Production Research Priorities- Endophyte Ill-thrift

By

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Abstract

Ingestion of endophyte alkaloids leads to alteration in homeostasis in ruminants under certain conditions. Neurological effects are often present as indicated by a staggers syndrome. In hot environments or where animals are stressed by exertion they may succumb to heat stress. The major economic effects of endophyte toxicosis is a poorly defined ill thrift, particularly on perennial rye grass [PRG] pastures.

Ill thrifts are widespread in grazing ruminants and are associated with many causes including parasitism, disease, deficiencies of minerals and protein in the feed and the ingestion of toxic components of plants. Some of these ill thrifts have a number of symptoms in common. It is suggested that there may be common underlying cause associated with increased demand for amino acids [or metabolisable protein, MP] to support the immune system and tolerance to stresses.

Under hot environmental conditions endophyte alkaloids decrease the animal's capacity to divert blood to the periphery and thus cool the body core; the circadian pattern of body temperature is elevated leading to greater potential for heat stress[Figure 1]. Repeated hyperthermic events in grazing ruminants may cause tissue damage in the organs of the splachnic bed through either direct effects of raised tissue temperatures or through ischemia or both. Heat stress has been associated with increased damage to the barrier functions of the gastrointestinal tract and a stimulation of the immune system through ingress of endotoxins and lipopolysaccharides. Hyperthermia may launch an immune response or amplify an already active immune stimulation as would be present in animals with intestinal parasites. The cascade of humeral and metabolic changes that occur may be similar in disease and endophyte alkaloid induced heat stress. This involves the impact of cellular changes on the organism's adaptation to high environmental temperatures and the enhanced body temperatures that occurs in ruminants consuming E+ grasses as compared to E- grasses. Heat accumulation and heat damage to proteins in tissues leads to synthesis of heat shock proteins [HSPS]. Their synthesis may also increase the overall requirements for essential amino acids by the ruminant.

HSPS have a number of roles but are essentially chaperones that protect or recover denatured protein structure that is essential in maintenance of homeostasis. They are also involved in the modulation of cellular immune response. Their accumulation in tissues leads to both heat tolerance and acclimatization to hot environments by animals.

Synthesis of HSPS and triggering of the immune response could increase protein requirements beyond the potential absorption of amino acids from the diet and provide an underlying common cause [ffor amino acid deficit] for a number of ill thrifts. In this respect Matzinger's model of immune function based on the ability to detect and address danger is more descriptive of events envisioned here then one which recognises only the non self nature of foreign materials in the body.

Research priorities are suggested for 1] surveying the incidence of ill thrift to obtain an estimated economic cost; 2] to establish any relationships between heat stress and endophyte alkaloid ingestion and immune activation[production of defence molecules] and the need to repair damaged tissues; 3] studies of the implications of increasing MP intake in animals showing heat sensitivity on PRG pastures; 4] to further investigate the causative agents in endophyte toxicity: their site of absorption as effected by disease and climatic conditions; 5] the potential to prevent the absorption of alkaloids by dietary sequestering agents such as bentonite; 6] further studies, which are needed to understand the interactions of sub clinical disease particularly intestinal parasites, and ingestion of endophyte alkaloids in sheep subject to hot conditions.

In all studies it will be critical to consider the impact of E+ pasture on productive animals [e.g. young animals or breeding animals in late pregnancy or early lactation]. Some emphasis is given to the potential carry over effects of apparent [or relative] protein deficit induced by consumption of endophyte alkaloids at critical development stages. For example the incidence of endophyte toxicosis in the third trimester of pregnancy that may lower productivity in the subsequent generation of lambs or calves and in a period following weaning both of which may compromise development of the immune system.

Introduction

Although the ingestion of endophyte alkaloids, and ergot alkaloids in particular, have been implicated in considerable production losses in ruminants grazing perennial rye grass [PRG] pastures, few researchers in the animal industries are either aware of their presence in grasses or consider the implications they may have in confounding their results.

Considerable signs of ill-thrift and toxicosis in ruminants grazing Tall Fescue pastures in the southern states of the United States of America and in sheep and cattle on PRG pastures, largely in New Zealand but also in a number of European countries and in areas of South America, have alerted the scientific communities in those countries to the potential production losses from the ingestion of grasses with endophytic fungal associations. The awareness has resulted in some prioritisation of research within the effected areas.

