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Tannins in tropical tree fodders fed to small ruminants: A friendly foe?☆

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ABSTRACT

Livestock production systems worldwide rely largely on conventional feedstuffs. The current world food crisis highlights the need to improve the use of local resources for animal nutrition, such as fodder trees and shrubs. The detrimental effects of tropical tannin-rich plants (TRP) on animal production have been frequently described. In contrast, their potential benefits have long been neglected. This paper presents the potential positive effects of tropical TRP on small ruminants either as source of feed or as nutraceuticals with anthelmintic (AH) properties. It also analyses the host behavioral and physiological adaptations associated with exploitation of those tannin-rich resources. Both sheep and goats preferred a mixture of plants even when tannin-free forage was available. Moreover, the preference for TRP by goats and hair sheep were mainly associated with the digestible fraction of fiber and to a less extent with tannin content, which implies that they do not necessarily select against TRP. The addition of polyethylene glycol did not modify the preference or intake of TRP by goats and sheep. Evidence of physiological adaptation to TRP is presented and discussed. Both, experienced hair sheep and goats had saliva with tannin binding capacity, enabling both species to eat higher quantities of TRP which could lead to a higher availability of tannins in the gastrointestinal tract. Tannins in the gastrointestinal tract could be an AH against gastrointestinal nematodes (GIN). Indeed, *in vitro* and *in vivo* studies have shown AH effects of tannins from TRP, suggesting their possible use as natural anthelmintics against GIN. This paper supports the change in the current view of tannins in TRP as anti-nutritional compounds. If adequately managed, TRP can be a valuable component of sustainable small ruminant production systems.

1. Introduction

Although goat and sheep industries make marginal contributions to the world meat and milk markets, their products are important commodities for resource-poor human populations. To feed the increasing human population will require a substantial increase in animal products by 2030 (FAO, 2008). Historically, to achieve their potential contributions, grains and improved forages have been used as feed supplements for small ruminants at different levels.

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However, the present world food crisis is causing people to re-consider the use of alternative feeds for animal production. While fodder trees and shrubs have not been an important source of human food, they can be a significant source of nutrients for ruminants in both harsh (Makkar, 2003) and biodiverse environments.

Tropical trees and shrubs are important sources of proteins for small ruminants. However, their high levels of plant secondary metabolites (PSM), including tannins, and/or their high fiber contents are often considered important factors limiting their use (Ben Salem et al., 2005; Sandoval-Castro et al., 2005). The concept of tannins being anti-nutritional, harmful and/or toxic to ruminants is common (Mueller-Harvey, 2006). However, tannins might also have beneficial effects on ruminant physiology, production and health (Ramírez-Restrepo et al., 2005; Hoste et al., 2006). Differences between the beneficial and detrimental effects amongst the diverse ecological niches depend on: (i) the type of vegetation (Iason and Villalba, 2006), (ii) the nutritional background/experience of the animals to use a specific vegetation (Villalba et al., 2002; Baraza et al., 2005; Sorensen et al., 2005; Provenza, 2006), and (iii) the existence of adaptation mechanisms (Shimada, 2006; Costa et al., 2008). Also most data illustrating the benefits from CT intake in small ruminants have been acquired on tannin-rich temperate legumes (Ramírez-Restrepo et al., 2005; Hoste et al., 2006; Waghorn, 2008). In contrast, the intake of tropical TRP has been frequently pointed out as detrimental for ruminants.

Native vegetation from the tropical forest of the Yucatan Peninsula has more than 260 legume species (Flores et al., 2006), some of which contain high levels of crude protein, low to moderate neutral detergent fiber (NDF) and variable levels of PSMs, including tannins (Ayala-Burgos et al., 2006). Animals foraging such chemically diverse environments likely show different response to the intake of tannins due to the development of different behavioral and/or physiological strategies to harvest nutrients and/or medicinal compounds as well as to counteract PSM. This review aims at presenting recent data obtained with tropical TRP using the flora of Yucatan as a model to explore: preferences and intake of tannin-rich species in goats and sheep with and without polyethylene glycol (PEG); the possible existence of adaptive mechanisms related to the presence of tannin-binding proteins in the saliva of goats and sheep; and the potential properties of native TRP as anthelmintics against gastrointestinal nematodes.

