Plant secondary metabolites: antiparasitic effects and their role in ruminant production systems

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The purported antiparasitic properties of plant secondary metabolites (PSM) have been the cause of controversy amongst the scientific community. Despite long-standing knowledge of the prophylactic and therapeutic properties of PSM-rich extracts, which comes mainly from ethnoveterinary sources, the scientific evidence of the antiparasitic effects of PSM is inconsistent. In the first part of the present paper the causes of this controversy are addressed, and the evidence available on the antiparasitic effects of PSM is critically examined. The focus is on examples of the antiparasitic activity of PSM against helminth nematodes. The conclusion is that PSM can have antiparasitic properties, which depend on their structure, level of ingestion and availability within the gastrointestinal tract of the animal. The second part is an appraisal of the potential role of PSM for parasite control in ruminant production systems. Despite their antiparasitic properties, PSM consumption does not necessarily have positive consequences in parasitised herbivores, as excessive consumption of PSM can adversely affect herbivore fitness and survival, through their anti-nutritional properties. For this reason it is suggested that the antiparasitic properties of PSM should be assessed at the same time as their anti-nutritional effects. The same measure, e.g. the performance of parasitised hosts, should be used when assessing these properties. The assessment of the costs and benefits suggests that parasitised herbivores can benefit from the long-term consumption of PSM only if the antiparasitic benefits outweigh the anti-nutritional costs of PSM. In addition, it is proposed that parasitised animals might be able to benefit from PSM consumption even if their performance is impaired, as long as the latter is a short-term compromise that leads to long-term benefits.

Bioactive plants: Gastrointestinal parasites: Helminths: Plant secondary metabolites

Gastrointestinal parasitism has been classified as a major health and welfare problem for ruminants. Parasitism, especially by helminth parasites, impairs health by causing inappetance, diarrhoea, anaemia and, in severe cases, death (Table 1). In addition to compromising health and welfare, parasitism impairs productivity and results in poor growth and reproduction, e.g. the reduction in body-weight gain by ≤70% within a few weeks of infection (Coop *et al.* 1982). Since the 1960s, when the first anthelmintic drugs for gastrointestinal parasite control became available, chemo-prophylaxis has been the main method of control. However, the emergence of resistance to anthelmintic drugs, which is now a worldwide phenomenon (Jackson & Coop, 2000), together with the increased awareness of consumers about drug residues that potentially enter the food chain, has stimulated investigation into alternative approaches to the control of gastrointestinal parasitism in ruminants. Alternative approaches to the use of anthelmintics that are currently under investigation include: biological control of nematodes by using nematophagous fungi (Larsen, 1999); breeding livestock for nematode resistance (Gray, 1997); nutrient supplementation of parasitised herbivores (Houdijk *et al.* 2001); development of vaccines against helminths (Smith, 1999); the consumption of bioactive forages (Coop & Kyriazakis, 2001). The purpose of the present paper is to consider further the use of bioactive forages to control parasitism in ruminant production systems. Bioactive forages are those that contain compounds that are active against pathogens; in the case of parasitism, the pathogens are the parasites. Although in many cases the active compounds of the bioactive forages are still unidentified, in those cases

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Table 1. Epidemiologically-important gastrointestinal nematodes of sheep, goats and cattle, their site of infection and main effect on the parasitised host (adapted from Houdijk & Athanasiadou, 2003)

	Site of infection	Nematodes	Consequences of parasitism
Sheep, goats	Abomasum	*Haemonchus contortus*	Anaemia
		Teladorsagia circumcincta	Reduced food intake
	Small intestine	*Nematodirus battus*	Dehydration
		Trichostrongylus colubriformis	Reduced food efficiency
		Trichostrongylus vitrinus	Reduced food efficiency
Cattle	Abomasum	*Haemonchus placei*	Anaemia
		Ostertagia ostertagi	Reduced food intake
	Small intestine	*Cooperia oncophora*	Reduced food efficiency
		Cooperia punctata	

where they have been identified they are plant secondary metabolites (PSM).

