Natural bioactive compounds for livestock health and production

Knowledge and Opportunity Audit

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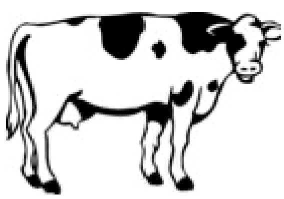

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EXECUTIVE SUMMARY

Plants have been used throughout history for their medicinal properties. This use has often focused on human health but plants have also been, and still are, applied in ethnoveterinary practice and animal health management.

In recent times the use of synthetic chemicals has become prevalent. Public awareness of the potential environmental and health risks associated with heavy chemical use has also increased. This has put pressure on regulatory bodies to reduce the use of chemicals in agriculture. The most striking example of this is the 2006 banning of antibiotics in animal feed by the European Union. Moves such as this has increased the drive to find alternatives to synthetic chemicals and research has again turned to the use of plant bioactives as a means of improving animal health.

Literature evidence suggests that there is the potential to use plants to enhance animal health in general and ruminants in particular. There are certain areas of animal health research where the focus has been on ruminant-specific production issues. Active areas of research for plant bioactives and ruminant health include feed intake and behaviour, wool growth, carcass composition, milk yield, reproductive efficiency, foam production/bloat control, methane production and nematode control.

There are a number of ways to approach bioactive discovery and this report discusses several strategies. Plants and their bioactives can also be delivered in numerous ways including, in situ grazing, supplemental feeding (eg. hay, silage) and as a drench (with the bioactives in various degrees of purity). The best mode of delivery is difficult to predict a priori and will depend on factors such as palatability, efficacy, toxicity and the levels of bioactives that are produced by the plants.

Much of the research carried out to date focuses on temperate plants, usually of European origin. The Australian native flora is an untapped resource that may provide an envrionmentally sensible and ecologically responsible solution to several ruminant health issues.

Key recommendations from this report are:

- The greatest value to the Australian producer would come through the development of plant bioactives for nematode control and bloat reduction.
- Native plant libraries, along with waste streams from key agricultural crops, should be investigated for bioactivity in these areas.

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1 Introduction

Plants produce an astounding array of organic chemicals with enormous structural diversity. Many of the phytochemicals are essential for plant growth and development and are sources of food for animals. Others have evolved in response to ecological pressure. These biologically active compounds are necessary for the plants that produce them but also for humans and other animals who have exploited them in many ways (Brielmann et al, 2006). This report focuses on the use of plants for their medical or pharmaceutical properties with a particular focus on plants that may be utilised for ruminant animal health.

Natural products, in various forms have been used in human and animal medication, for millennia. Ethnopharmacognsy is an example of how native peoples utilise plants for cures, and in many cases the treatments have been validated by modern science (e.g. artmesia for malaria, willow bark for fever (asprin)). In modern pharmaceutics natural products continue to play an important role. According to a recent survey by David J. Newman, Gordon M. Cragg, and Kenneth M. Snader of the National Cancer Institute (2003), 61% of the 877 small-molecule new chemical entities introduced as drugs worldwide during 1981–2002 can be traced to or were inspired by natural products. These include natural products (6%), natural product derivatives (27%), synthetic compounds with natural-product-derived pharmacophores (5%), and synthetic compounds designed on the basis of knowledge gained from a natural product (that is, a natural product mimic; 23%). In certain therapeutic areas, the productivity is higher: 78% of antibacterials and 74% of anticancer compounds are natural products or have been derived from, or inspired by, a natural product. The majority of these natural chemicals have been validated in animal models (generally rodent) but ruminant growers have subsequently taken up many, e.g. the β-lactam antibiotics.

There is increasing interest in, and research on, alternative bioactives for livestock both for general animal health and for specific problems such as methane production and bloat. A recent publication demonstrated the utility of lovastatin (a microbial natural product) to inhibit the in vitro growth and production of methane in strains of *Methanobrevibacter* isolated from the rumen. The compound did not inhibit growth of species of rumen bacteria that are essential for fermenting cellulose, starch and other plant polysaccharides (Miller and Wolin, 2001). Phytochemicals, plant compounds, have been used throughout history to treat both human and animal illness. Chapter 3 presents an overview of plant bioactives in the literature and in Chapter 3 we examine models for bioactive discovery.

A brief environmental and literature scan reveals a large amount of evidence for natural alternatives to synthetic anthelmintics and antibiotics. Many of these are phytochemicals, including saponins, essential oils, tannins, lignans, proteins and peptides, alkaloids, proanthocyanins, terpenes and flavonols. Often they are delivered to the animals as crude additives in feed. The evidence for animal health benefit ranges from anecdotal to clinically validated. This evidence is reviewed in Chapter 4.

2 Plant bioactives and models for discovery

2.1 Plant Bioactives in the Literature

Plants have been used for their bioactive properties throughout human history, resulting in a large body of knowledge regarding traditional use of plants in western medicine as well as other medical systems. Modern western medicine continues to use plants as a source of new drugs and modern techniques allow much greater understanding of the way these plant compounds work. Though generally human centric, there is much study world wide to discover novel plant bioactives for disease.

There is no doubt that past knowledge and the literature is a useful guide for developing therapeutic approaches. However, even a cursory search of the literature reveals a daunting amount of information on plant metabolites but with relatively little work done for ruminant health. Table 2.1 presents a summary of the results of a literature search that examines specific classes of plant compounds. Plant metabolites were searched based on structure type (terpene, alkaloid, lipid, carbohydrate, aromatic, saponin, tannin) and then each class examined for reports of bioactivity, specifically antibiotic or anthelmintic activity. The results were further refined to focus on ruminant specific research. There are some limitations and redundancy in this data but it highlights the large number of publications discussing plant metabolites and their antibiotic and anthelmintic activities. Manual inspection of each refined reference further reveals that some of the articles are 'false positives' in that they do not necessarily focus on ruminant health (e.g. some mention bovine serum albumin in the abstract). Figure 2.1 portrays this information visually and shows the areas of greatest study in terms of ruminants.

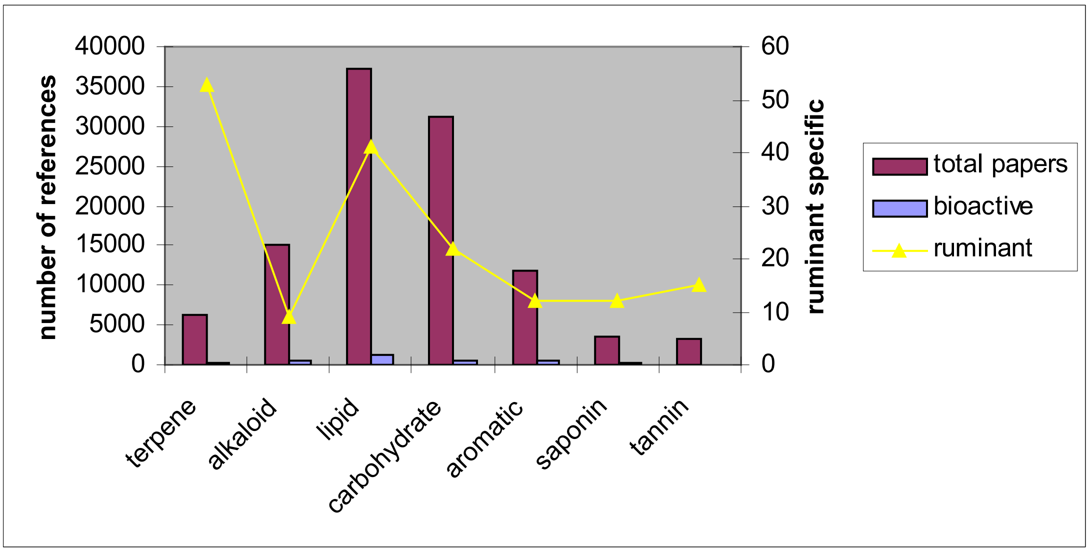
Table 2.1. Plant compounds and associated data

Compound Class	***Number of References***[a]			
	total	bioactive	ruminant	relevant
terpene	6410	339	53	19
alkaloid	15195	617	9	3
lipid	37351	1338	41	19
carbohydrate	31137	582	22	6
aromatic	11786	447	12	2
saponin	3464	309	12	8
tannin	3275	125	15	12
total	*108618*	*3757*	*164*	*69*

[a]Search strategy detailed in Appendix 1.

This analysis demonstrates that there are plant compounds of almost every conceivable chemical class with some sort of bioactivity reported and relatively few studies have been done in ruminants. Many of the studies that have been carried out in ruminants have been qualitative rather than quantitative in terms of plant chemistry (this will be discussed further in Chapter 3. For example, although there has been work done on the beneficial effects of some saponins, the chemical nature and quantity in the plant material has rarely been considered. This is problematic since small changes in chemical structure can lead to large changes in activity and these effects are not easy to predict.

Figure 2.1. Literature analysis of plant compounds for ruminant health



Given the enormous volume of literature reporting plant chemistry, and the even greater number of plants that have not been investigated for bioactivity, how can we find the plants that may be most beneficial for a particular problem? The next section, Pathways to Bioactive Discovery, examines this question and compares different models of bioactive discovery and validation.

2.2 Pathways to Bioactive Discovery

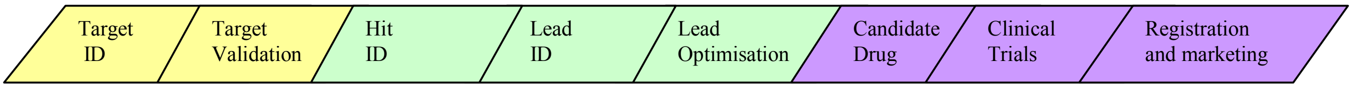
There are a number of approaches to bioactive discovery and there is no, one correct way, of carrying out bioactive discovery. This section examines different models for bioactive discovery. Some of the 'lessons learnt' from decades of drug discovery are highlighted. Perhaps one of the most fundamental of these relates to gross chemical structure. Structure similarities, particularly gross similarity, are at best, a guide to biological activity. This alone is not enough to determine activity since it fails to take into account the three-dimensional structure of the compound and how this influences function. A biologically active compound generally interacts with a molecular target that is not only three-dimensional but also potentially highly flexible. This necessitates the through testing of even closely related molecules and is in fact the premise behind structure activity relationship modelling (SAR), a model which is discussed in more detail later.

The first model to consider, and arguably one of the most successful, is the pharmaceutical model of discovery. In this context the term pharmaceutical also applies to the medicines used for animal health. An overview of the process employed by most major pharma companies is described below. The process generally takes 12 years and can cost, on average, $800M. The most time consuming and costly process is clinical trials.

2.2.1 Pharmaceutical Model

Research in the pharmaceutical industry usually follows a defined path with a number of critical steps along the chain, each with critical review and 'stop/go' decision points built in. This 'value chain process' is summarised in Figure 2.2 below.

Figure 2.2. Pharmaceutical value chain for bioactive discovery



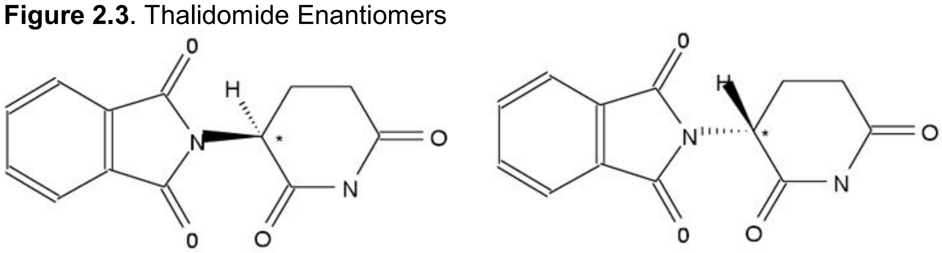
Target identification and validation typically involves the identification of a medical need and the search for a relevant biological target, for example a specific enzyme involved in cancer. There are numerous strategies for hit identification as well. For the majority of the large pharmaceutical companies today this involves High Throughput Screening (HTS). HTS allows the screening of hundreds of thousands of compounds or natural product extracts against the specific target. Compounds or extracts that are active are called 'hits'. This process is designed to rapidly focus attention on the most biologically relevant materials in a compound or extract library. Lead identification examines the hits in more detail. For synthetic compounds this involves synthesis of analogues of the active compounds. Typically for natural product extracts this involves the isolation and identification of the biologically active molecules in the extract. Lead optimisation is the process whereby the compounds can be altered to enhance activity or improve other properties such as oral bioavailability and ADME (absorption, distribution, metabolism and excretion) profiles. This process usually involves animal studies. The best of the 'leads' are nominated as candidate drugs and taken to clinical trials. A successful outcome from the clinical trial will lead to the registration of the compound as a NCE (new chemical entity) through the Food and Drug Administration (FDA) in the United States and other regulatory bodies.

In the hit to lead process there are numerous strategies that can be employed to reduce the time and to enhance the likelihood of finding a safe and effective drug. Such strategies include early assessment of DMPK (drug metabolism and pharmacokinetics), toxicity testing and, for natural product drug discovery, dereplication. Dereplication is a generic term used to describe chemical screening approaches that are employed to ensure that the same bioactive is not being chased in multiple samples.

2.2.2 Structure Activity Relationships (SAR) and Pharmacophore Modelling

Small changes in the chemical structure of compounds can have dramatic effects biologically. Perhaps the most well reported (and most tragic) example of this is thalidomide. Thalidomide is racemic – it contains both left- and right-handed isomers in equal amounts. One enantiomer is effective against morning sickness. The other is teratogenic, and causes birth defects. Enantiomers are mirror images of each other (Figure 2.3) (Knightley et al, 1979).

Figure 2.3. Thalidomide Enantiomers



(S)-thalidomide – teratogenic (R)-thalidomide – non-toxic

Small molecular changes can also confer positive changes. The β-lactams are extremely successful antibiotics. The original penicillin molecule has been structurally altered to produce a range of related antibiotics with enhanced properties including activity, and to overcome resistance.

What this means is that it is extremely difficult to predict the activity of a molecule based on its structure. In the lead optimisation process many molecules with small structural changes are synthesised and examined. This gradually allows development of a picture of how to change the molecule to enhance activity. That is, the process leads to an understanding of structure activity relationships (SAR). This understanding can allow the development of a pharmacophore model. In the case of the penicillin derivatives, it is the β-lactam functionality that is the key pharmacophore. Leaving this intact and altering other parts of the molecule can enhance the properties of penicillin. For well-understood classes of molecules, such as the penicillins, these pharmacophore models are extremely useful.

2.2.3 Structure Based Drug Design

Structure based drug design is a computational intensive activity that relies on having molecular structure information of the target protein (usually obtained through crystallography, NMR or homology modelling). This method lets the scientist design molecules that fit in the active site of a protein. Chemical synthesis of the molecules with a high-calculated activity is important to test the model. The approach has a couple of drawbacks. The major limitation is that the computational models do not account well for protein dynamics and flexibility. Generally only a small portion of the protein will be modelled – usually the active site and the model is often based on a static representation. This is because the calculations are already extremely time consuming and also because there is still much to be learnt about protein dynamics. A good example of this is the study of a natural product. Dysinosin A is a large natural product and a potent inhibitor of thrombin. Initial consideration of the available computational models suggested that the molecule would not bind well to the protein. However, follow up crystallography demonstrated that the protein changed its structure significantly to accommodate the larger molecule (Carroll et al, 2002). This information could then be used to develop a new model for computation.

2.2.4 Animal System Models

These are observational driven studies and can have their origin in ethno-veterinary practice or epidemiology based observations. The literature review, “A literature review on plant bioactive compounds for health and productivity in ruminants” highlighted many of these studies. At their best, these studies involve statistically significant numbers of animals and introduce the feed in a measurable and producible fashion. This information is presented in the next chapter.

2.3 Recommendations for Ruminant Studies

The rumen is complex and studies from monogastric animals are not necessarily directly relevant to ruminants. This implies that validation of any bioactives must occur in relevant, i.e. ruminant, animal system models. It would not be practical, from a time or cost perspective, to test all potentially useful plants in animal models. This implies that a pre-screening process is required. The most logical approach for identifying plants of interest is to follow the pharma model. The type of assay will determine how this is best performed. The desired or optimal delivery mode for individual plants will determine the type of animal studies that will need to be carried out.