2. Preferences and intake of native tannin-rich tree fodders by goats and sheep

Compared to temperate countries, livestock under tropical conditions face variable conditions in terms of feed quality. Trees and shrubs contain high levels of lignin and PSMs (i.e. tannins) (Sandoval-Castro et al., 2005). Indeed, some plants have low crude protein (CP) and/or energy content which, in combination with the presence of PSMs, might detrimentally affect animal performance (Waghorn, 2008; Ben Salem et al., 2003). However, the toxicity of PSMs (i.e. tannins) has often been reported without considering the nutrient profile of the feed. The latter is crucial information as excess nutrients, nutrient imbalances and toxins can all limit feed intake or give a satiation sensation to animals (Provenza, 2006). Under natural conditions, it is rare that grazing animals avoid specific forages (Iason, 2005). Usually, the PSM intake is regulated within limits that are tolerable by the herbivores (Provenza, 1995, 1996). They ingest many feeds containing PSMs and use both, behavioral strategies and/or physiological adaptations, to counteract their negative effects (Provenza, 2006). The way plants are provided (as a single feed or in cafeteria trials), the relationship between tannins and nutritive value (crude protein, energy, fiber), the nutritional status of animals and their experience of the diet developed during previous ingestions of TRP may result in better intake or tolerance of plant materials. Even more, these plants may be useful to small ruminants in certain circumstances.

Even though small ruminants can spend a high proportion of their time browsing TRP (Rios and Riley, 1985), little information is available on the effects of tannins on goats and sheep preference and intake of these plants, especially when offered simultaneously. Preference and selection studies represent a useful tool to evaluate tropical TRP. In such studies, it is important to distinguish whether the choice made by herbivores reflects the concentration of PSMs, the availability of nutrients, or both factors interacting in the plants (Iason and Villalba, 2006).

The ways TRP are fed (as a single feed or cafeteria) can influence preference and intake by small ruminants (Provenza et al., 2009). Rogosic et al. (2006) reported a higher biomass intake when goats were offered the choice of tree shrubs with high tannin content and diverse metabolite compounds than when offered a single plant. A meta-analysis from different preference studies performed in Yucatan, Mexico using tropical TRP with the same characteristics in their nutritive values for sheep and/or goats showed several similarities (Alonso-Díaz et al., 2008a, 2009a; Hernández-Orduño et al., 2008a; Revaud, 2007).

- *Animals do not seem to select against TRP and they favour diversity in food consumption*: When foliage from four plants (three tannin-rich plants and one with high nutritive value and low tannin content) was offered *ad libitum* to animals with browsing experience, 50–63% of the dry matter (DM) intake was comprised of TRP foliage (Revaud, 2007; Alonso-Díaz et al., 2008a, 2009a; Hernández-Orduño et al., 2008a). Duncan et al. (2003) suggested that toxin dilution could be an important reason why herbivores select a mixed diet. Indeed, the quality of toxins in foods and the availability of nutrients may all interact to modify intake (Mote et al., 2007). Animals eating a mixed diet might face different mixtures of PSMs, which are usually eliminated from the body via different metabolic pathways. If animals restrict their diet to a plant rich in a single PSM, a single pathway might become saturated. Consequently, the nutritive cost (protein and energy) to eliminate this specific PSM could be higher. In conclusion, intake of a mixed diet suggests the existence of a trade-off between ingesting nutritious feed and spreading the risk from PSM ingestion.

