

## Symposium on ‘Plants as animal foods: a case of catch 22?’\*

# The role of plant secondary metabolites in mammalian herbivory: ecological perspectives

Glenn Iason

Macaulay Institute, Craigiebuckler, Aberdeen AB15 8QH, UK

Plant secondary metabolites (PSM) have many ecological functions, but have long been considered as defences against pathogens or herbivores (vertebrate or invertebrate), reducing the likelihood and extent of attack. However, mammalian herbivores ingest many foods containing PSM and use both behavioural methods and physiological strategies to limit their negative effects. Most physiological counter-adaptations are inducible in response to ingested PSM, providing efficient protection against toxic effects. Possible positive effects of PSM include antioxidant and anthelmintic properties and complex formation between protein and condensed tannins that protects dietary protein from degradation by the symbiotic microflora of foregut fermenters, increasing its utilisation by the animal. This protein effect is probably only beneficial to animals under a narrow range of nutrient-rich conditions found mainly in agricultural systems. There are many examples of PSM causing food avoidance or reducing food intake, but there is as yet relatively little evidence for positive selection of them by herbivores. Although the feedback mechanisms relating the post-ingestive consequences of PSM to subsequent foraging behaviour are beginning to be understood, knowledge of the integration of behavioural and physiological strategies for regulating the effects of PSM is relatively poor. The opportunities for learned avoidance of PSM may be restricted in animals with complex diets that cannot associate a particular feedback signal with a given food type. A greater emphasis on the study of subclinical effects of PSM rather than acute effects, on pharmaco-kinetic studies in relation to behavioural studies and on the use of realistic experimental models is advocated.

### Plant secondary metabolites: Toxic effects: Counter-adaptation: Foraging behaviour

#### The evolution and ecology of plant secondary metabolites

Plant secondary metabolites (PSM) have no known function in the primary metabolism of plants. They were formerly considered to be a means of depositing excess C fixed by photosynthesis, but the early suggestion that they mediate a range of specific ecological interactions between species (Fraenkel, 1959) sparked a wealth of subsequent research on these roles (see Rosenthal & Berenbaum, 1991; Appel, 1993). Given the enormous diversity of PSM

and the way in which their structure determines their function, it is unsurprising that these specific effects can also combine to affect broader aspects of ecosystem function. Often the same chemical characteristics of PSM can influence and integrate different aspects of ecosystem function, such as the determination of plant-litter quality via plant–herbivore interactions and the degradation characteristics of that litter (McInnes *et al.* 1992; Pastor & Naiman, 1992).

There are clearly numerous reasons why the range of PSM as a whole could have evolved, but they are unlikely

\*Other papers from this symposium have been published in *Proceedings of the Nutrition Society* (2004), 63.

**Abbreviation:** PSM, plant secondary metabolites.

**Corresponding author:** Dr Glenn Iason, fax +44 1224 311556, email g.iason@macaulay.ac.uk

to have arisen, or to have been maintained, for a single selective evolutionary advantage. Even single compounds may act in many ways. It is widely accepted that many PSM act as plant defences against herbivores (Palo & Robbins, 1991; Rosenthal & Berenbaum, 1991) and pathogens (Kosuge, 1969), and these functions are likely to interact, as wounding by herbivores can predispose plants to pathogenic attack (Hatcher *et al.* 2004). It has been suggested recently that some phenolic PSM protect plants from photo-induced oxidative damage (Close & McArthur, 2002), a function that does not preclude other hypothesised roles, such as defence against herbivores. The hypothesised co-evolutionary arms race between herbivores and PSM (Feeny, 1991) is supported by examination of insect foraging strategies in relation to host plant toxins (Cornell & Hawkins, 2003). Although it is not known whether the evolution of specific PSM, or PSM in general, has been influenced by mammalian herbivory, in some circumstances, such as arctic communities in winter, the main herbivores impacting on the vegetation are mammals (Bryant & Kuropat, 1980), and an effect on the evolutionary fitness of plants seems likely. The two-way relationship between PSM and herbivory over shorter ecologically-relevant time-scales is emphasised by considering plants' responses in their secondary metabolism to herbivore damage. There are two main mechanisms by which herbivory can lead to induced responses in the PSM content of plants. One mechanism is by the tissue removal effecting changes in source–sink relationships for major nutrients and resources within plants such as C and N (Honkanen *et al.* 1999). This mechanism will in some cases lead to phytochemical changes associated with subsequent growth responses that are predictable by the resource limitation or growth–differentiation balance hypotheses for PSM in plants (Bryant *et al.* 1991; Herms & Mattson, 1992). A second mechanism is a direct and short-term response in secondary metabolism to herbivore damage that is mediated by the cascading effects of signalling compounds such as jasmonic acid or salicylic acid (van Dam *et al.* 2003). There has been relatively little work on these chemical responses in the context of mammalian herbivory.

### Plant secondary metabolites and mammalian herbivory

The role of plant toxins in mammalian herbivory has been specifically addressed by Freeland & Janzen (1974), who drew together disparate toxicological observations to predict the impact of PSM on diet selection and the nutrition of herbivorous mammals. Unfortunately, subsequent work attempting to explain the occurrence and distribution of PSM in plants has shifted emphasis away from the chemically-defined entities considered by Freeland & Janzen, (1974) towards consideration of broad groupings of PSM such as condensed tannins or total phenolics. PSM were considered as either 'quantitative' digestibility reducers present in 'apparent' (predictable and readily detectable) plants or 'qualitative' toxins found in 'unapparent' (e.g. ephemeral) plants (Feeny, 1976; Rhoades & Cates, 1976). It is now clear that single PSM can act on mammals as both digestibility reducers and toxins (Foley & McArthur, 1994).

Although the role of dose-dependent 'quantitative' PSM (e.g. condensed tannins that act in the gut as digestibility reducers) has been well studied, the categorising of lower-molecular-weight toxins that are readily absorbed as 'qualitative' or 'non-quantitative' implies that mammals either can or cannot deal with the compounds. This classification has probably diverted the attention of ecologists away from the necessary investigations of the dose dependency of the effects of the 'qualitative' PSM and their role in foraging by mammalian herbivores (Foley *et al.* 1999).

Plant secondary metabolites can act in many ways against mammals, including as toxins or digestibility reducers, modifying their food quality and their foraging behaviour (Palo & Robbins, 1991). They may in some cases be beneficial to mammals (for example, see Athanasiadou & Kyriazakis, 2004). The present review aims to draw together recent developments in the knowledge of how PSM act on mammalian herbivores, and focuses on how their negative effects are counteracted by behavioural and physiological processes. It identifies important current questions and areas of future research, and potentially fruitful approaches to addressing these questions.

