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Exploitation of tannins to modulate rumen ecosystem and ruminants performance: A review

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ABSTRACT

Tannins are polyphenolic polymers of relatively high molecular weight with the capacity to form complexes mainly with proteins due to the presence of a large number of phenolic hydroxyl groups. They are widely distributed in nutritionally important forage trees, shrubs and legumes, cereals and grains, which are considered as anti-nutritional compounds due to their adverse effects on intake and animal performance. However, tannins modulate rumen fermentation favorably such as reducing protein degradation in the rumen, prevention of bloat, inhibition of methanogenesis and increasing conjugated linoleic acid concentrations in ruminant-derived foods. The inclusion of tannins in diets has been shown to improve body weight and wool growth, milk yields and reproductive performance. This review discusses the effects of tannins on nutrient metabolism, rumen fermentation, and microbial populations bacteria, protozoa, fungi and archaea), metabolism of tannins, microbial tolerance mechanisms to tannins, inhibition of methanogenesis, and performance of animals. This review seeks to highlight the practical significance of tannins on ruminant nutrition in developing countries. It will also review approaches to counter the negative effects of tannins.

Key words: Antinutritional compounds, Bloat, Methanogenesis, Rumen microbes, Ruminants, Tannins

Animal husbandry is one of the important activities of farmers in the developing countries including India and availability of feed resources is identified as a major constraint. The interaction of the diet, microbes and animal host that determine the net supply of nutrient to the host are complex (Van Soest 1994). An interesting challenge for scientists in the field of Animal nutrition is the introduction of alternative feedstuffs that could overcome the problems of environmental harshness and production costs. At the same time, the maintenance of animal health, production and its quality is also essential.

The term plant secondary metabolites describe a group of chemical compounds present in plants that are not involved in the primary biochemical processes of plant growth and reproduction. The function of these secondary metabolites in plants is to protect the plants against infection, predation and restricting grazing by herbivores. Browse trees are important sources of livestock feed in the tropics and are well adapted to adverse agro-ecological conditions also. Therefore, most browse trees contain secondary metabolites such as tannins, alkaloids, saponins, cyanogenic glycosides, oligosaccharides, etc. that substantially limit their utilization by ruminants (Getachew *et al.* 2002). Tannins are among the most important anti-nutritional compounds in most browse trees. High levels of tannins are known to exert negative effects, including animal productivity, reduction of voluntary feed intake and nutrient digestibility as well as impairment of ruminal fermentation (Mueller-Harvey 2006). For rumen microbial adaptation and degradation of those secondary compounds, sufficient evidences in the literature are available to support (McSweeney *et al.* 2003).

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Chemistry of tannins

Tannins are defined as naturally occurring water-soluble polyphenols of varying molecular weight that differ from other natural phenolic compounds in their ability to precipitate proteins from solutions. Tannins are a heterogeneous group of high molecular weight phenolic compounds with the capacity to form reversible and irreversible complexes with proteins (mainly), polysaccharides (cellulose, hemicellulose, pectin, etc.), alkaloids, nucleic acids and minerals (Schofield *et al.* 2001).

Tannins are usually of 2 major types: hydrolysable and condensed tannins, and are having both adverse as well as beneficial effects depending on their concentration and nature besides these other factors such as composition of the diet, species and physiological state of animals (Makkar 2003).

Condensed tannins are termed proanthocyanidins because HCl/butanol treatment releases bright red anthocyanadin chloride. Proanthocyanidins are phenyl propanoid polyphenols and are categorized by the type of monomer they contain (flavan-3-ols or flavan-3, 4-diols) into catechins or leucanthocyanidins. Condensed tannins are polymers of flavanol (flavan-3-ol) units, linked by carbon-carbon bond which is not susceptible to degrade anaerobic enzymatic action. It is heterogeneous in nature and both size and structure affects their reactivity and impact on digestion (Mueller-Harvey 2006). Condensed tannins conserve nitrogen in an ecosystem, has credibility in low fertility environment because CT always increase the faeces nitrogen content and decrease urinary nitrogen output.

Hydrolysable tannins having different structure to condensed tannins, comprising a central sugar attached to several gallic acid groups. Their structure is also highly variable with different types and numbers of sugars and a range of cross linkages between gallic acids and other phenolics (Mueller-Harvey 2006). The astringent effect and nutrient binding affinity of tannins present in tree leaves generally affect their intake and digestion in animals. When HT are metabolized the final products such as gallic acid, pyrogallol, resercinol, other phenolic acids and their derivatives become toxic to animals (Makkar 2003).

