Review. Tannins and ruminant nutrition

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Abstract

Tannins are a complex group of polyphenolic compounds found in a wide range of plant species commonly consumed by ruminants. They are conventionally classified into two major groups: the hydrolysable and the condensed tannins. Although for a long time tannins were thought to be detrimental to ruminants, their effect may be either beneficial or harmful depending on the type of tannin consumed, its chemical structure and molecular weight, the amount ingested, and the animal species involved. High concentrations of tannins reduce voluntary feed intake and nutrient digestibility, whereas low to moderate concentrations may improve the digestive utilisation of feed mainly due to a reduction in protein degradation in the rumen and a subsequent increase in amino acid flow to the small intestine. These effects on nutrition are reflected in animal performance.

Key words: hydrolysable tannins, condensed tannins, rumen degradation, digestibility, animal production.

Resumen

Revisión. Taninos y nutrición de rumiantes

Los taninos son un grupo muy diverso de compuestos fenólicos que aparecen en una amplia variedad de plantas consumidas por los rumiantes. Tradicionalmente se han dividido, de forma simplista, en hidrolizables y condensados. Aunque durante mucho tiempo se pensó que los taninos eran negativos para los rumiantes, su efecto puede ser beneficioso o perjudicial dependiendo del tipo de tanino, de su estructura y peso molecular, de la especie animal que los consuma y, de modo fundamental, de la cantidad ingerida. Se sabe que el consumo de cantidades elevadas reduce la ingestión voluntaria y la digestibilidad de los nutrientes, en tanto que el consumo de cantidades pequeñas o moderadas puede mejorar la utilización digestiva, debido, principalmente, a una reducción de la degradación ruminal de la proteína y, en consecuencia, a una mayor disponibilidad de aminoácidos susceptibles de ser absorbidos en el intestino delgado. Estos efectos sobre la nutrición se ven reflejados en el rendimiento productivo de los animales.

Palabras clave: taninos hidrolizables, taninos condensados, degradación ruminal, digestibilidad, producción animal.

Tannins: structure and chemical properties

Definition and classification

The tannins are a group of plant secondary compounds which have been known and used by Man for centuries. Their name comes from the French *tan* meaning the bark of the holm oak and other trees used in tanning.

From a chemical point of view it is difficult to define tannins since the term encompasses some very diverse oligomers and polymers (Harborne, 1999; Schofield *et al.,* 2001). It might be said that the 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, etc. (McLeod, 1974; Mole and Waterman, 1987; Mangan, 1988; Mueller-Harvey and McAllan, 1992; Van Soest, 1994; Giner-Chavez, 1996; Schofield *et al.*, 2001).

The tannins have traditionally been divided into two groups: the condensed and the hydrolysable tannins. Hydrolysable tannins (HT) are made up of a carbohydrate core whose hydroxyl groups are esterified with phenolic acids (mainly gallic and hexahydroxydiphenic acid). The condensed tannins (CT), or proanthocyanidins, are non-branched polymers of flavonoids units (flavan-3-ol, flavan-3,4-diol), and usually have a higher molecular weight than the HT (1000-20000 Da compared to 500-3000 Da) (McLeod, 1974; Mueller-Harvey and McAllan, 1992; Mueller-Harvey, 1999).

Although this division of the tannins is the most widely accepted, many authors believe it does not fully reflect their chemical complexity (Mole and Waterman, 1987; Mueller-Harvey and McAllan, 1992; Van Soest, 1994; Mueller-Harvey, 1999).

Chemical properties

The tannins of different plant species have different physical and chemical properties (Mangan, 1988), and therefore they have very diverse biological properties (Zucker, 1983).

The high affinity of tannins for proteins lies in the formers' great number of phenolic groups. These provide many points at which bonding may occur with the carbonyl groups of peptides (McLeod, 1974; Hagerman and Butler, 1991; Leinmüller *et al.*, 1991; Hagerman *et al.,* 1992).

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 (McLeod, 1974; Zucker, 1983; Mangan, 1988; Hagerman and Butler, 1991). With respect to tannins, the factors promoting the formation of complexes include their relatively high molecular weight and their great structural flexibility (McLeod, 1974; Hagerman and Butler, 1991; Mueller-Harvey and McAllan, 1992). The proteins that show the most affinity for tannins are relatively large and hydrophobic, have an open, flexible structure and are rich in proline (Kumar and Singh, 1984; Hagerman and Butler, 1991; Hagerman *et al.*, 1992; Mueller-Harvey and McAllan, 1992).

The complexes formed between tannins and proteins or other compounds are generally unstable. 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 nu-

cleophilic 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 distribution of tannins in nature

The tannins are widely distributed throughout the plant kingdom, especially among trees, shrubs and herbaceous leguminous plants (McLeod, 1974; Perevolotsky, 1994). The range of species over which these compounds are found has grown as detection techniques have improved.

Despite the general idea that tannins are only found in plant species from tropical or arid/semi-arid areas (Giner-Chavez, 1996; Balogun *et al.*, 1998), they are found in those of other regions. For example, many species with appreciable amounts of tannin grow in environments with an Atlantic or Mediterranean influence. One might highlight 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*), Rosaceae (*Crataegus*, *Rosa*, *Rubus*) and Salicaceae (*Salix*) among others (Terril *et al.*, 1992; Jackson *et al.*, 1996; Barry and McNabb, 1999; Frutos *et al.*, 2002; Hervás *et al.*, 2003b).

In general, tannins are more abundant in the parts of the plant that are most «valuable» to it, e.g., new leaves and flowers (which are more likely to be eaten by herbivores) (Terril *et al.*, 1992; Van Soest, 1994; Álvarez del Pino *et al.*, 2001). Numerous reports illustrate the effects of environmental and seasonal factors as well as of phenological development. Very briefly, high temperatures, water stress, extreme light intensities and poor soil quality increase the tannin content of plants (Rhoades, 1979; Van Soest, 1994). According to Iason *et al.* (1993), 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.

Effect of tannins in ruminant nutrition

Tannins can be beneficial or detrimental to ruminants, depending on which (and how much) is consumed, the compound's structure and molecular weight, and on the physiology of the consuming species (Hagerman and Butler, 1991). It is important to remember that all the quantities mentioned in this revision should be taken with great caution since different analytical methods, and especially different standards (e.g., quebracho, tannic acid, catequin, cyanidin, delphinidin, or internal standards from the plant itself etc.) can provide very different —and therefore ambiguous— results (Giner-Chavez, 1996; Schofield *et al*., 2001).

