15 Deleterious Substances in Grazed Pastures

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Deleterious substances can reduce the nutritive value (NV) of a forage or pasture for grazing animals. These substances are often toxins, which increase mortality, decrease productivity or reduce the quality or wholesome nature of animal products and hence toxins can be deleterious to the animal, the consumer or both. The range of toxic substances is extensive, especially in native or unimproved forages or shrubs. Improved pastures, the main focus of this chapter, also have diverse toxins from fungal (mycotoxins), plant (phytotoxins) and bacterial origins. In addition to affecting sheep, many toxins also affect cattle and non-ruminants.

This chapter reviews forage toxins from a grazing perspective, identifies some major causes of ill health and poor productivity in sheep, considers the impact of intensive grazing practices upon their incidence and discusses the role of the rumen and tissues in altering the toxicology of some compounds. The list of substances that are deleterious to sheep is extensive. It is also important to appreciate that, even in improved pastures, ingestion of weed species can occur, especially in times of feed deficit.

Two main categories of toxins will be considered in some detail; fungal alkaloids, associated with temperate grasses, and oestrogenic compounds, often associated with clovers. Consideration will also be given to photosensitizing agents, cyanogens, phenolic compounds (tannins), bloat and nitrite (NO_2^-) poisoning. Most toxins associated with weeds will not be discussed in detail, as this topic has been comprehensively reviewed by Cheeke (1998).

Why are Plants Toxic?

Protection against herbivory is often suggested as the reason forages produce or are associated with toxic substances, but the threat to plants from insect and microbial predation is often greater than consumption by large

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animals. In some instances, plants may have evolved with fungi to reduce the impact of insect damage. For example, *Neotyphodium lolii* infection of perennial ryegrass (*Lolium perenne*) affords protection against the Argentine stem weevil (*Listronotus bonariensis*) and other insects, but has tremorgenic effects (staggering) on sheep (Easton, 1999). Likewise, the presence of condensed tannins in some legumes (e.g. *Lotus* spp.) protects against consumption, but, given a choice, sheep prefer leaves containing high concentrations of tannin to stems containing more structural fibre but less tannin.

Some innocuous plant components can become toxic following exposure to the rumen microflora, e.g. the production of equol from formononetin present in red clover (*Trifolium pratense*), with consequent reduced fertility. More often rumen microflora deactivate potentially toxic plant compounds.

Selection and Use of Improved Varieties

Past improvements in temperate forages have focused on agronomic factors, such as dry matter (DM) yield, disease resistance, seasonal growth and persistence, with little attention to NV (Woodfield, 1999). The use of *in vitro* digestibility and the advent of near-infrared reflectance spectroscopy (NIRS) have improved selection for nutritional characteristics (Casler, 1999), but until recently toxins in forages have received little attention. Notable exceptions include cyanoglycosides in white clover, oestrogenic compounds in subterranean and red clover and alkaloids in phalaris. Animal disorders associated with fungal endophytes have also been the focus of considerable international research for the past decade.

Intensive temperate agriculture has contributed to pastoral toxicity problems. Attempts to maximize profit by grazing high-producing monocultures or binary mixtures, aided by liberal fertilizer and irrigation, have created ideal conditions for fungal growth. The reduction in dietary choice and intensive grazing may force animals to eat more dead matter accumulated at the canopy base. The dead matter, stems and sheaths of grasses have poor NV and contain the highest concentrations of fungi in swards (Di Menna *et al.*, 1992). In addition, when grazing pressure is high, the reduced choice may lead animals to select pasture weeds containing high levels of toxins, such as ragwort (*Senecio jacobaea*) or Paterson's curse (*Echium plantagineum*).

Veterinary Indications of Toxicosis

Instances of ill health in livestock are often due to either inadequate diet (quantity and quality) or ingestion of excessive quantities of toxic material. Understanding the toxicosis also requires a knowledge of the diet eaten, as distinct from that which is on offer. The effects of some toxicoses are easily recognized (e.g. photosensitivity), but in many instances toxins cause sub-

clinical conditions, indicated only by diminished appetite and poor productivity (e.g. endophyte toxicity). Other toxicoses are apparent only through diminished fertility in apparently healthy animals.

A diverse range of deleterious substances is found in pastures (Table 15.1). Alkaloids and glycosides account for many deleterious effects but even fundamental compounds, such as chlorophyll and protein, can be toxic. Alkaloids are of fungal or plant origin and are classified on the basis of heterocyclic ring structures; many are used in human medicine. Glycosides are derived from plants, and the active (aglycone) portion is released by enzyme action following cell damage; their effects can be exacerbated by trampling, frost or chewing.

Aetiology and Metabolism of Toxic Compounds

Once selected, the plant is chewed with copious saliva and about 60% of cell contents are released and exposed to a diverse array of ruminal bacteria, protozoa and fungi, which may either decrease or increase the toxicity of ingested compounds. In general, ruminants are more tolerant of toxins than non-ruminant animals (Cheeke, 1998), suggesting that deactivation is the more prevalent outcome, but there are notable exceptions. For example, oestrogenic compounds impair fertility following rumen modification of formononetin present in subterranean and red clovers. Similarly, when the tropical legume leucaena (*Leucaena leucocephala*) is consumed, the toxic amino acid mimosine is degraded to goitrogenic dihydroxypyridine compounds, unless bacteria capable of degrading mimosine and its metabolites (e.g. *Synergistes jonesii*) are present in the rumen. Other examples of increased toxicity include conversion of ingested nitrates (NO_3^-) to nitrites (NO_2^-) and hydrolysis of cyanogenic glycosides to release cyanide.

Toxins can also impair microbial or rumen function; for example, dietary condensed tannins can result in significant reductions in fibre digestibility (Barry and Manley, 1984) and voluntary intake. Toxins such as saponins may also cause intestinal damage (irritants or lesions), especially of the microvilli, or inhibition of enzyme activity. Effects of trypsin inhibitors derived from beans are well known, but diarrhoea in sheep affected by pyrrolizidine alkaloids derived from ryegrass endophyte fungi suggests impaired absorption or hypermotility, leading to a rapid digesta passage to the faeces.

Dietary toxins that affect sheep may not affect other ruminants, and vice versa. Goats are more tolerant of dietary tannins than sheep, while sheep are more tolerant of ragwort toxin than cattle. These differences may be attributable to ruminal and/or postabsorptive characteristics of different species.

