

Chapter 8

Dietary Tannins on Microbial Ecology of the Gastrointestinal Tract in Ruminants

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Abstract This review discusses the effects of tannins on nitrogen metabolism in the rumen and intestine, microbial populations (bacteria, protozoa, fungi and archaea), metabolism of tannins, microbial tolerance mechanisms to tannins, inhibition of methanogenesis, ruminal biohydrogenation processes and performance of animals. The discrepancies in responses of tannins among different studies are attributed to the different chemical structures (degree of polymerization, procyanidins to propdelphinidins, stereochemistry and C–C bonding), different concentrations of tannins, and type of diets. An establishment of structure-activity relationship would be required to explain differences among studies and obtain consistent beneficial tannin effects. This paper reviews progress with plant tannins occurring in both temperate and tropical forages for fulfilling the objective of mode of action of tannins, rumen microbial activity and rumen metabolisms.

Keywords Tannins • Microbial ecology • Gastrointestinal tract • Metabolism • Rumen fermentation • Resistance mechanisms • Ruminants

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8.1 Introduction

Tannins (hydrolysable and condensed 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 ubiquitously distributed in nutritionally important forage trees, shrubs, legumes, cereals and grains. Intake of feeds and forages containing high concentration of tannins in animals may show adverse effects on feed intake, nutrient utilization and animal performance. However, tannins may modulate rumen fermentation favourably, such as decreased protein degradation in the rumen, prevention of bloat, inhibition of methanogenesis and increased concentrations of conjugated linoleic acid in ruminant-derived foods (Mangan 1988; Kumar and Vaithyanathan 1990; Aerts et al. 1999; Barry and McNabb 1999; McSweeney et al. 2001a; Min et al. 2003; Mueller-Harvey 2006; Waghorn 2008; Patra and Saxena 2010). All these beneficial responses are mediated through the modulation of microbial populations in the rumen. Besides, tannins may exert antimicrobial effects in the intestine.

Tannins were primarily considered as anti-nutritional biochemicals due to their adverse effects on feed intake and nutrient utilization (Kumar and Vaithyanathan 1990). Nevertheless in recent years, they have been recognized as useful phytochemicals for beneficially modulating the rumen microbial fermentation. The effects of tannins on ruminant production have been published in the past, which primarily focused on the adverse effects of tannins on animal system, with some discussion on their positive effects on protein metabolism and prevention of bloat (Mangan 1988; Kumar and Vaithyanathan 1990; Aerts et al. 1999; Barry and McNabb 1999; McSweeney et al. 2001a; Min et al. 2003; Mueller-Harvey 2006; Waghorn 2008). This chapter focuses on the effects of tannins on ruminal microbial populations that affect N metabolism, methanogenesis and ruminal biohydrogenation process in the rumen.

8.2 Chemistry and Occurrence of Tannins

Tannins are water soluble polyphenols of relatively high molecular mass and have capacity to form complexes mainly with proteins, to a lesser extent with carbohydrates due to the presence of a large number of phenolic hydroxyl groups. They are usually classified into two major groups: hydrolysable tannins (HT) and condensed tannins (CT).

The HT is complex molecules with a polyol as a central core such as glucose, glucitol, quinic acids, quercitol and shikimic acid that is partially or totally esterified with a phenolic group, i.e. gallic acid (3,4,5-trihydroxy benzoic acid; gallotannins) or gallic acid dimer hexahydroxydiphenic acid (ellagitannins) (Haslam 1989; Fig. 8.1). The resulting phenolic groups may further be esterified or oxidatively crosslinked to yield more complex HT (Haslam 1989). Hydrolysable tannins are susceptible to

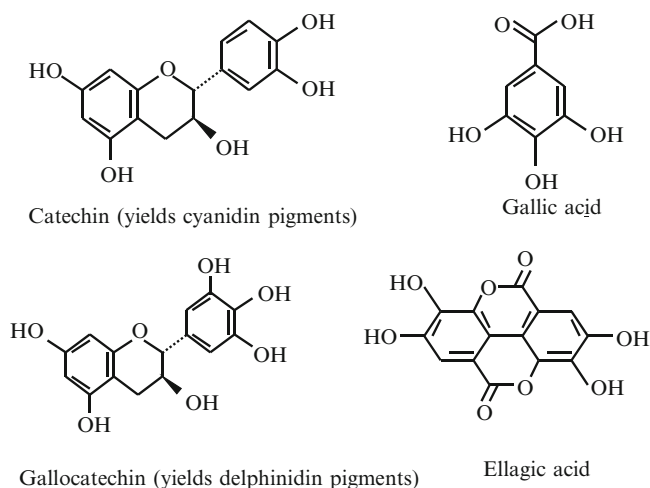


Fig. 8.1 Monomeric units of condensed (catechin and gallo catechin) and hydrolysable tannins (gallic and ellagic acid)

hydrolysis by acids, bases or esterases yielding polyol and the constituent phenolic acids (Haslam 1989).

The CT or proanthocyanidins are mainly polymers of the flavan-3-ol (epi) catechin and (epi) catechin units, which are linked by C4–C8 and C4–C6 interflavonoid linkages (Ferreira et al. 1999; Hagerman and Butler 1989). Many other monomers of CT, e.g. profisetinidins, probinetidins and proguibortinidins are also present (Haslam 1989). For example, quebracho tannins are of largely profisetinidin type (Hemingway 1989). In procyanidins and prodelfinidins, C4–C8 and C4–C6 linkages with a ratio of about 3:1 are more common and majority of tannins in these classes are of mixed stereochemistry with 2,3 *cis* to 2,3 *trans* ratios between 9:1 and 5:5 (Seigler 1998). The number of monomeric units can vary and this determines the degree of polymerisation from di-, tri- and tetraflavonoids to higher oligomers. These can then produce a large numbers of chemical structures, which in turn could produce different biological properties (Waghorn 2008). The CT is degraded to monomeric anthocyanidins (e.g. cyanidin and delphinidin) pigments upon treatment with acid butanol reaction (Porter et al. 1986; Haslam 1989). The CT can react by hydrogen bonding with plant protein to form stable and insoluble CT-protein complexes at pH 3.5–7.0, which dissociate and release protein at pH <3.5 (Jones and Mangan 1977).

Tannins are ubiquitously distributed in nutritionally important forage trees, shrubs and legumes, cereals and grains that often limit their utilization as feedstuffs. Generally, tannin concentrations are greater in vulnerable parts of the plants, i.e. new leaves and flowers (Terrill et al. 1992; Frutos et al. 2004) and various factors such as temperature, light intensities, water and nutrient stress, soil quality and topography influences the concentrations of tannins in plants (Frutos et al. 2004). Both HT and CT may occur in the same plant, but some plants may contain

predominantly HT whereas others contain CT (Haslam 1989). Again, although CT in plants represents a mixture of different monomeric units, a particular type of CT may be predominant in a particular plant, which may explain different physiological effects and animal performance (Waghorn 2008). For instance, CT from *Lotus corniculatus* (birdsfoot trefoil) predominantly has catechin subunits (67%), i.e. procyanidins type CT with average molecular mass of 1,900 (Foo et al. 1996), whereas CT from *Lotus pedunculatus* (big trefoil) has epigallocatechin subunits (64%), i.e. prodelphinidin type CT with average molecular mass of 2,200 (Foo et al. 1997). It has been noted that low molecular weight CT oligomers are more reactive and have higher protein precipitating capacities than high molecular weight polymeric tannins (Butler and Rogler 1992).