Voluntary feed intake

Until fairly recently, most researchers believed that the consumption of tannins reduced voluntary feed intake. However, we now have much more information, and are able to make more refined statements about tannins, their doses and their effects on the species that consume them, etc.

It would appear that the consumption of plant species with high CT contents (generally > 50 g kg^{-1} of dry matter, DM) significantly reduces voluntary feed intake, while medium or low consumption (< 50 g kg^{-1} DM) seems not to affect it (Barry and Duncan, 1984; Barry and Manley, 1984; Waghorn *et al*., 1994a).

Barry and McNabb (1999) indicated that the negative effect of consuming *Lotus pedunculatus* (CT content > 50 g kg^{-1} DM) on voluntary feed intake in grazing sheep is not seen when the same animals consume *L. corniculatus* (which has only 34-44 g CT kg^{-1} DM). Hervás *et al*. (2003c) dosed sheep intraruminally with different quantities of quebracho CT extract (0, 0.5, 1.5 and 3.0 g kg^{-1} liveweight, LW, per day, equivalent to 0, 28, 83 and 166 g kg^{-1} DM consumed in the diet) and found that all the animals ate everything offered them, except for those that had received the highest dose (eq. 166 g kg^{-1} DM). In these sheep, voluntary feed intake was practically nil after 5 or 6 days.

The effect of HT has also been reported variable, mainly dependent on the quantity consumed. McSweeney *et al*. (1988) observed no significant reduction in voluntary feed intake in sheep whose diet included *Terminalia oblongata,* a species low in HT (34 g kg^{-1} DM). However, a reduction did occur when the same animals were fed *Clidemia hirta,* a shrub with a high HT content (> 50 g kg^{-1} DM). Frutos *et al*. (2004) found no reduction in voluntary feed intake among sheep provided a feed containing soya bean meal treated with HT (20.8 g HT kg^{-1} DM of feed). However, in an experiment with sheep fed 8 g of tannic acid per kg liveweight, voluntary feed intake fell drastically after 24 h (from 18 to 2.5 g DM kg^{-1} LW) (Zhu *et al*., 1992).

Three main mechanisms have been suggested to explain the negative effects of high tannin concentrations on voluntary feed intake: a reduction in feed palatability, the slowing of digestion, and the development of conditioned aversions.

A reduction in palatability could be caused through a reaction between the tannins and the salivary mucoproteins, or through a direct reaction with the taste receptors, provoking an astringent sensation (McLeod, 1974). Many herbivore species base their diet on plant species with high levels of tannins, and proteins rich in proline are found in their saliva (Robbins *et al*., 1987; Austin *et al*., 1989; McArthur *et al*., 1995; Foley *et al*., 1999). These have a high capacity to bind with tannins (Kumar and Singh, 1984; Hagerman and Butler, 1991). The tannin-proline-rich protein complexes formed, unlike other protein-tannin complexes, are stable across the whole pH range of the digestive tract. This might cancel their negative effect on palatability, and therefore on feed intake, and improve the digestion of tannin-rich feeds (Robbins *et al*., 1987; Austin *et al*., 1989; McArthur *et al*., 1995; Narjisse *et al*., 1995).

It would seem very likely that, throughout evolution, herbivores would have developed different adaptive mechanisms for the consumption of tannin-rich plants (Robbins *et al*., 1987; Leinmüller *et al*., 1991; Hagerman *et al*., 1992; Narjisse *et al*., 1995). Browsing animals secrete proline-rich proteins constantly, while sheep, for example, only produce them when consuming plants rich in tannins (Robbins *et al*., 1987; Austin *et al*., 1989). In cattle, however, no increase in the production of such proteins has been observed in response to tannin ingestion, although other proteins with high affinity for these polyphenols have been found in their saliva (Makkar and Becker, 1998).

With respect to the second possible mechanism, Narjisse *et al*. (1995) infused tannins directly into the rumen to determine whether factors independent of palatability were responsible for the reduction in voluntary feed intake. Slowing the digestion of dry matter in the rumen impairs the emptying of the digestive tract, generating signals that the animal is 'full' and

providing feedback to the nerve centres involved in intake control. In agreement with some authors, this could influence voluntary feed intake more than a reduction of palatability (Waghorn *et al.,* 1994a).

The third mechanism is based on the identification of negative post-prandial consequences following the ingestion of tannins, and the subsequent development of conditioned aversions (Waghorn, 1996). The microorganisms of the rumen play a fundamental role in the nutrition of ruminants. It would therefore seem probable that the post-prandial consequences of ingesting tannin-rich feeds are mediated by factors relating to microbial fermentation (see below).

Digestibility of the diet

Numerous articles exist on the ability of tannins to reduce the digestibility of the diet. Tannins mainly exert this effect on proteins, but they also affect other feed components to different degrees (Kumar and Singh, 1984). Their main effect 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 (McLeod, 1974; Mangan, 1988; Hagerman *et al.,* 1992; Mueller-Harvey and McAllan, 1992).

Evidently, the modifications of the digestibility caused by tannin ingestion are mainly associated with changes in the ruminal fermentation pattern, along with changes in intestinal digestibility. The two subsections below discuss these effects, but it is worth mentioning here the repeatedly published conclusion that «one of the most clear pieces of evidence showing that tannins reduce the digestibility of feed is the increase in faecal excretion of nitrogen with increased dietary tannin content». Numerous examples of this argument exist, such as that in which sheep fed only carob (*Ceratonia siliqua*) leaves (tannin concentration = 50 g kg^{-1} DM) lose liveweight and excrete more protein in their faeces than they consume (Silanikove *et al.,* 1994). It is important to realise, however, that the consequences of tannin ingestion include increased secretion of endogenous proteins such as salivary glycoproteins, mucus and digestive enzymes, and increased desquamation of intestinal cells (Mehansho *et al.*, 1987; Waghorn, 1996). This increase in faecal nitro-

gen could therefore be an increase in metabolic faecal nitrogen, i.e., nitrogen of endogenous origin that does not represent a fall in the amount of protein absorbed from feed.

Ruminal fermentation

The reduction of ruminal protein degradation may be the most significant and well-known effect of tannins (e.g., McLeod, 1974; Mangan, 1988; Hagerman *et al.,* 1992; Mueller-Harvey and McAllan, 1992). The affinity of tannins for these molecules is very great, and the pH of the ruminal medium favours the formation of tannin-protein complexes. In general, this reduction in protein degradation is associated with a lower production of ammonia nitrogen and a greater non-ammonia nitrogen flow to the duodenum (Barry and Manley, 1984; Waghorn *et al.*, 1994b; Waghorn, 1996).

