

# Tannins in forage plants and their role in animal husbandry and environmental sustainability: a review

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## Abstract

The literature about tannins, polyphenolic secondary metabolites of plants, with both beneficial and adverse function according to their concentration and chemical structure, is vast and often conflicting. Tannins in forages have often been described as antinutritional factors, but this review aims to update information on beneficial effects on animals and the environment. Although research on the relation between tannins and animal production and health, for example, dry-matter intake, digestibility, rumen fermentation and diseases, has mainly focused on condensed tannins, this review also discusses potential benefits from the use of hydrolysable tannins as a feed additive. Attention is given to the use of tannins in the mitigation of methane emissions from ruminants in forage-based feeding systems and as a natural and ecologically friendly resource for improvement of nutrient utilization and environmental sustainability in meat and dairy farming.

**Keywords:** tannins, legumes, fodder crops, ruminant nutrition, methane emissions

## Introduction

Tannins are a heterogeneous group of polyphenolic polymers of varying molecular weight and complexity. Plant phenolics and tannins are synthesized to meet ordinary physiological demands but also as a response to biotic and abiotic stresses (Alonso-Amelot *et al.*, 2007). Tannins can be classified into two subgroups, condensed tannins (CT; also called proanthocyanidins)

and hydrolysable tannins (HT; Hagerman *et al.*, 1992). Both classes have been described as having adverse or beneficial effects, depending on tannin concentration and structure, plant source and animal species, and physiological state and diet. Condensed tannins are expected to bind proteins with a high affinity, providing protection from degradation by rumen microbes (Waghorn, 2008). Forage containing CT has been reported to minimize the detrimental effects associated with a heavy load of internal parasites (Niezen *et al.*, 1995). Consumption of plants containing CT may have a direct effect on gastrointestinal nematode abundance and also exert an indirect effect through improved protein supply (Niezen *et al.*, 1995; Barry *et al.*, 2001), with positive consequences for animal performance (Athanasiadou *et al.*, 2000). Defined concentrations of forage CT [20–50 g kg<sup>-1</sup> of dry matter (DM)] can be used to increase the efficiency of protein digestion and animal productivity in forage-fed ruminants and has been used to develop more ecologically sustainable systems of disease control under grazing (Barry and McNabb, 1999). Condensed tannins are also thought to function as protective factors in plants by prevention of predation by herbivores (Barbehenn and Constabel, 2011) and invasion by pathogenic bacteria, fungi and insects (Barry, 1989).

Plant species from multiple regions have been described as having anthelmintic activity, and many are currently used as part of traditional veterinary practices (Bullitta *et al.*, 2007). However, few of these have been tested experimentally. Also, the possibility of selected natural plant products as growth promoters for livestock could provide cheaper and safer alternatives to synthetic compounds. Building on many years of tannin research, considerable effort is now being directed towards exploiting the beneficial effects of tannins in livestock feeding. Thus, this review is a timely update on the current use of tannins in feed-stuffs and their effects in animals. Even if there are different classifications of tannins (complex tannins,

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gallotannins, ellagitannins, phlorotannins; Nonaka, 1989; Khanbabaee and Van Ree, 2001; Salminen and Karonen, 2011), focus is given to the CT and HT of important forage species and their effects on animal productivity and health.

### Tannin quantification and localization in plants

The various methods available for the analysis of tannins and related references are listed (Table 1), but technical details are not described in this study. Instead, we discuss the broader methodological principles of the different approaches. For example, plant tannin content is often difficult to compare between laboratories because of the different methods or standards used during quantification. The lack of suitable standards is a problem for colorimetric procedures such as vanillin–HCl and butanol–HCl. According to Schofield *et al.* (2001), a suitable method for CT content determinations is the butanol–HCl assay, but the standard must be extracted and purified from the same plant materials under study, preferably the same accession (Waghorn, 2008). The most commonly used standards for CT determination are quebracho, cyanidin and delphinidin in the butanol–HCl assay, and catechin in the vanillin–HCl assay. The lack of appropriate commercially available standards and CT heterogeneity across plant species makes the choice of standard in CT quantification problematic. Because CTs from various plant sources differ chemically and in the degree of polymerization, the use of external reference standards might be inappropriate. In NMR analyses of commercial quebracho tannins and tannins previously purified from mimosa, the presence of interfering compounds (probably polysaccharides linked to the CT polymers) was shown to contribute

to incorrect quantification of CT in the plant materials (Tava, 2005). On this basis, delphinidin was suggested as a standard, as this substance was the most abundant constituent from the acid-catalysed cleavage of CT from almost all the tested plant species, sulla (*Hedysarum coronarium*), sainfoin (*Onobrychis viciifolia*), edible lotus (*Lotus edulis*), grey birdsfoot trefoil (*Lotus cytisoides*) and clustered birdsfoot trefoil (*Lotus ornithopodioides*). Molle *et al.* (2003, 2009) have expressed the concentration of CT in *H. coronarium* relative to leucocyanidin, while Häring *et al.* (2007) have used big trefoil (*Lotus uliginosus*) tannin as the standard for the quantification of tannins in *O. viciifolia*, *Lotus corniculatus* and *Cichorium intybus* (chicory). In our experience, isolation of the standard from the species under study is technically difficult and requires specialist laboratory equipment. This approach is also problematic when making comparisons between species. This problem can be addressed by the use of a commercially available standard such as delphinidin, which is also useful when comparing data between laboratories.

Although colorimetric methods are useful for reporting the presence (CT is not found in all plants) and quantity of CT, they do not provide detail of the finer chemical structure, information that is critical when discussing tannins in the context of biological activity.

A modified butanol–HCl method has been developed for the determination of extractable, protein-bound and fibre-bound CT in forages, and this approach is recommended for CT quantification in freeze-dried forage samples and samples with low CT content (Terrill *et al.*, 1992a). Moreover, the distribution of free, protein-bound and fibre-bound tannins can vary depending on factors such as total CT content, age of the plant and climatic and nutritive conditions (Frutos *et al.*, 2002). Changes in the relative