- *In cafeteria trials, plant fiber and crude protein (CP) concentrations explain preference better than tannins*: To understand the main factors governing plant consumption, we explored the relationships between DM intake of sheep or goats and the various chemical components of each plant. Goats and sheep preferred plants with higher digestibility as illustrated by the fact that DM intake was positively and significantly correlated with the digestible fraction of fiber (cellulose and hemicellulose) in every study (Alonso-Díaz et al., 2008a, 2009a). However, the contents of total tannins and total phenols were the second or third best predictors of preference. On the other hand, high crude protein content (in two experiments with sheep) was negatively correlated with DM intake. In cafeteria trials, sheep seemed to reduce their selection of plants with very high contents of CP (from 150 to 300 g of CP/kg DM), perhaps due to the adverse effects of excess ammonia (Provenza, 2006), and the need for energy to eliminate excess N in the urine (Van Soest, 1994). This evidence suggests a possible role of high CP content as a limiting factor for the consumption of tropical legume trees and shrubs. Both species showed a similar preference pattern when they were offered the same plant foliages (Alonso-Díaz et al., 2008a, 2009a). Thus, in spite of possible differences in intake and digestion between sheep and goats, they might have a similar pattern of behavior toward feeds with high digestibility (Hadjigeorgiou et al., 2003). Hence, it seems that animals would rather select positively for the digestible material rather than mainly avoiding tannin ingestion, at least at the typical levels found in plants. In spite of the apparent limited role of tannins in the preference of animals, it is also true that they will obtain benefits from its consumption. In the case of browsing animals, tannins may bind protein and prevent its rumen degradation (by-pass protein supply).
- *Comparatively, goats eat larger quantities of TRP foliage than sheep (both with browsing experience)*: This was especially evident when the choice of foliage was restricted to only TRP material (Revaud, 2007; Alonso-Díaz et al., 2008a, 2009a; Hernández-Orduño et al., 2008a). In a similar way, when high quality forage (*Medicago sativa*) was offered to goats and sheep, the DM intake and digestibility were similar for both species. However, in the presence of a plant with lower quality (*Acacia saligna*) DM digestibility and metabolizable energy intake were higher in goats than in sheep (El-Meccawi et al., 2008). These results suggest that goats have higher ability than sheep to counteract the negative effects of tannins in tropical TRP.

The information discussed above was obtained from cafeteria studies of a short interval (4 h) and with animals supplemented with a concentrate feed (155 g CP/kg DM and 11.0 MJ/ME/kg DM) and chopped *Pennisetum purpureum* grass. Thus, care should be taken before extrapolating these results to complex ruminant systems or when different feeding systems are used. Low quality foliage from trees and shrubs as a sole feed (without grain, grass or another type of fodder) can cause negative effects on small ruminants. For example, when a single TRP plant (*Piscidia piscipula* or *Lysiloma latisiliquum*) was offered as a sole feed during 5 days, sheep and goats ate low quantities of TPR and lost weight (Alonso-Díaz et al., 2009b).

These preference studies have useful ecological implications as they might reflect the strategies small ruminants have developed to use vegetation and to cope with PSM under natural conditions. In addition, the information generated from preference and intake studies might have practical implications to design feeding strategies involving the use of tropical TRP. For instance, it would be desirable to employ feeding strategies that include several TRP rather than a single plant as it would increase total fodder intake and thus, improve the animal performance. Also, it could be possible to feed fodders selected to complement nutrient supply as well as the amount and type of PSM. This will again lead to improved feed consumption and animal performance.

3. Influence of PEG on preferences of goats and sheep for tropical tannin-rich plants

In the preference studies previously described, tannins were not the most important factor that explained preference and intake of TRP by goats and sheep with browsing experience. Therefore, polyethylene glycol (PEG) was used to confirm these results. The addition of PEG, which is a tannin blocking agent, has been used in livestock feeding to enhance the intake of TRP and/or to alleviate the tannin detrimental effects in ruminants (Silanikove et al., 2006). Also, PEG has been used to study the intake/utilization of diets containing high levels of CT (Quebracho) (Villalba and Provenza, 2001; Villalba et al., 2002). In general, positive results, with regard to improved intake, were found when tannin content in the diet or plant (as a sole feed) was above 50 g/kg DM (Makkar, 2003) or when a single source of tannins was offered to small ruminants (Silanikove et al., 2006).

In contrast, results of our preference trials using tropical sheep and goats showed that, when several tropical plants were offered (three or four species of TRP), the addition of PEG did not increase the overall consumption of TRP (Revaud, 2007; Hernández-Orduño et al., 2008a). These results confirm that animal preferences were mainly regulated by the digestible fraction of fiber rather than the tannin content of plants. It is important to mention that the tannin content of the plants used in those trials was less than 50 g/kg DM (measured with the butanol HCl assay; Makkar, 2003) and the level of CP in the plants was high (between 120 and 300 g of CP/kg DM). Also, animals of both species had browsing experience on native vegetation rich in TRP. Other preference studies have shown that the effect of PEG is related to the number of plants offered (Rogosic et al., 2008). As the number of plants increases, the lesser the effect of PEG. In summary, the effect of PEG seems to depend on factors such as:

- *The protein content in the diet*: Proteins, in a similar fashion as PEG, can bind to tannins (Makkar, 2003). Thus, it is possible that PEG may have less effect under the conditions of tropical forests, which are abundant in protein-rich legume fodders, as compared to conditions where the vegetation is not as rich in terms of CP content.

