Use of Tannins as Organic Protectants of Proteins in Digestion of Ruminants

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Journal of Livestock Science (ISSN online 2277-6214) 4: 67-77

Date of receipt:.18.3.2013; Date of acceptance: 14.07.2013

Abstract

Protection of proteins is essential for productive ruminants, where the protein requirement of these animals cannot be met from microbial protein synthesis. There has been considerable interest in reducing ruminal degradation of proteins. There is a growing interest in the possible use of Condensed Tannins (CT) as protectant of protein in the ration of animals. Tannins are a heterogeneous group of high molecular weight phenolic compounds and divided into two groups: the condensed and the hydrolysable tannins. The high affinity of tannins for proteins is due to the presence of great number of phenolic groups. These provide many points at which bonding may occur with the carbonyl groups of peptides. The tannins are widely distributed throughout the plant kingdom. There is an increasing demand at the consumer level for organic animal products like milk and meat. Tannins as organic protein protectants can be used at safer levels in livestock farming for sustaining the production and health of domestic ruminants.

Keywords: tannins; rumen; microbial degradation; ruminants; protected proteins

Introduction

Protein in ruminant diets is poorly utilized because of extensive breakdown in the rumen, which may exceed microbial protein synthesis. Degradation often results in wastage of dietary proteins, particularly in productive ruminants such as growing animals, which have high protein requirements. Protection of proteins is essential for productive ruminants, where the protein requirement of these animals cannot be met from microbial protein synthesis. There has been considerable interest in reducing ruminal degradation of proteins. Studies have indicated that feeding proteins, which are resistant to microbial breakdown in the rumen but available in the post-ruminally, significantly increased growth rate and production of milk and milk protein. Various treatments such as heating (Glimp *et al*, 1967) and formaldehyde treatments (Ferguson *et al*., 1967; Dutta and Agrawal, 2000) have been used to protect proteins from rumen degradation and thereby to provide by-pass protein to the lower tract. However, these treatments may impair the subsequent intestinal availability of some amino acids, notably lysine, cysteine, tyrosine and leucine (Schonhusen *et al.*, 1986). The use of formaldehyde and other chemicals to protect proteins from ruminal degradation has no scope in organic animal farming (IFOAM, 2006). It is therefore essential to explore alternative protectants of protein to improve protein utilization and animal productivity.

In this context, there is a growing interest in the possible use of Condensed Tannins(CT) as protectant of protein in the ration of animals. CT (proanthocyanidins) form complexes with proteins that are stable over the pH range of 3.5–7.0, but dissociate in the abomasum (pH below 3.5) (Getachew *et al*., 2000) and anterior duodenum (Perez-Maldonado *et al*., 1995). Complexation protects proteins from microbial hydrolysis and deamination in the rumen (Perez-Maldonado and Norton, 1996) and increases the availability of feed proteins for digestion and more amino acids are absorbed postruminally (Min *et al*., 2003).

Tannins: structure and classification

Tannins are secondary plant products, found in cell walls or harboured within vacuoles in stems, bark, leaves, flowers, or seeds in dicotyledonous plants (Barry 1989). They were originally recognised and used in the tanning industry for tanning hides into leather due to their ability to bind and cross-link proteins in the hides. Bate-Smith and Swain (1962) proposed a definition for tannins as "water-soluble phenolics having molecular weights between 500 and 3000 that can precipitate alkaloids and proteins" (Mcleod,1974). Tannins are classified as either hydrolyzable or condensed (Fig. 1). Hydrolyzable tannins consist of polyphenols (gallic acid and/or hexahydroxydiphenic acid) ester-linked to a hexose moiety, and they can be hydrolysed by heating with weak acid. In contrast, the condensed tannins can be oxidatively degraded only by hot mineral acid. Condensed tannins are polymers of flavan-3-ol (e.g., catechin) or flavan-3,4-diol (proanthocyanidins) linked by C–C or C–O–C bonds to yield compounds of varying molecular weight (Leinmuller *et al.* 1991). Condensed tannins are synthesised from precursors from the acetate and shikimic acid pathways, as is lignin. The anabolic pathway was described in detail by Waterman and Mole (1994). Condensed tannin polymers vary tremendously in their constituent monomers, stereochemistry, polymer size, and intermolecular linkages, in addition to the dynamics of their location, concentration and composition throughout the life of the plant. These attributes all influence the facility of CT to interact with other molecules. Because of the heterogeneity of their polymerization (linear and branched) and their linkages with other plant constituents, CT are often difficult to separate and quantify. The CT of two species of lotus exhibit differing plant protein binding capacities, which is believed to arise from differences in the stereochemistry of their monomers (McNabb *et al*. 1998).

