Proceedings of the Nutrition Society (2005), **64**, 403–412 DOI:10.1079/PNS2005449

A meeting of the Nutrition Society hosted by the Scottish Section with support from the British Society of Animal Science was held at the Royal Botanic Garden, Edinburgh on 19 April 2004

Symposium on 'Plants as animal foods: a case of catch 22?'*

Biochemistry of plant secondary metabolites and their effects in animals

T. Acamovic† and J. D. Brooker

Avian Science Research Centre, Animal Health Group, Scottish Agricultural College, West Mains Road, Edinburgh EH9 3JG and Avian Science Research Centre, Animal Health Group, Scottish Agricultural College, Ayr KA6 5HW, UK

Plant secondary metabolites, which include a wide variety of phytochemicals, have always been constituents of the diets of man and other animals. Although a high proportion of these phytochemicals have been considered to be of little value in plants (although this view is changing), they have frequently been shown to have adverse effects on animals when ingested. The effects depend to a great extent on the chemistry of the compounds, their concentration in the diet and the amount consumed, and are further dependent on the health status of the animals. Traditionally, most studies of the effects of these compounds on animals have focused on their adverse effects and how to alleviate them. However, recent public concern about the use of synthetic compounds in animal diets to enhance performance and health and welfare issues, coupled with changes in regulations on the use of synthetic medicaments, has stimulated interest and research in the use and effects of phytochemicals in the diets of farmed animals. Phytochemicals vary in their chemistry but can be divided into hydrophilic and hydrophobic compounds, of which a wide variety of polyphenolic and terpenoid compounds, as well as alkaloids, carbohydrates and non-protein amino acids, invoke special interest. The chemistry, biochemistry and mechanisms of action of these compounds in plants and their effects in animals when ingested will be explored.

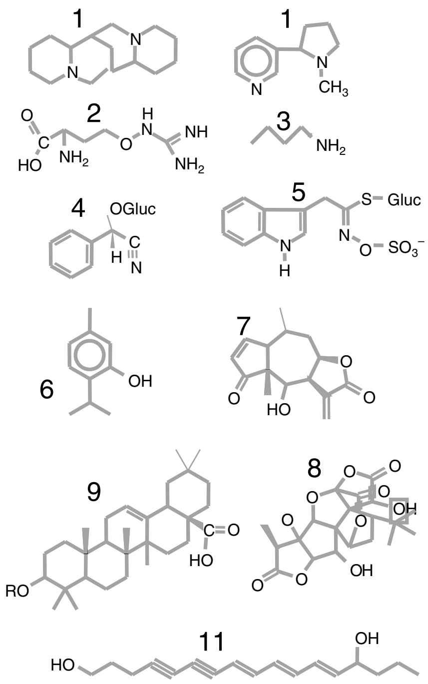
Phytochemicals: Effects in animals

As plant secondary metabolites (PSM) are an extremely large group of compounds, a comprehensive overview of their biochemistry, bioactivity and chemistry is not possible in a relatively short review paper. The importance of PSM in ecology, human foods and animal feeds, and as pharmaceuticals with chemical and biochemical attributes has already been described in some detail (D'Mello & Devendra, 1995; D'Mello, 1997; Brooker, 2000; Harborne, 2001; Pfannhauser *et al*. 2001; Acamovic *et al*. 2004; Nash, 2004). The present paper will discuss some aspects of the biochemistry and chemistry of PSM, referring to some specific compounds in more detail, in particular their occurrence and effects in animal feedstuffs.

PSM, also known as phytochemicals, represent a diverse group of natural products (Harborne, 2001; Wink, 2004), some of which may be nutritionally valuable but many of which have no nutritional value or antinutritional properties. Although precise numbers are at best an estimate, of the ≥100 000 different compounds of natural origin that have been described, ≥80 000 are derived from plants. Many of these compounds have been isolated and their structures have been elucidated using GC, GC–MS, HPLC, HPLC–MS, NMR or X-ray diffraction methods (Harborne, 1993; Wink, 1999*a*,*b*). However, it is likely that in the future many more compounds derived from well-known plant species as well as from more novel plant species will be isolated and identified.

Abbreviations: CT, condensed tannins; GIT, gastrointestinal tract; PSM, plant secondary metabolites.
*Other papers from this symposium have been published in *Proceedings of the Nutrition Society* (2004), **63**, 621–639 and *Proceedings of the Nutrition Society* (2005), **64**, 123–131.
†**Corresponding author:** Dr T. Acamovic, fax +44 1292 525 098, email thomas.acamovic@sac.ac.uk

	No. of natural products
With N	
1. Alkaloids	12 000
2. Non-protein amino acids	600
3. Amines	100
4. Cyanogenic glycosides	100
5. Glucosinolates	100
Without N	
6. Monoterpenes	1 000
7. Sesquiterpenes	3 000
8. Diterpenes	2 000
9. Triterpenes, Saponins, Steroids	4 000
Tetraterpenes	350
10. Flavonoids	2 000
11. Polyacetylenes	1 000
12. Polyketides	750
Phenylpropanes	1 000



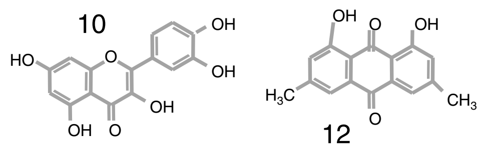


Fig. 1. Estimated range of plant secondary metabolites. Gluc, glucose; R, alkyl group. (Adapted from Wink 2004.)

The increased interest in phytochemicals in animal diets has been prompted by the disapproval and decline in the use of 'in feed' antibiotics, the removal of animal proteins from the diet and thus the increased variety and inclusion levels of vegetable protein sources. Furthermore, PSM in the diets of man could potentially have both beneficial and detrimental effects (Farhan & Cross, 2001; Hollis & Wargovich, 2001; Nash, 2004). Thus, there are increasing numbers of novel plant species and by-products that are being identified and studied for their potential use in the pharmacological, medical and agricultural industries.

A general overview of the range of PSM is shown in Fig. 1 (from Wink, 2004), which includes an estimate of known secondary metabolites and examples of the chemical structures for some of the classes. Frequently, the compounds that have been identified, such as the alkaloids and amino acids, are relatively simple molecules and are present in plants at <10 g/kg (Acamovic *et al.* 2004). However, there are numerous other structurally much more complex compounds that have physiological effects in animals. These compounds, which can be as important as, and in some cases more important than, the simple monomeric compounds, include proteins, peptides, carbohydrates and polyphenols (e.g. tannins). Some carbohydrates, e.g. monosaccharides, oligosaccharides and polysaccharides (e.g. arabinoxylans and β-glucans) are frequently present in plants and can have adverse effects on animals. They may also be considered in some circumstances as PSM and are sometimes present in concentrations >100 g/kg (Bach Knudsen, 1997).