- *Tannin concentration in the diet*: The effect of PEG is evident when the level of tannins in the diet is high (Makkar, 2003). Although PEG may be more effective as tannin levels increase (Titus et al., 2000), the effect of PEG is also positive even with low to moderate levels of tannins. A measurement of the biological activity (BA) of tannins should be considered simultaneously. However, only few studies have measured the BA.
- *Interactions between PSMs*: When animals have access to a diversity of plants, they can ingest different molecules of the same type of PSM (i.e., tannins), as well as other types of PSMs (saponins, alkaloids, etc.) (Provenza et al., 2009). In such cases, the effect of PEG on tannins may be less evident (Rogosic et al., 2008). The effectiveness of PEG to block tannins may be low when other forages are superior in nutritive value or have low levels (or different types) of secondary metabolites (Titus et al., 2000).
- *Physiological adaptation of animals*: Animals with high levels of proline-rich protein in their saliva (Austin et al., 1989), or any other tannin-binding salivary proteins (TBSPs), may mimic the effect of PEG. Thus, the addition of PEG could be redundant. The presence of TBSPs can be the result of animal adaptation to a tannin-rich environment as discussed below.

Mueller-Harvey (2006) stated that "because tannins interact with overall animal nutrition in such a variable manner and because PEG is relatively expensive, its benefits should be examined on each specific situation". According to current evidence, PEG does not affect preference and/or does not increase TRP consumption in short-term studies with the tropical plants of Yucatan.

4. Adaptation of animals to tannin-rich plants with emphasis on proline-rich saliva

Performance of small ruminants fed trees and shrubs depends on animal species and breed as well as plant species (Papanastasis et al., 2008). However, even within breeds, the response of animals to TRP could be influenced by their experience or degree of specialization/adaptation to a particular ecological niche (Provenza et al., 2003). For example, animals browsing/grazing in a vegetation rich in tannins might develop diverse mechanisms to attenuate the adverse effects of these plants and increase their intake. Several authors suggest that the physiological adaptation to PSMs is an induced response to their presence in feed (Iason, 2005; Clauss et al., 2005; Costa et al., 2008).

Ecological theory predicts that specialist mammalian herbivores consume greater quantities of PSM, loose less body mass, experience fewer signs of toxicity and maintain a more positive energy balance on their preferred plant (Sorensen et al., 2005). Specialists also use less expensive detoxification mechanisms and eliminate PSM from the body more efficiently than generalists. In the case of tannins, it is possible that their diverse structural composition and/or interactions between different PSMs make it difficult to prove this ecological theory in small ruminants. Nevertheless some evidence of adaptive response mechanism has been found in sheep and goats with browsing experience.

- *Evidence of reaction between tannins and the saliva obtained from browsing goats and sheep*: In small ruminants, TBSP-rich saliva is one of the main adaptive mechanisms. However, such mechanism remains controversial. TBSP-rich saliva is probably the first line of defense developed/expressed by mammalian species against tannins (Skopec et al., 2004; Mueller-Harvey, 2006; Shimada, 2006). Amongst these TBSPs, the proline-rich proteins and histatins have a strong affinity for tannins (Shimada, 2006). Animals exploiting ecological niches with high levels of tannins in the diet may produce increased levels of salivary TBSPs to modulate their effects. In fact, salivary TBSPs are an inducible mechanism which adapts to the presence of PSM in the feed (Clauss et al., 2005; Costa et al., 2008). The specificity between salivary TBSPs and tannins is an adaptive response of mammalian species. At the animal level, the expression of this protective system could be influenced by nutritional status, especially given the protein and energy cost of harvesting nutrients in some ecosystems.

We hypothesized that adult goats and hair sheep with experience browsing native vegetation rich in proteins and tannins could produce salivary TBSPs as an adaptive response. A first step towards validating this idea was to study the reaction between saliva from either sheep or goats and tannins. Fresh saliva samples were collected from adult goats ($n = 42$) and hair sheep ($n = 51$). All the animals had 2–5 years of browsing/grazing experience in the vegetation of a tropical forest with abundant TRP.

