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End of Symposium

## REVIEW

## **Tannins: Their Adverse Role in Ruminant Nutrition**

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This review attempts to provide a current summary of literature concerning the chemistry of tannins and their various adverse effects upon ruminant feed quality. These include the chemical nature of hydrolyzable and condensed tannins, their occurrences in numerous feeds and fodders, their interaction with proteins, and subsequent effects upon voluntary feed intake, dry matter and protein digestibility, and rumen metabolism. In addition, the processing of tannin-rich ruminant feed for their increased utilization is also discussed.

Tannins present in many important forages, several agricultural wastes, agroindustrial byproducts, and fodder tree leaves not only affect the feed quality adversely but also cause toxicity. Several episodes of loss of livestock from easting oak acorns have occurred in Europe (Wolter, 1974) in the American southwest (Sandusky et al. 1977; Keeler et al., 1978), and in South Africa (Naser et al., 1982), owing to the fact that oak leaves and twigs are eaten heavily at times when little else is available. Further, the inexorable scarcity of livestock rations in developing countries has made it obligatory to incorporate tannin-rich feeds in the livestock ration. Enormous work has been done to overcome the ruminants' nutritional problems associated with the presence of a high quantity of tannins in feeds and fodders; however, the literature is scattered and the last comprehensive review on the subject by M. N. McLeod appeared in 1974. Therefore, the present review attempts to compile and summarize the recent studies on the adverse effect of tannins upon ruminants' feed quality and rumen enzymatic reactions and various methods for their removal from ruminants feed. The beneficial effects of tannins such as the prevention of bloat and protection of protein against rapid ruminal degradation deserve a review of their own and are not included here.

Chemistry of Tannins. The term tannin referred originally to substances with the ability to tan leather. It is now generally used to include any naturally occurring compound of high enough molecular weight (500-3000) and containing a large number of phenolic hydroxylic groups (one to two 100 molecular weight) to enable it to form effective cross-links with protein and other molecues (Swain, 1979). The hydrolyzable and condensed tannins are two groups of these compounds widely distributed in the plant kingdom, which may be differentiated by their structure and reactivity toward hydrolytic agents (Freudenberg, 1920; Haslam, 1966). The chemistry of the two groups have been extensively reviewed (Swain, 1979; Haslam, 1981).

The potential livestock feeds rich in hydrolyzable tannins are green pods of Ceratonia siliqua (Joslyn et al., 1968), leaves of Quercus robus (Feeney and Bostock, 1968), acorns of Quercus incana (Vijjan and Katiyar, 1973), acorns of European Oak (Quercus pedunculata) Singleton, 1981), and deoiled sal (Shorea robusta) seed meal (Kumar,

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1980). However, the principal forage tannins are usually of a condensed type (McLeod, 1974, 1982). The presence of condensed tannins have been demonstrated in legume pasture species (Bell et al., 1965; Burns et al., 1967; Osbourn et al., 1971; Milic et al., 1972; Bate-Smith, 1973; Jones et al., 1976; Sarkar et al., 1976; Foo et al., 1982), in sorghum grain (Strumeyer and Malin, 1975; Gupta and Haslam, 1978), in the tree leaves (Lohan and Negi, 1981; Haslam, 1981; Negi, 1982), and in the agroindustrial waste products like sal seed meal (Negi, 1982).

Condensed tannins have been observed to possess a wide range of molecular weights. Jones et al. (1976) found that the molecular weights of condensed tannins isolated from the five pasture legume species ranged from 5800 to 13 200. Molecular weights in the region of 1700–2000 have been observed for the polymeric procyanidin of sorghum grains (Gupta and Haslam, 1978). Foo et al. (1982) determined the structure of condensed tannins of common fodder legumes and showed that most legume condensed tannins had an approximately normal distribution of molecular weight with number average values of 2000–4000.