This research has been mainly limited to studies with the two grass species. However, there are numerous grass species that harbour endophyte fungi some have been associated with sudden death and staggers syndromes [see for examples Powell and Petroski 1992]. An example in Australia is Rough Hedgehog grass [*Echinopogan ovatus*] [Miles *et al* 1998] in the Tenterfield area of Northern NSW. The latter grass is widely distributed throughout Australia excepting the Northern Territory and has been observed to induce staggers and sudden death in sheep [see Cleland 1912]

The main symptoms in ruminants ingesting endophyte alkaloids have been discussed in various publications [see Spiers *et al* 2005; Oliver 2005] and in the current proceedings. The symptoms often vary with plant growth pattern, stage of maturity of the plant, physiological state of the animal and most importantly prevailing climate and include:

- Neurological e.g. agitation, staggers and recumbency
- Apparent inability to maintain body temperature. Animals seeking shade for longer periods of time reducing grazing time or standing in water in troughs or open water areas. Heat stress symptoms are often more obvious where animals are subjected to more exercise then normal such as yard management practices
- Ill-thrift where the only signs are a reduced feed intake and sub-optimal production
- Apparent digestive problems indicated by soft moist faeces through to sticky diarrhoea which soils the rump and in sheep, is associated with increased fly strike
- Lowered reproductive efficiencies
- High post natal death of lambs
- An increased risk of abortion and agalactia in mares
- Wool growth and wool quality must be effected where feed intake is lowered by the presence of endophyte in PRG fodders. This is emphasised because it is

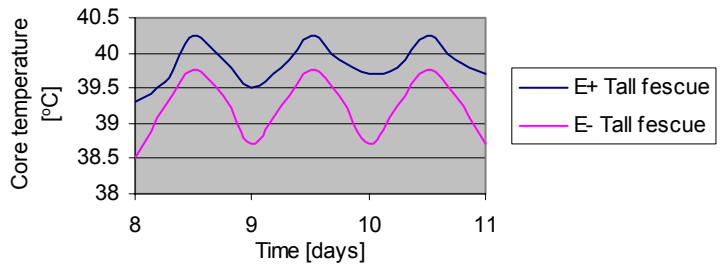
almost totally neglected area of study [wool growth in endophyte toxicosis] although anecdotal evidence is provided by Reed *et al* [2005].

It has generally been assumed that the symptoms are a result of the direct effects of endophyte alkaloids on the animal [Oliver 2005]. It has been tacitly accepted that environmental factors are involved in the damaging effects of ingestion of alkaloids since the worst effects are apparent in hot environments or during the hot season[e g summer slump][see Spiers *et al* 2005]. Cattle and sheep grazing endophyte infected PRG pastures in southern Australia have apparently succumbed from heat stress or by misadventure from attempting to get into water bodies because of heat stress[Reed *et al* 2005]. Heat stress in these cases is a result of high environmental temperatures and humidity and lowered ability of the thermoregulatory mechanisms that increase blood flow to the periphery where it would be dissipated by evaporative cooling. The pathogenesis of endophyte toxicosis in animals is due in part to the effects of alkaloids [ergovaline being the most likely but lysergic acid amide and lolitrem are also implicated] on the endothelium of blood vessels, resulting in thickened blood vessels, increased vascular tone and slowed blood flow or sludging of blood in arterioles and veins leading to ischemia and inability to control body temperature [Oliver 2005].

Ill thrift

Ill –thrift in domestic animals is simply defined as a sub optimala level of production based on the quality and quantity of feed available to the animals. The ill-thrift in cattle and sheep associated with ingestion of endophyte alkaloids [decreased weight gain, decreased reproductive efficiency and lowered milk production] may be a consequence of subtle changes in feed intake, digestion, metabolism and partitioning of nutrients. Similar ill-thrifts appear to be associated with intestinal parasite in grazing animals [see Besier and Love 2003] or ill thrift of pigs and poultry especially where there is poor hygiene control [Williams *et al* 1997; Klasing, 1988; Lipperheide *et al* 2000] or from poor housing conditions where calves are placed under some form of psychological stress such as being housed in pens with slippery floors [Alsemgeest *et al* 1995]. The immune system has been implicated in these ill thrift syndromes. The role of the immune system is now seen as more then simply a mechanism to rid the body of foreign or non self substances but also plays an interactive role with neural and endocrine system in the regulation of homeostasis [see Husband 1995].

Figure 1 Core temperature during exposure to cycling heat stress [5 hours at 33°C and 5h at 25°C] for cattle consuming E- and E+ tall fescue diets.



