

Developing a conceptual model of possible benefits of condensed tannins for ruminant production

L. O. Tedeschi^{1,2†}, C. A. Ramírez-Restrepo² and J. P. Muir³

¹Department of Animal Science, Texas A&M University, College Station, TX 77843-2471, USA; ²CSIRO Animal Food and Health Sciences, James Cook University, Townsville, QLD 4814, Australia; ³Texas A&M AgriLife Research, Texas A&M University, 1229 North U.S. Hwy 281, Stephenville, TX 76401, USA

(Received 14 November 2013; Accepted 11 March 2014; First published online 1 May 2014)

Enteric methane (CH₄) emissions from ruminants have compelled a wide range of research initiatives to identify environmental abatement opportunities. However, although such mitigations can theoretically be attained with feed additives and feeding strategies, the limited empirical evidence on plant extracts used as feed additives does not support extensive or long-term reductions. Nevertheless, their strategic use (i.e. alone or combined in a simultaneous or consecutive use) may provide not only acceptable CH₄ abatement levels, but also relevant effects on animal physiology and productivity. Condensed tannins (CT) represent a range of polyphenolic compounds of flavan-3-ol units present in some forage species that can also be added to prepared diets. Methods to determine CT, or their conjugated metabolites, are not simple. Although there are limitations and uncertainties about the methods to be applied, CT are thought to reduce CH₄ production (1) indirectly by binding to the dietary fibre and/or reducing the rumen digestion and digestibility of the fibre and (2) directly by inhibiting the growth of rumen methanogens. On the basis of their role in livestock nutrition, CT influence the digestion of protein in the rumen because of their affinity for proteins (e.g. oxidative coupling and H bonding at neutral pH) that causes the CT-protein complex to be insoluble in the rumen; and dissociate in the abomasum at pH 2.5 to 3.0 for proteolysis and absorption in the small intestine. CT may also reduce gastro-intestinal parasite burdens and improve reproductive performance, foetal development, immune system response, hormone serum concentrations, wool production and lactation. The objectives of this paper are to discuss some of the beneficial and detrimental effects of CT on ruminant production systems and to develop a conceptual model to illustrate these metabolic relationships in terms of systemic physiology using earlier investigations with the CT-containing legume Lotus corniculatus. Our conceptual model indicated four complex and long-lasting relationships (digestive, toxicological, physiological and morphological) that can alter the normal biology of the animal. These relationships are interdependent, integrative, and sometimes, complementary to each other. This conceptual model can be used to develop mechanistic models to improve the understanding of the interaction between CT and the ruminants as well as to guide research initiatives of the impact of polyphenol-rich foods on human health.

Keywords: condensed tannins, methane, modelling, physiology, ruminant

Implications

This paper reviews potential benefits of condensed tannins (CT), including mitigation of methane emission (a potent greenhouse gas), reduction of ruminal degradation of amino acids and reduction of gastro-intestinal parasitic infestation among many other benefits to cattle, sheep and goats; and proposes a conceptual model to understand the inter-relationships of the mechanisms of action of these benefits within the animal. Our conceptual model lays the foundation needed to develop a simulation model to understand the basic dynamics of CT in the ruminant.

Introduction

Condensed tannins (CT) are one of the plant secondary compounds under scrutiny for use within ruminant ecosystems as a feed additive. The presence of CT in plant leaves has been associated with inherent deterrent mechanisms against micro- and macroorganisms (Feeny, 1976; Heil *et al.*, 2002; Forkner *et al.*, 2004). Interest in the beneficial effects of CT is prominently featured in discussions of plant potential feed additives for methane (CH₄) abatement (Tavendale *et al.*, 2005; Beauchemin *et al.*, 2007; Ramírez-Restrepo *et al.*, 2010a) and anthelmintic (Aerts *et al.*, 1999; Ramírez-Restrepo *et al.*, 2004) effects in place of more potent chemical products (i.e. synthetic anthelmintic drenches).

† E-mail: luis.tedeschi@tamu.edu

CT, also known as proanthocyanidins, are large complex polyphenolic compounds of flavan-3-ol units (i.e. catechin, epicatechin, gallicocatechins and epigallocatechin) that form dimers and higher oligomers, which mainly contain four to eight linkages (Swain, 1979; Xie and Dixon, 2005). CT have the ability to bind proteins, metal ions and polysaccharides to render them unavailable to ruminal microbes (Mueller-Harvey and McAllan, 1992; Hedqvist *et al.*, 2000; Tedeschi *et al.*, 2011b). Methods have been developed that attempted to quantify and fractionate plant CT (Hagerman and Butler, 1978; Terrill *et al.*, 1992; Giner-Chavez *et al.*, 1997). However, these purely chemical, quantitative methods have a limited ability to predict the biological activity of specific CT from a given species (Martin and Martin, 1982; Makkar *et al.*, 1999) and biological assays are needed for this purpose (Ammar *et al.*, 2004; del Pino *et al.*, 2005).

In their review, Ramírez-Restrepo and Barry (2005) reported that chicory (*Chicorium intybus*), bird's foot trefoil – 'birdsfoot trefoil' – (*Lotus corniculatus* L. cultivar Grassland Goldie) and sulla (*Hedysarum coronarium*) were the most advantageous CT-containing forages in promoting growth and development in lambs. These forage species increase reproductive rates in ewes, increase milk production in ewes (Barry *et al.*, 1999; Ramírez-Restrepo *et al.*, 2005a) and dairy cattle (Woodward *et al.*, 2006) and reduce CH₄ emissions (Waghorn *et al.*, 2002; Woodward *et al.*, 2004). However, *L. corniculatus* stands out after considerable amount of research owing to its high nutritive value, adaptability to contrasting soil types and droughts, relatively high productivity compared with other legumes and sustainable ruminant performance (Wen *et al.*, 2002; Barry *et al.*, 2003; Ramírez-Restrepo *et al.*, 2004; MacAdam *et al.*, 2011). Nevertheless, despite all this research, the systemic links associated with the metabolic effects of CT in *L. corniculatus* on animal physiology remain unresolved.