Tannin–Protein Interaction. Tannins react with protein and form protein-tannin complexes. In the case of vegetable tannage itself, the protein collagen chains are cross-linked by appropriate polyphenols to give leather (Haslam, 1981). Four types of bonds have been suggested to participate in the formation of protein-tannin complexes: hydrogen-bond formation between phenolic and ketoimide groups of the protein in an arrangement analogous to that of the  $\beta$ -pleated sheet (Haslam, 1974), ionic bonds between the phenolate anion and the cationic site of protein molecule (Loomis, 1974), covalent links formed by oxidation of polyphenols to guinones and subsequent condensation with a nucleophilic group (-NH<sub>2</sub>, -SH, -OH) in the protein (Loomis, 1974; Haslam, 1979), and hydrophobic interaction between the aromatic ring structure of phenolic compounds and hydrophobic regions of protein (Loomis, 1974; Hagerman and Butler, 1980). The process of complex formation-brought about in part by H bonding and in part by hydrophobic bonding--is normally reversible, and both protein and polyphenols can in principle be recovered unchanged from the complex; however, the protein and polyphenol are brought into contact under some conditions (e.g., alkaline pH,  $O_2$ ), then the polyphenols may become oxidized (to a quinone), and the oxidized form may then form covalent linkages with protein, making the association irreversible (Haslam, 1982). The factors that determine the relative affinities of proteins for the tannin were studied by Hagerman and Butler (1981), who showed that proanthocyanidins efficiently precipitated one protein in the presence of a large excess of another protein. The specificity of interaction was an inverse function of size, conformation, and charge of the protein molecule. Proline-rich protein had very high affinities for tanning due to their open conformation and their capacities to form strong hydrogen bonds. However, the affinity of tannin for the protein precipitation has been observed to increase regularly in the polyphenolic series from those with a molecular weight of 576 and to those with a molecular weight beyond 1134 (Bate-Smith, 1973). The minimum molecular weight is about 350 for effective protein precipitation. In the condensed series this would begin with dimeric flavonoids as also suggested by Butler (1982) that bird repellency in sorghum may be due to short oligomers. In hydrolyzable tannin no fewer than two gallic acid precursor units or one ellagic acid precursor unit would be the theoretical minimum; when the molecular weight is quite large, more than 5000, the condensed tan-

nins becomes so poorly soluble in physiological solutions. even in absence of protein, that they have little leatherforming ability or astringent taste. Haslam (1974) observed that the polyphenol-protein complex formation that results in the precipitation is formed by cross-linking of separate protein molecules by the phenols and the latter's tanning capacity, although broadly related to molecular size, is primarily dependent on the number of separate sites in the molecule able to associate with the protein. The possible separate site in the natural polyphenols was suggested as o-dihydroxy and trihydroxy phenolic nuclei for association with protein, and the isolated phonolic groups did not seem to participate to any significant extent. It should be noted that condensed and hydrolyzable tannins display many structural similarities such as Odihydroxy and trihydroxy nuclei, which all project toward the outer surface of molecular structure.

McManus et al. (1981) studied protein-polyphenol complex formation by equilibrium dialysis and microcalorimetry and proposed a theory for precipitation of protein. At low protein concentration the polyphenol associates at one or more sites on the protein surface to give a monolayer that is less hydrophilic than the protein itself. Aggregation and precipitation then ensue. When the protein concentration is high, the relatively hydrophobic surface layer is then formed by complexation of the polyphenols on the protein and by cross-linking of different molecules by the multidentate polyphenols.

Effect of Tannins upon Feed Utilization. Tannins tend to depress the nutritive value of fodder for ruminants by reducing its voluntary feed intake and digestibility (McLeod, 1974).