Colditz [2002] suggests that environments where the sum of environmental and immunological stressors on an animal is high, nutrients are diverted away from accretion in muscle, meat and wool to liver anabolism and host defence mechanisms. Grazing

animals are subject to continuous immunological stimuli, particularly from gastro intestinal parasites and environmental stress that may be compounded by ingestion of toxins such as alkaloids. There appears to be potential for major involvement of the immune response in the syndrome of ill thrift on PRG pastures. The concept is that increased core temperature of grazing animals and inability to reduce deep body temperature within normal cyclical changes may release a cascade of events that ultimately leads to lowered feed intake and ill thrift.

The evidence for a rise in core temperature being a major determinant of ill thrift in cattle consuming endophyte infected forage is clear. Core body temperature of cattle given diets of E+ or E- tall fescue fluctuated as shown in Figure 1[smoothed data] after being in controlled temperature rooms with a daily cycling heat stress of 5 hours at 33^{o}C and 5 hours at 25^{o}C for 11 days [see for complete data Snyder *et al* 1998].The idealised data shown in Figure 1 [from Snyder *et al* 1998] indicates the two important trends.

1. That mean, daily core temperature of the cattle were elevated by heat stress; with cattle on E+ fescue having the highest core temperature at any time.
2. The cattle on E + fescue had reduced amplitude in their core body temperature and both a higher minimum and maximum core temperature.

Cattle that were consuming the E+ fescue had much reduced control over body temperature in the cooler period. The temperature fluctuations experienced by cattle in these studies was not as great as might be expected in the PRG pasture areas of Australia where both maximum and minimum day temperatures may be at times 8^{o}C higher.

In the rest of this article background evidence and suggestions are made for research that will

- Identify the economic cost of ill thrift and in particular relate plant and animal factors with environmental conditions to provide potential early warning systems of ill thrift or heat stress.
- Examine some of the possible underlying causes of ill thrift and areas for research.
- Study the options for control of endophyte alkaloid absorption or metabolism in the rumen or the animal.

Economic assessment of production losses

Initial studies should attempt to relate environmental variables with PRG growth, endophyte concentrations and animal production. Variables that should also be monitored include:

- Climatic factors, mainly temperature, humidity, radiation heat load and wind force.
- Plant factors, mainly pasture maturity, quality in terms of mineral and protein content, endophyte alkaloid content.
- Animal factors, the variables to consider are breed [as some breeds are more able to dissipate heat by sweating than others] weaner live weight and stage of pregnancy and lactation in ewes, indication of parasite load in animals [FEC] and disease incidence [Johnes, scabby mouth etc] and supplementation practices This assumes that further research will clarify the interactions and provide an early warning system for prediction of ‘bad’ endophyte seasons [eg 1986 and 2002].

- Blood samples may be monitored for symptoms of immune stimulation. Analysis including measurements of cytokinines, endotoxin and amino acid profiles.

Production studies

Background

From the limited research in Australia and the more comprehensive research undertaken in New Zealand and also from research in the US on tall fescue, it seems safe to suggest that cattle and sheep grazing the 6 million hectares of PRG in Australia are at risk from endophyte toxicosis. Production losses will vary from insidious low productivity to death of livestock [see Reed *et al* 2005 and also Foot *et al* 1994]. Two years 1986 and 2002 were years where livestock death rates were highest [Reed *et al* 2005]. In these years 'out of season' rain allowed either prolonged grazing of green PRG or a large volume of PRG dry pasture was available into the seasons when environmental temperatures are highest and concentrations of alkaloids in pasture plants are also the highest[late summer autumn period]. Rain at this time would be associated with higher then normal humidity and those years were also apparently associated with a high incidence of intestinal parasitism and scouring. Stock that survived showed poor weight gain, wool growth, wool strength and lowered reproduction rate extending through winter into spring. Parasite problems and scouring were severe relative to other years. Some properties apparently observed up to a 15% reduction of fleece weight of individual sheep.

A recent New Zealand farm survey indicated that there was a 62% incidence of ill thrift in sheep grazing PRG. The ill thrift was associated with; gastrointestinal parasites [45%], undiagnosed factors [36%] and presence of Fusarium saprophytic fungal toxins [19%]. In the same survey ill thrift in young bulls [36% in farms surveyed] was associated with gastrointestinal parasites [30%], Fusarium fungus toxicity [20%] and undiagnosed factors [50%] [Litherland *et al* 2004].