The liver is the first and most important organ able to intercept and detoxify metabolites absorbed from the gastrointestinal tract. Hepatic detoxification is often brought about by inducible, non-specific mixed-function oxidases (MFO), which are able to oxidize, alter or reduce toxi-

Table 15.1. Common toxins in temperate pastures (grasses, legumes, weeds).

Toxin	Sources	Structure	Principal effects on sheep
Alkaloids	Synthesized by plants and fungi	Usually basic, synthesized from amino acids and classified on heterocyclic ring structure	Often very toxic, causing irreversible liver damage
Pyrrolizidine	Ragwort, fescue endophyte Paterson's curse	Based around two five-sided rings	Irreversible liver damage, hepatic copper accumulation
Indole	Ergots in fescue, phalaris and ryegrass endophyte	Derivatives of tryptophan based around five- and six-sided rings	Vasoconstriction, hyperthermia, poor performance, incoordination
Quinolizidine	Lupins	Two six-sided rings	Respiratory paralysis, damage to central nervous system
Indolizine	Fungi in red clover	Five- and six-sided rings	Profuse salivation
Glycosides	Plant metabolites	Carbohydrate linked to a glycone moiety	Wide-ranging, dependent on properties of aglycone
Cyanogenic	White and sub. clovers, sorghum	Ruminal hydrolysis yields cyanide	Cellular respiratory inhibitor
Goitrogenic	Brassicas	Glucosinolates yielding (iso)thiocyanates, nitriles	Reduces thyroid function, goitre, poor growth
Saponins	Legumes (lucerne)	Steroid or triterpenoid aglycone	Foaming ruminal contents, bloat
Nitropropanol	Vetches (*Astragalus* spp.)	Yields 3-nitro-1-propanol	Methaemoglobinaemia, nerve damage
Isoflavones	Red and subterranean clovers	Flavanoid based on three six-sided rings	Oestrogenic activity, infertility
Cytoplasmic protein	Plant cytoplasm	Soluble, non-toxic unless in excess	Can cause bloat, high ammonia production, exacerbates effects of liver damage
Phylloerythrin	Metabolite of chlorophyll	Cyclic structure comprising five-sided rings	Photosensitization after liver damage
S-methylcysteine sulphoxide	Brassicas	Amino acid derivative	Haemolytic anaemia
Oxalates	Weed species (e.g. *Rumex*)	Oxalic acid	Impaired mineral (esp. calcium) absorption
Condensed tannin	Some legumes (e.g. lotus)	Polymerized flavonoid units	High concentrations impair rumen microbial activity and amino acid absorption
Hypericin	St John's wort	Multiple six-sided rings	Photosensitization

cants to less harmful compounds. A principal component of MFO is the cytochrome P450 system, which hydroxylates many toxic substances. This system alters reactive functional groups on toxins (e.g. OH, SH, NH_2) to facilitate conjugation and urinary excretion. Some toxins can impair liver function – for example, pyrrolizidine alkaloids from sources such as ragwort or the mycotoxin sporodesmin. Damage reduces liver capacity for utilizing absorbed nutrients or detoxifying ammonia from high-protein diets and increases susceptibility to other toxins. Some toxins are unaffected by the liver enzymes and pass into the systemic circulation to affect a wide range of organs.

Phytotoxicoses in Improved Temperate Forages

Plant varieties with potentially toxic metabolites have occasionally been released. Very successful programmes have been undertaken to reduce the phyto-oestrogen content of subterranean clover and red clover, as well as cyanoglycosides in white clover (Stern *et al.*, 1983; Caradus and Woodfield, 1997; Rumball *et al.*, 1997), but the most basic component of plants, chlorophyll, can still cause photosensitization in some instances. The use of animal trials in field and laboratory conditions will minimize the likelihood of further errors.

Photosensitization

Photosensitization can be broadly categorized into primary and secondary types. Primary photosensitization is due to the ingestion and absorption of photodynamic agents that reach the skin through the systemic circulation. Examples of plants in this category include St John's wort (*Hypericum perforatum*), found in many roadside pastures and waste areas, and buckwheat (*Fagopyrum esculentum*), grown as either a grain or a forage crop. Other examples include celery (*Apium* spp.) and spring parsley (*Petroselinum crispum*), grazed in rangelands (Cheeke, 1998).

Photosensitivity symptoms are most severe in light (non-pigmented) skin, typical of sheep. Absorption of light photons excites photodynamic agents in the skin (e.g. phylloerythrin (also known as phytoporphyrin)) to yield free radicals, which react with dermal proteins and cell membranes. This results in extreme sensitivity of the affected skin, which is alleviated by avoidance of sunlight, but secondary effects include reddening, serous oozing and thickening, sloughing and necrosis of the skin. Animals may refuse to feed and ewes may prevent lambs from sucking sensitive teats.

Ruminants are most commonly affected by secondary photosensitization. This is associated with liver damage, so that photodynamic compounds are not detoxified in sufficient quantity by the liver and pass into the systemic circulation. The most common situation is probably due to phylloerythrin, which is a ruminal degradation product of chlorophyll,

usually removed by the liver and excreted in the bile. When liver function or biliary flow is compromised, the phylloerythrin initiates photodynamic reactions in the skin. The effects may be immediate, if the forage contains sufficient chlorophyll, or delayed until animals are grazing lush green pasture. Toxins capable of causing liver damage include sporodesmin from the fungus *Pithomyces chartarum*, which can be very common in New Zealand improved pastures in warm humid weather, or the *Phomopsis leptustromiformis* parasite common to *Lupinus* species.

Other forms of photosensitization, not necessarily associated with liver disease but of an unknown aetiology, include photosensitization in lambs fed lucerne (*Medicago sativa*), either fresh or as hay, burr medic (*Medicago polymorpha*), bird's-foot trefoil (*Lotus corniculatus*), clovers (*Trifolium* spp.) and cicer milkvetch (*Astragalus cicer*) (Parton *et al.*, 2001). Photosensitization without liver damage is well known with lambs grazing rape, with oedema of the ears and neck and sometimes necrosis of the ears.