Voluntary Feed Intake. From the biological point of view, the importance of tannins lies in their effectiveness as a repellent to predators whether animals or microbial. In either case the relevant property is "astringency" (Bate-Smith, 1973, 1981), rendering the tissue unpalatable by precipitating salivary protein or by immobilizing enzymes, impeding the invasion of the tissue of the host by the parasites (Harborne, 1976). Unpalatability due to astringent tannins leads to reduction in the voluntary feed intake. Reports of grazing tests of Sericea lespedeza with sheep (Wilkins et al., 1953) and steers (Donnelly, 1954) showed that animals consumed more of the low tannin containing plants than those of high tannin containing plants. Donnelly and Anthony (1969) reported that the tannin level required for rejection by grazing animals is about 20 mg/g of dry matter. Jokl and Carlson (1982) reported that the leaves of Eucalyptus saligna and Lecytis pisonis were not acceptable to grazing animals due to high phenolic contents. Sal seed cake as such was found to be unpalatable to the animals but could be accepted when mixed with some palatable concentrate (Patel et al., 1972). Jones et al. (1976) isolated condensed tannins from legume pasture species and studied their molecular size distribution and composition (delphinidin/cyanidin ratio), for relating them with palatability. They concluded that palatability was in order of their prodelphinidin content and showed that Trifolium arvenese and Trifolium affine were less palatable than the Coronilla varia. Tannins also diminish the permeability of the gut wall, by reacting with the outer cellular layer of the gut (Mitjavila et al., 1977), so the passage of the nutrients through the gut wall is reduced. All these factors may adversely affect the voluntary feed intake by the ruminants.

*Effect upon Digestibility.* Tannins are the limiting factors in many plant forages and agricultural and industrial waste products of low biodegradability. Tannins

| Table I. | Tannin ( | Content and | Crude | Protein | Digestibility | of | Various | Tree L | eaves |
|----------|----------|-------------|-------|---------|---------------|----|---------|--------|-------|
|----------|----------|-------------|-------|---------|---------------|----|---------|--------|-------|

| species               | % tannin<br>content | % crude<br>protein<br>digestibility | references  |
|-----------------------|---------------------|-------------------------------------|---|
| Grewia optiva         | 0.00                | 72.0                                | Lohan et al. (1980)                                 |
| Ailanthus excelsa     | 0.25 (a)            | 16.2(b)                             | (a) Seghal (1981); (b) Singh and Patnayak (1977)    |
| Albizia lebbeck       | 0.73                | 67.0                                | Lohan et al. (1980)                                 |
| Ficus glomerata       | 0.76                | 60.0                                | Lohan et al. (1980)                                 |
| Moras alba            | 0.80                | 71.0                                | Lohan et al. (1980)                                 |
| Ficus benghalensis    | 0.98                | 21.0                                | Lohan et al. (1980)                                 |
| Bauhinia veriegata    | 1.21 (a)            | 10.7 (b)                            | (a) Lohan et al. (1980); (b) Kehar et al. (1955)    |
| Aegle marmelos        | 2.21                | 71.0 `´                             | Lohan et al. (1980)                                 |
| Leucaena leucocephala | 1.53 (a)            | 16.7 (b)                            | (a) Lohan et al. (1980); (b) Upadhaya et al. (1974) |
| Acacia catechu        | 1.54                | 24.0                                | Lohan et al. (1980)                                 |
| Gymnosporia soinosa   | 2.01 (a)            | 2.6 (b)                             | (a) Sehgal (1981); (b) Mohan et al. (1977)          |
| Quercus incana        | 2.56                | 57.0 <sup>°</sup>                   | Lohan et al. (1980)                                 |
| Prosopis cineraria    | 2.92 (a)            | 4.4 (b)                             | (a) Sehgal (1981); (b) Bhandari et al. (1979)       |
| Zizyphus nummularia   | 4.30 (a)            | 5.5 (b)                             | (a) Daniel et al. (1978); (b) Nath et al. (1969)    |
| Terminalia bellirica  | 6.42                | 10.0                                | Lohan et al. (1980)                                 |
| Eugenia Jambalana     | 7.57                | 1.0                                 | Lohan et al. (1980)                                 |