As the term implies, PSM are plant compounds that are not strictly essential to the main functions of the plants, such as their growth and reproduction. They have been associated, however, with plant defence mechanisms against herbivory (Harborne, 1999; Karban *et al*. 1999). Plant parts or extracts rich in PSM have been used to combat parasitism for many centuries, and in many parts of the world such products are still used for this purpose (International Institute of Rural Reconstruction, 1994). There is evidence of the antiparasitic effects of PSM against helminths, such as nematodes, and also against protozoa and external parasites (International Institute of Rural Reconstruction, 1994). Despite the long-standing, but mainly anecdotal, evidence of the antiparasitic effects of PSM-rich plants, there is controversy amongst parasitologists, nutritionists and animal scientists about their efficacy. The main reason for this controversy is that there is no generally accepted evidence on the antiparasitic effects of PSM. Thus, before assessing the potential role of PSM for parasite control, it is first necessary to critically evaluate the evidence for the antiparasitic properties of PSM. It will be shown that PSM can exert antiparasitic effects, the extent of which depends on their chemical structure, the level of ingestion and the site of parasitism.

In the second part of the paper it will be argued that even though PSM may have antiparasitic properties, these properties do not necessarily translate into beneficial consequences for parasitised ruminants. The reason is that the majority of PSM also have anti-nutritional properties. While most PSM deter herbivores from ingesting plants, there are other PSM that act as toxins and cause temporary or permanent physiological changes in herbivores (Bernays *et al*. 1989; Harborne, 1999). In order to determine the consequences of PSM consumption, it is proposed that the antiparasitic benefits and the anti-nutritional costs of PSM consumption should be considered together. It will be suggested that future research should include this simultaneous evaluation of the positive and negative effects of PSM; this approach is currently rare. In the final part of the paper the research that is required will be further explored before PSM consumption for parasite control is incorporated into ruminant production systems.

Antiparasitic properties of plant secondary metabolites: fact or fiction?

In ethnoveterinary medicine, which draws inspiration from traditional practice, there seems to be a plant or plant extract suitable for treating each parasitic disease (International Institute of Rural Reconstruction, 1994). For example, seeds of garlic (*Allium sativum*), onion (*Allium cepa*) and mint (*Mentha* spp.) have been used to treat animals that suffer from gastrointestinal parasitism, whereas extracts of the tobacco plant (*Nicotiana tabacum*) have been used to treat the skin of livestock afflicted with the external parasite that causes scabies (Guarrera, 1999). Leaves, dried flowers and oil from *Chenopodium ambrosioides*, a shrub that originated from Central America and has been distributed around the world, have all been used as anthelmintics since the early 1900s (Guarrera, 1999). Table 2 shows examples of plants that have been reported by ethnoveterinary sources to have antiparasitic properties. Although ethnoveterinary reports are the main source for the widely-held perception of the antiparasitic properties of plants, they offer little quantitative evidence of such antiparasitic effects, which would be necessary for the incorporation of such plants in parasite control strategies. The evidence from quantitative studies, which may be either *in vitro* or *in vivo*, and from ethnoveterinary reports is sometimes contradictory (Githiori *et al*. 2002; Ketzis *et al*. 2002). The inconsistency has led to doubt about the antiparasitic effects of PSM. Here, the grounds for this contradiction will be explored. The focus will be on the two issues believed to be the main causes of the scientific confusion: first the apparent conflict between the evidence coming from *in vitro* and *in vivo* studies; second the inconsistent results of *in vivo* studies.

Conflicting evidence between *in vitro* and *in vivo* studies

Scientific evidence supporting the antiparasitic properties of PSM comes mainly from *in vitro* studies. The main advantage of using *in vitro* assays in this context is that purified compounds can be tested; other advantages include their low cost and rapid turnover. Testing purified compounds allows their activity to be reliably quantified without the interference of other plant components or nutrients. *In vitro* assays allow assessment of the effects of

Table 2. Examples of plants that have been reported by ethnoveterinary sources to be effective against gastrointestinal parasites

Plant or forage	Parasite class	Continent of origin	Suspected active compound	Reference
Hedera helix	Cestode	Europe	Saponins	Julien *et al.* (1985)
Punica granatum	Helminth*	Europe	Polyphenols	Guarrera (1999)
Artemisia vulgaris	Helminth*	Europe	Tannins	Guarrera (1999)
Macleaya cordata	Nematode	Asia	Isoquinoline alkaloids	Satou *et al.* (2002)
Paeonia suffriticosa	Nematode	Asia	Gallotannins, condensed tannins	Mohamed *et al.* (2000)
Mallotus philippinensis	Cestode	South-eastern Asia	Glycosides	Akhtar & Ahmad (1992)
Sesbania sesban	Nematode	South-eastern Asia, Africa	Condensed tannins	Ibrahim (1992)
Balanites aegyptiana	Nematode	Africa	Saponins	Ibrahim (1992)
Chenopodium ambrosioides	Nematode	Central America	Ascaridol	Guarrera (1999)

*The parasite class was not identified in these studies.