Condensed tannins can be classified further on the basis of the hydroxylation patterns of their constituent rings. For example, proanthocyanidins are subdivided into procyanidins and prodelphinidins according to the hydroxylation pattern on the B-ring. Interflavan bonds between monomers at the C-4 and C-8 or C-4 and C-6 positions extend and branch the polymer (Haslam 1989). *Lotus corniculatus* CT consist of relatively homogeneous polymers of epicatechin-type procyanidin units, whereas *L. pedunculatus* CT contain a heterogeneous mix of catechin and epi-catechin-type prodelphinidin monomers (McNabb *et al.* 1998). The CT of other forages in the same study were identified either as predominantly procyanidin, such as in dock (*Rumex obtusifolius*), or as predominantly prodelphinidin, as in sainfoin, sulla (*Hedysarum coronarium*), and white clover (*Trifolium repens*). Those two Lotus species also differ in the polymer sizes of their CT. *Lotus corniculatus* CT have molecular weights (MW) of 1800 to 2100 and comprise six or seven monomers (Foo *et al.* 1996), whereas *L. pedunculatus* CT have an average MW of 2200 and a

polymer size of eight units (Foo *et al.* 1997). Seed coat CT isolated from alfalfa (cv. Beaver) consist primarily of procyanidin units with an average degree of polymerization of 6.5 (Koupai-Abyazani *et al.* 1993a). Sainfoin (cv. Melrose) CT ranged in MW from 1800 to 3300, and had polymer sizes of six to eight monomers and a delphinidin:cyanidin ratio of 88:12 (Koupai-Abyazani *et al.* 1993b). Studying on the developmental changes in sainfoin CT (Koupai-Abyazani *et al.* 1993b) and comparing CT from 26 accessions and species of Onobrychis, (Koupai-Abyazani *et al.* 1993c), Koupai-Abyazani and co-workers concluded that these plants cannot be classified on the basis of CT chemistry. This diversity in chemical structure of CT influences their binding capacities. Their nutritional effects are the result of a complex chemical interaction and ruminant physiology and behaviour during grazing. Astringency values are ascribed to CT based on the amount of protein precipitated per unit weight. Astringency is also the term used to describe the un palatability of feeds containing high levels of tannin leading to reduced feed intake in high-tannin feeds, and protects plants from herbivores (Windham *et al*. 1990).

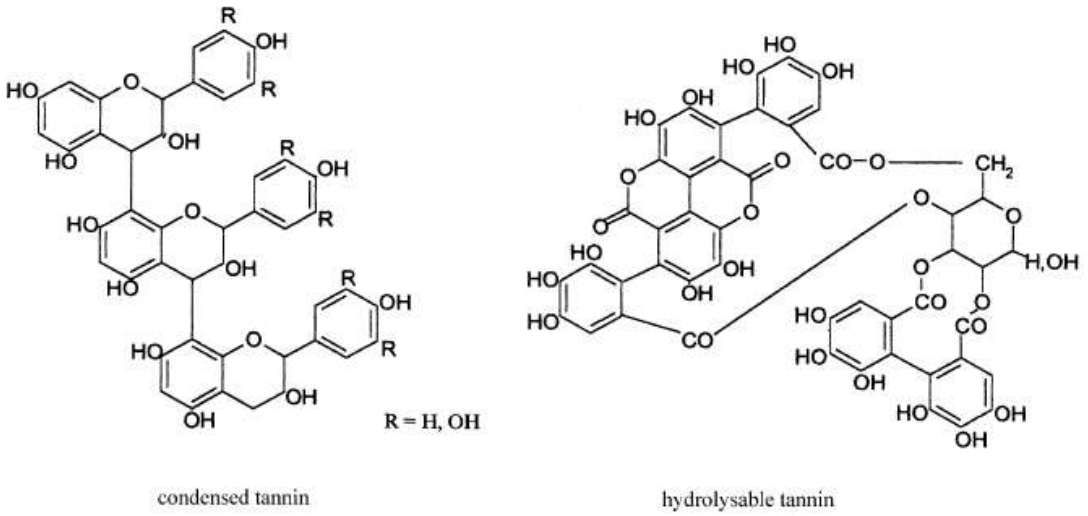

Figure 1: Chemical Structure of Condensed tannin and Hydrolysable tannin (Min *et al*., 2003)