**Table 1** Available assays for the determination of total polyphenols and tannins in plants.

Assay name	Assay type	Use for	References
Folin–Ciocalteu	Colorimetric	Polyphenols	Folin and Ciocalteu (1927)
Prussian blue	Colorimetric	Polyphenols	Price and Butler (1977)
Butanol–HCl	Colorimetric	Condensed tannins	Porter <i>et al.</i> (1986)
Vanillin–HCl	Colorimetric	Condensed tannins	Broadhurst and Jones (1978)
Rhodanine	Colorimetric	Gallotannins (hydrolysable tannins)	FAO/IAEA (2000)
Wilson and Hagerman	Colorimetric	Ellagitannins (hydrolysable tannins)	Wilson and Hagerman (1990)
Ytterbium precipitation	Gravimetric	Polyphenols	Reed <i>et al.</i> (1985)
Protein precipitation	Precipitation	Tannins	Hagerman (1987)
HPLC	Chromatographic	Polymers up to 7–8 units	Cheynier <i>et al.</i> (1999), Lazarus <i>et al.</i> (1999), Salminen <i>et al.</i> (2001, 2004)
NMR	Spectroscopy	Structure of tannins	Okuda <i>et al.</i> (1989)

Modified from Schofield *et al.* (2001).

proportions of bound and unbound CT fractions in the rumen and in the post-rumen tract are complex (Perez-Maldonado and Norton, 1996). Thus, it is unlikely that the nutritional effects of CT can be predicted from the distribution of such fractions in the plant itself (Frutos *et al.*, 2002). The modified butanol-HCl method for the determination of total CT (extractable and bound forms) should allow greater application, particularly for dried forages or processed feeds, but should be followed by further *in vitro* or *in vivo* evaluation, such as animal feeding trials (Wolfe *et al.*, 2008). Given the current methodological challenges, all tannin quantification studies should be treated with caution. For example, it is not unusual for different analytical methods and standards (e.g. quebracho, internal standard, tannic acid, catechin, delphinidin, cyanidin, etc.) to produce drastically different results. The butanol-HCl method provides greater sensitivity than the vanillin-HCl reaction and is considered most suitable for preliminary screening of CT. The rodanine assay (specific for gallotannins) and Wilson-Hagerman (1990) assay (specific for ellagitannins) are most suitable for the screening of HT. From our experience, colorimetric methods are rapid and simple to perform, making them suitable for preliminary screening. However, subsequent chromatographic and spectroscopic assays are required for the characterization of the finer chemical structure.

Tannins are widely distributed in nutritionally important forage and grain legumes, cereals and shrubs and can be found in the cell walls and vacuoles of stem, bark, leaf, flower and seed cells of many dicotyledonous plants (Barry, 1989). The content of CT is depleted in the vacuoles of mature leaves entering senescence, suggesting that plants may recycle these compounds (Lees *et al.*, 1995). Vacuolar CT does not appear to have an active role in plant metabolism; however, disruption of the vacuole during ingestion by herbivores enables the CT to interact with plant, microbial and animal proteins (McMahon *et al.*, 2000).

Tissue-specific localization has been observed in a number of forage species. The localization of CT in different plant parts of forage legumes is summarized in Table 2. The content of tannins varies with the growth stage of species (Piluzza *et al.*, 2000; Häring *et al.*, 2007; Molle *et al.*, 2009; Theodoridou *et al.*, 2010, 2011; Guglielmelli *et al.*, 2011) and in the different organs of plants (Piluzza *et al.*, 2000; Molle *et al.*, 2003; Theodoridou *et al.*, 2010). The dynamics of CT concentration in growing plants is important for the nutrition and health of ruminants. However, for Mediterranean forage species, there are few reports detailing the changes in CT content in different tissues and across different phenological stages.

There have been cases of conflicting reports on the presence of CT in certain plants. For example, CT has been reported in forage chicory (Terrill *et al.*, 1992a; Molan *et al.*, 2000; Häring *et al.*, 2007, 2008), but was not detected by other studies on the same species (Bullitta and Piluzza, 2005; Tava, 2005; Sulas *et al.*, 2007). Similarly, modest CT content was reported for *Astragalus cicer* (cicer milkvetch; Williams *et al.*, 2011), although previous studies have found little or no tannin in the same species (Davis, 1973; Broderick and Albrecht, 1997).

Woody species are indispensable sources of animal feed in southern Europe, particularly in areas with dry to semi-dry Mediterranean climates (Papanastasis *et al.*, 2008) where animals consume significant amounts of shrubs to meet their nutrient requirements. Young shoots of the shrub legumes *Cytisus purgans* (andorra broom), *Cytisus scoparius* (scotch broom), *Genista florida* and *Genista occidentalis* show low levels of CT ( $<6.5 \text{ g kg}^{-1} \text{ DM}$ ), generally considered unlikely to significantly affect nutrient digestion in ruminants (Frutos *et al.*, 2002). However, high CT content has been detected in the shoots and flowers of *C. scoparius* and *G. florida* samples collected in June. It is therefore accepted that inflorescences can be heavily defended against herbivory by the accumulation of plant secondary compounds such as tannins (Roberts and Olson, 1999).

The HT and CT concentrations in the leaves of *Chamaecytisus palmensis* (tagasaste) have been found to vary between accessions. Moreover, it was shown that varietal selection and harvesting management could be used to modulate tannin and alkaloid levels in this perennial leguminous forage shrub (Assefa *et al.*, 2008).

Considering that CT levels vary widely across plant species, as well as tissues and developmental stage, together with variability arising from method and standard choices, it is necessary to establish common procedures to compare results from different laboratories. Moreover, HT and CT data should not be compared as these are different compounds and their levels are determined using different analytical methods.

We recommend the creation of defined procedures for sample collection and storage (known to affect tannin extraction rate), as well as clear guidelines for standard choice. For example, the standard should be the most abundant constituent after the acid-catalysed cleavage of CT from the plant species under study.

## Beneficial effects on ruminants

The presence of CT in forage species may provide a practical means of protecting dietary forage protein