Therefore, the objective of this paper was to review the effects of CT from *Lotus* spp., primarily *L. corniculatus*, to characterize and develop a conceptual model of the possible beneficial and detrimental effects of CT on ruminant production biosystems.

Characterization of CT

Lotus spp. are perennial long-day herbaceous plants from the *Fabaceae* family that are found across all continents (Beuselinck and Grant, 1995) and *L. corniculatus* is the most variable species in the *Lotus* genus (Turkington and Franko, 1980; Jones and Turkington, 1986). The cross-pollinated perennial legume contains a moderate concentration of CT (1 to 4 g/100 g DM) that does not depress dry matter (DM) intake (DMI) significantly but improves ruminant digestion (Barry *et al.*, 1986b). Using ¹³C nuclear magnetic resonance and acid-catalysed degradation in the presence of excess phloroglucinol, Foo *et al.* (1996) reported an average molecular mass of the CT from *L. corniculatus* in the range 1800 to 2100 Da and a predominance of epicatechin units (67%) in the procyanidin-type polymer. In a subsequent study,

Foo *et al.* (1997) found that the average molecular mass of the CT from *Lotus pedunculatus* was 2200 Da, with the polymer being of the prodelphinidin type, with epigallocatechin (64%) as the major extender unit.

This variability in chemical structure led Sivakumaran *et al.* (2006) to report that CT in *Lotus americanus*, rhizomatous *L. corniculatus* 'creeping selection' and *L. pedunculatus* had predominantly prodelphinidin units, whereas the CT from *Lotus angustissimus* and non-rhizomatous *L. corniculatus* consisted primarily of procyanidin units; equal amounts of prodelphinidin and procyanidin units were found in *Lotus parviflorus* and *Lotus suaveolens*.

Relationship between CP and CT

CP and CT averages of *Lolium perenne* and *L. corniculatus* from nine studies compiled by Ramírez-Restrepo and Barry (2005) is shown in Figure 1. The trend in this figure indicates that CT does not follow the CP pattern for *L. perenne* and *L. corniculatus*. For instance, while the CT concentration in *L. corniculatus* diminishes from late spring to fall season, the CP concentration decreases up to summer but then increases towards early fall (Ramírez-Restrepo and Barry, 2005). On the other hand, Ramírez-Restrepo *et al.* (2006b) used *L. corniculatus* in an indoor metabolic trial to demonstrate a similar decreasing pattern of CP and CT concentrations across vegetative, flowering and mature stages. The greatest concentration of CT in *L. corniculatus* was found in late spring and early summer and the CT for grass was <0.5 g/100 g DM (Figure 1). In contrast, Cooper *et al.* (2014) found that CT concentrations during seed set were stable for *Sericea lespedeza* but decreased in *Desmodium paniculatum*, an herbaceous legume dependent on epizoochory. Epizoochory uses the presence of mammalian hair for seed dispersal whereas most legumes, such as *S. lespedeza*, are more dependent on gravity, wind or water. Deduction indicates that *D. paniculatum* has evolved to diminish forage CT during seed set in order to attract potential herbivorous seed carriers.

Environmental effects on CT

In addition to the length of time (e.g. grazing period) animals stay on CT-containing forages, some studies reported seasonal animal responses. Seasonal climate variations may in fact alter CT concentrations, composition of CT fractions, chemical structure in the plant (Gebrehiwot *et al.*, 2002; Ramírez-Restrepo *et al.*, 2006b) and other secondary constituents (Van Soest, 1994). The effectiveness of CT may be altered by such effects depending on the forage source. Oscillations in CT concentration are also related to plant stress because of herbivore selection, soil and temperature variability, water and nutrient availability, and competition with other plants, among others (Barry *et al.*, 1986b; Muir, 2011). In a study evaluating the response of *L. corniculatus* to environmental change drivers, Carter *et al.* (1999) reported an increase in plant CT in response to increased carbon dioxide exchange rate, whereas CT decreased with drought stress and as growth temperatures increased to 25°C.

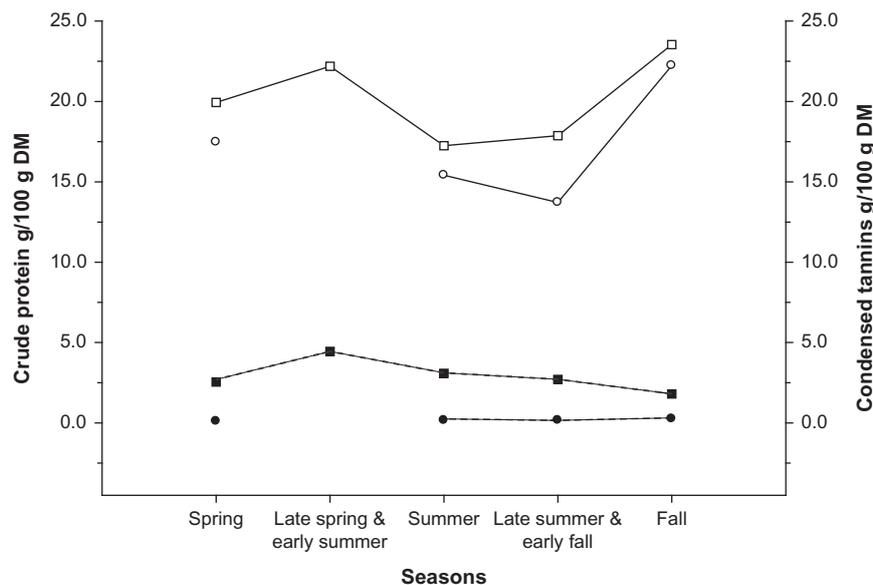


Figure 1 CP (open symbols and solid lines) and condensed tannins (solid symbols and dashed lines) concentrations (g/100 g of Dry matter) of *Lolium perenne* (circles) and *Lotus corniculatus* (squares) during three temperate seasons. Missing data for late spring and early summer for *L. perenne*. Adapted from Ramírez-Restrepo and Barry (2005).