Weaner ill thrift is not confined to areas where PRG is established. It is a common problem in areas such as the Yass and Northern NSW, where young sheep tend to only maintain weight over the period early December through to mid February in an area with limited PRG pastures. On PRG pastures in the same area, however, there are clear signs of endophyte toxicosis in sheep [personal observations]

There is constant reference in the literature to poor growth and wool growth accompanied at times by diarrhoea in sheep and poor growth in cattle under many different pasture conditions. In these same areas there are often low reproductive rates in ewes and low survival of particularly lambs. Ill –thrift obviously has many causes including disease problems such as Johnes disease, gastrointestinal parasites and nutritional deficiencies of protein, trace or macro minerals and at times vitamins and the ingestion of alkaloids from grasses having saprophytic and endophytic fungal associations The question posed here is 'is there a common link or similar underlying cause in many of the syndromes?' .

Lessons from ill thrift in ruminants infected with intestinal parasites

The immune system has been viewed as an effector organ reacting to environmental antigenic challenge. The defensive responses, of which, are designed to eliminate foreign substances [non self] from the body as efficiently as possible and then returns to surveillance mode as soon as the substances have been eliminated. Husband [1995] has

suggested that considerable affector contribution by the immune system and it is a sensory organ and a regulator of metabolism that interacts with the neuroendocrine response to deliver homeostasis. In the latter respect, Matzinger [2001] has proposed that the primary activators of the animal's immune system are in response to events connoting 'danger'. It appears feasible that in ruminants stressed by the effects of ingestion of endophyte alkaloids that the immune system may be stimulated by the effects of the alkaloids or by the consequences of associated heat stress or both. These maybe also magnified by an established stimulation of the immune system by disease and parasitism.

Colditz [2002] suggests that a major development in the understanding of the immune process has been the appreciation that the immune system modulates homeostatic regulation of many physiological systems. Immune activation impacts on behaviour, feed intake, metabolism and an instant increase in demand for essential amino acids for synthesis of a range of defence molecules that provides the innate immunity and for proliferation of lymphocytes. For rapid cell growth in any tissue [e.g. lymphocytes, gut epithelial and wool follicle] there is a high demand for glutamine that appears to provide purine and pyrimidine for nucleic acid synthesis and also energy [see Colditz 2002]. The extra requirements for essential amino acids and glutamine may substantially increase the animal's total requirements for amino acids over short periods [hours]or over longer periods [days] where the effects are more protracted.

The increase in faecal parasite egg count in sheep in the immediate post partum period [the peri-parturient rise in parasite egg production] is only curtailed when the metabolisable protein [MP] requirements are increased above accepted standards by 30% [see Sykes and Greer 2003]. In weaner lambs the poor live weight gain in parasitised sheep is returned close to the live weight gain of non parasitised lambs on the same diet when MP is increased by roughly the same amount [van Houtert *et al* 1995]. The underlying reasons for this apparent increase in essential amino acid requirements are discussed in a series of reviews published in Australian Journal of Experimental Agriculture 43[12] November 2003 1383-1488. The immune response[white cell proliferation and acute phase protein synthesis], repair of damaged intestinal tissue, leakage of proteins into the intestine that are fermented in the lower colon rather then being digested and absorbed and production of mucin are all involved.

Endophyte toxicosis, heat stress and the immune response.

Endophyte induced heat stress. Thermoregulation of ruminants is brought about by events that increase blood flow to the periphery carrying heat from the core to the skin surface where it is more readily dissipated by sweating [and also panting]. This increases the temperature gradient, dissipating heat and protecting the splanchnic bed organs from heat damage. In animals stressed by environmental temperature and humidity, increased peripheral circulation normally dissipates sufficient heat to maintain core temperature with in the normal limits. However, the increase in peripheral blood flow is prevented in animals suffering endophyte toxicosis by the direct action of alkaloids on the blood vessels. When thermoregulation by this route is blocked then core temperatures will continue to rise until the body comes into equilibrium with heat absorbed from the environment plus heat produced in metabolism. Body temperature runs out of control when a critical temperature is reached when metabolism is increased and body temperature rises precipitously [Spiers *et al* 2005]. A modest core temperature rise could