**Table 2** Condensed tannin localization in forage, grain and shrub legumes.

Species	Localization	References
<i>Astragalus cicer</i>	Whole plant	Williams <i>et al.</i> (2011)
<i>Bituminaria bituminosa</i>	Stems, leaves	Sumbele <i>et al.</i> (2012), Ventura <i>et al.</i> (2012)
<i>Hedysarum coronarium</i>	Stems, leaves, flowers, whole plant, leaf blades, petioles	Terrill <i>et al.</i> (1992a), Piluzza <i>et al.</i> (2000), Molle <i>et al.</i> (2009), Piluzza and Bullitta (2010)
<i>Lotus corniculatus</i> var. <i>japonicus</i>	Stems, petioles, flowers	Morris <i>et al.</i> (1993)
<i>L. corniculatus</i>	Whole plant, roots, leaves, stems	Gebrehiwot <i>et al.</i> (2002), Häring <i>et al.</i> (2007, 2008), Acuña <i>et al.</i> (2008), Marshall <i>et al.</i> (2008)
<i>Lotus pedunculatus</i>	Whole plant	Häring <i>et al.</i> (2008)
<i>Lotus tenuis</i>	Shoots	Acuña <i>et al.</i> (2008)
<i>Lotus uliginosus</i>	Leaves, stems, shoots	Gebrehiwot <i>et al.</i> (2002), Acuña <i>et al.</i> (2008), Marshall <i>et al.</i> (2008)
<i>Medicago sativa</i>	Seed coat	Koupai-Abyazani <i>et al.</i> (1993)
<i>Onobrychis viciifolia</i>	Whole plant, leaf blades, petioles, stems, racemes, leaves, roots	Lees (1993), Lees <i>et al.</i> (1995), Borreani <i>et al.</i> (2003), Häring <i>et al.</i> (2007, 2008), Piluzza and Bullitta (2010), Azuhniwi <i>et al.</i> (2011), Guglielmelli <i>et al.</i> (2011), Theodoridou <i>et al.</i> (2011)
<i>Trifolium repens</i>	Flowers	Burggraaf <i>et al.</i> (2003)
<i>Trifolium pratense</i>	Flowers	Barry (1985)
<i>Vicia amphicarpa</i>	Seeds	Berger <i>et al.</i> (2003)
<i>Vicia faba</i>	Seed hulls, seed coat	Jansman <i>et al.</i> (1995), Duc <i>et al.</i> (1995), Merghem <i>et al.</i> (2004), Vilariño <i>et al.</i> (2009)
<i>Vicia macrocarpa</i>	Seeds	Berger <i>et al.</i> (2003)
<i>Vicia nigra</i>	Seeds	Berger <i>et al.</i> (2003)
<i>Vicia sativa</i>	Seeds	Berger <i>et al.</i> (2003)
<i>Ceratonia siliqua</i>	Fruits	Priolo <i>et al.</i> (2000)
<i>Chamaecytisus palmensis</i>	Buds, leaves, stems, bark	Edwards (2000), Assefa <i>et al.</i> (2008)
<i>Cytisus purgans</i>	Shoots	Frutos <i>et al.</i> (2002)
<i>Cytisus scoparius</i>	Shoots	Frutos <i>et al.</i> (2002)
<i>Genista florida</i>	Shoots	Frutos <i>et al.</i> (2002)
<i>Genista occidentalis</i>	Shoots	Frutos <i>et al.</i> (2002)
<i>Mimosa hostilis</i>	Leaves	Guimarães-Beelen <i>et al.</i> (2006)

from ruminal degradation, thus increasing plant protein uptake in the small intestine with implications for animal performances.

### Effect of CT on intake and protein digestibility

Moderate CT concentrations (<50 g kg<sup>-1</sup> DM) enhance forage nutritive value in grazing ruminants by reducing protein degradation by rumen bacteria and increasing protein degradation in the intestine, without depressing rumen fibre digestion or voluntary intake (Aerts *et al.*, 1999; Min *et al.*, 2003). When present in large quantities (>50 g kg<sup>-1</sup>), CT can adversely affect nutrition of grazing herbivores by reducing intake and inhibiting protein digestibility (by inhibiting digestive enzymes or by direct systemic toxicity), leading to a reduction in feed intake, adverse effects on rumen fermentation and significantly depressed digestibility of almost all nutrients (Barry, 1989).

The affinity of tannins for proteins and amino acids can be variable. For example, proline-rich proteins are tightly bound; in fact, proline-rich salivary mucoproteins are considered to be an evolutionary adaptation of some herbivores to a tanniferous diet. Condensed tannins may also complex with carbohydrates (such as those present in glycoproteins), but usually with a lower affinity than for proteins (Barry, 1989). Condensed tannins may bind to more than one site on a protein and change the conformation of the substrate protein, thus protecting the protein in the ruminant digestive tract by inducing steric interference and preventing enzymatic hydrolysis. Normally, CT can react by hydrogen bonding with plant proteins in the near-neutral pH range to form CT-protein complexes. These complexes are stable and insoluble in the rumen (pH 3.5–7.0), but dissociate and release protein at pH <3.5 (Jones and Mangan, 1977). Such complexes are presumed to dissociate during passage through the more

acidic environment of the abomasum or in the alkaline environment of the small intestine. Condensed tannins present in forage plants are able to protect dietary proteins against degradation in the rumen and increase amino acid availability for absorption in the small intestine of ruminants (Wang *et al.*, 1996a). The widely held assumption that protein will be released from the CT-protein complex in the acid environment of the abomasums and subsequently be digested in the small intestine is, therefore, overly simplistic (McNabb *et al.*, 1998). It is unclear what happens to the tannins when they are released post-rationally and whether they subsequently bind to endogenous animal proteins. For example, quebracho tannins do not generate ruminal escape protein, but reduce protein absorption dramatically in the small intestine (Komolong *et al.*, 2001). This effect may have been caused by quebracho tannins binding to endogenous protein, rather than feed protein. It has been shown that 25 g kg<sup>-1</sup> CT in carob (*Ceratonia siliqua*) can be harmful, but 80 g kg<sup>-1</sup> in sainfoin is beneficial (Mueller-Harvey, 2006). The assumption that CT dietary concentrations <50 g kg<sup>-1</sup> are beneficial can be attributed to recommendations originating from feeding trials with *Lotus* species and may not be applicable to other feeds.

*In vitro* studies have also shown that the affinity of CT for protein can vary depending on the plant source (Osborne and McNeill, 2001; Schofield *et al.*, 2001). This raises the possibility that tannin-protein complexes (TPCs) containing particularly astringent CT may not fully dissociate post-rationally. Thus, some CTs may not improve the supply of digestible protein. Andrabi *et al.* (2005) describe an attempt to develop an *in vivo* assessment of TPC dissociation post-rationally, in which CTs were purified from *Acacia aneura* (mulga, Australian native forage tree) and *Leucaena pallida* (guaja, Mexican native forage tree) and dosed into sheep. The results show no detectable effect of CT type on the *in vivo* digestibility of plant proteins in sheep. Protein digestibility was uniformly high, indicative of complete dissociation of the TPC.

Condensed tannins from *L. corniculatus* were found to reduce the true digestibility of plant methionine and cysteine in the small intestine of sheep. The proportion digested in the proximal part of the small intestine was reduced, increasing the proportion digested in the final third of the small intestine (Wang *et al.*, 1996a).

Condensed tannins in birdsfoot trefoil and sulla can be used to increase the absorption of essential amino acids from the small intestine, whereas CTs in big trefoil and sainfoin produce no net increase, probably due to differences in CT structure and reactivity (Min *et al.*, 2003). It has been reported that when *H. coronarium* is grazed by sheep at flowering, dietary CT can

exert negative effects on DM and protein digestibility (Molle *et al.*, 2009). It has been suggested that this decrease in DM is a result of combined protein digestibility with lower fibre degradation (Barry and McNabb, 1999).