3 Literature Review - "Plant bioactive compounds for health and productivity in ruminants."

Summary and Conclusion

This literature review indicates that bioactive compounds in plants can be utilised by livestock for beneficial means. Secondary plant compounds can have an effect on feed intake, milk production, wool growth, carcass composition and growth. Many of the studies in ruminants to date have targeted specific classes of bioactives such as tannins and saponins. The focus of most ruminant research has been on rumen micro flora modification for a reduction in methane emission and enhanced growth. There have been several ruminant focused studies investigating the use of plants for nematocides, though the focus has again been on the effects of polyphenolics. However, there are plant bioactives of almost every chemical class that have demonstrated nematocidal or antibacterial activity suggesting that this could be a fertile area for future research. This review highlights the need for more controlled in vivo research to validate plant bioactivity.

"All substances are poisons; there is none that is not a poison. The right dose differentiates a poison from a remedy"….. Paraclesus 1493-1591

Introduction

Mainstream animal production relies heavily on the use of pharmaceuticals. Many of these products are developed through research and development for human pharmaceutics. Natural products are an important source of new drugs and drug leads in the pharmaceutical industry. For the animal market many of the currently used antimicrobial, feed additive antibacterial, endectocide and anticoccidial drugs are either natural products or synthetics based on natural products (Ruddock, 2000). The majority of these natural products are produced from the fermentation broth of microorganisms, though plants have also been an important source of bioactives. There is increasing public concern regarding the use of pharmaceuticals in the animal industry. Much of this has been as a result of the emergence of drug resistance. A particular area of criticism has been in the use of antibiotics as growth promoters and the associated risk of developing antibiotic resistance in human pathogens (Barton, 2000). This is not a new issue and in 1969 the Swann report resulted in the withdrawal of β-lactams from feed in the UK (Ruddock, 2000). However, this increasing trend has led to a closer examination of plants for animal health. In Western culture plants in the livestock industry have largely been considered as a source of nutrition or potential source of toxicity. Increasingly there is the realization that plants may offer non-nutrient performance enhancing factors that benefit animal production (Greathead, 2003). This realization has resulted in increased research, with the number of publications in this area increasing over the last 8-10 years. The research area is of sufficient significance to warrant focus in the journal 'Animal Feed Science and Technology'. In 2005, issues one and two were dedicated to "Phytochemicals in Livestock Production Systems". Specific programs to investigate the use of plants for animals have also been developed. For example, the banning of feed antibiotics by 2006 in the European Union (EU) prompted investment in the EU-Replace program which aims to screen 500 plants for a range of activities, including antibacterial, nematocidal and immune stimulating effects (EU-Replace, 2006).

This paper reviews the use of plants or their extracts to enhance ruminant health. Evidence from various sources, including in vitro and in vivo experiments and ethno-veterinary studies will be discussed. Not considered here are potential natural products derived from organisms other than plants. That is, bacteria and fungi are not covered in this review. There is already substantial evidence for the success of microbes in this area, and indeed, many of the antibiotics and helminthics used today are either microbial natural products or derivatives there of (e.g. avermectins and milbemycins from *Streptomyceses* species). Live organisms such as fungi have also been used for in situ nematode control. The commercially available DiTera contains the fungus *Myrothecium* spp whilst Paecil, which contains *Paecilomyces lilacinus*, has been used as a soil drench, the fungus being a nematode egg parasite (Ghisalberti, 2002). Potential biological mechanisms of control, such as this, will not be considered further in this review.

It is worth noting that plant bioactives is still an under-explored area of research and in many cases although biological activity has been observed, the natural phytochemicals responsible for the activity have not been identified. For example, a compilation of plants with nematocidal activity produced in 1997 contained 150 entries and for most the active agents have yet to be identified (Ghisalberti, 2002). Identification of the bioactive agent would require systematic fractionation and biological testing of plant extracts. Once pure compounds were obtained analytical techniques such as mass spectrometry (MS) and nuclear magentic resonance spectroscopy (NMR) could be employed to elucidate the chemical structure of the actives. These tools would also be of value in ensuring that plant material contains consistent levels of any bioactive compounds.

As has been mentioned, animal pharmaceutics are often derived from studies for human medications and for these studies ruminants are usually not the focus of bioactive investigation. There have been an extraordinary number of plant metabolites with antibiotic activity reported. A literature search using the terms "antibiotics from plants", yielded over 5000 references. The majority of the compounds/plants identified in these articles will never have been specifically tested against ruminants. Indeed, adding the words "and studies in ruminants" reduces the number of references to 30, and only a small proportion of these is relevant to the topic. Indeed, for ruminants, there is very little literature that focuses on plants as alternatives to antibiotics. In ruminant health the focus has been on bioactive effect of plants on ruminal flora rather than on specific pathogenic bacteria. This is perhaps understandable, since many of the desirable effects of antibiotics used as growth stimulants act through modification of the ruminal microbe population.

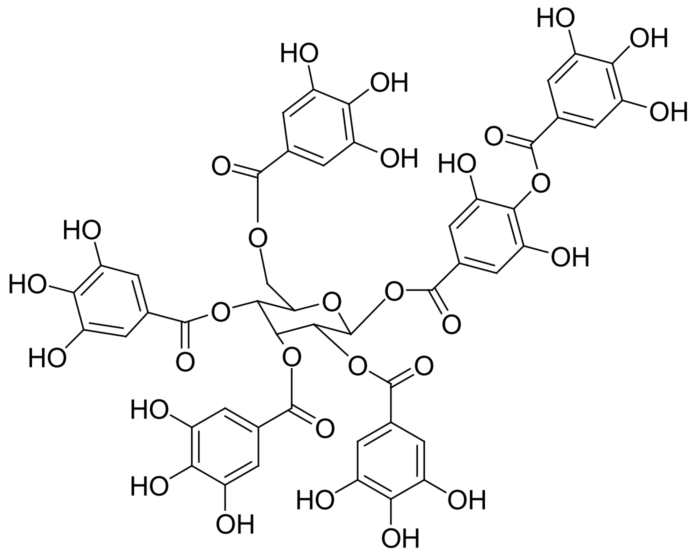
Delivery of bioactives is also considered here. The form the bioactive presented to the animal will affect not only bioavailability but also cost of delivery. Options for delivery range from growing the plant in field, through to application as hay, to dosing with either pure material or concentrated plant extract. In many ways the simplest of these is infield plant production, however there are numerous considerations as highlighted in a recent review, "Arguably the simplest method of delivering bioactive plant secondary metabolites to animals outdoors would be to grow the relevant plants in a field and then let the animals graze them in a controlled manner, assuming they are palatable." However the efficiency of such a method is doubtful, since despite the crude control of intake via controlled grazing, there would be no control on dosage due to the interplant variation in secondary plant metabolite (SPM) content. Methods of uniformly stressing crops of plants to ensure uniformity of SPM and perhaps even invoking the production of certain metabolites should be investigated (Greathead, 2003).

3.1 Bioactive Compounds and their Effects on Production

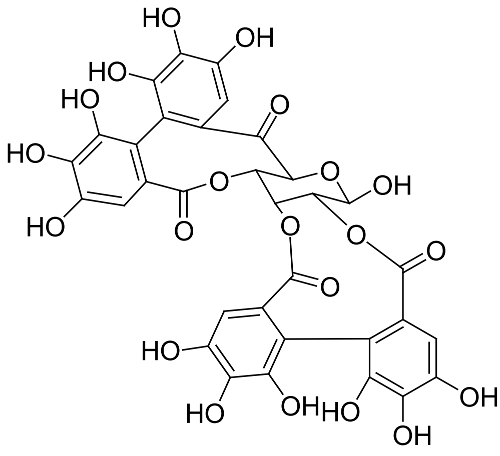
3.1.1 Feed Intake and Behaviour

Feed intake and animal-feeding behaviour is governed by many factors including availability, palatability and feed back mechanisms. Tannin containing plants have been the subject of significant research effort. The recent review by Irene Mueller-Harvey (2006) is an excellent summary of this work. Condensed tannins may be beneficial in the diet but at certain levels begin to affect feed intake. This level varies considerably, depending on the chemical nature of tannin and the animal species studied. The evidence is mixed, sometimes conflicting and often difficult to interpret in an objective manner since the actual tannin composition is not always well described. An additional complicating factor is the different physiological responses to tannin amongst ruminants. For example, deer saliva has tannin-binding proteins that are not found in sheep. The two animals also metabolise tannins of different structure classes in a different manner. In both deer and sheep, hydrolysable tannin is broken down shortly after consumption and there are no diminished protein absorption effects. However, condensed tannins (CT) are recovered almost entirely from deer faeces, but only 60% is recovered from sheep faeces, suggesting some absorption. Importantly gallotannins from different sources also had different effects on protein digestibility suggesting that both gross and subtle differences in tannin chemistry must be considered when assessing the effect of tannin on ruminants (Robbins et al, 1991; Hagerman et al, 1992). Typical tannins are depicted in Figure 3.1. The gallotanin (**1**) and ellagitanin (**2**) are members of the so-called hydrolysable tannins, whilst the proanthocyandin, (**3**), is one member of the condensed tannin family.

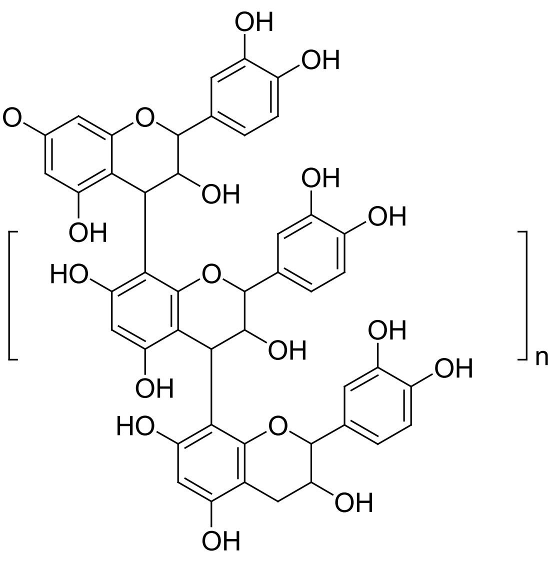
Figure 3.1. Examples of tannin chemical structures



1. Gallotannin



2. Ellagitannin



3. Proanthocyanidins (condensed tannins)

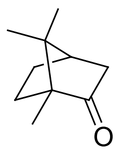
Condensed tannins (CT) present in a number of plant species may inhibit the activity of ruminal microorganisms. The level required in the diet varies and levels in plants can vary significantly due to environmental parameters. Barry (1985) demonstrated that condensed tannin levels in lotus are dependent upon fertility of the soil. Barry (1985) demonstrated conclusively that high concentrations of CT prevent maximum expression of live weight gain in young sheep. This result was predominately mediated through a depression in feed intake. These results are in contrast to those obtained with growing sheep grazing the same cultivar in high fertility soils. Under these conditions CT in *Lotus pendunculatus* contains 20 g/kg dry matter (DM) of CT and it is believed that at this concentration a beneficial effect is seen. Polyethylene glycol (PEG) increases feed intake when sheep are fed CT containing diets. PEG prevents binding of CT to protein so this suggests that the effect is due to polyphenolic plant metabolites.

Dietary CT *per se* can be considered as nutritionally deleterious and a net benefit only occurs with ruminants given fresh forage diets when the tannins react with forage proteins and reduce their solubility. The ideal amount of CT in a ruminants' diet would therefore be the minimum amount of CT necessary to render the plant protein insoluble, (20 – 40 g /kg DM is believed to be the ideal CT concentration in Lotus sp. (Barry, 1985)).

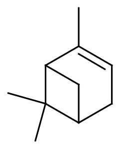
Tannins are not the only plant metabolites that are implemented in change of dry matter intake (DMI). DMI was increased for steers fed supplemental betaine compared to control steers resulting in increased fat deposition in the betaine-supplemented group (Loest et al, 2002).

Feed intake can be altered by palatability, as in the case of tannin, but physical properties are also important. Thorns or excessively rough material can effect forage intake, particularly of potentially important leguminous shrubs. Behaviour and feed intake may also be effected by aroma. Estell et al. (1998) demonstrated that terpene volatiles could effect feed intake in sheep. Varying levels of camphor (**4**) and α-pinene (**5**) (Figure 3.2) were implicated in differential use of 'tarbush' by ruminants. Knowledge of specific chemical interactions with feed intake may therefore ultimately lead to mechanisms for altering feeding behaviour.

Figure 3.2. Volatile terpenes



4. Camphor



5. α-pinene

3.1.2. Wool Growth

Wool growth is sensitive to the absorption of protein and overall health of the animal. The presence of condensed tannins in lotus and sainfoin in the diet of sheep may be expected to contribute to increased amino acid absorption and nitrogen retention. A 55 day feeding study carried out in New Zealand suggested that sheep grazing lotus showed improved reproduction and also increased wool production (Min et al, 1999). Analysis of plasma suggests that the effect was due to an increase in essential amino acids, particularly branched chain amino acids. This was achieved without increased voluntary feed intake. Polyethylene glycol (PEG) supplementation negated the benefit of feeding on Lotus, suggesting that the tannin is responsible for the positive results. This may, at least in part, be due to the higher metabolizable energy of Lotus compared to pasture. A similar study carried out over two years under commercial dryland farming conditions showed that such effects were greatest in years with exceptionally dry autumn periods (Ramirez-Restrepo et al, 2005).

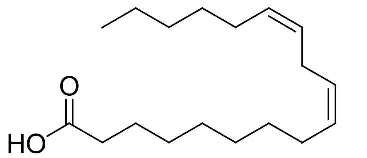
3.1.3. Growth and Carcass Composition

Nutritional studies on animal growth have often centred on an understanding of macronutrients (protein, fat, carbohydrate), however it is becoming apparent that plant bioactives effect not only animal growth but also carcass composition. In meat animals this has significant implications for consumer acceptance. Feed additives of natural origin namely betaine and conjugated linoleic acid can improve the fat:lean ratio in some circumstances (Sillence, 2004).

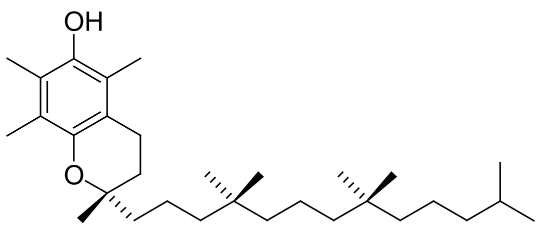
Betaine is a naturally occurring amino acid derivative (tri-methyl glycine) found in many plant and invertebrate species. Physiologically, betaine has an important osmoregulatory action and can serve as a methyl group donor via S-adenosyl-methionine. When incorporated into pig diets, betaine has been reported to improve growth performance by reducing the maintenance energy requirement of the animal (Schrama et al, 2003, Suster et al, 2004). This occurs through reducing the need for sodium/potassium pumping to maintaincellular osmolarity. In addition, dietary betaine has been reported to increase protein deposition and carcass leanness (Fernandez-Figares et al, 2002; Matthews et al, 2001a,b) and decrease back-fat (Cadogan et al, 1993). Betaine can also improve water holding capacity and reduce drip loss in meat (Dunshea et al, 2005). There have been fewer studies in ruminants, but there is some evidence that dietary betaine can reduce heat stress and improve feed intake and growth performance in beef cattle (M. Mottram, personal communication). Also, dietary betaine can improve the integrity of gut mucosal cells and reduce the severity of some enteric infections in poultry (Mathews and Southern, 2000; Klassing et al, 2002). It is possible that dietary betaine, either as a supplement or from plants naturally high in betaine, may provide a number of benefits to ruminant species.

Saturated fat from red meat in the diets of consumers has been associated with an increased risk of developing coronary heart disease and colorectal cancer. This has resulted in the dietetic recommendation to decrease red meat intake (Eynard and Lopez, 2003). Essential fatty acids such as conjugated linoleic acid (CLA) (**6**) (Figure 3.3) and other polyunsaturated fatty acids (PUFAs) have been demonstrated to have anti-carcinogenic, antithrombogenic and antiatherogenic properties. PUFAs also exhibit anti-oxidant effects in meat products which may enhance colour and extend shelf life, providing advantages for both retailers and consumers. Antioxidants in the form of selenium, vitamin E (**7**) (and related tocopherols), flavonols such as quercetin and larger polyphenolics such as tannins, have been demonstrated to have diverse biological effects some of which can be related to the reduction of free radicals. In animal studies high intake of compounds such as vitamin E correlates with reduction of placental retention and mastitis in dairy cattle, but also a greater stability of meat colour (Demeyer et al, 2004).

