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Review:

Utility of the browser's behavioural and physiological strategies in coping with dietary tannins: Are exogenous tannin-inactivating treatments necessary?

V. Mlambo[#], U. Marume & C.S. Gajana
Department of Animal Science, Faculty of Agriculture, Science and Technology, North-West University,
P Bag X2046, Mmabatho 2735, Mafikeng, South Africa

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Abstract

The desire to reduce feeding costs in small-ruminant production systems has led to increased reliance on non-conventional locally available browse products as protein supplements. Browse products contain variable quantities of tannins, whose nutritional effects on the animal can be positive or negative. Because of the lack of rapid evaluation techniques and methods that can differentiate, accurately, between potentially beneficial and harmful tannins, most researchers employ a 'safety first' approach in which tannin-inactivating treatments are applied to browse products to protect the animal and enhance feed utilization. This is despite the fact that browsing herbivores are known to have various behavioural and physiological strategies to cope with a number of anti-nutritional plant compounds, which include tannins. In this paper, the authors explore the rationale behind recommending the use of tannin-neutralization strategies when feeding animals with browse leaves and fruits. Are browsing herbivores' own coping strategies sufficient to protect them from suboptimal nutrition and possible toxicity caused by tannins or is intervention always required? In an attempt to answer this question, this review presents the current state of knowledge of tannins in ruminant nutrition before summarizing the strategies that browsing herbivores use to cope with tannins and their potential utility in various rearing systems. Finally, the utility of exogenous tannin inactivation strategies and the animals' own coping strategies are compared. Feeding scenarios are identified in which exogenous inactivation strategies may be worthwhile.

Keywords: Condensed tannins, proline-rich salivary protein, post-ingestive feedback, tannin inactivation, microbial adaptations, polyethylene glycol

[#] Correspondence: Victor.Mlambo@nwu.ac.za; victormlambo@yahoo.co.uk; victormlambo@icloud.com

Introduction

Herbivores, whose normal diet contains tannins, have been shown to possess a variety of coping strategies that protect them from negative nutritional and health effects (Estell, 2010; Ammar *et al*., 2011). The effectiveness of these coping strategies under various feeding systems is mostly unknown, and is confounded by the complex structure-activity relationships in tannins (Barry & McNabb, 1999; Santos-Buelga & Scalbert, 2000; Mueller-Harvey, 2006). As a result, attempts have been made to design, investigate and implement exogenous strategies that modify tannin levels and their *in vivo* reactivity to aid the herbivore in utilizing tannin-containing feedstuffs.

Tannins are a highly heterogeneous group of phenolic compounds that are produced by plants as part of their secondary metabolism. Over the years, several researchers (Makkar, 2003; Mlambo *et al*., 2004; Mueller-Harvey *et al*., 2007) have attempted to define the nutritional effects of tannins in browsing herbivores that encounter these compounds in their diets. The nutritional consequences of these compounds are ambiguous, with positive and negative effects being reported (Mueller-Harvey, 2006). This is attributed mostly to the high structural diversity of tannins, which determines their nutritional consequences. Greater attention has been paid to the role of tannins in ruminant nutrition, because of increased use of non-conventional locally available browse products as protein supplements in semi-intensive and extensive small-ruminant production systems (Aganga & Tshwenyane, 2003; Mlambo *et al*., 2004; Rogosic *et al*.,

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2008). Indeed, the roles of trees and shrubs as feed resources in semi-arid regions of the world need no further emphasis.

Most researchers recommend the collection and storage of browse products such as fruits for later use in drier seasons. Under these feeding conditions, there is concern that the animals' coping strategies could be insufficient and negative effects of tannins might reduce or negate the benefit of supplementing animals with tannin-containing browse products. As a result, a number of interventions that were designed to neutralize tannins and related compounds have been investigated and recommended, particularly the use of polyethylene glycol (PEG) (Gilboa *et al.*, 2000; Silanikove *et al.*, 2001; Villalba & Provenza, 2002; Smith *et al.*, 2003; Mlambo *et al.*, 2004; 2007; 2011; Mlambo & Mapiye, 2015), despite the fact that herbivores that routinely consume tannin-containing feedstuffs have been shown to possess strategies to circumvent negative dietary effects of tannins. These strategies include avoidance mechanisms (the bitter rejection response as postulated by Glendinning (1994)), secretion of proline-rich salivary proteins (PRPs), ruminal microbial physiological responses and hepatic detoxification activity. Therefore, the question is whether animals' own coping strategies are sufficient to mitigate suboptimal nutrition and avoid possible toxicity caused by tannins. A follow-up question is whether it is prudent to invest time and money on strategies to neutralize possible anti-nutritional effects of tannins in free-ranging and confined browsers? In an attempt to shed some light on these questions, this review paper explores the efficacy of herbivores' natural coping strategies, and juxtaposes the findings with the reported effectiveness of exogenous tannin-neutralizing approaches in mitigating the negative effects of condensed tannins.