### Effects of plant secondary metabolites on mammals

The most successful PSM would render the plant so unpalatable as to be never eaten. However, it is commonly observed that most plants are eaten to some extent despite containing some form of putative chemical or physical defence. So, from the plant's viewpoint PSM may be effective by reducing the probability of herbivory or the amount eaten by a herbivore (Augner, 1994). Similarly, from the mammalian herbivore's viewpoint the main question does not concern avoidance, rather how do herbivores regulate their intake of PSM within limits that are tolerable (Foley *et al.* 1999). The effects of, and counter-adaptations to, PSM are initially considered as physiological and behavioural, although the final analysis suggests that these two interact and should not be separated.

#### *Modes of action of plant secondary metabolites and counteraction by mammals*

*Toxic plant secondary metabolites (negative).* Toxic PSM generally enter the animal via absorption from the gastrointestinal tract and exert their effects via their lipophilicity, causing them to reside in membranes disrupting membrane function, or they enter the cells. Toxic PSM can as a group exert a plethora of negative effects on animals, such as induction of cysts and tissue damage, and ultimately lead to death (Scheline, 1991). For example, some phenolics can be carcinogenic (Singleton, 1981), and damage to the intestinal wall has been shown in small mammals following ingestion of tannic acid (Mitjavila *et al.* 1977; Freeland *et al.* 1985b). Such damage is bound to interact with the metabolic functions of the affected tissue. Pyrrolizidine alkaloids can also be carcinogenic and hepatotoxic (Cheeke, 1988, 1994), and other alkaloids are neurotoxic (Panter *et al.* 1988).

Detoxification in mammals is largely a result of the action of mixed function oxidase enzymes that catalyse

the oxidation of lipophilic compounds. In the 'phase I' reactions the cytochrome P450 enzymes add functional groups to toxins, increasing their polarity and thereby facilitating excretion in solution via the urine or, for large molecules, their return to the gut in the bile (Rozman & Klaassen, 2001). These enzymes are now classified into several subfamilies within which there is broad functional similarity, although specific catalytic characteristics depend on their precise amino acid sequences (Guengerich, 1997). The so-called phase II reactions of detoxification effect the conjugation of the PSM with other molecules to form conjugates that have increased polarity (Scheline, 1991). It is known that the main detoxifying chemical reactions can occur in a range of tissues, including the intestinal epithelium (Powell *et al.* 1974; Smith & Watkins, 1984; Watkins *et al.* 1987; Scheline, 1991), but most of the work on both phase I and phase II reactions in mammals has focused on the endoplasmic reticulum of the liver as the main site of action (Scheline, 1991; Cheeke, 1994). In many cases this focus on liver metabolism is likely to be well founded. However, the lack of consideration of the detoxification functions in the gastrointestinal epithelium is surprising considering that this tissue represents the first tissue to be encountered by ingested PSM. This omission may be a result of the inadequacy of *in vitro* cellular models in which to study the processes (Carrière *et al.* 2001). It has been recently hypothesised that P450 acts in the gut epithelium in concert with P-glycoproteins, an efflux system that actively pumps lipophilic substances from cells, thereby together preventing absorption and further effects of toxins (Hunter & Hirst, 1997; Sorensen & Dearing, 2003). This process implies that faecal excretion of plant secondary metabolites, which is often attributable to gut microbial effects (Boyle & McLean, 2004), or biliary excretion of detoxification products, may also be a result of active processes in the gut wall. This challenging hypothesis remains to be tested.

Although conjugation with a range of moieties is possible, including with glucuronic acid, sulfate, glycine and glutathione (Scheline, 1991), the conjugate considered most often in studies of mammalian herbivory is glucuronic acid, perhaps because it is the easiest to quantify (Foley *et al.* 1999). Many PSM do not undergo phase II reactions, e.g. the eucalypt terpenes that are mainly secreted in an unconjugated form by common brushtail possums (*Trichosurus vulpecula*; McLean *et al.* 1993). When assessing the costs of detoxification, consideration of the range of possible conjugations is essential as the costs vary greatly among them; sulfates are energetically the most costly (Brattsten, 1979). The substrate and other costs of the phase II reactions would be expected to vary with the nutritional status of the animal, e.g. glycine (an amino acid) and glutathione (a tripeptide).

*Pro-oxidants and antioxidants (positive or negative).* Many phenolics can theoretically act against animals that ingest them by being oxidised and generating free radicals such as superoxide and other free radicals that can attack DNA and inactivate enzymes (Appel, 1993). Conversely, some flavonoids such as quercetin can, depending on the conditions, act as antioxidants; their unsaturated C-ring structure scavenges free radicals produced from

normal oxidative metabolic processes, thereby limiting oxidative damage (Kahl, 1991; Hollman & Katan, 1997; Alessio *et al.* 2002). Despite great interest from the medical world because of the possible use of phenolics for treatment and prophylaxis against mutagenesis and carcinogenesis, the possible advantages of phenolics have not been specifically shown to be a determinant of mammalian herbivore foraging behaviour or food intake.

*Digestibility inhibition (negative).* Digestibility inhibition is a well-studied series of processes, in which PSM act within the gut of animals by either forming a complex with a food substrate and preventing degradation, e.g. condensed tannins binding with proteins on mastication, or inhibiting digestive degradation by the animal's endogenous or microbially-derived digestive enzymes, e.g. proteinase inhibitors (Ryan, 1979). The complex interactions of condensed tannins during ingestion and digestion have been comprehensively reviewed elsewhere (Foley *et al.* 1999), and have been shown in particular to reduce apparent N digestibility in all twenty-six studies of foregut- and hindgut-fermenting species of placental mammals in which the diets offered contained condensed tannins. As their name implies hydrolysable tannins can be hydrolysed to aglycones that are readily absorbed and need to be dealt with in the same way as other absorbable toxins and non-tannin phenolics, although they may also possess protein-binding activity (Hagerman *et al.* 1992). Dietary tannins can be counteracted by some herbivores, particularly those whose diet is tannin-rich, by the production of salivary tannin-binding proteins. These have a high affinity for, and preferentially bind with, dietary tannins on mastication, rendering them ineffective in forming complexes with dietary proteins, and reducing their negative effects on protein digestibility (Robbins *et al.* 1991; McArthur *et al.* 1995). Further partial counter-defence against dietary tannins may be provided by microbial populations able to degrade tannin-protein complexes present in the hindgut of some species of herbivore (Osawa & Sly, 1992).