However, the tannin chemistry in relation to animal nutrition is less useful because there are some compounds, such as catechin gallates, which have properties of both the hydrolysable and condensed tannins. Catechin gallates are nutritionally important because they are toxic to some rumen microflora (Mueller-Harvey 2006).

Role of tannin in ruminants

Beneficial effect of tannins: The performance of animal is higher with diet containing low levels of tannins, mainly because the feed protein protection from ruminal degradation leading to increase in the flux of essential amino acids (EAA) to duodenum and enhancing absorption of EAA to blood. Condensed tannins in small quantities are useful as they prevent bloat, protect proteins and prevent establishment of gastrointestinal parasites (Min and Hart 2003). The condensed tannin in *Lotus corniculatus* (20–45 g CT/kg DM) fed to sheep reduces rumen forage protein degradation due to reversible binding of proteins and to reducing the populations of proteolytic rumen bacteria (Min *et al*. 2003).

Makkar (2003) revealed that formation of complex with condensed tannins and proteins which are stable over the pH range of 3.7–7.0, but start dissociated in the abomasum and anterior duodenum. This protects proteins from microbial hydrolysis and deamination in the rumen and increases feed proteins availability for post rumen absorption. Animals consuming forages with moderate concentration of tannins were also associated with less emission of methane. Dey *et al.* (2007) reported that lambs supplemented with varying levels of CT (1%, 1.5% and 2%) of the diet had positive nitrogen balance irrespective of dietary treatment.

Detrimental effect of tannins: At higher level (>5%) tannins in feed reduced digestibility of fibre in the rumen by inhibiting the activity of anaerobic bacteria and fungi. Tannins may affect rumen function indirectly by decreasing rumen ammonia level through reduced protein degradation in the rumen. Sheep can adapt to a diet containing *Acacia* leaves, indicating that there are rumen microflora that detoxify their deleterious effects. The utilization tannin rich feeds by goat are better than sheep (Landau *et al.* 2000). Min *et al*. (2003) reported that high forage condensed tannin concentration (> 55g CT/kg DM) generally reduces voluntary feed intake and digestibility of nutrient and depress body and wool growth in grazing ruminants.

It is well established that tolerance capacity to tannins in ruminants are higher than non-ruminants because they have unique properties such as extra mastication of feed, large amount of saliva production and rumen fermentation (Smith *et al*. 2005). The variation in response to tanniniferous diets within the same animal species which largely depend on the physiological state of the animals to adapt to high tannin levels in the diet. The condensed tannin has more profound digestibility-reducing effect than hydrolysable tannin; however the HT may cause varied toxic manifestations due to hydrolysis in the rumen. Hydrolysable tannin is degraded in the rumen by the action of microflora, as a results the absorption of degraded products by the animal cells enhance the of phenolics concentration in the blood stream, which is beyond the capability of the liver to detoxify them and produce hepato-toxicity (Makkar 2003).

Effect of tannins on in vitro *rumen fermentation:* The productions of volatile fatty acids during in vitro fermentation were highly reduced when the substrate contained more than 6% condensed tannin. Work is in progress on the microbial degradation of tannins to improve the nutritive value of tannin-rich feeds. Makkar *et al*. (1994) used *Sporotrichum pulverulentum* for *in vitro* fermentation of oak leaves *(Quercus incana)* and found that the total phenol's CT content and protein precipitating capacity by 58, 66 and 65%, were decreased respectively.

The estimation of carboxymethyl cellulase (CMCase), xylanase and protease was done as described by Agarwal *et al.* (2000). Singh *et al.* (2001) used an *in vitr*o syringe system and found that the tannic acid degraded to their monomers such as gallic acid, pyrogallol and resorcinol in 24, 48 and 72 h, respectively, by mixed microflora of ruminal fluid from cattle that had no prior exposure to tannin-rich forage. Aguilar *et al*. (2004) worked on fungal strains of *A. niger* and *Penicillium spp.* isolated from Mexican deserts and found that they are able to degrade catechins. These fungi can use catechins as a sole carbon source and can degrade 76–79% catechin when included at a concentration of 3 g/l in the medium.

Patra *et al.* (2008) studied the effect of *Populus deltoids* (poplar) and *Quercus incana* (oak) leaves on *in vitro* gas production and methanogenesis and reported significant depression in gas production as well as methanogenesis with the addition of both leaf extract. Hess *et al.* (2008) found that the species specific condensed tannin properties play a major role in affecting rumen fermentation parameters. Despite the similar condensed tannin concentrations in *F. macrophylla* and *L. leucocephala*, these two legumes differed considerably in their fermentation properties. On the other hand, the two provenances of *C. calothyrsus* varied substantially in their condensed tannin concentration but showed similar fermentation properties.