Clinical or subclinical photosensitization represents an important cost to the sheep industry. Liver damage will reduce potential productivity of the flock, even after regeneration, and render these animals more susceptible to mild challenges in the future. It is essential to provide shade and remove the flock from the source of photosensitization. Prevention through avoidance of photosensitizing agents or hepatotoxins is paramount but may be difficult when the feed and toxin are inseparable.

Phyto-oestrogens

Many legumes contain compounds that bind weakly to the mammalian oestrogen receptor. These compounds impair the fertility of ewes and, to a lesser extent, cattle, but have little effect on male animals. Very high concentrations of phyto-oestrogens in subterranean clover can cause classical 'clover disease' in ewes, with dramatic signs, including prolapse of the uterus, dystocia, mammary development in ewes and wethers, enlarged bulbourethral glands of wethers (Plate 15.1) and very low lambing rates. Such obvious clinical problems are now rare, but low-level infertility appears to be widespread.

Two types of infertility have been described in sheep. Ewes grazing oestrogenic pasture have suppressed ovarian function, so that twinning rate and even ovulation itself are suppressed. The ability to conceive may be reduced through impaired sperm transport through the cervix and sometimes increased embryonic mortality is reported. However, the most significant effect is reduced twinning rate. Smith *et al.* (1979) reported that feeding ewes material containing 25 mg kg^{-1} coumestans reduced their ovulation rate by 25%. Indeed, the only effect of phyto-oestrogens may be a reduced number of twins born 5 months after grazing the oestrogenic pasture. Since twinning rate also depends on nutritional status, such losses would not be observed unless the phyto-oestrogenic content was being monitored. This form of infertility is temporary, resolving within 4–6 weeks after the ewes are removed from the oestrogenic pasture.

Plate 15.1. Enlarged bulbo-urethral gland in a wether sheep as a result of grazing pasture containing a highly oestrogenic variety of subterranean clover (*Trifolium subterraneum*). The enlargement was sufficient to cause some pressure necrosis on the skin overlying the gland.

The second form of infertility in ewes occurs after grazing oestrogenic pastures for periods of at least 4 months. This infertility is both permanent and cumulative, becoming worse with each year of exposure. The major clinical sign is failure to conceive; ovulation and twinning rates are normal but damage to the cervix prevents normal sperm transport in permanently infertile ewes. A low level of this infertility is widespread in Western Australia (Adams *et al.*, 1988), but has not been described elsewhere. The highly seasonal rainfall in Western Australia has resulted in strong dependence on subterranean clover as the pasture legume, and many of the original subterranean clover varieties had high formononetin concentrations.

Diagnosis of infertility due to phyto-oestrogens is not difficult; however, most cases of reproductive loss due to phyto-oestrogens are not diagnosed. Relationships between oestrogenicity and reproductive loss are reasonably well established, so it is possible to estimate the degree of reproductive loss due to phyto-oestrogens by measuring the oestrogenic activity in the feed (Adams, 1995). The most reliable means of estimating oestrogenicity is through bioassay, because phyto-oestrogens may undergo extensive metabolism. The metabolic pathways vary between ruminants and non-ruminants, so it is better to assess oestrogenicity in the species of interest. For sheep, measuring the increase in teat length of wethers over a 7–10-day period provides a quick, cheap and sensitive bioassay, although it is not very precise. However, the chemical identity and metabolism of the common phyto-oestrogens have now been described, so chemical assay is a useful guide to diagnosis. These diagnostic methods are suitable for temporary infertility, but permanent infertility may occur even years after the sheep have been exposed to oestrogenic pasture, so measurement of pasture oestrogenicity is only useful as supporting evidence. Accurate diagnosis of permanent oestrogenic infertility in ewes depends on identification of histopathological changes in the cervix (Adams, 1990).

Oestrogenic compounds appear to increase the resistance of the plant against disease. The significant oestrogenic compounds involved in infertility are isoflavones and coumestans. Concentrations of the oestrogenic isoflavones are under genetic control, as indicated in Table 15.2, so that their concentration depends primarily on the clover variety. Environmental conditions, such as temperature and nutrient supply, have a lesser effect on isoflavone concentrations. Varieties of subterranean clover and red clover have been developed that contain sufficient genistein or biochanin A to maintain competitiveness in the sward, but are low in formononetin so as to minimize reproductive problems (Rumball *et al.*, 1997).

Table 15.2. Summary of major oestrogens in pasture.

Class of phyto-oestrogen	Chemical compound	Control in the plant	Plant species affected
Isoflavone	Genistein Daidzein Biochanin A Formononetin	Genetic control	Subterranean clover Red clover Soybean Berseem clover
Coumestans	Coumestrol 4′-Methoxycoumestrol Repensol Trifoliol Sativol	Response to disease	Lucerne Medics White clover Soybean
Fungal oestrogens	Zearalenone Zearalenol	*Fusarium* fungi	Dead plant material

In contrast to the isoflavones, the concentration of coumestans depends primarily on the response by plants to environmental conditions, particularly attack by insects or fungi. Genotype is important only to the extent that it determines the overall sensitivity of the plant to infestation. The environmental impact is illustrated by the findings of Hall (1984) that samples of lucerne grown in inland Australia had low coumestrol concentrations, whereas most lucerne samples grown in more humid coastal regions of Australia contained sufficient coumestrol to cause reproductive problems in sheep. In annual medics, coumestans usually accumulate during the senescence and death of the plant, so concentrations are higher in dry pastures.

Isoflavone phyto-oestrogens are extensively metabolized in the rumen (Cox and Braden, 1974). After an adaptation period of 7–10 days, the rumen flora are able to break down genistein, biochanin A and daidzein to non-oestrogenic metabolites. As a result, these compounds have only a short-term effect on fertility of sheep. However, formononetin is transformed to the oestrogenic isoflavan equol, which is absorbed by the animal from the rumen, so that in ruminants formononetin is the major isoflavone of concern. Coumestans undergo little metabolism in the rumen, so the animal is less able to adapt to their presence.

Fusarium fungi growing on moist dead plant material may produce the oestrogenic compound zearalonone. This causes prolonged oestrus, lowered ovulation rates and temporary infertility in a high proportion of ewes. Although zearalonone is also used as a growth promotant, the presence of this compound in animal products can adversely affect trade with some countries.