in feed diminish the digestibility of the dry matter (Burns and Cope, 1974) and of the nitrogen (VanSoest, 1981). However, the chemical and biochemical nature of tannins seems to have an effect upon protein digestibility as there are reported anomalies in the digestibility of proteins of tree leaves vis-a-vis their total tannin content (Table I). These anomalies might be explained by partitioning the total tannin content in hydrolyzable and condensed form and by determining their protein precipitation capacity and degree of polymerization. Reid et al. (1982) reported that condensed tannin in the neutral detergent fiber of cassava limits its utility as a forage. In vivo dry matter digestibility of S. lespedeza was 58.5 and 64.5%, respectively, in high- (5.2-7.3%) and low-tannin (2.5-2.9%) plants (Donnelly and Anthony, 1970). In vitro digestibility of C. varia (Burns and Cope, 1974), S. lespendeza (Cope and Burns, 1971), and Sorghum forage (Arora et al., 1975) had a negative correlation with tannin content. The low digestibility of crude protein of Acacia albida and Adasonia digitata has also been attributed to the presence of the high quantity of tannins (Diagayete, 1981). Hibberd et al. (1982) and Buckley et al. (1983) studied the in vitro dry matter disappearance of several sorghum and faba bean varieties, respectively, and found that variation was due to the presence of tannins. Robb (1976) reported a negative protein digestibility in sal (Shorea robusta) meal that was attributed to the presence of the high quantity of tannins. Tannins present in sal seed meal (SSM) formed complexes not only with the protein of SSM but also with other dietary proteins as well (Negi, 1982). If binding dietary protein were the only direct effect of dietary tannins, then supplementation of diet with extra protein should eliminate it. Protein supplementation does markedly alleviate the growth-depressing effect of tannic acid (Glick and Joslyn, 1970). However, a large quantity of protein would be required to annul the effect of dietary tannins, as Hagerman and Butler (1978) showed that for total incorporation of tannins in tannin protein complex, at least twice as much protein as tannin (by weight) was required.

Apart from this, tannin in ruminant feed also results in a low milk yield (Jagadale et al., 1976), reduction in the availability of sulfur (Garner and Hurwood, 1976), toxic degenerative changes in the intestine, liver, spleen, and kideny (Gupta et al., 1977), mucus appearance in the urine (Mudgal and Sampath, 1969), and fatal constipation (Lohan et al., 1979). Therefore, tannin-rich feed sources for ruminants should be restricted or fed with caution as also suggested by Negi (1982).

The reduced digestibility of tannin-rich feeds can also be explained on the basis of the inhibition of digestive enzymes (Bressani and Elias, 1979). Tannins are a potent inhibitor of digestive enzymes due to their capacity to bind with enzyme proteins as well as with the substrate. The inhibition of trypsin by tannins of oak leaf (Feeney, 1969), carob pods (Tamir and Alumot, 1969), leucerne (Milic et al., 1972), Vicia faba (Griffith, 1979), and field beans (Griffith and Mosley, 1980) has been reported.

The phenolic compounds can affect the enzymes by either (a) reducing the solubility of the enzyme protein by forming insoluble protein-phenolic complexes (Williams, 1963) or (b) inhibiting the enzyme activity by forming a soluble but inactive enzyme-inhibitor complex (Loomis and Battaile, 1966; Zanobini et al., 1967). Therefore, competitive and noncompetitive reaction kinetics can be visualized for the inhibition of enzymes by tannins. Hall (1966) observed that the degree of inhibition of pectinesterase was proportional to the tannic acid concentration and with the increase in substrate concentration the inhibition decreased. The inhibition was reversible. Noncompetitive reaction kinetics have been reported in galactosidase (Goldstein and Swain, 1965), trypsin (Tamir and Alumot, 1969), amylase (Tamir and Alumot, 1969; Davis and Hoseney, 1979), and lipase (Griffith, 1979). However, a kinetic study of the inhibitory activity of the standard leaf protein concentrate (LPC) sample prepared from Italian rye grass, fescue, and quinoa indicated that, in each instance, there was mixed inhibition (Humphries, 1980). The resistance, to enzyme attack of the substrate complexed with tannin has been illustrated by Feeney (1969).