PSM on the development of parasite larvae, their mobility and their ability to feed. For example, condensed tannin extracts reduce the migration of third-stage larvae of *Trichostrongylus colubriformis*, a sheep nematode of economic importance in temperate climates (Lorimer *et al.* 1996). This finding has been taken as evidence of the anthelmintic effects of this extract. Similarly, *in vitro* use of a commercially-available condensed tannin plant extract causes reduced survival and development of sheep and rat nematode larvae (Athanasiadou *et al.* 2001; Butter *et al.* 2001). Other *in vitro* studies have shown that both purified condensed tannins and terpenoids from several legumes reduce the mobility and consequent migration ability of ovine nematode larvae (Molan *et al.* 2000, 2003). However, *in vitro* testing of the antiparasitic properties of PSM can be criticised as being inappropriate, for two main reasons. First, the majority of *in vitro* assays have been developed for completely different purposes, i.e. the identification of anthelmintic-resistant strains of nematodes and to indicate the efficacy of chemically-synthesised anthelmintic compounds. Although strong correlation has been reported between *in vitro* assays and *in vivo* evidence for anthelmintic efficacy and drug resistance, it is not yet clear whether a similar relationship would also be applicable for PSM testing. Second, *in vitro* assays are mainly performed with the free-living stages rather than the parasitic stages of nematodes, because the former are easier to obtain and the parasitic stages of nematodes cannot be maintained alive outside their host for >24 h, which is often an inadequate period for *in vitro* testing. Thus, it is often questionable whether the *in vitro* assays with pre-parasitic stages are relevant to *in vivo* conditions.

There is some further justification for this criticism, because it is often difficult to reproduce by *in vivo* experimentation the dose-related evidence of the antiparasitic activity of PSM clearly demonstrated *in vitro*. The apparent conflict of evidence may be partly related to a difference in the PSM concentrations used *in vitro* and those achieved *in vivo*. In general, there are two main issues that arise during *in vitro* testing, i.e. the PSM to use and the concentrations to test it at. A source of PSM is chosen usually on the basis of evidence in the literature. Crude or purified extracts from the plant are then used for *in vitro* testing. This procedure may not give results that

are always relevant to the *in vivo* situation. An example is related to the possible antiparasitic properties of the condensed tannins contained in chicory (*Cichorium intybus*). Purified condensed tannins from chicory have shown *in vitro* inhibitory activity against deer nematodes (Molan *et al.* 2003). However, the condensed tannin content of chicory, at approximately 0·5 g/kg DM is very low, similar to that of grass and clover (Barry, 1998). As a consequence, it is unlikely that the concentrations that have been shown to be successful *in vitro* can actually be achieved *in vivo*. Thus, although this example demonstrates the antiparasitic activity of the condensed tannins present in chicory, it is highly unlikely that the antiparasitic effects of chicory observed *in vivo* (Hoskin *et al.* 1999) are actually a result of the condensed tannins it contains.

A new methodology for testing the antiparasitic properties of plants *in vitro* has recently been developed. In contrast to the approach described earlier, there is no need for the initial identification either of the active PSM or of the concentrations to be tested. For example, the inhibitory activity of forages rich in PSM on the feeding and migration ability of an intestinal parasite has been tested *in vitro* by using rumen fluid taken from sheep grazing pure stands of PSM-rich plants (S Athanasiadou, O Tzamaloukas, I Kyriazakis, F Jackson and RL Coop, unpublished results). This material has been used in order to relate the PSM concentrations utilised *in vitro* to those observed *in vivo*. It is appreciated that the use of rumen material for PSM testing may also have its disadvantages. For example, the possible lack of PSM activity in the rumen material does not necessarily mean lack of activity in general, as PSM might become active in the lower parts of the gastrointestinal tract (see p. 634). Despite this limitation, this method seems to have more advantages than the approach described earlier.

