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Tannins Revisited - Changing Perceptions of their Effects on Animal System

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ABSTRACT

Tannins are a prominent class of compounds which constitute the plant secondary metabolites group. The two major structural groups of tannins are hydrolyzable tannins and condensed tannins. Due to their widespread nature and ability to complex with proteins and other biomolecules, they exert both harmful and beneficial effects on organisms. Hydrolyzable tannins have toxic effect on the animals that feed on forages rich in these tannins. High concentrations of dietary condensed tannins (6-12% DM) depress voluntary feed intake, digestive efficiency and animal productivity. In contrast, forages containing moderate concentrations of condensed tannins (2-4% DM) can exert beneficial effects on protein metabolism in ruminants especially sheep, by slowing degradation of dietary protein to ammonia by rumen microorganisms and increasing protein outflow from the rumen. This results in the increased absorption of amino acids in the small intestine and ultimately leads to increases in lactation, wool growth and live weight gain, without changing voluntary feed intake of the animal. Dietary condensed tannins can also contribute to improved animal health by reducing the detrimental effects of internal parasites in small ruminants and the risk of bloat in cattle. Therefore, forages containing moderate concentrations of condensed tannins can increase sustainability and productivity in intensive grazing systems through increasing the efficiency of animal production, decreasing urinary nitrogen excretion and reducing chemical inputs such as anthelmintics and detergents to control rumen bloat in cattle.

Key words : Hydrolyzable tannins; Condensed tannins; Negative effects; Positive effects

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INTRODUCTION

Tannins are water-soluble polyphenolic compounds of varying molecular weight, found as secondary compounds in pteridophytes, gymnosperms and angiosperms including some leguminous plants (Bhat *et al.* 1998). Based on their structures and properties, they are distributed into two major classes - hydrolysable tannins (HT) and condensed tannins (CT). Hydrolysable tannins are composed of esters of gallic acid (gallotannins) or ellagic acid (ellagitannins) with a sugar core which is usually glucose and are readily hydrolysed by acids or enzymes into monomeric products. Condensed tannins are hydrolytically cleaved to anthocyanidins and related compounds and are more correctly called proanthocyanidins or polyflavanoids. The structure and chemistry of tannins are complex, with variability in their stereochemistry, molecular size and polymeric form producing tannins specific to each plant in which they are found. As tannins are present in large number of feeds and forages, they are one of the most common anti-nutritional factors. These polyphenolic polymers are of relatively high molecular weight and have the capacity to form complexes with carbohydrates and proteins. Low molecular weight CT oligomers are more reactive and have higher protein precipitating capacities than high molecular weight polymeric tannins (Butler and Rogler, 1992). Formation of complexes of tannins with nutrients, especially proteins, has both negative and positive effects on their utilisation (Reed, 1995).

Tannins, especially HT, when present in plants can, in general, adversely affect herbivore nutrition by reducing intake, protein digestibility, inhibiting digestive enzymes or by direct systemic toxicity (Kumar and Singh, 1984). This leads to a reduction in their feed intake, adversely affects rumen fermentation and significantly depresses digestibility of almost all the nutrients. HT are toxic and cause poisoning in animals if sufficiently large amounts of tannin-containing plant material, such as leaves of oak (*Quercus* spp.) and yellow-wood (*Terminalia oblongata*) are consumed. (Garg *et al.* 1992; Fillipich *et al.* 1991). Tannins inhibit the activity of enzymes of rumen microbes (McLeod, 1974; Martin and Akin 1988; Makkar *et al.* 1988; Bae *et al.* 1993). CT in high concentrations have been shown to inhibit extracellular endoglucanase activity of *Fibrobacter succinogenes* (Bae *et al.* 1993) and extracts of condensed tannins from *Onobrychis viciifolia* reduced growth and proteolytic activity of *Butyrivibrio fibrisolvens, Ruminobacter amylophilus* and *Streptococcus bovis* (Jones *et al.* 1994).