Effect of Tannin-Rich Feeds upon Rumen Metabolism. Tannins have been found to influence rumen metabolism in general. The influence of tannins on various ruminal enzymatic reactions has been studied in detail. It was reported that a water-soluble substance in the leaves of *S. lespedeza* inhibited the enzymatic hydrolysis of cellulose (Smart et al., 1961; Cope et al., 1971) and reduction in cellulase activity was proportional to the concentration of the inhibitor present (Smart et al., 1961). Lyford et al. (1967) observed that this inhibitor was a part of the tannin fraction and was shown to be a polymer of delphinidin. Henis et al. (1964) studied the effect of aqueous extracts of carob pods upon the growth and morphology of microbes and found that their tannin fractions exerted both bacteriostatic and bactericidal effects upon Cellvibrio fulvus-a cellulolytic bacteria-and it was suggested that the site of action of tannins on sensitive microbes was the cell envelope. Tagari et al. (1965) demonstrated that inhibition of proteolysis and microbial synthesis occurred in the artificial rumen due to the presence of tannins in carob pod extracts, whereas the ammonia production remained unchanged. However, Leroy et al. (1964) reported that addition of aqueous tannin solution to groundnut and soya bean meals, when incubated with ruminal content in vitro, significantly reduced ammonia release without affecting cellulose digestibility. It may be noted that in the first case (Tagari et al., 1965) carob tannins were added directly to the ruminal content in vitro medium whereas in the second experiment (Leory et al., 1964) tannins were allowed to react with protein prior to in vitro incubation. Tripathi (1978) noted that the breakdown of groundnut protein was inhibited in the goat rumen by the addition of tannic acid. Pala (Zizyphus nummularia) leaf tannin has also been reported to have a inhibitory effect upon in vitro ruminal proteolysis of casein (Kumar and Singh, 1984). A poor protein disappearance rate due to high phenolic content of spent tea leaves by in vivo ruminal microbial fermentation has been observed by Jayasuria et al. (1982).

Benoit and Starky (1968) observed that urease was inhibited by wattle tannin. The inhibition of rumen fluid urease activity by acqueous extracts of oak (*Quercus incana*) leaves was reported by Lohan et al. (1981), and the inhibition was proportional to the content of inhibitor in the reaction mixture. However, Singh and Arora (1980a) demonstrated that SSM tannin did not affect the rumen urease activity; instead, more ammonia values were found in the presence of a high concentration of tannins. These contradictory findings can be explained on the basis of their respective methods of studies. Benoit and Starky (1968) and Lohan et al. (1981) allowed the tannins to react with enzyme prior to the addition of substrate, whereas Singh and Arora (1980a) added tannins after the addition of the substrate.

Tannin inhibited the gas (McGinty, 1969) and the volatile fatty acid (VFA) production in rumen (Singh, 1977, 1978). Sadanandan and Arora (1979) found that ruminal VFA, microbial DNA, and RNA decreased with increase in the tannic acid concentration in the diet. Phosphate utilization by rumen microbes is also reported to decrease with an increase in tannin concentration (Sadanandan and Arora, 1975). This type of microbial inhibition may have negative or positive effects on the animal depending upon the situation. Microorganism-dependent cellulose digestion is inhibited, and utilization of poor-quality feed by the ruminant is impaired. Sheep fed sorghum silage with 18.7 g of tannin/kg had depressed digestion of crude fiber and less microbial activity in the rumen when compared with sheep fed with maize silage containing 6.6 g/kg tannin (Ben-Ghedalia and Tagari, 1977). The threshold of toxicity of tannic acid added directly to rumen contents in fistulated animals was 3-5% in cattle but 8-10% in goats, apparently because the goat produced an active tannase in the rumen mucosa (Bejovic et al., 1978).

Methods of Removal or Inactivation of Tannins. Since plant tannins hinder utilization of fodders for livestock, it will be desirable to reduce the level of tannins. In most of the plant kingdom tannin content is simply inherited by one or two genes and it is not difficult to eliminate the tannin factor (Ma and Bliss, 1978; Marshall et al., 1981). A question then arises whether in selecting for low tannin content a breeder may lose important agronomic advantages of the high-tannin line. High-tannin sorghum with an open penicle structure appear to prevent bird depredation (Harris, 1969). Tannins are also responsible in plant resistance to pathogens and insects (Feeney, 1976; Schultz and Baldwin, 1982). Another desirable characteristic of tannins is weather resistance, in particular retardation of preharvest seed germination and molding (Harris and Burns, 1970). Therefore, a number of methods have been tried to overcome the nutritional problems associated with high-tannin ruminant feeds. However, the parallel information about all forages is scanty but the reviewed literature may provide a basis for extending research on this line.