8.3 Effects of Tannins on Microbial Nitrogen Metabolism

The quantity and quality of protein flowing to the intestine is one of the major factors determining the productivity of ruminant livestock. The protein reaching the abomasum consists of a mixture of dietary and microbial protein. The flow of protein from the rumen to abomasum depends on proteolysis by rumen microorganisms and the efficiency of microbial protein synthesis in the rumen. When ruminants are fed on a high quality fresh forages containing high concentration of N (25–35 g/kg DM), most of the proteins become rapidly soluble in the rumen and are degraded by rumen micro-organisms resulting in surplus levels of ammonia (20–35%) which is absorbed from the rumen and excreted in urine (Ulyatt et al. 1975). Tannins may improve the protein metabolism in the rumen by reducing protein degradation via formation of insoluble tannin-protein complexes and decreasing the solubility of protein (Tanner et al. 1994; Min et al. 2000; Fig. 8.2). Preincubation of total leaf protein with the rumen bacteria *Streptococcus bovis*, isolated from sheep rumen fluid resulted in a rapid degradation of the large subunit protein (Rubisco) within 2 h of incubation (Fig. 8.3). This was followed by further gradual degradation of Rubisco over the remaining 24 h incubation. Preincubation of bacterial cells with CT extracted from *L. corniculatus* prior to the addition of total soluble leaf protein markedly reduced the rate proteolysis of large and small subunits of Rubisco. This study shows that CT binds to both Rubisco (plant protein) and bacterial cells, and both interactions lead to a decrease in the degradation rate of the large and small subunits of Rubisco.

An increase in dietary protein flow to the abomasum may also be due to the inhibitory effects of tannins on proteolytic bacteria and proteolytic enzyme activity. Besides, tannins might sometime also affect the efficiency of microbial protein synthesis. Tamarind (*Tamarindus indica*) seed husk (containing 14% tannins) improved the efficiency of microbial protein synthesis *in vitro* (Bhatta et al. 2001). Quebracho tannins also increased microbial protein synthesis in a lucerne diet fed to sheep at dosages of 2% and 3% of DM, but not at 3% of DM (Al-Dobaib 2009). However, some studies reported that the microbial protein outflow from the rumen was little affected due to feeding of tannin-containing forages (McNeill et al. 2000; Min et al. 2003).

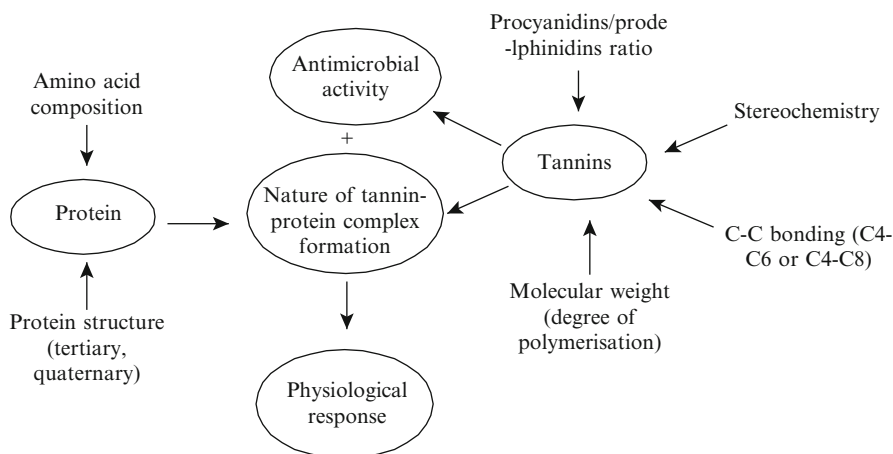


Fig. 8.2 Factors that may affect formation and dissociation of tannin-protein complex and antibacterial activities of tannins in the gastrointestinal tract (Adapted from Patra and Saxena 2011)

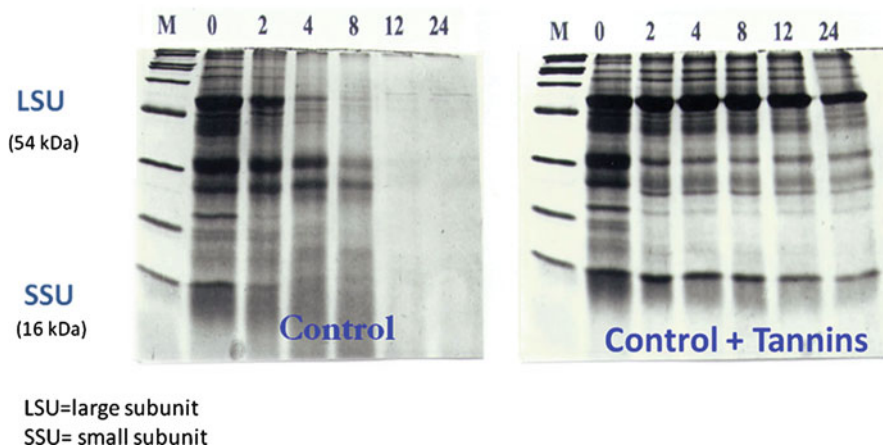


Fig. 8.3 The degradation of the large subunit (LSU) and small subunit (SSU) of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) during *in vitro* incubation of total leaf protein extracted from white clover (*Trifolium repens*) with *Streptococcus bovis* rumen bacterial strains (10⁸ cells inoculated) and condensed tannins extracted from *Lotus corniculatus* (birdsfoot trefoil). Incubations were performed with (b, CT-active) and without CT (a, CT-inactive). Samples were removed prior (0 h) and after 2, 4, 8, 12, and 24 h of incubation. The sample on the left side of each gel contains the broad range molecular weight (MW) markers (Bio-RAD, Hercules, CA; Min, 2012, unpublished data)

Proteolytic bacteria were present in relatively high numbers (10⁸/g of digesta) in sheep fed on *Calliandra* leaves (McSweeney et al. 1999). The changes in the activities of proteolytic bacteria and the protease enzyme by tannins could mediate nitrogen metabolism in the rumen (Tanner et al. 1994). A reduction in proteolysis might be attributed to the direct effects of CT on microbial proteolytic enzyme activity or