Tannins and the nutrition of herbivores

Tannins are a complex group of polyphenolic compounds that occur in many feeds such as fodder legumes, browse leaves and fruits (Hedqvist *et al.*, 2000). There are two major groups of tannins: hydrolysable tannins (HTs) and condensed tannins (CTs) (Figure 1). HTs are made up of a carbohydrate core whose hydroxyl groups are esterified with phenolic acids (mainly gallic and hexahydroxydiphenic acid) (Mueller-Harvey & McAllan, 1992). CTs or proanthocyanidins are non-branched polymers of flavonoids units (flavan-3-ol, flavan-3,4-diol), and usually have a higher molecular weight than HTs (1000–20000 Da compared with 500–3000 Da) (Mueller-Harvey, 1999). HTs tend to attract less attention from animal nutritionists than CTs, presumably because they are easily broken down in the digestive system of animals. However, HTs of low molecular weight and products of HT hydrolysis may be the cause of toxicity in animals. Terblance (1967) reported fatalities in goats that had consumed *Acacia nilotica* fruits, which are known to contain high levels of epigallocatechin gallates (Mlambo *et al.*, 2007).

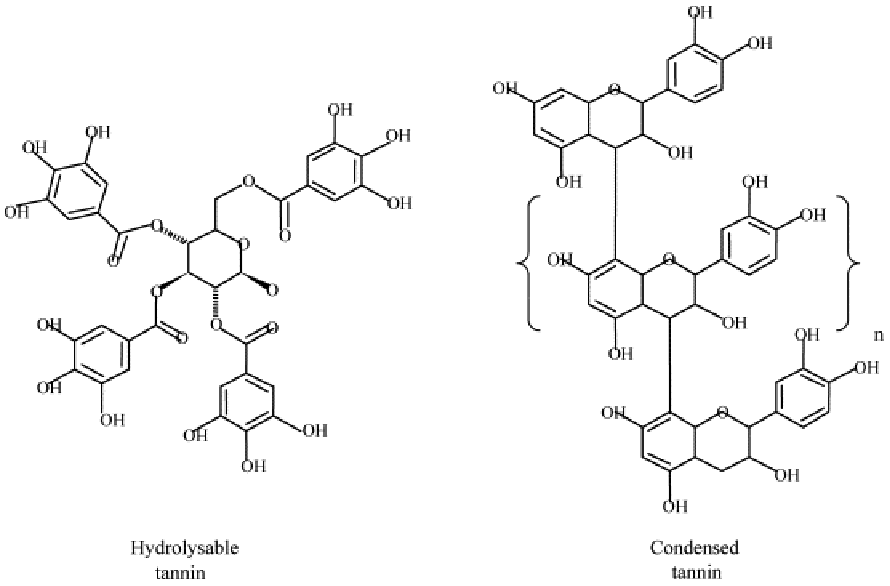

Figure 1 Diagrammatic representation of hydrolysable and condensed tannins (Krause *et al.*, 2005).

Numerous attempts have been made to predict the effect of tannins on animal nutrition and health, with variable success. This is hardly surprising, given that the nutritional effects of tannins depend on factors such as concentration, molecular weight and structure, as well as animal factors. Beneficial tannin effects include reduction in internal parasite load (Athanasiadou *et al*., 2000; 2001; Hoskin *et al*., 2000), prevention of frothy bloat when animals consume pastures that are rich in soluble proteins (Griffiths, 1991), improved nitrogen utilization efficiency (Mlambo *et al*., 2004; Waghorn, 2008), reduced enteric methanogenesis, and reduced nitrogen pollution through animal waste. On the other hand, astringency and negative post-ingestive effects of tannins cause reduced voluntary feed intake in the short term (Aganga & Tshewenyane, 2003). Silanikove *et al*. (1997) reported an inverse relationship between tannins and the degradation rate and digestibility of substrates in the digestive tract of ruminants. This ultimately reduces the amount of nutrients absorbed from the digestive tract. Other negative effects of tannins include toxicity, erosion of gastrointestinal tract and even animal deaths (Terblance *et al*., 1967). It is primarily the negative effects of tannins on voluntary feed intake and nutrient digestion that have resulted in indiscriminate use of exogenous tannin-inactivating treatments of tannin-containing feeds.