Water Treatment. Simple soaking, washing, and boiling with water have been shown to remove up to 80% tannins from sal seed meal (Singh and Arora, 1978b; Panda et al., 1979). However, the dry matter loss was 18-23%, which could carry away the water-soluble nutrients. Reichert et al. (1980) observed that imbibing the whole sorghum grain with 25% water by weight, for 9 days, reduced the tannin content from 3.63 to 0.3%. However, Price et al. (1980) demonstrated that by boiling high-tannin sorghum grain in water did not improve its nutritional quality, which could be due to unextractability of condensed tannins. Bate-Smith (1975) showed that condensed tannin such as procyanidin A could not be extracted in water due to its insolubility, and McLeod (1974) concluded that the principle forage tannins are of the condensed type.

Alkali Treatment. Recent work for removal/reduction/inactivation of the tannin level from two ruminant feeds is documented in Table II. Data presented in Table II clearly demonstrate that from sal seed meal, tannins can be removed maximally up to 74 and 100% by NaOH and  $Ca(OH)_2$  treatment, respectively. Armstrong et al. (1974) observed that the digestibility of protein of bird-resistant sorghum grain was 4.35% and, when the tannin was extracted by alkali, the value rose to 17.8%. Improvement in protein digestibility (Chavan et al., 1979) and dry matter digestibility (Reichert et al., 1980) of high-tannin sorghum grain after alkali treatment has also been reported. Singh and Arora (1978a) showed a better utilization of alkalitreated sal seed meal (AT-SSM) in a preliminary study on bullocks fed on the wheat straw. In a later study, Singh and Arora (1980b) observed that incorporation of 45% AT-SSM in the concentrate mixture provided a better growth rate in growing crossbred calves when compared with the incorporation of untreated SSM, but the feed/ gain ratio was found to be lowered. Negi (1982) suggested that the residual tannins in the SSM after alkali treatment were condensed tannins that might bind irreversibly with SSM protein and, therefore, exerted more harmful effects. Another explanation may be that sodium hydroxide can oxidize the tannin molecule. Oxidation converts hydrogen-bond donor hydroxyls into acceptor quinone carbonyls. This decreases protein binding by the usual tannin mechanism but may give substitution reactions with lysine amino or cyteine sulfhydryl groups, and so tannins bind dietary protein irreversibly. It is possible, therefore, that tannin after alkali treatment might not influence the enzymatic reactions and microbial activity; however, the irreversibly bound dietary protein will remain unutilized at the same time. Calcium hydroxide treatment was also shown to be ineffective, when feeding trials with goat revealed that treated SSM produced the usual deleterious effects such as lowered dry matter, energy, and protein

| Table II. | Removal | l of Tannins by | Various Alkali | Treatments | (Recent Work) |
|-----------|---------|-----------------|----------------|------------|---------------|
|-----------|---------|-----------------|----------------|------------|---------------|