The reviews that have appeared in the past on effect of tannins on ruminant nutrition have laid stress on their anti-nutritional nature (McLeod, 1974; Kumar and Singh, 1984; Makkar *et al.* 1987; Mangan, 1988; Kumar and Vaithiyanathan, 1990). Increasing attention has recently been given to CT as there is a growing realisation about their role in human and animal well-being. A lot of work has been published on the positive effects of feeding tanniniferous forages having low levels CT to ruminants (Reed, 1995; Lowry *et al.* 1996; Barry and McNabb 1999). These findings warrant a fresh look at the present scenario on effects of tannin feeding on the nutritional status of ruminants. This review focuses on the emerging area of the beneficial effects of condensed tannins at low to moderate level of intake on ruminant productivity.

Chemical structure of condensed tannins

Condensed tannins or proanthocyanidins are derived from the polymerization of flavan-3-ol-units, and they normally occur in plant vacuoles. The flavan-3-ol monomer units can be linked by C4/C9 or C4/C6 interflavanoid linkages which effects the shape of the CT polymer chain (Ferreira *et al.* 1999). The number of monomeric units can vary and this determines the degree of polymerization from bi-, tri- and tetraflavanoids to higher oligomers. These can then produce an infinite variety of chemical structures which in turn effect the biological properties of the CT.

Effects of condensed tannins on voluntary feed intake, digestion process and utilization efficiency of digested nutrients

The positive effects of CT are depicted in Figure 1. CT are secondary plant compounds that can bind with protein by hydrogen bonding at near neutral pH (pH 4.0-7.0) to form CT-protein complexes, but dissociate and release protein at pH <3.5 (Jones and Mangan 1977). They are released during mastication, bind with feed and salivary proteins forming insoluble complexes at rumen pH and are released from the complex in the abomasum. Much work has been conducted upon the effects of CT on nutrient supply of sheep fed fresh forage diets, using controlled indoor experiments with individually fed animals. Barry and Duncan (1984) found a substantial depression of 27% in the voluntary