The effect of tannins on protein degradation is basically a reduction in the immediately degradable fraction, and a reduction of the fractional rate of degradation (Aharoni *et al.*, 1998; Frutos *et al.*, 2000; Hervás *et al.*, 2000).

Though tannins mainly exert their effects on proteins, they also have effects on carbohydrates, particularly hemicellulose, cellulose, starch and pectins (Barry and Manley, 1984; Chiquette *et al.*, 1988; Leinmüller *et al.*, 1991; Schofield *et al.*, 2001). For a long time, the effect of tannins on the degradation of fibre was seen as a secondary anti-nutritional effect. However, several studies have shown that fibre degradation in the rumen can be drastically reduced in animals that consume tannin-rich feeds (e.g., Barry and McNabb, 1999; McSweeney *et al.*, 2001; Hervás *et al.*, 2003a).

The mechanisms by which tannins reduce ruminal degradation of different dietary components are not entirely clear. Among the most accepted are substrate privation (Scalbert, 1991; McAllister *et al.*, 1994b; McMahon *et al.*, 2000), enzyme inhibition (Barry and Manley, 1984; Bae *et al.*, 1993; Jones *et al.*, 1994) and direct action on rumen microorganisms (Leinmüller *et al.*, 1991; Scalbert, 1991). With respect to the first of these, several authors have reported that tannins prevent —or at least interfere with— the attachment of rumen microorganisms to plant cell walls, and it is well known that such attachment is essential for degradation to occur (Chiquette *et al.*, 1988; McAllister *et al.*, 1994a). Further, the formation of complexes with proteins and carbohydrates renders these nutrients

inaccessible to microorganisms (Mangan, 1988; Mueller-Harvey and McAllan, 1992). Tannins are also chelating agents, and this could reduce the availability of certain metallic ions necessary for the metabolism of rumen microorganisms (Scalbert, 1991).

With respect to enzyme inhibition, tannins can react with microbial (both bacterial and fungal) enzymes, inhibiting their activity (Makkar *et al.*, 1988; Mueller-Harvey and McAllan, 1992; McAllister *et al.*, 1994b; McSweeney *et al.*, 2001). Several authors (Leinmüller *et al.*, 1991; O'Donovan and Brooker, 2001) indicate that tannins alter the activity of bacterial proteolytic, cellulolytic and other enzymes, but it is important to point out that the binding of tannins to enzymes – whether bacterial or endogenous – does not necessarily imply their inhibition (Makkar *et al.*, 1988). With respect to fibrolytic enzymes, CT more easily inhibit the activity of hemicellulases than cellulases (Waghorn, 1996). This is possibly due to the fact that the latter are associated with bacterial cell walls while the hemicellulases are extracellular and therefore more sensitive (Van Soest, 1994). This would explain why the majority of researchers report a greater reduction in the degradability of hemicellulose in the presence of tannins (Barry and Manley, 1984; Waghorn *et al.*, 1994a; Hervás *et al.*, 2003a). However, this can vary depending on the tannin in question (McAllister *et al.*, 1994a).

Finally, tannins might have a direct effect on ruminal microorganisms, e.g., by altering the permeability of their membranes (Leinmüller *et al.*, 1991; Scalbert, 1991). Nonetheless, some rumen microorganisms can tolerate tannins (Nelson *et al.*, 1998; O'Donovan and Brooker, 2001). The degree of tolerance is specific to the microorganism in question, explaining the different susceptibility of bacterial strains. It also depends on the tannin, and the differences between HT and CT in this respect are notorious. Though few tolerant rumen microorganisms have been described, it is very likely that their true diversity is much greater than currently known (McSweeney *et al.*, 2001).

Several species of the ruminal microbiota respond to the presence of tannins by changing their morphology (Bae *et al.*, 1993; Jones *et al.*, 1994; McAllister *et al.*, 1994a). Chiquette *et al.* (1988) observed a thick glycocalyx on ruminal bacterial walls in response to high levels of CT from *L. corniculatus*, which did not occur when the concentration of the same compounds was lower. This phenomenon is similar to the secretion of glycoproteins in the saliva (Scalbert, 1991) for neutralising the action of tannins.

With respect to the inhibition of enzyme activity, apart from different sensitivities at different concentrations (Jones *et al.*, 1994), O'Donovan and Brooker (2001) indicate that proteolytic bacteria, which are initially sensitive to tannins, can, after a short period of adaptation, respond by modifying their metabolism. This is only one example of how ruminal bacteria with proteolytic and cellulolytic activity can continue to function when tannin levels are not too high (Jones *et al.*, 1994).

Several microbial enzymes have been identified which can metabolise tannins (O'Donovan and Broker, 2001), especially HT. The degradation of CT via the cleavage of carbon-carbon bonds has not been demonstrated even *in vitro*, and it seems very unlikely that such an event could occur in the anaerobic environment of the rumen (McSweeney *et al.*, 2001). Among the bacteria able to use HT are *Streptococcus caprinus* (*S. gallolyticus*), which produces pyrogallol (a product of tannic acid degradation) when gallate decarboxylase activity increases (O'Donovan and Brooker, 2001).

Intestinal digestibility

Although it has been suggested that CT may increase intestinal digestibility of organic matter (McSweeney *et al.*, 1988), many authors report that tannins exert a negative effect on nutrient absorption from the small intestine (Driedger and Halfield, 1972; Silanikove *et al.*, 1994 and 2001; McNabb *et al.*, 1998), which could be due to the persistence in the intestine of tannin-protein complexes which failed to dissociate in the abomasum, to the formation of tannin-digestive enzyme complexes or new tannin-dietary protein complexes, or to changes in intestinal absorption due to the interaction of tannins with intestinal mucosa.

Though tannin-protein complexes dissociate at pH < 3.5 (the pH of the abomasum), McNabb *et al.* (1998) indicate that the pH at the beginning of the intestine (≈5.5) might allow tannin-protein complexes to reform, and therefore impede digestion. Kumar and Singh (1984) suggest that tannins might also be able to inhibit the digestive enzymes because of their ability to bind to them to form insoluble complexes (or soluble but inactive complexes). Silanikove *et al.* (1994) mention the inhibition of the activity of some digestive enzymes (trypsin and amylase) because of CT. Nevertheless, the idea that the tannin-caused reduction in the

intestinal digestibility of proteins is owed to their ability to inhibit the digestive enzymes is questionable. After their dissociation from proteins in the abomasum, the tannins might once again bind to dietary proteins in the intestine (Mole and Waterman, 1987; Blytt *et al.*, 1988). Mehansho *et al.* (1987) uphold that tannins have the opportunity to form complexes with a wide variety of dietary proteins long before coming into contact with the digestive enzymes.