PSM have been extensively studied because of the adverse effects that they have when ingested by animals (Colegate & Dorling, 1994; D'Mello, 1997; Cheeke, 1998; Garland & Barr, 1998; Acamovic *et al.* 2004). However, more recently, the beneficial effects of PSM in animals (and man) have also been investigated (Douglas *et al.* 1995; Kinghorn & Kennelly, 1997; Pfannhauser *et al.* 2001; Cross *et al.* 2004; James *et al.* 2004; Nash, 2004; Bento *et al.* 2005). A classic example of a compound that was initially considered as problematic when consumed by animals is mimosine. When ingested it tends to reduce performance in animals, causes physiological changes and induces alopaecia (Crounse *et al.* 1962; Reis *et al.* 1975). However, the induction of alopaecia has been considered a potentially beneficial effect in some circumstances for chemically defleecing sheep.

While the effects on animals are a function of the nature of the compound, other contributing factors include the concentration in the diet, the amount consumed, the action within the gastrointestinal tract (GIT), absorption, transformation and excretion from the animal.

Biosynthesis and storage of plant secondary metabolites

Biosynthesis of PSM is organ-, cell- or development-specific in almost all higher plant species. In most cases the pathways, and indeed the genes involved in their synthesis, are tightly regulated and may be linked to environmental, seasonal or external triggers. Cellular sites of synthesis are compartmentalised in the plant cell, with the majority of pathways being at least partially active in the cytoplasm. However, there is some evidence that compounds such as alkaloids, quinolizidines, caffeine and some terpenes are synthesised in the chloroplast (Roberts, 1981; Wink & Hartmann, 1982). The biosynthesis of

https://doi.org/10.1079/PNS2005449 Published online by Cambridge University Press

protoberberine occurs in cell vesicles (Amann *et al*. 1986) and coniine and some amines are synthesised in mitochondria (Roberts, 1981; Wink & Hartmann, 1981). The synthesis of lipophilic compounds is usually associated with the endoplasmic reticulum, as are many of the post-synthetic modifications such as hydroxylation.

Although PSM are often detected throughout the plant, their initial site of synthesis is often restricted to a single organ such as roots, fruits or leaves. Thereafter, they can be transported around the plant via the phloem or xylem or by symplastic or apoplastic transport and stored in a number of different tissues. The site of storage often depends on the polarity of the compounds, with hydrophilic compounds such as alkaloids, glucosinolates and tannins being stored in vacuoles or idioblasts, whilst lipophilic compounds such as the terpene-based essential oils are stored in trichomes, glandular hairs, resin ducts, thylakoid membranes or on the cuticle (Wiermann, 1981). For some compounds that are present in the plant as defence barriers, e.g. alkaloids, flavonoids, cyanogenic glycosides, coumarins, storage may be in the epidermis itself (Wiermann, 1981; Wink, 1993; Wink & Roberts, 1998; Harborne, 2001). Storage may be tissue- or cell-specific (Guern *et al*. 1987), with flowers, fruits and seeds being rich sources of many PSM, especially in annual plants. In perennial species PSM are present in high levels in bulbs, roots, rhizomes and bark of the roots and stems.

PSM may not be the end products of metabolism, but may have a regular rate of turn over (Barz & Koster, 1981). N-containing PSM such as alkaloids, cyanogenic glycosides, non-protein amino acids, NSP and protease inhibitors are stored by the plant and are metabolised at germination to serve as N or C sources for the developing seedlings (Wink & Witte, 1985). There is also a turnover of carbohydrates (e.g. oligosaccharides) and lipids during germination. The concentration of some PSM, such as quinolizidine alkaloids and some monoterpenes, have also been shown to vary in a diurnal fashion, suggesting an interplay between synthesis and turnover, and active transcription of the genes involved.

Although PSM have been used for thousands of years in human medicine, for flavouring, as stimulants and hallucinogens, as fragrances in cosmetics and household fresheners and as therapeutic agents, their native function in plants remains contentious (Harborne, 2001). The primary use of many PSM is probably for plant defence. Whereas animals and birds can evade predation by relatively rapid movement, plants do not have this capability, and so have evolved elaborate mechanisms of protection. PSM are generally thought to be present in plants primarily for defence purposes and this view has been extended on the basis of some convincing evidence (Fraenkel, 1959; Erlich & Raven, 1969; Levin, 1976; Harborne, 1993, 2001; Ralphs *et al*. 2004). The proposed functions include: defence against grazing herbivores and insects; defence against micro-organisms, including bacteria, fungi and viruses; defence against other plants competing for nutrients and light; protection against the damaging effect of UV light.

However, PSM perform other roles including: acting as volatile attractants to promote pollination by birds and insects; colouring for the purpose of either camouflage or attraction; signals to promote colonisation by beneficial symbiotic micro-organisms such as mycorrhizal fungi and N-fixing rhizobia. They may also have a possible nutritional role, particularly the N-containing PSM, during germination of seeds. Interestingly, plants also regulate the synthesis and storage of PSM so that the more vulnerable tissues such as fruits and young leaves contain higher concentrations of PSM than senescing tissues (Wink, 2004). The physical location of structures such as trichomes, which serve as the sites of synthesis and storage of essential oils, also provides support for a defence role for these compounds. Trichomes are located on the surfaces of leaves, and are usually the first point of contact for browsers or insect predators. Tannins are usually located in leaf vacuoles beneath the epidermal surface. The volatile nature of the essential oils or the astringent and bitter taste of tannins and alkaloids respectively can be a clear deterrent to predators (Saunders & Conn, 1978; Harborne, 2001; Ralphs *et al*. 2004).

Bioactivity of plant secondary metabolites

The bioactivity of PSM has been described extensively in the literature (Wink, 1993, 1998, 2000; Colegate & Dorling, 1994; D'Mello, 1997; Cheeke, 1998; Garland & Barr, 1998; Acamovic *et al*. 2004). Furthermore, paradoxically, the very evolution of bioactive defence compounds in plants has produced compounds that may have many other beneficial effects in biotechnology, pharmacy and medicine. The structures of many PSM have been shaped to interact with many different molecular and cellular targets, including enzymes, hormone receptors, neurotransmitter receptors and transmembrane transporters, and can thus mimic a response at the corresponding molecular target (Fig. 2). There is hardly any cellular target that some PSM cannot modulate. Thus, plants produce a wide range of bioactive substances, and many of these substances are already in widespread use in the

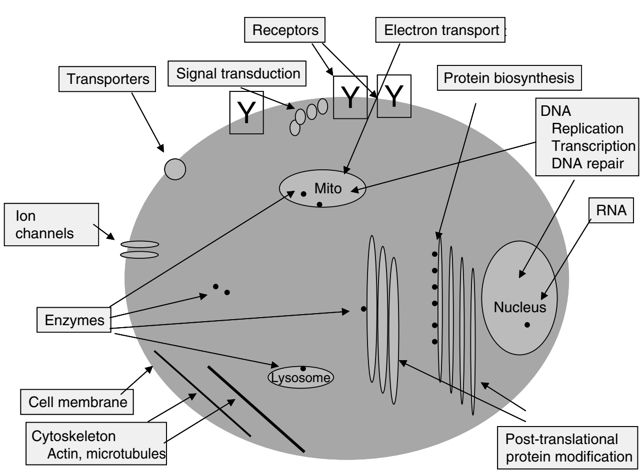


Fig. 2. Summary of potential cellular targets for plant secondary metabolites. Mito, mitochondrion. (Adapted from Wink, 2004.)

https://doi.org/10.1079/PNS2005449 Published online by Cambridge University Press