Figure 3.3. CLA and Vitamin E



6. conjugated linoleic acid



7. Vitamin E

Conjugated linoleic acid (CLA) is a fatty acid that decreases body fat and increases lean tissue. Ostrowska et al (1999) has demonstrated that feeding CLA to pigs decreases back fat by up to 25% and increases in protein deposition have been reported. Raw materials in the diets of ruminants may have an influence on the fatty acid composition of fat and muscle tissue by both the amount and composition of lipids in each ingredient (Bas and Morand-Fehr, 2000). Pasture is a rich source of CLA and PUFA in the diet of ruminants, however knowledge regarding the fat content and composition of forages available to grazing animals and the subsequent conversion into meat products is limited. Promising data (Table 3.1) would indicate significant variation in fatty acid composition for different forage species and phenological growth stages.

Temperature has also been suggested as a significant source of variation in PUFA concentrations in milk (Collomb et al, 2002). However it is more likely that plant species such as C_3 plants are responsible for compositional changes in meat and milk products. The conversion of fats from forage to animal tissues has been noted as being influenced by age, sex, lactation, level of fattening and fatty acid composition and previous dietary fat intake (Bas and Morand-Fehr 2000). The potential exists to produce animal products that are inherently healthier via increased levels of PUFA and CLA when grazed on certain pasture species compared to other pastures or grain feeding (Table 3.1). Management factors that maximise the concentration of PUFA and CLA in pasture based diets and the subsequent deposition in meat products of grazing ruminants await investigation.

3.1.4 Milk

Condensed tannin containing forages such as *L. corniculatus* has been shown to increase milk yield in ewes in the spring and summer. The *L. corniculatus* spp. contained 44.5 g/kg DM CT that is close to the reported limit for a beneficial effect from this species. Milk yield of cattle and sheep is a critical factor for survival and growth of the young. There have been reports of increased milk yield with supplementation of nicotinic acid in lactating dairy cows. Caffeine has been demonstrated to increase mammary gland development, increase milk yield and growth rates of the young in mice (Sheffield, 1991) and pigs (Li and Hacker,1995), however, there is no data available for ruminants.

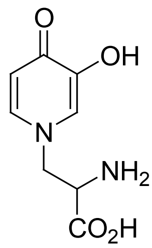
3.1.5 Reproductive Efficiency

In situ grazing of high condensed tannin containing pastures such as *Lotus corniculatus*, have been suggested to yield higher reproductive efficiency in sheep compared to animals grazed on grass alone. Ramerez-Restepo et al. (2005) suggested that the grazing of ewes before mating for up to 42 days on *Lotus corniculatus* increased ovulation rate of ewes resulting in increased multiple births for ewes that were mated on *L. corniculatus*. However, greater liveweight and liveweight gain of sheep from the *L. corniculatus* group could be explained by the higher OMD (organic matter digestibility), DOMD (dietary organic matter digestibility) and ME (metabolizable energy) values for *L. corniculatus* pasture and not just to higher condensed tannins in the *L. corniculatus* pasture *per se*. This is an area where further research would be beneficial. One approach would be to feed a control group of animals PEG in the diets. This would allow researchers to assess whether or not the effect was due to tannin.

3.1.6 Defleecing Agents

A number of chemicals have been studied as potential defleecing agents in Merino sheep (Reis et al. 1978; Reis 1978). Mimosine (**8**) (Figure 3.4) a bioactive compound of *Leucaena leucocephala* has been demonstrated to be effective in stopping the growth of wool, allowing subsequent manual removal of the fleece, though the compound is also toxic at high levels. *Leucaena leucocephala* has been the subject of considerable investigation since the fast growing leguminous tree is a source of both human and animal nutrition in India. The nutrition value is high; similar to alfalfa forage (D'Mello andThomas, 1977). Nutritive state of the sheep effects the breakdown of mimosine by the liver. There are also indications that such approaches may be used in Angora goats and sheep (Reis et al. 1999). The toxicity of the compound suggests that such a solution would be of little value in grazing animals since it would be extremely difficult to control the amount of plant material and hence the amount of mimosine that each animal was ingesting. In addition, it is highly likely that the plant's production of mimosine is environment dependent. This would imply that each plant would need to be analysed to ensure that animals were ingesting safe and effective levels. For defleecing using mimosine, a more effective approach would be to administer the compound in a concentrated dose at the correct time of year. Such a dose could be administered as a drench of either concentrated plant extract or purified mimosine. Either approach would require careful assessment of the levels of mimosine in the drench.

Figure 3.4. Mimosine - A defleecing agent



8. Mimosine

3.2 Bioactive Compounds and their Effects on Rumen Environment

3.2.1 Bacterial Populations

Antibiotic activity is one of the simplest and most important bioactivities to test for and there is a large body of literature reporting on research in this area. Plants have long been a rich source of antibiotics and an extraordinary number of plant metabolites with antibiotic activity have been reported. A literature search using the terms “antibiotics from plants”, yielded over 5000 references. The majority of the compounds/plants identified in these articles will never have been specifically tested against ruminants. Indeed, adding the words “and studies in ruminants” reduces the number of references to 30, and only a small proportion of these is relevant to the topic. The majority of plant-derived antibiotics tested specifically for ruminants are tested in order to assess the effect on the ruminal flora. The aim is generally either to assess safety, since ruminants derive much of their nutrition through bacterial gut fermentation antibiotics can have a deleterious effect on animal health, or to reduce the Gram positive bacteria that may be associated with less desirable gut metabolism. Recently, it was demonstrated that the forage species *Dorycnium rectum* contained a range of proanthocyanidins that had varying effects on ruminal bacteria. The plant is unusual compared to other temperate forage legumes since it contains CT of a very high degree of polymerisation. Some species of bacteria were more sensitive to certain structures than others and some, such as *P. anaerobius*, were extremely sensitive to both high and low molecular weight polymers (Sivakumaran et al, 2004).

Tannins are not the only metabolites that effect ruminant flora, and indeed it is likely that many of the nematocides reported in Table 3.3 will also effect bacterial populations to an extent (though this is largely untested). For example, essential oils have demonstrated antibiotic activity (Wallace et al. 2002), though this is under-explored in ruminants. In addition this area of research is further limited by a concentration on in vitro studies with few in vivo studies done with grazing animals.

Foam Production / Bloat Control

Pasture bloat is a costly disorder, particularly for cattle grazing on high protein improved pastures (Tanner et al. 1995). Frothy bloat is caused by the capture of ruminal gases in a polysaccharide slime layer and causes an inability of the animal to release gas pressure which is formed as a result of ruminal fermentation (Tanner et al. 1995). Proanthocyanidins have been demonstrated to reduce foam production in vitro in a dose dependant manner (Tanner et al. 1995). Similarly, Waghorn and Jones (1989) demonstrated an absence of bloat in cows fed dock (*Rumex obtusifolius*) at 10% of dry matter while consuming a lucerne based diet. In addition bloat scores in steers have been reduced by feeding Sainfoin herbage (*Onobrychris Viciifolia*) at 10-20% dry matter (McMahon et al. 1999). Although it is possible to co-cultivate a bloat susceptible sward such as lucerne or clover with a CT containing plant such as Sainfoin, Lotus or Dock, consistent intakes of both plants by ruminants must be verified and production traits assessed before full recommendation to producers. A summary of evidence for plant bioactivity in this area is presented in Table 3.2.

This is an area where additional research could have significant industry benefits. Mixed grazing systems or segregated feeding paddocks could reduce the incidence of bloat. For animals such as dairy cattle plant supplements could be added to their diet during milking. Research into additional plants that could be of use in situ and in lots would be a priority. In addition, it is still unclear if tannin is entirely and exclusively responsible for this benefit. Any research in this area would require extensive chemical analysis to ensure that tannin levels and types are qualitatively and quantitatively described across feeding studies.

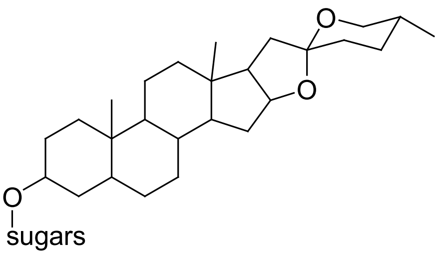
Methane production

Methane is produced as a by-product of the digestive process and represents a loss of feed energy (2-12%) from the diet (Pen et al, 2006). Methane is also one of the primary greenhouse gasses and livestock production is the major source of anthropogenic methane (Wood and Knipneyer, 1998). Both tannins and saponins have received attention for their ability to reduce methane production. Legumes containing condensed tannins decrease gas formation and microbial deamination because of plant–protein interactions. Reducing methane emissions and ruminal protein degradation could result in decreased metabolic energy losses and gaseous nitrogen emissions. Tannins in many plants may reduce ruminal protein breakdown and increase duodenal protein flow when provided at moderate doses (Carulla et al. 2005). However, when given to animals at higher doses they may also adversely affect animal performance. Carulla et al. (2005) supplemented *Acacia mearnsii* tannins at a level of approximately 0.025% of the diet DM and significantly reduced methane emissions by 13%. However, the replacement of grass by legumes demonstrated no advantage in reducing methanogenisis. The research in this area raises the interesting possibility of supplemental feeding with feed that incorporates tannin-nutrient complexes as a mechanism to provide high value feed whilst lowering methane production.

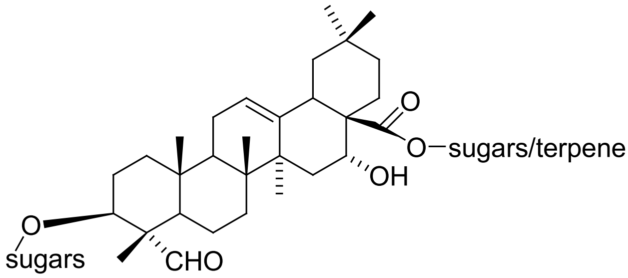
Saponins are an important class of plant metabolites that show enormous structural diversity. Essentially they are terpene glycosides the composition of which can vary in the core terpenoid (sterol derivatives to triterpenes) and also the number, type and substitution pattern of the glycoside residues (Figure 3.5). They have numerous biological effects and some can demonstrate highly selective and potent biological activities.

Hess et al. (2003) found that the fruits of *S.saponaria* reduced methane production in an in vitro culture by 11% in grass-alone and legume supplemented diets. In vitro fermentation experiments using the ruminal fluid of Holstein cows (Pen et al, 2006) demonstrated that *Yucca schidigera* extract (YSE) decreased methane production whereas *Quillaja saponaria* extract (QSE) did not. Protozoal numbers decreased in both cases (56% with YSE, 41% with QSE). The authors suggest that the chemical nature of the saponins may be responsible for the differing activities. Yucca saponins have a steroidal (**9**) nucleus whereas Quillaja saponins have a triterpenoid nucleus (**10**) (Figure 3.5). It would be interesting to see if these in vitro results could be translated to in vivo activity and if the saponins can be administered via plant feed rather than dosing with an extract as in this procedure.

Figure 3.5. Examples of saponin chemical structures



9. Yucca saponin base structure



10. Quillaja saponin base structure

Saponins effect the ruminal flora in other ways. In culture isolates it was demonstrated that YSE stimulates the growth of *Prevotella ruminicola* and suppresses the growth of *Streptococcus bovis*. The antimicrobial effect is most pronounced against gram positive bacteria, similar to the action of ionophores. The impact on the complex ruminal bacterial populations is difficult to assess and is also dependant on overall population numbers and variation (Cheeke, 2000).

Other studies have demonstrated the reduction of methane by plant extracts, without identification of the active agents. Broudiscou et al. (2000) investigated the effect of 13 plant extracts in continuous culture. They observed little effect on protozoa numbers but showed that *L. officinalis* (lavender) and *S. virgaurea* promoted the extent of fermentation and that *E. arvense* and *S. officinalis* (sage) had a possible inhibitory effect on methane production. Though the plants were selected for their high flavonoid content, it cannot be assumed the flavonols were responsible for activity. Patra et al. (2006) recently studied the in vitro effect of five plants (*Acacia concinna*, *Terminlaia chebula*, *Terminalia beleerica*, *Emblica officinalis* and *Azadirachta indica*) extracted with solvents of varying polarity (water, methanol and ethanol). Their results showed *T.chebula* could be used to reduce methane production. It was interesting to note that these researchers also observed that a decrease in protozoa counts *(A. concinna, A, indica and T.chebula*) does not necessarily mean a decrease in methane production.

A similar study (Sliwinski et al, 2002) comparing *Yucca schidigera* extract (YSE) to *Castanea sativa* wood extract (CSE; containing hydrolysable tannins and lignan) in in vitro rumen models showed effects on methane production only at very high levels. In vivo studies with fistulated lambs from the same authors concluded that there was a weak potential of YSE and CSE to favourably modify nitrogen turnover in the rumen, in the metabolism of the animal and in manure during storage. Sliwinski et al. (2002_b) further concluded that the effects on methanogenesis were inconsistent between in vitro and in vivo data. The authors were using commercial products and suggest the cause for variation between these results and others is the variability in chemical constituents in each extract. This draws attention to studies using plants where levels of bioactives are unknown (Patra et al, 2006) and where these levels are likely to fluctuate with season and location. It also highlights the need for thorough chemical analysis to enable meaningful comparisons across in vitro or in vivo studies.

3.3 Bioactive Compounds and their Effects on Livestock Health

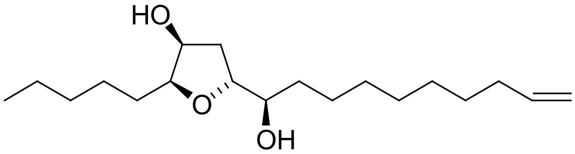
3.3.1 Nematodes

Nematodes are a diverse group of organisms with some 30,000 described species. Approximately 50% of these are marine, 15% are animal parasites, 10% are plant parasites and 25% are free living (Ghisalberti, 2002). Anthelmintic resistance in GI nematodes is an increasing problem, though modern pasture management techniques including pasture rotation, harrowing, regular manure removal, and "worm and move" programs can be of help in parasite control (Nguyena et al, 2005). It has been shown that alternate pastures such as *Lotus corniculatus* (birdsfoot trefoil) and *Chicorium intybus* (chicory) can reduce the nematode load in ruminants. There is question as to whether this effect is due to plant structure effects (i.e. higher grazing so less reinfection in animals through manure ingestion) or by the biological activity of the polyphenolic phytochemicals in the forage (Marley et al, 2005; Marley et al, 2006a; Marley et al, 2006b; Ramirez-Restrepo and Barry, 2005).

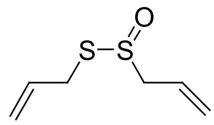
The action of secondary metabolites as antinematodals has been the subject of recent reviews exploring both in vitro and in vivo effects of plant constituents (Ghisalberti, 2002; Githiori et al., 2006). A large number of plants have documented nematocidal activity, though for the majority the bioactives responsible for this activity remain unidentified (Ghisalberti, 2002). This portion of the review includes a more general assessment of bioactives since in many cases the use of a plant covers animals and plants. Many of the plants summarised in Table 3.3 have traditional use. For example the juice of the marigold flower was used to kill worms in humans since the 1st century AD and is traditionally used in India as an agricultural pest control (Ghisalberti, 2002). It has been suggested that the separation of nematodes into free-living and parasitic is less relevant than how much the species has in common and so an indication for plant bioactives against free-living soil nematodes may also indicate activity for in vivo ruminant control.

The putative bioactives fall into a wide range of chemical classes (Figure 3.6). Lipids (fatty acids to complex derivates such as tetrahydrofurans), phenolics (simple stilbenes to complex tannins), alkaloids and terpenes (ranging from essential oils to glycosoylated triterpenes – saponins) have all been identified as nematocides. In the case of the simple fatty acids the presence of the acid moiety seems to be important. Lipids such as linoleic and oleic acids, with LD50s of 5-25 ppm are inactive when tested as the methyl ester (Ghisalberti, 2002). Other structural moieties have also been identified as important. For example in the polyenes, such as those obtained from *Erigeron philadelphicus* (daisy) are most potent when the compounds contain a ketone conjugated to a triple bond, aryl or ester group. In some cases in vivo activity is not matched by in vitro activity. A metabolite and biosynthetic analogs from the brown alga*, Notheia anomala*, were potent nematocides with LD50s 1-10ppm, a level of activity comparable to that of commercially available levamisole and closntel (Capon et al, 1998). However, when tested in vivo in infected sheep there was no evidence of efficacy for the purified metabolites. Almost certainly this is due, in part, to the hydrophobicity of the purified compounds. It is possible that in a different matrix the metabolites would be more bioavailable. Interestingly, the converse may also be true. When the nematocidal effects of *D. rectum* in lambs was studied in New Zealand the effects measured in vitro generally under-estimated effects measured under field conditions.