The desire to improve feed intake and enhance nutrient bioavailability has resulted in the use of exogenous tannin-neutralizing treatments, particularly in feeding systems in which tannin-rich feedstuffs are used as protein supplements. Under feeding conditions in which the basal diet is protein deficient, the primary objective should be to meet the nitrogen requirements of rumen microbes. The presence of tannins in protein forages is a constraint to attaining optimal ruminal nitrogen concentration for maximum microbial activity. The use of tannin-inactivating compounds seems to be the easiest and fastest way to resolve this problem. However, contradictory results were obtained when these inactivating compounds were used. The challenge for scientists is that currently there are few, if any, rapid methods to differentiate reliably between potentially beneficial and harmful tannins (Muller-Harvey *et al*., 2007). Table 1 presents a summary of the nutritional benefits obtained through neutralizing tannins with PEG. These findings have encouraged the widespread use of detannifying strategies in which non-conventional tannin-rich forage is used. This may be a waste of scarce resources, given that animals' adaptive mechanisms may adequately neutralize the nutritional threat posed by tannins. In addition, some of the tannins that are 'removed' from the diet could be beneficial to the animal (Mlambo *et al*., 2004).

Table 1 Polyethylene glycol (PEG) as a diagnostic tool for *in vivo* effects of tannins in goats

Reference	Rate of PEG	Diet offered	Findings
Mkhize *et al*., 2015	20 g/animal/day	Free-ranging goats. The rangeland is dominated by *Acacia karroo*, *Acacia tortilis*, *Ziziphus mucronata*, and some *Euclea* species. *Lippia rehmannii* and *Tarconanthus camphoratus* herbs	Dosing animals with PEG increased use of tannin-containing woody plants. Unsupplemented goats spent more time grazing than browsing
Fagundes *et al*., 2014	60 g/animal/day	Tifton 85 hay, which was replaced by *flemingia* leaf hay at 0%, 12.5% and 25% rates	PEG increased intake and digestion of tannin-rich flemingia leaf hay. No effect was observed on milk yield
Narvaez *et al*., 2011	0.05% - 0.3% body weight/day	Fresh evergreen branches (leaves and stems) of condensed tannin-rich *Arctostaphylos canescens* Eastw	Dosing with PEG increased feed intake, nutrient digestibility and weight gain
Mlambo *et al*., 2004	20 g PEG/100 g *Dichrostachys cinerea* fruit	Mature and ripe *Dichrostachys cinerea* fruits (200 g/animal/day) milled through a 4-mm screen. 600 g/day grass hay	PEG treatment resulted in excessive protein degradation in the rumen leading to high urine N loss and low N retention values. Untreated fruits promoted higher N retention values

Until the early 2000s, it was understood that high concentrations of tannins tended to have negative nutritional effects, whereas low to moderate concentrations had beneficial effects. Barry (1989) suggested that a condensed tannin level at 2% - 4% of DM in the diet would increase protein flow into the duodenum, resulting in improved growth. Later, Barry & McNabb (1999) reported that CTs at concentrations lower than 50 g/kg DM did not affect most ruminal fermentation parameters. An upper limit of 5 g CT/100 g DM was proposed by Min *et al*. (2003) as a safe level. However, more recent findings on *in vivo* tannin activity indicate that dependence on the quantity of tannins as a predictor of *in vivo* effects is unreliable. Using *Dichrostachys cinerea* fruits containing nearly 50% DM of tannins, Mlambo *et al*. (2004) demonstrated that neutralizing the tannins with PEG negatively affected the nutrient retention capacity of indigenous Matabele goats. Untreated fruits promoted higher nitrogen retention values, suggesting that even at high levels, some tannins still have beneficial effects on animal nutrition. In later studies with tannins from *D. cinerea* and other browse plants that are common in semi-arid regions, Mueller-Harvey *et al*. (2007) revealed that while the quantity of tannin is an important factor, the structure of the tannin matters more. Thus, several factors influence whether tannins exert positive or negative effects on browsers. Frutos *et al*. (2004) identified some of these factors as types of tannin, chemical structure and molecular weight of tannin, amount ingested, and animal species.