Mechanism of action

The tannins of different plant species have different physical and chemical properties and therefore they have very diverse biological properties. The high affinity of tannins for proteins is due to the presence of great number of phenolic groups. These provide many points at which bonding may occur with the carbonyl groups of peptides. The formation of such complexes is specific, both in terms of the tannin and protein involved, the degree of affinity between the participating molecules residing in the chemical characteristics of each. With respect to tannins, the factors promoting the formation of complexes include their relatively high molecular weight and their great structural flexibility. The proteins that show the most affinity for tannins are relatively large and hydrophobic, have an open, flexible structure and are rich in proline. The complexes formed between tannins and proteins or other compounds are generally unstable (McNeill *et al*, 1998). The bonds uniting them continually break and re-form.

Kumar and Singh (1984) suggested that complexes could come about through four types of bond: 1) hydrogen bonds (reversible and dependent on pH) between the hydroxyl radicals of the phenolic groups and the oxygen of the amide groups in the peptide bonds of proteins, 2) by hydrophobic interactions (reversible and dependent of pH) between the aromatic ring of the phenolic compounds and the hydrophobic regions of the protein, 3) by ionic bonds (reversible) between the phenolate ion and the cationic site of the protein (exclusive to HT), and 4) by covalent bonding (irreversible) through the oxidation of polyphenols to quinones and their subsequent condensation with nucleophilic groups of the protein. For a long time it was believed that the formation of tannin-protein complexes was owed mainly to hydrogen bonds. However, it is now known that hydrophobic interactions are important.

The main effect of tannins on proteins is based on their ability to form hydrogen bonds that are stable between pH 3.5 and 8 (approximately). These complexes —stable at rumen pH— dissociate when

the pH falls below 3.5 (such as in the abomasum, pH 2.5-3) or is greater than 8 (for example in the duodenum, pH 8), which explains much about the activity of tannins in the digestive tract. This reduction in protein degradation is associated with a lower production of ammonia nitrogen in the rumen and a greater non-ammonia nitrogen flow to the duodenum.

Distribution of tannins in nature

The tannins are widely distributed throughout the plant kingdom, especially among trees, shrubs and herbaceous leguminous plants (Perevolotsky, 1994).

The genera belonging to the families of the Betulaceae (Betula), Cesalpinaceae (Ceratonia), Cistaceae (Cistus), Cupresaceae (Juniperus), Ericaceae (Calluna, Erica, Vaccinium), Fagaceae (Castanea, Quercus), Leguminaceae (Cytisus, Genista, Lathyrus, Lotus, Medicago, Onobrychis, Trifolium), Poaceae (Holcus, Hordeum, Lolium, Sorghum, Triticum) are rich in tannins(Barry and McNabb, 1999 ; Hervás *et al*., 2003). Tannins are more abundant in the parts of the plant that are most valuable to it, e.g., new leaves and flowers (Terril *et al.*, 1992). Numerous reports illustrate the effects of environmental and seasonal factors as well as of phenological development. High temperatures, water stress, extreme light intensities and poor soil quality increase the tannin content of plants. Seasonal variation (which clearly correlates with phenological stage) is owed to the different demand for nutrients. During their growth period, when plants produce a lot of biomass, few resources are available for synthesis of phenolic compounds. However, during flowering, when growth is reduced, excess carbon may be available for tannin synthesis.

Treatments to protect dietary protein from ruminal degradation

Effect on DMI:

The comparable ($P > 0.05$) intake by lambs irrespective of dietary treatments are in agreement with the earlier observations that moderate levels (1–4%) of CT in the diet from various plant sources exerted no significant effect on feed intake (Wang *et al*., 1996a,b; Bhatta *et al*., 2000; Komolong *et al*., 2001).