| chemical used   | feed                                 | maximum<br>reported<br>reduction<br>in tannin<br>content, % | references              |
|---|--------------------------------------|---|-------------------------|
| NaOH  | sal seed (Shorea robusta) meal (SSM) | 68-74   | Wah et al. (1977)       |
|   | sorghum grain Br-54                  | 99  | Price et al. (1979)     |
|   | high-tannin sorghum grain IS 2825    | 86  | Chavan et al. (1979)    |
|   | high-tannin sorghum grain            | 86  | Reichert et al. (1980)  |
| КОН   | high-tannin sorghum                  | 87  | Chavan et al. (1979)    |
| Ca(OH),   | SSM                                  | 100   | Wah et al. (1977)       |
| lime water  | SSM                                  | 100   | Sinha and Nath (1982a)  |
| NH₄OH   | sorghum grain Br-54                  | 94  | Price et al. (1979)     |
| -   | high-tannin sorghum grain IS 2825    | 80.5  | Reichert et al. (1980)  |
| Na <sub>2</sub> CO <sub>3</sub>   | SSM                                  | 57.51   | Singh and Arora (1978b) |
| 2 5   | sorghum grain IS 2825                | 83  | Chavan et al. (1979)    |
| K <sub>2</sub> CO <sub>3</sub>  | Br-54 sorghum                        | 90  | Price et al. (1979)     |
| NaHCO,  | SSM                                  | 49.88   | Singh and Arora (1978b) |
| mogadi soda (Na <sub>2</sub> CO <sub>3</sub> ,<br>NaHCO <sub>3</sub> , 2H <sub>2</sub> O) | sorghum grain                        | 57  | Mundi et al. (1981)     |

digestibility and white flakes in the urine (Sharma et al., 1977).

There are reports that loss of dry matter (20-70%) occurs after alkali treatment for removing tannins (Wah et al., 1977; Singh and Arora, 1978b). This would leach out the soluble nutrients in the ruminants feed and fodder. Loss of cysteine occurs when soyabean is treated with 0.1 N NaOH (Badenhop and Hackler, 1973). It may be noted that cysteine is an important ingredient of the diet of sheep for wool. Lignins present in plant cell wall are solubilized by alkali treatment and may have an additional effects upon rumen fermentation as a polyphenolic inhibitor (VanSoest, 1981). Therefore, NaOH treatment for removing tannin should be studied with extreme caution.

Gandhi et al. (1975) processed the sal seed meal with  $NH_3$  to depolymerize tannins and observed that the processed meal was nontoxic and more palatable and loss of dry matter did not occur. Price et al. (1978) also reported the improvement in nutritional quality of high-tannin sorghum grain by moistening it with  $NH_4OH$ . Treatment with  $NH_3$  increased the N content of sorghum grain by 17.2% even after the treated grain had been ground and exposed to air until no smell of  $NH_3$  could be detected (Ford and Hewitt, 1979). Therefore, the treatment of high-tannin feed with ammonia may be advantageous to the ruminants, which could essentially lead to an increase in the NPN content. However, its economics has to be explored before incorporating it in the field conditions.

Treatment of high-tannin sorghum grain with NaHCO<sub>3</sub> reduced the tannin content, but harmful effects could not be overcome as the rats fed treated high-tannin sorghum grain gained even less weight than rats given untreated grain (Price et al., 1980). In sal seed meal the dry matter loss by Na<sub>2</sub>CO<sub>3</sub> and NaHCO<sub>3</sub> treatment ranged from 19 to 26% (Singh and Arora, 1978b). Treatment with mogadi soda (Na<sub>2</sub>CO<sub>3</sub>, NaHCO<sub>3</sub>, 2H<sub>O</sub>), of tannin-rich sorghum grains have been found to increase the digestibility of organic matter, starch, and crude protein (Mundi et al 1981; Mundi and Thomke, 1981).

Removal by Addition of Adsorbents. Tannins could be removed by the addition of certain adsorbents such as polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG) to which they bind more strongly than proteins (Loomis, 1974). Rayudu et al. (1970) showed that this property of binding tannins was operative in vivo, because PVP markedly reduced the growth-depressing effect of tannic acid in chicks. Jung and Fahey (1981) studied ruminal in vitro digestion of forages by using PVP to remove the forage phenolics that appeared to increase cellulose and protein digestibilities of alfa alfa (*Madicago sativa*) but not that of crownvetch (*Coronilla varia*). Jokl and Carlson (1982) observed that the tree leaf protein concentration had a low nutritive value due to associated phenolics but the value was enhanced by the addition of PVP.

Feeding experiments with sheep showed that condensed tannin of sainfoin inhibited the release of soluble protein in the rumen and, when PEG-4000 was added, a large quantity of protein was released (Reid et al., 1974). Jones and Mangan (1977) demonstrated in vivo experiments with sheep and with in vitro studies that PEG breaks the complex of protein with condensed tannin.