The apparent conflict between the evidence from *in vitro* and *in vivo* studies could be a result of the difference in the conditions prevailing in the *in vitro* assays compared with those present in the gastrointestinal tract of grazing herbivores. It could be the case that as a result of the conditions present in the gastrointestinal tract a PSM that has activity *in vitro* could be inactive *in vivo*. An example is a condensed tannin extract that shows strong antiparasitic activity against abomasal nematodes of sheep

in vitro (Athanasiadou *et al.* 2001), but when this extract is tested *in vivo* no effect on the abomasal parasite burden of parasitised animals is observed (Athanasiadou *et al.* 2001). This difference could have arisen because of the difference in the conditions *in vitro* and *in vivo*. Condensed tannins are polyphenolic compounds with a high affinity for proteins (Mueller-Harvey & McAllan, 1992). The majority of condensed tannins present in the abomasum of sheep fed on tannin-rich forages have been found in complexes with protein and are thus probably unavailable for action against parasites (Terrill *et al.* 1994). In the small intestine, on the other hand, the complexes formed between condensed tannins and protein are expected to break down and free the tannins for action against parasites (Hagerman, 1989; Hagerman & Butler, 1991). Support for this explanation is provided by good agreement between the *in vitro* and *in vivo* assessments of the antiparasitic activity of condensed tannins against intestinal nematodes (Athanasiadou *et al.* 2001). It is likely, therefore, that under certain circumstances parasitised animals might not be able to experience the beneficial effects of anthelmintic compounds observed *in vitro* (Fig. 1). In addition, the habitat niche occupied by the parasite in the gastrointestinal tract may reduce the contact between parasite and the PSM. In *in vitro* studies the parasite will always be in direct contact with the PSM. Lack of contact could account for the absence of activity of PSM *in vivo* against certain stages of *Teladorsagia circumcincta*, an abomasal nematode of sheep that usually parasitises the glands rather than the lumen of the abomasum (Urquart *et al.* 1996).

In conclusion, *in vitro* tests can provide a valuable means for large-scale plant screening, when *in vivo* screening would be unrealistic because of time and resource constraints. It is, however, important that *in vitro* results should always be validated *in vivo* before making generalised and firm conclusions on the antiparasitic properties of PSM.
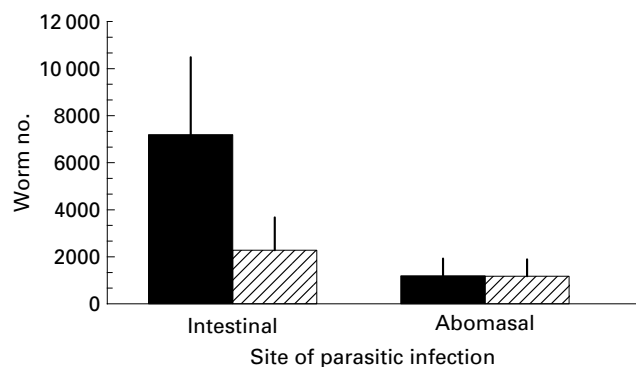
Fig. 1. Total worm population recovered in sheep infected with either an intestinal (*Trichostrongylus colubriformis*) or an abomasal (*Haemonchus contortus*) nematode species on day 1 of experiment. On day 28 sheep were drenched with 80 g commercially-available condensed tannin extract (Quebracho extract)/kg food intake (▨). Undrenched controls are also shown (■). The values are back-transformed means and 95% CI represented by vertical bars. (From Athanasiadou *et al.* 2001.)

The failure of *in vivo* studies to be consistent

Two methods have been used to investigate the antiparasitic effects of PSM *in vivo*. The first method involves indoor supplementation studies. Animals with no previous experience of parasites are infected with a given number of a specific parasite species. They then receive either a PSM supplement or no supplement. In these conditions, in which the level of the parasitic infection, the immune and nutritional state of the animals and particularly the PSM amount consumed are all controlled, the evidence for the antiparasitic properties of PSM is strong and abundant. For example, supplementation of parasitised goats with an extract from *Mallotus philippinensis*, which is rich in glycosides, results in an 80% reduction in the mixed-nematode parasitic burden compared with unsupplemented controls (Akhtar & Ahmad, 1992). Sheep parasitised with an intestinal nematode and given access to a tannin-rich food show a 50% lower nematode egg excretion and parasite worm burden than sheep offered a tannin-free food (Athanasiadou *et al.* 2000). In some cases the antiparasitic efficacy of PSM-rich extracts is similar to that of broad-spectrum anthelmintic drugs; Satrija *et al.* (1994) almost eliminated intestinal nematodes within 7 d of supplementation with PSM.