Nutrient utilization

The nutritional effects of tannins are associated with their ability to bind with proteins (dietary and enzymes), structural carbohydrate polymers found in plant cell walls and minerals with an overall effect of lowering the bioavailability of nutrients at specific sites in the gastro-intestinal tract (Ndluvo, 2000). However, other workers have reported that tannins are beneficial to ruminants at low concentration because they protect plant proteins from degradation in the rumen (Waghorn and Shelton, 1992; Wang *et al*., 1994). In the present study the addition of CT up to 1.5% (*i.e.* 1.5 and 1%) of supplement does not seem to interfere with the microbial activity or total tract digestibility of nutrients. A depressing effect on DM digestibility at 2% CT level may be due to associative effects of CT and higher percentage of structural carbohydrates contributed by *F. infectoria* leaves. This may probably be due to interference of CT with microbial attachment or depressing cellulolytic bacterial population (McAllister *et al*., 1994; McSweeney *et al*., 1998).

Significantly higher (linear, $P < 0.001$) N retention as percentage of absorbed N (an indicator of availability of amino acid-N at tissue level) was observed in animals given CT protected concentrate which could have been due to better amino acid availability and apparent biological value of CT protected diets as suggested by Barry and McNabb (1999). Comparisons of duodenal nitrogen flows per unit nitrogen intake in animals fed forages which contain different levels of tannin indicated a dose responsive increase in the efficiency of capture of microbial protein where the tannin is present and cross-links with the forage protein making it less rapidly released during microbial fermentation (Barry and McNabb 1999). An increased N retention in sheep given tanniferous feeds at moderate levels (1–4%) due to lowered nitrogen excretion through urine has been reported earlier (Waghorn et al., 1994b; Ngwa *et al*., 2002). This protects the protein from microbial hydrolysis and deamination in the rumen and increases the proportion of dietary amino acids available for post-ruminal absorption.

Microbial Nitrogen Supply

One of the basic goals of protein nutrition in ruminants is to optimise dietary protein use in order to maximize animal growth and milk production per unit of protein consumed. The nutritional effects of tannins are associated with their ability to bind with proteins (dietary and enzymes), structural carbohydrate polymers found in plant cell walls and minerals with an overall effect of lowering the bioavailability of nutrients at specific sites in the gastro-intestinal tract (Ndluvo, 2000). However, other workers have reported that tannins are beneficial to ruminants at low concentration because they protect plant proteins from degradation in the rumen (Waghorn and Shelton, 1992). Increased N retention in sheep given tanniferous feeds at moderate levels (1–4%) due to lowered nitrogen excretion through urine has been reported earlier (Waghorn *et al*., 1994b; Ngwa *et al*., 2002). This protects the protein from microbial hydrolysis and deamination in the rumen and increases the proportion of dietary amino acids available for post-ruminal absorption. Hence, 1.5% CT is the optimum level at which there are enough tannins to exert noticeable effects. Similar trends of increased growth in sheep supplemented with CT were reported by Ngwa et al. (2002). At appropriate concentration, the CT reduced the degradation of sulphur amino acids (SAA) in the rumen, increases the irreversible loss of cystine from plasma and increased the flow of cystine to body synthetic reaction (McNabb et al., 1993; Wang et al., 1994) and thereby improves the performance of lambs. With respect to HT, in 1972 Driedger and Halfield managed to reduce the in vitro ruminal protein degradability of soya bean meal through treatment with tannic acid. Its effect on intestinal digestibility however, was not very consistent.

Pace *et al*. (1993) observed that the CT of quebracho had a greater reduction in the degradability of soya bean meal than commercial tannic acid. Frutos *et al*. (2000) treated soya bean meal with different doses (0, 1, 4.7, 9, 13 and 20%) of tannic acid or commercial quebracho CT extract, and significantly reduced the extent of crude protein degradation in the rumen. The effect was significant even at the lowest dose. With respect to the intestinal digestibility of the non-degraded protein, no negative effects were seen until the 13% dose was reached with tannic acid and until the 20% dose was reached in the quebracho CT treatment. Kariuki (2004) reported that supplementation of legumes containing condensed tannins in the diet of sheep significantly improved the release of previously bound feed protein post ruminally in the intestine.