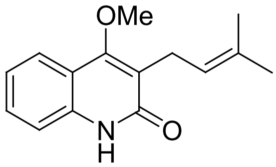
Figure 3.6. Examples of nematocides from plants



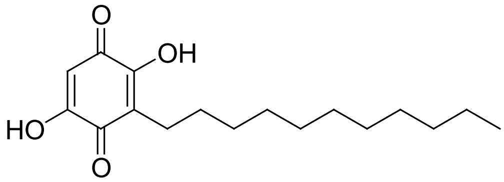
11. tetrahydrofuran from *N. anomola*



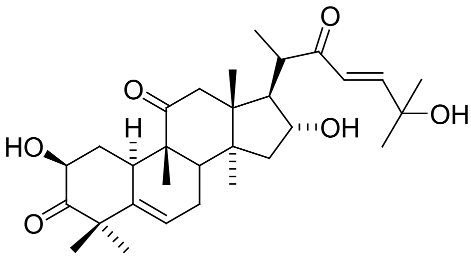
12. Allicin from garlic



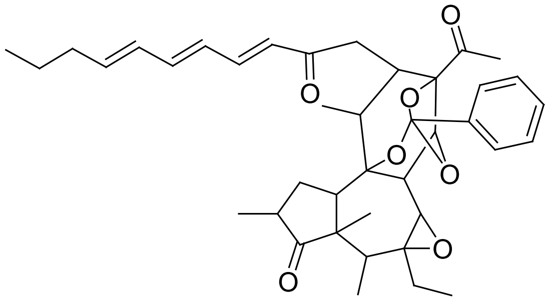
13. atanine from *Evodia ruteacarpa*



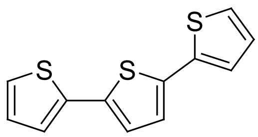
14. Embelin from *Embelia schimperi*



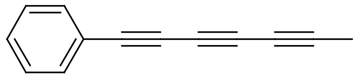
15. Curcurbitacin from *Cucumis sativus* (cucumber)



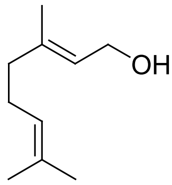
16. Odoratrin from *Daphne odora*



17. α-tertthienyl from Marigolds



18. Polyacetylene



19. Geraniol

The level of reported bioactivity varies greatly and in some cases it is difficult to assess the level of activity since the specific compound responsible for activity may be unidentified and the plant used in the study may have an unspecified amount of the material. It has already been noted that the structure of tannins is important for their biological activity in other areas and the same is true for their nematocidal properties (Mueller-Harvey et al, 2005). Similarly, the species specific effects of tannins have been noted between sheep and goats whereby the nematode load has been reduced in sheep but not goats (Max et al, 2006). Synergistic effects have been observed and validated in several cases, particularly with respect to essential oils and lipids (Ghisalberti, 2002). The evidence for efficacy is mixed, but in many cases convincing. This is also one area of bioactives chemistry where there have been several studies on ruminants. A summary of these observations is presented in Table 3.3.

In the Australian context, information on African and Indian forages may be particularly relevant. Many of the leguminous trees studied in Africa and India have Australian counter parts. Though further study would be required, this may represent an opportunity for ecologically sustainable nematode control from temperate to subtropical regions in Australia. Many of the browse feed studies that relate to tannins are focused on examining nutritional effects rather than specific bioactive properties (Aschalk et al, 2000; Singh and Makkar, 2002) and few studies have taken place in Australia. The forages that have been studied are often not ideal for the majority of Australian conditions. CT-containing forages such as sulla (Niezen et al, 2002), Lotus sp (Min et al, 1999; Molan et al., 2000) and sainfoin (Paolini et al, 2003) are all perennial cool season legumes and whilst they show some efficacy they would not be ideal for all Australian conditions. In contrast the warm season *Sericea lespedeza (Lespedeza cuneata)*, which is adapted to infertile, acid soils and warm climatic conditions (Shaik et al, 2006) may be a good option for some parts of Australia. This species can also be used as hay and has been investigated as a feed for lambs (Lange et al, 2006) where it has a demonstrated effect on worm fecundity. Though the activity has been attributed to the CT content the plant also contains bioactives including the nyctinastic metabolite potassium lespedezate, a phenolic glycoside (Shigemori et al, 1990). Lange et al, (2006) also note the additional benefits of use of the plant as hay compared to forage, in that it allows storage of bioactives until peak infective seasons and also may improve palatability due to changes in tannin chemistry.

Research into the nematocidal properties of Australian plants is warranted. There is ample evidence that plants do contain compounds effective against nematodes, however those that would be applicable to growth or farming systems in the Australian environment have not been well studied. Nematodes are responsible for considerable profit loss through their effect on animal health and resistance to current anlthelmintics is an increasing problem.

3.4 Safety and Environmental Considerations

The anti-nutritional activity of tannins is well documented but the potential negative effect of many substances has not been investigated in any detail. Before the introduction of any new feed, in-field toxicity and nutritional effects would need to be evaluated. The outcomes of such studies may also suggest the best way to deploy the bioactive containing material. For example, if in situ feeding of the plant proves to be detrimental, the plant may still be of use for supplemental feeding where the appropriate dose is more easily monitored. The plant may be provided fresh or dried, as hay or pellets.

Another important consideration is the environmental risk of establishing the use of non-native plants. The plant *Leucaena leucocephala,* that produces the compound Mimosine, mentioned earlier for defleecing, has noxious weed status in several countries. A risk assessment of *Leucaena leucocephala* for Australia was prepared by Pacific Island Ecosystems at Risk (PIER) and a recommendation to reject the plant for import was made (Global Invasive Species Database, 2006). By contrast the forage plant *Sericea lespedeza* has been considered as a potential forage crop in Australia (Australian New Crops, 2001) though it is a designated noxious weed in several states of the USA (Kansas State Research and Extension, 2005).

3.5 Conclusion

The use of plant bioactives for animal health is an area of increasing research importance. Many of the studies in ruminants to date have targeted specific classes of bioactives such as tannins and saponins. The focus of most ruminant research has been on ruminal flora modification for a reduction in methane emission and enhanced growth. There have been several ruminant focused studies investigating the use of plants for nematocides, though the focus has again been on the effects of polyphenolics. However, there are plant bioactives of almost every chemical class that have demonstrated nematocidal or antibacterial activity suggesting that this could be a fertile area for future research. One of the problems in assessing much of the available literature is a lack of chemical analysis for the feeds, despite evidence of structure activity relationships in both tannins and saponins. Additionally, many studies have relied on in vitro analysis via ruminal fermentors. This review suggests that plants may indeed be beneficial for animal health, whilst at the same time, highlights the need for more controlled in vivo research to validate plant bioactivity.

Of the many possible avenues for research, investigation into anthelmintics and plants to control bloat seem to offer the most advantage with respect to current Australian farming practice.

Table 3.1. Literature supporting fatty acid variation in forage and grain based diets for ruminants.

	Pasture	Species	Supplement	PUFA %	CLA%	SF%	Comments
Velasco et al. 2004	15% Trifolium spp. 45 % Lolium, Bromus, & Agrostis sp, 40% Compositae. (Oak-wooded pasture land)	Lambs	barley or conc	10.56% - 13.48%		51%-55%	Study assessed the influence of weaning and supplementation type on fatty acid analysis. Significant difference for weaning and W x F interaction.
French et al. 2000	(rotationally grazed grassland) Irish study – assume C3 grass	Cattle	no	4.14% - 5.35%	0.37%-1.08%	44%-48%	Study assessed the influence of grass, grass silage or concentrates on fatty acid composition of I.M. fat of beef steers. Significant difference for increasing level of grass in the diet on increasing PUFA and CLA and reducing saturated fat in muscle tissue.
Rowe et al. 1999	Cynodon dactylon pasture v grain Brazilian study	Lambs	no	5.36% v 4.74%	0.69% v 0.28%	55% v 49%	Study assessed the difference between grain fed v C4 pasture. Significant difference for PUFA, CLA and saturated fats for pasture fed animals.
Gatellier et al. 2005	Non descript ‘summer' pastures French study	Cattle	no	5.74% - 9.18%	1.55% - 0.83%		Study assessed steers, cows and heifer carcasses at an abattoir based upon pasture or conc+pasture classification. Significant difference in PUFA for ‘pasture fed'.
Fraser et al. 2004	Lucerne, Red clover and perennial Ryegrass	Lambs	no		1.09%-1.33%		Study assessed finishing lambs on different pastures and their subsequent effects on carcass quality. No sig effects for CLA however significant difference in PUFA:SF for red clover v rye or lucerne.
Rhee et al. 2003	Non descript rangeland v intensively fed grain diet. Broom weed, klein grass, three awn, silver, tobosa grass and sideoats	Lambs	sorghum and lucerne meal Whole cotton	4.98 – 9.33	0.45-1.33%	41%-46%	No diff found between treatments but compounded by feeding oilseeds to all treatment groups. Demonstrates significant difference between pasture species for all fats.
Dewhurst et al. 2001	Lollium spp. Compared *L. perenne, L. multiforum, L. x boucheanum*	Lollium sp	no	Significant genetic effects on the level and pattern of concentration of fatty acids in grasses. Potential to breed high lipid grasses			
Elgersma et al. 2003	Lollium sp. *L. perenne*	Lollium	no	Comparison of the fatty acid composition of fresh and ensiled perennial ryegrass affected by cultivar and regrowth Significant difference between fresh and ensiled grass			

CLA= conjugated linoleic acid, PUFA= poly unsaturated fatty acids, SF= saturated fats

Table 3.2. Literature supporting diets for ruminal modification or immunomodulation

Common Name	Botanical Name	Active compound	Action	Reference
Cinnamon	Cinnamomium spp.			
Birdsfoot trefoil	Lotus corniculatus	Proanthocyanidins/condensed tannins	Destabilise plant protein foams	Tanner et al. 1995 (invitro)
	Onobrycius viciifolia	Proanthocyanidins/condensed tannins	Bloat safe	
	Astragular cicer	Proanthocyanidins/condensed tannins	Bloat safe	
Dock	Rumex obtusifolius	Proanthocyanidins/condensed tannins	Bloat safe	Waghorn and Jones 1989
trefoil	Lotus pendunculatus	Condensed tannins	Anti-methanogen	Tavendale et al. 2005 (in vitro)
Lucerne	Medicago sativa	Crude protien /low NDF	Anti-methanogen	
Mulga	Acacia aneura	Proanthocyanidins/ condensed tannins	Protien binding / anti-nutritional	Miller et al. 1997 (in Vivo)
Yellow wood	Terminalia oblongata	Hydrolysable tannins – Gallic acid	Toxic dependant on nutritional state	Murdiati et al. 1991
Yellow wood	Terminalia oblongata	Hydrolysable tannins (0.9g/kg Bwt)	Not toxic when fed with Stylosanthes sp	McSweeney et al. 1988
Acacia mearnsii	Acacia mearnsii	Condensed tannins	Anti-methanogen, decrease rumen N and urea	Carulla et al. 2005
Birdsfoot trefoil	Lotus corniculatus	Condensed tannins	Decreased growth of proteolytic bacteria	Min et al. . 2002 (in vitro)
Calliandra	Calliandra calothyrsus	Proanthocyanidins	Decrease purines with increasing dose	Mbugua et al. 2005
Leucaena	Leucaena leucocephala	Mimosine	Depilatory agent – Goats	Reis et al. 1999 in vivo
	Leucaena glauca	Mimosine	Depilatory agent – sheep	Hegarty et al. 1964 (in vivo)
	Quillaja saponaria – Molina	Saponins	Immuno-stimulant	Francis et al. 2005
	Glycyrrhiza radix	Saponins	Anti viral	
	Quillaja saponaria	Saponins	Increase body mass gain in fish, increased food conversion ratio	Francis et al. 2002
Soapberry tree	Sapindus saponara	Saponins	Decrease rumen protozoa; decrease rumen NH4 and methane conc; decrease prop:acetate	Hess et al. 2003
Birdsfoot trefoil	Lotus corniculatus	Condensed tannins	Increase reproductive efficiency; liveweight	Ramirez-Restrepo et al. 2005b
Garlic; Cimmamon; Yucca; Anise; Oregano; Capsicum; Cinamaldehyde		Essential oils	Rumen pH dependant – 5.5 significant effects for lower acetate and higher proprionate for Ani, ORE, GAR, CAP, CDH and YUC. Anti-methanogenic at low pH	Cardozo et al. 2005.

Table 3.3. Plants with Nematocidal Activity

Plant Cited	Putative Bioactive (if known)	Chemical Class (if known)	Target Organism (where specified)	In vitro	In vivo	Reference
Allium sativum (garlic)	allicin	Thiosulfonates	Haemonchus contortus	x	goat	(Githiori et al, 2006)
Alnnona squamosa		anthraquinone terpenoids	Haemonchus contortus		goat	(Githiori et al, 2006)
Artemisia herva-alba	santonin	terpene	Haemonchus contortus		goat	(Githiori et al, 2006)
Calotropis procera		Triterpenoids, anthocyanins, alkaloids	Haemonchus contortus		sheep	(Githiori et al, 2006)
Canavalia brasiliensis			Haemonchus contortus		goat	(Githiori et al, 2006)
Carica papaya	Benzyl isothiocyanate	isothiocyanate	Haemonchus contortus		goat	(Githiori et al, 2006)
Chenopodium ambrosioides	Ascaridole	terpene peroxide	Haemonchus contortus		goat	(Githiori et al, 2006), (Ghisalbertil, 2002)
Chrysophyllum cainito			Haemonchus contortus		bovids	(Githiori et al, 2006)
Hymenaea courbaril			Haemonchus contortus		goat	(Githiori et al, 2006)
Menta spp			Haemonchus contortus		goat	(Githiori et al, 2006)
Momordica charantia			Haemonchus contortus		goat	(Githiori et al, 2006)
Musa acuminate			Haemonchus contortus		goat	(Githiori et al, 2006)
Tinospora rumphii			Haemonchus contortus		goat	(Githiori et al, 2006)
Butea monosperma	Sterols, palasonin	terpene	Caenorhabditis elegans	x		(Githiori et al, 2006)
Combretum spp.	Phenantherenes	aromatics	Caenorhabditis elegans	x		(Githiori et al, 2006)
Cymbogon martini	Geraniol	terepene	Caenorhabditis elegans	x		(Githiori et al, 2006)
Evodia ruteacarpa[1]	Atanine β	alkaloid	Caenorhabditis elegans	x		(Githiori et al, 2006)
Ocimum sanctum	Eugenol	phenolic	Caenorhabditis elegans	x		(Githiori et al, 2006)
Taverniera abyssinica		Phytoalexins (various classes)	Caenorhabditis elegans	x		(Githiori et al, 2006)
Terminalia macroptera		terpene	Caenorhabditis elegans	x		(Githiori et al, 2006)
Acacia auriculiformis			Ascaris lumbricoides	x		(Githiori et al, 2006)
Albizia lebbek			Ascaris lumbricoides	x		(Githiori et al, 2006)
Apium graveolens			Ascaris lumbricoides	x		(Githiori et al, 2006)
Artemesia santonica	Santonin	terpene	Ascaris lumbricoides	x		(Githiori et al, 2006)
Cassia obtusifolia			Ascaris lumbricoides	x		(Githiori et al, 2006)
Inula helenium	Alantalactone	terpene	Ascaris lumbricoides	x		(Githiori et al, 2006)
Carica papaya	Benzyl isothiocyanate	isothiocyanate	Ascaris suum	x		(Githiori et al, 2006)
Mentha cordifolia	β-Sitosterols, glucosides	terpene	Ascaris suum	x		(Githiori et al, 2006)
Carica papaya	Benzyl isothiocyanate	isothiocyanate	Ascaridia galli	x		(Githiori et al, 2006)
Albizia anthelmintica			Heligmosomoides polygyrus	x		(Githiori et al, 2006)
Embelia schimperi	Embelin	hydroxy quinone	Heligmosomoides polygyrus	x		(Githiori et al, 2006)
Alstonia boonei			Heligmosomoides polygyrus	x		(Githiori et al, 2006)
Nauclea latifolia		akaloids, saponin	Heligmosomoides polygyrus	x		(Githiori et al, 2006)