### Effect of tannins on rumen fermentation

The effect of tannins on ruminal bacteria depends upon the bacterial species as well as the type and source of tannin (Patra and Saxena, 2011). A list of studies about the effects of different plant species containing CT on different rumen microorganisms is presented in Table 3.

Min *et al.* (2002) report that when sheep diet was changed from perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) at pasture (not containing CT) to *L. corniculatus* (32 g CT kg<sup>-1</sup> DM), populations of the proteolytic rumen bacteria (*Clostridium proteoclasticum*, *Eubacterium* sp., *Streptococcus bovis* and *Butyrivibrio fibrisolvens*) decreased. Condensed tannins extracted from *Lotus pedunculatus* were more effective at reducing the degradation of Rubisco (fraction-1 leaf protein) by rumen microorganisms than CT extracted from *L. corniculatus* (Molan *et al.*, 2001), possibly due to differences in CT chemical structure.

Condensed tannins (100–400 µg mL<sup>-1</sup>) from *L. corniculatus* have been shown to inhibit extracellular endoglucanase activity of *Fibrobacter succinogenes in vitro* (Bae *et al.*, 1993). Extracts of CT from *O. viciifolia* (<600 µg mL<sup>-1</sup>) reduced growth and proteolytic activity of *B. fibrisolvens* and *S. bovis* (Jones *et al.*, 1994). Moreover, CT may reduce bloat by inhibiting growth of microorganisms that produce a dextran slime (e.g. *S. bovis*) that increases the viscosity of ruminal fluid causing frothy feedlot bloat (Cheng *et al.*, 1976). An absence of tannin-resistant rumen bacteria can reduce protein degradation in the rumen, thus increasing the quantity of protein digested in the small intestine and reducing bloat, all potentially increasing animal production.

Microorganism tolerance for tannins depends upon the species and tannin properties (Frutos *et al.*, 2004). An *in vitro* study on the effects of CT from *L. corniculatus* on the growth and proteolytic activity of eleven ruminal isolates reported that some strains of rumen microorganisms, such as *C. proteoclasticum* and *Ruminococcus albus*, show a transient increase in growth at low (50–100 µg mL<sup>-1</sup>) but not at high (>200 µg mL<sup>-1</sup>) concentrations of CT, while nine ruminal strains were found to be sensitive to CT at low concentrations (Min *et al.*, 2005a). *In vivo* growth assays have demonstrated that *Streptococcus gallolyticus* 2-2 is tolerant to tannic acid and CT from calliandra (*Calliandra calothyrsus*) and acacia (*Acacia angustissima*).



**Table 3** Effects of plant species containing condensed tannins on rumen microorganisms.

Plant species	Experiments/ animals	Rumen microorganisms	Effects on rumen		References
			microorganisms	Controls	
<i>Acacia angustissima</i>	<i>In vivo</i> /sheep	<i>Streptococcus bovis</i>	S1	PEG, <i>Medicago sativa</i>	Krause <i>et al.</i> (2005)
<i>A. angustissima</i>	<i>In vivo</i> /sheep	<i>Streptococcus gallolyticus</i>	R1	PEG, <i>M. sativa</i>	Krause <i>et al.</i> (2005)
<i>Calliandra calothyrsus</i>	<i>In vivo</i> /sheep	<i>S. bovis</i>	S1	PEG, <i>M. sativa</i>	Krause <i>et al.</i> (2005)
<i>C. calothyrsus</i>	<i>In vivo</i> /sheep	<i>S. gallolyticus</i>	R1	PEG, <i>M. sativa</i>	Krause <i>et al.</i> (2005)
<i>Lotus corniculatus</i>	<i>In vivo</i> /sheep	<i>Clostridium proteoclasticum</i> , <i>Eubacterium</i> sp., <i>S. bovis</i> , <i>Butyrivibrio fibrisolvens</i>	S2	PEG, <i>Lolium perenne</i> , <i>Trifolium repens</i>	Min <i>et al.</i> (2002)
<i>L. corniculatus</i>	<i>In vitro</i>	<i>Fibrobacter succinogenes</i>	S1	Medium without CT	Bae <i>et al.</i> (1993)
<i>L. corniculatus</i>	<i>In vitro</i>	<i>C. proteoclasticum</i> , <i>Ruminococcus albus</i>	S1	PEG	Min <i>et al.</i> (2005a)
<i>Onobrychis viciifolia</i>	<i>In vitro</i>	<i>B. fibrisolvens</i> , <i>S. bovis</i>	S1	Medium without CT	Jones <i>et al.</i> (1994)
<i>O. viciifolia</i>	<i>In vitro</i>	<i>Ruminobacter amylophilus</i> , <i>Prevotella ruminicola</i>	R2	Medium without CT	Jones <i>et al.</i> (1994)

S1, growth inhibition; S2, population decrease; R1, tannin tolerant; R2, tannin resistant.

In particular, *S. gallolyticus* 2-2 is tolerant to a range of tannins (including both HT and CT) with an apparent higher resistance exhibited with the smaller molecular weight HT (tannic acid). Exopolysaccharide has been hypothesized to be produced by *S. gallolyticus* in response to tannins and may form a protective layer around its cells. Tannin-resistant *S. gallolyticus* is more abundant than *S. bovis* in the rumen in the presence of tannins, indicating that the dominance of tannin-resistant organisms may have significant nutritional consequences for the animal (Krause *et al.*, 2005). This is particularly noticeable when ruminants naturally graze forages with high tannin content and are able to digest plant species that contain tannin concentrations higher than 6%.

### Effect of CT on animal production

The nutritional effect of CT in the ruminant diet depends upon the concentration, structure and molecular weight of the tannin (Barry, 1989; Wang *et al.*, 1994), and the type of CT present in forages may greatly influence voluntary feed intake and animal performance. Diets containing as little as 26 g kg<sup>-1</sup> from carob pulp resulted in a decrease in growth rate of lambs, from 140 g to <50 g per day (Priolo *et al.*, 2000). In contrast, the CTs in *H. coronarium* (72 g kg<sup>-1</sup> of DM) did not affect daily gain of lambs (Douglas *et al.*, 1999). According to Lascano *et al.* (2003), the predominance of prodelphinidins in the extractable tannin fractions from different accessions of *Calliandra calothyrsus* was associated with greater feed intake and digestion, compared with samples where procyanidins

were predominant. Undoubtedly, different plant species contain complex mixtures of tannins, and not all tannins have the same feeding effects.