Getachew *et al.* (2008) revealed that the types and level of tannins affect rumen fermentation and protein degradation accordingly. Gallic acid did not exert significant influence on *in vitro* dry matter and protein degradation and have little impact on nitrogen utilization. Agarwal *et al.* (2009) also reported that the increased methanogen population with a decrease in *in vitro* feed degradability. Kumar *et al.* (2011) revealed that the feeding of *Streptococcus gallolyticus* strain TDGB 406 as probiotic was effective in both in vitro as well as in vivo conditions without affecting the rumen fermentation pattern and enzyme activity of animals. However, increased the population of tannin degrading/tolerating bacteria in the rumen without affecting other microbial profiles, which showed positive effect on animal fed on high tannins containing (oak leaves) as a sole source of roughages. Chaudhary *et al.* (2011) reported that the feeding of live culture of Isolate-6 (direct fed microbials, DFM) did not show any effect on the rumen fermentation pattern viz. pH, production of NH3-N, TCA ppt -N, and activities of CMCase, xylanase, avicelase and protease.

Effect of tannin rich feeds on rumen microbes: Tannins inhibit activity of rumen microbes directly, by complex formation with the bacterial cell envelop, or indirectly by reducing the protein nitrogen and sulphur availability for their use. McSweeny *et al*. (1999) reported that the population of *Ruminococcus spp*. and *Fibrobacter spp*. were reduced but fungi, protozoa and proteolytic bacteria were less affected when animals fed on tannin rich *Calliandra calothyrsus*. Dietary tannins can adversely affect fermentation pattern by inactivating ruminal enzymes system and by bacteriostatic and bactericidal activities (Faixova and Faix 2005).

Animals developed resistance to tannins, when regularly browse on condensed tannin containing plants, at least partly through the presence of tannin resistant ruminal microorganisms. Tannins also induce changes in the morphology of several species of ruminal bacteria. Electron microscopy examination indicated that sainfoin proanthocyanidins bound to cell coat polymers of *Streptococcus bovis, Butyrivibrio fibrisolvens, Prevotella ruminicola* and *Ruminobacter amylophilus* but abnormal cell growth and division was observed only in *S. bovis and B. fibrisolvens* (Jones *et al*. 1994). Hydrolysable tannins are apparently metabolized by the ruminal microflora to gallic acid, which is neither hepatotoxic nor nephrotoxic to animals. However, pyrogallol the decarboxylated product of gallic acid is produced in high concentration in the rumen of sheep and causes methaemoglobinaemia. Tannic acid can be absorbed through intact or injured gastrointestinal tract and ultimately cause kidney and liver necrosis. Singh *et al.* (2011) reported that the goat isolate number 6 (Accession no. HM771331) was tolerant to the phenolic monomers, exhibited tannase activity and improved in vitro true digestibility of tannin rich pakar leaves. The isolate appears to have a potential to be used as microbial feed additive to improve the utilization of poor quality tannin containing feeds by the ruminants.

There are certain experiments in which tannins fed to ruminants have shown marked effects on rumen protozoa and other ruminal microorganisms. Tannic acid exerted a more marked inhibitory effect than was obtained with gallic acid, ellagic acid, catechin or epicatechin in a continuous batch culture system inoculated with mixed rumen organisms. Interestingly, gallate initially reduced cellulolysis, but after several culture transfers, appeared to enhance cellulose breakdown. Gallate can be degraded by some rumen bacteria, and the stimulatory effect on cellulolysis in mixed culture may have resulted from the supply of vitamins or other growth factors to cellulolytic bacteria in the incubations. The different compounds tested inhibit cellulolysis and zoospore attachment to cellulose by pure cultures of the rumen anaerobic fungus *Neocallimastix frontalis* strain RE1. However, gallic acid, ellagic acid and catechin has more inhibitory effect to cellulolysis than was tannic acid, and ellagic acid having the most inhibitory effect to zoospore attachment, perhaps indicating the involvement of different cell-surface receptors in these processes. Tannic acid inhibited the activity of rumen fungal and bacterial xylanases, and the enzyme assay system was used to compare the effectiveness of binding agents in protection against tannin binding. PEG 8000 was the most effective binding agents tested in the xylanase-tannic acid system. Further work with pure cultures and cloned enzymes of rumen microbes could lead to a mechanistic understanding of the effects of phenolic compounds on the rumen fermentation, facilitating the development of new approaches to alleviating their deleterious effects.