*Protection of protein from microbial degradation (positive).* The same processes that inhibit digestive protein degradation can in some circumstances have positive effects. The binding of condensed tannins with proteins depends on the specific chemical characteristics of both reactants and is pH dependent (Hagerman & Butler, 1981; Hagerman *et al.* 1992). For tannins of certain forage species, at low concentrations, the formation of complexes with protein in the forestomach of ruminants can limit the degradation of protein by rumen bacteria and deliver more essential amino acids for absorption in the small intestine, where the tannin-protein complexes dissociate as a result of the change in pH (Barry & Manley, 1984; Barry *et al.* 1986; Min *et al.* 2003). This process can therefore be viewed as the condensed tannin protecting the protein from microbial degradation in the rumen. However, although this means by which protein by-passes the rumen may be an important advantage in agricultural conditions where the ruminants' diets may be of high quality (protein and energy), these conditions are likely to occur only very rarely in wild, free-ranging or extensively-managed ruminants, which have mixed lower-quality diets.

*Anthelmintic effects (positive).* The positive nutritional role of condensed tannins, proposed to be functional in ruminant livestock, may underlie their suggested effectiveness as anthelmintics. This effect is hypothesised to function either via the enhanced amino acid absorption that increases the effectiveness of the immune system, or via the condensed tannins directly inhibiting parasite larvae (Barry *et al.* 2002; Marley *et al.* 2003; see Athanasiadou & Kyriazakis, 2004). If the effect is a result of a general enhancement of the immune system, then future work may demonstrate positive effects of condensed tannins against other pathogens.

*Depression of the activity of symbiotic microbial populations (negative).* Many herbivorous mammals that lack cellulase enzymes have populations of symbiotic microorganisms in their gastrointestinal tracts, which ferment cellulose to volatile fatty acids that are absorbed and used as an energy source by the host (Van Soest, 1982). The activity of these microbial populations can be depressed by ingested PSM (Wallace, 2004), although microbial populations can adapt to become tolerant to PSM such as hydrolysable tannins (Odenyo & Osuji, 1998). Furthermore, the presence of a foregut fermentation system is hypothesised to represent an important anti-PSM strategy, providing a medium for microbial detoxification of PSM before they reach the animal's tissues (Freeland & Janzen, 1974; Palo, 1987), e.g. the metabolism of pyrrolizidine alkaloids by the rumen microflora of sheep (Cheeke, 1994). However, conversely, in some cases the process of herbivore foraging can render compounds more toxic or harmful; for example, rumen microbial populations can toxify as well as detoxify some PSM, and the hydrolysis of phenolic glycosides in the rumen can increase their toxicity (Smith, 1992). The action of plant or animal enzymes on precursors that are brought together on mastication can yield toxic products; for example, the action of plant cyanogenic glycosides in generating HCN from  $\alpha$ -hydroxynitriles, or the formation of isothiocyanates or nitriles from glucosinolates catalysed by plant myrosinase (Duncan, 1991). The action of the rumen microbiota on S-methyl cysteine sulphoxide in brassicas yields toxic dimethyl disulphide (Earl & Smith, 1982). On the other hand, in some cases the hydrolysis of phenolic glycosides can yield deconjugated sugar moieties that can themselves be fermented (Murray *et al.* 1996).

*Disruption of the sodium mineral balance (negative).* Disruption of the Na mineral balance in response to ingestion of diets rich in PSM has been recorded in a range of lagomorphs (Pehrson, 1983; Reichardt *et al.* 1984, 1990; Iason & Palo, 1991) and mice (Freeland *et al.* 1985*b*). This effect has been attributed to PSM-induced Na wasting via the urine (perhaps a result of impaired kidney function in the lagomorphs), or to greater faecal loss following poor absorption or enhanced salivary or intestinal mucosal cycling of Na to the gut (Freeland *et al.* 1985*b*). Foley *et al.* (1995, 1999) consider the Na loss in lagomorphs to be related to the general phenomenon of acidosis induced by the detoxification of PSM in many species. The phase I and II detoxification reactions generally lead to the formation of organic acids, the excretion of which may

be associated with the loss of cations such as Na that accompanies the excretion of phosphate used to buffer the urinary pH.

*The energy costs of detoxification (negative).* The energy costs of detoxification are hypothesised to be small (Brattsten, 1979), and the effects of PSM on whole-animal energy metabolism have been rarely measured. This shortcoming is surprising given the importance of energy as a common currency in animal foraging studies (Stephens & Krebs, 1986). The energetic effects of infusion of low-molecular-weight phenolics into the gastrointestinal tract of sheep, after controlling for their effects on reduced food intake and digestibility, are quite small (Iason & Murray, 1996). However, infusion into the peripheral circulation shows increased energy metabolism, which has been explained in terms of a toxic effect in the animal tissues (Singleton & Kratzer, 1969). Gallic acid ingestion increases the BMR of voles (*Microtus pennsylvanicus*; Thomas *et al.* 1988), although a depressed energy metabolism following ingestion of some monoterpene PSM has been recorded (Boyle & Dearing, 2003).

*Diuresis (negative).* Diuresis results from the ingestion of the monoterpene  $\alpha$ -pinene and creosote resin by woodrats (*Neotoma* spp.; Dearing *et al.* 2001). This water loss is an integral part of the general processes of detoxification and excretion of PSM, but its importance in the determination of the nutritional ecology of animals occupying water-scarce environments such as deserts or high-latitude zones in winter is unexplored.

#### *Summary of the effects and counter-adaptations to plant secondary metabolites*

The conclusion is that in most cases the effects of PSM are negative rather than positive in relation to mammalian herbivores (Table 1). However, under some special circumstances particular effects of protein protection from digestion, anthelmintic effects or antioxidant effects may be beneficial to the herbivore. It has been suggested that PSM such as tannins and saponins can interact with one another, one nullifying the effect of the other (Freeland *et al.* 1985*a*), although examples of such interactions are scarce in living systems. *In vitro* stimulation of cellulose digestion by phenolics and inhibition by tannins of enzyme-catalysed cyanogenesis from cyanogenic glycosides have been demonstrated and positive nutritional benefits have been hypothesised (Goldstein & Spencer, 1985; Mole & Waterman, 1985). However, their relevance to the nutrition of mammals has not yet been demonstrated. The potentially-beneficial effects of ingestion of PSM must be balanced against the potentially-negative effects of the same compound or set of compounds. The list of potential positive effects of PSM on mammalian herbivores is quite short and as yet relatively unexplored, particularly the anthelmintic and antioxidant functions. This position contrasts starkly with the broad range of benefits from PSM that may be gained by insects, e.g. host finding, location of oviposition sites, phagostimulation and sequestering toxins to protect themselves from predation by higher trophic levels (see Feeney, 1991; Appel, 1993).