lead to mild to extensive heat damage to tissues of the splanchnic bed. The intestinal mucosa is likely to be the most sensitive to heat damage as it is a major contributor to total energy utilisation and sequesters 45-65 % of total body uptake of essential amino acids [McRae *et al* 1997]. Barrier function impairment in the gut by heat damage and/or compounded by the presence of parasites could allow ingression of endotoxins and lipopolysaccharides derived from gram negative bacteria from the rumen and maybe the lower gut that would result in a major immune response. Oliver [2005] has related a serum inflammatory response to the clinical signs of toxicity related to the potential damage inflicted on epithelial cells by exposure to ergot alkaloids with release of inflammatory mediators. Inflammation with narrowing of the lumen of blood vessels in localised areas of the gut and other organs of the splanchnic bed may also result in tissue damage from anoxia. Lytic cell death and increased epithelial cell permeability would induce the immune response. Lytic cell death as against normal cell death and turnover, particularly in gut tissues is postulated to be a major signal connoting danger in Matzinger [2005] view of the immune response.

Heat stress in the absence of endophyte alkaloids. It is interesting here to relate the present concept of heat stress in the absence of alkaloid toxicity where multi organ dysfunction occurs through the ischemia of these organs because of diversion of blood flow to the periphery [Jessen 2001]. During heat load, blood flow rate to the gut may be reduced by 40-50%.The barrier functions of the gut are believed to be disrupted or damaged by lack of oxygen allowing, leakage of proteins into the lumen and ingress of endotoxins and lipopolysaccharide with stimulation of the immune response [Jessen 2001;Gisolfi and Mora 2000].This can occur with as little as 1^{o}C rise in core temperature in the rabbit [Butkow *et al* 1984] which suggests that damage to the gut lining occurs at a relatively mild degree of heat load.

Cronje [2005] has recently reviewed the literature on heat stress and has firmly argued for the application of a new paradigm in humans and domestic animals "that places damage to the tissues of the gut as the pivot through which the adverse effects of heat load are promulgated" The cascade of events includes ischemic damage to the gut wall permitting entry of endotoxin; endotoxin initiation of reperfusion injury; endotoxin precipitation of septic shock and multiple organ injury. The body responds to damage to critical protein molecules by producing heat shock proteins[HSPS] that stabilise protein structure and remove the effects of denaturation and reduce the effects of inflammatory cytokinines [see Cronje 2005].

Climate induced compared to endophyte alkaloid induced heat stress. Heat stress induced by endophyte alkaloids is not accompanied by diversion of blood to the body surface. To fit with Cronje's summation of the cascade of detrimental effects resulting from high environmental temperatures, direct damage to the gut epithelial cells would have to be the pivotal cause of stimulation of the immune response. Under these circumstances frequent tissue damage from periodic ischemia or high blood temperatures could result in extensive damage to tissues of the splanchnic bed and stimulation of the immune system. These same events are the primary cause of the relative protein deficiency in sheep with intestinal parasites [Stear *et al* 2003; Coop and Sykes 2002; Sykes and Greer 2003] which is corrected by increasing MP intake [Van Houtert *et al* 1995].

Endophyte toxicosis and HSPS

Exposure to heat stimulates the intracellular production of 'HSPS' [HSP] that bind denatured protein and restore their functionality [they often termed protein chaperones]. Moseley [1994] demonstrated that accumulation of HSPS in an epithelial cell culture reduced a heat-induced increase in cell permeability. Using both cellular systems and studies in the intact organism, Moseley's research group has identified the following important issues

- Gut injury and the loss of epithelial barrier integrity are the early, and perhaps pivotal events in the pathogenesis of heat stress.
- Cells and animals become endotoxin tolerant after accumulation of HSPS following heat stress.
- The endotoxin tolerance maybe direct or tolerance to cytokine or inhibition of cytokine production by inflammatory cells [Moseley 1997].

Even mild heat damage to the tissues of the splanchnic bed including the gastrointestinal tract could require increased amounts of essential amino acids initially for HSPS synthesis and also for repair of damaged cells. Protein synthesis to meet the immune response may add to the requirements whether this is triggered by heat damage or presence of parasites or both.

In animals that have limited availability of MP, priority for essential amino acids is diverted away from tissue accretion or amino acids are mobilised from skeletal tissues [Lobley *et al*. 2001].During inflammation amino acids are mobilised from skeletal muscle to supply the needs for synthesis of new protein in the liver, the immune system and the target tissues [Colditz 2002]. The balance of essential amino acids required by the immune system [and for HSPS synthesis?] appears to be different to the array found in skeletal muscle. This leads to surplus amino acids being deaminated and oxidised in the liver [see Lobley *et al* 2001]. In response to an endotoxin induced inflammation in sheep the plasma amino acid concentrations are reduced by 30-40%. The aromatic amino acids increase in plasma but threonine and methionine are reduced by up to 80% and the challenge lifts both the rate of protein synthesis in and the number of circulating lymphocytes by 3 fold [Lobley *et al* 2001].