to the indirect effects on rumen metabolite concentration that can regulate proteolytic activity in some bacteria (Waghorn et al. (1994a, b). Rate of proteolysis by bacteria varies among bacterial species in the presence of CT. Min et al. (2005) reported that the proteolytic bacterial cultures without CT (from *L. corniculatus*) or with CT+ polyethylene glycol (PEG; which binds to tannins thus decreasing the effects of tannins) degraded large (LSU) and small (SSU) of Rubisco protein rapidly (6–8%/h). Cultures of *Prevotella ruminicola* C21a degraded both the LSU (11.3%/h) and SSU (9.8%/h) when grown in the presence of 1.5 g CT/L. Bacterial strains *S. bovis* B315 (11.5%/h) and strain NCFB 2476 (10.6%/h), *Eubacterium* sp. C12b (5.1%/h) and C124b (5.5%/h) degraded SSU more effectively than the LSU (2.6%, 2.3%, 3.8%, and 2.6%/h, respectively) in the presence of 1.5 g CT/L. *Fibrobacter succinogenes* S85, *Ruminococcus albus* 8, *P. ruminicola* 23, *Clostridium proteoclasticum* B316^T, *Butyrivibrio fibrisolvens* WV1 and C211a had low (0.3%/h) to moderate (3–4%/h) rates of proteolysis when CT was included in the medium (Min et al. 2005). The CT has been shown to inhibit endogenous enzyme activity (Oh and Hoff 1986; Horigome et al. 1988). It is suggested that at higher tannins/protein ratios, the inhibition of proteolysis is likely due to coating of the protein surface by the polyphenolic compounds (McManus et al. 1981), hence, leading to interference with the interaction of enzyme and substrate. Jones et al. (1994) studied the protease activity of four strains of ruminal bacteria as affected by CT from *Onobrychis vicifolia*. Total protease activity in cultures of *B. fibrisolvens* and *S. bovis* was reduced by 48% and 92%, respectively, at a concentration of 25 mg of CT/L; whereas, the total protease activity in cultures of *P. ruminicola* was 36% higher in the presence of 100 mg of CT/L than in the control. CT did not inhibit the protease activity of *Ruminobacter amylophilus* (formerly *Bacteroides amylophilus*) cultures at concentrations below 100 mg/L. The cell-associated protease activity in *S. bovis* was similar to that of the control up to 25 mg CT/L, but the activity decreased considerably (32% of control) at 50 mg of CT/L. In contrast, there was no reduction in the cell-associated protease activity of *P. ruminicola* at any CT concentration up to 300 mg/L of CT.

Information on fungus on the degradation of tannin-protein complex is limited. A fungus, *Aspergillus niger* var Tieghem having tannin-protein complex-degrading activity was isolated from the faeces of such cattle fed largely on oak leaves (Bhat et al. 1996). In some animals, microbes responsible for degrading the tannin-protein complex have ecological advantages by cleaving the protein moiety from the complex. Tannin-protein complex degrading *S. bovis* biotype I and enterobacteria that digest tannin-protein complexes has been isolated from the faeces of koalas, *Phascolarctos cinereus* (Goldfuss), which almost solely fed on eucalyptus leaves with higher tannin content (Osawa 1992).

8.4 Effects of Tannins on Ruminal Methanogenesis

Methane produced during anaerobic fermentation in the rumen represents a 2–15% feed energy loss and contributes to greenhouse effects in the environment. Therefore, reducing methane emission has been a great interest for ensuring the sustainability

of ruminant production. Methane is produced normally during fermentation of feeds by methanogenic archaea. The removal of ruminal protozoa can also reduce methane production as some population of methanogens remains attached with protozoa (Hess et al. 2003). Tannin-containing forages and tannin extracts have been demonstrated to decrease methane production both *in vivo* and *in vitro* (Patra and Saxena 2010). The inhibitory effects of tannins on rumen methanogenesis have been ascribed due to direct effects on methanogenic archaea, protozoal associated methane production and indirectly through a depression of fiber digestion in the rumen. Animut et al. (2008a) also observed that feeding of different levels of Kobe lespedeza (*Lespedeza striata* K) decreased methane production linearly in goats and it has been attributed to the presence of CT (Animut et al. 2008b). Tavendale et al. (2005) reported that methane production (ml/g DM) at 12 h for *Medicago sativa* (alfalfa) containing negligible amount of CT (25 ml) was higher than *L. pedunculatus* (17.6 ml) that contains 0.10% CT and after addition of PEG increased methane production for *L. pedunculatus* (17%), but not for *M. sativa*. They also investigated the inhibitory effects of extractable CT fractions from *L. pedunculatus* on the common rumen methanogens *Methanobrevibacter ruminantium* strains YLM-1 and DSM1093. Oligomeric CT fractions did not have any influence on both strains, as determined by methane production measurements. The polymeric fraction completely inhibited methane production. Inhibitory effects in broth culture for strain YLM-1 were bacteriostatic, while strain DSM1093 did not recover growth, as indicated by methane production, even upon prolonged incubation. In a plate assay, the zone of inhibition with the polymeric fraction remained after a further week of incubation. Similarly, Huang et al. (2011) showed that the CT fraction with higher molecular weight (1,349 Da) isolated from *Leucaena* hybrid forage had pronounced effect on *in vitro* methane (62% inhibition) followed by lower molecular weight fractions. Besides, direct inhibitory to methanogens, tannins have been shown to lower protozoal numbers (Patra and Saxena 2009, 2011), which may also decrease protozoal associated methanogenesis (Finlay et al. 1994).

8.5 Effects of Tannins on Ruminal Biohydrogenation

Increasing the concentrations of conjugated linoleic acids (CLA) in foods derived from ruminants has been of recent interests in many researches due to its beneficial effects on health such as anti-cancer properties, reduced risks for cardiovascular diseases, reduction of body fat accretion and modulation of the immune system. The CLA represents *cis* and *trans* isomers of linoleic acid (C18:2) with conjugated double bonds, e.g. *cis*-9, *trans*-11 C18:2 (also called rumenic acid and a predominant isomer in meat and milk) and *trans*-10, *cis*-12 C18:2 (Bauman et al. 1999). The CLA concentrations in ruminant-derived foods can be increased by nutritional and management practices that facilitate higher fore-stomach output of CLA and vaccenic acid (*trans*-11 C18:1) for absorption and incorporated into animal tissues (Bauman et al. 1999). Many ruminal bacteria species of the genera *Butyrivibrio*, *Ruminococcus*, *Treponema-Borrelia*, *Micrococcus*, *Megasphaera*, *Eubacterium*,

Fusocillus and *Clostridium* are known to be associated in ruminal biohydrogenation of unsaturated fatty acids (Harfoot and Hazlewood 1997; Maia et al. 2007; Durmic et al. 2008). *Butyrivibrio* group are most active species among the group A bacteria, which form CLA from linoleic acid, while few species of bacteria such as *Fusocillus* spp. and *C. proteoclasticum* (group B) converts vaccenic acid to stearic acid (Maia et al. 2007; Paillard et al. 2007; Durmic et al. 2008). Therefore, it has been suggested that selective inhibition of group B bacteria without affecting group A bacteria may provide more vaccenic acids and CLA (Harfoot and Hazlewood 1997; Durmic et al. 2008).

The ability of plant extracts including tannins to modify the fatty acid composition of ruminant-derived food products (i.e. milk or meat) has received great attention recently. Sivakumaran et al. (2004) demonstrated that all three fractions (i.e. low, medium and high molecular weight) of proanthocyanidins from *Dorycnium rectum* forage (a perennial legume shrub) inhibited the growth of *C. proteoclasticum* at concentrations of 100, 200 and 300 mg/L of *in vitro* medium, whereas, its low and medium molecular weight fractions inhibited the growth of *B. fibrisolvens* at these concentrations, while the high molecular weight fraction stimulated the growth of *B. fibrisolvens* at the concentration of 100 mg/L. The effects of tannins on biohydrogenation by rumen microbial populations have been discussed in details in Chap. 9.