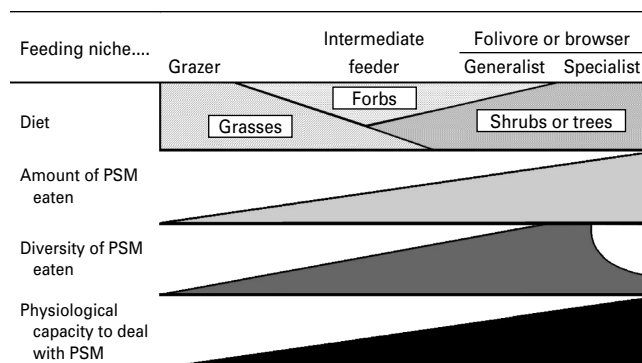
**Table 1.** Summary of negative and positive effects of plant secondary metabolites (PSM) on mammalian herbivores

Negative effects of PSM (toxicity)	Physiological counter-adaptations	Positive effects of PSM
Tissue damage	Detoxification system, e.g. MFO, conjugation and excretion	
Energy expenditure	Detoxification and excretion	
Oxidative damage	Antioxidant system	Antioxidant function
Acidosis	–	
Na imbalance	–	
Diuresis	–	
Digestive inhibition, e.g. substrate binding, enzyme inhibition, antimicrobial effects	Tannin-binding salivary proteins	Anthelmintics Protection of protein Interaction with other PSM

MFO, mixed-function oxidases.

### Comparative studies of plant secondary metabolite–mammal interactions

A framework for understanding the range of nutritional strategies in relation to the feeding strategies of ungulates refers to grazers, intermediate feeders and browsing strategists (Hofmann, 1989), which have an increasing likelihood of ingestion of PSM. However, this classification also applies to a wider range of mammalian species and to their likely responses to PSM in available food (Fig. 1; McArthur *et al.* 1991). There are several examples of negative effects being less or counter-adaptations to PSM being present or better developed among species that commonly ingest food types rich in the particular PSM. Mountain hares (*Lepus timidus*) that commonly browse woody plants do not suffer the extreme urinary Na losses that the European hares (*Lepus europaeus*; a grazer) suffer when ingesting phenolic extracts of birch (*Betula pendula*; Iason & Palo, 1991). The salivary tannin-binding proteins tend to be present in browsing herbivores, but not in



**Fig. 1.** The continuum of mammalian foraging strategies from grazers to browsers and folivores, which have increasing use of plants containing plant secondary metabolites (PSM). (Redrawn from McArthur *et al.* 1991.)

grazing herbivores (Austin *et al.* 1989; Mole *et al.* 1990). Specialist folivorous marsupials have greater liver microsomal metabolism of leaf monoterpenes (Pass *et al.* 2001, 2002) and dietary specialist woodrats maintain lower plasma circulating levels of  $\alpha$ -pinene than generalist woodrats (Sorensen & Dearing, 2003).

### Integration of physiological effects of plant secondary metabolites with foraging behaviour

#### Feedback mechanisms

A hierarchy of routes by which PSM affect foraging behaviour has been identified. These routes range from pre-ingestive stimuli (e.g. taste and smell) to post-ingestive mechanisms that involve feedback from reduced digestibility, or to post-absorptive effects of PSM (Foley *et al.* 1999). The pre-ingestive mechanisms are likely to act rapidly (e.g. via stimulation of the trigeminal nerve) to determine whether or not a food is eaten, whereas the post-ingestive mechanisms would involve a lag before being sensed and take longer to affect foraging. The effects of digestibility reducers such as condensed tannins are probably perceived by the herbivore as a forage of lower quality.

Food intake and diet composition can be modified by herbivores learning to associate negative effects, such as malaise as a result of emetics, with particular food types (Provenza, 1995, 1996), and these conditioned food aversions have also been shown using secondary metabolites (Kyriazakis *et al.* 1997, 1998). A role for previous learning as a determinant of food choices is particularly likely for PSM, since negative stimuli produce stronger learning responses than positive ones (Ginane *et al.* 2004); although it should be remembered that herbivore diet selection is in many cases probably the result of influences of both the nutrient and PSM content of the available foods (Foley *et al.* 1999; Villalba *et al.* 2002). However, although these learning processes are probably effective where stimuli are strongly and clearly associated with a particular food, animals foraging in heterogeneous environments may receive a complex set of temporally-overlapping signals from PSM. This outcome may limit their ability to associate particular stimuli with particular forages and thereby restrict the power of associational learning (Duncan & Young, 2002). It is hypothesised that this effect may be most acute in foregut-fermenting herbivores, in which the direct effects of ingested PSM are buffered by the enlarged forestomach, and in larger herbivores that process PSM more slowly than smaller animals because of their slower mass-specific metabolic rates (Freeland, 1991).

#### Intake regulation

Although some forages are avoided almost completely by herbivores, it has been argued that these situations are rare, and that PSM intake is considered to be regulated to within limits that are tolerable by the herbivore (Provenza, 1996). This form of regulation implies an interaction between behavioural and physiological mechanisms, which are only beginning to be understood. Both ecological responses to PSM and the physiological and pharmacological

mechanisms on which they are based are likely to be threshold phenomena with ranges of sensitivity and tolerance (Appel, 1993). Such a threshold effect has been demonstrated in the case of cattle responses to increasing doses of alkaloids in larkspur (*Consolida ajacis*; Pfister *et al.* 1997). Considerable progress on the key relationships between the concentration of PSM and food intake, the consequent dose of the PSM ingested and identification of thresholds of plasma concentration has recently been made using arboreal marsupial folivores. Development and application of this pharmaco-kinetic approach (Boyle & McLean, 2004) in conjunction with behavioural studies of foraging (Wiggins *et al.* 2003) in this and other appropriate model systems is advocated. In general, as the concentration of a PSM in the forage increases the intake decreases, and where this response has been measured it has been shown to be either linear or curvilinear (Foley *et al.* 1999; Lawler *et al.* 2000; Stapley *et al.* 2000; Boyle & McLean, 2004). However, relatively few other studies have investigated a sufficient range of concentrations to quantify the precise relationship. There is some evidence of temporal cyclicity in the intake response to ingested PSM, i.e. the animals follow periods of ingestion of PSM-containing food with periods of low intake that permit plasma toxin levels to decrease below a threshold level (Pfister *et al.* 1997; Stapley *et al.* 2000).