Changes in the permeability of the intestinal wall caused by the reaction between tannins and the membrane proteins of the intestinal mucosal cells, and the resulting reduction in intestinal absorption, may also lie behind reduced intestinal digestibility (McLeod, 1974; Silanikove *et al.*, 2001).

However, it is important to bear in mind that the majority of studies affirming that tannins negatively affect intestinal digestibility have been performed *in vitro*. Several authors indicate that these tests do not take into account factors such as the presence of bile salts (Blytt *et al.*, 1988), which could act as detergents and prevent the binding of tannins to digestive enzymes.

In any event, a moderate reduction of the intestinal digestibility would not necessarily prevent from a greater nitrogen retention. Ruminants can benefit from dietary CT when the increases in protein flow from the rumen exceed the reduction in the absorption of amino acids from the intestine (Waghorn, 1996).

Effect of tannins on animal production

Since tannin consumption can affect voluntary feed intake and its digestive utilisation, there are likely to be consequences on the productivity of the animals that consume them.

In general, high tannin intakes have a clear negative effect on productivity; nutrient availability is reduced because of the complexes formed between tannins and several types of macromolecules, voluntary feed intake and digestibility are reduced, the digestive physiology of the animal may be impaired, and there may be mucosal perturbations, etc.

Barry (1985) observed a significant reduction in the gain of liveweight in lambs fed *L. pedunculatus* (which has a high CT content; 76-90 g kg^{-1} DM). However, some authors indicate that the continued ingestion of tannins might lead to a partial adaptation to these compounds, with the disappearance —or at least the attenuation— of their harmful effects (Barry, 1985; Silanikove, 2000).

In any event, the importance of the quantity consumed is receiving more and more recognition since tannins in several types of forage can have beneficial effects in moderate amounts (Aerts *et al.*, 1999; Barry and McNabb, 1999; Min *et al.*, 2003; Waghorn and McNabb, 2003). The intake of under 50 g CT kg^{-1} DM (10 - 40 g kg^{-1} DM) improves the digestive utilisation of feed by ruminants, mainly because of a reduction in ruminal protein degradation and, as a consequence, a greater availability of (mainly essential) amino acids for absorption in the small intestine (Schwab, 1995; Barry and McNabb, 1999; Min *et al.*, 2003).

Barry and Manley (1984), by comparing with predicted values for non-tannin-containing diets, report positive effects on the retention of nitrogen in lambs fed *L. corniculatus* (< 50 g CT kg^{-1} DM). Similarly, Driedger and Hartfield (1972) report increases in nitrogen retention in lambs fed soya bean meal treated with HT.

Wang *et al.* (1994 and 1996a) observed that the grazing of *L. corniculatus* (34 g CT kg^{-1} DM) reduced feed intake but increased the gain in liveweight, carcass weight, and dressing proportion, compared with a group supplemented with polyethylene glycol (PEG), which binds to tannins and inactivates them. Montossi *et al.* (1996) published similar results. These authors observed a 23% improvement in liveweight gain when lambs grazed *Holcus lanatus* (4.2 g CT kg^{-1} DM).

With respect to milk production, Wang *et al.* (1996b) report an increase of 21% during mid and late lactation in sheep fed *L. corniculatus* (44.5 g CT kg^{-1} DM) *vs.* sheep dosed with PEG. They also report significant increases in the efficiency of milk production, increased protein and lactose production, and a decrease in the fat content of the milk. This increased concentration of protein might be explained by the greater availability of intestinal amino acids, especially of methionine and lysine, which are thought to limit milk production. The greater concentration of lactose can be explained by greater glucose supply; most lactose synthesis in the mammary gland relies directly on blood glucose, and in ruminants gluconeogenesis mainly involves propionic acid and amino acids. Thus, a greater availability of amino acids would contribute to greater synthesis of glucose. The latter authors showed that the increase in lactose concentration occurred without modification of the molar proportions

of volatile fatty acids - which confirmed this was due to the action of tannins. The reduction in the concentration of fat was attributed to a simple dilution effect as the concentrations of lactose and protein increased.

Several authors (Wang *et al*., 1994; Min *et al*., 1999 and 2003), using PEG for comparisons, indicate that the grazing of *L. corniculatus* (30-35 g CT kg^{-1} DM) increases wool production by 10-14%, which they attribute to a greater absorption of essential amino acids (especially sulphur amino acids) in the intestine. Montossi *et al*. (1996) also observed that grazing on *H. lanatus*, with its much lower CT concentration (4.2 g CT kg^{-1} DM), increased wool production by 10%.

With respect to the effect of CT on reproductive efficiency, Min *et al*. (1999) observed that sheep grazing *L. corniculatus* (17 g CT kg^{-1} DM) increased their production of lambs by 25% due to increased rates of ovulation and a subsequently increased lambing percentage, possibly related to protein utilisation.

Treatments to avoid the negative effects of tannins

Numerous papers offer information on how to reduce or even avoid the negative effects of tannins in certain feeds. This information is especially useful in impoverished areas with few plant resources and where the majority of available species are rich in tannins. For example, wetting the feed with water or alkaline solutions can separate these phenolic compounds from the most nutritive parts, thus reducing their activity. Treatments with wood ash, as a good and cheap source of alkali, or urea have also been commonly used. «Chopping the leaves and then storage» has been found as an easy practical application by farmers. In this process, tannin inactivation seem to be due to oxidation of tannins and polymerisation to higher inert polymers (Makkar, 2001).

More recent (and more studied) alternatives include treatment with polyethylene glycol (PEG), poly-vinyl-polypyrrolidone, calcium hydroxide, etc. (Murdiati *et al*., 1990; Makkar *et al*., 1995; Ben Salem *et al*., 1999 and 2000; Makkar, 2001). Some of these tannin-binding agents, for instance the PEG, have been reported to prevent the formation of tannin-protein complexes and even to be able to displace protein from a pre-formed tannin-protein complex. In line with this, PEG has been widely used in research to study the effects of tannins on ruminal fermentation (see e.g., Makkar *et al*., 1995; Getachew *et al*., 2000; Hervás *et al*., 2001). This compound is also utilized in husbandry (see e.g., Ben Salem *et al*., 1999, 2000; Gilboa *et al*., 2000) to treat tannin-rich feedstuffs, that provide large amounts of fodder in arid and semi-arid regions, in order to improve their digestive utilization by ruminants.