Formalin Treatment. Daiber (1975) and Kemm et al. (1981) showed that formalin treatment of sorghum grain changes polyphenols (tannins) to nonreactive resins, and Reichert et al. (1980) observed that 0.3% formalin reduced tannin by 88.98% from high-tannin sorghum, which could increase its nutritive value. But in an experiment with sheep, formaldehyde-treated bird-resistant sorghum grain did not affect the organic matter intake, intake of digestible matter, dry matter digestibility, organic matter in rumen, and ruminal ammonia concentration in comparison to the untreated sorghum grain diet (Pienaar and Renton, 1980). Detailed experiments to study the effect of HCHO treatment on tannin-rich feeds are therefore necessary to arrive at a conclusion that will be relevant for the animal production.

Urea Supplementation. If the interaction of dietary protein with tannins leading to the formation of indigestible protein-tannin complexes were the direct effect of dietary tannins, then supplementation of the diet with extra protein should eliminate it. But a large quantity of protein would be required to annul the effect as Hagerman and Butler (1978) showed that for total incorporation of tannins in tannine protein complex, at least twice as much as protein as tannin (by weight) would be required. In ruminants diet urea can replace part of the proteins. Therefore, urea supplementation with tannin-rich food has been tried in a few cases. Deoiled sal seed meal (DSSM) has a negative crude protein digestibility (Robb, 1976). Rai and Shukla (1977) observed that CP digestibility of the concentrate containing 20% DSSM was not affected in the presence of 10% urea. Sinha and Nath (1982b) observed that urea supplementation made the DSSM palatable and enhanced the digestibility of nutrients, especially that of crude protein and crude fiber. Buckley et al. (1983) found no difference between the ruminal IVDMD of tannin-rich faba beans (Diana) and tannin-free beans in the presence of excess urea. However, the same quantity of urea could

not annul the deleterious effect of the higher quantity of tannins in Hertz Freya faba beans. Therefore, a quantitative relationship between tannins and urea for improving the feed quality has to be worked out. Savon et al. (1973) in an attempt to protect protein by tannic acid in a high molasses urea diet, could not observe any significant difference between ruminal pH, NH<sub>3</sub>, and VFA of protected and unprotected samples. Urea supplementation with tannin-rich feeed can improve the feed quality by providing the extra N source and by its chemical activity. Urea destabilizes the hydrogen bonds and hydrophobic interactions (Nozaki and Tanford, 1963), which participate in the formation of the protein-tannin complex. Therefore, the use of urea may render protein free from the complex, for its further utilization by animals.

Summary and Research Needs. The high quantity of tannins present in many important forage crops, agricultural waste, byproducts, and tree leaves hampers their utilization as a ruminant feed. Tannins quickly combine with proteins in the diet, salivary protein, digestive enzymes, mucus secretion, and microorganisms and adversely affect the rumen metabolism. This may lead to griping diarrhea or constipation, reinforcing food avoidance reactions in the presence of tannins. The tannins are seen as plant protectants having this sort of "toxic" effect. The risk to unconfined animals having a choice of diet seems very small. It is not small, however, when high-tannin food is the only choice, as has been proved by the fatalities in animals. Therefore, in areas where high-tannin forages form an important source of feed, the tannin-protein interactions are creating a nutritional problem of applied nature.

Significant progress in removing the tannins from ruminant feed has been made in recent years. However, some aspects of their economical field application remains to be elucidated. First, the cost of chemicals used may be detrimental to the farmers; second, loss of dry matter during the removal process may not be in the economical interest of the livestock industry.

Determination of the procyanidin content of various feeds and forages, as it affects the platability, exploration of the range of molecular weight and degree of polymerization of tannin that determine the protein precipating capacity of tannins, studies on enzyme inhibition kinetics, and specific interaction of plant proteins with tannins leading to the formation of complex and breaking the complex in vivo by some possible common ruminants feed component are some of the applied areas of future research.

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