EFFECTS OF TANNINS IN RUMINANT: A Review

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Abstract: Tannins are very diverse chemically, are widely found in plant material and can have diverse effects on animals which consume them. This mini-review has the aim to investigation and discusses information available in the scientific literature on the effects of condensed tannins on ruminant nutrition. Polyethylene glycol (PEG) has been used in most experiments to deactivate condensed tannins (CT). The effects of dietary CT on nutrition have been studied principally with tannins from temperate legume pastures. At low concentrations, CT appear to increase intake because of a reduced protein degradation in the rumen and better amino acids supply to, and absorption from, the small intestine. CT from *Lotus corniculatus* increased milk yield, protein and lactose percentages, and reducing fat percentage. Regarding carcass characteristics, CT from temperate plants, at low concentrations, have increased carcass leanness and reduced carcass fatness. This can be due to improved protein nutrition due to an increased flow of protein and essential amino acids to the intestine. Condensed tannins from *Acacia cyanophylla* had no significant effects on lamb tissue repartition. Meat quality has been evaluated after lambs received diets containing CT from this shrub species or *Ceratonia siliqua* pulp with or without PEG. In both cases, lean color appeared to be greatly affected by dietary CT, being darker in the animals that receive PEG supplementation. The effect of CT on meat color could be linked with a reduced myoglobin synthesis although iron absorption seems not to be affected by CT in ruminant.

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1. Introduction

Feeding costs is one of the major problems in the economic balance of the ruminant farmers. In most of the world regions, animal feed production is difficult and farmers purchase expensive concentrates. In many cases a problem in utilization or these alternative feeds is the presence of anti-nutritional factors such as condenses tannin (CT) and phenolic compounds found in many forage plants [10, 16].

CT in some plant species have been associated with a number of adverse conditions. Examples include Lespedeza cuneata [34, 16], Acacia aneura [24, 16], *Lotus pedunculatus* grown under wet cold conditions [1, 16], *Calliandra calothyrsus* [32, 16] and *Ceratonia siliqua* [26, 16].

Negative effects include reduced absorption of some minerals [37, 16], reduced rumen protein utilization [6, 16, 1] voluntary intake [16, 27], and microbial activity in the rumen [16, 20] and toxic effects reflected by damage of kidney and liver [10, 16].

In the rumen, CT bind with plant proteins, reducing their availability for rumen microflora and the host animal. Consequently, the rate and extent of fiber digestion is also reduced with consequent reductions of voluntary intake, metabolizable energy availability and amino acid (AA) absorption. CT reacts preferentially with polyethylene glycol (PEG) and the supplementation of PEG has been largely used to eliminate and to measure the effects of CT [24, 16].

There is a large body of scientific literature on various aspects of tannins. This short review seeks to highlight the practical significance of tannins on ruminant nutrition in less developed countries using this literature and the experiences of the staff of the Natural Resources Institute. It will also review approaches to countering the negative effects of tannins, with particular reference to the use of tannin-binding agents. This review does not seek to be comprehensive and where appropriate key references are given containing further details.

2. STRUCTURE

The complex tannin is a series of compound first isolated from a Fagaceus plants, and now found to occur widely in the plants containing both hydrolysable and condensed tannins. The isolation of these tannins is almost invariably achieved by combination of sephadex LH-20 dextran gel and reverse-phase chromatographies. The former chromatography allows separation from more mobile lower-molecular-weight polyphenolics, while the latter is effective for the separation of each structural isomer (Fig1).

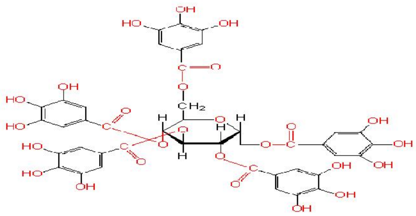

Figure 1: structure of Tannin

METHODOLIGIS AVAILABE

To match the range of tannins, there is a very diverse range of chemical and biochemical assays which has been used to measure the tannin contents of plants. Several methods use the chemical properties of tannins in colorimetric assays. These assays vary considerably in specificity. Their properties and merits have been reviewed by Hagerman and Butler [2, 16]. The most widely used colorimetric assays are given below:

- General measurements of tannins (total phenols) Folin Denis assay Prussian blue assay
- Measurements of condensed tannins Vanillin assay Acid butanol assay

There are several published procedures and modifications to these assays. Based on the recommendations of Hagerman and Butler [2, 16], the Prussian blue assay using the Price and Butler [16, 23] procedure and the acid butanol assay by the Porter et al [16, 22] procedure are used at NRI. These assays are usually used to measure polyphenols which have been extracted in solvent, often aqueous acetone. However, the acid butanol method is also used to measure non-extractable tannins. Again there are various procedures but they involve the treatment of a residue (after extraction) with acid butanol. Terrill *et al.* [16, 36] have developed a method which sequentially extracts free, protein-bound and fiber-bound condensed tannins.