Condensed tannins increased liveweight gains in sheep consuming *H. coronarium* compared with those consuming lucerne or perennial pasture (Terrill *et al.*, 1992b; Niezen *et al.*, 1995). This was also true in sheep consuming *L. corniculatus* when compared with those receiving polyethylene glycol (PEG), which deactivates CT (Douglas *et al.*, 1995; Decandia *et al.*, 2000). Other effects of CT include reduced carcass fatness in sheep consuming *L. pedunculatus*, in comparison with those consuming the non-CT-containing lucerne. Also, increased milk production and wool growth were observed in sheep grazing on *L. corniculatus*, when compared with sheep treated with PEG (Wang *et al.*, 1996b; Min *et al.*, 1998). Min *et al.* (2003) report that the action of CT in lactating ewes grazing *L. corniculatus* had no effect upon milk secretion in early lactation, but increased secretion rates of whole milk, lactose and protein (by 21, 12 and 14%, respectively) during mid- and late lactation. The same authors also report that the beneficial effects of CT in *L. corniculatus* for wool production occur in the range of 22–38 g kg<sup>-1</sup> DM. Wang *et al.* (1996c) found that the action of CT from *L. corniculatus* (35 g kg<sup>-1</sup> DM) increased wool growth in lambs and milk production in lactating ewes without affecting voluntary feed intake, milk protein or lactose or reducing fat percentage.

Vasta *et al.* (2008) reported that goats grazing on CT-rich grass and Mediterranean shrubs or bushland (mainly *Mirtus communis*, *Pistacia lentiscus*, *Quercus ilex*, *Arbutus unedo*) had a higher conjugated linoleic acid

(CLA) and trans-vaccenic acid content in milk fat, compared with animals grazing only grass. However, compounds other than CT could be involved in CLA production when Mediterranean shrubs are offered to dairy sheep and goats. Priolo *et al.* (2005) found that when fresh sulla was offered to lambs as the sole diet, meat CLA and trans-vaccenic acid content were not affected by the supplementation of PEG, suggesting that the amount of CT in the sulla diet ( $18 \text{ g kg}^{-1} \text{ DM}$ ) was probably not sufficient to affect the activity of ruminal microorganisms responsible for the biohydrogenation of linoleic and linolenic acids. Vasta *et al.* (2008) review the quality of meat and milk from sheep and goats offered alternative feed, as a replacement for concentrates. Among alternative feed resources, tannin-containing feeds result in meat of a lighter colour and tend to increase milk yield and protein content. Luciano *et al.* (2009) found that the inclusion of quebracho tannins in the concentrate offered to sheep improved the colour stability of fresh lamb meat during extended refrigerated storage; this was attributed to the positive effects of dietary tannins on haem pigment concentration and metmyoglobin formation during the storage period. However, the mechanism by which dietary tannins can affect the myoglobin concentration and its redox state is unclear.

The study of the nutritional effects of tannins is complicated because of their great structural diversity, and this difficulty has led to considerable confusion in the literature when determining their benefits and/or toxicity (Mueller-Harvey, 2006). Tannins are compounds that need to be studied individually because their origin, chemical structure and biological properties are greatly diverse, and this approach is recommended for a better understanding of contrasting results obtained from CT of different plant species.

### Condensed tannins effect on animal diseases

Grazing ruminants are subject to a number of diseases caused by rumen gas formation (bloating) and internal parasite infection. Bloat is caused by very high solubility of forage proteins that produce stable foam in the rumen that traps rumen gases (Pinchak *et al.*, 2005). Therefore, altering the rate of proteolysis, biofilm formation and gas production in the rumen may be central for bloat mitigation in animals at pasture.

The presence of trace amounts of CT in most of the common grasses and legumes grazed in temperate agriculture ( $1\text{--}2 \text{ g kg}^{-1} \text{ DM}$ ) is considered too low to affect protein solubility and degradation in the rumen (Min *et al.*, 1999). The minimum plant CT concentration needed to make forages bloat-safe has been proposed to be  $5 \text{ g kg}^{-1} \text{ DM}$  or greater (Li *et al.*, 1996). However, high CT concentrations ( $>55 \text{ g kg}^{-1} \text{ DM}$ )

generally reduce forage intake and digestibility and depress rates of body and wool growth in grazing ruminants. Commercially available quebracho CT extract has been shown to increase animal production and reduce bloat potential (Min *et al.*, 2003, 2006), but elevated doses of quebracho can produce negative effects, impairing fibre digestion and causing toxicosis in sheep (Hervas *et al.*, 2003). The increase in anthelmintic resistance by sheep gastrointestinal nematodes suggests a need for alternative methods for the control of such parasites (McKenna *et al.*, 1995). Plants containing CT have been used to reduce soil nematode populations (Taylor and Murant, 1966), and plant CT is able to affect nematodes in the gastrointestinal tract of sheep (Niezen *et al.*, 1998). A summary of studies on the anthelmintic effect of different plant species containing CT is presented in Table 4.

Lambs grazing CT-containing forages (*H. coronarium* and *L. pedunculatus*) are more tolerant to parasite infection and display increased growth and lower gut-worm burdens than lambs grazing the non-CT-containing forage (lucerne; Niezen *et al.*, 1995). Moreover, the CTs extracted from *L. pedunculatus*, *L. corniculatus*, *H. coronarium* and *O. viciifolia* have anthelmintic-like activity against deer lungworm and gastrointestinal larvae (Molan *et al.*, 2000), suggesting these forages may be used as an alternative method for controlling internal parasites in deer. Other work has reported a reduced establishment of *Ostertagia circumcincta* when lambs were fed *L. pedunculatus* rather than ryegrass, but the mechanism for this response is unknown (Niezen *et al.*, 1998). Molan *et al.* (2002) suggest that CT may be able to break the life cycle of nematodes and thus reduce the contamination of pastures with viable eggs, thereby decreasing the need for anthelmintics as the principal method of control.

Patterns of strongyloid nematode parasites in lambs grazing *L. corniculatus* suggest a decrease in the degree of parasite control from the abomasum to the rectum. This might be due to differences in the susceptibility of nematode species to CT and changes in the CT molecule after release of protein at  $\text{pH} < 3.5$  in the abomasums, thus accounting for the reduced effect of CT on gastrointestinal nematode parasites from the abomasum to the large intestine (Ramirez-Restrepo and Barry, 2005).