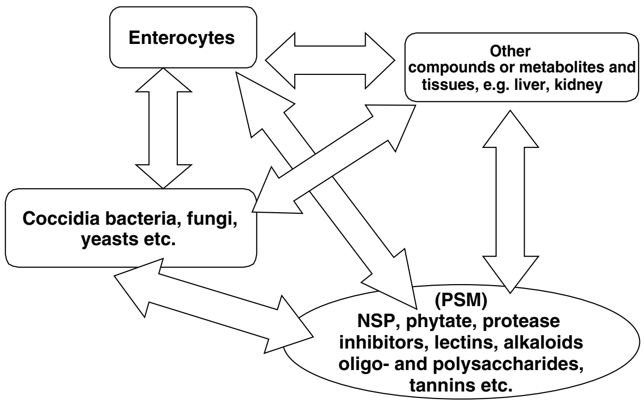


Fig. 3. Interactions between plant secondary metabolites (PSM) and components within animals.

pharmacological, medical and agricultural industries, while others are under development. In many cases PSM, e.g. the terpenoid essential oils, can be more effective than chemically-synthesised pure compounds because they are a complex mixture of components. Their complexity enables the PSM to interact with multiple molecular targets and, thus, it is more difficult for target micro-organisms or herbivores to develop any effective response because resistance at different targets would be required.

Defence against herbivores or pathogens is not necessarily constitutive. Wounding and infection can trigger several events in plants (e.g. the release of glucosinolates or cyanogenic glycosides), which can work together with secreted enzymes (e.g. β-glycosidases) to assist in protecting the plant from attack. Many antibacterial and antifungal compounds (phytoalexins) are produced in plants as a result of infection, and tannins are often produced in response to browsing by herbivores (Baldwin, 1994). However, just as plants have evolved defence mechanisms for their protection, many animals, micro-organisms and parasites have evolved parallel mechanisms to overcome these defences (Hartmann & Witte, 1995). There is considerable interaction between ingested PSM and tissues, enzymes and other compounds within the animal (Fig. 3). The interaction during absorption, deposition and metabolism, and excretion are highly dependent on the physico-chemical attributes of the compounds involved and their susceptibility to transformation. When ingested the relevant PSM can pass through the animal unchanged or combine with bile salts and be excreted in the faeces. They can also interact with tissue and other compounds within the GIT, pass through and be excreted. The original compound can also be either absorbed directly or transformed within the GIT and then absorbed and deposited. If absorbed, the compounds can undergo further transformation, usually to increase hydrophilicity, and then excretion via the kidneys in the urine. This process has been clearly demonstrated for glucosinolates, which are transformed and excreted as mercapturic acids after degradation by myrosinase and conjugation with glycine, cystine and glutamic acid under the influence of the relevant amino acid transferases (Timbrell, 1992).

Physico-chemical factors that are extremely influential in PSM when ingested are: molecular size and architecture; pH of the environment; hydrophilicity; lipophilicity; charge and polarity; ability to form micelles; solubility. Aspects of these factors have been well described and discussed (Timbrell, 1992; Cheeke, 1998; Harborne, 2001); however, it is appropriate to give an overview with some relevant examples. In general, the smaller the molecule and the greater the hydrophilicity, the greater is the likelihood of absorption of the compound from the GIT when ingested.

Cellulose and NSP are well utilised by ruminants (after degradation by fungi and bacteria) but not by poultry. The molecules are too large to be absorbed through the GIT and, in the case of ruminants, are degraded by micro-organisms within the rumen before absorption of the metabolites. The degradation or lack of degradation of such large molecules may alter the microflora within the GIT and may be advantageous or disadvantageous (Spring, 2004; Apajalahti *et al*. 2004). For example, the presence of NSP within the lower GIT of poultry and pigs has been shown to be disadvantageous and can cause gastrointestinal problems by altering the profile of micro-organisms in the GIT. These effects may include changes in the immune response of the animals, which may be productively disadvantageous (Boros *et al*. 2002; Apajalahti *et al*. 2004; Humphrey & Klasing, 2004; Kelly, 2004). Similarly, when poultry are fed different diets with or without extracted *Camelina sativa* meal or with different lupin (*Lupinus* spp.)-seed meals and cereals the microbial ecology of the GIT changes (Cowieson *et al*. 2000; Gilbert *et al*. 2000; Apajalahti *et al*. 2004), as measured by differences in the cytosine:guanidine of the different bacteria. (Apajalahti *et al*. 1998, 2004). In some instances the presence of fructo-oligosaccharides and manan-oligosaccharides, which may be produced within the GIT, has proved to be beneficial in reducing the presence of pathogenic bacteria and improving the health of the GIT. This outcome may be a result of alteration of the pH and/or interference with the ability of micro-organisms to attach to the epithelial tissue within the GIT (Spring *et al*. 2000; Houdijk *et al*. 2002; Chen *et al*. 2003; Spring, 2004).

Proanthocyanidins, although hydrophilic and water soluble, are not absorbed from the GIT and with other tannins they can alter microflora populations, reduce attachment of fungi and bacteria to substrates, increase endogenous losses and damage the GIT in animals (Mansoori & Acamovic, 1998*a*,*b*; McSweeney *et al*. 2001; Bento *et al*. 2005). They have also been shown to interact with parasites within the GIT (Athanasiadou & Kyriazakis, 2004). Hydrolysable tannins undergo ready degradation because of ester linkages to the glucose moiety, and the degradation products are absorbed from the GIT and cause toxicity (Cheeke, 1998). Similarly saponins, which are highly hydrophilic and surface active, can be absorbed either directly or as micelles. These compounds can affect intestinal parasites as well as the epithelial tissue within the GIT (Johnson *et al*. 1986). Amino acids are amphoteric and thus their solubility in aqueous solvents is highly pH dependent; their effects on animals therefore depend on the pH of the environment in

https://doi.org/10.1079/PNS2005449 Published online by Cambridge University Press

which they exist. Similarly, alkaloids tend to have high acid dissociation constants (>7) and their solubility and thus toxicity (or otherwise) is therefore highly dependent on the pH within the GIT. Amino acids such as mimosine and canavanine, which are toxic to animals and man and can influence microbial activity, may be incorporated into protein (Ferraz de Oliveira *et al*. 1994; Harborne, 2001) or degraded (in the case of mimosine) to a more toxic compound. Similarly, pyrizolidine and other alkaloids, as well as glucosinolates and cyanogenic glycosides, are also metabolised to more toxic compounds within the animal (Cheeke, 1998; Acamovic *et al*. 2004). Compounds such as gossypol from cottonseed and erucic acid from rapeseed, and sapogenins from saponin degradation, are lipophilic and thus must be absorbed after micelle formation and by active transport (Oakenfull & Sidhu, 1983; Oakenfull, 1986; Timbrell, 1992; Cheeke, 1998).