8.6 Effects of Tannins on Rumen Microbial Populations

8.6.1 Bacteria

Phenolic compounds are used as antimicrobial agents in an array of products, including food, paint, leather, metal working fluids, textiles and petroleum (Marouchoc 1979). The ability of an array of phenolic compounds, e.g. ferulic acid, CT (tea catechins), oleuropein, HT (ellagic acid) and p-coumaric acid, to inhibit the growth of bacteria (*Salmonella enteritidis*, *Staphylococcus aureus*, *Listeria monocytogenes*) and fungi in milk has been reported (Payne et al. 1989; Tassou and Nychas 1994, 1995; Rosenthal et al. 1997, 1999; Apostolidis et al. 2011). A number of studies showed that tannins also selectively inhibit the growth of microorganisms in the digestive tracts depending on the types of tannins. A long time ago, Tagari et al. (1965) reported that cellulolytic and proteolytic activities were inhibited in the presence of carob tannins (mainly CT types) in artificial rumen. The gallotannins strongly inhibited cellulolytic activity, but had slight effect on proteolysis (Tagari et al. 1965). Similarly, CT of *L. corniculatus* reduced the populations of *C. proteoclasticum*, *B. fibrisolvens*, *Eubacterium* spp. and *S. bovis* in sheep (Min et al. 2002). In a pure culture study, CT of *L. corniculatus* inhibited the growth of *F. succinogenes* at a concentration of 400 mg/L, but had no appreciable inhibitory effect on the growth at concentrations below 400 mg/L (Bae et al. 1993). The effects of tannins on ruminal

bacteria are reported to be species-dependent. Jones et al. (1994) studied the effects of CT of the legume sainfoin (*O. viciifolia*) on growth and proteolysis by four strains of ruminal bacteria. They observed that growth and protease activities of *B. fibrisolvans* and *S. bovis* were reduced by CT, but had little effect on a strain of *P. ruminicola* and *R. amylophilus*. Sivakumaran et al. (2004) reported that antibacterial activity of proanthocyanidin from leaves of the forage legume *D. rectum* ranged from the very sensitive to *R. albus* and *Peptostreptococcus anaerobius* to the less sensitive to *Clostridium aminophilum*, *C. proteoclasticum* and *B. fibrisolvans*. Addition of 200, 400 and 600 mg CT/L significantly reduced the growth rate of most bacterial strains (*S. bovis* NCFB 2476 and B315, *B. fibrisolvans* strains WV1 and C211, *P. ruminicola* 23, *Prevotella*-like strain C21a, *Eubacterium* spp. C12b and C124b and *F. succinogenes* S85) except *C. proteoclasticum* B316^T and *R. albus* 8, which showed transient increases in their growth rate at low (50–100 mg/L), but not at high (>200 mg/L) concentrations of CT. Morphological changes occurred in the bacteria that were inhibited by tannins, whereas, an extracellular material produced by *P. ruminicola* probably protected the bacteria from the direct action of tannins (Jones et al. 1994). Similarly, addition of phlorotannins from *Ascophyllum nodosum* (brown seaweed) at 0.5 mg/mL to rumen cultures inhibited the growth of *F. succinogenes*, but had minimal effect on *Ruminococcus flavefaciens* and *R. albus*, whereas, the growth of *Selenomonas ruminantium*, *S. bovis*, *R. amylophilus*, and *Prevotella bryantii* was stimulated (Wang et al. 2009).

The shifts of microbial populations in the digestive tracts generally occur in animals fed on tannin-containing diets because of selective inhibition of bacteria and tolerance of some bacteria to tannins. Plumb et al. (2000) demonstrated that members of the *Cytophaga-Flexibacter-Bacteroides* group predominated over members of the low G+C Gram-positive bacteria in both the sheep and the goats (78% versus 21%, and 82% and 11%, respectively) fed with *A. aneura* forage, while members of the low G+C Gram positive bacteria predominated (74% versus 25%) in the sheep fed grass. In a rat model, Smith et al. (2005) noted that the proportion of the low G+C Gram positive bacteria decreased, while the *Bacteroides-Prevotella-Porphyromonas* group and *Enterobacteriaceae* predominated in faeces of rats fed an *A. angustissima* tannin-containing diet. The levels of lactobacilli increased, whereas, the numbers of total bacteria and Bacteroidaceae decreased in the feces of 30-days-old pigs fed 0.2% tea polyphenols (Hara et al. 1995). Furthermore, Rosenthal et al. (1997, 1999) reported that tea CT (catechins) and ferulic acid inhibit the growth of pathogenic bacteria (coliforms and *Salmonella*) with little effect on lactic acid bacteria.

Tannins may also reduce the archaeal populations and/or activity directly or indirectly in the rumen depending upon the concentrations and type of tannins (Patra and Saxena 2010, 2011). There are also species and strain differences of methanogens showing sensitiveness to different molecular fractions of CT (Tavendale et al. 2005). The number of methanogens in the *Methanobacteriales* order decreased quadratically with increasing doses of CT from *Leucaena leucocephala* leaves (Tan et al. 2011a). The tannins modify the diversity of methanogens in the rumen probably due to greater sensitivity of some methanogens to tannins. For instance, Tan et al. (2011b) reported that the 16S rRNA gene library of the CT-fed animals had

lower proportions of archaea from the orders *Methanomicrobiales* (32% vs. 16.9%) and *Methanobacteriales* (8.5% vs. 1.7%) as compared to those found in the CT treatment clone library in both orders (32% and 8.5%, respectively).

8.6.2 Fungus

Muhammed et al. (1995) investigated the effects of tannic acid, ellagic acid, gallic acid and catechin on rumen fungus *Neocallimastix frontalis* strain RE1. All these compounds inhibited the cellulolysis and zoospore attachment to cellulose by *N. frontalis*. Gallic acid, ellagic acid and catechin showed more inhibitory effect to cellulolysis than tannic acid. However, ellagic acid was most inhibitory to zoospore attachment, perhaps indicating the involvement of different cell-surface receptors in this process. Paul et al. (2003) reported that fungus could grow at tannic acid concentration up to 20 g/L and the growth was not appreciably affected up to 10 g/L concentration acid. However, fibre-degrading ability of rumen fungi may be less sensitive to the inhibitory effects of CT compared to cellulolytic bacteria (McSweeney et al. 1998). Salawu et al. (1999) showed that quebracho tannins (5% of DM) reduced cellulase and xylanase activities of the rumen microbes and total rumen protozoa in sheep fed a grass-barley diet.

8.6.3 Protozoa

Effects of tannins on rumen protozoa are variable. Newbold et al. (1997) investigated that tannins were not responsible for the anti-protozoal activity of *Sesbania sesban*. Salem et al. (1997) observed a linear increase in protozoal numbers in rumen fluid of sheep fed on a lucern-hay based diets by addition of increased proportion of *Acacia cyanophylla* Lindl. foliage, which contained 4.5% CT. Similarly, CT present in *L. corniculatus* and *Hedysarum coronarium* (sulla) increased protozoal numbers in the rumen of sheep (Chiquette et al. 1989; Terrill et al. 1992). There are many reports indicating inhibitory effect of tannins on rumen protozoa. Makkar et al. (1995a) reported that quebracho CT (0.1–0.4 mg/mL media) significantly reduced the numbers of total protozoa, entodiniomorphs and holotrichs, the effect being higher on holotrichs in Rusitec system. Monforte-Briceno et al. (2005) screened the defaunating properties of 15 multipurpose tree fodders, which generally contain tannins. Out of the 15 plants, inhibitory effect on protozoa was due to *Acacia farnesiana*, *Calliandra calothyrsus* and *Lysiloma latisiliquum* that contained CT higher than 1% of dry matter. Tannins extracted with ethanol and methanol from *Terminalia chebula* decreased total protozoa as well as large and small entodiniomorph (Patra et al. 2006). Tannins contained in kobe lespedeza also decreased protozoal number linearly in goats without affecting the total and cellulolytic