Max *et al.* (2009) demonstrate that *Acacia mearnsii* tannin drenches have significant anthelmintic activity against important nematodes in sheep but not in goats, despite both hosts being infected by the same species. It has also been suggested that goats are more resistant to the detrimental effects of tannins (Narjisse *et al.*, 1995). Thus, it is possible that some of the bio-active tannins administered to goats were neutralized

**Table 4** In vivo and in vitro experiments to check anthelmintic effect of condensed tannin-containing plants on ruminant species.

Plant species	CT		Host species	Parasites	Effects on nematode populations	Controls	References
	Inhibitory concentration $\mu\text{g mL}^{-1}$ (in vitro)	Plant concentration $\text{g kg}^{-1}$ (in vivo)					
<i>Acacia meurnsii</i>	–	§	Sheep	<i>Haemonchus contortus</i> , <i>Trichostrongylus vitrinus</i> , <i>Oesophagostomum columbianum</i>	+++	Placebo drench (in water)	Max <i>et al.</i> (2009)
<i>Doryenium pentaphyllum</i>	39	–	Sheep	<i>Trichostrongylus colubriformis</i>	+++	Without CT §§	Molan <i>et al.</i> (2002)
<i>Doryenium rectum</i>	41	–	Sheep	<i>T. colubriformis</i>	+++	Without CT	Molan <i>et al.</i> (2002)
<i>Hedysarum coronarium</i>	53	–	Sheep	<i>T. colubriformis</i>	+++	Without CT	Molan <i>et al.</i> (2002)
<i>H. coronarium</i>	–	15.8	Sheep	<i>T. colubriformis</i>	=	<i>Lolium perenne</i> <i>Trifolium repens</i>	Athanasiadou <i>et al.</i> (2005)
<i>Lotus corniculatus</i>	78	–	Sheep	<i>T. colubriformis</i>	+++	Without CT	Molan <i>et al.</i> (2002)
<i>L. pedunculatus</i>	40	–	Sheep	<i>T. colubriformis</i>	+++	Without CT	Molan <i>et al.</i> (2002)
<i>L. pedunculatus</i>	–	15.9	Sheep	<i>T. colubriformis</i>	=	<i>Lolium perenne</i> <i>Trifolium repens</i>	Athanasiadou <i>et al.</i> (2005)
<i>Onobrychis viciifolia</i>	41	–	Sheep	<i>T. colubriformis</i>	+++	Without CT	Molan <i>et al.</i> (2002)
<i>O. viciifolia</i>	–	14.9	Sheep	<i>T. colubriformis</i>	=	<i>L. perenne</i> / <i>T. repens</i>	Athanasiadou <i>et al.</i> (2005)
<i>Rumex obtusifolius</i>	36	–	Sheep	<i>T. colubriformis</i>	+++	Without CT	Molan <i>et al.</i> (2002)
<i>H. coronarium</i>	–	35.1	Lambs	<i>T. colubriformis</i> , <i>Teladorsagia</i> spp.	=	<i>Medicago sativa</i>	Niezen <i>et al.</i> (2002)
<i>L. pedunculatus</i>	–	56	Lambs	<i>Ostertagia circumcincta</i> , <i>T. colubriformis</i>	+++	<i>L. perenne</i> , PEG	Niezen <i>et al.</i> (1998)
<i>O. viciifolia</i>	–	20	Lambs	<i>T. colubriformis</i>	=	Grass hay	Rios-De Alvarez <i>et al.</i> (2008)
<i>Schinopsis</i> spp.	–	50	Lambs	<i>T. colubriformis</i>	+++	Uninfected	Butter <i>et al.</i> (2000)
<i>Desmodium intortum</i>	–	n.a.	Goats	<i>H. contortus</i>	+	PEG	Debela <i>et al.</i> (2012)
<i>H. coronarium</i>	–	26	Goats	<i>T. colubriformis</i> , <i>Teladorsagia circumcincta</i> §§§, <i>Trichostrongylus vitrinus</i> , <i>Trichuris</i> spp.	=	<i>M. sativa</i>	Pomroy and Adlington (2006)
<i>Lespedeza cuneata</i>	–	64.7	Goats	<i>H. contortus</i>	+++	<i>Cynodon dactylon</i>	Terrill <i>et al.</i> (2007)
<i>L. cuneata</i>	–	36	Goats	<i>H. contortus</i> , <i>T. circumcincta</i> , <i>T. colubriformis</i> ,	+++	<i>C. dactylon</i>	Shaik <i>et al.</i> (2006)
<i>L. cuneata</i>	–	152	Goats	<i>H. contortus</i> , <i>T. colubriformis</i> , <i>Nematodius</i> spp.	+++	<i>Digitaria ischaemum</i> , <i>Festuca arundinacea</i>	Min <i>et al.</i> (2005b)
<i>L. cuneata</i>	–	46	Goats	<i>H. contortus</i>	+++	<i>Secale cereale</i> <i>D. ischaemum</i>	Min <i>et al.</i> (2004)
<i>Sesbania sesban</i>	–	n.a.	Goats	<i>H. contortus</i>	+	PEG	Debela <i>et al.</i> (2012)
<i>H. coronarium</i>	–	35	Deer	<i>Dictycaulus</i> sp.	=	<i>M. sativa</i>	Hoskin <i>et al.</i> (2000)

§, oral drench at 1 g kg<sup>-1</sup> body weight; §§, 0  $\mu\text{g mL}^{-1}$  of CT; §§§ also known as *Ostertagia circumcincta* +++; high reduction in faecal egg counts (FECs); ++, medium reduction in FEC; +, low reduction in FEC; =, no effect vs. control.



or inactivated before they could reach or act on the nematodes. In fact, sheep and cattle seem to be more sensitive to tannins than goats, possibly due to differences in tolerance and oral detection of these compounds (Lamy *et al.*, 2011). In contrast, promising results from feeding unground and ground hay from *Lespedeza cuneata* to gastrointestinal nematode-infected sheep and goats have been reported (Shaik *et al.*, 2004, 2006; Dyches *et al.*, 2006; Lange *et al.*, 2006). Goats fed *L. cuneata* hay had an 80% reduction in faecal egg count, approximately 70% fewer adults of *Haemonchus contortus* in the abomasums and fewer *H. contortus* larvae in faecal cultures, compared with those that were fed *Cynodon dactylon* hay (Shaik *et al.*, 2006). Terrill *et al.* (2007) found that pelleting *L. cuneata* hay enhanced its anthelmintic effectiveness against gastrointestinal nematode of goats and could potentially increase the utility of this forage as a natural deworming agent for small ruminants.