Browser coping strategies

Plants produce diverse mixtures of biochemicals, particularly secondary compounds that provide herbivores with challenges in accessing nutrients for survival and reproduction. While some compounds may be beneficial, most can be detrimental to rumen microbial populations, interfere with digestion processes, cause intestinal damage and result in metabolic toxicity post absorption (Rogosic *et al*., 2008). Browsers therefore cope with these toxic plant compounds through behavioural responses, physiological mechanisms and microbial adaptations.

Behavioural responses

Behavioural responses refer to animal strategies to reduce or eliminate consumption of anti-nutritional plant compounds through behavioural changes. These behavioural changes, which vary according to production system (free-ranging or intensive), include avoidance and aversion (pre-ingestive cues, and regulating intake below a threshold via conditioned antipathies) (Burritt & Provenza, 2000), neophobia (Owen, 1992), differential and cautious sampling (selecting plants or plant parts with lower concentrations) (Dziba & Provenza, 2008), increasing diet breadth, food imprinting (learning from parents), associative learning (social interactions and feedback) (Duncan & Young, 2002; Estell, 2010), altering the frequency and length of feeding bouts (cyclic consumption) and plant secondary metabolite complementarity (Rogosic *et al*., 2008).

Avoidance and aversion occur when animals perceive and respond to flavours and flavour intensities created by the integration of odour, taste and texture of the feed. This allows them to detect and avoid certain plants or plant parts, thus modifying feeding behaviour accordingly (Burritt & Provenza, 2000; Estell, 2010). Avoidance can be innate, where animals are apparently genetically programmed with knowledge of plant palatability, and thus are attracted to sweet flavours and repelled by bitter flavours (Owen, 1992). In this case, animals avoid or are reluctant to eat feeds that are high in antifeedants without prior experience, a condition normally referred to as neophobia. However, there is a dearth of information to confirm this. Nevertheless, goats may demonstrate an aversion to certain plant biochemicals through learning from post-ingestion consequences or feedback. In goats, the consumption of some toxic plants may elicit post-ingestive stimuli to the brain about the effect(s) the feed is having on the animal (Provenza, 1995). This causes a negative feedback effect, culminating in the animal making an unconscious association between plant flavour (taste and odour) and the resultant negative digestive feedback (Duncan & Young, 2002; Estell, 2010). This ultimately results in avoidance or aversion (hedonic shift) from that feed because of past negative associations. With some toxins that supposedly taste bitter (e.g. alkaloids, saponins and cyanogenic glycosides) or provoke an astringent sensation when eaten (e.g. tannins), a bitter rejection response could be elicited, consisting of a suite of withdrawal reflexes and negative affective responses (Glendinning, 1994). Nonetheless, bitter response thresholds vary independently of toxicity thresholds, indicating that the bitter rejection response can be elicited by a harmless bitter food.

Feed imprinting and associative learning are behavioural ways in which goats cope with plant secondary metabolites. Feed imprinting provides offspring with an efficient means of optimizing their subsequent behaviours towards certain feeds. Provenza *et al.* (1993) observed that young animals learn from their mother’s example to eat preferred feeds and avoid feeds with toxins. However, while the mother is an important source of information for young animals, post-ingestive consequences are probably more critical (Provenza *et al*., 1993). Learning can occur through associational cues that alert an animal to an aversive compound that is imperceptible prior to consumption and post-ingestive effects (Moore *et al*., 2004; Estell, 2010). Goats, like other domestic livestock, are social animals. They frequently observe one another and modify their diet selection according to what their grazing companions are eating (Thorhallsdottir *et al*., 1990). Learning can also take place through trial and error. However, trial and error learning can be risky as sampling toxic plants is imprecise, and errors while sampling could be debilitating or lethal (Provenza *et al*., 1992).