Microorganisms have different tolerance capacity to tannin and differed among the groups also. Rumen fungi, proteolytic bacteria and protozoa are more resistant to tannin as compared to other microbes. The protozoal population was decreased as reported by Singh et al. (2011) in goats fed on pakar leaves. Vliwisky *et al*. (2002) reported significant decrease in bacterial count by adding hydrolysable tannin in the diet of lamb @ 1 and 2 g/kg DM, whereas, protozoal population remained unaffected. Patra *et al.* (2006b) observed reduced protozoal numbers (total, small and large entodiniomorphid) in *in vitro* syringe system with the addition of tannin rich harad extracts at the level of 0.5 ml/30 ml incubation medium but not at 0.25 ml level.

Paul *et al*. (2003) studied the effect of different phenolic monomers on biomass and hydrolytic enzymes from an anaerobic fungus *Piromyces spp.* and reported that the degradation of phenolic monomers by anaerobic fungi isolated from nilgai and also suggested the tannin tolerance of anaerobic fungi can play an important role in fibre degradation in the ruminants. Tannins can disrupt membrane function of microorganism by inhibiting electron transport chain and oxidative phosphorylation. Goel *et al*. (2005) reported a 10–20 times increase in the chain length of ruminal streptococci grown in a medium containing 4% tannic acid.

Min *et al*. (2005) found that when the diet of sheep changed from perennial rye grass/white clover pasture (which does not contain CT) to *L. corniculatus* (32 g CT/kg DM), the population of proteolytic rumen bacteria *Clostridium proteoclasticum*, *Eubacterium sp., S. bovis, and B. fibrisolvenes* were decreased from 1.6×10^7, 2.7×10^8, 2.7×10^6 and 1.2×10^6 to 1.1×10^7, 1.5×10^8, 1.6×10^6 and 1.0×10^6 per ml, respectively. They reported that some strains of rumen microorganisms, such as *C. proteoclasticum* and *R. albus,* were resistant to condensed tannin in leaves at a level of 200 µg/ml. Agarwal *et al.* (2008) revealed that the microbial profile of goats was studied by RT-PCR using MESA GREEN qPCR Master Mix (2X) for SYBR Assay I dTTP (EUROGENTEC) and specific primers for total bacteria, *Fibrobacter succinogenes, Ruminococcus flavefaciens* and methanogens. Singh *et al.* (2009) reported that the rumen protozoa, fungi and cellulolytic bacteria were reduced however tannin degrading/tolerating bacteria were increased in goat rumen fed on pakar (*Ficus infectoria*) leaves.

Effect of tannins on methane emission: Puchala *et al.* (2005) worked on CH_4 emission relative to dry matter intake by goats fed *Lespedeza cuneata* (177 g CT/kg DM) 0.55 of that by goats consuming a mixture of grasses (*Digitaria ischaemum* and *Festuca arundinacea*; 5 g CT/kg DM) and found that relative reduction in methane production. The reason of depressed fiber degradation could be due to a reduced number of cellulolytic bacteria, formation of tannin-cellulose complexes and impaired bacterial adhesion to substrate and fibrolytic activity of rumen microbes.

Goetsch *et al.* (2008) reported that condensed tannins from different sources had disparate influence on nitrogen metabolism, but similar effects on rumen microbial CH_4 emission by goats, possibly by altering the activity of methanogens, though change in actions of other bacteria and/or protozoa may also be involved. Thus, various tannins sources could be used in future strategies to reduce ruminal CH_4 emission. Patra *et al.* (2008) suggested that the condensed tannins containing forage decreased CH_4 emission by goats regardless of its level and the effect per unit of CT increased with decreasing CH_4. However, the impact of condensed tannins on CH_4 emission appeared attributable to changes in archea activity, but which might also involve alterations of protozoal activity. This suggests that relatively low dietary levels of condensed tannins could be employed to decrease methane emission without any marked detrimental effects on other conditions such as total tract nitrogen metabolism.

Degradation of tannin by rumen microbes

Microbial tannase: Tannin acyl hydrolase is commonly referred as tannase, an enzyme which form gallic acid in an aqueous solution of tannins, where grew two fungal species later identified as *Penicillium glaucum* and *Aspergillus niger* (Lekha and Lonsane 1997). Tannase catalyses the hydrolysis reaction of the ester bonds present in the gallotannins, complex tannins, and gallic acid esters. The enzyme is used in food and beverage processing; however, the practical use of this enzyme is at present limited due to insufficient knowledge about its properties, optimal expression, and large-scale application.