Ocimum gratissimum	Oleanolic acid	terpene	Heligmosomoides polygyrus	x		(Githiori et al, 2006)
Piliostigma thonningii		Tannins, alkaloids	Heligmosomoides polygyrus	x		(Githiori et al, 2006)
Adhatoda vesica		Alkaloids, glycosides	mixed GI infections		sheep	(Githiori et al, 2006)
Albizia anthelmintica		Sesquiterpene, kosotoxins	mixed GI infections		sheep	(Githiori et al, 2006)
Ananas comosus (pineapple)	Bromelain	mixture proteolytic enzymes	mixed GI infections		sheep, bovid	(Githiori et al, 2006)
Annona squamosa (sugar apple)		Anthraquinone terpenoids	mixed GI infections		goat, bovid	(Githiori et al, 2006)
Azadirachta indica (neem)	Azadirachtin	terepene	mixed GI infections		sheep, bovid	(Githiori et al, 2006), (Chitwood, 2002), (Ghisalberti, 2002), (Marley, 2005)
Chenopodium ambrosioides (Mexican tea)	Ascaridole		mixed GI infections		sheep	(Githiori et al, 2006)
Chrysanthemum cinerariaefolium	Pyrethrins		mixed GI infections		sheep	(Githiori et al, 2006)
Caesalpinia crista			mixed GI infections		sheep	(Githiori et al, 2006)
Embelia ribes			mixed GI infections		sheep	(Githiori et al, 2006)
Fumaria parviflora			mixed GI infections		sheep	(Githiori et al, 2006)
Hagenia abyssinica			mixed GI infections		goat	(Githiori et al, 2006)
Hildebrandtia sepalosa			mixed GI infections		sheep	(Githiori et al, 2006)
Khaya anthotheca			mixed GI infections		bovids	(Githiori et al, 2006)
Khaya senegalensis			mixed GI infections		sheep	(Githiori et al, 2006)
Maerua edulis			mixed GI infections		sheep	(Githiori et al, 2006)
Myrsine africana	Benzoquinone		mixed GI infections		sheep	(Githiori et al, 2006)
Nauclea latifolia		Resin, tannins, alkaloids	mixed GI infections		sheep	(Githiori et al, 2006)
Solanum aculeastrum			mixed GI infections		bovids	(Githiori et al, 2006)
Terminalia glaucescens		Anthraquinone	mixed GI infections		bovids	(Githiori et al, 2006)
Vernonia anthelmintica			mixed GI infections		sheep	(Githiori et al, 2006)
Vernonia amygdalina			mixed GI infections		bovids	(Githiori et al, 2006)
Medicago sativa (lucerne)			mixed GI infections		sheep	(Marley et al, 2005)
Trifolium pratense (red clover)			mixed GI infections		sheep	(Marley et al, 2005)
Trifolium repens (white clover)			mixed GI infections		sheep	(Marley et al, 2005)
Lolium perenne (rye grass)			mixed GI infections		sheep	(Marley et al, 2005)
Lotus corniculatus (birdsfoot trefoil)	CT	plant structure, phenolic	mixed GI infections		sheep sheep	(Marley et al, 2006a), (Marley et al, 2006b), (Ramirez-Restrepo, 2005)

Chicorium intybus (chicory)	CT, sequiterpene lactones,	phenolic, terpene, plant structure	mixed GI infections		Sheep sheep and deer	(Marley et al, 2006a) (Ramirez-Restrepo et al, 2005)
Lolium perenne (rye grass)			mixed GI infections		sheep	(Marley et al, 2006a)
Tagetes sp (marigolds)		polythienyls isothiocyanate	plant		plant/soil	(Chitwood, 2002) (Chitwood, 2002)
Brassicaceae		glucosinolates		x		(Chitwood, 2002), (Ghisalberti, 2002)
Sorghum sudanese/bicolor	dhurrin	cyanogenic glycosides		x		(Chitwood, 2002)
Manihot esculenta (cassava)	linamarin etc	cyanogenic glycosides		x		(Chitwood, 2002)
Asteraceae e.g. Rudbecia hirta (black eyed susan)		polyacetylenes		x		(Chitwood, 2002)
Physostigma venenosum (calabar bean)	physostigmine	alkaloid		x		(Chitwood, 2002)
Sophora flavescens[a]	Monocrotaline, Nmethyl cytisine, anagyrine, matrine, sophocarpine, sophoramide	alkaloid				(Chitwood, 2002)
decomposing rye numerous plants	butyric acid	fatty acids				(Chitwood, 2002)
Iris japonica iris	2-undecylenic acid, linoleic acid	fatty acids				(Chitwood, 2002), (Ghisalberti, 2002)
Mucuna (velvet bean)	1-triacontanol triacontanyl tetracosanoate		M. incognnita	x		(Chitwood, 2002) (Chitwood, 2002)
Ocimum basilicum (basil)	eugenol, menthol, cineole, geraniol	terpene oils	H. cajani			(Chitwood, 2002)
O. sanctum [a]						(Chitwood, 2002)
Mentha piperatum (peppermint)						(Chitwood, 2002)
Callistemon lanceolatus (bottle brush)						(Chitwood, 2002)
Eugenia caryophyllata (clove)			broad spectrum			(Chitwood, 2002)
Cymbopogon caesius (kachi grass) [a]			sting nematodes	x		(Chitwood, 2002) (Cox et al, 2006)
Pinus massoniana	humulene	terpene	B. xylophilus	x		(Chitwood, 2002)
Daphne odora	odoracin, odoratrin	terpene	A. besseyi	x		(Chitwood, 2002) , (Ghisalberti, 2002)
Quassia amara [a]		terpene		x		
Hannoa undulata	chaparrinone, glaucarubolone and klaineanone.	Terpene	M. incognnita			

solanaceae	tomatine, chaconine	terpene (glycoalkaloids)	P. redivivus, M. incognita			
Asparagus adescendens (shrub)	asparanin I and B	terpene (glycoalkaloids)				
Albizia chinensis [a]	albichinin II	terpene glycoside				
Acacia concinna [a]	sonunin III	terpene glycoside				
Acacia auriculiformis (black wattle) Oz	acaciaside A/B	terpene glycoside				
Dioscorea deltoidea [a]	protodioscin , deltoside	terpene glycoside				
Bacopa monniera herb [a]	jujubogenin glycosides	terpene glycoside				
Lantana camara	Lantanilic acid, camaric acid and oleanolic acid	terpene glycoside				
Ocimum gratissimum (clove or tree basil) [a]	oleanolic acid	terpene				
Cucumis sativus (cucumber)	curcurbitacins	terpene		x	mice	
	pelargonic acid					(Ghisalberti, 2002)
Helenium sp (asters) roots	pentayne	polyacetylenes		x		(Ghisalberti, 2002)
Carthanmus tinctorius (safflower)		polyacetylenes		x		(Ghisalberti, 2002)
Cirsium japonicum (thistle)		polyacetylenes		x		(Ghisalberti, 2002)
Erigeron philadelphicus (daisy) also known as fleabane		polyacetylenes		x		(Ghisalberti, 2002)
Angelica pubescens [a]		polyacetylenes				(Ghisalberti, 2002)
Nothia anomala (brown alga)		tetrahydrofurans		x		(Ghisalberti, 2002)
Annonaceae (large plant family inc. pawpaw, custard apple)		acetogenins		x		(Ghisalberti, 2002)
Anacardiaceae (cashew, mango etc)	cardol	phenolic lipid			rats	(Ghisalberti, 2002)
E. grandis (fresh eucalyptus leaves)	G-inhibitors e.g. β-triketone	polyketide			goats	(Ghisalberti, 2002)
Zanthoxylum sp (Z. liebmannianum)	sanshool					(Ghisalberti, 2002)
Butea frondosa [a]	palasonin	terpene		x		(Ghisalberti, 2002)
Curcuma longa (turmeric)	tumerone	terpene		x		(Ghisalberti, 2002)
Solanum tuberosum (potato)	rishitin	terpene		x		(Ghisalberti, 2002)
Gossypium hirsutum (cotton)	gossypol	terpene		x		(Ghisalberti, 2002)
Melia sp (Meliaceae)Melia	limonoid 28-deacetyl	terpene				(Ghisalberti, 2002)

azedarach (cape lilac, white cedar)	sendanin					
Phaseolus vulgaris (kidney bean)	glycinoeclepin	terpene		x		(Ghisalberti, 2002)
Solanum incanum Chinese herb also trad use nigeria	solamargine	terpene			rats	(Ghisalberti, 2002)
Streblus asper [a]	cardenolides – asperoside and sterbloside	terpene		x		(Ghisalberti, 2002)
Piper betle [a]	chavibetol (betel-phenol; 3-hydroxy-4-methoxyallylbenzene)	propenylphenols		x		(Ghisalberti, 2002)
Acorus gramineus (sweet-flag)	asarone	Shikimate pathway (phenolics)		x		(Ghisalberti, 2002)
Phaseolus lunatus (Lima bean)	coumestrol	Shikimate pathway (phenolics)		x		(Ghisalberti, 2002)
Pycanthus angolensis (trad use Cameroon)	dihyro-guaiaretic acid	Shikimate pathway (phenolics)		x		(Ghisalberti, 2002)
Pinus massoniana	ligans	Shikimate pathway (phenolics)				(Ghisalberti, 2002)
Zingiberaceae (turmeric, cardomon, ginger)	diarlyheptanoids inc. curcumin I-III	Shikimate pathway (phenolics)		x		(Ghisalberti, 2002)
Zingiber officinale (ginger)	gingerol, shogaol	Shikimate pathway (phenolics)		x		(Ghisalberti, 2002)
Quisqualis indica (trad use China)	potassium quisqualate	Amino acid derived		x	human	(Ghisalberti, 2002)
Asparagus officinalis	asparagusic acid	Amino acid derived		x		(Ghisalberti, 2002)
Laminariales (brown alga)	laminine	Amino acid derived				
Digenea simplex [a] also found in alga Alsidium helminthocorton, Centrocerus clavulatum Caloglossa liprieurii.	Kainoid amino acids (e.g. kainic acid)	nonproteogenic acids			human	(Ghisalberti, 2002)
Chondria armata	domoic acid	nonproteogenic acids				(Ghisalberti, 2002)
Dorycnium rectum	CT tannin	phenolic	Trichostrongylus colubriformis		Lambs	(Niezen et al, 2002)
Sericea lespedeza	CT tannin		Haemonchus contortus		Goats	(Shaik et al, 2006)
Sericea lespedeza	CT tannin		Haemonchus contortus		lambs	(Lange et al, 2006)

[a]Plant has traditional use

4 Current Research & Development Activities

Though there are many facets to bioactive discovery (including high throughput screening, in silico modelling, structure-activity modelling etc.) there are two main models that we can explore in relation to ruminant health systems.

4.1 Pharma Model

The pharmaceutical drug discovery model outlined in Section 2.2.1, has been very successful in delivering bioactives for human health. Large companies use the same model for plant health and animal health bioactive discovery. One such company is Syngenta. Syngenta (a large agribusiness company formed in 2000 when Novartis merged with AstraZeneca's agribusiness) employs high throughput screening (HTS) along with the other strategies described to discover bioactives for plants and animals. In Australia, Novartis Animal Health (pre-Syngenta) worked with several Australian natural product researchers to discover natural anthelmintics.

There are two large EU programs aimed at developing practical alternatives to antibiotic use in animal feed and replacing the use of synthetic antibiotics in animals (Replace-EU, 2006). These are large-scale projects involving seven to ten member countries and are well funded.

Project 1: RUMEN-UP
This work involves the collection of plants and screening them for various activities.
Progress so far is difficult to gauge however, a recent report highlighted 22 plants identified so far with promising activity (see Table 1, Appendix 2) (Wallace, 2005). These plants will be further investigated for in vivo efficacy and to determine the plant chemical/s responsible for the activity.

Project 2: FEED FOR PIG HEALTH:
Development of Natural Alternatives to Anti-microbials for The Control of Pig Health and Promotion of Performance. Project Funding: 4 million euro (Cordis, 2006).
This project has been running for more than a year. Phase one of the project involved recollection of sufficient quantities of the 500 plants/plant extracts to be tested. Phase two of the project, investigated the potential of the plants/plant extracts in the suppression of infections caused by micro-organisms such as *E. coli* 0149:K88, *C. perfringens*, *Lawsonia intracellularis* and nematodes. Studies to investigate the immune stimulatory effects of the plants/plant extracts on fish were completed.The group have now agreed on a list of plant species that have demonstrated potential. Appendix 3 lists the compounds/plant extracts that the group will focus on. Further work will include in vivo analysis and nutrient retention studies (Feed for Pigs, 2006).

Both of these programs have utilised what is essentially the pharma model of discovery i.e. Identify screening targets, build an extract library, screen the extracts and identify the active extracts. The next steps they propose also fit with this schema i.e. in vivo evaluation (for efficacy and safety), identification of bioactive compounds and recommendations for use.

These EU research groups summarise the steps as:

- Phase 1: Establish a plant collection of potentially relevant materials (500 samples)
- Phase 2: Develop robust assays and assess plants for in vitro activity (suppression of infection, nutrient retention, food safety)
- Phase 3: In vivo validation of effect, identification of bioactives, assess safety
- Project evaluation and recommendations for industry.

The main difference between pharma design and this approach is the type of bioassays used. The RUMEN-UP and EU-Replace assays, although in vitro, are unlikely to be high through put. On a much smaller scale this sort of research is being replicated in many universities around the world, though in general the aim is to find plant compounds for human use.

In Australia the only similar research (with respect to ruminants) is the CSIRO/UWA collaboration which focuses on the use of Australian plants to reduce acidosis. The screening involves the identification of antibacterial agents toxic to lactic acid producing bacteria. A recent report indicated the identification of three plants with specific activity. Further investigation of these plants will include in vivo testing and identification of bioactives.

4.2 Animal System Models

This sort of research is being carried by out various groups around the world as exemplified in the literature report, section 3. Some of the most notable are summarised below:

Dr Chris Grainger, DPI Victoria: The effect of tannin on dairy cow heath and milk production. This project is mainly aimed at reducing greenhouse gases and energy losses associated with the break down of excess protein in dairy cows grazing high protein pasture in Spring. Mechanisms involve anti-protozoal activity as well as physical binding of protein by condensed tannins. These studies are virtually all being conducted in vivo and utilise sophisticated whole animal calorimetry and milk yield responses.

Dr Dean Revell, CSIRO Livestock Industries WA: Browse feeding for health – self medicating sheep.

Professor Tom Barry: Massey University Institute of Veterinary, Animal & Biomedical Sciences: Ruminant nutrition, forage feeding value, effect of plant secondary compounds including condensed tannins on nutritive value. Development of grazing systems. The nutrition and productivity of farmed deer. Drought feeding of livestock.

R John Wallace, Rowett Research Institute, Bucksburn, Aberdeen, Scotland: Natural products to manipulate ruminal and intestinal fermentation and protein and hydrogen metabolism in the digestive tract of ruminants and man. Research at the Rowett Research Institute is now largely focussed on human nutrition and functional foods but John Wallace is, at least in the short term, still continuing to use in vitro and artificial rumen fermentors to conduct screens on plant products that may have bioactive or greenhouse reducing effects. These studies aim to provide candidate molecules or plants for animal studies.

IGER: There are also broader groups which collectively have projects of relevance. For example the Institute of Grassland and Environmental Research (IGER) in the UK carries out research aiming to optimise ruminant health. The focus of the research is generally forage based but they also utilise novel technologies, such as metabolomics, to investigate systems. Metabolomics is another of the ‘omic’ sciences and examines the metabolites of an organism as the down stream result of the interaction between genome and environment. Metabolomics techniques can be employed to investigate animal response to medication or feed (Rochfort, 2005).