One of the drawbacks of using tannins as additives to protect protein rich feeds is the possibility of their degradation by rumen microorganisms. In such cases the treated feeds would be just as vulnerable to ruminal degradation as untreated feeds. In the experiment of Frutos *et al.* (2000), the intraruminal administration of quebracho CT extract to sheep for 60 days did not increase the capacity of the microorganisms to degrade tannins and it was also reported that the proanthocyanidins, or CT, cannot be degraded in the rumen (McSweeney *et al*., 2001). However, the consumption of small quantities of HT in soya bean meal (20.8 g /kg DM) by Merino sheep under practical finishing conditions showed that these compounds were neither toxic nor had any negative effect on animal performance (Frutos *et al*., 2004). Dey *et al.* (2008) observed that on feeding condensed tannin rich leaves of *Ficus infectoria* at the level of 1.5% in the diet of lambs significantly improved nitrogen (N) utilization and nutrient retention.

Effect on nutritive value

McNeill *et al.* (1999) indicated that dietary protein complexed with tannins was made available in abomasum and digested in intestine, but tannins released from the protein-tannin complexes may react with non-dietary protein (including digestive enzymes) as it passes along the intestines, thus counteracting the benefits of by-pass dietary protein. This may explain the decrease in TDN content as consequence of decreased OM digestibility when the level of CT increased to 2.0% of supplement. The findings suggest that plane of nutrition was not affected adversely with CT supplementation in conformity with the earlier reports (Terrill *et al.*, 1992; Waghorn *et al.*, 1994a,b; Wang *et al*., 1996a,b). The palatability of browse species was closely related to the concentration of tannins. There appears to be a threshold of tannin contents (approximately 5%) below which no adverse effect was evident on nutrient intake and utilization.

Effect on FCR and ADG

Improvement in ADG and FCR on supplementation 1.5% level CT in lambs was reported by Dey *et al*. (2008) and improved growth rate in sheep (Ngwa *et al.*, 2002). At appropriate concentration, the CT reduced the degradation of sulphur amino acids (SAA) in the rumen, increases the irreversible loss of

cystine from plasma and increased the flow of cystine to body synthetic reaction (McNabb *et al.,* 1993; Wang *et al.,* 1994) and thereby improves the performance of lambs.

Wool growth and quality

Min *et al.* (2001) reported an increase in fleece weight in condensed tannin supplemented groups indicating that CT may have enhanced effect on the absorption of sulfur containing amino acids. This is similar to earlier observations that CT in Lotus increased the efficiency of wool production (Min *et al*., 1998; Ramirez-Restrepo *et al*., 2004). The wool quality in term of fibre length (mm) and fibre diameter (mm) was similar ($P < 0.05$) in lambs irrespective of dietary treatments. Present observations are in agreement with the earlier report of Wang *et al*. (1996a), but contrary to some reports describing beneficial (higher staple length) effect of CT on these parameters in grazing sheep (Min *et al*., 2001; Ramirez-Restrepo *et al*., 2004). This could be due to difference in concentration and chemical composition of CT, which affect its biological activity (Aerts *et al*., 1999). Further, in the present experiment local non-descript breed of sheep was used and they are known for inferior (carpet) quality wool production.

Beneficial effects of condensed tannins on ruminants

Ruminal Escape Protein

In the rumen, up to 70% of soluble forage proteins may be degraded (Barry and Manley 1984), yielding ammonia as a byproduct of amino acid deamination. Ammonia in excess of that used for microbial protein synthesis crosses the rumen wall to the blood, however there is an energetic cost for its subsequent conversion to urea in the liver. Reduction of excess ruminal ammonia concentrations is desirable because it minimizes nitrogen losses and may prevent reproductive problems in cows associated with high levels of plasma urea nitrogen (Elrod and Butler 1993; Ferguson 1996). Condensed tannins have been shown to lower soluble protein and ammonia-N levels in ruminal fluid (Barry *et al.* 1986; Chiquette *et al.* 1989; McMahon *et al.* 1999) and to promote greater nitrogen retention by reducing urea excretion (Egan and Ulyatt 1980) and by increasing urea recycling to the rumen (Waghorn *et al*. 1994). In the Rusitec, use of ammonia N by bacteria increased as the proportion of sainfoin in a mixed alfalfa:sainfoin diet increased, presumably because feed protein was unavailable for bacterial metabolism (McMahon *et al*. 1999). Increased efficiency of N utilization in the rumen was also considered to be responsible for the reduced urinary N excretion by sheep fed *L. pedunculatus*, as compared to sheep fed lotus treated with PEG (Barry *et al*. 1986).