Presently, tannase is known to be a ubiquitous enzyme of the microbial world. It is produced both as membrane bound and extracellular forms (Deschamps *et al.* 1983). Brooker *et al.* (1994) isolated *S. caprinus* from the ruminal contents of feral goats browsing on tannin-rich *Acacia species*, which gave clearing zones on tannic acid protein agar medium. Tannase activity has been observed for the first time in an anaerobic ruminal bacterium isolated from goats browsing on tannin-rich forage (Skene and Brooker 1995). These workers have reported the presence of this enzyme in *S. ruminantium* sub-spp. *ruminantium*. Perez-Maldonado and Norton (1996) observed substantial degradation and disappearance of labeled condensed tannin from *D. intortum* in the gastrointestinal tract of sheep and goats.

The major commercial applications of the tannases is in the elaboration of instantaneous tea or of a corn liquor and in the production of gallic acid (Belmares *et al.* 2004), the latter being an important intermediary compound in the synthesis of the antibacterial drug, trimethroprim, used in the pharmaceutical, food and beverage industry while gallic acid is a substrate for the chemical or enzymatic synthesis of propyl gallate, a potent antioxidant.

Aerobic breakdown: The production of extracellular tannase (tannin acyl hydrolase) by bacterial cultures with simultaneous release of gallic acid and glucose was reported for the first time by Deschamps *et al.* (1983). Strains of *Bacillus pumilus*, *B. polymyxia*, and *Klebsiella planticola* produced tannase with chestnut bark used as a sole carbon source. The gallic acid monomers as readily utilized as substrates by oxidative breakdown in to simple aliphatic acids, which then enter the citric acid cycle. Prior to ring cleavage, gallic acid is converted to pyrogallol by the action of gallate decarboxylase.

Anaerobic breakdown: The anaerobic decomposition of gallic acid, the monomer of hydrolysable tannins, occurs by different mechanisms. The first step is decarboxylation of gallic acid to form pyrogallol which is then isomerized to phloroglucinol by pyrogallol-phloroglucinol isomerase and to dihydrophloroglucinol by phloroglucinol reductase. Dihydrophloroglucinol is then converted to 3-hydroxy-5-oxohexanoate (HOHN) by dihydrophloroglucinol hydrolase (Brune and Schink 1992).

Murdiati *et al.* (1992) worked on the ruminal degradation of tannic acid in sheep by monitoring the appearance of its metabolites in various body fluids. Some studies showed the sequential appearance of metabolites namely gallic acid, pyrogallol and resorcinol/phloroglucinol after 24, 48 and 72 h of incubation, respectively. Phloroglucinol was not observed to appear at any stage of incubation. The thin layer chromatography of 96 h indicated the disappearance of resorcinol, apparently due to its degradation to some unknown metabolite(s). Resorcinol is normally known to be undegraded in the ruminal system. After accumulation in the rumen it is excreted as resorcinol glucuronides and other conjugates. The appearance of degraded products, as observed by TLC for different time intervals, was confirmed by HPLC pattern for the corresponding period (Singh *et al.* 2001). Our studies showed that the appearance of phenolic metabolites such as gallic acid and pyrogallol only after 24, 48, 72 and 96 h of incubation, respectively and thin layer chromatography indicated that pyrogallol was not disappeared.

Hiura *et al.* (2010) found that the colonies of *Streptococcus spp.* from rumen fluid were successfully established by immobilizing rumen bacteria in Ca-alginate gel beads. The gel beads allowed growth of *S. macedonicus* EC-D140 with exhibited an efficient ability to digest tannic acid. Gallic acid released from hydrolysable tannins may play a physiological role in the survival of sika deer in the Shiretoko Peninsula in winter, when less feed is available and cold weather conditions predominate.

Isolation of tannin degrading microbes and fate of tannins in rumen: Brooker *et al.* (1994) isolated a gram positive facultative anaerobe from the rumen liquor of feral goats browsing on *Acacia spp*. leaves, which could grow in a medium containing 2.5% tannic acid. The bacterium was named as *Streptococcus caprinus*. It was not a major inhabitant of the rumen and was present at a population density of 2×10^6 per ml of rumen fluid. Several ruminal microorganisms that can degrade/tolerate phenolic monomers have been isolated by different workers from different species of animals (McSweeney *et al*. 1999; and Lukose 2004). Nelson *et al*. (1998) isolated six strains from the rumen fluid of Sardinian sheep (*Ovis aries*), Honduran and Colombian goats (*Capra hircus*), White tailed deer (*Odocoileus virginianus*) and Rocky Mountain elk (*Cervus elaphus nelsoni*). Out of that four of the isolates belonged to the genus *Streptococcus*, most closely related to *S. bovis* and *S. gallolyticus*.