Feeding value of CT

Lotus spp. studies conducted by Niezen *et al.* (1995 and 1998) and Leathwick and Atkinson (1995 and 1998) showed reduced parasite infestation and dag¹ formation, respectively. Using a dryland farming approach, Ramírez-Restrepo *et al.* (2004) confirmed in two consecutive spring experiments that weaning BW, average daily gain (ADG) and wool production of lambs grazing birdsfoot trefoil was greater than those grazing a mixture of perennial ryegrass (*L. perenne*) and white clover (*Trifolium repens*) pasture. They also reported that, compared to those on pure grass pasture, ewes and their lambs grazing *L. corniculatus* had reduced dag formation. The reduction in dag formation could also be attributed to a lower content of endophyte toxins ergovaline or lolitrem B in *L. corniculatus* compared with grass forages (Ramírez-Restrepo *et al.*, 2004).

According to Fletcher *et al.* (1999), alkaloids tend to increase faecal moisture, increasing the probability of dag formation in wool sheep. In agreement with other reports (Waghorn *et al.*, 1987; Douglas *et al.*, 1995; Wang *et al.*, 1996), Ramírez-Restrepo *et al.* (2004) assigned the increased animal performance to reduced microbial degradation of forage protein in the rumen by CT with a consequent increase in amino acid (AA) availability and absorption in the small intestine. Later, Ramírez-Restrepo and Barry (2005) reinforced the sparing effect of CT on AA proteolysis in the rumen to explain the correlation with increased AA absorption in the small intestine. Furthermore, Ramírez-Restrepo and Barry (2005) also reported a possible benefit (i.e. positive correlation) of dietary CT concentration in *L. corniculatus* on ewe fertility owing to increased grazing period of *L. corniculatus*.

This increased fertility was caused by a linear increase in ovulation rate as mating ewes were grazing *L. corniculatus* for longer periods of time during late summer or early fall resulting in a 16% increase in number of lambs born and weaned (Ramírez-Restrepo *et al.*, 2005a). These topics are discussed further in the next section.

However, it is not clear if the observed improvements in most of these experiments were solely owing to CT concentration or to increased organic matter (OM) digestibility (i.e. metabolisable energy) of a legume compared with a grass and consequently increased DMI or a combination of both (Ramírez-Restrepo *et al.*, 2006a). The effect of CT-containing forages on the improved performance of grazing animals is also influenced by other factors such as the proportion and digestibility of fibre and the content of protein, non-structural carbohydrates, and lignin. For example, lambs consuming willow (*Salix* spp.) fodder blocks (i.e. combined 6000 trees/ha and unimproved herbage grown under tree canopies; silvo-pastoral grazing system) had lower ADG compared with those lambs grazing a mixture of perennial ryegrass/white clover pasture (92 v. 134 g/day, respectively), which was likely owing to lower DMI as reported by Ramírez-Restrepo *et al.* (2010b). Most legumes also contain other secondary substances (potentially defensive compounds) that can have anti-nutritional characteristics and can reduce voluntary DMI (Van Soest, 1994). The consideration of all factors that may influence DMI is not only important in illustrating the possible benefits of feeding CT-containing (i.e. and effective) legumes, but also important for building conceptual models of forages characteristics on the metabolism of growing ruminants and their reproductive indices. However, conclusive data, in large part, are still absent or have not been fully elucidated.

¹Dag is the matted wool in the hindquarters of a sheep that is caused by the accumulation of faeces surrounding the anal region.

Potential benefits of CT

Ruminal CH₄ production

The carbon footprint contribution of ruminant production to the environment has received considerable attention by stakeholders and public policy makers around the world. Consequently, it has compelled research organizations and institutes to seek CH₄ abatement opportunities to mitigate the environmental impact of livestock production systems. Means of reducing CH₄ emissions can be classified into five categories, including feed additives, genetic selection, physiological modifications, rumen manipulation and management practices (Ramírez-Restrepo C. A., personal communication). Genetic selection of ruminants for low emissions of CH₄ has concomitantly arisen with the selection for efficiency via residual feed intake (Hegarty *et al.*, 2007). Although there is little evidence, this reduction in CH₄ emission is likely the result of reduced daily DMI instead of efficient animals having a different CH₄ emission per DM consumed (g CH₄/kg DM per day) (Waghorn and Hegarty, 2011).