Cyanogens

Cyanogens are ubiquitous in plants, but are only considered to be cyanogenic if the concentration exceeds 10 mg kg^{-1} fresh weight (Davis, 1991). Common cyanogenic forages include sorghum (*Sorghum vulgare*), Sudan grass (*Sorghum sudanense*), their hybrid 'Sudax', cynodon grasses (e.g. Bermuda grass) and white clover (*Trifolium repens*). Some weed species also contain very high cyanogenic glycoside concentrations (Parton *et al.*, 2001).

Cyanide is released from the glycosides by hydrolysis subsequent to rupture of cellular vacuoles (containing the glycosides) in the presence of cytosolar hydrolytic enzymes. Hence foliar damage caused by wilting, trampling, chewing or freezing will enhance the release of cyanide. The near-neutral pH of the rumen is optimal for enzyme activity, so ruminants are more sensitive to cyanide than non-ruminants. However, cyanide is readily detoxified, so toxicity occurs only when intake is rapid and excessive.

Hydrogen cyanide (HCN) is readily transported into animal cells, where it inactivates the respiratory cytochrome oxidase system. This effectively halts oxygen utilization, resulting in anoxia in all tissues, with brain and heart failure the primary causes of death. Signs of cyanide poisoning are dyspnoea (laboured breathing), excitement, staggering, convulsions and coma. These symptoms occur only when the cyanide absorption is

very high and exceeds the tissue capacity for detoxification through conversion to thiocyanate, which is excreted in the urine. The lethal level is about 2 mg HCN kg^{-1} body weight (BW) in ruminants.

Methods for minimizing the risk of cyanide poisoning vary with the type of forage on offer. Avoidance of cyanogenic weeds (such as *Poa aquatica*) is obvious, while the toxicity of Sudan grass and sorghum is lower after flowering. White clovers with reduced cyanide levels are available. Cyanide concentrations in grasses tend to be highest in new foliage and are increased by nitrogenous fertilizers and some herbicides. Frosting and wilting increase the cyanide content and the rate of ingestion has a major impact on the likelihood of toxicosis. Hungry animals should not be given free access to these forages, and treatment – usually removal from toxic pastures – must be initiated very rapidly to prevent deaths of affected animals (Parton *et al.*, 2001).

Condensed tannins

Detrimental effects of condensed tannins in temperate improved swards are rare, except under poor growing conditions, which result in high tannin concentrations in the DM and reduced choice for the grazing animal. Condensed tannins occur mainly in dicotyledonous plants, especially *Lotus* spp., sulla (*Hedysarum coronarium*), sainfoin (*Onobrychis viciifolia*), dock (*Rumex obtusifolius*), lespedeza (*Serecia lespedeza*) and leucaena (*L. leucocephala*). They comprise up to 100 g kg^{-1} of DM in temperate forages but may reach 300 g kg^{-1} of DM in tropical forages and shrubs (Jackson *et al.*, 1996). They are often present in succulent portions of plants (leaves), which are sought after by grazing ruminants, especially when grasses are of poor quality.

When dietary concentrations exceed about 40–80 g kg^{-1} of DM, animal growth can be impaired due to low intake, but deaths occur only after prolonged grazing of forage having concentrations in excess of 100 g kg^{-1} of DM. The hydroxyl moieties in condensed tannins bind with plant, microbial and animal proteins, reducing the efficiency of microbial digestion in the rumen and also reducing absorption of amino acids from the intestine. Effects are more serious in non-ruminants than in ruminants (Waghorn *et al.*, 1999).

Distinct from condensed tannins are the hydrolysable tannins. These are readily hydrolysed to yield potentially toxic compounds (e.g. gallic acid), but they are not present in pastures, only in tree leaves and browse.

Recent studies have demonstrated the beneficial attributes of low concentrations of dietary condensed tannins for sheep (Waghorn *et al.*, 1999). Tannins are able to reduce excess losses of protein to microbial degradation in the rumen by reducing proteolysis, so a higher proportion of plant protein reaches the intestine and increases net amino acid absorption relative to equivalent diets without tannin. Low levels of condensed tannins will also reduce the incidence of bloat by preventing the production of a stable foam in the rumen following the rapid ingestion of high-quality forage, particularly legumes.

Oxalates

Oxalates in species such as sheep sorrel (*Rumex acetosella*), buffel grass (*Cenchrus ciliaris*), setaria (*Setaria sphacelata*) and sour sob (*Oxalis pes-caprae*) have caused poisoning and deaths in sheep (Seawright, 1982). Oxalates occur as oxalic acid or as salts in these plants and are largely degraded to carbonate and formate in the rumen, or precipitated as the calcium salt. However, when ingested in sufficient quantity, oxalates may be absorbed and reduce plasma calcium concentration. Absorbed oxalates also damage capillaries, leading to pulmonary oedema and oxalate-crystal precipitation in the kidneys. Symptoms of toxicity resemble hypocalcaemia, with staggering and recumbence.

Tissue damage and death occur after prolonged feeding on forages containing oxalate, or feeding diets containing an excess of 20 g kg^{-1} oxalate in the DM (McKenzie *et al.*, 1988). Death usually results from kidney damage.

Nitrate–nitrite

Nitrate–nitrite toxicity is a common and often lethal consequence of forage having high concentrations of nitrate (NO_3^-) in the DM (5–10 g kg^{-1}). This is usually associated with highly fertilized soils, especially when moisture causes rapid plant growth following a dry period. Nitrates accumulate in stems of rapidly growing plants, especially in overcast conditions (which favour nitrogen uptake but not photosynthesis). Nitrite poisoning has been associated with ryegrass species, brassicas (rape, turnip, choumoellier), as well as lucerne, barley, wheat and maize. Water containing over 200 mg kg^{-1} NO_3^- is also potentially hazardous. Toxicosis is brought about by intraruminal conversion of NO_3^- to NO_2^-, which is usually further reduced to ammonia, unless concentrations are very high, in which case absorbed NO_2^- associates with haemoglobin to form methaemoglobin, resulting in death through tissue anoxia. Horses and other non-ruminants are less susceptible to NO_3^- poisoning, because they do not convert NO_3^- to NO_2^- in the digestive tract. Nitrate poisoning can be very rapid or take several hours, depending on the source of NO_3^-. Pregnant animals may abort. Treatment is to limit access to high-NO_3^- feeds.