In another study in Indonesia, Wiryawan *et al*. (2000) isolated five types of tannin degrading bacteria, which tolerated 3% tannic acid or 1% condensed tannin in their growth medium and reduced tannin content by 52% in 72 h of growth. Inoculation of these bacteria into the rumen of un-adapted goats at the rate of 3×10^{11} cfu/ml was improving feed digestibility and live weight gain in the animals. Some studies have also been shown that tannin tolerant bacteria could be isolated from the rumen fluid of animals previously exposed to tanniniferous feeds (Smith and Mackie 2004). Chaudhary *et al.* (2009) isolated tannin degrading/tolerating bacteria from the rumen liquor goat fed on tannin rich tree leaves by culture enrichment method and phylogenetic analysis of isolates showed similarities with *Streptococcus gallolyticus.*

Hiura *et al*. (2010) revealed that *Streptococcus macedonicus* EC-D140 grown in the Ca-alginate beads was major hydrolysable tannin-degrading bacterium in sika deer. Some ruminal bacteria of sika deer in winter avoid production of pyrogallol in the rumen fluid, but preferably provided gallic acid actively which is polyhydroxybanzoic acid monomer and recognized as an antioxidant. Gallic acid may affect volatile fatty acid production of rumen microbiota, but we have no evidence for this.

Streptococcus gallolyticus is observed ubiquitously in the rumen of many animals browsing on tannin-rich forage legumes. Biochemical studies have shown that this bacterium metabolizes gallic acid to pyrogallol, although it does not metabolize pyrogallol, and produces extracellular polysaccharide (EPS) in response to tannins in the growth medium. Induction of EPS appears to be a bacterial defense mechanism that permits the bacterium to maintain its population when related species are dying. *Selenomonas ruminantium* K2 grown in the presence of hydrolysable or condensed tannins as a sole carbon source and secretes a unique enzyme tannase. A number of other tannin-resistant bacterial species, including *Lactobacillus*, *Butyrivibrio* and *Enterobacteriacae* have been isolated, however their mechanisms of tannin resistance are not known. The enzymes like alkaline phosphatase and aminopeptidase-N activities are inhibited, intestinal microvilli structure is disturbed and tissue fragility signs are evident. These studies indicate that the protein-tannin complex formation may effect intestinal as well as rumen function and that microbial interactions in the rumen may reduce but not eliminate tannin toxicity.

The microflora present in rumen of animals could degrade gallic acid, pyrogallol, phloroglucinol and quercetin to acetate and butyrate. In the ruminal system, HOHN is converted to HOHN-CoA by the enzymatic action of HOHN-CoA transferase, which is further transformed to acetate and butyrate by the activity of ruminal bacteria as sequential enzymatic action of b-hydroxybutyryl-CoA dehydrogenase, butyryl-CoA dehydrogenase, acetyl-CoA acetyl transferase, enoyl-CoA hydrase, phosphate acetyl transferase and acetate kinase. Resorcinol is not degraded further by ruminal bacteria and is excreted as a urinary phenolic conjugate (Murdiati *et al.* 1992). Nelson *et al.* (1995) observed the breakdown of tannins, gallic acid, pyrogallol, ferulic acid and p-coumaric acid by a novel ruminal bacterial isolate.

The tannic acid first degraded to gallic acid monomers are readily utilized as substrates by oxidative breakdown to simple aliphatic acids, which then enter the citric acid cycle. Prior to ring cleavage, gallic acid is converted to pyrogallol by gallate decarboxylase. The aerobic breakdown of flavonoid compounds derived from CT occurs through two pathways. The first degradation pattern is marked by the cleavage of the heterocyclic ring of catechin, a flavan-3-ol, to phloroglucinol carboxylic acid and protocatechuic acid. Phloroglucinol carboxylic acid, by decarboxylation and scission of the aromatic rings by various oxygenases, finally forms b-ketoadipate, an aliphatic acid, through intermediates like phloroglucinol, resorcinol, hydroxyhydroquinone and maleyl acetate. Protocatechuic acid is also converted to b-ketoadipate through b-carboxy cis, cis muconate and catechol pathways. Quercetin, a flavonol, is broken into phloroglucinol and 3, 4–dihydroxyphenyl acetate through the second pathway of flavonoid degradation. The former ends up as b-ketoadipate, while the latter is not degraded further.