#### Diet choice

There are many correlative studies that have identified PSM as determinants of diet choice by mammals, but the current view is that the choices made by herbivores reflect both the concentration of PSM and nutrients in available food plants (Whiten *et al.* 1991; McArthur *et al.* 1993; Barton & Whiten, 1994). Examples of positive selection for PSM are rare. However, the preference for natural forages by roe deer (*Capreolus capreolus*) is positively correlated with the presence of protein-binding phenolics (Tixier *et al.* 1997). Although roe deer exhibit lower rates of ingestion of diets containing high concentrations of hydrolysable tannins than a control diet with no tannins, they eat relatively more of an artificial diet to which moderate levels of hydrolysable tannins have been added than the control diet (Verheyden-Tixier & Duncan, 2000). These results probably arise from physiological adaptations of this species to ingestion of tanniferous diets, including the large salivary glands that secrete saliva containing a tannin-nullifying protein (Duncan *et al.* 1998).

A basic tenet of the Freeland & Janzen (1974) hypothesis is that a herbivore's ingestion of a particular food source is limited by its ability to detoxify and excrete specific secondary metabolites. A reduction in intake is likely to lead to a herbivore seeking alternative foods. Several studies support the resultant prediction that herbivores choose mixed diets in order to minimise the effects of particular PSM, thereby avoiding nutritional limitation as a result of saturation of specific detoxification pathways. Both brush-tail possums and lambs are able to eat more food when given the choice of foods with different PSM added than they can when offered no choice or a diet containing only one of the PSM (Wiggins *et al.* 2003; Villalba *et al.* 2004).

It remains a major challenge for future research to translate these known and hypothesised physiological relationships from intake regulation into herbivore food choices under field conditions and to generate behaviourally-based predictable management of plant communities by herbivores (Provenza *et al.* 2002).

#### The costs and benefits of herbivore counter-adaptation to plant secondary metabolites

Generalisations about the costs that detoxification of PSM imposes on animals are difficult, given the diversity of PSM and the range of different metabolic effects of even a single compound. The absolute costs of any one of the physiological avoidance or elimination strategies are likely to be small (Iason & Murray, 1996), but are hypothesised to vary with the nutrient intake, becoming excessive when nutrient intakes are below maintenance levels (Illius & Jessop, 1995). The fact that the cytochrome P450 system and the salivary tannin-binding proteins are inducible on exposure to the PSM stimulus (Mehansho *et al.* 1983; Pass *et al.* 2001) suggests that these systems are efficient, in that their maintenance carries at least some costs that are not borne unless they are necessary, i.e. when the diet contains PSM (Table 1). Regardless of their magnitude, the physiological costs of counter-adaptations should be weighed against their benefits. Animals that merely ingest PSM and bear the effects of reduced digestibility, or chronic or acute toxicity, could suffer major physiological and (evolutionary) fitness costs. The extent of chronic and acute toxicity in wild herbivores is unknown, but acute toxic effects observed in animals typically occur when a system such as an agricultural system contains a non-native animal or plant species that lacks a shared co-evolutionary history. This outcome is a testimony to the efficacy of evolved herbivore counter-adaptations to PSM (Feeney, 1991).

The costs of seeking higher-quality food elsewhere because of the presence of PSM are not easily quantified, but involve increased costs of foraging as a result of greater movement and energy expenditure, as well as the feeding interruptions that reduce the short- and long-term rate of food intake. In addition to compromising nutritional status, if PSM cause greater search times and ranging behaviour, then animals are also likely to incur a greater risk of predation. These hypothesised effects of PSM are poorly quantified in mammals.

#### Acknowledgements

This work was funded by the Scottish Executive Rural Affairs Department and has benefitted from helpful discussions with Bill Foley, Stuart McLean, Juan Villalba and Alan Duncan, who also provided helpful comments on the manuscript.

#### References

- Alessio HM, Hagerman AE, Romanello M, Carando S, Threlkeld MS, Rogers J, Dimitrova Y, Muhammed S & Wiley RL (2002) Consumption of green tea protects rats from exercise-induced oxidative stress in kidney and liver. *Nutrition Research* **22**, 1177–1188.