bacterial counts (Animut et al. 2008a). Benchaar et al. (2008) did not observe any effect on protozoal numbers in dairy cattle fed quebracho tannins (CT concentrations of 70%, 150 g/day) probably due to addition of a low dosage in the diet. Although rumen protozoa contribute to fiber digestion, there are studies demonstrating increased digestibility of fibrous feeds and the potential for improvement in ruminant productivity maintaining the defaunated state because protozoa increase microbial protein turnover in the rumen, which reduces the efficiency of protein utilization in ruminants (Wallace and McPherson 1987). Protozoal associated methanogenesis accounts for about 37% of rumen methanogenesis (Finlay et al. 1994). It has been suggested that many of the negative effects of defaunation may be transitory and disappear as the bacterial and the fungal populations increase and occupy the niches previously filled by the protozoa (Williams and Coleman 1997). Therefore, removal of protozoa has been a target of rumen microbiologists for rumen manipulation. The anti-protozoal effects of tannins might be beneficial for improving protein utilization and inhibiting methanogenesis in the rumen. However, the mechanisms of inhibition of rumen protozoa by tannins are not clearly known.

Feeding of tannin-containing forages may also show beneficial effects on shedding of pathogenic microbes in faeces depending upon dietary levels and sources of tannins. Min et al. (2007) demonstrated that chestnut tannins (15 g of tannins per day) infused intra-ruminally to steers fed on Bermuda grass hay diet decreased fecal shedding of *Escherichia coli*. Similarly, Berard et al. (2009) reported that tannin-containing sainfoin (*O. viciifolia*) forage (1.2% CT) reduced fecal *E. coli* numbers compared with alfalfa hay in cattle. However, feeding of tanniferous Sericea lespedeza (*Lespedeza cuneata*) forage to goats did not affect fecal shedding of *E. coli* and total coliform numbers in goats (Lee et al. 2009). There are contradictory reports on the effects of tannins on beneficial bacteria in the intestine. Hara et al. (1995) reported that a diet containing 0.2% tea tannins for 2 weeks resulted in significantly increased levels of lactobacilli and a decrease in the levels of total bacteria and *Bacteroidaceae* in the feces of pigs. In contrast, Salem et al. (2010) from *in vitro* study suggested that phenolic compounds present in *Acacia saligna* may be inhibitory to lactobacilli in the intestine.

8.6.4 Mechanisms of Antimicrobial Effects of Tannins

Several mechanisms have been proposed to explain the antimicrobial properties of tannins. The antimicrobial effects of phenolic compounds are probably related to the inhibition of bacterial enzymes, alterations in cell wall permeability, an increase in the hydrogen ion activity of the microbial environment, a reduction in the surface and/or interfacial tension and perhaps chelation of essential minerals, particularly iron with a concomitant impairment of the microbial oxidative metabolic system (Chung et al. 1998). The antimicrobial activities of tannins are ascribed to the interactions of goats tannins with the extracellular enzymes secreted and

the cell wall of bacteria causing morphological changes of the cell wall, destabilization of cytoplasmic and plasma membranes, direct action on microbial metabolism through inhibition of oxidative phosphorylation, deprivation of substrates for microbial growth and chelation of cations by tannins reducing its availability to microbes (Kumar and Vaithyanathan 1990; Scalbert 1991; Jones et al. 1994; Smith et al. 2005). Catechins have been shown to cause leakage from liposomes disrupting membrane integrity (Ikigai et al. 1993). *F. succinogenes* grown on *L. corniculatus* CT (100–300 mg/L) had large amounts of surface materials, which were suggested to the formation of tannin-protein complex on the cell surface (Bae et al. 1993). Cell associated and extracellular endoglucanase activities of *F. succinogenes* were inhibited by CT (0.1–0.4 mg/mL) from *L. corniculatus* under *in vitro* conditions (Bae et al. 1993). It has been stated that tannins from carob pod extract change the morphology of bacteria to produce antimicrobial activity (Heins et al. 1964). Similarly, CT fractions from *O. vicifolia* leaf caused morphological changes in tannin-sensitive bacteria *S. bovis* and *B. fibrisolvans* indicating the action of CT on the cell wall (Jones et al. 1994). Chung et al. (1998) demonstrated that tannic acid inhibited the growth of some human intestinal bacteria, but not lactic acid bacteria. In their studies, addition of iron in the medium restored the growth of *E. coli*, which indicates that the iron-chelating properties of tannins contributed to the inhibitory activities. Although methylgallate and propylgallate inhibited the growth of the intestinal bacteria, iron was not responsible for the growth inhibition (Chung et al. 1998). However, cellulolytic activity of rumen inoculum was not found to be affected by chestnut tannins (Zelter et al. 1970). This indicated that different sources of tannins might have different anti-microbial effects. Flavonols with a trihydroxy B ring (gallo-catechins) have generally more inhibitory effects on the non-rumen microbial species than catechin having a dihydroxy B ring (Sakanaka et al. 1989). It has been further suggested that the toxicity of tannins towards micro-organisms correlates with their molecular weight. Therefore, the toxicities of epicatechin gallate and epigallocatechin gallate towards *Clostridium botulinum* were greater than that of their ungalated counterparts – epicatechin and epigallocatechin (Okuda et al. 1985). McAllister et al. (1994) reported that CT of *L. corniculatus* caused a considerable detachment of *F. succinogenes* S85 from colonised filter paper after a 30 min exposure. Inclusion of 30% *Calliandra* leaves in the diet significantly reduced rumen cellulolytic bacteria including *F. succinogenes* and *Ruminococcus* spp. without affecting the total protozoal population, fungi and proteolytic bacteria (McSweeney et al. 1998, 2001b).

8.7 Tannins: Fiber Digestion by Micro-organisms

Tannins have profound inhibitory effects on the fibrolytic bacterial populations in the rumen at high doses depending upon the type of tannins, thus reducing the fiber digestion and decreases fiber digestibility. The presence of *Calliandra* tannins in the

diet (2–3% tannin) reduced the population of fibre degrading bacteria (McSweeney et al. 2001a). The major fiber degrading bacteria in the rumen such as *F. succinogenes*, *R. albus* and *Ruminococcus flavefaciens* have been found to be inhibited by tannins although degree of inhibition varied among the studies depending upon the dose and type of tannins. For instance, Singh et al. (2011) noted that feeding of *Ficus infectiria* leaves containing 8–12% CT at 50% of the diet of goats did not decrease the number of *F. Succinogenes*, but reduced the number of *R. flavefaaciens*. Bae et al. (1993) studied the effects of CT extracted from *L. corniculatus* on the fiber degrading *F. succinogenes*. The proanthocyanidin from leaves of *D. rectum* was very sensitive to *R. albus*, but were less sensitive to *B. fibrisolvens* (Sivakumaran et al. 2004). Phlorotannins at 0.5 mg/mL inhibited the growth of *F. succinogenes*, but had minimal effect on *R. flavefaciens* and *R. albus* (Wang et al. 2009). Addition of 200, 400 and 600 mg CT/L significantly reduced the growth rate of *B. fibrisolvens* and *F. succinogenes* (S85); however the growth rate of *R. albus* transiently increased at low (50–100 mg/L), but not at high (>200 mg/L) concentrations of CT (Min et al. 2005). The CT reduced the extracellular endoglucanase activity at concentrations as low as 25 mg/L; whereas, cell associated endoglucanase activity increased at CT concentration of up to 300 mg/L and then decreased at 400 mg/L. The exposure of *F. succinogenes* to CT appears to cause the formation of tannin-protein complex on the cell surface, which is suggested to interfere with the adhesion process of bacterial cells to the cellulose.