Biochemical assays can be used to measure the activity or capacity of tannins to bind and precipitate protein. This is really a measurement of tannin activity and it has been suggested by Hagerman and Butler [22, 16] that these types of assays give better indicators of the biological activity of tannins than chemical assays. Again there are several procedures available. At NRI, the radial diffusion assay of Hagerman [22, 16 and 39] is used as it does not require sophisticated equipment, making it potentially suitable for use in less developed countries.

More sophisticated chemical techniques suchas high performance liquid chromatography (HPLC) have been used to identify particular tannins, especially lower molecular weight polyphenols [16, 19] but also for separating tannins of different molecular weight [16, 29]. The detailed analysis of the higher molecular weight tannins is, however, very challenging.

Relationships between tannin assays and their anti-nutritive properties

Considering the volume of literature on tannin assays, there is comparatively little on the effectiveness of the different assays as indicators of anti-nutritive effects. This is because such trials are difficult to conduct. The nutritive value of tannin ferrous feeds is due to all its components and isolating the effect of tannins is not straightforward. There are no entirely suitable compounds which can be readily obtained and added to feeds as experimental models. With such a diverse group of compounds there is a considerable risk that model compounds may be of limited value. Tannin-binding agents have been used to inactivate tannins thus giving indications of what effects the tannins are having.

[16, 30] correlated crude protein content to the proportion of digestible protein for feeds such as grasses and agriculturally-produced legumes and grains with very low tannin contents. Using the correlation equation obtained to predict protein digestibility and comparing this to the measured protein digestibility of tanniniferous feeds consumed by mule deer, an estimate of the reduction in digestible protein due to Tannins were obtained. This was found to be highly correlated with the activity of the tannins as assayed by a protein precipitation assay. [5, 16] used predictive equations developed by Robbins et al. [30] to predict successfully the protein and dry matter digestibility of tanniniferous forage leaves from seven species and one sample of twigs when fed to black-tailed deer, confirming that a protein precipitation assay gave a useful estimate of the effect of tannins In vivo.

McKey et al. [17] have also found that total phenols (by the Folin Denis assay) and extractable condensed tannins (by acid butanol) correlated negatively with the dry matter digested by rumen inoculum during 96 hours incubation. Data were quoted for 30 species of trees from which samples of mature leaves had been taken. For 72 West African fodder trees and shrubs, Rittner and Reed [16, 31] found that in vitro protein degradability was negatively correlated with total phenols (by ytterbium precipitation Assay) and extractable condensed tannins (by acid butanol). However, the behavior of some species deviated greatly from that indicated by the correlation studies. Wood and Plumb [25, 16] found strong correlations between total phenols (Prussian blue assay) and the inhibition by tannins from Bolivian fodder tree leaves of *In vitro* fermentation (gas production) by rumen microbes. Similar correlations were found using protein precipitation by the radial diffusion assay, but there was no significant correlation with the acid butanol assay. Other workers have had less success in finding correlations. Makkar *et al.* [13] were unable to find significant correlations between total phenols (Folin Denis assay), condensed tannins (by the vanillin assay) and protein precipitation with *in sacco* dry matter loss for leaves of 10 species of trees from India.

Khazaal and 0rskov [8, 16] found that the increase in gas production resulting from treating eleven leaf samples with the tannin binding agent PVPP was not related to total phenols (by Folin Denis and by a gravimetric assay), extractable condensed tannins (by acid butanol and vanillin assays) and total condensed tannins (by acid butanol assay). Mole and Waterman [16, 18] found little correlation between chemical assays for total phenols (by the Folin method), condensed tannins (by the vanillin method), hydrolysable tannins (by various methods) and biochemical activities as assayed by a protein precipitation method and cellulose inhibition.