The essential amino acid requirements for synthesis of HSPS in ruminants have not been measured but could be relatively high. A heat stress plus inflammation in ruminants would multiply the need for essential amino acids. It is also possible that production of HSPS may have precedence for essential amino acids and therefore lower the immune response to inflammation or *vice versa.*

Important consequences of excessive immune activation include lymphocyte proliferation, production of pro inflammatory cytokinines and activation of the acute phase response, activation of heat shock protein synthesis, fever and inappetence. In the advent of low essential amino acid availability from the diet this may then incur amino acid resorption from muscles, redirection of nutrients away from synthesis of tissues, milk and wool [Colditz 2002] Protein[essential amino acid] requirements would therefore be increased in heat stressed animals. MP requirements of the animal, following a return to thermo-neutrality, would continue to remain high in order to meet essential amino acids required to replace damaged and mobilised tissue proteins. In addition there appears to be a delayed response in HSPS synthesis to heat stress. Cronje and colleagues

[see Cronje 2005] measured the HSPS in leucocytes from cattle exposed to daily cyclic heat load over five days. Only a modest rise in HSPS occurred initially from the first to the fourth day but increased at a rapid rate thereafter despite a return of the animal to room temperatures after day five. This may indicate a time lag in the synthesis of HSPS following a hyperthermic incidence.

Potential impact of physiological state on animal response to ingestion of endophyte alkaloids

Parasite and disease incidence, growth phase and stage of pregnancy or lactation may increase the animal's response to endophyte induced hyperthermia leading to further increased requirements for MP, which in the absence of increased supply could direct essential amino acids away from tissue accretion resulting in poor growth and milk production, low conception rates and survival of lambs and would also impact on wool production [see Leng 2003]. The effects would be greatest when essential amino acid requirements of animals are high as in the last trimester of pregnancy and early lactation. Low birth weights of lambs are highly related to their mortality in the first few days after birth. Birth weight is increased in lambs from ewes on native pastures by increased availability of MP in the last trimester of pregnancy [Stephenson and Bird1987]. Subjecting ewes to heat stress during pregnancy directly reduces lamb birth weight [Alexander 1984]. Feeding supplemental bypass protein [cottonseed meal] to pregnant sheep grazing pastures containing PRG significantly increased survival of lambs from multiparous ewes [Hinch *et al* 1996]. Thus the interactions of ingestion of endophyte infected forage, incidence of hyperthermia and availability of MP in pregnant animals are important priority area for research. This is especially so since there are indications that the immune system of lambs is compromised by low birth weight [see Cronje 2003].

Milk yield is also highly influenced by MP intake [see Clay and Satter 1979]. Heat stress reduces feed intake of cattle but milk yield is depressed some 2-4 days later when feed intake has recovered [Maust *et al* 1972] This may be related to a time lag for an heat induced increased need for MP. The high requirement for growth of the foetus in late pregnancy and the competition for MP with the immune system are also illustrated by the apparent high MP requirements in late pregnancy for colostrum production. An increase in MP in ewes in late pregnancy on good quality diets almost doubles colostrum yield [Robinson *et al* 2002].

Growth rates of lambs are not impeded by intestinal parasite burden when fed a diet with high availability of MP but when MP availability is low, intestinal parasites decrease growth considerably [van Houtert *et al* 1995;Datta *et al* 1998]. Lambs with intestinal parasites but supplemented to increase MP intake for 9 weeks post weaning had apparent greater immune competence in subsequent years [Datta *et al* 1999]. From the analogy of these effects of parasites or heat stress on the animal, it can be assumed that periods of ill thrift could have life time consequences for animals.

Parasite infestation and heat stress could significantly interact to stimulate the immune system response and parasitised animals would potential have less capability to respond to heat and vice versa. The longer the immune stimulation, the greater the likelihood of a depression of the immune response through exhaustion of supply of essential amino acids from skeletal muscle and glutamine synthesis from glucose and non essential amino acids [Lobley *et al* 2001]. In support of this Saker *et al* [1998] observed that steers that grazed

E+ tall fescue had lowered immune function which was associated with copper deficiency.