Livestock develop a negative nitrogen balance and lose weight and body condition consuming tannin-rich diets (>55 g CT/kg DM), unless supplemented with non-protein nitrogen, carbohydrate and minerals. Phenolic compounds also interact with salivary and mucosa associated proteins producing astringency which is reflected in reduced feed intakes in grazing animals. The prolonged exposure to tanniniferous diet, some rumen microbes acquire mechanism to deal with tannins. Such mechanisms include microbial extra-cellular polysaccharide secretions that reduce the effects of tannins on microbes and production of tannin degrading enzymes (Krause *et al*. 2005).

Condensed tannins are clearly a double edged sword for feeding value. They are not digested by the microflora and the carbon skeleton is not absorbed, so high concentrations of CT reduce the DM potentially available for digestion. Condensed tannin protects protein from ruminal degradation, but same time can also inhibit absorption of the protected protein. Min *et al.* (2005) reported a greater affinity between condensed tannins and bacterial cells than with plant proteins, and cellulolytic bacteria were affected more than cellulolytic fungi.

The composition of the rumen bacterial population, determined by fluorescence *in situ* hybridization and real-time polymerase chain reaction (RT-PCR), has been observed to be affected by feeding young oak leaves (Belenguer *et al.* 2008). A dose-dependent negative effect of feeding tannin containing oak leaves on ruminal fermentation of conventional feeds, such as the grass hay, but also improved fermentation of tannin containing feeds, in bulls fed moderate quantities of oak leaves, which suggests adaptation of the rumen microbial population. Detrimental effects indicate that the high level of tannins in the rumen of bulls on the highest dose of oak leaves would have exceeded the capacity of the microorganisms to resist or detoxify them (Doce *et al.* 2009).

Role of tannins on ruminant performances

Animals grazing on forage based diets are subjected to have nutritional and metabolic diseases. It has been found that tannin containing legumes fed to ruminants markedly reduced rumen gas production by precipitating the stable forage protein foam and prevents bloat. In general, high amount of tannin intakes exert a clear negative effect on productivity and nutrient availability to animals because of the complexes formed between tannins and different types of macromolecules. The voluntary feed intake and digestibility of animals are reduced, the digestive physiology of the animal may be impaired, and there may be mucosal perturbations occurred.

Therefore, some researchers indicated that the continuous ingestion of tannins containing forages might lead to a partial physiological adaptation to these compounds by reducing their harmful effects. Polyethylene glycol (PEG) is an excellent binding agent which form complexes with condensed tannin without interfering the palatability and digestion of feeds consumed by animals.

However, the feeding of small quantities of hydrolysable tannin in soya bean meal (20.8 g kg-1 DM) by Merino sheep under practical feeding conditions showed that these compounds were neither toxic nor had any negative effect on animal performance (Frutos *et al*. 2004). Therefore, more research is needed in this area since hydrolysable tannin is easily hydrolyzed and their effect could easily be nullified by the rumen microorganisms.

Molan *et al.* (2000) found that condensed tannin extracted from forages had the ability to inhibit the development of *Trichostrongylus colubriformis* eggs to infective larvae and to reduce larval motility also. They also suggested that the forages containing condensed tannin may have the ability to break the life cycle of sheep nematodes and reduces pasture contamination with infective larvae. This may reduce dependence on anthelmintic drugs as the main method of controlling internal parasites in grazing ruminants.

Priolo *et al.* (2008) revealed that tannin-containing feeds cause converse effects on meat and milk fatty acid composition, depending on the quantities of condensed tannins ingested by the animals. The color of meat is evidently paler when condensed tannins are present in the diet of goats and ewes.

Animal performances: The consortium of ruminal microorganisms from adapted animals is potentially transferable to non adapted animals and is reported to improve the crude protein digestibility of high tannin diets. Many browsers produce proline-rich salivary proteins (PRP) that bind dietary tannins and inactivate them. Cattle and sheep are devoid of PRP therefore, decrease in DMI due to astringent taste mechanism associated with tannins may not occur in sheep and cattle (Makkar 2003).

Puchala *et al.* (2005) found increased dry matter intake and reduced methane emissions in Angora does fed *Lespedeza cuneata* (CT containing forage) vs. a mixture of *Digitaria ischaemum* and *Festuca arundinacea*. He also suggested that the late lactation dairy cows consuming *Lotus corniculatus* (tannin containing forage) had higher DMI and lower methane per unit milk solids yield compared to cows fed ryegrass silage. Frutos *et al.* (2004) worked on finishing lambs consuming a high-energy ration (14.2 MJ GE/kg DM) with chestnut hydrolysable tannin and reported no effect of on dry matter intake and feed conversion efficiency of animals. There are some exceptions to suppress DMI of animals by tannin but in some cases there is an increase in

DMI due to tannin supplementation (Beauchemin *et al.* 2007). A seventy percent forage ration supplemented with quebracho condensed tannin fed to cattle, found no adverse effect on dry matter intake and average daily gain Beauchemin *et al.* (2007). Krueger *et al.* (2010) reported that the supplementation of hydrolysable tannin and condensed tannin at low levels does not have any detrimental effects on animal performance as well as other economically important traits and would make an interest for further research on tannin effects on food borne pathogens.