Relevant IGER projects include: “Developing approaches to the use of forage legumes in upland environments to enhance biodiversity and produce balanced quality ruminant feed, Michael Fothergill” , “Use of the dairy cow metabolome in plasma and milk to improve health, fertility and nutrient utilisation for milk production, Jon Moorby”, “Plant-derived antimicrobial activities for use in animal feed: identification by screening, production and optimisation of plant growth conditions using hydroponic technologies (ERDF KEF), Phillip Morris”. Dr Christina

Marley, at the Aberystwyth IGER site, has also published extensively in the area of the effect of different forages on livestock production and examined the effect of these on parasite load in lambs.

AgResearch New Zealand: Similarly to the research at IGER, AgResearch in New Zealand is a diverse organisation but several projects within it are of relevance. Work is focused on several strategies to improve ruminant health. This includes the use of plants as replacements for antibiotics. In the 2005 annual report AgResearch reported a project under the Food & Health Group aimed to identify and determine the chemical structures of a number of novel plant and bacterial molecules that have dual function against the pathogenic bacteria and parasites that cause coccidiosis and necrotic enteritis; conditions that significantly reduce productivity in the intensive animal industries. Research is also dedicated to the reduction of methane emission by ruminants and has included studies on various pasture species. Key researchers in these areas included Harry Clark, Garry Waghorn, Keith Joblin and Warren McNabb.

4.3 *Evaluation of Research Models*

An evaluation of research methodologies outlined in 2.2.1 and 2.2.4 is presented in Table 4.1.

Table 4.1. Comparison of Models for Bioactive Research

Evaluation Factor	*Pharma Model*	*Animal System Model*
Throughput (number of molecules/extracts that can be investigated)	Very high to medium depending on the assay	Very Low
Initial Screening Cost	Low	High
Follow up Screening Cost (animal assay)	High	Incorporated in above
Time for initial results	Low	High

Both models offer valuable methods for determining bioactives. The best method depends on the project scope. For example, if a few, highly promising plants were to be investigated then the animal system model could be applied. If large numbers of plants needed to be investigated this would not be practical and the pharma model would need to be utilised.

For a focus on in situ use an early combination of models may be appropriate. For example, plants would need to be screened for activity and toxicity in vivo then animal response in terms of forage acceptance and palatability could be assessed. At the same time the bioactives could be isolated and their structure determined. Establishment of in field testing for consumption would then immediately allow assessment of compound levels in different plants in the real environment and assess the plant response to grazing stress.

5 Social Drivers for the Replacement of Antibiotics in Animal Feed

Human health and safety concerns are ultimately behind the push to reduce the use of antibiotics in animal production. There is increasing public concern regarding the use of pharmaceuticals in the animal industry. Much of this has been as a result of the emergence of drug resistance. A particular area of criticism has been in the use of antibiotics as growth promoters and the associated risk of developing antibiotic resistance in human pathogens, though there is still considerable debate about the science behind these concerns (Barton, 2000). This is not a new issue and in 1969 the Swann report resulted in the withdrawal of β-lactams from feed in the UK (Ruddock, 2000).

The regulatory push to reduce the level of antibiotic use in animals is strongest in the European Union with legislation to remove antibiotics in animal feed in effect as of January 2006. In the United States there has been less regulation but there has been a reduction in antibiotic use in animal feed through a number of mechanisms.

Lobby groups have had a significant impact and one of the most active in this area in the USA is "Keep Antibiotics Working". The organization describe themselves as "a coalition of health, consumer, agricultural, environmental, humane and other advocacy groups with more than nine million members dedicated to eliminating a major cause of antibiotic resistance: the inappropriate use of antibiotics in food animals." The group lobbies regulators (FDA), law makers (federal government), medical bodies and animal producers (KAW, 2006).

In 2003, the World Health Organization (WHO) released a report finding that the use of antibiotics in animal feeds could be reduced without serious implications and encouraged countries to follow Denmark's example. Denmark stopped the use of antibiotics in feed in 1999. The report added credence to WHO's long standing view that the use of drugs in healthy animals should be curtailed (Grady, 2003).

As public concern is raised by NGOs large companies such as McDonalds have taken notice. In 2003 McDonald's Corporation announced plans that called for its suppliers worldwide to phase-out animal growth promotion antibiotics that are used in human medicine. The company released a "Global Policy on Antibiotics" that defined a set of standards for McDonald's direct meat suppliers. In a press release from the company antibiotic resistance and the associated social issues were defined as being behind the move, "As a company committed to social responsibility, we take seriously our obligation to understand the emerging science of antibiotic resistance, and to work with our suppliers to foster real, tangible changes in our own supply community, and hopefully beyond," said Frank Muschetto, Senior Vice President of Worldwide Supply Chain Management at McDonald's Corporation. "McDonald's is asking producers that supply over 2.5 billlion pounds of chicken, beef and pork annually to take actions that will ultimately help protect public health" (McDonalds Coporation, 2003).

The company's purchasing power has influenced their suppliers. By 2005 all of the chicken meat suppliers with direct relationships with the company had eliminated the use of human antibiotics as growth promoters. Tyson Foods, Perdue Farms and Foster Farms, which combined produce a third of the chickens consumed by Americans, acknowledged that they had voluntarily taken most or all of the antibiotics out of what they feed healthy chickens. (Burros, 2005)

Other major purchasers, Wendy's, Dairy Queen and Burger King, have adopted similar strategies towards sourcing their products.

This is not to say regulatory action is unimportant in the USA. In July 2005, the Food and Drug Administration (FDA) banned the use of the antibiotic Baytril in poultry because of concerns that it could lead to antibiotic-resistant infections in people (Associated Press, 2005). In May 2006 the US House of Representatives passed an amendment to allocate $1 million to the Food and Drug Administration's Center for Veterinary Medicine. The money will be used to assure the safety of animal drugs with respect to antibiotic resistance.

In Australia, the increasing general concern led to the formation of JETACAR – Joint Expert Advisory Committee on Antibiotic Resistance. This group reported in 1999 that there was evidence for the emergence of antibiotic resistant bacteria through agricultural practice. The report made several recommendations including the establishment of a surveillance system to monitor the emergence of resistant bacteria. As a result, a pilot surveillance program for antimicrobial resistance was set up for 2003-2004. The program was supported by the Department of Agriculture, Fisheries and Forestry (DAFF) and focused on livestock species where antimicrobials are used in feed or water.

A National Health and Medical Research Council (NHMRC) Expert Advisory Group on Antibiotic Resistance (EAGAR) was established to provide continuing scientific and technical advice on antibiotic resistance and related matters.

Given the strong regulatory environment of the EU, and the increasing moves of the USA to reduce the use of antibiotics in animal feed, it seems highly likely that such restrictions will eventually be seen in Australia.

6 Regulatory Frameworks

Animal feed is subject to regulation in many countries including the United States, the European Union and Australia.

6.1 USA

In the USA animal feed is regulated by the Center for Veterinary Medicine (CVM). CVM is a section of the Food and Drug Administration (FDA) which in turn is part of the U.S. Department of Health and Human Services. CVM is responsible for ensuring animal feed is safe and appropriately labeled. This applies to dietary supplements, such as vitamins and minerals, and any other added materials.

In 1994, Congress passed the Dietary Supplement Health and Education Act (DSHEA). DSHEA created a new category of substances with changes in the associated regulatory framework. The main effect was to remove some ingredients from regulation as food additives which required pre-market approval. This created an increase in the number of animal feed products sold with additives for health benefits (generally as a flow on from those used in human products). In 1996 CMV determined that this act did not apply to animal feed and this has been tested successfully in court. This means even if a substance is marketed as a dietary supplement for humans, the substance still falls into the earlier regulatory framework for animals and must be considered a food, food additives, a new animal drug or have GRAS (generally recognized as safe) status. There are many products on the market in the US which are therefore technically illegal. However, these violations are of low enforcement priority (Grassie 2002).

CVM works in association with the Association of American Feed Control Officials (AAFCO). AAFCO brings together regulators across the USA and Canada. They offer advice to CVM. Examples of relevance are advice in 2002 and 2003 to remove comfrey and kava respectively, from animal feeds due to concern about the potential toxicity.

Interestingly, AAFCO, had formed a subcommittee to specifically address the issue of botanicals and herbs in feed. However, the Botanicals and Herbs Committee received no submissions for ingredient definitions. The committee recommended to the AAFCO Board of Directors that it be disbanded (replaced with a single investigator) until such a time as there was more demand (Anon, 2002).

CVM publish a list of approved food additives and GRAS substances in Title 21, Part 570 – 584 of the Code of Federal Regulations (see Appendix 4). Any substance that does not fall into these tables cannot be used without thorough safety testing and pre-market regulatory approval. Included in section 582.10: Spices and other natural seasonings and flavorings, are a large number of herbs, including some that may be of value for animal health, for example marigolds (anthelmintic activity).

These regulations would apply to any stock feed, including that designed to be supplemental to grazing.

6.2 European Union

In Europe, animal feed is ultimately regulated by the European Commission though it acts on recommendations from European Food Safety Authority (EFSA). EFSA carries out evaluations when regulatory approval is sought. Feed additives are defined as "products used in animal nutrition for purposes of improving the quality of feed and the quality of food from animal origin, or to improve the animals' performance and health, e.g. providing enhanced digestibility of the feed materials."

The regulations are strict and safety assessments must include environmental analysis as well as potential to negatively impact human and animal health. EFSA may also require maximum residue limits (MRLs) and a market-monitoring plan. (EFSA, 2006)

The basic legislation was foreshadowed in the European Commission's white paper on food safety. In 2003 this white paper resulted in a formal regulatory framework (European Parliament and Council Regulation (EC) No 1831/2003). It was this regulation that resulted in the phasing out of antibiotic feed additives from January 2006. It also introduced new provisions regarding labeling and packaging of feed additives.

Authorization must be sought for any additive. Additives are broadly grouped into five categories, Table 6.1.

Table 6.1 Feed Additive Categories in the EU

Category	*Examples*
Technological additives	preservatives, antioxidants, emulsifiers, stabilising agents, acidity regulators, silage additives
Sensory additives	flavours, colorants
Nutritional additives	vitamins, minerals, amino acids, trace elements
Zootechnical additives	digestibility enhancers, gut flora stabilizers
Coccidiostats and histomonostats	

As for the USA, these regulations would apply to any stock feed, including that designed to be supplemental to grazing.

6.3 Australia

In Australia animal feed is regulated by The Australian Pesticides and Veterinary Medicines Authority (APVMA). APVMA is a government authority responsible for the assessment and registration of pesticides and veterinary medicines and until 2003, was known as the National Registration Authority for Agricultural and Veterinary Chemicals (NRA).

In 2001 APVMA released guidelines for stock food and stock food additives. If no therapeutic, performance enhancing or productivity claims are made then the feed does not require APVMA registration. Medicated stock feeds also do not require registration as long as the veterinary chemicals they contain are registered and the product is labeled with the appropriate instructions for the additive. There is a schedule outlining what are acceptable functional claims for various accepted feed additives (Appendix 5).

Herbal medicines and nutraceuticals for animals require registration.

APVMA also provide a database of active agents that are approved for use (APVMA, 2006a).

Of potential relevance to this investigation is a list of IP protected ingredients. This includes several plant products such as neem and rosemary oil. (APVMA, 2006b).

Any stock feed, including that designed to be supplemental to grazing, and making functional health claims would need to be registered.

7 Opportunities for Substitution of Synthetic Chemicals by Plant Bioactives

The previous sections clearly indicate the direction of national and international research with respect to the use of plant bioactives for animal health. Sections 3 and 4 described research across a range of applications. The majority of the research has focused antibiotics and anthelmintics.

In summary, bioactives from plants may be substituted for:
- Antimicrobials as growth promoters (but unlikely to replace antibiotics in extreme cases of infection)
- Anthelmintics, particularly in a pasture setting.

In addition, plant bioactives may also enhance animal health via a capacity to:
- Reduce production of methane (one likely mechanism of action is through an antimicrobial effect on specific ruminal bacteria, but this is an area that would benefit from additional research)
- Improve nitrogen metabolism (enhanced growth).

Bioactives incorporated into the food chain are also likely to have benefits for consumers and greater acceptance because the mode of delivery is natural:

- Colour preservation in meat
- Healthy meat – antioxidants, CLA for heart health.

The best evidence for efficacy exists for anthelmintics and for increased productivity through modification of the rumen. In Australia there is the opportunity to leverage relevant experience in India, Asia and the southern parts of the USA to identify Australian leguminous plants that will enhance animal health and also environmental health. Such plants are likely to be effective in what is currently less productive land. There is considerable work being done with respect to temperate legumes for grazing (including GM efforts to produce tannin rich varieties in plants such as white clover). Complementary approaches to identify high value forage plants may enable solutions for a range of Australian environments. Given this evidence it appears that such approaches could have a high chance of delivering real benefits to industry.

There is also opportunity to develop new antibiotics from plants but this would require additional research. A starting point would be an in-depth review of plant antimicrobials – a review, which was beyond the scope of the current work. Two clear classes of bioactive could be assessed here – those for topical application and those for systemic application. The information already in the literature would provide a good starting point for assessment of topical applications. There are many such metabolites reported, since in vitro anti-microbial activity is one of the easiest bioassays to develop. There are many novel antibiotics (both antibacterials and antifungals) reported from plants each year, some of them extremely potent. Much more basic research aimed at ruminants would be needed to ensure safety of potent systemic plant bioactives.

8 Potential Delivery Mechanisms to Animals of Bioactive Compounds

The potential delivery mechanisms range from a purified plant bioactive (in a capsule or injectable form) to in situ grazing on plants in the paddock. In between these extremes are options such as:

- Drenching with partially purified and concentrated plant extract
- Drenching with crude plant extract.
- Incorporation of processed crude plant material or extract into feed pellets or solution
- Application of fresh plant material to the paddock/feed lot
- Application of partially processed plant material to the paddock/feed lot e.g. plant meal
- Application of preserved plant material to paddock/feed lot e.g. hay, dried material
- Growth of plants in the field for browsing.

The most appropriate delivery method is impossible to predict *a priori*. The best delivery option will depend on compound stability, potency, bioavailability and safety. Economics of delivery will also be of key importance.

The nature of delivery also is dependent on the type of plant the bioactives are sourced from. For example, there is evidence that bioactives from agricultural waste streams such as citrus waste and chickpea hulls, contain several classes of bioactive compounds including essential oils, tannins, saponins and polyphenolics. It would not be possible to provide the majority of these for in situ foraging but the material could almost certainly be applied to the paddock in a number of ways including as preserved plant material. There is also the potential that the compounds would be suitable for purification and use as a drench.

In terms of regulations, interpretation of the data presented in section 6, indicates that any product, regardless of what it is will need to be thoroughly tested before any therapeutic statement could be made.

9 Gaps and Opportunities for Research

There are a few major gaps in the research being conducted. The focus in the EU seems to be largely, though not exclusively, on plants that have GRAS status eg certain herbs. Logically enough, other plants prioritized are those that have a long history of cultivation in Europe (e.g. *Prunus avium*, wild cherry).

In New Zealand and Australia the majority of the work in health for ruminants has focused on certain graze and forage plants such as clover and grasses. There is only one project that has examined the use of Australian natives for improvement. Further research in this area is warranted. A particular opportunity here would be to examine natives from the various grazing regions of Australia for biological activity. This may allow incorporation of some of these plants into the diet via free-foraging in the paddock. The dried plant material or extract of these plants may also be of use for more direct delivery to the animals. The study of environmentally suitable plants for both in situ foraging and processing is a gap in the current research.

For any plant based delivery of medicinal compounds, the growth conditions that effect compound production must be well understood. For in situ grazing this is particularly important since a plant grown in one part of the country may produce significantly different levels of bioactives compared to the same species of plant grown in another part of the country. If the levels of compound production are not well understood, then even if the animals graze consistently they may be receiving vastly different levels of bioactives. This may result in a reduction in desirable effects, or worse, in the case of over production it may result in toxicity. Again, this highlights the need to understand precisely what the bioactive is and how the levels can be measured.

The majority of farmed animals in Australia, with the possible exception of those on large cattle ranges in WA, QLD and NT, are not exposed to a wide variety of plants to feed on. Behavioural studies would need to be carried out to assess the level of consumption for various animals with any plant to be used in situ. There is some evidence that animals must be taught to eat the plants that are of benefit to them. Similarly, even if the plant is to be delivered in a dried form such as incorporation into a hay-based feed, animal studies would need to ensure that the plant was palatable to animals and that they were receiving safe and effective levels of compound. This type of research would be essential to obtain regulatory authorisation for any product baring a functional statement.