- Appel HM (1993) Phenolics in ecological interactions: the importance of oxidation. *Journal of Chemical Ecology* **7**, 1521–1552.
- Athanasiadou S & Kyriazakis I (2004) Plant secondary metabolites: antiparasitic effects and their role in ruminant production systems. *Proceedings of the Nutrition Society* **63**, 631–639.
- Augner M (1994) Should a plant always signal its defense against herbivores? *Oikos* **70**, 322–332.
- Austin PJ, Suchar LA, Robbins CT & Hagerman AE (1989) Tannin-binding proteins in saliva of deer and their absence in saliva of sheep and cattle. *Journal of Chemical Ecology* **15**, 1335–1347.
- Barry TN, Hoskin SO & Wilson PR (2002) Novel forages for growth and health in farmed deer. *New Zealand Veterinary Journal* **50**, 244–251.
- Barry TN & Manley TR (1984) The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 2. Quantitative digestion of carbohydrates and proteins. *British Journal of Nutrition* **51**, 493–504.
- Barry TN, Manley TR & Duncan SJ (1986) The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 4. Sites of carbohydrate and protein digestion as influenced by dietary reactive tannin concentration. *British Journal of Nutrition* **55**, 123–137.
- Barton RA & Whiten A (1994) Reducing complex diets to simple rules: food selection by olive baboons. *Behavioural Ecology and Sociobiology* **35**, 283–293.
- Boyle RR & Dearing MD (2003) Ingestion of juniper foliage reduces metabolic rates in woodrat (*Neotoma*) herbivores. *Zoology* **106**, 151–158.
- Boyle RR & McLean S (2004) Constraint of feeding by chronic ingestion of 1,8-cineole in the brushtail possum (*Trichosurus vulpecula*) *Journal of Chemical Ecology* **30**, 757–775.
- Brattsten LB (1979) Biochemical defense mechanisms in herbivores against plant allelochemicals. In *Herbivores: Their Interactions with Plant Secondary Metabolites*, pp. 199–270 [GA Rosenthal and DH Janzen, editors]. New York: Academic Press.
- Bryant JP & Kuropat PJ (1980) Selection of winter forage by subarctic browsing vertebrates: the role of plant chemistry. *Annual Reviews of Ecology and Systematics* **11**, 261–285.
- Bryant JP, Reichardt PB, Clausen TP, Provenza FD & Kuropat PJ (1991) Woody plant-mammal interactions. In *Herbivores: Their Interaction with Secondary Plant Metabolites*. vol. 2 *Ecological and Evolutionary Processes*, pp. 344–370 [GA Rosenthal and MR Berenbaum, editors]. London: Academic Press.
- Carrière V, Chambaz J & Rousset M (2001) Intestinal responses to xenobiotics. *Toxicology in vitro* **15**, 373–378.
- Cheeke PR (1988) Toxicity and metabolism of pyrrolizidine alkaloids. *Journal of Animal Science* **66**, 2343–2350.
- Cheeke PR (1994) A review of the functional and evolutionary roles of the liver in the detoxification of poisonous plants with special reference to pyrrolizidine alkaloids. *Veterinary and Human Toxicology* **36**, 240–247.
- Close DC & McArthur C (2002) Rethinking the role of many plant phenolics – protection from photodamage not herbivores? *Oikos* **99**, 166–172.
- Cornell HV & Hawkins BA (2003) Herbivore responses to plant secondary compounds: a test of phytochemical coevolutionary theory. *American Naturalist* **161**, 507–522.
- Dearing MD, Magnione AM & Karasov WH (2001) Plant secondary compounds as diuretics: an overlooked consequence. *American Zoologist* **41**, 890–901.
- Duncan AJ (1991) Glucosinolates In *Toxic Substances in Crop Plants*, pp. 124–147 [JPF d'Mello, CM Duffus and JH Duffus, editors]. Cambridge: The Royal Society of Chemistry.
- Duncan AJ & Young SA (2002) Can goats learn about foods through conditioned food aversions and preferences when multiple food options are simultaneously available? *Journal of Animal Science* **80**, 2091–2098.
- Duncan P, Tixier H, Hofman RR & Lechner-Doll M (1998) Feeding strategies and the physiology of digestion in roe deer. In *The European Roe Deer: The Biology of Success*, pp. 91–116 [R Andersen, P Duncan and JDC Linnell, editors]. Oslo: Scandinavian University Press.
- Earl CRA & Smith RH (1982) Dimethyl disulphide in the blood of cattle fed on brassicas. *Journal of the Science of Food and Agriculture* **34**, 23–28.
- Feeney P (1991) The evolution of chemical ecology: contributions from the study of herbivorous insects. In *Herbivores: Their Interaction with Secondary Plant Metabolites*. vol. 2, *Ecological and Evolutionary Processes*, pp. 1–44 [GA Rosenthal and MR Berenbaum, editors]. London: Academic Press.
- Feeny PP (1976) Plant apparency and chemical defense. *Recent Advances in Phytochemistry* **10**, 1–40.
- Foley WJ, Iason GR & McArthur C (1999) The role of plant secondary metabolites in the nutritional ecology of mammalian herbivores: how far have we come in 25 years? In *Nutritional Ecology of Herbivores*, pp. 130–209 [H-JG Jung and GC Fahey, editors]. Savoy, IL: American Society of Animal Science.
- Foley WJ & McArthur C (1994) The effects and costs of allelochemicals for mammalian herbivores: an ecological perspective. In *The Digestive System in Mammals: Food, Form and Function*, pp. 370–391 [DJ Chivers and P Langer, editors]. Cambridge: Cambridge University Press.
- Foley WJ, McLean S & Cork SJ (1995) Consequences of biotransformation of plant secondary metabolites on acid-base metabolism in mammals – a final common pathway? *Journal of Chemical Ecology* **21**, 721–774.
- Fraenkel GS (1959) The raison d'être of secondary plant substances. *Science* **129**, 1466–1470.
- Freeland WJ (1991) Plant secondary metabolites: biochemical coevolution with herbivores. In *Plant Defenses Against Mammalian Herbivory*, pp. 61–81 [RT Palo and CT Robbins, editors]. Boca Raton, FL: CRC Press Inc.
- Freeland WJ, Calcott PH & Anderson LR (1985a) Tannin and saponins: interaction in herbivore diets. *Biochemical Systematics and Ecology* **13**, 189–193.
- Freeland WJ, Calcott PH & Geiss D (1985b) Allelochemicals, minerals and herbivore population size. *Biochemical Systematics and Ecology* **13**, 195–206.
- Freeland WJ & Janzen DH (1974) Strategies in herbivory by mammals: The role of plant secondary compounds. *American Naturalist* **108**, 269–287.
- Ginane C, Duncan AJ, Young SA, Elston DA & Gordon IJ (2004) Herbivore diet selectivity on response to simulated variation in nutrient rewards and plant secondary metabolites concentrations of food plants. *Animal Behaviour* (In the Press).
- Goldstein WS & Spencer KC (1985) Inhibition of cyanogenesis by tannins. *Journal of Chemical Ecology* **11**, 847–858.
- Guengerich FP (1997) Comparisons of catalytic selectivity of cytochrome P450 subfamily enzymes from different species. *Chemico-Biological Interactions* **106**, 161–182.
- Hagerman AE & Butler LG (1981) The specificity of proanthocyanidin-protein interactions. *Journal of Biological Chemistry* **256**, 4494–4497.
- Hagerman AE, Robbins CT, Weerasuriya Y, Wilson TC & McArthur C (1992) Tannin chemistry in relation to digestion. *Journal of Range Management* **45**, 57–62.
- Hatcher PE, Moore J, Taylor JE, Tinney GW & Paul ND (2004) Phytohormones and plant-herbivore-pathogen interactions: Integrating the molecular with the ecological. *Ecology* **85**, 59–69.