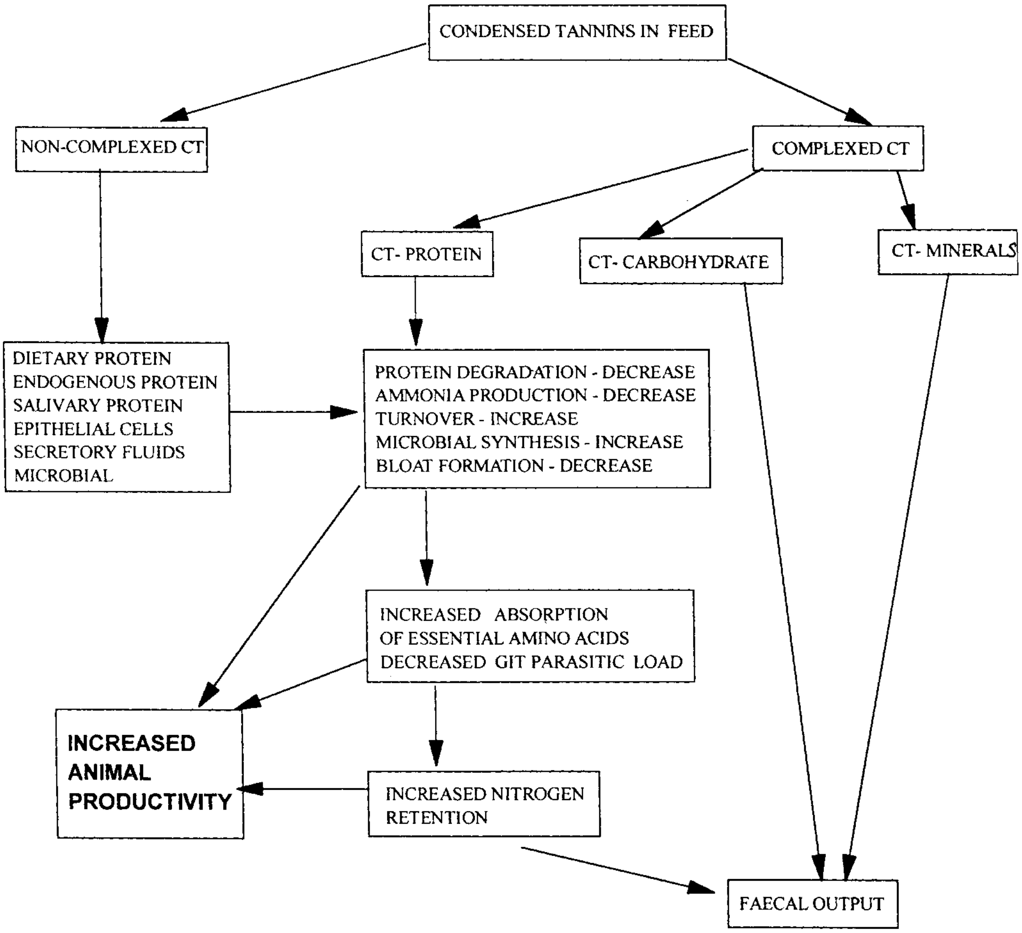

Fig 1. Positive effects of condensed tannins at low to moderate levels

feed intake (VFI) of sheep which grazed on *Lotus penduncuIatus* having high dry matter CT concentrations (6.3-10.6 %). Smaller depressions of 12% in VFI were produced by 5.5% CT in *Lotus penduncuIatus* (Waghorn *et al.* 1994). However, medium CT concentrations in sulla (4.5%) and in *Lotus corniculatus* (3.4- 4.4 %) had no effect upon VFI (Terrill *et al.* 1992; Wang *et al.* 1996a,b).

Duodenal non-amino nitrogen (NAN) flow can be used as an index of protein nitrogen leaving the rumen. With different cultivars of ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*), which contain only traces of CT, duodenal NAN flow is only about 0.75 of nitrogen intake, illustrating the extensive absorption of ammonia from the rumen. However, with *Lotus* spp. duodenal NAN flow increased linearly with increasing CT content and equalled nitrogen intake at ~ 4% CT concentration. McNabb, *et al.* (1996) attributed this effect to the action of CT in *Lotus* spp. in slowing the rates of both solubilization and degradation of forage proteins by rumen microbes, especially that of the principal leaf protein, ribulose-biphosphate carboxylase/oxygenase (Rubisco).

Waghorn, *et al.* (1987, 1994) found a difference due to CT level on the apparent absorption of essential amino acids (EAA) from the small intestine of sheep fed on fresh *L. corniculatus* (2.2% CT) and L. *pedunculatus* (5.5% CT). The CT of *L. corniculatus* increased both abomasal flow and the net absorption of EAA from the small intestine (53 and 59% respectively), with no effect on apparent digestibility in the small intestine, while CT of L. *pedunculatus* increased abomasal flow by 30% in sheep fed on this forage. However, this was counteracted by reduced apparent digestibility in the small intestine, with there being only a 10% increase in apparent absorption of EAA from the small intestine. These findings with *L. pedunculatus* could be due to differences in CT concentration with *L. corniculatus* and the different reactivity of CT of the two species with plant proteins due to dissimilarities in their CT structure (Foo *et al.* 1996, 1997).

McNabb, *et al.* (1998) were of the opinion that the differences in CT structure between *L. corniculatus* and *L. penduncuIatus* were insuffcient to cause any appreciable difference in the *in vitro* precipitation of Rubisco when their CT extracts were reacted with total soluble leaf protein from white clover. However, the CT extract from *L. penduncuIatus* was more effective at reducing Rubisco degradation by ruminal microbes than the CT extract from *L. corniculatus* (Aerts *et al.* 1999b). These findings suggest that protein precipitation by the CT may be more responsive to the relative molecular mass of the CT, and to a lesser extent, effected by the prodelphinidin content, whereas the effect of CT on the degradation of protein by ruminal microorganisms may be more responsive to difference in the flavan-3-ol composition of the CT. This aspect, besides the effects of the CT in not releasing some amino acids in the small intestine, increasing endogenous protein secretion or inactivating digestive enzymes, requires further investigation..