8.8 Resistance of Gut Micro-organisms to Tannins

8.8.1 Tannin Tolerant Microbes

Initially, many strains of *Streptococci* that degraded tannin-protein complexes were isolated from the feces of koalas and other animals (Osawa 1990; Osawa and Sly 1991). These strains, which ferment mannitol, were conventionally classified as *S. bovis* biotype I strains to distinguish them from biotype II strains, which do not ferment mannitol. Osawa and Walsh (1993) demonstrated that many *S. bovis* biotype I strains produce an enzyme, tannase, which hydrolyzes tannins to release gallic acid. Subsequently, it was noted that all strains of *S. bovis* that exhibited galate decarboxylase activity belonged to a single DNA homology group described as *Streptococcus gallolyticus* (Osawa et al. 1995a). *S. gallolyticus* had been isolated from several environments, including the feces of koalas, kangaroos, possums, cows, horses, pigs, dogs, and guinea pigs, as well as animals with bovine mastitis, human clinical sources, and the sheep rumen (Sly et al. 1997).

Several species of tannin tolerant or degrading bacteria have been isolated from *Enterobacteriaceae* and genera *Streptococcus* and *Selenomonas* from the gastrointestinal ecosystems of different animals including humans, laboratory and zoo animals (Table 8.1; Nelson et al. 1998; Odenyo and Osuji 1998; Skene and Brooker 1995; Odenyo et al. 2001), which enable animals to thrive in high tannin-rich diets.

Table 8.1 Tannin-tolerant bacteria isolated from different animals

Isolate	Animals	Morphology	Gram stain	Tolerance to tannins (g/L)	Tannase	References
<i>Streptococcus bovis</i> JB1	Cattle	Cocci	Positive	TA, 2; CT of <i>A. aneura</i> , 1.5	Negative	Odenyo et al. (2001)
<i>Streptococcus</i> ES1	Sheep	Cocci	Positive	TA, 30; CT of <i>A. aneura</i> , 4	Negative	Odenyo et al. (2001)
<i>Butyrivibrio</i> sp. EAT6	Antelope	Curved rods	Negative	TA, 60; CT of <i>A. aneura</i> , 8	Negative	Odenyo et al. (2001)
<i>S. ruminantium</i> D	Cattle	Curved rods	Negative	TA, 4; CT of <i>A. aneura</i> , 1.5	Positive	Odenyo et al. (2001)
<i>S. ruminantium</i> K2	Feral goat	Curved rods	Negative	TA, 20	Positive	Skene and Brooker (1995)
<i>Selenomonas</i> sp. EG4.2	Goat	Curved rods	Negative	TA, 70	Positive	Odenyo et al. (2001)
<i>Streptococcus galloyticus</i>	Koalas	Cocci	Positive		Positive	Osawa et al. (1995a)
<i>S. galloyticus (caprinus)</i>	Goats	Cocci	Positive	TA, 25	Positive	Brooker et al. (1994)
<i>Selenomonas</i> sp. ES3	Sheep	Curved rods	Negative	TA, 70; CT of <i>A. aneura</i> , 8	Positive	Odenyo and Osuji (1998)
<i>Selenomonas</i> sp. EG19	Goat	Curved rods	Negative	TA, 50–60; CT of <i>A. aneura</i> , 8	Positive	Odenyo and Osuji (1998)
<i>Selenomonas</i> sp. EAT2	Antelope	Curved rods	Negative	TA, 50–60; CT of <i>A. aneura</i> , 8	Positive	Odenyo and Osuji (1998)
<i>Streptococcus</i> sp. KN1	Goat	Cocci	Positive	TA, 30; <i>Desmodium</i> tannins, 0.8	–	Nelson et al. (1998)
<i>Streptococcus</i> sp. KN2	Sheep	Cocci, chain	Positive	TA, 2; <i>Desmodium</i> tannins, 0.7	–	Nelson et al. (1998)
<i>Streptococcus</i> sp. KN3	Goat	Cocci, single	Positive	TA, 2; <i>Desmodium</i> tannins, 0.6	–	Nelson et al. (1998)
<i>Streptococcus</i> sp. TW1	Elk	Cocci, chain	Positive	TA, 8; <i>Desmodium</i> tannins, 0.6	–	Nelson et al. (1998)
<i>Eubacterium</i> sp. TW2	Elk	Rod	Positive	TA, 2; <i>Desmodium</i> tannins, 0.5	–	Nelson et al. (1998)
<i>Escherichia</i> sp. KN4	Deer	Rods	Negative	TA, 2; <i>Desmodium</i> tannins 0.4	–	Nelson et al. (1998)

TA tannic acid, CT condensed tannins

The tannin-tolerant bacteria are present in the digestive tracts of animals irrespective of the geography, climate and host (Nelson et al. 1998). Tannin-tolerant bacteria could be both Gram positive and negative with cocci, straight or curved rods types. Brooker et al. (1994) isolated a HT and CT tannin-resistant *Streptococcus caprinus* (a subjective synonyms of *S. gallolyticus*; Sly et al. 1997) from feral goats browsing *Acacia aneura* (containing 11–14% tannins), which was absent in domestic goats and sheep. *S. caprinus* was able to grow in the medium containing 2.5% (w/v) tannic acid. *S. gallolyticus* and *S. caprinus* produces lactate, and small amount of acetate and ethanol and were unable to grow in the presence of ammonia only (Brooker et al. 1994; Osawa et al. 1995a). The trans-inoculation of these bacteria using rumen fluid from feral goats to sheep fed on *Acacia* improved DM intake and nitrogen digestibility in sheep and the population was maintained as long as the animal was fed the *Acacia* diet. Another anaerobic diplococoid bacterium, isolated from the rumen of goat fed on *Desmodium ovalifolium* (contains 17% CT), was able to grow in the presence of up to 30 g of tannic acid and 4 g of *Desmodium ovalifolium* CT per litre of media and this bacterium degraded tannic acid to pyrogallol (Nelson et al. 1995). Nelson et al. (1998) isolated six tannin tolerant isolates from sheep, goat, mountain elk and white-tail deer. The 16S rRNA sequence analysis showed that four of the isolates (isolated from goat, sheep and elk) were members of the genus *Streptococcus*, and were most closely related to *S. bovis* and *S. gallolyticus*. One of the other isolates (elk), a Gram positive rod, clustered with the clostridia in the low G+C group of Gram positive bacteria. The sixth isolate (deer), a Gram negative rod, was a member of the family *Enterobacteriaceae* in the gamma subdivision of the class *Proteobacteria*. Similarly, Odenyo and Osuji (1998) isolated three strains of tannin-tolerant bacteria related to *Selenomonas* sp. from sheep, goat and antelope, which were able to grow in media containing 50 g/L of a tannin extract and 50–70 g/L of tannic acid. The presence of tannins tolerant bacteria in the digestive tracts may depend upon the previous exposure to tannins. Tannin tolerating bacteria were not isolated from sheep, goats, cows, which had no history of tannin consumption (Brooker et al. 1994; Nelson et al. 1998).