After absorption most compounds are transformed in the liver into compounds with more hydrophilic properties and then they are excreted, primarily in the urine (Timbrell, 1992; Cheeke, 1998). Often the more hydrophilic compounds are conjugated with glucose, glucuronic acid or S-containing compounds via glutathione and then excreted (Timbrell, 1992; Cheeke, 1998). The loss of energy and essential nutrients such as the S amino acids and a potential compromise of their antioxidant defences are extremely costly to the animals and result in greater susceptibility to disease (Timbrell, 1992; Bladeren *et al*. 1993; Cheeke, 1998; Humphrey & Klasing, 2004; Kelly, 2004). Tannins and inositol phosphate esters have also been shown to increase endogenous losses from animals, including mineral losses. These losses are likely to occur by chelation of the minerals within the GIT of the animal (Mansoori & Acamovic, 1998*a*; Cowieson *et al*. 2004).

Tannins, which are produced by many woody plants (e.g. *Acacia aneura*), deter browsing by ruminants because of their astringent taste and antinutritive properties (Harborne, 2001). However, in animals adapted to these plants (e.g. feral goats), tannin-binding salivary proteins are secreted by the animal (Landau *et al*. 2000), and many micro-organisms in the intestinal tract are either resistant to the inhibitory effects of tannins or metabolise the tannins and utilise the energy derived for their own growth. Microbial enzymes such as gallate decarboxylase and tannin acyl hydrolase have been reported to be synthesised in many tannin-tolerant micro-organisms in response to exposure to tannins (Skene & Brooker, 1995; O'Donovan & Brooker, 2001). Thus, it would appear that PSM are not inactive waste products of plant metabolism, but are compounds that are bioactive, are produced in response to specific signals and provide an important link between the plant, its potential predators and the environment in which they both live.

The effects seen in animals when PSM are ingested are frequently a result of the structural similarity between the PSM and molecules that occur naturally within the animal, e.g. mimosine and 3,4-dihydroxyphenylalanine, and canavanine and arginine (Fig. 4). Such similarities in structure allow the PSM to interfere in enzyme function and in the synthesis of protein and other essential compounds (Harborne, 2001).

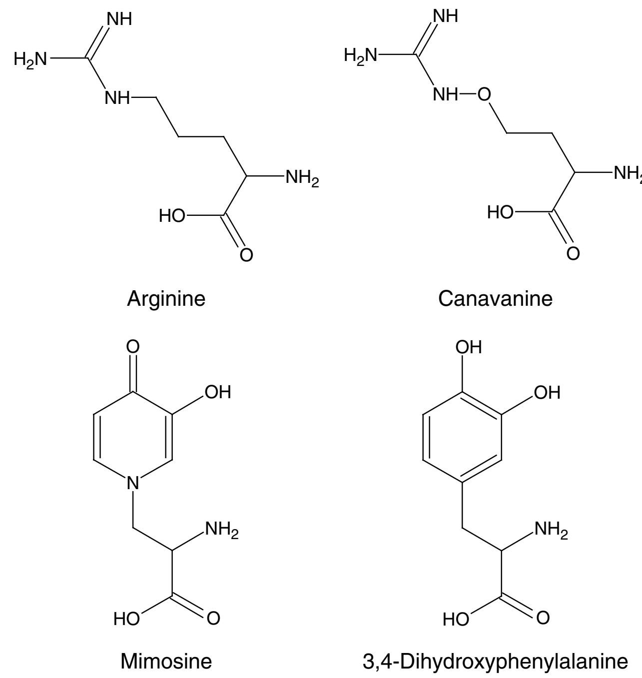


Fig. 4. Structures of some plant secondary compounds that are similar to amino acids found in animals.

Tannins

Tannins derive their main biochemical properties from an ability to interact with and precipitate protein at neutral pH. They are a complex group of water-soluble polyphenolic compounds that have similar physical and chemical properties, and thus react similarly, but to varying extents, with other compounds. Their chemical similarity and susceptibility to O_2 can lead to difficulties in their analytical measurement (Makkar, 2003), which can frequently impede the understanding of their effects in animals.

There are two biosynthetically-distinct classes of tannins, hydrolysable tannins (esters of gallic or ellagic acid and glucose) and condensed tannins (CT; proanthocyanidins), both of which can have antinutritional and toxic properties when consumed by animals (Mansoori & Acamovic, 1998*a*,*b*; Salawu *et al*. 1999; Mupangwa *et al*. 2000, 2003; Harborne, 2001; McSweeney *et al*. 2001; Min *et al*. 2003). Tannins have been observed to have effects within the GIT, but the degradation products of hydrolysable tannins can be absorbed and cause toxicity. However, CT, when present in forages in moderate concentrations (20–40 g/kg DM) can exert beneficial effects on protein metabolism in ruminants, by slowing the rapid microbial degradation of dietary protein and increasing protein outflow from the rumen, thus increasing the absorption of amino acids in the small intestine of the animal. It is clear that the digestibility of the amino acids in the lower gut of ruminants is reduced by the presence of tannins; an effect also seen in single-stomach animals. The improvement in overall digestibility of amino acids is almost entirely a result of the increased flow of protein to the duodenum despite a reduced digestibility coefficient

(Salawu *et al*. 1999; Mupangwa *et al*. 2003). Thus, potentially there could be an increase in lactation, wool growth, reproductive performance and live-weight gain without changing voluntary feed intake (Min *et al*. 2001, 2003). Dietary CT may also contribute to animal health by reducing the detrimental effects of internal parasites in sheep and the risk of bloat in cattle (Niezen *et al*. 1998). In contrast, high dietary CT concentrations (>50 g/kg DM) depress voluntary feed intake, digestive efficiency and animal productivity (Aerts *et al*. 1999).

Plants of the same species can vary in CT content (Koupai-Abyasani *et al*. 1993; Douglas *et al*. 1995; Heering *et al*. 1996; Hedqvist *et al*. 2000) and composition (Foo *et al*. 1982) depending on region and season of growth. *Calliandra calothyrsus* is a shrub legume that occurs throughout the tropics and sub-tropics, and is potentially a valuable livestock forage because of its high protein content and digestibility. However, there are reports of low digestibility for some accessions, but not others, and many researchers have related this disparity to the variable concentrations of tannins (Kumar & Singh, 1984; Ahn *et al*. 1989; Salawu *et al*. 1997, 1999; Mupangwa *et al*. 2000). An evaluation of more than twenty calliandra accessions for their nutritive value and CT content has shown that CT contents range from a trace (<10 g/kg DM), to low (10–30 g/kg DM), medium (40–60 g/kg DM) or high (>60 g/kg DM; Balogun, 1998). In *C. calothyrsus*, as with other browse species, high levels of CT have generally been correlated with low digestibility.

Profiles of calliandra CT, fractionated into monomeric, oligomeric and polymeric components, have been carried out and correlated with DM digestibility data in order to understand the contribution tannins make to the variation in digestibilities across different accessions and develop a model for predicting the effect of tannins on DM digestibility (Rakhmani & Brooker, 2005). There is a negative correlation between oligomers, flavonols and flavonol glycosides and DM digestibility *in vitro*, and a positive correlation between the polymeric proanthocyanidins and DM digestibility *in vitro*. Since these results refer particularly to *in sacco* digestibility of calliandra leaf, they relate mainly to microbial activity in the rumen. Interaction between flavonols and protein is not necessarily excluded because flavonols and oligomers are not associated with the insoluble fraction. However, the interaction is likely to be weak or the complexes are of low molecular size and remain in solution, and may be subject to microbial and enzymic attack in the digestive tract. Nevertheless, it is clear that considerations of CT bioactivity and predictions of forage digestibility should not take account only of total proanthocyanidin levels.