Practical use of tannins

Treatments to protect dietary protein from ruminal degradation

One of the basic goals of protein nutrition in ruminants is to optimise dietary protein use in order to maximise animal growth and milk production per unit of protein consumed (Schwab, 1995). As mentioned several times, tannins could protect dietary proteins from ruminal degradation.

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 provoked a greater reduction in the degradability of soya bean meal than commercial tannic acid, but in general the results obtained were very variable and depended on many factors.

Hervás *et al*. (2000) and 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.

One of the drawbacks of using tannins as additives to protect protein rich feeds is the possibility of their degradation by rumen microorganisms. If this were to happen, 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. These results

were expected; most authors believe that the proanthocyanidins, or CT, cannot be degraded in the rumen (McSweeney *et al*., 2001).

Two problems arise with respect to the use of HT. Though *in vitro* tests have shown their efficacy as additives for reducing dietary protein digestion in the rumen, their use runs up against the apparently general belief that these compounds are toxic to animals (Spier *et al*., 1987; Zhu *et al*., 1992). However, the consumption of small quantities of HT in soya bean meal (20.8 g kg^{-1} 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). However, more research is needed in this area since HT are easily hydrolysed and their effect could easily be nullified by the rumen microbiota.

Although somewhat obvious, it is worth pointing out that proper management of natural tannin-containing resources (e.g., selective grazing or supplementing the diet with the right kind of shrubs) could provide the same beneficial effects with respect to protein degradation.

Bloat prevention

It is well documented that bloat occurs when grazing ruminants consume large quantities of leguminous plants (e.g., alfalfa or clover). The gases produced in the rumen during fermentation cannot be released in the normal way since they are trapped in a persistent foam caused by the rapid release of soluble proteins during chewing and ruminal degradation. However, when these animals graze on leguminous plants containing CT (for example *Onobrychis viciifolia*) this does not occur (Mangan, 1988; Aerts *et al*., 1999; Barry and McNabb, 1999; McMahon *et al*., 2000). The substitution of a small amount (approximately 10%) of ingested alfalfa DM by *Onobrychis viciifolia* provides unquestionable benefits in the prevention of bloat (McMahon *et al*., 1999 and 2000). The problem of this strategy is, however, the low persistence of this plant species in mixed cropping with alfalfa. The possibility of genetically modifying alfalfa to produce CT has been suggested on several occasions and has been the subject of several studies (see the review by McMahon *et al*., 2000). However, the difficulty of the molecular techniques required has made progress slow.

Very recently, the preliminary results of a study on the ruminal fermentation of transgenic alfalfa were published. The *Lc* gene of maize was introduced into alfalfa to induce the synthesis of CT (Wang *et al*., 2003). The modification of the alfalfa decreased its initial rate of degradation in the rumen, but not the extent of degradation. This offers an interesting way to help to prevent bloat.

Control of internal parasites

The tannins of numerous plant species help to control certain internal parasites of animals, for example the nematode *Trichostrongylus colubriformis* (Butter *et al*., 2000). It is speculated that the positive effect on the host animal might be associated with a direct negative effect on the parasites themselves plus an indirect effect in the form of increased availability and digestive utilization of protein (Niezen *et al*., 1995; Min and Hart, 2003). The literature has several examples of this in sheep and goats grazing *L. corniculatus* or *Hedysarum coronarium* (Robertson *et al*., 1995) and after having ingested quebracho CT (Butter *et al*., 2000) etc.

Toxicity

Although a review of the toxicity of these compounds is beyond the scope of this review, a few points should be made. Several reports exist on the toxic effect of HT, but there are very few indeed on CT. It has long been known that the toxicity of tannins is related to their molecular size (McLeod, 1974) since tannins with high molecular weights cannot be absorbed. This might explain the high toxicity of low molecular weight tannins from *Quercus* species in rats, while the larger, high molecular weight tannins of *Lespedeza cuneata* seem not to be toxic.

Several articles describe intoxications by HT (Spier *et al*., 1987; Zhu *et al*., 1992, Plumlee *et al*., 1998). These are mainly characterised by anorexia, depression, ruminal atony, hepatic and renal failure, ulcers along the digestive tract, and severe gastroenteritis. The intensity of the lesions appears to depend on the dose and structure of the tannin consumed (Zhu *et al*., 1992, Plumlee *et al*., 1998). However, the majority of these works are descriptions of intoxications that occurred naturally, and it is therefore hard to know what tannin —and how much of it— was involved.

With respect to CT, very high intakes appear to be necessary for animals to suffer serious intoxication (Hervás *et al*., 2003c).

Finally, it is important to point out that intoxications caused by tannins usually only occur when animals are obliged to eat tannin-rich feed because of the lack of alternative plant resources (Spier *et al*., 1987).

References

AERTS R.J., BARRY T.N., McNABB W.C., 1999. Polyphenols and agriculture: beneficial effects of proanthocyanidins in forages. Agr Ecosyst Environ 75, 1-12.

AHARONI Y., GILBOA N., SILANIKOVE N., 1998. Models of suppressive effect of tannins. Analysis of the suppressive effect of tannins on ruminal degradation by compartmental models. Anim Feed Sci Tech 71, 251-267.

ÁLVAREZ DEL PINO M.C., FRUTOS P., HERVÁS G., GÓMEZ A., GIRÁLDEZ F.J., MANTECÓN, A.R., 2001. Efecto del contenido de taninos en la degradación ruminal *in vitro* de varios órganos de especies arbustivas. ITEA, Prod Anim 22, 355-357.

AUSTIN P.J., SUCHAR L.A., ROBBINS C.T., HAGERMAN A.E., 1989. Tannins-binding proteins in saliva of deer and their absence in saliva of sheep and cattle. J Chem Ecol 15, 1335-1347.

BAE H.D., McALLISTER T.A., YANKE J., CHENG K.J., MUIR A.D., 1993. Effects of condensed tannins on endoglucanase activity and filter paper digestion by *Fibrobacter succinogenes* S85. Appl Environ Microb 59, 2132-2138.

BALOGUN R.O., JONES R.J., HOLMES J.H.G., 1998. Digestibility of some tropical browse species varying in tannin content. Anim Feed Sci Tech 76, 77-88.

BARRY T.N., 1985. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 3. Rates of body and wool growth. Brit J Nutr 54, 211-217.

BARRY T.N., DUNCAN S.J., 1984. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 1. Voluntary intake. Brit J Nutr 51, 485-491.

BARRY T.N., MANLEY T.R., 1984. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 2. Quantitative digestion of carbohydrates and proteins. Brit J Nutr 51, 493-504.

BARRY T.N., McNABB W.C., 1999. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. Brit J Nutr 81, 263-272.