The second method used to investigate the antiparasitic effects of PSM involves grazing the animals on PSM-rich forages, which is more relevant to normal agricultural practice. It is the conflicting evidence from these studies that casts major doubt on the antiparasitic effects of PSM. For example, sheep grazing on birdsfoot trefoil (*Lotus* spp.; a forage that is rich in condensed tannins) have been shown to reduce their level of parasitism in some studies (Marley *et al.* 2003), but not in others (Niezen *et al.* 1998a,b). Similar inconsistency has been reported when sheep graze sulla (*Hedysarium coronarium*), another tannin-rich forage (Niezen *et al.* 1995, 2002). One of the reasons for the conflicting evidence from grazing studies might be the variable PSM content of the forages. Both PSM concentration and structure can vary between experimental sites and years, and even within the same grazing period (Barry & MacNabb, 1999). These variations occur because PSM production depends on many factors, such as the extent of herbivory, weather conditions and pathogen infestation (Hagerman & Butler, 1991). As the antiparasitic properties of PSM are likely to be related to their structure and content in the plants, the large variability in the antiparasitic effects of PSM observed in grazing studies is likely to be directly related to this variation. The control over the amount of PSM ingested by parasitised herbivores in supplementation studies may result in reduced variability in the antiparasitic activity of PSM and a consequently smaller extent of inconsistency than that seen in grazing studies.

In addition to the variation in PSM content and structure, the evidence from different grazing studies might be seen to be in conflict for artificial reasons, as it may be the outcome of inappropriate comparisons. In grazing studies the levels of parasitism are measured in animals grazing on forages rich in PSM and in controls. The forages may also differ in characteristics other than

their PSM contents. When sheep are grazed on birdsfoot trefoil (expected to have 40–100 g condensed tannins/kg DM according to Barry & Duncan, 1984) and grass with traces of condensed tannins (Niezen *et al*. 1998*b*), the sheep on birdsfoot trefoil show a lower level of parasitism (a lower number of nematode eggs excreted in the faeces) than sheep on grass. As the crude protein content of the birdsfoot trefoil would be expected to be approximately twice that of the grass (Barry & Duncan, 1984), this difference could contribute to the difference in parasitism (Coop & Kyriazakis, 1999). In addition, the difference could be a result of the difference in the levels of condensed tannins or the protein content, or neither of these factors. There are other examples in the literature that may be open to similar interpretation (Niezen *et al*. 1993, 1994; Robertson *et al*. 1995).

The view that the beneficial effects of birdsfoot trefoil in the study of Niezen *et al*. (1998*b*) are a result of its nutritional effects is supported by the work of Niezen *et al*. (1998*a*). They reported that sheep grazing on birdsfoot trefoil show a similar level of parasitism to those grazing on lucerne (*Medicago sativa*; a forage low in tannins). In this case parasitised sheep grazing on birdsfoot trefoil are not expected to have any nutritional advantages compared with those grazing on lucerne, as the protein intake is expected to be similar in both cases. Providing that the content and structure of the condensed tannins in birdsfoot trefoil is similar in both studies, then the lack of a reduction in parasitism in the Niezen *et al*. (1998*a*) study supports the view that previously reported beneficial effects of birdsfoot trefoil (Niezen *et al*. 1998*b*) may have been a result of the nutritional benefits of birdsfoot trefoil rather than the antiparasitic effects of condensed tannins contained in it. In grazing studies it is difficult to draw definite conclusions on the potential antiparasitic properties of PSM, as the contents of other nutrients as well as that of PSM may be variable. Thus, caution is required when comparisons are made between PSM-rich and control forages. The great advantage of supplementation studies is that parasitised animals are given access to foods that can be nutritionally identical, with the exception of their PSM contents. Thus, it is probably more appropriate to draw conclusions on the antiparasitic properties of PSM from supplementation studies rather than from grazing studies.