The effects of CT upon rumen fermentation of carbohydrate and protein has been explained by the concept of 'free tannin' developed by Barry and Manley (1986), and is defined as the CT not precipitated in high-speed centrifugation of plant mascerates. The plant proteins precipitated 90% of the CT and 10% was free in solution upto a total CT concentration of about 9%, whereas increments in total CT concentration above 9% were all released as 'free tannin'. Thus for *Lotus* spp. almost all the CT reacted with proteins in the host plant until the binding capacity of this system had been saturated (at ~ 9% CT). It has been suggested that insoluble CT functions through reducing plant protein degradation in the rumen, whereas free CT can react with and inactivate microbial enzymes, explaining why high levels of free CT reduce rumen carbohydrate digestion. This concept also explains why mixing CT-containing and non CT-containing temperate forages seldom produces a beneficial outcome (Beever and Siddons, 1986), as the CT will preferably react with proteins in the forage of the CT-containing plant. It also explains why there is limited transfer of CT-induced protein-precipitating activity through rumen fluid from CT-containing to non CT-containing plants. Beneficial effects of forage mixing can only be expected if the content is extremely high and the protein content relatively low in the CT-containing plant, thus releasing some 'free' CT to bind with proteins in the non CT-containing plant. These conditions occur with some tropical legume forages and legume shrubs, especially if grown under low soil-fertility conditions, and some advantage may occur if they are added as supplement to a diet of non CT-containing tropical grasses.

Wang, *et al.* (1996a) investigated the action of CT in lactating ewes rearing twin lambs that were grazing *L. corniculatus*. CT had no effect upon milk secretion in early lactation but increased the secretion rates of whole milk, lactose and protein by 21, 12 and 14% respectively, during mid-and late-lactation The diet selected was also of high nutritive value. containing 3.6% N and 4.4% CT, with an organic matter digestibility of 0.73. Milk protein concentration was unaffected by CT, but action of CT reduced milk fat content by ~ 1%. These results have implications for the dairy industry, as a nutritional treatment which simultaneously increase the efficiency of milk protein production whilst reducing fat content would be very desirable for human nutrition. Experiments of this type with dairy cows were conducted by Woodward *et al.* (1999) and they found that cows fed on *L. corniculatus* had higher milk yields than those fed on *L. corniculatus*+PEG, ryegrass or ryegrass+PEG, indicating that CT contributed to 42% of the increased milk yield that resulted from feeding *L. comiculatus* rather than ryegrass. CT had no effect on forage intake but accounted for all the increase in herbage conversion efficiency. It also caused a 57% increase in the milk protein of cows fed *L. corniculatus* when compared with milk from cows fed other forage preparations. In *L. corniculatus* fed cows, CT caused lowering of milk fat level but there was no effect on lactose content. However, herbage and CT had no effect on the casein or whey protein concentrations. Bhatta, *et al.* (2000) observed an increase in body weight and milk protein content of cross-bred dairy cows fed 7.5% tamarind seed husk, a tannin-rich agricultural by-product.

For studying the effect of CT on ovulation rate, trials were carried out with ewes grazing perennial ryegrass/white clover pasture and *L. corniculatus,* containing 0.1 and 2.3% CT respectively, Mean ovulation rates of ewes fed on *L. corniculatus* were greater than those of ewes grazing pasture, showing a clear response to CT (Min *et al.* 1999). It was also seen that action of CT increased lambing percentage through increasing fecundity with no effect on fertility.

The emerging role of condensed tannins in organic livestock farming

Ruminants grazing forage diets are subject to a number of diseases, some of which have a nutritional component. Two such conditions are rumen frothy bloat in cattle and internal parasitic infections in grazing sheep, cattle, deer and goats. Both are currently controlled by regular oral administration of chemicals - detergents in the former condition to disperse the foam and anthelminitic drenches in the latter case to kill the parasites. These remedies control both conditions in the short term but have long-term problems. First, they treat the symptoms and not the cause. Second, they cause consumer concerns about sustained use of chemicals and possible product residues, leading to longer withholding periods. Finally, in the case of anthelmintics, sustained regular use over many years has led to the development of parasites that are resistant to the control measures. Therefore, there is a need for development of new control measures that are more nutritionally based and ecologically sustainable.

Bloat is caused by very high solubility of forage proteins leading to the development of a stable foam in the rumen, and is very prevalent in cattle fed on legumes, especially in spring (Mangan, 1959). Because of their protein-precipitating properties, grazing of CT-containing legumes has long been known to eliminate bloat (Jones *et al.* 1973). However, the minimum plant CT concentration needed to make forage bloat-safe was not known. This has recently been proposed to be 0.5 % or greater (Li *et al.* 1996). Most common legumes and grasses used in temperate agriculture have CT concentrations well below this value, and both conventional plant breeding and genetic engineering techniques are now being examined to try and increase these levels (Aerts *et al.* 1999a).