BARRY T.N., MANLEY T.R., DUNCAN S.J., 1986. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 4. Sites of carbohydrate and protein digestion as influence by dietary reactive tannin concentration. Brit J Nutr 55, 123-137.

BEN SALEM H., NEFZAOUI A., BEN SALEM L., TISSERAND J.L., 1999. Different means of administering polyethylene glycol to sheep: effect on the nutritive value of *Acacia cyanophylla* Lindl. foliage. Anim Sci 68, 809-818.

BEN SALEM H., NEFZAOUI A., BEN SALEM L., TISSERAND J.L., 2000. Deactivation of condensed tannins an *Acacia cyanophylla* Lindl. foliage by polyethylene glycol in feed blocks. Effect on feed intake, diet digestibility, nitrogen balance, microbial synthesis and growth by sheep. Livest Prod Sci 64, 51-60.

BLYTT H.J., GUSCAR T.K., BUTLER L.G., 1988. Antinutritional effects and ecological significance of dietary condensed tannins may not be due to binding and inhibiting digestive enzymes. J Chem Ecol 14, 1455-1465.

BUTTER N.L., DAWSON J.M., WAKELIN D., BUTTERY P.J., 2000. Effect of dietary tannin and protein concentration on nematode infection (*Trichostrongylus colubriformis*) in lambs. J Agr Sci 134, 89-99.

CHIQUETTE J., CHENG K.J., COSTERTON J.W., MILLIGAN L.P., 1988. Effect of tannins on the digestibility of two isosynthetic strains of birdsfoot trefoil (*Lotus corniculatus* L.) using *in vitro* and *in sacco* techniques. Can J Anim Sci 68, 751-760.

DRIEDGER A., HATFIELD E., 1972. Influence of tannins on the nutritive value of soybean meal for ruminants. J Anim Sci 34, 465-468.

FOLEY W.J., IASON G.R., McARTHUR C., 1999. Role of secondary metabolites in the nutritional ecology of mammalian herbivores: how far have we come in 25 years? In: Nutritional ecology of herbivores (Jung H.J.G. and Fahey G.C.Jr., eds.). American Society of Animal Science, Illinois (USA), pp. 130-209.

FRUTOS P., HERVÁS G., GIRÁLDEZ F.J., FERNÁNDEZ M., MANTECÓN A.R., 2000. Digestive utilization of quebracho-treated soya bean meal in sheep. J Agr Sci 134, 101-108.

FRUTOS P., HERVÁS G., RAMOS G., GIRÁLDEZ F.J., MANTECÓN A.R., 2002. Condensed tannin content of several shrub species from a mountain area in northern Spain, and its relationship to various indicators of nutritive value. Anim Feed Sci Tech 95, 215-226.

FRUTOS P., RASO M., HERVÁS G., MANTECÓN A.R., PÉREZ V., GIRÁLDEZ F.J. 2004. Is there any detrimental effect when a chestnut hydrolyzable tannins extract is included in the diet of finishing lambs? Anim Res 56, 127-136.

GETACHEW G., MAKKAR H.P.S., BECKER K., 2000. Effect of polyethylene glycol on *in vitro* degradability and microbial protein synthesis from tannin-rich browse and herbaceous legumes. Brit J Nutr 84, 73-83.

GILBOA N., PEREVOLOTSKY A., LANDAU S., NITSAN Z., SILANIKOVE N., 2000. Increasing productivity in goats grazing Mediterranean woodland and scrubland by supplementation of polyethylene glycol. Small Ruminant Res 38, 183-190.

GINER-CHÁVEZ B.I., 1996. Condensed tannins in tropical forages. Doctoral Thesis. Cornell University. Ithaca, NY, USA.

HAGERMAN A.E., BUTLER L.G., 1991. Tannins and lignins. In: Herbivores: their interactions with secondary plant metabolites, Vol I: The chemical participants, (Rosenthal G.A. and Berenbaum M.R., eds.), Academic Press, NY (USA), pp. 355-388.

HAGERMAN A.E., ROBBINS C.T., WEERASURIYA Y., WILSON T.C., McARTHUR C., 1992. Tannin chemistry in relation to digestion. J Range Manage 45, 57-62.

HARBORNE J.B., 1999. An overview of antinutritional factors in higher plants. In: Secondary plants products. Antinutritional and beneficial actions in animal feeding (Caygill J.C. and Mueller-Harvey I., eds.). Nottingham Univ Press, UK, pp. 7-16.

HERVÁS G., ÁLVAREZ DEL PINO M.C., GIRÁLDEZ F.J., MANTECÓN A.R., FRUTOS P., 2001. Effect of two types of tannin, in the presence or absence of PEG, on in vitro rumen fermentation in goats. Proc of the 9th Seminar of the FAO-CIHEAM Sub-Network on sheep and goat nutrition, nutrition and feeding strategies of sheep and goats under harsh climates, Hammamet (Tunisia), 8-10 November. Institut National de la Recherche Agronomique de Tunisie, INRAT (Tunisia), p.57.

HERVÁS G., FRUTOS P., GIRÁLDEZ F.J., MANTECÓN A.R., ÁLVAREZ DEL PINO M.C. 2003a. Effect of different doses of quebracho tannins extract on rumen fermentation in ewes. Anim Feed Sci Tech 109, 65-78.

HERVÁS G., FRUTOS P., SERRANO E., MANTECÓN A.R., GIRÁLDEZ F.J., 2000. Effect of tannic acid on rumen degradation and intestinal digestion of treated soya bean meals in sheep. J Agr Sci 135, 305-310.

HERVÁS G., MANDALUNIZ N., OREGUI L.M., MANTECÓN A.R., FRUTOS P., 2003b. Evolución anual del contenido de taninos del brezo (*Erica vagans*) y relación con otros parámetros indicativos de su valor nutritivo. ITEA, Prod Anim 99A, 69-84.

HERVÁS G., PÉREZ V., GIRÁLDEZ F.J., MANTECÓN A.R., ALMAR M.M., FRUTOS P., 2003c. Intoxication of sheep with quebracho tannin extract. J Comp Pathol 129, 44-54.

IASON, G.R., HARTLEY, S.E., DUNCAN, A.J. 1993. Chemical composition of *Calluna vulgaris* (Ericaceae): do responses to fertilizer vary with phenological stage? Biochem Syst Ecol 21, 315-321

JACKSON F.S., McNABB W.C., BARRY T.N., FOO Y.L., PETERS J.S., 1996. The condensed tannin content of a range of subtropical and temperate forages and the reactivity of condensed tannin with ribulose-1,5-*bis*-phosphate carboxylase (Rubisco) protein. J Sci Food Agr 72, 483-492.