The formation of tannin–protein complexes leads to haze development or turbidity (Horne et al., 2002) such that strong tannin–saliva reactions results in lower transmittance values due to turbidity. This reaction (turbidity) is highly specific to proline-rich saliva (Asano et al., 1982). In our studies, turbidity of the sheep and goats' saliva remained unchanged during measurements performed every 15 min for 2 h. However, when sheep or goats' saliva were mixed with tannic acid, the turbidity showed a strong dose-dependent reaction ($r = 0.99$ for sheep, and $r = 0.99$ for goats; $P < 0.05$) (Alonso-Díaz et al., unpublished data). Thus, our results support the evidence of presence of TBSPs in the saliva of tropical goats and hair sheep. It is worth to indicate that higher turbidity values were found in goat saliva than in hair sheep saliva. Currently, we are analyzing lyophilized saliva of goats and sheep by HPLC to establish which proteins are present in these two small ruminant species. These results are in contrast with previous evidence indicating that sheep lacked TBSP (Austin et al., 1989). In the case of goats the presence of TBSP was suspected (Van Soest, 1994) but to our knowledge this has not been confirmed.

The presence of salivary TBSPs in experienced sheep and goats probably modifies the astringency and post-ingestive effects of TRP (Mantz et al., 2009). Therefore, animals can ingest large amounts of tannin-rich feeds which otherwise might prove to be less accepted/ingested. In small ruminants, the fate of tannin–protein complexes through the gastrointestinal tract has not been explored. Tannin–protein complexes resulting from the combination with bovine albumin or leaf proteins are insoluble. On the other hand, complexes between tannins and salivary proteins are soluble. Thus, Mueller-Harvey (2006) posed a key question: Are tannins in the soluble complexes more readily washed out of the rumen than those that are insoluble? Considering the pH dependent nature of tannin–protein interactions (stable at pH near neutrality and disassociate as pH becomes more acidic), we hypothesized that TBSP–tannin complexes are soluble in the lower tract of experienced animals ingesting higher amounts of TRP. Then, the remaining question would be: would tannins released along the gastrointestinal tract be active and useful for the control of gastrointestinal nematodes (GIN)? It is important to mention that the effects arising from different tannins (with different chemical structures) will result in different outcomes. However, this paper is not reviewing the fate of different tannin molecules.

5. Anthelmintic effect of tropical tannin-rich plants

One of the most explored beneficial effects of TRP during the last 10 years is the AH effect of some PSMs against GIN of small ruminants. Most studies have focused on temperate legumes whose AH effects were mainly attributed to condensed tannins (CT) (Hoste et al., 2006; Terrill et al., 2007, 2009). However, legumes from temperate areas cannot be exploited worldwide. In some areas, e.g. the Mediterranean region, the pastoral production systems rely on the exploitation of TRP in browse by goats and sheep. Similar situations can be found in many tropical areas where browsing is an essential component of the production system. The woody plants and/or bushes composing the browse vegetation belong to various botanical families, and their biochemical composition is usually much more complex than that of temperate legume forages. A combination of CT, hydrolysable tannins, polyphenols, and other PSMs (i.e. alkaloids, saponins, etc.) can be found in those plants, making the rational exploitation of these resources less straightforward. This explains the need for specific studies on the plants composing the browse in tropical areas.

Recent literature reviews focused on the possible role of different bioactive plants, which contain various types of PSM, as alternative AHs (Min and Hart, 2003; Hoste et al., 2006, 2008; Athanasiadou et al., 2007). Those reviews have proposed future actions intended to improve the likelihood of using these plants in sustainable parasite management strategies. During the last 5 years a number of trials were undertaken using TRP from tropical regions to improve our understanding of how these plants can be used to control GIN. Some of these trials and results are presented to illustrate the research in this field (Table 1). All these trials illustrate the AH effects of some tropical plants against GIN of ruminants studied both *in vitro* and *in vivo*.

5.1. In vitro studies

In vitro studies are useful for screening the AH effects of PSM, as well as providing models to investigate the mechanisms of action of PSMs on nematodes (Paolini et al., 2004; Brunet and Hoste, 2006). A number of *in vitro* techniques has been developed to study the AH effect of plant extracts on a variety of parasite species and/or stages (from eggs to adults) (Hoste et al., 2008). These techniques are relatively simple, low cost, require limited amounts of biological material, and usually do not require slaughtering of animals (Hoste et al., 2008).