Among non-ruminants some resistance to low-to-moderate concentrations of tannins has been developed, e.g. some insects have thicker peritrophic membranes in the intestinal tract and mice, rats and deer show hypertrophy of the salivary gland, including secretion of tannin-binding proline-rich proteins (Makkar & Becker, 1998). In some feral ruminants, particularly goats and camels, tannin-resistant rumen microbial populations have been described (Brooker *et al*. 1994), with the ability of feral goats and camels to digest tannin-containing forages being ascribed, at least in part, to the action of these micro-organisms. Several tannin-tolerant or tannin-degrading bacterial species have now been isolated from a variety of sources worldwide (McSweeney *et al*. 2001), and the existence of these micro-organisms appears to be a general phenomenon in animals adapted to a diet containing high levels of tannins.

The sensitivity of domesticated ruminants to tannins has been variously described as being a result of the formation of protein–tannin complexes, the inhibition of microbial action in the rumen, the sequestration of minerals in insoluble complexes, or potentially damaging effects on intestinal function (Makkar *et al*. 1995). Decreased voluntary feed intake may be associated with astringency caused by the formation of tannin–salivary protein complexes in the mouth or signals of gut distension resulting from tannin interactions with proteins of the gut wall (D'Mello & Devendra, 1995). Tannins may also inhibit gut enzyme activity and affect gut permeability, causing decreased passage of nutrients through the gut wall (Walton *et al*. 2001). N balance studies have demonstrated an increase in faecal N, often ascribed to undigested complexes between tannins and feed or microbial N. However, in some cases faecal N content is greater than N availability in the feed, and Barry (1989) has suggested a compensatory tannin-induced increase in microbial growth. It has been demonstrated that when tannins or tannin-containing materials are administered orally to chickens endogenous losses are increased substantially, presumably a result of interaction between the tannin and the epithelial tissue within the GIT and also the microflora within the GIT (Muhammed *et al*. 1994; Mansoori & Acamovic, 1998*a*,*b*; Acamovic & Stewart, 2000; Bento *et al*. 2005). This interaction is likely to account, at least in part, for the invariable reduction in apparent digestibility coefficients of N and amino acids, and metabolisable energy found in animals that consume tannins. Increased loss of endogenous material from animals is extremely costly in terms of the energy associated with the synthesis of the compounds that are excreted. However, no studies with ruminants have clearly demonstrated whether faecal N, present in tannin complexes, is derived from forage, micro-organisms or is from endogenous sources.

Tannin–protein interactions are pH dependent and it has been proposed that tannin–protein complexes formed in the rumen are hydrolysed in the abomasum (Hagerman & Carlson, 1998). However, it is not clear whether the hydrolytic products can recomplex with the protein in the neutral–alkaline conditions of the lower gut. If the proteins are not bound, the tannins may have important antinutritive effects by causing changes in intestinal structure and inhibiting nutrient digestion and absorption in the small intestine. While there may be free reactive tannins in the lower GIT that may bind to endogenous proteins, it is clear that there must be some interaction between proteins and tannins in the lower GIT because it is well demonstrated that N digestibility is reduced in the presence of tannin (Salawu *et al*. 1997; Mansoori & Acamovic, 1998*b*; Mupangwa *et al*. 2003; Bento, 2004). When intestinal structure and brush-border enzyme production was investigated in sheep fed a diet of mulga (*Acacia aneura*), striking structural and functional changes were found in the abomasum and small intestine of these animals as compared with animals on control diets (Robins & Brooker, 2005). These data demonstrate the multifunctional effect of tannins, i.e. protein binding by the larger polymers and histopathological effects of the smaller flavonols and flavonol glycosides, while histochemical and biochemical measurements of enzyme activity in the intestine demonstrate a tannin-dependent inhibition of activity.

Essential oils

Aromatic essential oils have been known since antiquity to possess biological activity, including antibacterial, antifungal, antiviral and anti-inflammatory effects. These oils can also be active against higher organisms such as nematodes, helminthes, insects etc. Generally, they have terpenoid structures and their effect is the result of the combination of all their constituents, which in some oils may number >100 compounds. Some constituents in themselves are bioactive, while others may affect physical variables such as absorption rates or bioavailability. In addition, the enantiomeric composition of various terpenes in different plant species can complicate the biological activity of particular oils.

One of the well-established properties of plant essential oils is their antimicrobial activity. They are active against a wide range of organisms, including food-spoilage organisms, potentially-pathogenic microbes of human, environmental or animal origin and some micro-organisms in the GIT of animals. Deans & Ritchie (1987) have tested fifty different plant essential oils against twenty-five genera of bacteria. More than thirty oils were found to be inhibitory to ten or more of the test organisms. Similar studies carried out with essential oils from various aromatic plant species (Piccaglia *et al*. 1993), have shown that the most active components of the oils are thymol, carvacrol, p-cymene, γ-terpinene, 1,8-cineole, *cis*-ocimene, camphor, linalool, terpinene-4-ol, thujone, limonene, α-bisabolol and chamazulene. Antifungal and antioxidant activities of essential oils have also been established (Svoboda & Greenaway, 2003). Thymol has been shown to reduce the numbers of coliforms within the digesta from chickens from about 1×10^{10}/g to about $1\times10^{8\cdot5}$/g (Cross *et al*. 2004). Thymol has also been demonstrated to reduce fermentation by micro-organisms from the GIT in chickens (Shanmugavelu *et al*. 2004), although other work has demonstrated little effect of a mixture of essential oil components (Lee *et al*. 2003). The variability of the effects of such supplements may be highly dependent on the environmental conditions in which animals are maintained.

Many essential oils have been tested for pharmacological and toxicological properties, and many are used as human medicaments. However, there is increasing interest in the potential agricultural importance of these compounds as possible alternatives for the antibiotics that have been used prophylactically in livestock feeds for several decades. Many essential oils have a bacteriocidal effect and could be used to control the digestive microbial ecosystem. Essential oils can affect rumen fermentation and decrease both the rate of deamination of amino acids and the degradation of protein supplements in Dacron bags; the latter effect may be associated with a decrease in the colonisation of substrates by rumen bacteria (Castillejos *et al*. 2005). However, although the essential oils look promising as alternatives to antibiotics, little information is available on the effective dose that can be used in animals without inducing toxic effects or imparting unwanted taints to meat or milk products. More research is needed in this area.

Potential for the use of plant secondary metabolites in agriculture

The present paper describes aspects of the biochemistry and chemistry as well as some of the disadvantages and advantages of PSM. It is obvious from their biochemistry that PSM have a wide range of biological activities and enormous potential for uses in agriculture that requires in-depth investigation and evaluation in the context of domesticated livestock production, particularly now that the use of conventional antibiotics is being reduced or eliminated from the diets of food and fibre-producing livestock. The complexity and breadth of the bioactivity of PSM have the potential to reduce the likelihood that micro-organisms or parasites will develop resistance, and their effectiveness is such that concentrations as low as 0·1 g/kg feed may be sufficient. In some cases they may already be components of feedstuffs that animals eat or can eat. Nevertheless, issues such as toxicity, photosensitivity, residues, taint, allergenicity and cost effectiveness still need to be addressed before these compounds will gain widespread acceptance in the agricultural industries. Furthermore their use as prepared compounds will need to be agreed by the registration authorities within the countries in which they will be used or in which the products from livestock will be sold and consumed.