- Hermes DA & Mattson WJ (1992) The dilemma of plants to grow or defend. *Quarterly Reviews of Biology* **67**, 283–335.
- Hofmann RR (1989) Evolutionary steps of ecophysiological adaptation and diversification of ruminants – a comparative view of their digestive system. *Oecologia* **78**, 443–457.
- Hollman PCH & Katan MB (1997) Absorption, metabolism and health effects of dietary flavonoids in man. *Biomedicine and Pharmacotherapy* **51**, 305–310.
- Honkanen T, Haukioja E & Kitunen V (1999) Responses of *Pinus sylvestris* branches to simulated herbivory are modified by tree sink/source dynamics and by external resources. *Functional Ecology* **13**, 126–140.
- Hunter J & Hirst BH (1997) Intestinal secretion of drugs. The role of P-glycoprotein and related drug efflux systems in limiting oral drug absorption. *Advanced Drug Delivery Reviews* **25**, 129–157.
- Iason GR & Murray AH (1996) The energy costs of ingestion of naturally occurring nontannin plant phenolics by sheep. *Physiological Zoology* **69**, 532–546.
- Iason GR & Palo RT (1991) Effects of birch phenolics on a grazing and a browsing mammal: a comparison of hares. *Journal of Chemical Ecology* **17**, 1733–1743.
- Illius AW & Jessop NS (1995) Modeling metabolic costs of allelochemical ingestion by foraging herbivores. *Journal of Chemical Ecology* **21**, 693.
- Kahl R (1991) Protective and adverse biological actions of phenolic antioxidants. In *Oxidative Stress: Oxidants and Antioxidants*, pp. 245–273 [H Sies, editor]. London: Academic Press.
- Kosuge T (1969) The role of phenolics in the host response to infection. *Annual Reviews of Phytopathology* **7**, 195–222.
- Kyriazakis I, Anderson DH & Duncan AJ (1998) Conditioned flavour aversions in sheep: the relationship between the dose rate of a secondary plant compound and the acquisition and persistence of aversions. *British Journal of Nutrition* **79**, 55–62.
- Kyriazakis I, Papachristou TG, Duncan AJ & Gordon IJ (1997) Mild conditioned food aversions developed by sheep towards flavors associated with plant secondary compounds. *Journal of Chemical Ecology* **23**, 727–746.
- Lawler IR, Foley WJ & Eschler BM (2000) Foliar concentration of a single toxin creates habitat patchiness for a marsupial folivore. *Ecology* **81**, 1327–1338.
- McArthur C, Hagerman AE & Robbins CT (1991) Physiological strategies of mammalian herbivores against plant defences. In *Plant Defenses Against Mammalian Herbivory*, pp. 103–114 [RT Palo and CT Robbins, editors]. Boca Raton, FL: CRC Press Inc.
- McArthur C, Robbins CT, Hagerman AE & Hanley TA (1993) Diet selection by a ruminant generalist browser in relation to plant chemistry. *Canadian Journal of Zoology* **71**, 2236–2243.
- McArthur C, Sanson GD & Beal AM (1995) Salivary proline-rich proteins in mammals: roles in oral homeostasis and counteracting dietary tannin. *Journal of Chemical Ecology* **21**, 663–691.
- McInnes PF, Naiman RJ, Pastor J & Cohen Y (1992) Effects of moose browsing on vegetation and litter of the boreal forest, Isle Royale, Michigan, USA. *Ecology* **73**, 2059–2075.
- McLean S, Foley WJ, Davies NW, Brandon S, Duo L & Blackman AJ (1993) The metabolic fate of dietary terpenes from *Eucalyptus radiata* in the common ringtail possum (*Pseudocheirus peregrinus*). *Journal of Chemical Ecology* **19**, 1625–1643.
- Marley CL, Cook R, Barrett J, Keatlinge R, Lampkin NH & McBride SD (2003) The effect of dietary forage on the development and survival of helminth parasites in ovine faeces. *Veterinary Parasitology* **118**, 93–107.
- Mehansho H, Hagerman A, Clements S, Butler L, Rogler J & Carlson DM (1983) Modulation of proline-rich protein biosynthesis in rat parotid glands by sorghums with tannin levels. *Proceedings of the National Academy of Sciences USA* **80**, 3948–3952.
- Min BR, Barry TN, Attwood GT & McNabb WC (2003) The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. *Animal Feed Science and Technology* **106**, 1–4.
- Mitjavila S, Lacombe C, Carrera C & Derache R (1977) Tannic acid and oxidized tannic acid on the functional state of rat epithelium. *Journal of Nutrition* **107**, 2113–2121.
- Mole S, Butler LG & Iason G (1990) Defense against dietary tannin in herbivores: a survey for proline rich salivary proteins in mammals. *Biochemical Systematics and Ecology* **18**, 287–293.
- Mole S & Waterman PG (1985) Stimulatory effects of tannins and cholic acid on tryptic hydrolysis of proteins: ecological implications. *Journal of Chemical Ecology* **11**, 1323–1332.
- Murray AH, Iason GR & Stewart C (1996) Effect of simple phenolic compounds of heather (*Calluna vulgaris*) on rumen microbial activity in vitro. *Journal of Chemical Ecology* **22**, 1493–1504.
- Odenyo AA & Osuji PO (1998) Tannin-tolerant ruminal bacteria from East African ruminants. *Canadian Journal of Microbiology* **44**, 904–909.
- Osawa R & Sly LI (1992) Occurrence of tannin-protein complex degrading Streptococcus species in feces of various animals. *Systematic and Applied Microbiology* **15**, 144–147.
- Palo RT (1987) Chemical defenses in a woody plant and the role of digestive systems of herbivores. In *Proceedings of the Symposium on Plant-Herbivore Interactions*. USDA Forest Service Intermountain Research Station General Technical Report INT-222, pp. 103–107 [FD Provenza, JT Flinders and ED McArthur, editors]. Ogden, UT: USDA Forest Service Intermountain Region.
- Palo RT & Robbins CT (editors) (1991) *Plant Defenses Against Mammalian Herbivory*. Boca Raton, FL: CRC Press.
- Panter KE, Keeler RF & Baker DC (1988) Toxicoses in livestock from the hemlocks (*Conium* and *Cicuta* spp.). *Journal of Animal Science* **66**, 2407–2413.
- Pass GJ, McLean S, Stupans I & Davies N (2001) Microsomal metabolism of the terpene 1,8-cineole in the common brushtail possum (*Trichosurus vulpecula*), koala (*Phascolarctos cinereus*), rat and human. *Xenobiotica* **31**, 205–221.
- Pass GJ, McLean S, Stupans I & Davies N (2002) Microsomal metabolism and enzyme kinetics of the terpene *p*-cymene in the common brushtail possum (*Trichosurus vulpecula*), koala (*Phascolarctos cinereus*) and rat. *Xenobiotica* **32**, 383–397.
- Pastor J & Naiman RJ (1992) Selective foraging and ecosystem processes in boreal forests. *American Naturalist* **139**, 690–705.
- Pehrson A (1983) Digestibility and retention of food components in caged mountain hares *Lepus timidus* during the winter. *Holarctic Ecology* **6**, 395–403.
- Pfister JA, Provenza FD, Manners GD, Gardner DR & Ralphs MH (1997) Tall larkspur ingestion, can cattle regulate intake below toxic levels? *Journal of Chemical Ecology* **23**, 759–777.
- Powell GM, Miller JJ, Olavesen AH & Curtis CG (1974) Liver as a major organ of phenol detoxication? *Nature* **252**, 234–235.
- Provenza FD (1995) Postingestive feedback as an elementary determinant of food preference and intake in ruminants. *Journal of Range Management* **48**, 2–17.
- Provenza FD (1996) Acquired aversions as the basis for varied diets of foraging ruminants on rangelands. *Journal of Animal Science* **74**, 2010–2020.
- Provenza FD, Villalba JJ & Bryant JP (2002) Foraging by herbivores: linking the biochemical diversity of plants to