Another gap that has received little research is the possible use of agricultural waste for functional feeding, though there is a history of use for basic supplemental nutrition. In the grains processing industries, waste streams result from a number of manufacturing processes. In some cases the value (usually feed value) is understood but the bioactive properties are less well understood. For example, barley is used in malting and brewing. After mashing (extraction of soluble sugars) the spent grain contains protein, fibre and other cell wall materials such as beta glucans. The material is used mainly for animal feed.

One promising waste stream could be pulses. Dehulling of pulses (lentils, fieldpeas and desi chickpeas) produces hulls that are a source of saponins and tannins. These hulls also contain other polyphenolics. It is well understood that this general class of compounds may be beneficial for animal health but there have been no specific or systematic studies of the pulse hull metabolites for animal health. The economics in extracting this is not known, though there is also potential that the hulls could be incorporated directly into a semi-processed animal feed.

Another source of antimicrobials is citrus waste. Grape seed (or grape mark) and citrus waste are available in great quantities (particularly in Victoria) and have in vivo activity. However, the efficacy of antimicrobial action of these two natural products in animal feeds has not been assessed.

10 Prioritized, Specific Research Proposals

Recommendations for future research are based on the research described in this document. The recommendations take into account the diversity of the Australian landscape. The strategies recommended also leverage Australian grains and horticultural production. Though there are several areas where the use of plant bioactives may assist in ruminant production systems, the evidence suggests the most value could be obtained by investing in additional research for bloat control and the discovery of anthelmintics.

10.1 Research Priorities

10.1.1 Anthelmintics

The emergence of resistant worms is a major threat to the Australian sheep industry. Currently worm control relies on treatment with anthelmintics from a narrow range of chemical classes (including benzimidazoles, levamisole and macrocyclic lactones). In their recent article Besier and Love (2003) state that by 2010 the potential cost to industry will be in order of $700 million pa. The same authors advocate integrated approaches to pest management and note that plants containing tannins and other bioactives could form part of this strategy. The evidence detailed in this report endorsees this view. The use of plants for nematode control, both in situ and for supplemental feeding, is an under explored area of research. Given the evidence that plants are capable of producing nematocides of many different structure classes, there is a strong probability that plant bioactives exist that would be effective for ruminant health and so would provide real benefits to industry.

10.1.2 Bloat

Bloat is a significant problem for ruminants, particularly when grazed on protein rich legume pastures that occur significantly in the southern states of Australia. There is good evidence that tannins can be effective in preventing bloat. The mechanisms for this effect and the structure class of the most beneficial plant chemicals are still not clear. In a recent review Muller-Harvey (2006) notes that “Although many plants with high levels of tannins produce negative effects and require treatments, others are very useful animal feeds. Our ability to predict whether tannin-containing feeds confer positive or negative effects will depend on interdisciplinary research between animal nutritionists and plant chemists. The elucidation of tannin structure-activity relationships presents exciting opportunities for future feeding strategies that will benefit ruminants and the environment within the contexts of extensive, semi-intensive and some intensive agricultural systems.”

10.2 Research Strategies

There are numerous research strategies that could be employed to address these needs (and this document describes approaches others have taken). In the Australian context there are two broad themes for which there is significant opportunity. These themes offer the potential for sustained competitive advantage for Australian producers. In the first case this is through access to unique flora, in the second through leveraging the agricultural production diversity in Australia.

Theme One: In situ foraging or supplemental feeding
Aim: Identify native Australian plants that will enhance ruminant productivity through animal feeding in the field.

Theme Two: Agricultural waste for enhancing ruminant health
Aim: Identify agricultural waste plants that will enhance ruminant productivity through delivery of plant bioactives to animals as supplemental feed or in concentrated form.

Both research themes have common elements and would follow the general procedure below:
- Identify plants of interest
- Develop assays e.g. ruminal fermentors, G-ve antimicrobial, anthelmintic
- Prepare extracts
- Screen extracts
- Assess activity, eliminate duplicate hits (i.e. the same compounds with the same activity in different plant extracts)
- Preliminary assessment of the nature of the bioactive
- Identification of the bioactive and associated in vitro activity levels
- Assess efficacy in vivo
- Assess effectiveness of different delivery systems.

For Theme One it is recommended that research be initiated by a preliminary assessment of what native plants are present in the regions of interest in Australia and if there is any chemical knowledge associated with these plants.

Establishing an effective bioassay and plant library are key steps in carrying out these projects. There are several organisations that have plant libraries, that is, physical collections of plant material from different Australian regions. These include most of the state herbariums and botanic gardens. In addition, there are a few organisations that have large collections in a format suitable for natural product studies.

These organisations include:

Cerylid Biosciences: Victorian based natural product biotechnology company. Cerylid is currently selling off all its assets. This includes a considerable plant library of some 28,000 samples. The library contains approximately 50g of dried, ground plant material obtained from Victoria, Tasmania, New South Wales, Queensland and the Northern Territory. The company is closing down and is selling assets. The plant library is currently for sale.

Southern Cross University: SCU is an academic organisation based in NSW and in February 1999, the Centre for Phytochemistry and Pharmacology was established there. Although they do not have a large library of native plants they work with Bioprospect and herbariums to obtain samples. Their current focus is on human health and the investigation of Asian traditional medicines and herbs for natural bioactives.

Natural Product Discovery: NPD was formed collaboratively between Griffith University in QLD and the UK-Swedish pharma company AstraZeneca. The organisation has a library composed of large numbers of organisms, including Australian native plants. The plants have been dried and ground and are ready for extraction and screening. The majority of the plants are from QLD (~20,000), PNG (~5500) and China (~6500). There is the potential to screen this library for actives. NPD also has the necessary staff expertise and infrastructure for the isolation and identification of bioactive natural products.

Any library of plants could also incorporate agricultural plants as suggested in Theme 2. Here the initial focus is suggested to be on pulses (which may have GRAS status and are known to contain bioactive compounds), citrus waste and grape mark.

Bioactivity Assays: The types of plant extracts that are suggested to be efficacious include tannin rich materials. For this reason testing of these plants in molecular assays **is not** recommended. Tannin rich extracts generally cause strong interference in such assays resulting in false positives. It is therefore suggested that the assay focus be on whole cells or organisms. E.g. nematode eggs through to parasitic life-stages.

Any research program would also need to consider that ruminants differ, not only in their browse and grazing habits, but in their metabolic response to phytochemicals.

10.3 Integrated Research Model

Ideally further research in this area would be carried out in a fully integrated environment where expertise existed across bioassay development, natural product chemistry, animal production and farming systems. Although it would be possible to carry out the research via separate institutes the integrated approach offers advantages in terms of time, cost and leverages the greater interaction that can occur between researchers with different expertise.

The analysis so far suggests that there is good evidence that plant secondary metabolites can effect ruminant digestion to increase efficiency, decrease acidosis and decrease methane production. There is a long history of use of plant bioactives for the treatment of intestinal parasites such as nematodes. The evidence for efficacy in ruminants is mixed but promising.

In conclusion there is increasing evidence that plant bioactives can be used to enhance health in ruminants and that the area of research is being actively pursued, particularly in the EU (including the UK) and New Zealand. The Australian native and agricultural environment offers potential advantages for research, with abundant and diverse plant sources for investigation. The research strategies proposed here aim to convert this phytochemical potential to economic benefit for the Australian growers.

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Appendix 1 Literature Search Summary

The below searches were carried out using Scifinder 2006 (a CAS product). A new 'explore by research topic' task was used for each class of compound with the results refined as below. This search was updated on 24/09/2006.

Alkaloids

5 Research Topic candidates were identified in CAPLUS.
using the phrase "alkaloids from plants"
Selected 3 of 5 candidate topics.
502 references were found containing **"alkaloids from plants"** as entered.
10240 references were found containing the two concepts **"alkaloids"** and **"plants"** closely associated with one another.
15195 references were found where the two concepts **"alkaloids"** and **"plants"** were present anywhere in the reference.
Research Topic Refine started
617 references were found when refined using the phrase **"bioactive or antimicrobial or anthelmintic or antibiotic"**
617 references were found (1 duplicate removed)

Research Topic Refine started
9 references were found when refined using the phrase **"ruminants or cow or deer or sheep"**
using the phrase "aromatic compounds, flavonols, from plants"

Aromatics

Selected 6 of 10 candidate topics.
36 references were found containing all of the concepts **"aromatic compounds"**, **"flavonols"** and **"plants"**.
4150 references were found containing the concept **"plants"**, and either the concept **"aromatic compounds"** or the concept **"flavonols"**. The concepts found were closely associated with one another.
11786 references were found containing the concept **"plants"**, and either the concept **"aromatic compounds"** or the concept **"flavonols"**. The concepts found were present anywhere (perhaps widely separated) within the reference.
9612 references were found containing the two concepts **"aromatic compounds"** and **"plants"**.
2210 references were found containing the two concepts **"flavonols"** and **"plants"**.
144 references were found containing both of the concepts **"aromatic compounds"** and **"flavonols"**.

Research Topic Refine started
447 references were found when refined using the phrase **"bioactive or antimicrobial or anthelmintic or antibiotic"**
447 references were found (0 duplicates removed)

Research Topic Refine started
12 references were found when refined using the phrase **"ruminants or cow or deer or sheep"**
13 Research Topic candidates were identified in CAPLUS.

Carbohydrate

using the phrase "oligosaccharide or glucosinolate or carbohydrate from plants"

Selected 8 of 13 candidate topics.
11929 references were found containing the concept **"plants"**, and at least one of the concepts **"oligosaccharide"**, **"glucosinolate"** or **"carbohydrate"**. The concepts found were closely associated with one another.
31137 references were found containing the concept **"plants"**, and at least one of the concepts **"oligosaccharide"**, **"glucosinolate"** or **"carbohydrate"**. The concepts found were present anywhere (perhaps widely separated) within the reference.
1311 references were found containing the two concepts **"oligosaccharide"** and **"plants"** closely associated with one another.
3558 references were found where the two concepts **"oligosaccharide"** and **"plants"** were present anywhere in the reference.
882 references were found containing the two concepts **"glucosinolate"** and **"plants"** closely associated with one another.
1296 references were found where the two concepts **"glucosinolate"** and **"plants"** were present anywhere in the reference.
9917 references were found containing the two concepts **"carbohydrate"** and **"plants"** closely associated with one another.
27342 references were found where the two concepts **"carbohydrate"** and **"plants"** were present anywhere in the reference.

Research Topic Refine started
582 references were found when refined using the phrase **"bioactive or antimicrobial or anthelmintic or antibiotic"**
582 references were found (0 duplicates removed)

Research Topic Refine started
22 references were found when refined using the phrase **"ruminants or cow or deer or sheep"**

Lipids
11 Research Topic candidates were identified in CAPLUS.

using the phrase "lipids or fatty acids from plants"
Selected 3 of 11 candidate topics.
1 reference was found containing **"lipids or fatty acids from plants"** as entered.
15216 references were found containing the concept **"plants"**, and either the concept **"lipids"** or the concept **"fatty acids"**. The concepts found were closely associated with one another.
37351 references were found containing the concept **"plants"**, and either the concept **"lipids"** or the concept **"fatty acids"**. The concepts found were present anywhere (perhaps widely separated) within the reference.

Research Topic Refine started
1338 references were found when refined using the phrase **"bioactive or antimicrobial or anthelmintic or antibiotic"**
1338 references were found (1 duplicate removed)

Research Topic Refine started
41 references were found when refined using the phrase **"ruminants or cow or deer or sheep"**

Saponins
10 Research Topic candidates were identified in CAPLUS.
using the phrase "saponin or saponins from plants"

Selected 6 of 10 candidate topics.
2139 references were found containing the concept **"plants"**, and either the concept **"saponin"** or the concept **"saponins"**. The concepts found were closely associated with one another.
3464 references were found containing the concept **"plants"**, and either the concept **"saponin"** or the concept **"saponins"**. The concepts found were present anywhere (perhaps widely separated) within the reference.
993 references were found containing the two concepts **"saponin"** and **"plants"** closely associated with one another.
2343 references were found where the two concepts **"saponin"** and **"plants"** were present anywhere in the reference.
1563 references were found containing the two concepts **"saponins"** and **"plants"** closely associated with one another.
3054 references were found where the two concepts **"saponins"** and **"plants"** were present anywhere in the reference.
3458 references were found (6 duplicates removed)

Research Topic Refine started
309 references were found when refined using the phrase **"bioactive or antimicrobial or anthelmintic or antibiotic"**
Research Topic Refine started
12 references were found when refined using the phrase **"ruminants or cow or deer or sheep"**

Tannin
5 Research Topic candidates were identified in CAPLUS.
using the phrase "tannin from plants"

Selected 3 of 5 candidate topics.
83 references were found containing **"tannin from plants"** as entered.
1291 references were found containing the two concepts **"tannin"** and **"plants"** closely associated with one another.
3275 references were found where the two concepts **"tannin"** and **"plants"** were present anywhere in the reference.
3271 references were found (4 duplicates removed)

Research Topic Refine started
125 references were found when refined using the phrase **"bioactive or antimicrobial or anthelmintic or antibiotic"**
Research Topic Refine started
15 references were found when refined using the phrase **"ruminants or cow or deer or sheep"**

Terpenes
5 Research Topic candidates were identified in CAPLUS.
using the phrase "terpenes from plants"

Selected 3 of 5 candidate topics.
43 references were found containing **"terpenes from plants"** as entered.

2329 references were found containing the two concepts **"terpenes"** and **"plants"** closely associated with one another.
6410 references were found where the two concepts **"terpenes"** and **"plants"** were present anywhere in the reference.

Research Topic Refine started
53 references were found when refined using the phrase **"ruminants or cow or deer or sheep"**
53 references were found (0 duplicates removed)

Appendix 2 RUMEN-UP

Table1. Plant materials identified as potential feed additives in RUMEN-UP

Botanical name	Description of the sample	Potential application
Arctostaphylos uva-ursi	Bear-berry; leaves and stem	Proteolysis
Bellis perennis	Daisy; whole plant, mainly leaves	Protozoa
Carduus pycnocephalus	Italian thistle; mixture of stems, leaves and flowers	Methane
Epilobium montanum	Broad leaved willowherb; foliage	Proteolysis
Eugenia caryophyllata	Clove; dried embryo seed	Protozoa, methane
Gentiana asclepidea	Gentian; leaf and stem	Protozoa
Gentiana lutea	Gentian; root	Protozoa
Helianthemum canum	Rock-rose; leaves and flowers	Proteolysis
Knautia arvensis	Field scabious, all overground	Proteolysis
Lactuca sativa	Garden lettuce; whole overground	Acidosis
Lonicera japonica	Japanese honeysuckle; leaves, stems and flowers	Protozoa
Lonicera japonica (flower)	Extract of flowers	Protozoa
ß-Myrcene	Essential oil compound	Protozoa
Olea europaea	Olive; dried leaves	Protozoa
Paeoniae alba radix	White peony; root	Methane
Peltiphyllum peltatum	Indian rhubarb; whole overground	Proteolysis
Populus tremula	Aspen; leaves and stem	Methane
Prunus avium	Wild cherry; mainly leaves and small stems	Methane
Rheum nobile	Sikkim rhubarb; leaves and stem	Methane
Salix caprea	Goat-willow; mainly leaves and small stems	Methane
Symphytum officinale	Comfrey; all over ground plant	Protozoa
Urtica dioica	Stinging nettles; whole plant	Acidosis

Appendix 3 Feed for Pigs

Table 1 Some of the plant extracts and natural substances (PENS) with potential to act as replacements for in-feed antimicrobials.

SUBSTANCE	MODE OF ACTION
Bacteria	Probiotic, gut health
Inulin	Prebiotic
Seaweed extracts	Immune system
Garlic	Immune system
Sanguinarine	Anti-inflammatory
Chlorella	Anti-inflammatory
Isoflavones	Anti-inflammatory
Carob Pulp	Anti-bacterial
Nucleotides	Anti-inflammatory
Thyme	Anti-oxidant

Appendix 4 CVM list of approved food additives

§ 582.30 Natural substances used in conjunction with spices and other natural seasonings and flavorings.