Most *in vitro* studies with tropical plants have focused on *Haemonchus contortus* with different concentrations of extracts (varying from μg/ml to mg/ml) (Table 1). Athanasiadou et al. (2007) pointed out the difficulty of relating the concentrations of the bioactive compounds found in the extract to those found in the plant. This is especially important when the AH screening is performed for plants with possible nutraceutical use. The criteria to define the concentration of the bioactive compound for *in vitro* trials must be based on scientifically valid grounds. For example, the doses used in several bioassays of temperate legumes were based on levels of free tannins in ruminal liquid as described by Terrill et al. (1994). Therefore, the quantity of tannins used for *in vitro* trials uses this criterion to set the doses to be evaluated (Molan et al., 2000, 2002; Paolini et al., 2004). However, the actual amounts of free tannins present in rumen contents and flowing to the lower gastrointestinal tract, after feeding on TRP fodder is still unknown. Some of those *in vitro* results showed consistent *in vivo* AH effects. In the case of the *in vitro* tests on tropical plant extracts, only a few studies relied on such criteria. The criterion used for temperate legumes was applied recently with extracts of a TRP (*Lysiloma latisiliquum*) well accepted by goats and sheep. At the concentration levels used, the AH effect showed a dose-dependent relationship against both *H. contortus* and *Trichostrongylus colubriformis* (Alonso-Díaz et al., 2008b,c). Also, a consistent AH effect was found *in vivo* against *H. contortus* and *T. colubriformis* in goats eating the same plant (Brunet et al., 2008). Even though the *in vitro* use of a dose that correspond to the levels of free tannins in ruminal liquid of temperate legumes seemed to work in tropical plants, it is still essential to investigate the fate of PSM throughout the gastrointestinal tract in small ruminants.

As previously mentioned, most *in vitro* trials focused on *H. contortus*, which is highly prevalent and highly pathogenic under tropical conditions. However, the inclusion of *T. colubriformis* in *in vitro* studies is useful, as differences in sensitivity exist among nematode species. Indeed, in tropical regions, GIN infections are mixed and involve *T. colubriformis* as an economically important species. So far, only a few studies with tropical plants have used *T. colubriformis* and *H. contortus* as models with the same plant extracts (Houzangbe-Adote et al., 2005a,b; Alonso-Díaz et al., 2008b,c). In general, consistent results have been obtained on abomasal and intestinal nematode species. However, these findings need to be corroborated by *in vivo* studies, especially because the environmental conditions in different parts of the gastrointestinal tract can influence the bioavailability of PSMs, the exposure of worms to the compounds and consequently their AH efficacy (Athanasiadou et al., 2007).

Several authors suggest the need to identify which PSMs are involved in the AH effect of tropical plants (Hoste et

Table 1
Anthelmintic effect of tropical fodder plants against gastrointestinal nematodes: *in vitro* and *in vivo* studies.