Parasitism of the abomasum and small intestine causes extensive protein losses in sheep (Kimambo *et al.* 1988), and re-directs protein synthesis away from skeletal muscles and into repair of gut tissues, leading to reduced N retention (MacRae, 1993). Increasing dietary protein intake and abomasal infusion of protein results in the animal being much better, able to tolerate these infections and improves N retention (Brown *et al.* 1991; Coop and Holmes, 1996), with the main effect of increased protein supply being to increase the rate of acquisition of immunity. Niezen, *et al.* 1993a, b, 1994 and 1996 initially observed that sheep fed natural diets rich in CT gained weight more rapidly than controls. Subsequent studies showed that lambs grazing CT-containing forages (*L. pedunculatus* and *Sulla*), are better able to tolerate parasitic infections than lambs grazing non CT-containing forage (lucerne) and have both increased growth and lower worm burdens (Niezen *et al.* 1995, 1998). Similar observations have been made in deer infected with gastrointestinal parasites and lungworms (Molan *et al.* 2000a,b; Hoskin *et al.* 2000). Butter, *et al.* 1998 and Athanasiadou, *et al.* 2000 observed a reduction in the burden of *Trichostrongylus colubriformis* in trickle-infected or single dose-infected sheep which consumed a CT containing extract incorporated in their feed, compared with infected sheep fed a standard ration. A 37-63% inhibition in migration of *T. colubriformis* larvae has been reported for CT extracts of seven forages (Molan *et al.* 2000c). The addition of polyethylene glycol (PEG) to the CT extracts eliminated 81-93% of their larval migration inhibition activity. A similar effect of PEG on CT activity has been observed in the case of goats grazing on CT-rich browse (Kabasa *et al.* 2000; Molan *et al.* 2000d). The faecal helminth egg counts in these free-ranging goats which was quite low in control, nearly doubled in animals administered PEG orally, thus negating the beneficial effect of CT in lowering the host's gastrointestinal parasitic burden. The above studies suggest that condensed tannins may have an effect on the populations of larval and/or adult worm population of gastrointestinal nematodes in ruminants.The question is whether this effect is a direct anthelmintic effect, or indirect, or a combination of both. An indirect metabolic effect could be mediated through the ability of tannins to bind to dietary protein in the buccal cavity and rumen, and thus increase the amount of undegradable protein passing to the small intestine for absorption (Woodward and Reed, 1997). Such an increase has been shown to improve the ability of ruminants to mount an effective immune response against gastrointestinal parasites (Coop and Kyriazakis, 1999). There may be involvement of two possible mechanisms. First, improved EAA supply from the action of the CT may counteract the protein loss caused by gut parasitism and may stimulate the immune system, and second the CT may directly react with and inactivate parasite larvae during passage through the gut. Forages containing CT may thus lead to the development of nutritionally-based and ecologically-sustainable systems for disease control in grazing animals and allow the reduction in the use of anthelmintic drugs.

Conclusions and future projections

The ideal CT concentration for ruminant nutrition has been suggested to be in the range of 2-4 % (Barry 1989; Waghorn *et al.* 1990). Improved estimation methods have shown the presence of trace amounts of CT (0.1-0.2 %) in most of the common grasses and legumes grazed in temperate agriculture. However, results to date show that this is too low to reduce protein solubility and degradation in the rumen, reduce bloat and gastrointestinal parasitism, and a low to moderate level of CT is suggested (Figure 1) i.e. a minimum concentration of 0.5% (for control of bloat) to 2-4% (for control of internal parasites). Robertson, *et al.* 1995 have proposed a system for controlling internal parasites in sheep with minimal anthelmintic usage in which CT-containing and CT-free plants are alternatively grazed. An agronomic evaluation of tanniniferous plants has also to be done as this will determine how they can be incorporated into ruminant production systems. By integrating CT-containing forages into intensive grazing systems in this way, a much more sustainable control of both internal parasites and bloat, with reduced dependence on chemicals and simultaneous increase in production efficiency of animals will be achieved. Evaluation of traditional plant breeding methods and genetic engineering techniques and their application for increasing CT concentration in common legumes such as white clover, red clover (*Trifolium pratense*) and lucerne offers exciting future possibilities. The effects of long term feeding of CT-containing forages on animals and their rumen microbial ecosystem also need to be investigated.

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