References

Acamovic T & Stewart CS (2000) Plant phenolic compounds and gastrointestinal micro-organisms. In *Tannins in Livestock and Human Nutrition*, pp. 127–130 [JD Brooker, editor]. Canberra, ACT: ACIAR.

Acamovic T, Stewart CS & Pennycott TW (editors) (2004) *Poisonous Plants and Related Toxins*. Wallingford, Oxon.: CAB International.

Aerts RJ, Barry TN & McNabb WC (1999) Polyphenols and agriculture: beneficial effects of proanthocyanidins in forages. *Agriculture Ecosystems and Environment* **75**, 1–12.

Ahn JH, Robertson BM, Elliott R, Gutteridge RC & Ford CW (1989) Quality assessment of tropical browse legumes: tannin content and protein degradation. *Animal Feed Science and Technology* **27**, 147–156.

Amann M, Wanner G & Zenk MH (1986) Purification and characterisation of (S) tetrahydroberberine oxidase from cultured *Coptis japonica* cells. *Phytochemistry* **37**, 979–982.

Apajalahti J, Kettunen A & Graham H (2004) Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. *World's Poultry Science Journal* **60**, 223–232.

https://doi.org/10.1079/PNS2005449 Published online by Cambridge University Press

Apajalahti J, Sarkilahti LK, Maki RR, Heikkinen JP, Nurminen PH & Holben WE (1998) Effective recovery of bacterial DNA and percent-guanine-plus-cytosine-based analyses of community structure in the gastrointestinal tract of broiler chickens. *Applied Environmental Microbiology* **64**, 4084–4088.

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.

Bach Knudsen KE (1997) Carbohydrate and lignin contents of plant materials used in animal feeding. *Animal Feed Science and Technology* **67**, 319–338.

Baldwin I (1994) Chemical changes rapidly induced by folivory. In *Insect Plant Interactions*, pp. 1–23 [BA Bernays, editor]. Boca Raton, FL: CRC Press.

Balogun RO, Jones JJ & Holmes JH (1998) Digestibility of some tropical browse species varying in tannin content. *Animal Feed Science and Technology* **76**, 77–88.

Barry TN (1989) Condensed tannins. Their role in ruminant protein and carbohydrate digestion and possible effects upon the rumen ecosystem. In *The Roles of Protozoa and Fungi in Ruminant Digestion*, pp. 153–169 [JV Nolan, RA Leng and DI Demeyer, editors]. Armidale, NSW: Penambul Books.

Barz W & Koster J (1981) Turnover and degradation of secondary products. In *The Biochemistry of Plants*. vol. 7, *Secondary Plant Products*, pp. 35–84 [EE Conn, editor]. Orlando, FL: Academic Press.

Bento MHL (2004) The effects of condensed tannins on rumen and post-rumen digestion of nutrients. PhD Thesis, University of Aberdeen.

Bento MHL, Acamovic T & Makkar HPS (2005) The influence of tannin, pectin and polyethylene glycol on attachment of ^{15}N-labelled rumen microorganisms to cellulose. *Animal Feed Science and Technology* (In the Press).

Bladeren PJ, Ploeman J & van Ommen B (1993) Glutathione S-transferase polymorphisms in relation to toxicity. In *Development and Ethical Considerations in Toxicology*, pp. 69–85 [MI Weitzner, editor]. London: Royal Society of Chemistry.

Boros D, Marquardt RR, Guenter W & Brufau J (2002) Chick adaptation to diets based on milling fractions of rye varying in arabinoxylans content. *Animal Feed Science and Technology* **101**, 135–149.

Brooker JD (editor) (2000) *Tannins in Livestock and Human Nutrition*. Canberra, ACT: ACIAR.

Brooker JD, Skene I, Clarke K, Blackall L, Muslera P & O'Donovan L (1994) *Streptococcus caprinus* sp. nov. A tannin resistant ruminal bacterium from feral goats. *Letters in Applied Microbiology* **18**, 313–318.

Castillejos L, Calsamiglia S, Ferret A & Losa R (2005) Effects of a specific blend of essential oil compounds and the type of diet on rumen microbial fermentation and nutrient flow from a continuous culture system. *Animal Feed Science and Technology* **119**, 29–41.

Cheeke PR (1998) *Natural Toxicants in Feeds Forages and Poisonous Plants*. Danville IL: Interstate Publishers.

Chen HL, Li DF, Chang BY, Gong LM, Dia JG & Yi GF (2003) Effects of Chinese herbal polysaccharides on the immunity and growth performance of young broilers. *Poultry Science* **82**, 364–370.

Colegate SM & Dorling PR (editors)(1994) *Plant Associated Toxins*. Wallingford Oxon.: CAB International.

Cowieson, AJ, Acamovic T & Bedford MR (2004) The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. *British Poultry Science* **45**, 101–108.

Cowieson AJ, Sarkilahti LK, Apajalahti JHA, Acamovic T & Bedford MR (2000) Caecal microflora in broilers fed diets containing camelina with and without enzyme supplementation. *Proceedings of XXI World's Poultry Congress*. CD ROM. Montreal, Quebec: World's Poultry Science Association; Canadian Branch.

Cross DE, Hillman K, Fenlon D, Deans SG, McDevitt RM & Acamovic T (2004) Antibacterial properties of phytochemicals in aromatic plants in poultry diets. In *Poisonous Plants and Related Toxins*, pp. 175–180 [T Acamovic, CS Stewart and TW Pennycott, editors]. Wallingford, Oxon.: CAB International.

Crounse RG, Maxwell JD & Blank H (1962) Inhibition of growth of hair by mimosine. *Nature* **194**, 694–695.

Deans SG & Ritchie G (1987) Antibacterial properties of plant essential oils. *International Journal of Food Microbiology* **5**, 165–180.

D'Mello JPF (1997) *Handbook of Plant and Fungal Toxicants*. Boca Raton, FL: CRC Press.

D'Mello JPF & Devendra C (1995) *Tropical Legumes in Animal Nutrition*. Wallingford, Oxon.: CAB International.

Douglas GB, Wang Y, Waghorn GC, Barry TN, Purchas RW, Foote AG & Wilson GF (1995) Liveweight gain and wool production of sheep grazing *Lotus corniculatus* and lucerne (*Medicago sativa*). *New Zealand Journal of Agricultural Research* **38**, 95–104.

Erlich PR & Raven PH (1969) Butterflies and plants: A study of coevolution. *Evolution* **18**, 586–608.

Farhan H & Cross HS (2001) Genistein action in prostrate cancer. In *Biologically Active Phytochemicals in Food*, pp. 28–31 [W Pfannhauser, GR Fenwick and S Kokhar, editors]. London: Royal Society of Chemistry.