A gamut of factors can influence the production of CH₄ in the rumen, including substrate and commercial or natural chemical products that decrease CH₄ production, but substrate fermentability is by far the greatest factor that affects the output of CH₄. Hence, when evaluating the emissions (or production) of CH₄ from different products, CH₄ output should be expressed per fermentable OM and fermentable carbohydrate bases rather than DM consumption. Ash, lipids (i.e. fat and fatty acids) and proteins may naturally restrict

the production of CH₄ in the rumen if their relative proportion is increased and daily DMI is kept the same. Literature review of experimental research (Tedeschi *et al.*, 2003 and 2011b; Cieslak *et al.*, 2013) has indicated that in current production systems such mitigation might be attained with feed additives and feeding strategies used alone or combined. Regardless of limitations in measuring CT and the generally unknown fate and transformations of the CT molecule through the ruminant digestive system (Terrill *et al.*, 1994), CT are thought to reduce CH₄ production in two ways. The first way is indirectly by binding to the dietary fibre and/or reducing the rumen digestion and apparent digestibility of the fibre (Barry and Manley, 1984; Barry *et al.*, 1986a), and the second way is directly by inhibiting the growth of methanogens (Tavendale *et al.*, 2005). After chewing unbound CT present in the vacuoles of the plant inactivates both microbial and digestive enzymes in the initial hydrolysis of large molecular weight carbohydrates such as cellulose and hemicelluloses (Benoit and Starkey, 1968; Barry and Duncan, 1984). In this environment, readily fermentable carbohydrates (e.g. water-soluble carbohydrates and pectin) are almost completely digested in the whole digestive tract, while their ruminal digestion is very slightly depressed by CT up to a concentration of 106 g/kg DM, as shown in Table 1 (Barry and Manley, 1984; Barry *et al.*, 1986b).

The mitigation of CH₄ owing to consumption of forage CT and possible mechanisms of action have been addressed in several scientific papers. Waghorn *et al.* (2002) evaluated six commonly grazed forages by sheep in New Zealand and

Table 1 Digestion of structural carbohydrates and readily fermentable carbohydrates (soluble CHO + pectin) in sheep fed *Lotus pedunculatus* differing in total condensed tannin (CT) and free CT contents due to applications of high, low and zero rates of polyethylene glycol (PEG/L tap water) on the diet¹

Compounds	Levels of PEG (MW, 3350 Da) ²			Fertility conditions ³	
	300 g	75 g	0	High	Low
Secondary compounds					
Total CT (g/kg DM)	14	45	95	46	106
Free CT (g/kg DM)	2	5	15	3	14
Structural carbohydrates					
Cellulose					
Apparent digestibility	0.74	0.75	0.75	0.78	0.63
Rumen digestion	0.73	0.72	0.69	0.69	0.53
Post-ruminal digestion	0.01	0.03	0.06	0.09	0.10
Hemicellulose					
Apparent digestibility	0.72	0.66	0.72	0.73	0.56
Rumen digestion	0.61	0.52	0.49	0.44	0.21
Post-ruminal digestion	0.11	0.14	0.23	0.28	0.35
Readily fermentable carbohydrates					
Apparent digestibility	0.98	0.98	0.97	0.95	0.93
Rumen digestion	0.83	0.77	0.89	0.80	0.78
Post-ruminal digestion	0.15	0.21	0.08	0.15	0.16

MW = molecular weight, DM = dry matter.

The effects were tested on forages harvested from high and low fertility soils resulting in varying CT concentration.

¹Apparent digestibility, rumen digestion and post-ruminal digestion are expressed as proportion of feed intake.

²Sprayed at 77 ml/kg fresh forage.

³Soil pH 5.3 v. 4.7, available P (µg/g soil) 18 v. 6 and S (µg/g soil) 12 v. 4 for high and low levels of soil fertility, respectively.

Adapted from Barry and Duncan (1984), Barry and Manley (1984), Barry *et al.* (1986b) and Ramírez-Restrepo (2004).

reported that lower levels of CH₄ production (g/kg DMI; CH₄ yield) were obtained for forages with high ratios of non-fibre carbohydrate to NDF and the presence of CT (sulla and *L. pedunculatus*). In an elegant factorial design, Woodward *et al.* (2004) compared the effectiveness of feeding ryegrass and *L. corniculatus*, with or without polyethylene glycol (PEG; MW 3350 Da; used to neutralize CT) to grazing dairy cows to reduce CH₄ production. Daily CH₄ production was similar between ryegrass with or without PEG and *L. corniculatus* with PEG (24.7, 24.2 and 22.9 g CH₄/kg DM per day, respectively). When PEG was not added to *L. corniculatus*, however, CH₄ production was reduced (19.9 g CH₄/kg DM per day) by 3 g/kg DM per day (13% reduction). The authors also indicated that the CT in *L. corniculatus* was responsible for more than 65% of the reduction in CH₄ production. The addition of PEG did not affect DMI or milk yield of the cows, but cows fed *L. corniculatus* produced 32% less CH₄/kg of milk solids (i.e. CH₄ intensity) compared with those fed ryegrass. These results confirmed that CT from *L. corniculatus* was in fact responsible for this reduction in CH₄ emissions because when the CT binding ability was neutralized, the CH₄ emission was similar to that observed for animals consuming only ryegrass.

The data from most studies with feed additives of plant origin (i.e. plant extracts) have shown small inhibitory effects on CH₄ production when expressed per unit of intake (Tedeschi *et al.*, 2011b), but their strategic use (i.e. alone or combined in a simultaneous or consecutive use) may provide significant abatement levels even in grazing conditions. Furthermore, as discussed by many related reviews, the results of *in vitro* studies need to be confronted with the results of *in vivo* studies to confirm the long-term efficacy (i.e. more than 3 months of duration) of such feed additives.