Species-specific toxicoses

Phalaris

Phalaris toxicoses are a significant problem in Australia, where sheep graze phalaris (*Phalaris aquatica*, formerly *Phalaris tuberosa*)-dominant pastures, and on *Phalaris arundinacea* (reed canary grass) pastures in the USA. The three unrelated toxicoses associated with *P. aquatica* include staggers, car-

diac sudden death and polioencephalomalacic sudden death (PE) (Bourke, 1998a). Potentially toxic concentrations of NO_3^- and cyanide have also been reported in *P. aquatica* pastures associated with sudden death in sheep (Bourke, 1992), so a range of situations, often affected by climate, may cause significant health problems for sheep. In contrast, toxicosis associated with *P. arundinacea* is not usually lethal, but high concentrations of hordenine have been associated with poor palatability and indole alkaloid concentrations exceeding 2 g kg^{-1} of the DM with diarrhoea and ill thrift (Cheeke, 1998).

Phalaris staggers is a nervous disorder that affects sheep and cattle, and is caused by methylated tryptamines and β-carboline alkaloids, which occur in all commercial phalaris varieties. While these alkaloids are present in phalaris plants throughout the year, the incidence and severity of phalaris staggers, the time of onset and the reversibility of symptoms are extremely variable. The most common control of phalaris staggers is through cobalt prophylaxis by slow-release cobalt ruminal pellets or pasture sprays (Bourke, 1998b), which facilitate rumen microbial capacity for detoxification. Selection for reduced toxin concentrations remains an option for long-term control. Low dimethyltryptamine alkaloid selections, such as var. Sirolan (Oram and Edlington, 1996), still caused phalaris staggers, due to elevated concentrations of *N*-methyltyramine alkaloids and β-carboline, which inhibit the hepatic monoamine oxidase system and reduce the animal's capacity to detoxify absorbed alkaloids (McKenna and Towers, 1984). Sheep affected by phalaris staggers can display tremors, twitching, head nodding, leg weakness, lack of coordination, collapse and struggling to rise. The nervous symptoms of phalaris staggers can persist from several days to several months, and death or permanent disability can occur (Bourke, 1998b).

Cardiac sudden death affects sheep and horses (Bourke, 1998a) and is caused primarily by *N*-methyltyramine (Anderton *et al.*, 1994). It is characterized by difficulty in breathing, sudden collapse and frequently death of a few sheep when mobs are gathered, moved or disturbed. Incidence is highest in autumn, coinciding with new-season regrowth of phalaris, particularly after drought. Long-term control may be provided by phalaris varieties with low *N*-methyltyramine content (Oram and Edlington, 1996). In the absence of such varieties, the best management is to minimize disturbances of affected mobs, since most animals will survive without ill effects.

Polioencephalomalacic sudden death affects sheep, resulting in the sudden death overnight of a high proportion of animals between 12 and 48 h of starting to graze toxic phalaris-dominant pastures (Bourke, 1998a). The toxin responsible for PE is not known; however, Bourke (1998a) suggests that it is probably a direct antagonist of either thiamine or pyridoxine, since it rapidly compromises the integrity of blood vessels supplying the central portion of the brain. Failure of these blood vessels to supply oxygen and nutrients results in rapid degeneration of the brain and ultimately death. The PE is often worst when very hungry mobs of

sheep are moved on to phalaris-dominant pastures with short new-season regrowth after periods of drought or frost.

Annual ryegrass

Annual ryegrass (*Lolium rigidum*) toxicity is a significant problem in South Australia and Western Australia and in South Africa. This neurological disease of sheep can be responsible for hundreds of deaths when grazing toxic pasture. The symptoms are superficially similar to ryegrass staggers (see below), with neurological disturbances, high-stepping gait and convulsions, but annual-ryegrass toxicity is lethal, involving damage to the brain (especially the cerebellum) from 2 days to 12 weeks after grazing toxic pasture (Chapman, 1989). Lesions are brought about by highly toxic glycolipids (corynetoxins), which inhibit the activity of enzymes responsible for *N*-glycosylation of glycoproteins (Jago and Culvenor, 1987).

Annual-ryegrass toxicity requires interaction between a plant nematode (*Anguina agrostis* or *Anguina funesta*) and a pathogenic bacterium (*Clavibacter toxicus*), possibly in association with a bacteriophage. When annual-ryegrass seedlings become infected with *A. agrostis*, the larvae are carried on the growing tip and burrow into the developing flower to form a gall, in which the adult nematode lays eggs. The eggs hatch into larvae, which can remain dormant for several years. The nematode is non-toxic and the galls are brown or black, unless the nematodes are infected with *Clavibacter* (possibly in association with a bacteriophage (Ophel *et al.*, 1993)), which produces corynetoxins. The presence of these bacteria is indicated by a yellow slime on the seed heads, which gives a yellowness to ryegrass fields and indicates a potentially toxic situation. Corynetoxins are not detoxified by rumen fermentation and there is no treatment for sheep suffering from annual-ryegrass toxicity.

No practical methods exist for controlling *Clavibacter*, and the best way to prevent annual-ryegrass toxicity is by breaking the nematode life cycle, either by eradicating annual ryegrass by cropping or by preventing flowering. The risk of toxicity in a sward is reduced when galls are shed late in the season and, provided stocking rates are low, it is possible to graze previously toxic pastures late in the season (Chapman, 1989).

Kikuyu

Kikuyu (*Pennisetum clandestinum*) is a widely grown tropical forage, which may contain a variety of deleterious substances, including oxalates, saponins and nitrates (Pienaar *et al.*, 1993). Outbreaks of kikuyu poisoning in sheep (and other animals) have been reported in New Zealand, Australia and South Africa. The aetiology is not understood and, although some evidence suggests mycotoxins may be responsible, other evidence suggests this is unlikely (Cheeke, 1998). Invasions of army worm have also been

implicated in kikuyu poisoning in some cases (Smith and Martinovich, 1973), but not others. Symptoms include salivation, sham drinking, cessation of ruminal and intestinal motility and severe dehydration. There is no effective treatment and mortality may be high.

Brassicas

Although not strictly pastures, brassica species, such as turnips, kale and rape, are sown with grass or used in grazing systems. All of these contain two sulphur-containing compounds (the amino acid *S*-methylcysteine sulphoxide (SMCO) and glucosinolates). The presence of these compounds, and especially their degradation products, can lower growth rates and lead to haemolytic anaemia (SMCO) and goitre or goitrogenic effects (glucosinolates).