Research requirements

The need is first to establish the response of the ruminants to sub lethal heat stress in relation to the requirements for MP and essential amino acids. The response to single isolated event, or to periodic hyperthermic events, as might occur in a hot season between night and day, should be included among the variables investigated.

If heat stress increases the requirements for MP, then as with the parasite -nutrition interactions, MP requirements are increased above the accepted standards. Feeding trials should be directed at developing response curves to increasing availability of MP with weaner and breeding livestock subject to regulated hyperthermia or in field situations where animals are likely to suffer hyperthermia on PRG pastures.

Previous studies of supplementation to increase MP intake of sheep

Supplementation to increase the MP intake with animals on endophyte infected grasses has received some attention from researchers. Supplementation of beef cows with grain or hay plus grain on high or low endophyte infected tall fescue hay had no effect on pregnancy rates in over wintering cows when it is cool and heat stress may not have occurred[Tucker *et al* 1989]. Forcherio *et al* [1995] examined the effects in beef cows of supplemental protein and energy when consuming mature tall fescue forage of very low protein content. There were no apparent differences in the effects of supplement on intake, digestion or microbial growth in the rumen. However, when bypass protein was fed to lactating cows on endophyte infected pasture, milk production was increased, but control animals on E- pastures were not included in the research. Although there has been some research that attempted to examine the benefits of increasing MP availability in ruminants the work is sparse and fragmentary and a comprehensive research programme should examine the interactions of the ingestion of alkaloids, thermal discomfort and MP availability.

Manipulating MP availability to ruminant animals.

Protein requirements of ruminants are met from the microbial biomass digested in the intestines and dietary protein that escapes hydrolysis and deamination by rumen microbes [see Preston and Leng 1986]. In ruminants on mature pasture with low protein and /or mineral content the efficiency of microbial growth in the rumen may be compromised; the ratio of protein to energy in the nutrients produced in the rumen will be below that normally produced in sheep on high quality pasture [Preston and Leng 1986]. The low ratio of MP in the metabolisable energy available to animals on mature leached PRG pasture could increase the susceptibility to endophyte induced ill thrift.

Supplementation of ruminants on such nutrient deficient forage may be effective in increasing the supply of MP and at the same time reduce the heat load by reducing the heat of fermentation in the rumen and the heat increment of feeding [Leng 1990]. Similarly supplementation with nutrients that increase the quality of the incubation medium in the rumen increases the resilience and resistance of sheep to intestinal parasites [Knox *et al* 1994; Knox 2003].

When feed is of a quality that ensures that requirements for essential microbial growth factors in the rumen are met, then MP availability can be enhanced by either feeding supplemental bypass protein or by manipulating the microbial growth efficiency by either feeding small amounts of bentonite [Fenn and Leng 1989] or by removing protozoa from the rumen [Bird and Leng 1984].

Summary of concept

In summary the consequences of stress induced by hyperthermia in ruminants on E+ pastures may be related to a syndrome of essential amino acid metabolism including some or all of the following; reduced feed intake, with possible loss of protein through breeches in the gut epithelium, diversion of protein to tissue repair and for immune defence molecule synthesis and synthesis of HSPS. It is hypothesised that there is an increased demand for essential amino acids or a relative protein deficiency in the animal. The effects are reduced productivity and poorer immune response that would increase disease problems.

Absorption of endophyte alkaloids.

Ergot alkaloids are apparently freely soluble or bound to plant fibrous materials in the rumen. Some release of the bound alkaloids occurs through microbial action in the rumen [see Hill 2005 for review]. Some degradation of ergovaline and ergonovine occurs through microbial action but ergovaline is relatively insoluble in rumen contents possibly bound to resistant plant structural components. It seems clear from recent studies that absorption of ergot alkaloids in healthy sheep grazing tall fescue pastures occurs from the rumen as shown by the relative concentrations of these in plasma from ruminal, gastric or mesenteric veins [Hill 2005]. The recent studies of Hill and co-workers suggest that alkaloid exiting the foregut to the abomasum is not absorbed. Hill [2005] has also suggested that lysergic acid is the main alkaloid metabolite excreted in the urine and that it is readily absorbed across the rumen wall. The main conclusion is that lysergic acid from the ingested forage or produced somewhere in the body is the toxic entity responsible for tall fescue toxicosis. Clearly such studies as undertaken by Hill are desirable with PRG endophyte alkaloids. Clarification of the principle toxin, together with the sites of absorption and clearance from the body are essential to develop possible techniques to reduce the absorption of alkaloids. If the rumen is the principal site of absorption of the toxic alkaloids, this improves the potential to use sequestering agents to enable the toxin to pass through the site of absorption. A number of such materials have been tested with other toxic alkaloids, for example with aflatoxin and have been found to considerably reduce its absorption by dairy cows [Diaz *et al* 1999]. Supplementation with bentonite reduced milk aflatoxin levels in dairy cows by over 60% [Diaz *et al* 1999].