AA absorption in the small intestine

Likewise, both the concentration and type of CT in the diet influence rumen protein digestion (McLeod, 1974; Reid *et al.*, 1974). The great affinity of CT for leaf proteins is based on two mechanisms, oxidative coupling, which is not reversible, and H bonding, which is reversible (McLeod, 1974; Jones and Mangan, 1977; Swain, 1979). Hydrogen-bonded CT-protein complexes are insoluble in the rumen at pH 6.5 to 7, but may become unstable in the abomasum at pH 2.5 to 3.0, which makes AA available for digestion and absorption in the small intestine at pH 8.0 to 9.0 (Waghorn *et al.*, 1987; Mangan, 1988; Waghorn *et al.*, 1994). In sheep grazing fresh *L. pedunculatus*, a CT-containing legume, the duodenal non-ammonia N (NAN) flow per unit total N intake was equal to total N intake at a CT concentration of ~40 g/kg DM, but the flow ratio reached 1.35 as dietary reactive CT increased to 110 g/kg DM (Barry and McNabb, 1999).

Research with sheep has shown different CT effects upon the digestion and absorption of AA among *Lotus* spp. (Table 2; Waghorn *et al.*, 1987 and 1994). CT in *L. corniculatus* increased both the flux of essential AA (EAA; 52%) and non-essential AA (NEAA; 14%) through the abomasum. A similar trend was observed in *L. pedunculatus*, but the magnitude of the response was lower than in *L. corniculatus*: 14% and 8% for EAA and NEAA, respectively. Rumen fermentation in sheep fed CT-containing *L. corniculatus* resulted in a smaller loss of EAA (14% of intake) than in NEAA (30% of intake). However, when PEG (CT binder) was also fed, a net loss of both EAA and NEAA of 44% and 39% of intake, respectively, was shown from the rumen.

In contrast, sheep fed CT-containing *L. pedunculatus* exhibited a net gain of EAA (17% of intake) and a small loss of NEAA (4% of intake) across the rumen, but when PEG was given, there was a lower net loss of both EAA and NEAA than

Table 2 The effect of condensed tannins (CT) in sheep fed fresh *Lotus corniculatus* (22 g/kg DM) and *Lotus pedunculatus* (55 g/kg DM) with or without polyethylene glycol (PEG) upon digestion of amino acids (AA)¹

Items	Non-essential AA				Essential AA			
	<i>L. corniculatus</i>		<i>L. pedunculatus</i>		<i>L. corniculatus</i>		<i>L. pedunculatus</i>	
	CT-active	PEG	CT-active	PEG	CT-active	PEG	CT-active	PEG
Intake (g/day)	97.9	97.9	87.6	98.9	98.9	98.9	91.6	103.5
Abomasal flow (g/day)	68.5	60.0	84.3	77.7	84.6	55.5	106.8	93.7
Proportion of intake	0.70	0.61	0.96	0.79	0.86	0.56	1.17	0.91
Apparent loss in the rumen (g/day)	29.4	37.9	3.3	21.2	14.3	43.4	-15.2 ²	9.8
Proportion of intake	0.30	0.39	0.04	0.21	0.14	0.44	-0.17 ²	0.09
Apparent absorption from SI (g/day)	37.4	41.3	50.8	57.2	58.8	36.2	71.0	73.5
Proportion of abomasal flow	0.55	0.69	0.60	0.74	0.70	0.65	0.66	0.78
Proportion of intake	0.38	0.42	0.58	0.58	0.59	0.37	0.78	0.71

SI = small intestinal.

Non-essential AA are alanine, asparagine, glutamate, glycine, proline and serine; Essential AA are histidine, isoleucine, lysine, phenylalanine, threonine, tyrosine (i.e. phenylalanine as an only source), valine and leucine. Arginine values are excluded from this comparison.

¹Effects of CT were assessed through intra-ruminal infusion of PEG (molecular weight of 3500 Da) into half the sheep fed each legume.

²Negative values denote a net gain of essential AA (0.17 of intake) from rumen fermentation.

Adapted from Barry and Blaney (1987), Waghorn *et al.* (1987 and 1994), and Ramírez-Restrepo (2004).

with *L. corniculatus*. In addition, the apparent absorption (i.e. proportional abomasal flow) of EAA with *L. corniculatus* was not different between the CT- and CT + PEG-fed sheep, but was affected by NEAA. However, CT depressed the apparent absorption of both EAA and NEAA in sheep fed *L. pedunculatus*. The effects of CT treatment on increasing the apparent absorption (i.e. proportion of intake) of EAA in the small intestine were greater for sheep fed *L. corniculatus* (59%) than in *L. pedunculatus* (10%). In contrast, CT reduced the apparent absorption of NEAA by 10% of intake for sheep fed *L. corniculatus*, but had no effect for sheep fed *L. pedunculatus*.

Such relationships have been extensively evaluated for their effect on ruminant productivity in temperate (Barry and McNabb, 1999; Ramírez-Restrepo and Barry, 2005) and tropical (Muir, 2011) regions. Not all CT are biologically active or equally reactive because of differences in the degree of biological activity among species (Naumann *et al.*, 2013a); thus, CT concentration and NAN flow interactions may be forage species specific.

Gastro-intestinal parasite suppression

CT consumption also affects ruminant nutrition indirectly by mitigating the negative effect of blood-consuming gastro-intestinal nematode parasites (GINP). Most anthelmintic effects of CT-containing forages on ruminants have been determined using GINP larval feeding or motility inhibition, larval exsheathment reduction (Novobilský *et al.*, 2011), larval identification from larval cultures, *postmortem* gastro-intestinal worm counting and faecal egg count (FEC) assays (Ministry of Agriculture, Fisheries and Food, 1986). Underestimation may be a problem with all of these. For example, the most widespread assay, conventional FEC, underestimated the intensity of infection possibly because of CT in the faeces of willow-fed sheep (Mupeyo *et al.*, 2011). Perhaps, a more likely explanation is that in the faeces CT bind indigestible plant matter and protein layers present on the nematode egg shells (Wharton, 1980) affecting their flotation and microscopic examination (Mupeyo *et al.*, 2011).