The SMCO can account for as much as 40–60 g kg^{-1} of the DM and is metabolized in the rumen to release dimethyldisulphide, which is absorbed and reduced to an inactive form by glutathione peroxidase. When excess dimethyldisulphide is absorbed, haemolytic anaemia develops, with reduced haemoglobin concentrations and the appearance of precipitated, oxidized haemoglobin granules in the erythrocytes (Heinz bodies). Symptoms of haemolytic anaemia may not develop until animals have grazed brassicas for 3–4 weeks, and include poor performance, loss of appetite, diarrhoea and jaundice. Extended exposure to high concentrations of SMCO will result in death and in liver and kidney damage in surviving sheep. Surviving animals will make a complete recovery 3–4 weeks after removal from brassica pasture.

The SMCO content of brassicas increases during winter and is exacerbated by nitrogenous fertilizers. Growth on low-sulphur soils has been reported to lower the SMCO content of brassicas (McDonald *et al.*, 1981); both a gradual introduction and supplementation with pasture are likely to minimize the toxic effects.

The glucosinolates are hydrolysed by plant or microbial enzymes in the rumen to yield glucose, as well as isothiocyanate, nitrile or thiocyanate, depending upon the structure of the aglycone portion of the molecule. These compounds may interfere with thyroid function through a range of reactions, and are exacerbated by low dietary iodine concentration, leading to goitre. Interference with thyroid function is manifest by reducing iodine uptake by the thyroid gland or interference with iodination of tyrosine and reducing thyroxine secretion.

Symptoms of glucosinolate toxicity are primarily those of iodine insufficiency; clinical signs include enlargement of the thyroid gland, and up to 60% of newborn lambs have died when their dams have been grazing kale (Sinclair and Andrews, 1958). Prolonged exposure to glucosinolates will reduce productivity, but exposures of several weeks may not be apparent in ewes, although newborn lambs may suffer from goitre (Grace, 1994).

Mycotoxicoses in Forages

Mycotoxins are secondary metabolites produced by fungi associated with vegetative herbage and dead litter. Active research into mycotoxins in forages grew from frequent widespread outbreaks of photosensitization due to facial eczema in sheep in New Zealand and led to the realization of the benefits to pastures as well as detrimental effects on animal production. For example, the ryegrass endophyte *N. lolii* provides protection for the plant from insect attack, improves drought tolerance and may also contribute to improved persistence by deterring excessive grazing. The endophyte is also responsible for ryegrass staggers, requiring researchers to select fungal strains that are able to protect the host plant without impairing animal performance. The extensive and ongoing research effort given to modifying and understanding the perennial-ryegrass endophyte (Woodfield and Matthew, 1999) indicates the complex associations between fungi and forage; simple elimination of fungi may be more detrimental to farming than the risks of toxicoses on animal performance.

Mycotoxins include a wide range of compounds but are usually aromatic, non-immunogenic hydrocarbons of relatively low molecular weight. The principal genera responsible for toxicoses in sheep grazing pasture (Table 15.3) are *Aspergillus*, *Penicillium* and *Fusarium* but also include *Claviceps*, *Stachybotrys*, *Alternaria*, *Myrothecium* and *Pithomyces*. Toxins must survive rumen degradation to be absorbed and affect a range of organs, including the liver, kidneys and nervous system, but also cardiac function, gastrointestinal function and reproduction. Some are carcinogenic or immunosuppressive.

Facial eczema

Facial eczema (pithomycotoxicosis) is the most important mycotoxicosis in New Zealand, where outbreaks affect both sheep and cattle (Smith, 1989). Facial eczema has also been reported from both coasts of the USA, Australia, southern areas of South Africa and parts of South America, France and the UK. The disease occurs in warm moist situations, which favour the growth of *Pithomyces chartarum* in pasture litter. Ingestion of these fungal spores results in rapid absorption of sporodesmin, often leading to severe photosensitization.

Sporodesmin is concentrated in the liver and bile. A series of glutathione-linked oxidation and reduction reactions of sporodesmin generate superoxide and other free radicals, leading to liver damage and secondary photosensitization. This damage prevents excretion of phylloerythin (from chlorophyll), leading to photosensitivity, and endogenous porphyrins accumulate, leading to jaundice. Even severe liver damage rarely leads to deaths and partial regeneration is common, but appetite is greatly reduced and animals become extremely sensitive to sunlight, seeking shade wherever possible. Symptoms of photosensitization include swelling and burning of the ears and head, skin sloughing and inappetence.

Table 15.3. Common mycotoxicoses affecting sheep grazing improved temperate pastures.

Disorder	Fungus	Principal toxin	Forage type/component	Mode of action	Outcome
Facial eczema	*Pithomyces chartarum*	Sporodesmin	Pasture litter	Hepatotoxin, secondary photosensitization	Inappetence, very poor performance, rarely fatal
Ryegrass staggers	*Neotyphodium lolii*	Lolitrem B, ergovaline	*Lolium perenne*, esp. sheath, stem, seed	Neurotoxin, tremorgen	Very poor growth, heat stress, diarrhoea
Tall-fescue toxicosis	*Neotyphodium coenophialum*	Ergovaline, ergopeptides, clavine alkaloids	*Festuca* spp.	Vasoconstrictor	Heat stress, very poor growth, dry gangrene
Fusarium infertility	*Fusarium* spp.	Zearalenone	Pasture litter	Hyperoestrogenism, testicular atrophy	Temporary infertility
Paspalum	*Claviceps paspali* *Claviceps purpurea*	Paspalinines, ergotomine, ergometrine, ergotoxine	Paspalum seed heads	Tremorgen, gangrene, internal bleeding	Staggers, loss of extremities
Kikuyu poisoning	?	?	Kikuyu grass		Depression, drooling, convulsions
Lupinosis	*Phomopsis leptostromiformis*		Lupin stubble	Hepatotoxin	Inappetence, secondary infections, deaths

The severity of the problem in New Zealand has led to a good understanding of conditions likely to trigger an outbreak of facial eczema, as well as procedures for reducing the impact of the toxin. Spores are produced most freely when relative humidity approaches 100%, day temperatures are between 20 and 24°C and night temperatures are over 14°C. These conditions favour rapid development of spores within a 48 h period, so that pasture spore counts are used to indicate danger periods. Counts in excess of 100,000 spores per g of fresh grass are considered dangerous. The severity is affected by a range of factors, including stocking rate, closeness of grazing, previous exposure and sheep breed.