Ferraz de Oliveira *et al*. (1994) In *Plant Associated Toxins: Agricultural, Phytochemical and Ecological Aspects*, pp. 195–200 [SM Colegate and PR Dorling, editors]. Wallingford, Oxon.: CAB International.

Foo LY, Jones WT, Porter LJ & Williams VM (1982) Proanthocyanidin polymers of fodder legumes. *Phytochemistry* **21**, 933–935.

Fraenkel G (1959) The raison d'etre of secondary substances. *Science* **129**, 1466–1470.

Garland T & Barr AC (1998) *Toxic Plants and Other Natural Toxicants*. Wallingford, Oxon.: CAB International.

Gilbert C, Sarkilahti LK, Apajalahti JHA, Acamovic T & Bedford MR (2000) The effect of lupin-based diets, with and without enzyme supplementation on microbial populations in caecal digesta analysed by DNA profiling. *Proceedings of XXI World's Poultry Congress*. CD ROM. Montreal, Quebec: World's Poultry Science Association; Canadian Branch.

Guern J, Renaudin JP & Brown CC (1987) The compartmentation of secondary metabolites in plant cell cultures. In *Cell Culture and Somatic Cell Genetics*, pp. 43–76 [F Constabel and I Vasil, editors]. New York: Academic Press.

Hagerman AE & Carlson DM (1998) Biological responses to dietary tannins and other polyphenols. *Recent Research Developments in Agricultural and Food Chemistry* **2**, 689–704.

Harborne JB (1993) *Introduction to Ecological Biochemistry*, 4th ed. London: Academic Press.

Harborne JB (2001) Twenty-five years of chemical ecology. *Natural Product Reports* **18**, 361–379.

Hartmann T & Witte L (1995) Chemistry, biology, and chemoecology of the pyrrolizidine alkaloids. In *Alkaloids; Chemical and Biochemical Perspectives*, vol. 9, pp. 155–233 [SW Pelletier, editor]. Oxford: Pergamon.

Hedqvist H, Harvey Mueller I, Reed JD, Krueger GC & Murphy M (2000) Characterisation of tannins and in vitro protein digestibility of several *Lotus corniculatus* varieties. *Animal Feed Science and Technology* **87**, 41–56.

Heering H, Reed JD & Hanson J (1996) Differences in *Sesbania sesban* accessions in relation to their phenolic concentration and high performance finger prints. *Journal of the Science of Food and Agriculture* **71**, 92–98.
Hollis D & Wargovich MJ (2001) Phytochemicals as modulators of cancer risk. In *Biologically Active Phytochemicals in Food*, pp. 13–20 [W Pfannhauser, GR Fenwick and S Kokhar, editors]. London: Royal Society of Chemistry.
Houdijk JGM, Hartemink R, Verstegen MWA & Bosch MW (2002) Effects of dietary non-digestible oligosaccharides on microbial characteristics of ileal chyme and faeces in weaner pigs. *Archives of Animal Nutrition* **56**, 297–307.
Humphrey BD & Klasing KC (2004) Modulation of nutrient metabolism and homeostasis by the immune system. *World's Poultry Science Journal* **60**, 90–100.
James LF, Panter KE, Gaffield W, Molyneux RJ & Weinzweig J (2004) Poisonous plant research: Biomedical applications. In *Poisonous Plants and Related Toxins*, pp. 361–376 [T Acamovic, CS Stewart and TW Pennycott, editors]. Wallingford, Oxon.: CAB International.
Johnson IT, Gee GM, Price K, Curl C & Fenwick GR (1986) Influence of saponins on gut permeability and active nutrient transport in vitro. *Journal of Nutrition* **116**, 2270–2277.
Kelly D (2004) Regulation of gut function and immunity. In *Interfacing Immunity Gut Health and performance*, pp. 93–105 [LA Tucker and JA Taylor-Pickard, editors]. Nottingham: Nottingham University Press.
Kinghorn AD & Kennelly EJ (1997) Medicinal applications of plant toxins. In *Handbook of Plant and Fungal Toxicants*, pp. 255–268 [JPF D'Mello editor]. Boca Raton, FL: CRC Press.
Koupai-Abyasani MR, Muir AD, Bohm A, Towers GH & Gruber MY (1993) The procyanidin polymers in some species of onobrychis. *Phytochemistry* **34**, 113–117.
Kumar R & Singh M (1984) Tannins their adverse role in ruminant nutrition. *Journal of Agricultural and Food Chemistry* **32**, 377–453.
Landau S, Silanikove N, Nitsan Z, Barkai D, Baram H, Provenza FD & Perevolotsky A (2000) Short-term changes in eating patterns explain the effects of condensed tannins on feed intake in heifers. *Applied Animal Behaviour Science* **69**, 199–213.
Lee KW, Everts H, Kappert HJ, Frehner M, Losa R & Beynen AC (2003) Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. *British Poultry Science* **44**, 450–457.
Levin DA (1976) The chemical defenses of plants to pathogens and herbivores. *Annual Review of Ecology and Systematics* **7**, 121–159.
McSweeney CS, Palmer B, McNeill DM & Krause DO (2001) Microbial interactions with tannins: nutritional consequences for ruminants. *Animal Feed Science and Technology* **91**, 83–93.
Makkar HPS (2003) *Quantification of Tannins in Tree and Shrub Foliage*. Dordrecht, Germany: Kluwer Academic Publishers.
Makkar HPS & Becker K (1998) Adaptation of cattle to tannins: role of proline-rich proteins in oak-fed cattle. *Animal Science* **67**, 277–281.
Makkar HPS, Blummel M & Becker K (1995) Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins, and their implication in gas production and true digestibility in in vitro techniques. *British Journal of Nutrition* **73**, 897–913.
Mansoori B & Acamovic T (1998*a*) The excretion of minerals from broilers fed tannic acid, polyethylene glycol and protein. In *Toxic Plants and Other Natural Toxicants*, pp. 101–105 [T Garland and AC Barr, editors]. Wallingford, Oxon.: CAB International.
Mansoori B & Acamovic T (1998*b*) The influence of tannic acid on amino acid digestibility by broilers. In *Toxic Plants and Other Natural Toxicants*, pp. 106–110 [T Garland and AC Barr, editors]. Wallingford, Oxon.: CAB International.
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**, 3–19.
Min BR, Fernandez JM, Barry TN, McNabb WC & Kemp PD (2001) The effect of condensed tannins in *Lotus corniculatus* upon reproductive efficiency and wool production in ewes during autumn. *Animal Feed Science and Technology* **92**, 185–202.
Muhammed S, Stewart CS & Acamovic T (1994) The effect of tannic acid on cellulose degradation, adhesion and enzymatic activity of rumen microo-organisms. *Proceedings of the Society of Nutritional Physiology* **3**, 174–176.
Mupangwa JF, Acamovic T, Topps JH, Ngongoni NT & Hamudikuwanda H (2000) Content of soluble and bound condensed tannins of three tropical herbaceous forage legumes. *Animal Feed Science and Technology* **83**, 139–144.
Mupangwa JF, Ngongoni NT, Topps JH, Acamovic T & Hamudikuwanda H (2003) Rumen degradability and post-ruminal digestion of dry matter, nitrogen and amino acids in three tropical forage legumes estimated by the mobile nylon bag technique. *Livestock Production Science* **79**, 37–46.
Nash RJ (2004) Remedies from nature. *Chemistry World* **7**, 20–23.
Niezen JH, Waghorn GC & Charleston WAG (1998) Establishment and fecundity of *Ostertagia circumcincta* and *Trichostrongylus colubriformis* in lambs fed lotus (*Lotus pedunculatus*) or perennial ryegrass (*Lolium perenne*). *Veterinary Parasitology* **78**, 13–21.
Oakenfull DG (1986) Aggregation of saponins and bile acids in aqueous solution. *Australian Journal of Chemistry* **39**, 1671–1683.
Oakenfull DG & Sidhu GS (1983) A physico-chemical explanation for the effects of dietary saponins on cholesterol and bile salt metabolism. *Nutrition Reports International* **27**, 1253–1259.
O'Donovan L & Brooker JD (2001) Extracellular polysaccharide biosynthesis as a mechanism of tannin resistance in *Streptococcus caprinus*. *Microbiology* **147**, 1025–1033.
Pfannhauser W, Fenwick GR & Kokhar S (editors) (2001) *Biologically Active Phytochemicals in Food*. London: Royal Society of Chemistry.
Piccaglia R, Marotti M, Giovanelli E, Deans SG & Eaglesham E (1993) Antibacterial and antioxidant properties of Mediterranean aromatic plants. *Industrial Crops and Products* **2**, 47–50.
Rakhmani S & Brooker JD (2005) Composition of condensed tannins from *Calliandra calothyrsus* and correlation with *in sacco* digestibility. *Animal Feed Science and Technology* (In the Press).
Ralphs MH, Gardner DR & Pfister JA (2004) Toxophenology and grazing risk models of tall larkspur. In *Poisonous Plants and Related Toxins*, pp. 575–581 [T Acamovic, CS Stewart and TW Pennycott, editors]. Wallingford, Oxon.: CAB International.
Reis PJ, Tunks DA & Hegarty MP (1975) Fate of mimosine administered orally to sheep and its effectiveness as a defleecing agent. *Australian Journal of Biological Science* **28**, 495–501.
Roberts MF (1981) Enzymic synthesis of coniceine in *Conium maculatum* chloroplasts and mitochondria. *Plant Cell Reports* **1**, 10–13.