Reduced parasitic infestations with *Haemonchus contortus*, *Teladorsagia* spp., *Nematodirus* spp., *Cooperia* spp., *Trichostrongylus* spp., *Chabertia ovina*, *Oesophagostomum* spp. and *Trichuris ovis* were reported for lambs grazing *L. corniculatus* without receiving anthelmintic drenches (Ramírez-Restrepo *et al.*, 2005b). Similar reduction of GINP infestation was observed in cattle and the most effective forage was *L. pedunculatus* followed by *Onobrychus viciifolia* and *L. corniculatus* (Novobilský *et al.*, 2011). The possible effects of CT present in *L. corniculatus*, *L. pedunculatus*, *H. coronarium* and *O. viciifolia* against GINP in small ruminants include (1) reduced egg laying and hatching; (2) development of egg to L₃ larval stage outside the ruminant; (3) motility of L₃ larvae; and (4) an improved host immunological competence (Molan *et al.*, 1999, 2000 and 2003; Hoste *et al.*, 2006).

However, the anthelmintic properties of CT from different legumes is not only parasite specific; it can also be compartment specific because CT varies in effectiveness in

different parts of the gastro-intestinal tract (Ramírez-Restrepo *et al.*, 2005b). There are some data suggesting a greater deleterious effect of CT on nematode population inhabiting the abomasum compared with the small intestine (Hoste *et al.*, 2006; Ramírez-Restrepo *et al.*, 2010b). The preceding information suggests that further studies are needed to unveil the underlying mechanisms of CT to restrain nematode proliferation and infestation in ruminants consuming CT-containing forages (Ramírez-Restrepo *et al.*, 2005b).

Effects on fertility and foetal development through endocrine regulation

Experimental evidence by Barry *et al.* (1986a) suggested that endocrine concentrations in sheep fed *L. pedunculatus* have shown that diets with the lowest value of CT have the highest plasma concentration of both 3,5,3'-tri-iodothyronine (T₃) and free T₃. Barry *et al.* (1986a) also reported that a linear, positive relationship existed between plasma growth hormone (GH) concentration (µg/l) and CT concentration (g/kg DM) in sheep fed *L. pedunculatus* with no alteration of plasma concentrations of other hormones. Effects of ovine GH concentration may be related to a reaction of free CT in inactivating gut-wall proteins by H bonding. Previous research with sheep (Davis *et al.*, 1970a and 1970b; Muir *et al.*, 1983) has shown that GH concentration is related to increased levels of plasma glucose, insulin and N gain. According to Muir *et al.* (1983) GH has an immediate, short-term insulin-like effect on adipose tissue yielding adipose tissue refractoriness to the insulin-like growth factor, which is followed by an extended, lipolytic response when GH is chronically increased.

Ramírez-Restrepo *et al.* (2005a) identified greater prenatal and postnatal lamb survival in ewes that grazed *L. corniculatus* during their mating and early pregnancy than in ewes that grazed perennial ryegrass/white clover pasture. Differences in ovulation rate, reproductive efficiency, pregnancy rate, fecundity, lambing rate and survival at weaning were not only associated with higher quality of the legume, but also to the forage CT effect upon uterine microenvironment, folliculogenesis, conception, implantation, early foetal growth and lamb viability. Given the association between the environment, nutrition and phenotype, lambs from the *L. corniculatus*-fed ewes were linked with a functionally modified epigenetic (i.e. heritable modifications in gene expression not associated to altered DNA sequences) mechanism in terms of carbohydrate and protein metabolisms (Barry *et al.*, 2004).

In a recent comprehensive review, Walker and Mitchell (2013) indicated that nutrition intervention influences a correlation between DNA methylation (i.e. epigenetic mechanism) and gene expression in the endometrium to promote folliculogenesis, uterine receptivity to embryonic attachment and growth in early stages, and offspring phenotype. It has also been reported that polyphenolic metabolites modulate the expression of genes related to cell adhesion and cytoskeletal organization (Chanet *et al.*, 2013). Furthermore, because pregnancy recognition in ruminants is accomplished by production of large amounts of interferon-tau (IFN- τ) by the

mononuclear trophectoderm of the blastocyst (i.e. days 12 to 14 of gestation; Stewart, 1992; Ott *et al.*, 1998), DNA methylation might be associated with transcriptional differences in the mutual response to this pathway (Walker and Mitchell, 2013). Although the experimental evidence needs to be tested at physiologically relevant conditions, this provides a potential explanation of the CT and/or their conjugated metabolites in blood circulation to improve reproductive performance and offspring survival through transcriptional regulation of gene expression.