Plant	Study	Nematode specie	Dose tested	AH effect	Component/confirmation	Authors
Leucaena leucocephala	*In vitro*	*Haemonchus contortus*	0.75–10 µg/ml	Yes	Tannins/separated by HPLC	Ademola et al. (2005)
Melia azedarach	*In vitro*	*H. contortus*	3.2–50 mg/ml	Yes	CT, alkaloids	Maciel et al. (2006)
Mangifera indica	*In vitro*	*H. contortus*	0.08–50 mg/ml	Yes	CT	Costa et al. (2002)
Brosimun alicastrum *L. leucocephala* *Acacia gaumerii* *Havardia albicans*	*In vitro*	*H. contortus*	150–2800 µg/ml	Yes	CT/no confirmation	Hernández-Orduño et al. (2008b)
Zanthoxylum zanthoxiloides	*In vitro*	*Trichostrongylus colubriformis* *H. contortus*	300–2500 µg/ml 125–1000 µg/ml	Yes Yes	ND/no confirmation ND/no confirmation	Houzangbe-Adote et al. (2005a,b)
Acacia pennatula *Piscidia piscipula* *L. leucocephala* *Lysiloma latisiliquum*	*In vitro*	*H. contortus* *T. colubriformis*	150–1200 µg/ml 150–1200 µg/ml	Yes Yes	Tannins/confirmed (PVPP) Tannins/confirmed (PVPP)	Alonso-Díaz et al. (2008b,c)
Acacia mearnsii	*In vivo* (sheep)	*T. colubriformis* *H. contortus* *O. colombianum*	18 g/kg LW	Yes	CT/no confirmation	Cenci et al. (2007)
L. leucocephala *Manihot sculenta* *Gliricidia sepium*	*In vivo* (sheep and mice)	*H. contortus*	7.5–15 mg/kg LW	Yes	CT/no confirmation	Rojas et al. (2006)
Acacia mollisima	*In vivo* (sheep)	*H. contortus* *T. colubriformis*	1.6 g/kg LW	Yes	CT/no confirmation	Minho et al. (2007)
Acacia karoo *Acacia nilotica*	*In vivo* (goats)	*H. contortus*	40% of inclusion in the diet	Yes	CT/no confirmation	Kahiya et al. (2003)
Lysiloma latisiliquum	*In vivo* (goats)	*H. contortus* *T. colubriformis*	1.4 mg of CT/kg LW	Yes	CT/confirmed (PEG)	Brunet et al. (2008)
ND	*In vivo* (goats)	ND	ND	Yes	Tannins/confirmed (PEG)	Kabasa et al. (2000)
Acacia cyanophylla	*In vivo* (sheep)	Several	ND	Yes	Tannins/confirmed (PEG)	Akkari et al. (2008a,b)
Acacia cyanophylla	*In vivo* (sheep)	Several	ND	Yes	Tannins/confirmed (PEG)	

ND = non-determined; CT = condensed tannins; PEG = polyethylene glycol; LW = liveweight; PVPP = polyvinyl polypyrrolidone.

al., 2006; Behnke et al., 2008). Most *in vitro* work involved plant extracts containing several PSMs. In the case of TRP, two common methodologies have been applied to corroborate the participation of tannins in the AH activity: (i) direct isolation of the active compound(s) by means of specific fraction separation using HPLC. This is a complex process which requires high-tech equipment and expertise (Athanasiadou et al., 2007). Based on such methods, Ademola et al. (2005) and Ademola and Idowu (2006) demonstrated that the tannin and polyphenol fractions of *L. leucocephala* extracts were related with the AH effect against *H. contortus*. (ii) The use of specific inhibitors for the suspected bioactive compound, such as PEG and/or polyvinyl polypyrrolidone (PVPP) for tannins (Hoste et al., 2008). The first approach is less common. The second option has been used frequently to confirm the role of tannins in temperate forages, but only a few trials on tropical plants have used this approach to confirm the role of tannins in the AH effect (Table 1).

Finally, it is important to mention that the content and bioactivity of PSMs in the plants can vary. The most common factors affecting variability due to environment include: (i) season, (ii) part of the plant, (iii) age/physiological stages of plant, and (iv) geographical biotype (source) of the plant (Hoste et al., 2006, 2008; Athanasiadou et al., 2007). It is important to explore the variability of polyphenolic compounds and their biological activity within nutraceutical species because this can be a major source of inconsistencies in the results.

5.2. *In vivo studies*

In vivo studies with tropical TRP have been performed in two ways: dosing the plant extracts (from one or several plants) directly to animals as a drench (phytotherapeutic drugs), and feeding animals with plant material as nutraceuticals. In temperate regions, the TRP have been tested mainly as nutraceuticals. The intake of fresh forage (Niezen et al., 1998), hay (Paolini et al., 2003; Lange et al., 2006; Hoste et al., 2005), silage (Heckendorn et al., 2006), and pellets (Terrill et al., 2007) can have an AH effect against GIN. In tropical regions, TRP have been mainly tested as phytotherapeutic drugs (Rojas et al., 2006; Cenci et al., 2007; Minho et al., 2007). One of the probable reasons is that some tropical plants are not well accepted when offered as a single feed (Alonso-Díaz et al., 2009b). However, as was previously discussed, different factors should be considered such as the full plant chemical composition (tannins, crude protein, energy, fiber), concentrate supplementation, nutritional status of animals and their experience developed during previous ingestions of TRP. Therefore, an essential step, after the assessment of the *in vitro* AH effect, is to study the best way to provide the plant as feed for small ruminants to obtain the desired AH effect. For example, the recent finding that TRP are better accepted by goats and sheep when offered in cafeteria (Alonso-Díaz et al., 2008a, 2009a) rather than as single plants (Alonso-Díaz et al., 2009b) provided the rationale to develop useful strategies to achieve an *in vivo* AH effect. So far, a similar strategy has been used by Osoro et al. (2007) using heather vegetation in goats.