Natural substances used in conjunction with spices and other natural seasonings and flavorings that are generally recognized as safe for their intended use, within the meaning of section 409 of the act, are as follows:

Common name	Botanical name of plant source
Algae, brown (kelp)	*Laminaria* spp. and *Nereocystis* spp.
Algae, red	*Porphyra* spp. and *Rhodymenia palmata* (L.) Grev.
Dulse	*Rhodymenia palmata* (L.)

§ 582.40 Natural extractives (solvent-free) used in conjunction with spices, seasonings, and flavorings.

Natural extractives (solvent-free) used in conjunction with spices, seasonings, and flavorings that are generally recognized as safe for their intended use, within the meaning of section 409 of the act, are as follows:

Common name	Botanical name of plant source
Algae, brown	*Laminaria* spp. and *Nereocystis* spp.
Algae, red	*Porphyra* spp. and *Rhodymenia palmata* (L.) Grev.
Apricot kernel (persic oil)	*Prunus armeniaca* L.
Dulse	*Rhodymenia palmate* (L.) Grev.
Kelp (see algae, brown).	
Peach kernel (persic oil)	*Prunus persica* Sieb. et Zucc.
Peanut stearine	*Arachis hypogaea* L.
Persic oil (see apricot kernel and peach kernel).	
Quince seed	*Cydonia oblonga* Miller.

§ 582.50 Certain other spices, seasonings, essential oils, oleoresins, and natural extracts.

Certain other spices, seasonings, essential oils, oleoresins, and natural extracts that are generally recognized as safe for their intended use, within the meaning of section 409 of the act, are as follows:

Common name	Derivation
Ambergris	*Physeter macrocephalus* L.
Castoreum	Castor fiber L. and C. canadensis Kuhl.
Civet (zibeth, zibet, zibetum)	Civet cats, *Viverra civetta* Schreber and *Viverra zibetha* Schreber.
Cognac oil, white and green	*Ethyl oenanthate*, so-called.
Musk (Tonquin musk)	Musk deer, *Moschus moschiferus* L.

§ 582.60 Synthetic flavoring substances and adjuvants.

Synthetic flavoring substances and adjuvants that are generally recognized as safe for their intended use, within the meaning of section 409 of the act, are as follows:

Acetaldehyde (ethanal).
Acetoin (acetyl methylcarbinol).
Aconitic acid (equisetic acid, citridic acid, achilleic acid).
Anethole (parapropenyl anisole).
Benzaldehyde (benzoic aldehyde).
N-Butyric acid (butanoic acid).
d- or *l*-Carvone (carvol).
Cinnamaldehyde (cinnamic aldehyde).
Citral (2,6-dimethyloctadien-2,6-*al*-8, geranial, neral).
Decanal (*N*-decylaldhehyde, capraldehyde, capric aldehyde, caprinaldehyde, aldehyde *C*-10).
Diacetyl (2,3-butandeione). Ethyl acetate. Ethyl butyrate.
3-Methyl-3-phenyl glycidic acid ethyl ester (ethyl-methyl-phenyl-glycidate, so-called strawberry aldehyde, C-16 aldehyde).
Ethyl vanillin.
Eugenol.
Geranoil (3,7-dimethyl-2,6 and 3,6-octadien-1-*ol*).
Geranyl acetate (geraniol acetate).

food use and/or animal feed use and will not affect its status for other uses not specified in the referenced regulation, pending a specific review of such other uses.

§ 582.10 Spices and other natural seasonings and flavorings.

Spices and other natural seasonings and flavorings that are generally recognized as safe for their intended use, within the meaning of section 409 of the act, are as follows:

Common name	Botanical name of plant source
Alfalfa herb and seed	*Medicago sativa* L.
Allspice	*Pimenta officinalis* Lindl.
Ambrette seed	*Hibiscus abelmoschus* L.
Angelica	*Angelica archangelica* L. or other spp. of Angelica.
Angelica root	Do.
Angelica seed	Do.
Angostura (cusparia bark)	*Galipea officinalis* Hancock.
Anise	*Pimpinella anisum* L.
Anise, star	*Illicium verum Hook. f.*
Balm (lemon balm)	*Melissa officinalis* L.
Basil, bush	*Ocimum minimum* L.
Basil, sweet	*Ocimum basilicum L.*
Bay	*Laurus nobilis* L.
Calendula	*Calendula officinalis* L.
Camomile (chamomile), English or Roman	*Anthemis nobilis* L.
Camomile (chamomile), German or Hungarian	*Matricaria chamomilla* L.
Capers	*Capparis spinosa* L.
Capsicum	*Capsicum frutescens* L. or *Capsicum annuum* L.
Caraway	*Carum carvi* L.
Caraway, black (black cumin)	*Nigella sativa* L.
Cardamom (cardamon)	*Elettaria cardamomum* Maton.
Cassia, Chinese	*Cinnamomum cassia* Blume.
Cassia, Padang or Batavia	*Cinnamomum burmanni* Blume.
Cassia, Saigon	*Cinnamomum loureirii* Nees.
Cayenne pepper	*Capsicum frutescens* L. or *Capsicum annuum* L.
Celery seed	*Apium graveolens* L.
Chervil	*Anthriscus cerefolium* (L.) Hoffm.
Chives	*Allium schoenoprasum* L.
Cinnamon, Ceylon	*Cinnamomum zeylanicum* Nees.
Cinnamon, Chinese	*Cinnamomum cassia* Blume.
Cinnamon, Saigon	*Cinnamomum loureirii* Nees.
Clary (clary sage)	*Salvia sclarea L.*
Clover	*Trifolium spp.*
Cloves	*Eugenia caryophyllata* Thunb.
Coriander	*Coriandrum sativum* L.
Cumin (cummin)	*Cuminum cyminum* L.
Cumin, black (black caraway)	*Nigella sativa* L.
Dill	*Anethum graveolens* L.
Elder flowers	*Sambucus canadensis* L.
Fennel, common	*Foeniculum vulgare* Mill.
Fennel, sweet (finocchio, Florence fennel)	*Foeniculum vulgare* Mill. var. *dulce* (DC.) Alex.
Fenugreek	*Trigonella foenum-graecum* L.
Galanga (galangal)	*Alpinia officinarum* Hance.
Garlic	*Allium sativum* L.
Geranium	*Pelargonium* spp.
Ginger	*Zingiber officinale* Rosc.
Glycyrrhiza	*Glycyrrhiza glabra* L. and other spp. of *Glycyrrhiza*.
Grains of paradise	*Amomum melegueta* Rosc.
Horehound (hoarhound)	*Marrubium vulgare* L.
Horseradish	*Armoracia lapathifolia* Gilib.
Hyssop	*Hyssopus officinalis* L.
Lavender	*Lavandula officinalis* Chaix.
Licorice	*Glycyrrhiza glabra* L. and other spp. of *Glycyrrhiza*.
Linden flowers	*Tilia* spp.
Mace	*Myristica fragrans* Houtt.
Marigold, pot	*Calendula officinalis* L.
Marjoram, pot	*Majorana onites* (L.) Benth.
Marjoram, sweet	*Majorana hortensis* Moench.
Mustard, black or brown	*Brassica nigra* (L.) Koch.
Mustard, brown	*Brassica juncea* (L.) Coss.
Mustard, white or yellow	*Brassica hirta* Moench.
Nutmeg	*Myristica fragrans* Houtt.
Oregano (oreganum, Mexican oregano, Mexican sage, origan)	*Lippia* spp.
Paprika	*Capsicum annuum* L.

Common name	Botanical name of plant source
Parsley	*Petroselinum crispum* (Mill.) Mansf.
Pepper, black	*Piper nigrum* L.
Pepper, cayenne	*Capsicum frutescens* L. or *Capsicum annuum* L.
Pepper, red	Do.
Pepper, white	*Piper nigrum* L.
Peppermint	*Mentha piperita* L.
Poppy seed	*Papaver somniferum* L.
Pot marigold	*Calendula officinalis* L.
Pot marjoram	*Majorana onites* (L.) Benth.
Rosemary	*Rosmarinus officinalis* L.
Rue	*Ruta graveolens* L.
Saffron	*Crocus sativus* L.
Sage	*Salvia officinalis* L.
Sage, Greek	*Salvia triloba* L.
Savory, summer	*Satureia hortensis* L. (Satureja).
Savory, winter	*Satureia montana* L. (Satureja).
Sesame	*Sesamum indicum* L.
Spearmint	*Mentha spicata* L.
Star anise	*Illicium verum* Hook. f.
Tarragon	*Artemisia dracunculus* L.
Thyme	*Thymus vulgaris* L.
Thyme, wild or creeping	*Thymus serpyllum* L.
Turmeric	*Curcuma longa* L.
Vanilla	*Vanilla planifolia* Andr. or *Vanilla tahitensis* J. W. Moore.
Zedoary	*Curcuma zedoaria* Rosc.

§ 582.20 Essential oils, oleoresins (solvent-free), and natural extractives (including distillates).

Essential oils, oleoresins (solvent-free), and natural extractives (including distillates) that are generally recognized as safe for their intended use, within the meaning of section 409 of the act, are as follows:

Common name	Botanical name of plant source
Alfalfa	*Medicago sativa* L.
Allspice	*Pimenta officinalis* Lindl.
Almond, bitter (free from prussic acid)	*Prunus amygdalus* Batsch, *Prunus armeniaca* L. or *Prunus persica* (L.) Batsch.
Ambrette (seed)	*Hibiscus moschatus* Moench.
Angelica root	*Angelica archangelica* L.
Angelica seed	Do.
Angelica stem	Do.
Angostura (cusparia bark)	*Galipea officinalis* Hancock.
Anise	*Pimpinella anisum* L.
Asafetida	*Ferula assa-foetida* L. and related spp. of Ferula.
Balm (lemon balm)	*Melissa officinalis* L.
Balsam of Peru	*Myroxylon pereirae* Klotzsch.
Basil	*Ocimum basilicum* L.
Bay leaves	*Laurus nobilis* L.
Bay (myrcia oil)	*Pimenta racemosa* (Mill.) J. W. Moore.
Bergamot (bergamot orange)	*Citrus aurantium* L. subsp. bergamia Wright et Arn.
Bitter almond (free from prussic acid)	*Prunus amygdalus* Batsch, *Prunus armeniaca* L., or *Prunus persica* (L.) Batsch.
Bois de rose	*Aniba rosaeodora* Ducke.
Cacao	*Theobroma cacao* L.
Camomile (chamomile) flowers, Hungarian	*Matricaria chamomilla* L.
Camomile (chamomile) flowers, Roman or English	*Anthemis nobilis* L.
Cananga	*Cananga odorata* Hook. f. and Thoms.
Capsicum	*Capsicum frutescens* L. and *Capsicum annuum* L.
Caraway	*Carum carvi* L.
Cardamom seed (cardamon)	*Elettaria cardamomum* Maton.
Carob bean	*Ceratonia siliqua* L.
Carrot	*Daucus carota* L.
Cascarilla bark	*Croton eluteria* Benn.
Cassia bark, Chinese	*Cinnamomum cassia* Blume.
Cassia bark, Padang or Batavia	*Cinnamomum burmanni* Blume.
Cassia bark, Saigon	*Cinnamomum loureirii* Nees.
Celery seed	*Apium graveolens* L.
Cherry, wild, bark	*Prunus serotina* Ehrh.
Chervil	*Anthriscus cerefolium* (L.) Hoffm.

Appendix 5. Acceptable Nutritional Messages in Australia

From : *Commonwealth of Australia Gazette No. 2 3 February 2004*
Agricultural and Veterinary Chemicals Code Act 1994 page 35

Schedule 1 - ACCEPTABLE NUTRITIONAL MESSAGES
VITAMINS
Acceptable 'nutritional messages'
Vitamin A (Retinol)
- Has a role in maintaining normal vision, skin, bones and muscles
- Has a role in maintaining normal growth processes
- Is involved in normal reproductive performance
- Has a role in maintaining integrity of skin and mucous membranes

Vitamin D
(D2 – Ergocalciferol)
(D3 - Cholecalciferol)
- Has a role in the absorption of calcium & phosphorous
- Has a role in normal growth and health of bones and teeth.

Vitamin E (Tocopherol)
- Is an antioxidant

Vitamin K (Menadione)
- Has a role in maintaining normal blood clotting processes

Vitamin C (Ascorbic Acid)
- Has a role in maintaining healthy cartilage, tendons and bone

Vitamin B1 (Thiamin)
- Has a role in the metabolism and maintenance of normal muscle and nerve function
- Has a role in assisting in the maintenance of normal appetite and bodyweight

Vitamin B2 (Riboflavin)
- Is required for normal general metabolism and growth
- Has a role in maintaining integrity of skin, mucous membranes

Vitamin B6 (Pyridoxine)
- Has a role in normal general metabolism, nervous system function and vision
- Is involved in red blood cell formation
- Has a role in maintaining normal healthy skin and vision

Vitamin B12 (Cyanocobalamin)
- Has a role in general metabolism, nervous and reproductive function
- Has a role in blood cell production

Folic Acid
- Involved in general metabolism
- Involved in the formation of red and white blood cells and haemoglobin
- Has a role in blood cell production

VITAMINS
Acceptable 'nutritional messages'
Pantothenic Acid
- Has a role in normal energy metabolism, reproduction, growth and nerve function
- Involved in transmission of nerve impulses

- Needed for health of skin and hair

Biotin
- Has a role in general metabolism
- Has a role in maintaining integrity of skin, hair and hooves

Choline
- Is involved in metabolism of fats
- Has a role in transmitting nerve impulses

Inositol
- Has a role in the metabolism of fats and integrity of hair coat
- Has a role in maintaining a normal healthy coat

Niacin
- Involved in general metabolism and red blood cell formation
- Has a role in maintaining normal healthy skin and hair condition

MINERALS
Acceptable 'nutritional messages'
Calcium
- Has a role in normal growth and maintenance of bones, teeth, nervous system, muscle function, blood clotting mechanism and cardiac function

Chromium
- Has a role in the regulation of glucose metabolism

Cobalt
- Is involved in the formation of vitamin B12 and subsequent formation of red blood cells and haemoglobin
- Has a role maintaining normal nerve cell function

Copper
- Has a role in iron metabolism, bone development, and maintenance of elastic connective tissue

MINERALS
Acceptable 'nutritional messages'
Iodine
- Has a role in normal thyroid function
- Is a component of thyroid hormones which regulate metabolic processes including hair growth

Iron
- Has a role in maintaining normal metabolism
- Is a component of haemoglobin in red blood cells

Magnesium
- Has a role in general metabolism, the formation of bone and teeth
- Is involved in maintenance of nervous function

Manganese
- Has a role in general metabolism, development of bone, cartilage and connective tissue
- Is involved in normal blood clotting
- Has a role in maintaining normal growth, reproduction and lactation

Molybdenum
- Has a role in general metabolism

Potassium
- Has a role in maintaining cellular integrity and healthy nerve and muscle function
- Is involved in the normal digestion and utilisation of dietary nutrients

- Has a role in muscular contraction, nerve function and relaxation of the heart muscle

Phosphorous
- Has a role in general metabolism and nerve function
- Is involved in the normal formation of bones, muscles and teeth

Selenium
- Has a role in preventing cellular oxidation
- Necessary for normal growth and fertility

Sodium and Chloride
- Has a role in maintaining normal electrolyte balance in body tissues during heavy exercise
- Has a role in recovery after strenuous exercise

Sulphur
- Has a role in general metabolism and protein synthesis
- Has a role in maintaining healthy hair, skin and hooves
- Has a role in maintaining normal healthy joints

Zinc
- Has a role in general growth and metabolism
- Is required for normal bone and cartilage development
- Is involved in maintaining the integrity of skin and mucous membranes, hair and hooves and in wound healing
- Has a role in maintaining a normal healthy coat.

AMINO ACIDS

Acceptable 'nutritional messages'

Arginine
- Has a role in promoting release of metabolic hormones – insulin, growth hormone
- Is involved in the immune response
- Is a component of urea cycle

Histidine
- Is involved in normal growth

Isoleucine
- Is involved in normal protein synthesis and energy production

Leucine
- Has a role in normal protein synthesis and energy production

Lysine
- Has a role in normal protein synthesis

Methionine
- Aids liver in detoxification mechanisms

Phenylalanine
- Has a role in normal protein synthesis

Threonine
- Required for normal growth, feed conversion and nitrogen balance in tissues

Tryptophan
- Has a role in normal growth
- Involved in synthesis of Niacin (vitamin B3)

Valine
- Has a role in normal energy metabolism and protein synthesis