Impacts on the immune system

Ramírez-Restrepo *et al.* (2010b) demonstrated that lambs consuming willow fodder blocks had a greater systemic antibody response triggered by the CT exemplified by increased clusters of differentiation CD21⁺ and $\gamma\delta$ differentiation TCR⁺ (TC receptor) lymphocyte subsets. According to Kabelitz and Wesch (2003), $\gamma\delta$ T-cells are present in all vertebrate species to bind antigens, much as antibodies do, because of their innate immune system function and structural similarities with immunoglobulin. The $\gamma\delta$ T-cells populate the blood and usually enrich lymphatic tissues, lungs, skin, reproductive tract and small intestine epithelia. Immune-physiological reproductive evidence suggests that increased numbers of $\gamma\delta$ T-cells in endometrial luminal epithelium are not only dependent upon conceptus stimulus (Tekin and Hansen, 2004) but also on systemic signals (i.e. maternal blood circulation; Majewski *et al.*, 2001; Oliveira *et al.*, 2012). In immune-modulating *in vitro* assays (Holderness *et al.*, 2007) with apple CT (i.e. procyanidin-type polymer), bovine CD8⁺ and $\gamma\delta$ T-lymphocytes demonstrated cell proliferation via upregulation of the interleukin (IL)-2R α chain expression and possibly endogenous concentration of phosphoantigens (Holderness *et al.*, 2007) and IFN- γ secretion (Percival *et al.*, 2008). It is also likely that protein mediators (i.e. limphokines) produced by epithelial uterine lymphocytes induce regulatory dynamics on IFN- τ and IFN- γ (Oliveira *et al.*, 2012). However, although the function of $\gamma\delta$ T-lymphocyte on early maternal–embryonic and embryonic–foetal interfaces is not clear, Holderness *et al.* (2007) and Jutila *et al.* (2008) suggested that CT (i.e. trimers and complex oligomeric-CT; Holderness *et al.*, 2007) are prominent $\gamma\delta$ T-lymphocyte agonists. Therefore, it may be possible that after ruminal fermentation, the CT molecule, the CT-protein bound fractions or CT-like compounds act on lymphocyte T-cell receptors, cytokine and chemokine synthesis and/or secretion to engage signal transduction. This might initiate the adaptive cellular host immune response to GINP and to some extent other immuno-modulatory, stimulatory, suppressive and/or tolerance effects on other systemic epitheliums. However, further studies must be performed to test these hypotheses.

Integration of mechanisms of action of CT

Recently, an *in vitro* study has suggested the CT concentration by itself may not be a reliable predictor of the CT

biological activity relative to the reduction of CH₄ production (Naumann *et al.*, 2013b). Therefore, other aspects of CT need to be considered. The effectiveness of CT-containing forages in promoting the benefits discussed above may depend on CT concentration in the forage consumed, reactivity (i.e. biological activity) of that CT (Barry and McNabb, 1999) as well as the metabolism of the ruminant animal. The reactivity of the CT may in turn depends on multiple factors, including plant species and elements that are inherent to their molecular structure, size, weight, polymer forming units (i.e. building blocks) and moieties (Mueller-Harvey, 2006; Naumann *et al.*, 2013a). Furthermore, early studies suggested that forage morphology may also impact parasitism levels in the animal independently of factors inherent in the CT (Ramírez-Restrepo and Barry, 2005). For instance, short grasses and legumes are more prone to larval infestation and intake by grazing animal than those grasses and legumes that present a high stand (i.e. greater grazing height; Georgi, 1985). Thus, the reasons that some forages perform better than others in decreasing parasitism are type and concentration of CT (and other secondary compounds) as well as pasture architecture (i.e. grazing horizon, proximity to soil, length of regrowth period during grazing rotations).

A conceptual model for the mechanisms of action of CT

Simulation modelling is a potentially valuable approach to assist research in agriculture that has consistently been underutilized (Tedeschi *et al.*, 2011a), most likely owing to lack of awareness of its capabilities and the notion of model development is complex and difficult. We propose the use of system dynamics (SD), a methodological approach that uses minimum mathematical formalism and focuses primarily on the development of intuition and conceptual understanding, without sacrificing the rigour of the scientific method (Sterman, 2000). The SD approach is a computer-aided modelling methodology that can be used to develop models for policy analysis and decision support systems (DSS) applied to problems arising in complex ecological dynamic systems characterized by interdependence, mutual interaction, information feedback and circular causality (Sterman, 2000; Tedeschi *et al.*, 2011a). The trend for increasing livestock production in tropical and subtropical regions and the reliance on these regions to produce high-quality food and energy crops warrants the development of DSS to address issues such as alternative production systems (including those using CT as a feeding strategy). These can assess the negative (if any) impacts livestock can have on greenhouse gas emissions and the environment, as well as the effects of climate change on livestock systems. Sterman (2001) pointed out that well-intentioned efforts to solve urgent problems very often create unintentional side effects owing to unforeseen outcomes or reactions to decisions. The development and application of models with strong scientific foundation not only allow for minimizing the occurrence of unforeseen pitfalls, but also permits research collaboration among individuals with different technical expertise.