Differential and cautious sampling (selecting plants or plant parts with lower concentrations), increasing diet breadth, altering the frequency and length of feeding bouts (cyclic consumption) (Perevolotsky *et al*., 2006; Dziba & Provenza, 2008) and consuming plants with complementary secondary metabolites are other ways in which goats can cope with the effects of secondary plant metabolites (Provenza *et al*., 1992; Estell, 2010). Cautious sampling enhances the consumption of secondary plant metabolites with toxic threshold limits and allows detoxification enzymes to be maintained at an induced state as a protective mechanism in the event of plant secondary metabolites consumption (McLean & Duncan, 2006). Cyclic consumptions and increasing dietary breadths may reduce the cumulative effects of secondary plant metabolites (Provenza *et al*., 1992). Additionally, ample time is allowed for the detoxification process to be completed before more toxic metabolites are consumed. It has been observed that goats can limit the effects of toxins in a single plant by mixing diets (Provenza, 1996). Variable chemical composition of toxins in different plant species suggests variable and different detoxifications pathways in animals. Marsh *et al*. (2006) therefore proposed the detoxification limitation theory, which assumed that animals could consume more toxin-containing forages of greater chemical diversity because detoxification is spread over more metabolic pathways, thereby reducing the constraints on enzymes and substrates. Rogosic *et al*. (2007) observed that complementary relationships among classes of secondary plant metabolites influence the toxicity of chemicals and amount of feed ingested. For example, in goats and sheep, the consumption of tannins and saponins simultaneously was observed to reduce the toxic effects of these chemicals and increase feed intake compared with the consumption of the two classes of chemicals in isolation (Rogosic, 2007; 2008; Estell, 2010).

From a synthesis of the literature, there is evidence that browsing herbivores are well equipped with behavioural responses that should allow them to overcome the challenges posed by secondary plant compounds in their diet. It is therefore tempting to conclude that scientists should not be concerned with the negative effects of dietary tannins when fed to browsers. However, most of these behavioural responses may be curtailed under restrictive rearing and feeding systems, hence their importance should be viewed in this light. Clearly, browsers benefit most from these behavioural strategies if they are allowed to express their natural feeding habits. Browsers reared under intensive production systems may not exhibit the whole range of behavioural strategies, as these would depend on what the animal was offered. Even for free-ranging browsers, behavioural strategies could be constrained by fluctuations in feed quantity and quality.

Physiological mechanisms

Complementary to changes in feeding behaviour, browsers possess physiological mechanisms that influence how they cope with secondary plant metabolite consumption (Estell, 2010). These mechanisms include the production of salivary proline-rich proteins (PRPs), microbial ruminal fermentation, and liver and stomach wall detoxification (Jason & Murray, 1996; Estell, 2010).

Proline-rich proteins are salivary proteins with high affinity for polyphenolic compounds such as tannins. In saliva, they constitute up to 70% of total parotid protein. PRPs can form stable complexes with tannins in consumed forages, consequently protecting animals from anti-nutritional effects (Mehansho *et al*., 1987). Part of this protection may be through diminished intestinal uptake of tannin caused by the insoluble tannin-PRP complexes. *In vitro* studies have shown that most insoluble tannin-PRP complexes formed under conditions in the mouth remain insoluble when exposed to environments similar to those of the stomach and the intestines, and hence intestinal absorption will not occur (Mehansho *et al*., 1987). This has implications for the protein economy of animals since PRP are lost in faeces and therefore this physiological response is taxing on animals' protein pools. Secretion of PRPs constitutes the first line of defence against ingested tannins in some rodents (Mehansho *et al*., 1987). This is supported by findings that feeding tannins to rats resulted in a decreased growth rate, but after a few days, the growth returned to normal concomitantly with a marked stimulation of PRP synthesis (Mehansho *et al*., 1983, 1985).

Goats are normally referred to as mixed feeders and have been hypothesized to produce PRPs as a means of coping with the consumption of tannins. However, Distel & Provenza (1991) detected no significant amounts of PRPs in goat saliva, regardless of whether they had been adapted to a high-tannin diet or not. Additionally, in grazing and strictly browsing ruminants, secretion of PRPs did not appear to be affected by dietary tannin levels (Makkar & Becker, 1998; Clauss *et al*., 2003). Nevertheless, recent observations indicate that when exposed to tannin-rich diets, goats have the ability to secrete PRPs that complex with tannins and reduce their detrimental effects (Mueller-Harvey, 2006; Alonso-Díaz *et al*., 2010). Gilboa *et al*. (1995) detected high amounts of proline (6.5%), glutamine (16.5%) and glycine (6.1%) in goat saliva when goats were exposed to tannin-rich feeds. These amino acids are known to enhance the affinity of proteins with tannins (Mehansho *et al*., 1987). These tannin-PRP complexes are generally stable and essentially inactivate tannins and reduce their absorption and toxicity (Shimada, 2006). All these observations explain why goats are able to consume higher amounts of browse than sheep and cattle, and are better able to cope with tannins than other domestic animals (Salem *et al.,* 2006; Rogosic *et al*., 2008; Utsumi *et al*., 2009).