The effects of facial eczema can be controlled by reducing the intake of spores or by treating animals with zinc to reduce liver injury. Administration of zinc must be 20–30 times the nutritional requirements and be given before the sporodesmin challenge. Treatment in New Zealand is now by slow-release intraruminal bullet, although drenching with a zinc oxide slurry is also effective. A daily dose of 25 mg Zn kg^{-1} BW (usually as ZnO) for sheep provides protection without inducing toxicity or residue problems in the animal product or pasture. Spraying pastures with benzimidazole fungicides will render them safe for grazing for 6 weeks, provided rainfall does not occur within 3 days of spraying. Lax grazing, to reduce intake of spores from dead material, may also alleviate the impact of toxicity, but administration of zinc salts has been the most effective means for providing protection for animals.

There are genetic differences between breeds and between individuals in their susceptibility to facial eczema. Merinos appear more resistant to sporodesmin than British sheep breeds (Smith *et al.*, 1980) and heritability of resistance to facial eczema has been calculated as 0.42, so that selection for improved resistance to facial eczema is a possible means for reducing the costs of this problem (Smith, 1989).

Ryegrass staggers

Ryegrass staggers affects animals grazing perennial-ryegrass (*L. perenne*)-dominant pastures and should not be confused with grass staggers (hypomagnesaemia) or annual-ryegrass toxicity. It is a significant problem in New Zealand and parts of Australia where perennial ryegrass is a dominant pasture species. Outbreaks of ryegrass staggers occur mainly in summer and autumn and the neurological impact on coordination, resulting in staggering, head shaking and collapse when sheep become excited, can result in significant mortality from misadventure. Wild-type *N. lolii* produces a range of alkaloids, including lolitrem B and ergovaline, which are responsible for tremorgenic reactions in sheep. Further, it is increasingly apparent that the tremorgens responsible for staggering are also associated with poor animal performance, health and well-being, previously described as ill thrift.

A higher proportion of perennial-ryegrass pastures in New Zealand are infected with *N. lolii* than is apparent in Europe (Easton, 1999). The fungal endophyte improves ryegrass persistence, partly through the effects of peramine, which inhibits feeding (and consequently egg laying and larval development) of the Argentine stem weevil (*L. bonariensis*). An extensive research effort has been undertaken by New Zealand scientists to identify beneficial and detrimental compounds produced by *N. lolii* and to select strains that protect the plant without compromising sheep health. These strains produce very low concentrations of lolitrem B but maintain a high production of peramine.

Endophyte is concentrated in the leaf sheath, reproductive stem and inflorescence with relatively low concentrations in the leaf blade. As new leaves form, the endophyte grows into the blade to only a small degree. The symbiotic association between ryegrass and endophyte is supported by the intracellular location of hyphae, so there are no external indications of the endophyte in the seed or other parts of the plant. Endophyte within the seed embryo maintains the infection in the new seedling, but the viability of the endophyte is reduced if seed is stored for long periods and is compromised by warm conditions prior to germination, so seed falling to the ground and remaining dormant for several months is likely to lose viable endophyte.

Health problems in sheep grazing perennial ryegrass with wild-type endophyte include reduced growth rates (or even weight loss), ryegrass staggers, increased diarrhoea, dags and fly strike, heat stress and reduced plasma prolactin concentrations (Table 15.4). Although ryegrass endophyte does not appear to compromise reproductive performance in ewes, any reduction in ewe feed intake will compromise lamb growth rate (Watson *et al.*, 1999).

Table 15.4. Impact of ryegrass staggers on sheep performance in New Zealand (from Fletcher *et al.*, 1999).

	Number of		Endophyte			
	Trials	Sheep	Wild	Nil	Difference	Significance
Spring grazing						
LW gain (g day^{-1})	3	280	126	168	42	*
Dags (0–5 scale)	2	160	1.5	0.5	–	*
Respiration rate (min^{-1})	3	280	88	79	9	n.s.
Body temperature (°C)	3	360	40.2	40.0	0.2	n.s.
Plasma prolactin (ng ml^{-1})	2	160	103	243	140	**
Summer/autumn grazing						
LW gain (g day^{-1})	5	330	41	112	71	**
Staggers (0–5)	4	300	3.1	0	–	**
Dags (0–5)	3	240	1.5	0.4	–	*
Fly strike (%)	2	180	23	2	21	**
Respiration rate (min^{-1})	3	192	99	73	26	*
Body temperature (°C)	4	300	40.6	40.2	0.4	*
Plasma prolactin (ng ml^{-1})	3	240	63	136	73	**

LW, live weight; *, significant at 5% probability; **, significant at 1% probability; n.s., not significant.

Control of ryegrass staggers is made difficult by the benefits that the endophyte provides for the ryegrass plant. Much of New Zealand farming is carried out in hilly environments best suited to ryegrass/clover pastures, so the strategy adopted in that country has been to select endophytes that produce peramine to benefit the host plant, but which do not produce significant quantities of lolitrem B or ergovaline. Selection of these strains has produced marked improvements in sheep performance (Table 15.4) at equivalent DM yields (Woodfield and Matthew, 1999). Introduction of non-toxic endophytes offers good opportunities for success, because the symbiosis between endophyte and ryegrass is very specific, only allowing a single strain per plant. The main potential problem remains contamination of pastures containing novel endophytes through germination of seed containing wild-type endophyte. This can occur through transfer in hay or after passage of seed through the animal (Hume, 1999).

Tall-fescue toxicoses

Tall fescue (*Festuca arundinacea*) also has an association with an endophyte fungus, *Neotyphodium coenophialum*, leading to a wide range of deleterious effects in grazing animals. These include dry gangrene of lower extremities, reduced feed intakes, low weight gains and milk production and very low plasma prolactin concentrations in sheep and cattle.