It is strongly recommended that comparisons across grazing and *in vitro* studies should be made with caution, as they might not always provide reliable information. It would be more appropriate and relevant to relate the antiparasitic properties of PSM to their structure and content across PSM-rich forages and studies. In other words, it would be more informative to suggest that condensed tannins at the x level and y structure contained in birdsfoot trefoil have antiparasitic properties rather than that birdsfoot trefoil has antiparasitic properties. Unfortunately, such essential information is usually absent from the description of PSM-rich forages used in grazing experiments, and thus comparisons are presently made at the level of the forage, rather than the PSM content and chemical structure.

Supplementation studies in which PSM consumption and experimental conditions can be managed have provided convincing evidence of the antiparasitic action of certain PSM. *In vitro* evidence provides further support that PSM such as condensed tannins, alkaloids and glycosides have the ability to exert antiparasitic properties. However, despite their potential positive effects, PSM consumption does not necessarily benefit parasitised herbivores, as their excessive consumption can result in detrimental effects related to their anti-nutritional properties. In the next part of the paper the conditions under which parasitised animals may be able to benefit from PSM consumption will be explored by taking into account both the positive and negative effects of PSM.

Positive and negative consequences of plant secondary metabolite consumption

The antiparasitic activity of PSM could be seen as an alternative way of controlling parasitic infections in agricultural systems. However, in addition to their antiparasitic properties, PSM are better known for their anti-nutritional properties. The consumption of PSM-rich plants by herbivores can result in reduced intake, weight loss, toxicity and death (Milgate & Roberts, 1995; Waghorn & McNabb, 2003). For example, condensed tannin consumption has been shown to be associated with reduced food intake, reduced digestibility and impaired rumen metabolism (Barry & McNabb, 1999; Min *et al*. 2003). Some types of condensed tannins are also responsible for mucosal toxicity and consequently a reduction in nutrient absorption (Reed, 1995; Dawson *et al*. 1999). Saponins have also been considered responsible for reduced food intake and growth impairment (Applebaum & Birk, 1979; Milgate & Roberts, 1995). They have also been associated with haemolytic action and bloat in ruminants. Excessive consumption of alkaloids, glycosides and terpenoids can result in lesions in the nervous system (Conn, 1979; Mabry & Gill, 1979). It is thus evident that in order for PSM to have any role in parasite control it is necessary to ensure that parasitised herbivores can obtain a net benefit from PSM ingestion. For this reason both positive (antiparasitic) and negative (anti-nutritional) effects of PSM consumption should be evaluated based on the same measures. The common measure that can be used for this purpose is the performance of parasitised hosts, because it is expected that both the level of parasitism and PSM consumption will affect host performance.

As a consequence of the antiparasitic effects of PSM, the performance of parasitised herbivores is expected to improve following the consumption of PSM (Coop *et al*. 1982; Sykes, 1994). Thus, the greater the reduction in the level of parasitism, the larger should be the improvement in the performance of parasitised herbivores. Many parasitological studies have taken this 'pharmacological' approach to evaluate the antiparasitic properties of PSM. The main aim of PSM administration has been the achievement of effective parasite control, without taking into account the potential detrimental effects of PSM consumption on host performance (Akhtar & Ahmad, 1992; Ketzis *et al*. 2002; Satrija *et al*. 1994). However, the consumption of PSM can also impair herbivore performance as a result of their anti-nutritional properties. Thus,

Table 3. The consequences of plant secondary metabolite (PSM) consumption on the performance of parasitised herbivores in relation to the performance of non-parasitised herbivores not given access to PSM*

Performance of non-parasitised herbivores (NPH; arbitrary units)	Performance of parasitised herbivores (PH)		Consequences of PSM consumption on parasitised herbivores
	− PSM (% NPH)	+ PSM (% NPH)	
100	80	60	Net cost (A)
100	80	80	Neither cost nor benefit (B)
100	80	81–100	Net benefit (C)

(A), When the performance of herbivores given is lower than that from their PSM-free counterparts, a net cost is expected from PSM consumption; (B), when the performance of PSM and PSM-free herbivores is similar, then parasitised herbivores obtain neither cost nor benefit from PSM consumption; (C), when the performance of PSM herbivores is improved compared with that of PSM-free herbivores, then the parasitised herbivores obtain a net benefit from PSM consumption.