After the tannin and other secondary metabolites enter the gut, they may be modified by rumen microbes (discussed below) or absorbed into the bloodstream, at which point liver detoxification becomes critical. The liver contains enzyme systems that metabolize nonpolar compounds, which are normally toxic, into water-soluble compounds that can be excreted through the urine (Pfister, 1999). Hervas *et al*. (2003) demonstrated hepatic detoxification function in response to incremental levels of dietary quebracho tannin extract in sheep. Although most detoxification takes place in the liver cells and the kidney, the stomach and intestinal mucosa, lungs and skin play critical roles (Zimmerman, 1978; Pfister, 1999). Liver detoxification has advantages over microbial detoxification in that, first, liver enzymes are under genetic control, and hence protection can be passed on from generation to generation. Second, liver enzyme systems are variable and can handle a diversity of toxins, and, third, toxin stimulation of liver enzymes is rapid, hence the enzymes become active at a slight elevation of toxin levels (Foley *et al*., 1999; Pfister, 1999). Liver detoxification, however, is dependent on the nutritional status of the animal and the provision of adequate dietary nutrients (protein, energy, including water) (Villalba *et al*., 2002; Utsumi *et al*., 2009). Protein and energy status are both critical for detoxification because processes such as synthesis of detoxification enzymes, modifications of the toxins for excretion, and maintenance of acid/base balance required protein and energy (Illius & Jessop, 1995; Foley *et al*., 1999).

Microbial adaptations

In addition to the production of PRPs, tannins can be degraded in the rumen via microbial pre-gastric fermentation (Freeland & Janzen, 1974). The utilization of toxic plants in browsers is made possible by the massive numbers of microbes in the rumen, where millions of microbes may be found per millilitre of rumen contents (Pfister, 1999; Duncan *et al*., 2000*)*. Some of these microbes are capable of degrading or detoxifying some plant toxins (Estell, 2010). For hydrolysable tannins and saponins, there seems to be a dependence on the detoxification process of these compounds in the rumen, probably because of degradation of these compounds by bacteria (Newbold *et al*., 1997; Teferedegne, 2000). Weimer (1998), however, reported that rumen microbial degradation occurs only where these toxic compounds are an energy source for microbes. Microbes must also inhabit a particular rumen niche that allows them to survive when the toxin is not present, and increase their population rapidly when the toxin enters the rumen (Pfister, 1999; Cardozo *et al*., 2004; Busquet *et al*., 2005). Conversely, microbial metabolism of some secondary plant metabolites can result in the conversion of innocuous substances into toxic compounds (Estell, 2010). For example, Kakes (1991) and Majak (2001) observed that microbial hydrolysis of cyanoglucosides and glucosinolates to sugars and aglycones releases toxic cyanide and thiocyanates, respectively.

Cardozo *et al.* (2004) and Busquet *et al.* (2005) observed that the induction of microbial adaptations lasts only a few days for some plant extracts, suggesting that ruminal microorganisms adapt to the compounds with time. Duncan *et al.* (1997) also noted that rumen microbial population could respond rapidly to dietary changes, and adaptation to secondary plant metabolites could occur in a few days. These adaptive mechanisms include the secretion of exo-polysaccharide (EPS) to form a protective layer around cells, dissociation of tannin-substrate complexes and formation of a thick glycocalyx or glycoprotein, which has high binding affinity for tannin (Nicholson *et al*., 1986; Chiquette *et al*., 1988; Smith *et al*., 2005). Mlambo *et al*. (2007) also provide evidence that rumen fluid from animals that consume tannin-rich feeds (adapted) is better able to ferment tannin-containing substrates *in vitro* than rumen fluid from unadapted animals. This points to the existence of adapted rumen microbes capable of modifying the anti-nutritional effects of tannins. Exo-polysaccharides have been shown to improve the survival of some species of *Lactobacillus* or to act as a protective barrier for some tannin-resistant ruminal *Streptococci* such as *Streptococcus gallolyticus* against the antimicrobial action of tannic acid (O`Donovan & Brooker, 2001). The EPS is not only a molecular sieve, but harbours enzymes (tannic acid hydrolase, gallate decarboxylase) in the matrix, which probably catalyses reactions that reduce access of potentially toxic substance to the microorganisms (Krause *et al*., 2005). Nevertheless, these protective functions have not been demonstrated for many *Lactobacillus* spp. Nicholson *et al.* (1986) demonstrated that glycoprotein component of some fungi spore mucilages, which are similar to animal microbial mucins, have exceptionally high affinity for binding some phenolic compounds and hence protecting them from the inhibitory effects of phenolics. However, the interaction between free tannins and microorganisms in the rumen is probably less than expected, because tannins often form complexes at ruminal pH with many organic compounds including polysaccharides, protein and minerals (McSweeney *et al*., 2001). Nonetheless, such interactions have been shown to result in net tannin effect loss of up to 78% in the rumen of goats fed *Calliandra calothyrsus* (Perez-Maldonado & Norton, 1996). This, in theory, enables goats to cope with tanniferous substances in feed.