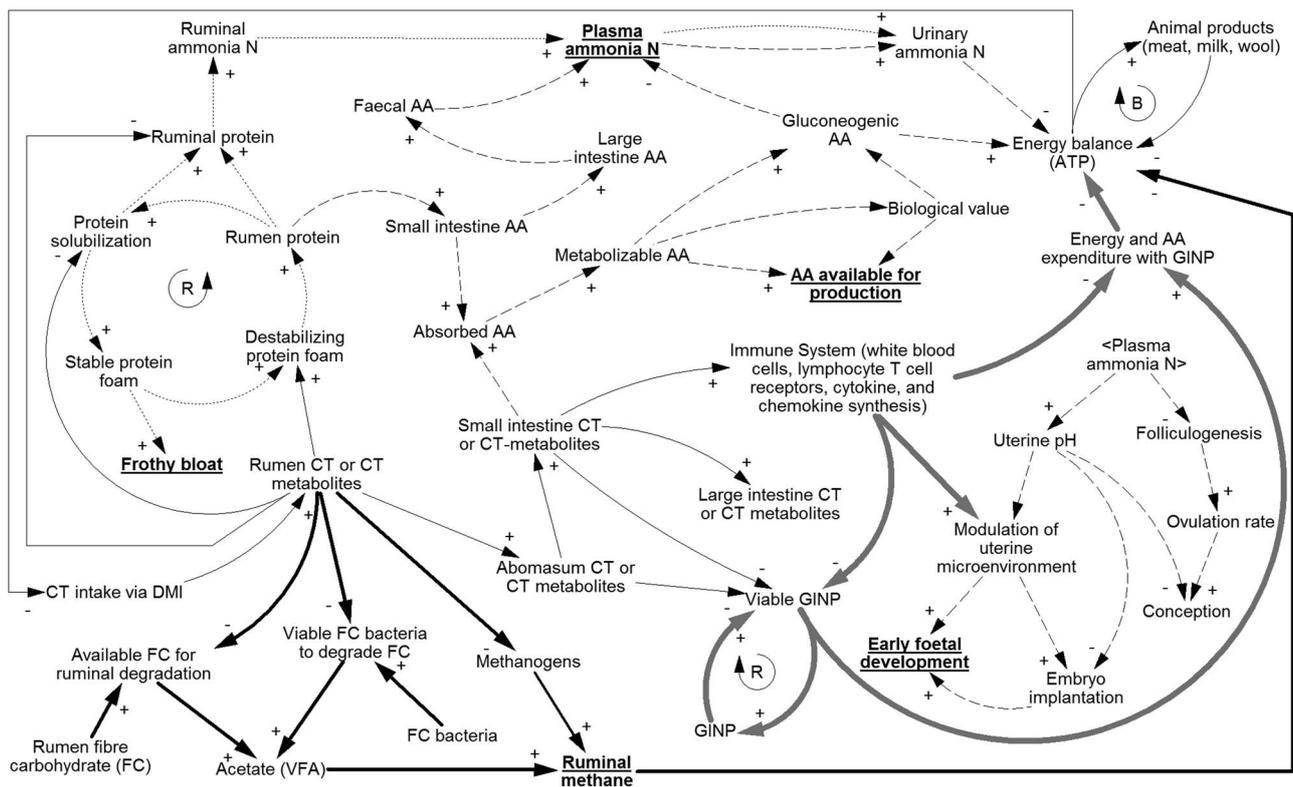


Figure 2 Five proposed mechanisms of action (bold, underlined variables) of condensed tannins (CT): amino acid (AA) sparing, ammonia production and embryonic development (long dashed arrows, centre and lower right regions), ruminal protein degradation and frothy bloat (short dashed arrows, upper left region), methane production (thick arrows, lower left), resistance to gastro-intestinal nematode parasite (GINP) and anthelmintic effect (thicker grey arrows, right region). R and B within an arrow circle indicate reinforcing and balancing loops, respectively, and the (+) and (-) signs at arrow heads indicate correlation direction between the variables. DMI = dry matter intake; FC = fibre carbohydrate; VFA = volatile fatty acids.

The first step in developing SD models is the statement of the problem and the identification of key variables (endogenous or exogenous to the problem) and their interrelationships, including feedback loops. The next step, which is not addressed in this paper, is the parameterization of the conceptual model.

As discussed above, the main problem with CT is that the unpredictability of their effects might be related to different mechanisms and modes of action. Figure 2 has five proposed mechanisms of action of CT for the protein (i.e. AA) ruminal sparing (decreased proteolysis), frothy bloat, ruminal CH₄, parasitic infestation and foetal development effects. In addition to what was discussed above, a decrease in ammonia formation was observed when *L. corniculatus* was fed to sheep (Waghorn *et al.*, 1987). The decrease in AA deamination and fewer short peptides are likely because of a lower proteolysis as CT exerts varying effects upon flows, loss and absorption of AA from the rumen to the small intestine. According to Li *et al.* (1996), 5 mg of CT/g of DM (i.e. 0.5 g/100 g of DM) is the minimum concentration necessary to prevent bloat (i.e. frothy bloat) in forage legumes. The effect is achieved by (1) destabilizing plant protein foams (Tanner *et al.*, 1995); (2) reducing protein solubility (Jones *et al.*, 1973); and (3) impairing the formation of stable protein foam and consequently reducing the incidence of bloat.

Besides the direct impact of CT on frothy bloat, ruminal CH₄, AA sparing, viable GINP and foetal development (Figure 2), these five mechanisms of action of CT were commonly and indirectly related to the 'energy balance' of the animal. We believe this critical feedback between 'energy balance' and 'CT intake' is responsible for creating complex dynamics and non-linear behavioural systems, but it is not clear which feedback loop dominates the system at a given time and at what frequency.

In conclusion, the consumption of CT by ruminants gives rise to complex and long-lasting relationships with various aspects of digestive, toxicological, physiological and morphological processes. The conceptual model structure indicated that, by nature, these relationships are integrative and sometimes complementary. A systematic approach will further our understanding of these relationships in a holistic manner, notably to account for possible benefits and detriments when different types and levels of CT are consumed by ruminants. We have provided a conceptual model delineating these relationships and discussed their strengths and weaknesses. We believe this conceptual model can guide new animal research programmes and assist the development of future mechanistic models that improve the understanding of the interaction between CT and the ruminant. This may also provide insight into prototype models for research initiatives of the impact of polyphenol-rich foods (e.g. green tea, grape and vegetables) on human health.

Acknowledgements

The authors acknowledge the Commonwealth Scientific Industrial Research Organisation (CSIRO) for provision of Sir Frederick McMaster Fellowship support to Associate Professor Luis Orlando Tedeschi, while in Australia. We would like also to express our appreciation to Emeritus Professor Tom Barry, Massey University; Professor Garry Waghorn, Dairy NZ; Associate Professor Jennifer MacAdam, Utah State University; and Assistant Professor Harley Naumann, University of Missouri for their insightful comments and invaluable criticisms that improved the quality of this manuscript.

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