Inoculation of tannin tolerant bacteria to animals fed high tannin-containing diets have shown to improve utilization of nutrients and performance of animals. For example, feral goats in Australia tolerated diets containing *Acacia aneura* (mulga; contains 5–25% CT) better than sheep tolerated such diets. Rumen fluid (Miller et al. 1995) or an *in vitro* cultured rumen inoculum (Miller et al. 1997) from goats improved nitrogen digestion in sheep fed *A. aneura*. Similarly, rumen inoculation with tannin-resistant Gram positive rod to sheep fed peanut skin CT (7.1%) showed a positive effect on nitrogen balance compared with sheep that did not receive this inoculum (Molina et al. 1999). Wiryawan et al. (2000) also demonstrated that inoculation of a pure culture of an uncharacterized tannin-resistant bacterium from Indonesian goats increased body weight gain over 40 days in goats changed from a grass diet to a 100% *Calliandra calothyrsus* diet (containing 6–10% CT), compared to the body weight gain of uninoculated goats. However, inoculation of a pure culture of tannin-resistant *S. caprinus* was ineffective to improve the digestion of *A. aneura* forage in sheep (Miller et al. 1996).

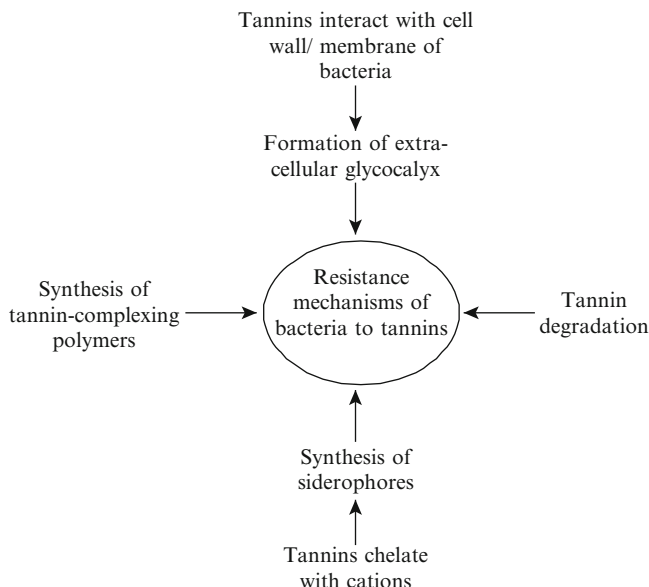


Fig. 8.4 Tolerance mechanisms of bacteria to dietary tannins (Adapted from Patra and Saxena 2011)

8.8.2 Resistance Mechanisms of Microbes to Tannins

Several mechanisms have been proposed for the resistance mechanisms of the bacteria to tannins (Fig. 8.4). Some bacteria grown in the presence of tannin secrete exo-polysaccharides (glycoproteins) that form a protective layer around the cells and thus protect the cells from the action of tannins (O'Donovan and Brooker 2001; Krause et al. 2005). Chiquette et al. (1988) demonstrated using transmission electron microscopy that ruminal bacteria produced a thick glycocalyx when grown in association with the high tannin containing *L. corniculatus* forage. The gene expression study in *E. coli* revealed that cytosolic and cell membrane damage caused by tannins may be overcome through an increase expression of cell envelope stress protein genes responsible for these protein synthesis (Zoetendal et al. 2008). Besides, the morphology of bacteria may be changed in the presence of tannins in media (O'Donovan and Brooker 2001). Goel et al. (2005) also observed that *Streptococcus* species isolated from non-adaptive cattle were converted from diplococi to an elongated chain of 40–50 cells with increasing concentrations of tannin acid in the media. Tolerance mechanism of bacteria to tannins may also involve degradation of tannins by secretion of tannase enzyme. The tannin-degrading microorganisms have been isolated from several species of ruminants and non-ruminants including sheep, goat, deer, horse, pigs and elk that have access to tannin rich forages (Sly et al. 1997; Odenyo and Osuji 1998). Odenyo et al. (2001) observed that some tannin-tolerant bacteria were not tannase-positive. Some intestinal bacteria such as

Lactobacillus and *Bifidobacterium* were inherently resistant to tannic acid because heme-containing enzymes are not present in these bacteria (Chung et al. 1998). Tannic acid has a strong iron binding capability, which depletes iron from media for the growth of bacteria having heme-containing enzymes (Chung et al. 1998). Besides, bacteria grown in the presence of tannins may synthesize siderophores (Scalbert 1991), which probably counteract the depletion of iron by tannins. Besides the development of tolerance mechanisms by the rumen bacteria, animals itself may develop tolerance to tannins by secreting increased amounts proline-rich proteins in the saliva (Mehansho et al. 1983). These proteins have strong affinity for tannins and may be constitutive or inducible depending upon the species of the animals (Mueller-Harvey 2006). The secretion of proline-rich proteins may diminish the responses of tannins to the N metabolism.

8.9 Metabolism of Tannins by Microorganisms in the Digestive Tract

The microorganisms of gastrointestinal ecosystems of animals are able to degrade HT and simple plant phenolic compounds to variable extent depending upon the types of phenolic compounds and species of microbes to counteract anti-nutritive effects of tannins (Fig. 8.5). Phenolic glycosides such as rutin and naringin, and flavonoid ring system of the common plant flavonoids (e.g. quercitrin, kaempferol and naringenin) are readily degraded by the rumen and intestinal micro-organisms by hydrolysis of the glycoside and cleavage of the heterocyclic ring to acetate, butyrate, di- and monohydroxyphenolics, and phloroglucinol (Simpson et al. 1969; Winter et al. 1989; Lowry and Kennedy 1996; Schoefer et al. 2003). The HT are primarily degraded by an enzymes, tannase (tannin acyl hydrolase, EC 3.1.1.20) by bacteria and fungi, which is active in galloyl residues of galloyl esters, hexahydroxydiphenoyl groups and other ellagitannins. It has both esterase and depsidase activities. The tannase-positive microbes have been reported in the cattle, sheep, goats, koalas, deer and humans, which consume tannin-containing diets (Skene and Brooker 1995; Osawa et al. 1995b, 2000). The ester bonds and depside bonds of HT are both cleaved in the rumen by *S. ruminantium* and *Streptococcus* spp. by tannase, (Skene and Brooker 1995; Nelson et al. 1998; Bhat et al. 1998; Goel et al. 2005) releasing glucose, gallic acid and ellagic acid. However, hydrolysis of tannic acid may sometime not involve the production of tannase (Odenyo et al. 2001). Gallic acid is then decarboxylated to pyrogallol and converted to resorcinol and phloroglucinol before cleavage of the phloroglucinol ring to acetate and butyrate by rumen micro-organisms (Murdiani et al. 1992; Bhat et al. 1998). Tsai and Jones (1975) isolated *Streptococcus* strains and three *Coprococcus* strains from the bovine rumen that were capable of degrading up to 80% of phloroglucinol within 2 days, where phloroglucinol was present as the only added carbon source. *Eubacterium oxidoreducens*, a strictly anaerobic rumen bacterium that degrades gallate, phloroglucinol, and pyrogallol to acetate and butyrate in the presence of hydrogen and formic