Utility of animals' coping mechanisms: Are exogenous interventions necessary?

Browsers' coping strategies are useful adaptive mechanisms that protect the animals from deleterious effects of tannins in their natural habitat. It is unlikely that detannification interventions would be necessary for animals that spend most of their time in their natural foraging habitat. Nevertheless, even under free-ranging rearing systems, fluctuations in feed availability may interfere with the ability of the browser to fully exercise its avoidance behaviours, leading to negative nutritional and health effects (Terblance *et al*., 1967). The utility of browser coping strategies under other feeding conditions remains unknown. Indeed, most of the scientific investigations involving the use of exogenous agents to ameliorate tannin effects are carried out under intensive and semi-intensive rearing systems. Under these systems, the normal foraging behaviour of browsers may be restricted and thus animals are unable to rely fully on their behavioural responses to escape the negative effects of tannins. Behavioural activities such as differential and cautious sampling, increasing dietary breadth, ensuring complementarity of plant secondary metabolites and associative learning may be curtailed under intensive feeding systems, as well as in rearing systems that isolate animals, such as the use of individual feeding pens. Under these conditions, it may be justifiable to explore the use of exogenous agents such as PEG to protect the animal from negative tannins effects. However, browsers' physiological and microbial adaptations would play a prominent role in protecting animals from the detrimental effects of tannins even under these feeding systems. The production of PRPs is the first line of physiological defence. While there is little disagreement about the affinity of PRPs with tannins, there is ambiguity about the fate of PRP-tannin complexes as they pass through the digestive tract. Failure of these complexes to dissociate in the digestive system makes this physiological response a drain on the animals' nitrogen pool since the PRPs are lost in faeces. Clearly, the consumption of high levels of tannins would result in greater nitrogen losses from the animal, which could be prevented by the use of exogenous tannin-inactivating treatments. Most scientific investigations and recommendations on the use of detannifying agents have been made under feeding conditions that expose the animal to unusually high levels of tannin-rich supplements. This indicates that researchers have adopted a safety-first approach, which may work, depending on the type of tannins present in the animals' feed. Scientific evidence indicates that *in vitro* ruminal fermentation of tanniferous forages still benefited from tannin inactivation with PEG, even though adapted rumen fluid, obtained from animals fed tannin-rich diets for 85 days, was used (Mlambo *et al*., 2007). While microbes in the adapted rumen fluid had acquired greater potency against tannins compared with those in unadapted rumen fluid, this was not sufficient to completely neutralize the inhibitory effects of tannins on ruminal fermentation. The ability of rumen microbes to adapt to dietary tannins has been extensively reported by McSweeney *et al*. (2001). However, indiscriminate inactivation of tannins is unlikely to improve the nutrition of browsers all the time, as seen in a study by Mlambo *et al*. (2004). In this study, better nitrogen retention figures were obtained in goats that were offered untreated *D. cinerea* fruits compared with those offered PEG-treated fruits. Laboratory tests for tannin bioactivity, such as protein precipitation assays, solution calorimetry and octanol-water partitioning coefficients, should inform decisions on whether exogenous tannin ameliorating strategies are necessary. However, even these tests may not accurately predict the nutritional or anti-nutritional effects of tannins.

Conclusion

In conclusion, animals' behavioural coping strategies may not be sufficient under certain feeding systems and conditions. When animals are exposed to higher than normal levels of tannins, and are unable to express their normal feeding behaviour, exogenous tannin-inactivating strategies may be justifiable. Yet, even under these feeding conditions, inactivating dietary tannins may reduce the nutritional quality of an animal's diet, particularly the efficiency of nitrogen utilization.

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