Robins C & Brooker JD (2005) The effects of *Acacia aneura* feeding on abomasal and intestinal structure and function in sheep. *Animal Feed Science and Technology* (In the Press).

Salawu M, Acamovic T, Stewart CS, Hovell DdeB & McKay I (1997) Assessment of the nutritive value of *Calliandra calothyrsus*: *in sacco* degradation and *in vitro* gas production in the presence of quebracho tannins with and without browse. *Animal Feed Science and Technology* **69**, 207–218.

Salawu M, Acamovic T, Stewart CS, Hvelplund T & Weisbjerg MR (1999) The disappearance of dry matter, amino acids and proanthocyanidins in the gastrointestinal tract from different fractions of calliandra leaves. *Animal Feed Science and Technology* **79**, 289–300.

Saunders GA & Conn EE (1978) Presence of the cyanogenic glycoside dhurrin in isolated vacuoles from sorghum. *Plant Physiology* **61**, 154–157.

Shanmugavelu S, Brooker JD, Acamovic T & Cowieson AJ (2004) Effect of thyme oil and garlic powder on microbial fermentation in various sections of the gastrointestinal tract of broilers. *British Poultry Science* **45**, S9–S10.

Skene I & Brooker JD (1995) Characterisation of the tannin acylhydrolase in the ruminal bacterium *Selenomonas ruminantium*. *Anaerobe* **1**, 321–327.

Spring P (2004) Impact of mannan oligosaccharides on gut health and pig performance. In *Interfacing Immunity Gut Health and Performance*, pp. 93–105 [LA Tucker and JA Taylor-Pickard, editors]. Nottingham: Nottingham University Press.

Spring P, Wenk C, Dawson KA & Newman KE (2000) The effects of dietary mannanoligosaccharides on cecal parameters and the concentration of enteric bacteria in the ceca of salmonella-challenged broiler chicks. *Poultry Science* **79**, 205–211.

Svoboda KP & Greenaway RI (2003) Investigation of volatile oil glands of *Satureja hortensis* L. (summer savory) and phytochemical comparison of different varieties. *International Journal of Aromatherapy* **13**, 196–202.

Timbrell JA (1992) *Principles of Biochemical Toxicology*. London: Taylor Francis Ltd.

Walton JP, Waghorn GC, Plaizier JC, Birtles M & McBride BW (2001) Influence of condensed tannins on gut morphology in sheep fed *Lotus pedunculatus*. *Canadian Journal of Animal Science* **81**, 605–607.

Wiermann R (1981) Secondary plant products and tissue differentiation. In *The Biochemistry of Plants*, vol. 7, pp. 85–116 [EE Conn, editor]. New York: Academic Press.

Wink M (1993) The plant vacuole – a multifunctional compartment. *Journal of Experimental Botany* **44**, Suppl., 231–246.

Wink M (1998) Mode of action of alkaloids. In *Alkaloids: Biochemistry Ecology and Medicinal Applications*, pp. 265–298 [MF Roberts and M Wink, editors]. New York: Plenum Press.

Wink M (editor) (1999*a*) Introduction: Biochemistry, role and biotechnology of secondary metabolites. In *Biochemistry of Plant Secondary Metabolism Annual Plant Reviews*, vol. 2, pp. 1–15. Boca Raton, FL: CRC Press.

Wink M (editor) (1999*b*) Introduction: Biochemistry, role and biotechnology of secondary metabolites. In *Biochemistry of Plant Secondary Metabolism Annual Plant Reviews*, vol. 3. pp. 1–16. Boca Raton, FL: CRC Press.

Wink M (2000) Interference of alkaloids with neuroreceptors and ion channels. In *Bioactive Natural Products*, vol. 11, pp. 3–129 [Atta-Ur-Rahman, editor]. Amsterdam: Elsevier.

Wink M (2004) Evolution of toxins and antinutritional factors in plants with special emphasis on Leguminosae. In *Poisonous Plants and Related Toxins*, pp. 1–25 [T Acamovic, CS Stewart and TW Pennycott, editors]. Wallingford, Oxon.: CABI publishing.

Wink M & Hartmann T (1981) Properties and subcellular localisation of L-alanine aldehyde aminotransferase: Concept of an ubiquitous plant enzyme involved in secondary metabolism. *Zeitschrift für Naturforschung* **36**c, 625–632.

Wink M & Hartmann T (1982) Localisation of the enzymes of quinolizidines alkaloid biosynthesis in leaf chloroplasts of *Lupinus polyphyllus*. *Plant Physiology* **70**, 74–77.

Wink M & Roberts MF (1998) Compartmentation of alkaloid synthesis, transport and storage. In *Alkaloids: Biochemistry Ecology and Medicinal Applications*, pp. 239–258 [MF Roberts and M Wink, editors]. New York: Plenum Press.

Wink M & Witte L (1985) Quinolizidine alkaloids as nitrogen source for lupin seedlings and cell suspension cultures. *Zeitschrift für Naturforschung* **40**c, 767–775.