There are over 15 million ha of endophyte-infected pastures in North America, and fescue toxicity is also important in Australasia and Argentina. The endophyte benefits the plant through pest and drought resistance and better tolerance of adverse soil and environmental conditions and results in a greener, more vigorous plant than endophyte-free fescue. The endophyte is located primarily in the sheath and inflorescence, rather than the leaf. It has no reproductive phase and, like *N. lolii*, is transmitted via infected seeds.

Although ergovaline and clavine alkaloids are important toxins, the fungus produces a range of modified ergopeptides, as well as loline alkaloids (pyrrolizidine alkaloids). This diversity may account for the greater toxicity of fescue endophyte compared with ryegrass endophyte (Lane *et al.*, 1999) and effects on thermoregulation, reproduction and lipid metabolism (Cheeke, 1998). The vasoconstriction leads to tissue anoxia (dry gangrene) and hyperthermia, due to insufficient capacity for heat loss. Sheep appear less affected by fescue toxicosis than cattle, perhaps because their principal route for heat loss is respiratory rather than through the skin. Nevertheless, hyperthermia is common, with reduced intakes, lameness and loss of extremities under severe conditions.

Fescue toxicity can be prevented by either reducing intake of toxic fescue or feeding non-toxic fescue. Avoidance of seed heads (containing the highest concentrations of alkaloid) can reduce toxicity, as will dilution of fescue with non-toxic forages. Hay made before seed development and treatment of hay by ammoniation have been shown to reduce toxicity. Ensiling tall-fescue forage does not affect its ergovaline content.

Endophyte-free fescue can be grown under good climatic conditions, but in many regions of the USA the presence of endophyte is essential for persistence and forage growth. Selection of endophyte strains containing peramine to deter insects, but with low ergovaline concentrations, represents a promising means for achieving both persistence and animal performance.

Paspalum staggers

Paspalum staggers has been reported in Australia, New Zealand, South Africa, the USA and parts of Europe where *Paspalum dilatatum* (dallis grass) is grown. Paspalum seed heads are frequently infected by *Claviceps paspali*, which produces tremorgens, leading to incoordination, head tremors and collapse when disturbed (Botha *et al.*, 1996). These symptoms are similar to those of ryegrass staggers. Paspalum may also be infected with *Claviceps purpurea*, which grows on several grains and grasses and also forms sclerotinia (ergot bodies) in the seed heads. When sheep consume *C. purpurea*, they develop breathing difficulties, excessive salivation, diarrhoea and bleeding within the digestive tract (Cheeke, 1998), but the avoidance of seed heads, more typical of sheep than cattle, reduces the incidence of ergot poisoning, provided adequate leafy foliage is available.

Symptoms due to the tremorgenic paspalinine are more common than the gangrenous condition in sheep, and removal of sheep from infected pastures will enable complete recovery. This condition also resembles ryegrass staggers, in that rapid movement will induce collapse, but the condition is not lethal.

The gangrenous syndrome arises from derivatives of lysergic and isolysergic acids, including ergotamine (a central nervous system stimulant and depressor), ergometrine and ergotoxine, all of which are powerful arteriolar smooth-muscle constrictors. Arteriolar and capillary constriction results in bleeding and gangrene of the affected part, with clinical signs similar to those of fescue toxicity.

Forage lupins

Lupins can cause toxicity in sheep through either alkaloid poisoning or mycotoxicosis associated with grazing mature lupin stems or stubble. The quinolizidine alkaloid poisoning affects sheep to a greater extent than goats or cattle and can be a major cause of mortality in sheep in western USA. Toxicity increases after flowering, with seeds being especially toxic. Symptoms of toxicity include laboured breathing, with death by respiratory paralysis.

Lupin mycotoxicosis is caused by ingestion of toxins from *P. leptostromiformis*. The fungi reside mainly in lupin stems and become toxic after seed harvest, so sheep grazing lupin stubble are at the greatest risk of toxicity. The fungus has been reported in both wild, high-alkaloid lupin (*Lupinus cosentinii*) and commercially available sweet lupin (*Lupinus angustifolius*). Fungal growth is facilitated by warm, overcast, moist conditions. Stubble

may remain toxic for several months, with peak toxicity at the end of summer. Lupinosis has been reported in sheep in Europe, South Africa, the USA, Australia and New Zealand and is an important toxicosis in Australia, where lupins are used extensively as a fodder crop.

Symptoms of lupinosis include lack of appetite, loss of condition and lethargy. Deaths may occur within 2–4 days of sheep being introduced to toxic stubble. Chronic or subacute lupinosis, resulting from liver damage and consumption of lesser amounts of toxin over a long period, can result in a high proportion of a flock having poor performance, with increased susceptibility to disease and other toxins (e.g. photosensitivity). With acute toxicity, the liver is enlarged, fatty and yellow or orange in colour, in contrast to the small, hardened copper- or tan-coloured livers in sheep exposed to chronic toxicity. Liver copper concentrations are elevated, while zinc concentrations are depressed.

Lupinosis can be minimized by grazing stubble soon after seed harvest, before extensive fungal growth occurs. Grazing intensity should be low so that sheep are not forced to eat excessive stem, and animals should be removed from stubble in the event of rain, which increases fungal growth. Treatment should focus on improving appetite, while zinc administration will lower liver copper concentrations and help overcome inappetence.

Conclusion

Although this review has identified wide-ranging toxicoses that affect sheep grazing temperate forages, the advantages provided by yield, quality, palatability and persistence of these forages under a range of growing conditions far outweigh the occasional (and sometimes severe) health problems. These problems are nevertheless important and can affect reproduction and productivity and influence product quality for human usage. Future solutions need to focus on causes and prevention rather than treatment of toxicoses.

Improved communication and cooperation between animal and plant researchers, together with chemists, mycologists and molecular biologists, are essential for the development of improved forage varieties for animal production. Recent research involving teams with such expertise has identified causes of ill thrift, and breeders are producing varieties well suited to high-performance ruminants. However, producers may have to move away from pastures based on only one or two forage species if animal health and productivity are to be sustained with minimal veterinary intervention.

High stocking rates and minimum choice force sheep to eat lower pasture strata and increase the likelihood of toxicoses in flocks grazing intensive pastures. This emphasizes the difficulties faced by farmers attempting to maximize profitability in the face of the vagaries of climate and commodity prices. However, there is an increasing need to achieve this goal in a sustainable manner with good animal welfare, with the implication that toxicoses must be minimized in a consumer-driven market.

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