Reduced ruminal protein degradation associated with CT increases the quantity of NAN and amino acids reaching the small intestine (Waghorn *et al*. 1987a, b). Birdsfoot trefoil(*L. corniculatus*) containing 20 g CT / kg DM fed to sheep without PEG provided 50% more essential amino acids and 14% more non-essential amino acids to the small intestine than when the trefoil-fed sheep were treated with PEG (Waghorn *et al.* 1987b). Fractional absorption of essential amino acids in the small intestine of sheep fed the legume only was similar to that in sheep supplemented with PEG, but absorption of non-essential amino acids in the small intestine was reduced. However, the larger quantity of essential amino acids reaching the small intestine resulted in a greater apparent absorption (59 vs. 36 g/d) in sheep in which the forage CT were not inactivated by PEG (Waghorn *et al*. 1987a). Similar responses were obtained when sheep were fed *L. pedunculatus* containing over 50 g CT / kg DM supplemented with and without PEG (Waghorn *et al*. 1994).

The pattern of reduced nitrogen digestibility in the rumen accompanied by increased NAN flux to the small intestine is characteristic for forages containing CT (Waghorn *et al.* 1994; Wang *et al.*, 1996a; Barahona *et al.* 1997). Condensed tannins from *L. corniculatus* affected amino acid digestion in sheep fed lotus with and without PEG (Waghorn *et al*. 1987b). The proportion of non-sulphur amino acids degraded in the rumen was 22% in the control compared with 40% in PEG treated (CT-inactivated) sheep. Abomasal fluxes (as a proportion of DM intake) of valine, isoleucine, threonine, phenylalanine, histidine, tyrosine, lysine, arginine, glutamic acid, proline and glycine were higher with CT, and those of aspartic acid, serine and alanine were lower. Sheep receiving CT absorbed quantitatively more valine, threonine, isoleucine, leucine, tyrosine, phenylalanine, histidine and lysine from the small intestine than did those treated with PEG. Apparent digestibility of amino acids was lower in sheep fed CT, but quantitatively more amino acids reached the small intestine. Waghorn *et al.* (1987b) suggested that these

data reflect either an incomplete release of protein/amino acid from the tannin complex in the intestine, or a direct effect of CT on intestinal digestion or absorption of amino acids.

A specific study has also been conducted on the effects of CT on the site of digestion of Rubisco (Fraction 1 protein). McNabb *et al.* (1996) determined that although CT did not affect solubilization of Rubisco in the rumen, the rate and amount of ruminal degradation of Rubisco from *L. pedunculatus* in sheep were reduced by CT, and intestinal digestion was increased. Digestion of Rubisco in the small intestine of control sheep was 26%, compared with only 4% in sheep receiving PEG in conjunction with the lotus, but total tract digestibility was similar between groups. Because of the predominance of Rubisco among forage proteins, these data reveal that CT can dramatically increase the quantity of dietary protein available for digestion in the small intestine.

The extent of CT-mediated reduction in ruminal protein degradation *in vitro* is generally proportional to tannin content (Broderick and Albrecht 1997). Ruminal escape values calculated from *in vitro* protein degradation rates for a number of forages were 22% (alfalfa), 18% (*L. corniculatus*), 38% (*L. pedunculatus*), 54% (sainfoin) and 60% (lespedeza). Degradation rates varied among species with similar tannin contents, implying that the chemical properties of the tannins, as well as their quantity, influenced protein degradation. These experiments show that low levels of CT increase the amount of protein and amino acids reaching the small intestine because less protein is degraded in the rumen (Waghorn 1990) suggesting that it may be possible to feed an optimum level of CT to increase bypass protein without impairing intake or digestibility. Protein solubility in the rumen of cattle was reduced by as little as 0.17% dock (*R. obtusifolius*) CT in the diet (Waghorn and Jones 1989). At 50 to 60 g/ kg DM, CT doubled the amount of plant protein leaving the rumen (Waghorn *et al.* 1990), but at a cost of reduced total tract digestibility and intake.

Conclusion

An increasing number of consumers demanding healthy and natural foods have pushed organic livestock farming that is reputed to be environment friendly, sustaining animals in good health, with high welfare standards and prohibit routine use of growth promoters, animal offals or any other chemicals and additives to livestock rations. Condensed Tannins (CT) are organic protectants of protein in the ration of animals. The proper management of natural tannin-containing resources (e.g., selective grazing or supplementing the diet with the right kind of shrubs containing condensed tannins) could provide beneficial effects with respect to protein degradation.

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