*It is assumed that consumption of PSM will lead to a reduction in parasitism.

PSM can have a role in parasite control only if parasitised herbivores can obtain a net benefit on their performance from PSM ingestion. This net benefit can only be achieved if the antiparasitic (positive) effects of PSM outweigh the anti-nutritional (negative) consequences on host performance.

Whether the antiparasitic effects of PSM can outweigh their anti-nutritional consequences in relation to the performance of the parasitised host depends on the strength of the effects of PSM. Table 3 shows the three possible consequences of PSM consumption by parasitised herbivores. First, if the cost from the anti-nutritional activity of PSM on the performance of parasitised herbivores is greater than the benefit from its antiparasitic activity, then parasitised herbivores are expected to show a net cost, i.e. a reduction in performance, from the PSM consumption (Table 3; case A). For example, two recent studies (Athanasiadou et al. 2000; Butter et al. 2000) have reported that sheep parasitised with T. colubriformis have a lower body-weight gain than their non-parasitised counterparts. Both studies have also shown that parasitised sheep given access to a food that contains condensed tannins reduce their level of parasitism, but also reduce their final body weight by 20 % when compared with parasitised sheep offered access to a tannin-free food. Similarly, the administration of a condensed tannin extract at 80 g/kg food intake to sheep infected with the same nematode results in a reduced level of parasitism, but also in penalties on the performance of sheep when compared with their PSM-free infected counterparts (Athanasiadou et al. 2001). However, when the same condensed tannin extract is administered at 40 g per kg food intake, the level of parasitism is reduced, but the performance of these sheep is not impaired. Thus, in the latter case parasitised sheep obtain neither a benefit nor a cost from PSM consumption in terms of their performance (Table 3; case B). This lack of a net effect on host performance is probably attributable to the fact that the anti-nutritional effects of PSM offset a potential benefit on host performance mediated through the reduced level of parasitism.

On the other hand, if the antiparasitic activity of PSM has greater consequences on the performance of parasitised hosts than its anti-nutritional activity, then parasitised herbivores should be able to obtain a net benefit, i.e. an improved performance, from the consumption of PSM (Table 3; case C). Despite a diligent search of the literature, no examples of supplementation studies have been found to support the latter possibility. In supplementation studies in which performance measurements have been recorded it seems that the performance of parasitised herbivores is either impaired or remains unaltered compared with unsupplemented controls. It is unfortunate that the majority of supplementation studies that have most robustly demonstrated the antiparasitic effects of PSM do not also report the effects of PSM on host performance. Thus, it is not possible to draw useful conclusions on the potential use of PSM for parasite control in ruminants.

In contrast to supplementation studies, there is a large body of evidence suggesting that grazing on PSM-rich forages can result in a reduced level of parasitism and improved performance of parasitised hosts (Niezen et al. 1995, 1998b; Hoskin et al. 1999; Marley et al. 2003). Although parasitised hosts have undoubtedly obtained a benefit following the consumption of PSM-rich forages in the studies mentioned earlier, it is not always clear how this benefit has been achieved. It has already been mentioned that PSM-rich forages are usually leguminous and therefore nutritionally advantageous compared with conventional grazing forages. Consequently, the improved performance of parasitised hosts grazing on such forages could be attributable to either the nutritional benefits or the antiparasitic activity of the PSM-rich forages. The majority of the grazing studies have not been designed to distinguish between the different effects of the PSM-rich forages. Awareness on how such beneficial effects have been achieved would help to improve the understanding of the consequences of PSM consumption and determine the appropriate grazing strategies for incorporating PSM-rich forages for parasite control. The differentiation between the nutritional and antiparasitic effects of PSM-rich forages could be facilitated by the inclusion of parasitised and non-parasitised animals in the same studies. Any differences in the performance of non-parasitised animals grazing on PSM-rich and control forages would provide evidence of the nutritional effects of PSM-rich forages. In addition, as increased nutrient supply during the course of a parasitic infection has been shown to enhance immunity to parasites (Coop & Kyriazakis, 1999; Houdijk et al. 2001), short experimental studies that minimise the interactions between nutrition and immunity would also

assist in distinguishing the antiparasitic from the nutritional effects of PSM-rich forages (Athanasiadou *et al.* 2004; Tzamaloukas *et al.* 2004).