Hydrated sodium calcium aluminosilicate [a bentonite clay] mixed in an aqueous solution of ergotamine irreversibly bound 95% of ergotamine within the pH range 2.5-7.8 but the complex dissociated at pH 8 [Chestnut *et al* 1992]. If this applied to other alkaloids, particularly lysergic acid amide, then there is the possibility of a bentonite preferentially binding the toxins in the rumen. In ruminants grazing predominantly pasture rumen contents are generally buffered at about pH 6.5 and therefore bentonite maybe used to prevent absorption of some alkaloids.

However, Chestnut *et al* [1992] found that supplementing sheep on either endophyte free or infected fescue with bentonite had no effects on feed intakes and digestibility, respiration rates and plasma prolactin. In fact the animals on E+ fescue did not show typical signs of toxicosis. This promising line of research appears to have been abandoned because there appeared to be a depressed absorption of dietary Mg, Mn and Zn in sheep given the bentonite [Chestnut *et al* 1992].

These studies contrast with results from Australia; aluminosilicate, in particular bentonite –sodium when fed to ruminants have a stimulatory effect on the amount of microbial protein entering the small intestines indicated by increased wool growth [Fenn and Leng 1989,1990] and increased rumen bacterial protein flow to the small intestine [Ivan *et al* 1992]. Studies by Fenn and Leng [1989] indicated highly positive increases in weaner live weight gain and wool growth in year round grazing of sheep in the New England Tablelands from supplementation with 15g bentonite sodium daily. Following this research, supplementation of grazing cattle and sheep with bentonite has developed quickly, particularly in grazing areas where diarrhoea was an annual occurrence in weaners when new pasture growth commenced. Many thousands of tonnes of bentonite are being used in the Australian grazing industry annually [Leng personal observation]. Field observations suggest a decrease in the incidence of diarrhoea and responses in live weight gain and wool growth of weaners to feeding bentonite[in a block lick] that are apparently greater than those recorded in the original research by Fenn and Leng [1990]. The greatest amount of anecdotal evidence for productivity increases in sheep to bentonite supplementation comes from areas with PRG pastures [Backhouse JW and Leng RA personal observations].

There are opportunities for further studies on the potential for binding the more toxic endophyte alkaloids and to examine the possibility of using bentonite and other clays and perhaps some specific sequestering materials to prevent absorption of endophyte alkaloids

Suggestions that the barrier functions of the gut may be affected by heat stress and /or parasitism could have major implications on the site of absorption of alkaloids. The main question here is "is a leaky gut syndrome likely to alter the pattern of uptake of alkaloids post-rumen" and is important when studying the effects of dietary sequestering agents under practical grazing conditions where there are a number of interacting stresses.

Anthelmintic treatment and endophyte toxicosis

Anthelmintic treatments [specifically ivermectin treatment] have at times appeared to be effective in lowering the ill thrift associated with alkaloid absorption Good results were reported where ivermectin was given every 14 days but slow release capsules of ivermectin did not relieve an ill thrift syndrome [see Stuedemann and Seman 2005]. Low level parasite burdens are essential for maintaining the animals immune response [van Wyk [2001]. Low level parasite burdens [where the animal appears to be free of parasites as indicated by faecal egg counts] maybe reduced by continuous administration of anthelmintics. It appears feasible that the suppression of parasites, even when the parasite burden is very low, could have major impact on the response to endophyte toxicosis but would depend on the degree of stress endured by the animal. In this case continual administration of anthelmintics [see Knox 1995] may be more effective in eliminating parasites from the gut or reducing their immune activation and thus removing the need to

secrete immune proteins into the gut. This adds to earlier arguments relating to interactions of endophyte effects with the environmental effects.

Summary of the major area for research

Critical research to test whether bypass protein or MP availability is a major production constraint in animals with endophyte-induced ill-thrift is required. Experimental design is extremely difficult but initial studies should examine, in growth trials, the effects of plus or minus endophyte under high thermal load v thermoneutral v fluctuating temperature/ humidity conditions. The experimental animals should always be those with the greatest demand for MP.

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