We conclude that despite the large number of studies addressing anthelmintic activity of CT-containing forages, the underlying mechanisms remain poorly understood. Thus, further research is required for the development of new nutritionally and ecologically sustainable control measures.

### The effect of hydrolysable tannins on grazing ruminants

Some HTs are toxic and can cause poisoning in animals if sufficiently large amounts of tannin-containing plant material are consumed (Garg *et al.*, 1992). They are apparently metabolized by the rumen microflora to phenolic compounds such as gallic acid, which is neither hepatotoxic nor nephrotoxic to animals. The decarboxylated product of gallic acid (the pyrogallol) is produced in high concentrations in the rumen of sheep and causes methaemoglobinemia in these animals (Zhu *et al.*, 1995). Ellagitannin punicalagin has been identified as the toxic principle of *Terminalia oblongata* (yellow wood), responsible for liver necrosis in cattle and sheep (Clifford and Scalbert, 2000). In non-ruminant animals (or in ruminants if the rumen is bypassed), HT such as tannic acid can be absorbed through the gastrointestinal tract, leading to kidney and liver necrosis (Zhu *et al.*, 1995). In contrast, Hagerman *et al.* (1992) report that HT should not affect protein digestibility because HTs are more easily degraded than CT. They found that dietary quebracho (CT) diminished protein digestibility in deer and sheep, whereas a diet with commercial tannic acid (HT) did not affect protein digestibility. To evaluate the effects of tannins, deer and sheep were fed either artificial diets or natural forages. The two diets were found to produce different effects, perhaps because

commercial tannic acids are of a low molecular weight and heterogeneous. Tabacco *et al.* (2006) show that low levels of chestnut HT, applied prior to ensiling lucerne, are useful for reducing proteolysis in silages and could improve protein utilization (with a slight depression of organic matter digestibility).

The preference patterns for tannic acid and quebracho have been investigated using a series of preference trials with captive roe deer (*Capreolus capreolus*). Pelleted feed differed only in the respective tannin content, and tannic acid was preferred to quebracho. Moreover, the tannic acid preference pattern was biphasic, with an initial period of high preference followed by a stable period of a moderate preference – a finding that raises concerns about the validity of short-term preference trials in general.

*In sacco* and *in vitro* trials have shown that HTs decrease ruminal degradation of soya bean meal in sheep without detrimentally affecting its intestinal digestion (Hervas *et al.*, 2000). The consumption of small amounts of a chestnut HT extract included in the diet (20.8 g kg<sup>-1</sup> DM) of finishing lambs as a feed additive was not toxic for the animals and had no detectable detrimental effects on their productive performance (Frutos *et al.*, 2004). The effect of dietary HT has also been studied in non-ruminants. In poultry feeding, Schiavone *et al.* (2008) investigated the influence of a natural extract of sweet chestnut (*Castanea sativa*) wood (Silvafeed, ENC) rich in HT. The use of ENC did not influence the digestibility, nitrogen (N) balance or carcass quality, but it had a positive influence on growth performance. The use of HT to reduce rumen protein degradability is not well studied. Poncet and Remond (2002) found that the addition of low levels of chestnut tannin (HT) did not decrease the *in situ* degradability of pea seed (*Pisum sativum*) and did not significantly affect the N digestion of sheep diet.

Plants tend to produce complex mixtures of tannins and not all tannins have the same feeding effects. The study of tannins in animal production has primarily focused on CT, and little information is available on the effects of HT in livestock production. Clearly, further research is required to achieve a better understanding of the roles and utility of these compounds in livestock feeding.

### Effects of tannins on methane emissions from ruminants

Enteric fermentation in ruminants produces about 25% of the global anthropogenic emissions of methane (CH<sub>4</sub>), and in rural communities this proportion can be as high as 50%, with rumen fermentation accounting for >90% (Abberton *et al.*, 2008). There is public and political concern regarding the need to

reduce CH<sub>4</sub> emissions from livestock, and efforts are being made to achieve this. For countries that have signed the Kyoto protocol, reduction in methane production from grazing ruminants fed forage diets is an important research area (Ramirez-Rastrepo and Barry, 2005). A range of strategies to reduce CH<sub>4</sub> emission from enteric fermentation has been taken into account (Hopkins and Del Prado, 2008), and there have been reports of lower CH<sub>4</sub> emissions by ruminants consuming forages containing low or moderate levels of CT (Woodward *et al.*, 2001, 2002; Puchala *et al.*, 2005). Methane yields from sheep and cattle fed plant species of varying CT content are shown in Table 5. Tavendale *et al.* (2005) studied the inhibitory effects of CT from the forage legumes *L. pedunculatus* and *Medicago sativa* on CH<sub>4</sub>, hydrogen, total gas and volatile fatty acid formation in the presence and absence of the tannin-binding agent polyethylene glycol (PEG). They found that the accumulated CH<sub>4</sub> volume for *M. sativa* (12.5 mL) was higher than *L. pedunculatus* (8.8 mL) and that PEG increased CH<sub>4</sub> production for *L. pedunculatus* but not for *M. sativa*. In goats fed with the CT-containing forage *L. cuneata* (sericea lespedeza), a 30% reduction in CH<sub>4</sub> loss was observed relative to those fed a mixture of crabgrass (*Digitaria ischaemum*) and 'Kentucky 31' tall fescue (*Festuca arundinacea*) (Puchala *et al.*, 2005).

Direct ruminal intervention represents one possible approach for the control of ruminant CH<sub>4</sub> emissions (Williams *et al.*, 2010). Methanogens are a distinct group of organisms that form a normal component of

the rumen microbial ecosystem (Tavendale *et al.*, 2005). Hydrogen (H<sub>2</sub>) and CO<sub>2</sub> are the principal substrates used by rumen methanogens to produce CH<sub>4</sub>; therefore, compounds that directly inhibit the activity of methanogens are likely to reduce or eliminate CH<sub>4</sub> production (Baker, 1999). The action of CT against methanogenesis can be attributed to indirect effects such as reducing the H<sub>2</sub> production (and presumably reduced forage digestibility) and to direct inhibitory effects on methanogens (Tavendale *et al.*, 2005). Bhatta *et al.* (2009) found that tannins suppress methanogenesis by directly reducing methanogenic populations in the rumen or indirectly by reducing the protozoal population (thereby reducing symbiotically associated methanogens). In addition, they found that tannin sources containing both HT and CT were more potent in suppressing methanogenesis than those containing only HT.