A role for plant secondary metabolite-rich forages in parasite control: future research directions

The role for PSM-rich forages in parasite control could be considered as part of either a short-term or a long-term strategy. In the first case parasitised animals would be provided with PSM-rich forages for short periods of time, in order to reduce their level of parasitism, and then they would return to conventional grazing. This option, in which PSM-rich forages would act as 'de-worming' paddocks, would be extremely attractive if they are effective, as potential adverse effects of PSM consumption on the performance of parasitised hosts would only be for the short term. Indeed, if short-term PSM consumption could reduce the level of parasitism, as has already been the case for certain PSM (Paolini *et al.* 2003; Min *et al.* 2004; Tzamaloukas *et al.* 2004), then potential short-term penalties on animal performance could actually lead to long-term benefits. These long-term benefits would be translated as a lower level of parasitism in the grazing environment, thus lower parasite exposure and greater protection from parasites in subsequent grazing seasons. In addition to animals being transferred to 'de-worming' paddocks for parasite treatment, livestock could also be given the choice of grazing PSM-rich forages whenever they wished. There is already evidence suggesting that non-parasitised grazing animals can distinguish between and avoid plants rich in PSM, probably because of their anti-nutritional properties (Provenza *et al.* 1990; Villalba & Provenza, 2002). However, although no evidence is yet available to indicate whether parasitised domestic herbivores can select a diet rich in PSM in order to combat their parasitic infection (Hutchings *et al.* 2003), this approach remains an attractive possibility (Huffman, 2003). Evidence indicating the ability of animals to select a diet to combat parasitism, i.e. to self medicate, will facilitate the design of sustainable and efficient grazing systems for ruminant production that provide for parasite control.

Second, PSM consumption could also be considered as part of a long-term strategy in which parasitised animals would be given access to PSM-rich forages for most of the grazing season. Long-term grazing on PSM-rich forages could be considered advantageous in cases where short-term PSM consumption is not effective against parasites. However, in order for a long-term approach to be sustainable and beneficial for parasitised animals, the costs of long-term PSM consumption would need to be thoroughly determined. Long-term consequences of PSM consumption have been reported to be more severe than the short-term ones. For example, the consumption of certain PSM for periods longer than a few days seems to be associated with irreversible negative post-ingestive consequences (Reed, 1995; Silanikove *et al.* 2001). In the short term such negative consequences would have been reversible. Such problems arising from the long-term exposure to PSM may be overcome by offering sequentially a combination of PSM-rich forages to parasitised

animals for short periods of time. The combination of more than one PSM-rich forage could minimise penalties to host performance by dilution of the anti-nutritional effects of PSM and could offer even better parasite control through synergistic antiparasitic effects of PSM (e.g. through the effects of different PSM on different parasite species).

To date, ethnoveterinary and 'pharmacological' evidence of the antiparasitic properties of PSM have focused on the medicinal effects of PSM. The findings have led to the identification of active compounds and the synthesis of antiparasitic drugs, such as santonine and ivermectine (Clark, 1996). However, both sources of evidence have so far failed to report and take into account possible detrimental effects of PSM consumption on the performance of parasitised herbivores, attributed to their anti-nutritional properties. In the present paper the need for a holistic approach to the antiparasitic effects of PSM through simultaneous evaluation of the costs and benefits of PSM consumption has been emphasised. This knowledge is important for the incorporation of PSM-rich forages into grazing systems for parasite control. It should be noted, however, that even with this evaluation it is unlikely that complete parasite control can be achieved through the use of PSM-rich forages alone. No evidence available to date suggests complete parasite control as a result of PSM consumption similar to that achieved by the use of antiparasitic drugs, i.e. 100% reduction in parasites without adverse effects on animal performance. As a consequence, the development of non-chemical sustainable parasite control strategies will require a multi-angle approach, in which strategic use of bioactive forages is combined with nutritional control, genetic selection for increased resistance, vaccination, biological control and, whenever inevitable, the use of drugs to combat gastrointestinal parasitism.

Acknowledgement

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