Preferred indicators for the anti-nutritive effects of tannins

As may be inferred, there is no clear consensus as to which assays is the most reliable indicators of anti-nutritive effects. Correlations to some extent reflect the selection of samples. There is evidence indicating the general applicability of protein precipitation assays and the Prussian blue total phenols assay, although neither of these can distinguish between condensed and hydrolysable tannins.

Tannins can be modified during sample preparation. Harvested oak leaves appeared to increase in total phenol and tannin content after 48-72 hours, then decline to near their original levels within 120 hours [9, 16]. Hagerman [4, 16] reported variable effects of drying at 40°C and freeze-drying on extractable tannin contents, although oak leaf tannins have been found to be resistant to change when leaves were dried [15, 16]. Orians [21, 16] found that the condensed tannin content of willow leaves fell when samples were air-dried and recommended using a freeze-drier but without pre-freezing the sample to minimize changes to tannins. When Bolivian tree leaves were oven dried at 50°C for 16-24 hours losses of extractable tannins ranged from 19 to 70% compared to fresh leaves, depending on species (unpublished NRI data).

Thus the effect of tannins of sample preparation appears to depend on the precise nature of the tannins. While using a freeze-drier without pre-freezing may be the preferred method to dry samples, in many practical situations in less developed countries samples would have to be air-dried or oven-dried. The author suggests that for nutritive value assessment there may be advantages in using *In vitro* measurements of feed degradation rather than trying to correlate tannin assays to degradation characteristics. These techniques appear to be less prone to modifications resulting from sample preparation than the tannin assays and the data obtained will be more readily applicable than tannin assay data. While tannin measurement will continue to be extremely useful for research purposes, it may be less suitable for routine nutritive value assessment.

Occurrence of tannins in ruminant diets
Types of feeds which contain tannins

Tannins are found widely in plant material, in both leguminous and non-leguminous species. Of most significance to ruminant feeds is that many, if not nearly all, species of forage and browse legumes contain tannins. Kumar and D'Mello [11, 16] concluded that hydrolysable tannins were abundant in leaves, fruits, pods and galls of dicotyledons such as oak, chestnut and other species; condensed tannins are even more widespread. Some species contain both types of tannin. Makkar [14, 16] listed the polyphenolic contents of 62 species of trees and shrubs from India. The list included important fodder species such as various Acacias, Albizias, *Calliandra calothrysus, Ficus* sp., *Gliricidia sepium, Leucaena leucocephala,* various species of Prosopis and Quercus. Ritter and Reed [16, 31] studied 72 West African fodder tree and shrub species (from Benin, Niger and Nigeria) and found polyphenols in all the species studied. Average polyphenolic contents did not appear to be different in the climatic zones studied. Wood *et al.* [16, 39] studied 20 tree leaf fodders from Bolivia, 26 from West Africa and 24 from Colombia. Only 9 of the 70 samples were found to have no extractable tannins (as gauged by protein precipitation activity; unpublished data). Tannins are not restricted to tropical feeds; lotus, sainfoin and other temperate species have been found to contain condensed tannins [16, 37]. Thus, while not all tree and shrub fodders and browses contain tannins, most certainly do. The occurrence of tannins is not restricted to particular limited classes of plants or climatic zones. Tannins are therefore highly likely to be consumed in all Agricultural systems where trees and shrubs are used as livestock feed.

Tannins are not confined to just trees and shrubs. [19, 16].

