two legumes were mixed before or after homogenizing, probably because protein-tannin complexing occurs rapidly once the plant cells are disrupted.

Aspects of digestion in sheep fed fresh sainfoin or white clover are compared in Table 3. The animals were Romney Marsh wethers prepared with rumen and re-entrant duodenal cannulae. The methods of feeding, digesta collection and chemical analysis are given by Egan and Ulyatt (1974: in preparation). The main difference between fresh white clover and sainfoin lay in the relative proportions of digestible N that were digested in the stomach or intestines: there was appreciable loss of N from the stomach of sheep fed white clover and none from sheep fed sainfoin. The digestible N thus appeared more efficiently utilized from sainfoin. A very interesting comparison between the digestion of fresh sain-

TABLE 3: DIGESTION OF NITROGEN IN THE STOMACH AND INTESTINES OF SHEEP FED WHITE CLOVER AND SAINFOIN

	Feed		
	White Clover	*Sainfoin*	
	Fresh[1]	*Fresh*	*Dried*[2]
N intake (g/day)	34.3	27.8	25.6
N entering duodenum (g/day)	28.3	27.9	32.6
Faeces N (g/day)	5.9	6.6	12.6
Apparent digestibility of N (%) ...	82.8	76.3	50.8
Partition of ingested N (g/g N intake):			
Stomach	0.17	— 0.01	— 0.27
Intestines	0.66	0.77	0.78
Faeces	0.17	0.24	0.49

[1] Pooled data from MacRae and Ulyatt (1974) and Egan and Ulyatt (1974: in preparation).
[2] Thomson *et al.* (1971).

foin and dried sainfoin is also made in Table 3 using data for dried sainfoin given by Thomson *et al.* (1971). Compared with fresh sainfoin, dried sainfoin had a lower apparent N digestibility, higher faecal N loss and a very high apparent gain of N in the stomach. This last effect eludes explanation at this stage, but the low apparent digestibility and high faecal N suggest that during the drying process, the availability of the protein was reduced. This was probably not caused by heating *per se* as the digestibility of the tannin-free dried lucerne used in the same work (Thomson *et al.*, 1971) was not reduced to the same extent. Rather, it would seem more likely to be a specific effect, due to the formation of stable tannin-protein bonds during the drying of the sainfoin.

DISCUSSION

So far, we have been concerned with benefits that might be gained from the presence of tannins in plants. However, tannins are more widely known for their deleterious effects, and this reputation may well cloud the issues when the practical value of tannins to the farmer is being judged. Thus. tannins have been blamed for the low palatability and poor digestibility of forages such as *Lespedeza* (Donnelly, 1954; Donnelly and Anthony, 1969). For this reason interest in reducing the tannin content of *Lespedeza* has been expressed (Cope and Burns, 1971). Tannins have also been blamed for poisoning of stock by plants such as shin oak (*Quercus* spp.) (Pigeon *et al.*, 1962) and Queensland "supplejack" (*Ventilago viminalis*) (Pryor *et al.*, 1972). We believe that such a range of effects could simply reflect a range in concentration —

and possibly the kinds of tannin — present in the various plants. Further, it is not unlikely that some of the harmful effects attributed to tannins are in fact due to other plant constituents. Both these explanations must remain matters of conjecture until all species are analysed for both tannins and other toxic constituents using reliable methods of sample preparation and analysis. We would regard such a phytochemical and toxicological survey to be urgently needed.

Exploitation of the Beneficial Effects of Tannins

Even the most simple enquiry points clearly to the need to know much more about the system before the beneficial effects to be gained from tannins can be reliably exploited on the farm. The problems can be little more than listed here.

(1) *Plant Considerations*

Kind of Tannin: With the herbage available we have no choice in the tannin type. So far as any attempt to manipulate tannins in herbage species is concerned, we are uncertain which type of tannin best suits our purpose. It may well be that hydrolysable tannins are preferable because they are less likely to form irreversible bonds with proteins and other substances.

Concentration of Tannin: Because of the complexity of the systems involved, both plant and animal, *in vitro* experiments seem of limited value in determining optimum tannin concentrations. We believe this may be a weakness in the *Lespedeza* breeding programme which has been guided by such testing. From the work with sainfoin, it would appear that the level of tannin required can be quite low. However, if there is a narrow range between beneficial and deleterious effects (yet to be defined) the situation could well be complicated by factors such as variation in tannin content due to stage of growth of the plant, environmental factors, agronomic practices, and so on. An unknown factor is the possible development of strains of rumen micro-organisms capable of attacking tannin-nutrient complexes.

(2) *Animal Considerations*

Intake: Because of their astringency — an effect probably due to partial tanning of proteins or glycoproteins in the mouth — tannins might be expected to be distasteful. However, in contrast to *Lespedeza* species, sainfoin appears to present no palatability problems. At this laboratory we have found both sheep and cattle to eat sainfoin avidly, while

Osbourn *et al.* (1966) have noted unexpectedly high intakes of hay of common sainfoin (*O. sativa*) by sheep. It would appear, therefore, that the levels of tannins necessary to produce beneficial effects need not depress intake.

Effects of Continual Ingestion of Tannins: The effects on the animal of continual ingestion of low levels of tannins are not known at present. Of the toxicity of high levels of intake there is no doubt although the literature is confusing in details. We have been unable to find any reports which unequivocally deal with the toxicity of condensed tannins: all papers found have concerned tannic acid or its metabolites (*e.g.*, Cameron *et al.*, 1943; Dollahite *et al.*, 1962; Pigeon *et al.*, 1962; Krezanoski, 1966; Boyd *et al.*, 1965; Rayudu *et al.*, 1970).

The classical effects of tannic acid poisoning include haemorrhagic gastroenteritis, hepatic necrosis and nephritis and these effects have been reported in a wide range of animals — rodents, ruminants, poultry, horses and man. There is some disagreement as to whether or not tannins are absorbed as such but the effects on liver and kidney make it certain that toxic principles are indeed absorbed from the gut, and it has been claimed (*e.g.*, Krezanoski, 1966; Pryor *et al.*, 1972) that tannin can be detected in the blood.

(3) *Agronomic Considerations*

Overseas work has usually been concerned with prepared supplements containing tannins which are fed to housed animals. On economic grounds, we believe that if tannins are to make a valid contribution to New Zealand farming, it must be within a system of supplying the grazing animal forage which incorporates tannin-containing plants. Possibilities for achieving this ideal include breeding tannin-containing forms of traditional pasture legumes (Jones *et al.*, 1973), incorporation of tannin-containing species in mixed pastures, and cropping of tannin-containing species, *e.g.*, sainfoin. However, many practical and research problems must be overcome in the search for economically acceptable systems. Problems of plant breeding, agronomic performance, selective grazing and suitability of tannin-containing herbage for conservation are examples.

FUTURE RESEARCH

The potential rewards from a successful system for exploiting tannins should attract considerable practical interest in the future.

It will readily be appreciated, however, that much research will be required before such a system can be established and find practical application. Basic biochemical studies of the accumulation of tannins in pasture species, of the complexing of tannins with proteins and other plant constituents, the effects of feed processing or conservation on the availability of complexed nutrients are all needed. These must be supported by detailed studies of the fate of the complexes in the gut, particularly the interactions of gut pH and the stability of the complexes, the effects and fate of tannins released within the gut. These latter studies are needed to understand more fully the effects on nutrition and health of the animal, and to assess the possibility that "resistance" may develop. Work in several of these areas is being undertaken at this laboratory.

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