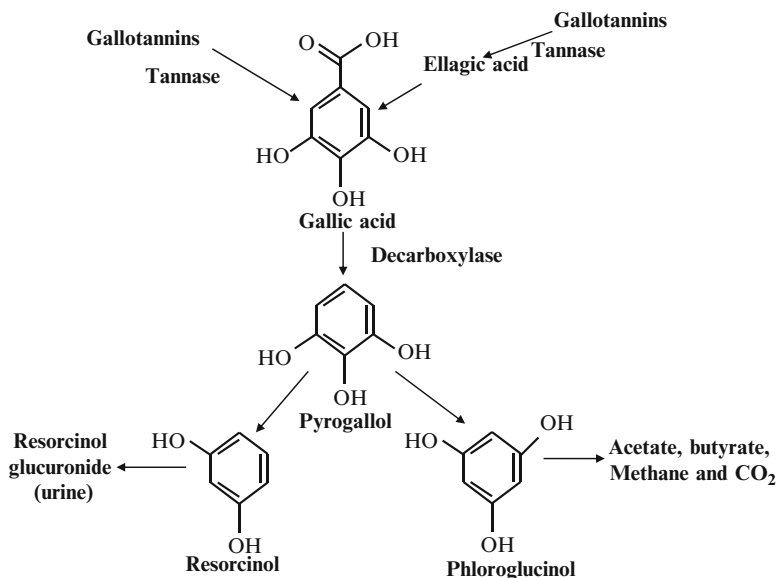


Fig. 8.5 Pathways for degradation of hydrolysable tannins in the rumen (Modified from Bhat et al. 1998)

acid (Krumholz and Bryant 1986). These simple monomeric compounds might be absorbed from the alimentary tracts and excreted through urine as conjugates. Sheep fed tannic acid and HT from leaves of *Terminalia oblongata* (containing ellagitannins) excreted glucuronides of resorcinol and 2-carboxy-2',4',4,6-tetrahydroxy diphenyl 2,2'-lactone as urinary metabolites (Murdiati et al. 1992). Compared to gallotannins, the ellagitannins are more difficult to be degraded by microbes than gallotannins due to their complex structures (Mingshu et al. 2006).

The colonic microbial populations also degrade HT to gallic acid, pyrogallol, phloroglucinol, and finally to acetic and butyric acid by sequential activities of different enzymes. *Enterococcus faecalis*, which inhabits gastrointestinal tracts of animals and humans has shown to produce tannase enzyme and degrade tannic acid to gallic acid, pyrogallol and resorcinol (Goel et al. 2011). The ellagitannins is degraded into ellagic acid and then transformed to urolithin B by colonic microbiota of humans and rats, which is detected as glucuronide form of urolithin B (Cerda et al. 2003). Catechin and gallo catechin can be degraded to velerolactone and phenylpropionic acid by intestinal micro-organisms, and glucuronic acid or sulphate conjugates of velerolactone have been observed to excrete through urine and faeces in rats and human (Hollman 2001; Mueller-Harvey 2006).

In contrast to HT, the metabolism of CT in the gastrointestinal tract is not clear. Some quantitative nutritional trials have shown apparent losses of 29.1% and 51.7% of CT from *Acacia barteri* and *Bauhania variegata*, respectively, up to 24 h of fermentation *in vitro* with no further increase in the degradability of CT after 24 h (Makkar et al. 1995b; Paul et al. 2006). With radioactively labeled CT,

Perez-Maldonado and Norton (1996) detected substantial losses of ^{14}C -labelled CT from *Desmodium intortum* in the gastrointestinal tract of sheep and goats. Wiryawan et al. (2000) noted that tannin degrading bacteria isolated from goats exposed to *Calliandra* tannins were able to decrease tannic acid and CT from the medium. It has been suggested that low molecular weight free CT or breakdown products of CT may be absorbed from the digestive tract (Perez-Maldonado and Norton 1996; Deprez et al. 2000; Serrano et al. 2009). For example, Deprez et al. (2001) observed that catechin, a proanthocyanidin dimer and trimer had similar permeability coefficients in intestinal cells *in vitro*, close to that of mannitol (a marker of paracellular transport); whereas, permeability of a oligomeric proanthocyanidin with an average polymerisation of six (molecular weight=1,740) was approximately ten times lower. However, the absorption of low molecular weight proanthocyanidins from the digestive tract depends upon their properties. Jimenez-Ramsey et al. (1994) showed that proanthocyanidins soluble in water and ethanol were absorbed from the intestine, while proanthocyanidin fractions soluble in aqueous acetone but insoluble in water and ethanol were not absorbed in chicks. Further, it has also been postulated that apparent disappearance of CT from the digestive tract can be a result of minor conformational changes in the reactive CT ring structure and consequently these polyphenols are not quantified by the analytical techniques (Terrill et al. 1994; Perez-Maldonado and Norton 1996; Waghorn 2008). There may also be an underestimation of CT due to low recovery of CT in aqueous phase as CT may strongly bind with dietary macromolecules in the intestine (Deprez et al. 2000; Serrano et al. 2009). Although, the cleavage of carbon-carbon bonds of flavan-3-ols ring system (e.g. catechin and epicatechin) of CT by rumen and intestinal microorganisms has not been confirmed (McSweeney et al. 2001a; Serrano et al. 2009), there is evidence from the studies in simple stomach animals that a small amount of CT may be degraded into aromatic acid metabolites by the large intestinal microbiota (Gonthier et al. 2003; Deprez et al. 2000).

The tannins that reach the large intestine could be degraded by colonic microorganisms. The studies on metabolism of tannins by intestinal microbiota in ruminants are limited. Colonic microbiota of humans apparently metabolized the polymeric CT extensively after 48 h of incubation under *in vitro* anaerobic conditions (Deprez et al. 2000). Phenylacetic, phenylpropionic and phenylbutyric acids were the main metabolites detected, although, they represented only 2.7% of the initial radioactivity from the substrate (Deprez et al. 2000).

8.10 Conclusions

Tannins interact with proteins predominately via hydrogen bonds forming tannin-protein complexes thus preventing degradation of protein in the rumen, and these tannin-protein complexes are dissociated in the abomasum releasing protein. Tannins might also inhibit the growth of proteolytic bacteria, which can reduce proteolysis.

These increase non-ammonia N flow in the intestine for absorption. Moderate concentrations (depending upon the type of tannins) of tannins in diets improve body weight and wool growth, milk yields and reproductive performance. However, these effects have not been consistently observed. These discrepancies among studies are attributed to the mode of tannin-protein interactions, which do not always work in an optimal mode depending upon the chemical structures of tannins and protein. While tannins may shift N metabolism from rumen to intestine, the effects of tannins on large intestinal protein metabolism and microbial populations are not known. Recently, inclusion of tannins in diets has been shown to enrich CLA content in meat and milk. Tannins can exert beneficial effect environmentally by shifting N excretion from urine to faeces and decreasing methane output. Tannins lower methane production probably by directly inhibiting the activities of methanogenic archaea and/or reducing the fiber digestion in the rumen. Not all types of tannins produce beneficial nutritional and environmental responses. The elucidation of structure-activity relationships would be required to obtain consistent beneficial tannin effects in ruminants.

Alternative internal parasites management and food-borne pathogen control strategies using plant containing tannins and polyphenolic extracts have recently been suggested. It seems possible that consumption of plant tannins may reduce gastrointestinal parasites numbers, food-borne pathogens populations and improve animal performance through direct and indirect mechanisms.

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