Strategies for alleviating the anti-nutritive effects of tannins

Tannin binding agents, notable polyethylene glycol (PEG) and polyvinylpyrrolidone (PVP) have been widely used as research tools to investigate the effects of tannins both *In vivo* and *in vitro.* Both compounds are commercially available in a range of molecular weights. Lower molecular weight polymers are highly water soluble and bind strongly to tannins. Jones and Mangan [7, 16] first used PEG (molecular weight, MW, 4000) to prevent the formation of sainfoin tannin and protein complexes and to release protein from the complexes. While PEG was able to prevent tannin-protein complex formation, its ability to release protein from such complexes related to the pH of the environment, amount of tannin in the complex and the age of the complex at the time of adding PEG. At pH 8.0 a maximum of only 30% of the protein was released from the complex by PEG. This may account for the variable responses to treating dried feed samples with tannin binding agents observed by Khazaal and 0rskov [8, 16] and Wood and Plumb [16, 38]. While PEG MW 4000 has been the most widely used tannin-binding agent, Makkar *et al.* [15, 16] showed that PEG MW 6000 was more effective in overcoming the effects of tannins at neutral pH *in vitro.* PEG was more effective at binding tannins than PVP. Some *in vivo* trials using PEG are reviewed below. Barry and Duncan [1, 16] sprayed lotus herbage with a 0.3 g g^{-1} solution of PEG (MW 3350) at a rate of 2.4 g PEG per g condensed tannin to overcome the effects of the tannin. The treatment increased apparent digestibility of nitrogen by 0.26 and also led to modest increases in cellulose and hemicellulose digestibility. Oral administration of PEG (MW 3350) at the rates of 40 and 60 g per day to lambs grazing on sulla and pasture was used by Terrill *et al.* [16, 36] PEG had no consistent effect on the sheep grazing tanniniferous sulla or (tannin-free) pasture. Silanikove *et al.* [16, 33] used PEG (MW 4000) at 12.5, 25, 32 and 50 g/day to supplement a sheep diet containing the tanniniferous carob leaves (containing about 20 g kg^{-1} total phenols). The PEG was administered each morning Mixed with a small amount of concentrate prior to feeding. Supplementation with 25 g per day PEG increased digestible organic matter intake by two-fold due both to increased intake and digestibility. 32 g per day PEG led to further apparent increases but these were not statistically significant; there were no further improvements with 50 g per day supplementation. Withdrawal of PEG resulted in a rapid return to the lower intakes and digestibility of the supplemented controls. Prichard *et al.* [16, 24] used 12 and 24 g per day of PEG (MW 4000) to supplement mulga browse consumed by sheep and reported a 56 and 78% (respectively) increase in intake but with little effect of dry matter digestibility. Protein digestibility did, however, increase. Wool growth increased by 166 and 178% respectively as a result of PEG, Thus treatment with tannin binding agents can be highly effective in overcoming the anti-nutritive effects of tannins leading to improved animal performance. However, their affects can be variable which may relate to the nature of the tannins, the nature of the tannin-feedstuff complexes which can form and the history of the feeds tested.

Makkar (1993) lists 28 agro-industrial by-products which might be use

Feed mixtures

Free-ranging animals can select their diets so as to avoid the worst effects of tannins. It is said that incidence of fatalities in which tannins have been implicated occur when animals are very hungry and are unable to select alternative feeds. In the cut and carry feeding systems of Nepal farmers there are some indications that mixtures of different tree fodders are used on occasions, possibly in part as a strategy of avoiding possible toxicity of excessive amounts of some fodder species. Using tree fodders as supplements to roughages, a common feeding practice, may coincidentally help limit the intake of tannins. However, this is an area which has been little investigated.

Conclusions on importance of tannins

Tannins are consumed in agricultural systems where tree, shrubs and forage legumes are used as livestock feed. Certain agricultural by-products such as some seed cakes and sorghum stoves also contain tannins. Therefore tanniniferous feeds are widely consumed by ruminants in less developed countries. Their importance does, however, vary considerably regionally, seasonally and even between farms. The major situations where tanniniferous feeds are important for ruminants are given below:

(a) Tanniniferous feeds are vitally important in arid and semi-arid areas in the dry season and during periods of drought. These types of feeds are often the major type of feed available under these circumstances and enable the animals to survive in periods of feed shortage.

(b) Tanniniferous tree leaves are a particularly important feed in upland forests, again particularly in dry seasons. Trees are used as sources of feed in forested regions in general.

(c) In regions of higher agricultural potential tanniniferous forage legumes can be an important part of the diet. Tanniniferous seed cakes may also be important in some regions. Such feeds are generally valuable sources of protein.

There are several possible strategies for alleviating the negative effects of tannins as suggested below:

(a) Using feed mixtures to minimise the intake of particular tannins may be beneficial and there are some indications that such practices are used in some feeding systems. It is, however, a very poorly understood approach and its potential effectiveness is unclear.

(b) Tannin-binding agents can be effective. In droughts (in particular) they could boost performance sufficiently to improve survival rates of ruminants. However, their commercial application has only recently begun and their usefulness is not clear. There is a case for reviewing the costs and benefits of agents such as PEG to see if such Supplements are likely to be of commercial interest.

(c) Microbial or enzymic treatments of feeds to degrade tannins may be developed in the longer term and warrant further investigation.

(d) In the longer term improved plant varieties with optimal types and levels of tannins could be produced, but the potentially vital positive effects tannins can have for plants must not be lost in reducing the negative effects on animals.

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