*Methanobrevibacter ruminantium* is a predominant rumen methanogen in grazing ruminants. Tavendale *et al.* (2005) found inhibitory effects of polymeric CT fractions from *L. pedunculatus* on the growth of *M. ruminantium* in broth culture. The effect of CT fractions on pure cultures of methanogens may be greater than the effect of CT-containing plants in mixed rumen fluid. In another study, it was found that quebracho-supplemented ruminal fluid incubated with minced wheat forage produced less gas and CH<sub>4</sub> *in vitro* (Min *et al.*, 2006).

Guglielmelli *et al.* (2011) found that sainfoin forage, at the stage between early and late flowering,

**Table 5** Methane emissions from ruminants fed with condensed tannin- or non-condensed tannin-containing forages.

Plant species	CT (g kg <sup>-1</sup> )	Experiments/ animals	Value	Methane emissions		References
				PEG control	Unit*	
<i>Cichorium intybus</i>	0.0	<i>In vivo</i> /sheep	16.2	n.a.	(g kg <sup>-1</sup> DMI)	Waghorn <i>et al.</i> (2002)
<i>Hedysarum coronarium</i>	35.0	<i>In vivo</i> /sheep	17.5	n.a.	(g kg <sup>-1</sup> DMI)	Waghorn <i>et al.</i> (2002)
<i>Leucaena leucocephala</i>	30.0	<i>In vitro</i> /cattle	5.5	14.9†	(mL g <sup>-1</sup> DM)	Tan <i>et al.</i> (2011)
<i>Lepedeza cuneata</i>	177.0	<i>In vivo</i> /goats	6.9	16.2‡	(g kg <sup>-1</sup> DMI)	Puchala <i>et al.</i> (2005)
<i>L. cuneata</i>	153.0	<i>In vivo</i> /goats	7.3	n.a.	(g kg <sup>-1</sup> DMI)	Puchala <i>et al.</i> (2012)
<i>L. striata</i>	151.1	<i>In vitro</i> /goats	10.9	26.2‡	(L per day)	Animut <i>et al.</i> (2008a)
<i>L. striata</i>	151.0	<i>In vivo</i> /goats	9.6	19.0	(L kg <sup>-1</sup> DMI)	Animut <i>et al.</i> (2008b)
<i>Lotus corniculatus</i>	26.2	<i>In vivo</i> /cows	19.9	23.8	(g kg <sup>-1</sup> DMI)	Woodward <i>et al.</i> (2004)
<i>Lotus pedunculatus</i>	66.0	<i>In vitro</i> /sheep	8.8	10.6	(mL per 0.5 g DM)	Tavendale <i>et al.</i> (2005)
<i>L. pedunculatus</i>	53.0	<i>In vitro</i> /sheep	11.5	13.8	(g kg <sup>-1</sup> DMI)	Waghorn <i>et al.</i> (2002)
<i>Medicago sativa</i>	0.0	<i>In vivo</i> /sheep	20.6	n.a.	(g kg <sup>-1</sup> DMI)	Waghorn <i>et al.</i> (2002)
<i>M. sativa</i>	<0.1	<i>In vitro</i> , sheep	12.5	12.0	(mL per 0.5 g DM)	Tavendale <i>et al.</i> (2005)
<i>Onobrychis viciifolia</i>	48.5	<i>In vitro</i> /dairy cows	8.1	n.a.	(mm per day)	Williams <i>et al.</i> (2011)
<i>Trifolium pratense</i>	0.3	<i>In vivo</i> /sheep	17.7	n.a.	(g kg <sup>-1</sup> DMI)	Waghorn <i>et al.</i> (2002)
<i>Trifolium repens</i>	–	<i>In vivo</i> /dairy cows	18.1	n.a.	(g kg <sup>-1</sup> DMI)	Lee <i>et al.</i> (2004)

n.a., not available; PEG, polyethylene glycol. \*Units as reported by each author. †Instead of PEG, a sample deprived from CT was used as control. ‡Instead of PEG, a grass species was used as control.

could reduce *in vitro* CH<sub>4</sub> production, highlighting the importance of plant phenological stage for CH<sub>4</sub> emission. Hydrolysable tannin extract such as gallotannins produced a 50% inhibition of CH<sub>4</sub> production with lower toxicity associated with monomers vs. polymers (Field and Lettinga, 1987).

Dual-flow continuous fermenters were used to determine effects on ruminal fermentation of CT-containing legume hays in total mixed rations (Williams *et al.*, 2011); the legume forages assessed contained a range of CT contents and differed in their effects on *in vitro* ruminal fermentation, particularly CH<sub>4</sub> and NH<sub>3</sub>-N production. Methane production was decreased by the forages birdfoot trefoil, sainfoin and cicer milkvetch (relative to lucerne); however, none of these inhibited *in vitro* ruminal fermentation. The alternative legumes assessed elicited positive impacts on CH<sub>4</sub> reduction and may have potential to improve nutrient utilization and environmental sustainability of dairy cattle production when substituted for lucerne in the diets of lactating dairy cows.

It is evident that the complexity and variability among trials/experimental conditions make it difficult to compare estimates of CH<sub>4</sub> emissions from ruminants (Table 5). Future studies are needed to identify the specific chemical component and mechanism responsible for antimethanogenic activity. Animal feeding trials are also needed to evaluate the long-term tannin effects on rumen microbial populations and animal performance. However, it is clear that plant secondary metabolites are able to reduce CH<sub>4</sub> emissions from enteric fermentation and represent a promising target for genetic improvements of forage plants (Abberton *et al.*, 2008).

## Conclusions

Despite much research, the mechanisms by which tannins exert their effects on animal health and performance are not completely understood. In addition, the use of different standards for determination of tannins often makes it impossible to compare results between studies. It is suspected that potential interactions between tannins and other primary and secondary plant compounds may be important for palatability and biological function. Therefore, it is important to utilize the appropriate tannin-containing plant species/varieties in ruminant feeding. The potential use of HT as a feed additive requires further investigation to provide a stronger basis for understanding the possible role of HT in animal feeding. Further research is necessary to determine the role of rumen microbial populations, to better utilize tannin-containing diets and to prevent intoxications in animals ill-adapted to HT consumption. The finding that CT can reduce

ruminant CH<sub>4</sub> emissions has important environmental implications. Future studies are required to evaluate the sustainability of CT supplementation in CH<sub>4</sub> mitigation and rumen methanogenesis, without detrimental effects on productivity or health. As a natural and ecologically friendly tool to improve nutrient utilization for meat and dairy farming, the exploitation of forages containing both CT and HT has an important role to play in agriculture. Further research is necessary, focused on the definition of appropriate strategies to better exploit tannin-containing plant species and/or varieties in ruminant feeding, thereby improving animal husbandry and contributing to environmental sustainability.

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