Alternative feed resources and carcass quality: It has been shown that the supplementation of poly ethylene glycol to animals given a tanniniferous diet, eliminates the effects of condensed tannin on meat quality. Among meat volatile compounds, skatole (3-methylindole) and indole could strongly affect flavor, because they are associated with a faecal odour. The deamination and decarboxylation of the amino acid tryptophan by the action of ruminal bacteria produce skatole (Deslandes *et al.* 2001).

Consumers usually associate with colour, brightness and freshness of meat according to their cultural background. It is well known that higher pH values produce meat darker in colour (Priolo *et al.* 2005). The mechanisms of action of condensed tannin on meat colour are still unclear. Some workers investigated the haematic parameters in animals fed condensed tannin containing diets. For instance, lambs and kids fed a diet containing condensed tannin from *Prosopis cineraria* found to be lower blood hemoglobin concentration than that of lambs and kids fed the same diets supplemented with PEG. The cattle offered a diet containing green tea, which has condensed tannin, had a lower iron blood concentration than animals fed a condensed tannin free treatment. Also in this case the meat of the green tea fed cattle was lighter in colour than that of the control animals (Kondo *et al.* 2004).

Frutos *et al.* (2004) reported no adverse effect of chestnut hydrolysable tannin (0.84 g tannin/kg BW) on lamb carcass traits fed a high-grain diet and there was no effect of HT supplementation on average daily gain, feed efficiency, and length of finishing period. They also found that weights of empty GIT components, skin and non-carcass fat depots did not differ between control and HT treated finished lambs. Other than that the chemical composition of the empty body weight was not different between control and HT treated lambs. Likewise, Kumar *et al.* (2005) reported that feeding high tannin sorghum to broilers did not affect carcass traits and proportional weights of visceral organs.

Schreurs *et al.* (2008) demonstrated that feeding condensed tannin containing forages to ruminants reduces indole and skatole formation in the rumen by reducing protein degradation and inhibiting the activities of proteolytic, indole and skatole forming rumen microbes. The reduction in the formation of indole and skatole in rumen is dependent on the concentration and structure of condensed tannin. The forages with a higher condensed tannin concentration tending to be more effective. The CT is also likely to reduce concentrations of other pastoral flavour compounds produced from forage protein fermentation in the rumen. Krueger *et al.* (2010) reported that there were no detrimental effects of tannins on offal, indicating that tannins supplementation may be a viable option in finishing beef cattle if bactericidal efficacy is established. Kumar *et al.* (2011) found that the feeding of bacterial isolate (TDGB 406) as probiotic increased DM digestibility. All the animals were in positive nitrogen balance. Improvement in BW gain, ADG and feed conversion efficiency in goats fed on oak leaves as one of the main roughage source.

The degree of tannin tolerance is specific to the microorganism in question, explaining the different susceptibility of bacterial strains. It also depends on the types and nature of tannins, and the differences between hydrolysable and condensed tannin in this respect are notorious. However, few tannin tolerant rumen microorganisms have been described; it is very likely that their true diversity is much greater than currently known.

Conclusion and future research priorities

The tanninolytic microorganisms has developed different strategies to counter the toxic effects of tannin metabolites and played important role in health as well as production of livestock in tropics. The future research should focus on the isolation and identification of potential tannin degrading (both hydrolysable and condensed tannin) microorganisms from different types of herbivore from different geographic areas. Genetic identification of these microbes and the genes which is responsible for tannin degradation could be another area of special interest. These researches are doing for developing probiotic that may modify or inhibit the toxicity of tannins within the rumen ecosystem. The potential isolates may then be exploited for the successful trans-inoculation and genetic manipulation of ruminal microflora to achieve long term protection against tannin toxicity. Chemical analyses should be complemented by measurements of binding capacity with plant proteins and effects on enzyme activity, because the relationships between chemical structure and astringency are not yet clearly defined. A focus on tolerance to hydrolysable and condensed tannin could have positive outcomes for sheep and goat production in hot climates but a combination of animal and management strategies is most likely to have the best outcome for producers.

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