Due to the complex chemical profile of tropical PSM, it is difficult to relate a specific bioactive compound with the AH effect. In spite of that difficulty, the use of specific tannin inhibitors has been proposed to evaluate the participation of tannins in the AH effect. A first report of the effect of PEG to demonstrate tannin's participation on the *in vivo* AH effect of TRP was described for goats browsing/grazing native vegetation (Kabasa et al., 2000). A similar situation was reported recently for sheep fed *Acacia cianophylla* foliage (Akkari et al., 2008a) or grazing pastures that contained it (Akkari et al., 2008b). Based on the same approach, the role of tannins has also been reported with *Lysiloma latisiliquum* used as a "cut and carry" fodder in goats (Brunet et al., 2008).

6. How to use tropical tanniniferous plants?

The use of tropical TRP could be considered at several levels of complexity but also different levels of control/certainty of the active plant (compound) to be distributed:

- *Browsing TRP-rich rangelands*: Paradoxically, this approach is both the simplest in terms of means of delivery and the most complex with regard to the certainty of achieving the objective. The complexity is directly related to plant biodiversity such that the level of uncertainty in the expected results is difficult to predict. However, this approach can be used by the majority of farmers with access to rangelands rich in TRP. In this approach animals express their ability to select plants for improving their nutrition and health status (self-medication). This implies we need to know how well, and under what circumstances, animals self-medicate (Villalba and Provenza, 2007).
- *Fodder bank for browsing*: The concept is to concentrate a large quantity of TRPs in a small size plantation. The difficulty is to keep animals confined for enough time to ensure TRP intake in the fodder bank is sufficient to obtain the desired results. The option of "cut and carry" and browsing a fodder bank will depend on the "trade-off" between labour for "cut and carry" vs. keeping the fodder bank.
- *Cut and carry supplements*: This method can be more easily incorporated into the activities of a farm. This approach must consider the positive effects originated from the nutritive value and the possible medicinal effect(s) as well as the possible negative effects on feed utilization. Although the level of inclusion of the plant materials can be accurate in the feed, the level of uncertainty in the results could be high. This uncertainty is the result of the variability found among and within tree species.
- *Herbal remedies*: This link could be an alternative to the industrial extractions, though the product is not likely to be as homogeneous and effective. As a result, the level of uncertainty of its effect(s) is higher than that of the next approach. For this link, it would be essential to have a clear methodology to produce a standardized and innocuous product/remedy using locally available plants.

- *Industrial production of plant extracts*: This link would be dominated by the pharmaceutical industry aiming at isolating and exploiting the active compounds in the plant(s) providing a product which is safe, homogeneous and effective for particular or multiple purposes (feed additive, anthelmintic, etc.). The price of this type of approach may render this product accessible only to some commercial farmers.

For the proper application at any level of use it is necessary to define many aspects involved: quality and homogeneity (source of material, species, part of the plant, age, interaction between plants or their components, etc.), the animals involved (species, age, browsing experience, physiological status, etc.), the processes (extraction/purification, dosage, management of the plant, etc.) and the extension work involved in disseminating the different technologies (the message, traditions, adaptation, adoption, etc.).

7. Conclusions

Goats and sheep with browsing experience in the tropical forests show adaptations to exploit such ecological niche. Firstly, they showed preference toward plants with higher digestibility, suggesting that animals select digestible material rather than avoid tannin ingestion. Secondly, both goats and sheep did not increase their intake of TRP fodder with the addition of PEG. Evidence of TBSP in the saliva of both species has been obtained. However, goats were able to eat more TRP than sheep. The capacity to eat TRP by goats and sheep can enable the use of these plants as non-conventional anthelmintics. In general, the information presented in this review widens the potential uses of TRP fodder in small ruminant production systems. However, as pointed out, the results depend on many factors related to the host, the plants and the environment. Therefore, any practical application will need to be at the farm level.

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