- herbivore culture and landscape diversity. In *Landscape Ecology and Resource Management: Linking Theory with Practice*, pp. 387–421 [JA Bissonette and I Storch, editors]. New York: Island Press.
- Reichardt PB, Bryant JP, Clausen TP & Wieland GD (1984) Defense of winter-dominant Alaska paper birch against snowshoe hares. *Oecologia* **65**, 58–69.
- Reichardt PB, Bryant JP, Mattes BR, Clausen TP, Chapin FS & Meyer M (1990) Winter chemical defense of Alaskan balsam poplar against snowshoe hares. *Journal of Chemical Ecology* **16**, 1941–1959.
- Rhoades DF & Cates RG (1976) Toward a general theory of plant antiherbivore chemistry. *Recent Advances in Phytochemistry* **10**, 168–213.
- Robbins CT, Hagerman AE, Austin PJ, McArthur C & Hanley TA (1991) Variation in mammalian physiological responses to a condensed tannin and its ecological implications. *Journal of Mammalogy* **72**, 480–486.
- Rosenthal GA & Berenbaum MR (editors) (1991) *Herbivores: Their Interaction with Secondary Plant Metabolites*. vol. 2, *Ecological and Evolutionary Processes*. London: Academic Press.
- Rozman KK & Klaassen CD (2001) Absorption, distribution, and excretion of toxicants. In *Casarett and Doull's Toxicology: The Basic Science of Poisons*, pp. 107–132 [CD Klaassen, editor]. New York: McGraw-Hill.
- Ryan CA (1979) Proteinase inhibitors. In *Herbivores: Their Interaction with Plant Secondary Metabolites*, pp. 599–618 [GA Rosenthal and DH Janzen, editors]. New York: Academic Press.
- Scheline RR (1991) *Handbook of Mammalian Metabolism of Plant Compounds*. Boca Raton, FL: CRC Press.
- Singleton VI (1981) Naturally occurring food toxicants: phenolic substances of plant origin in common foods. *Advances in Food Research* **27**, 149–242.
- Singleton VI & Kratzer FH (1969) Toxicity and related physiological activity of phenolic substances of plant origin. *Journal of Agricultural and Food Chemistry* **17**, 497–512.
- Smith GS (1992) Toxicification and detoxification of plant compounds by ruminants: an overview. *Journal of Range Management* **45**, 25–30.
- Smith GS & Watkins JB (1984) Biotransformations of xenobiotics in tissues of cattle and sheep. *Canadian Journal of Animal Science* **64**, Suppl., 278–280.
- Sorensen JS & Dearing MD (2003) Elimination of plant toxins by herbivorous woodrats: revisiting an explanation for dietary specialization in mammalian herbivores. *Oecologia* **134**, 88–94.
- Stapley J, Foley WJ, Cunningham R & Eschler B (2000) How well can common brushtail possums regulate their intake of Eucalyptus toxins? *Journal of Comparative Physiology* **170B**, 211–218.
- Stephens DW & Krebs JR (1986) *Foraging Theory*, p. 247. Princeton, NJ: Princeton University Press.
- Thomas DW, Samson C & Bergeron JM (1988) Metabolic costs associated with the ingestion of plant phenolics by *Microtus pennsylvanicus*. *Journal of Mammalogy* **69**, 512–515.
- Tixier H, Duncan P, Scehovic P, Yani J, Gleizes M & Lila M (1997) Food selection by European roe deer (*Capreolus capreolus*): Effects of plant chemistry and consequences for the nutritional value of their diets. *Journal of Zoology* **242**, 229–245.
- van Dam NM, Harvey JA, Wäckers FL, Bezemer TM, van der Putten WH & Vet LEM (2003) Interactions between above-ground phytophages and belowground induced responses against phytophages. *Basic and Applied Ecology* **4**, 63–77.
- Van Soest PJ (1982) *Nutritional Ecology of the Ruminant*. Corvallis, OR: O & B Books.
- Verheyden-Tixier H & Duncan P (2000) Selection for small amounts of hydrolysable tannins by a concentrate-selecting mammalian herbivore. *Journal of Chemical Ecology* **26**, 351–358.
- Villalba JJ, Provenza FD & Bryant JP (2002) Consequences of the interaction between nutrients and plant secondary metabolites on herbivore selectivity: benefits or detriment for plants? *Oikos* **97**, 282–292.
- Villalba JJ, Provenza FD & Guo-Dong Han (2004) Experience influences diet mixing by herbivores: implications for plant biochemical diversity. *Oikos* (In the Press).
- Wallace RJ (2004) Antimicrobial properties of plant secondary metabolites. *Proceedings of the Nutrition Society* **63**, 621–629.
- Watkins JB, Smith GS & Hallford DM (1987) Characterization of xenobiotic biotransformation in hepatic, renal and gut tissues of cattle and sheep. *Journal of Animal Science* **65**, 186–195.
- Whiten A, Bryce RW, Barton RA, Waterman PG & Heinzi SP (1991) Dietary and foraging strategies of baboons. *Philosophical Transactions of the Royal Society (London)* **334B**, 187–197.
- Wiggins NL, McArthur C, McLean S & Boyle R (2003) Effects of two plant secondary metabolites cineole and gallic acid, on nightly feeding patterns of the common brushtail possum. *Journal of Chemical Ecology* **29**, 1447–1464.