JONES G.A., McALLISTER T.A., MUIR A.D., CHENG K.J., 1994. Effects of sainfoin (*Onobrychis viciifolia* Scop.) condensed tannins on growth and proteolysis by four strains of ruminal bacteria. Appl Environ Microb 60, 1374-1378.

KUMAR R., SINGH M., 1984. Tannins: their adverse role in ruminant nutrition. J Agr Food Chem 32, 447-453.

LEINMÜLLER E., STEINGASS H., MENKE K.H., 1991. Tannins in ruminant feedstuffs. Biannual Collection of Recent German Contributions Concerning Development through Animal Research 33, 9-62.

MAKKAR H.P.S., 2001. Chemical, protein precipitation and bioassays for tannins, effect and fate of tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Proc of the 9th Seminar of the FAO-CIHEAM

sub-network on sheep and goat nutrition, nutrition and feeding strategies of sheep and goats under harsh climates, Hammamet (Tunisia), 8-10 November. Institut National de la Recherche Agronomique de Tunisie, INRAT (Tunisia), 60 pp.

MAKKAR H.P.S., BECKER K., 1998. Adaptation of cattle to tannins: role of protein-rich proteins in oak-fed cattle. Anim Sci 67, 277-281.

MAKKAR H.P.S., BLÜMMEL M., BECKER K., 1995. Formation of complexes between polyvinyl pyrrolidones or polyethylene glycol and tannins, and their implication in gas production and true digestibility in *in vitro* techniques. Brit J Nutr 73, 897-913.

MAKKAR H.P.S., SINGH B., DAWRA R.K., 1988. Effect of tannin-rich of oak (*Quercus incana*) on various microbial enzyme activities of the bovine rumen. Brit J Nutr 60, 287-296.

MANGAN J.L., 1988. Nutritional effects of tannins in animal feeds. Nutr Res Rev 1, 209-231.

McALLISTER T.A., BAE H.D., JONES G.A., CHENG K.J., 1994a. Microbial attachment and feed digestion in the rumen. J Anim Sci 72, 3004-3018.

McALLISTER T.A., BAE H.D., YANKE L.J., CHENG K.J., MUIR A., 1994b. Effect of condensed tannins from birdsfoot trefoil on the endoglucanase activity and the digestion of cellulose filter paper by ruminal fungi. Can J Microbiol 40, 298-305.

McARTHUR C., SANSON G.D., BEAL A.M., 1995. Salivary proline-rich proteins in mammals: roles in oral homeostasis and counteracting dietary tannin. J Chem Ecol 21, 663-691.

McLEOD M.N., 1974. Plant tannins - Their role in forage quality. Nutr Abst Rev 44, 803-812.

McMAHON L.R., MAJAK W., McALLISTER T.A., HALL J.W., JONES G.A., POPP J.D., CHENG K.J., 1999. Effect of sainfoin on *in vitro* digestion of fresh alfalfa and bloat in steers. Can J Anim Sci 79, 203-212.

McMAHON L.R., McALLISTER T.A., BERG B.P., MAJAK W., ACHARYA S.N., POPP J.D., COULMAN B.E., WANG Y., CHENG K.J., 2000. A review of the effects of forage condensed tannins on ruminal fermentation and bloat in grazing cattle. Can J Plant Sci 80, 469-485.

McNABB W.C., PETERS J.S., FOO L.Y., WAGHORN G.C., JACKSON S.J., 1998. Effect of condensed tannins prepared from several forages on the *in vitro* precipitation of ribulose-1,5-*bis*phosphate carboxilase (rubisco) protein and its digestion by trypsin (EC 2.4.21.4) and chymotrypsin (EC 2.4.21.1). J Sci Food Agric 77, 201-212.

McSWEENEY C.S., KENNEDY P.M., JOHN A., 1988. Effect of ingestion of hydrolysable tannins in *Terminalia oblongata* on digestion in sheep fed *Stylosanthes hamata*. Aust J Agr Res 39, 235-244.

McSWEENEY C.S., PALMER B., McNEILL D.M., KRAUSE D.O., 2001. Microbial interactions with tannins: nutritional consequences for ruminants. Anim Feed Sci Tech 91, 83-93.

MEHANSHO H., BUTLER L.G., CARLSON D.M., 1987. Dietary tannins and salivary proline-rich proteins:

interactions, induction and defence mechanisms. Annu Rev Nutr 7, 423-440.

MIN B.R., BARRY T.N., ATTWOOD G.T., McNABB W.C., 2003. The effect of condensed tannins on the nutrition of ruminants fed fresh temperate forages: a review. Anim Feed Sci Tech 106, 3-19.

MIN B.R., HART S.P., 2003. Tannins for suppression of internal parasites. J Anim Sci 81, E. Suppl. 2, E102-E109.

MIN B.R., McNABB W.C., BARRY T.N., KEMP P.D., WAGHORN G.C., McDONALD M.F., 1999. The effect of condensed tannins in *Lotus corniculatus* upon reproductive efficiency and wool production in sheep during late summer and autumn. J Agr Sci 132, 323-334.

MOLE S., WATERMAN P.G., 1987. Tannic acid and proteolytic enzymes: enzyme inhibition or substrate deprivation? Phytochemistry 26, 99-102.

MONTOSSI F.M., HODGSON J., MORRIS S.T., RISSO D.F., 1996. Effects of the condensed tannins on animal performance in lambs grazing Yorkshire fog (*Holcus lanatus*) and annual ryegrass (*Lolium multiflorum*) dominant swards. Proc N Z Soc Anim Prod 56, 118-121.

MUELLER-HARVEY I., 1999. Tannins: their nature and biological significance. In: Secondary plants products. Antinutritional and beneficial actions in animal feeding (Caygill J.C. and Mueller-Harvey I., eds.). Nottingham Univ Press (UK), pp. 17-70.

MUELLER-HARVEY I., McALLAN A.B., 1992. Tannins. Their biochemistry and nutritional properties. In: Advances in plant cell biochemistry and biotechnology, Vol. 1 (Morrison I.M., ed.). JAI Press Ltd., London (UK), pp. 151-217.

MURDIATI T.B., McSWEENEY C.S., CAMPBELL R.S.F., STOLTZ D.S., 1990. Prevention of hydrolysable tannin toxicity in goats fed *Clidemia hirta* by calcium hydroxide supplementation. J Appl Toxicol 10, 325-331.

NARJISSE H., ELHONSALI M.A., OLSEN J.D., 1995. Effects of oak (*Quercus ilex*) tannins on digestion and nitrogen balance in sheep and goats. Small Ruminant Res 18, 201-206.

NELSON K.E., THONNEY M.L., WOOLSTON T.K., ZINDER S.H., PELL A.N., 1998. Phenotypic and phylogenetic characterization of ruminal tannin-tolerant bacteria. Appl Environ Microb 64, 3824-3830.

NIEZEN J.H., WAGHORN T.S., CHARLESTON W.A.G., WAGHORN G.C., 1995. Growth and gastrointestinal nematode parasitism in lambs grazing either lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*) with contains condensed tannins. J Agr Sci 125, 281-289.

O'DONOVAN L., BROOKER J.D., 2001. Effect of hydrolysable and condensed tannins on growth, morphology and metabolism of *Streptococcus gallolyticus* (*S. caprinus*) and *Streptococcus bovis*. Microbiol 147, 1025-1033.

PACE V., SETTINERI D., CATILLO G., 1993. Influenza di trattamenti con tannini sulla digestibilità in vitro della farina di soia. Zootecnia i Nutricion Animali 19, 73-79.

PEREVOLOTSKY A., 1994. Tannins in Mediterranean woodlands species: lack of response to browsing and thinning. Oikos 71, 333-340.

PLUMLEE K.H., JOHNSON B., GALEY F.D., 1998. Disease in cattle dosed orally with oak or tannic acid. In: Toxic plants and other natural toxicants, (Garland T. and Barr A.C., eds.), CAB International, Wallingford, UK, pp. 549-553.

RHOADES D.F., 1979. Evolution of plant chemical defence against herbivores. In: Herbivores: their interactions with secondary plant metabolites (Rosenthal G.A. and Janzen D.H., eds.). Academic Press, NY, USA, pp. 3-54.

ROBBINS C.T., HANLEY T.A., HAGERMAN A.E., HJELJORD O., BAKER D.L., SCHARTZ C.C., MAUTZ W.W., 1987. Role of tannins in defending plants against ruminants: reduction in protein availability. Ecology 68, 98-107.

ROBERTSON H.A., NIEZEN J.H., WAGHORN G.C., CHARLESTON W.A.G., JINLONG M., 1995. The effect of six herbages on liveweight gain, wool growth and faecal egg count of parasitised ewe lambs. Proc N Z Soc Anim Prod 55, 199-201.

SCALBERT A., 1991. Antimicrobial properties of tannins. Phytochemistry 30, 3875-3883.

SCHOFIELD P., MBUGUA D.M., PELL A.N., 2001. Analysis of condensed tannins: a review. Anim Feed Sci Tech 91, 21-40.

SCHWAB C.G., 1995. Protected proteins and amino acids for ruminants. In: Biotechnology in animal feeds and animal feeding (Wallace R.J. and Chesson A., eds.). V.C.H. Press, Weinhein (Germany), pp. 115-141.

SILANIKOVE N., 2000. The physiological basis of adaptation in goats to harsh environments. Small Ruminant Res 35, 181-193.

SILANIKOVE N., NITSAN Z., PEREVOLOTSKY A., 1994. Effect of a daily supplementation of polyethylene glycol on intake and digestion of tannin-containing leaves (*Ceratonia siliqua*) by sheep. J Agr Food Chem 42, 2844-2847.

SILANIKOVE N., PEREVOLOTSKY A., PROVENZA, F.D. 2001. Use of tannin-binding chemicals to assay for tannins and their negative postingestive effects in ruminants. Anim Feed Sci Technol 91, 69-81.

SPIER S.J., SMITH B.P., SEAWRIGHT A.A., NORMAN B.B., OSTROWSKI S.R., OLIVER M.N., 1987. Oak toxicosis in cattle in northern California: clinical and pathologic findings. J Am Vet Med Assoc 191, 958-964.

TERRIL T.H., ROWAN A.M., DOUGLAS G.B., BARRY T.N., 1992. Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. J Sci Food Agric 58, 321-329.

VAN SOEST P.J. (ed.), 1994. Nutritional ecology of the ruminant, 2nd ed. Cornell Univ Press. Ithaca, NY, USA. 476 p.

WAGHORN G., 1996. Condensed tannins and nutrient absorption from the small intestine. Proc of the 1996 Canadian Society of Animal Science Annual Meeting, Lethbridge, Canada (Rode L.M., ed.). pp. 175-194.

WAGHORN G.C., McNABB W.C., 2003. Consequences of plant phenolic compounds for productivity and health of ruminants. Proc Nutr Soc 62, 383-392.

WAGHORN G.C., SHELTON I.D., McNABB W.C., 1994a. Effects of condensed tannins in *Lotus pedunculatus* on its

nutritive value for sheep. 1. Non-nitrogenous aspects. J Agr Sci 123, 99-107.

WAGHORN G.C., SHELTON I.D., McNABB W.C., McCUTCHEON S.N., 1994b. Effects of condensed tannins in *Lotus pedunculatus* on its nutritive value for sheep. 2. Nitrogenous aspects. J Agr Sci 123, 109-119.

WANG Y., DOUGLAS G.B., WAGHORN G.C., BARRY T.N., FOOTE A.G., 1996a. Effect of condensed tannins in *Lotus corniculatus* upon lactation performance in ewes. J Agr Sci 126, 353-362.

WANG Y., FRUTOS P., GRUBER M.Y., RAY H., McALLISTER T.A., 2003. Comparison of *in vitro* digestibility of parental and anthocyanin-containing *Lc*-transgenic alfalfa. Proc of the 2003 Canadian Society of Animal Science Annual Meeting. University of Saskatchewan, Saskatoon (Canada). 10-13 June, p.27.

WANG Y., WAGHORN G.C., DOUGLAS G.B., BARRY T.N., WILSON G.F., 1994. The effects of the condensed tannin in *Lotus corniculatus* upon nutrient metabolism and upon body and wool growth in grazing sheep. Proc N Z Soc Anim Prod 54, 219-222.

WANG Y., WAGHORN G.C., McNABB W.C., BARRY T.N., HEDLEY M.J., SHELTON I.D., 1996b. Effects of condensed tannins in *Lotus corniculatus* upon the digestion of methionine and cysteine in the small intestine of sheep. J Agr Sci 127, 413-421.

ZHU J., FILIPPICH L.J., ALSALAMI M.T., 1992. Tannic acid intoxication in sheep and mice. Res Vet Sci 53, 280-292.

ZUCKER W.V., 1983. Tannins: does structure determine function? An ecological perspective. Am Nat 121, 335-365.