Final report

Developing increased understanding, awareness and potential mitigation strategies for perennial ryegrass toxicosis in sheep production systems

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Abstract

Ryegrass pastures infected with the Wild-type (WT) endophyte *Neotyphodium lolii* produces several classes of alkaloids which when ingested cause perennial ryegrass toxicosis (PRGT). The effects of PRGT can be devastating, with serious outbreaks causing considerable losses and animal welfare problems. Recent estimates place the economic cost to the Australian Sheep Industry resulting from clinical, sub-clinical and indirect effects on animal performance, at $100 million dollars annually. Endophyte in ryegrass provides resistance to insect pests and confers agronomic advantages, and is therefore essential for enhancing the persistence and productivity of ryegrass. This project tested the novel endophytes AR1 and AR37 against wildtype (WT) endophyte and showed that there was no agronomic disadvantage from using them and that improved animal outcomes were possible. With respect to animal safety, the endophytes ranked AR1>AR37 >WT, with WT having been shown to have a range of negative productivity and physiological effects on sheep. The potential for utilizing rumen detoxifying agents to provide short term mitigation and/or preventative strategies for managing PRGT was clearly demonstrated. Modelling clearly demonstrated that in high PRGT risk areas, renovating pastures to eliminate PRGT was economically viable even if no improvement in stocking rate was achieved.

Executive summary

Ryegrass pastures infected with the wild-type (WT) endophyte *Neotyphodium lolii* produces several classes of alkaloids which when ingested cause perennial ryegrass toxicosis (PRGT). The effects of PRGT can be devastating, with serious outbreaks causing considerable losses and animal welfare problems. Recent estimates place the economic cost to the Australian Sheep Industry resulting from clinical, sub-clinical and indirect effects on animal performance, at $100 million dollars annually.

Alkaloids produced by endophyte in ryegrass provide resistance to insect pests and confer agronomic advantages, and are therefore essential for enhancing the persistence and productivity of ryegrass. Development of novel ryegrass endophytes that do not cause or have reduced animal health problems provides an important option to ensure improved persistence and production of ryegrass. In addition, there is potential for utilizing rumen detoxifying agents to provide short term mitigation and/or preventative strategies for managing PRGT.

Broad objectives of this project were:

- Assess the pasture performance and physiological and productivity responses of sheep grazing AR1, AR37 and WT endophyte-infected perennial ryegrass
- Characterize the interaction between known endophyte alkaloid intake and a range of conditions (temperature, nutrition plane) on the productivity and physiology of sheep.
- Develop an animal model and experimental approach to investigate the efficacy of currently available rumen detoxifying agents on mitigating PRGT. Assess the production and economic impact of grazing weaner sheep on commercial, WT perennial ryegrass pastures and evaluate the efficacy of novel rumen detoxifying agent.

Key findings from the grazing experiments were:

- Sheep grazing WT endophyte infected ryegrass experienced ryegrass staggers, had lower live weights, increased rectal temperatures and increased respiration rates. These effects were significant when they occurred but were variable and appeared to be dependent on a range of factors including seasonal conditions and feed availability. Interestingly, the most significant impact of WT endophyte occurred when a substantial feed wedge was built up in the previous spring and the forage subsequently consumed from mid-summer onwards – an often used strategy on farm.
- Sheep grazing AR1 novel endophyte infected ryegrass did not experience ryegrass staggers, elevated rectal temperatures, nor increased respiration rates.
- Sheep grazing AR37 did experience ryegrass staggers as equally severe as WT ryegrass however importantly it was generally later and slower to develop. Sheep production and physiology measures under AR37 were variable but not worse than WT, often intermediate between WT and AR1 and sometimes better than both AR1 and WT.
- This project demonstrated that WT ryegrass can have significant impacts on sheep productivity and physiology. With respect to animal safety the endophytes ranked as AR1>AR37>WT – with WT considered to be unsafe.

Key findings from controlled, short term indoor experiments are as follows:

- Ewes fed ergovaline at different stages of pregnancy had reduced feed intake which did not necessarily recover upon the withdrawal of ergovaline from the diet – even if this occurred in mid-pregnancy. Sheep on switched diets also lost weight at a quicker rate. Lamb growth rate also tended to be lower in the ergovaline and ergovaline/nil treatments. Milk production and mammary gland size was not reduced due to ergovaline intake as hypothesized.
- Low levels of ergovaline, similar to what might be found in pasture during winter, can increase heat load in sheep, even under thermoneutral conditions, but are not likely to adversely impact productivity.
- Feeding a low level of ergovaline and lolitrem B, individually and in combination, to growing crossbred and composite breed sheep for several weeks under thermoneutral conditions did not adversely affect animal productivity. However, rectal temperature and faecal moisture were found to increase when ergovaline and lolitrem B were fed in combination.
- Crossbred and composite breed sheep have different production and physiological responses when exposed to ergovaline. Crossbred ewes were unable to cope with the heat exposure as well as the composite breed ewes, with increased rectal temperature and skin temperature, no change in water intake and decreased dry matter intake (DMI). This difference in breed may be due to the higher proportion of Merino genetics found in the Crossbreds.
- Feeding a moderate level of PRG alkaloids decreased feed intake in Merino ewes and this was countered to some extent by the consumption of a commercial mycotoxin eliminator, Elitox®. Elitox, which binds and deactivates alkaloid toxins according to the manufacturer, may play a role in increasing the production response in sheep fed PRG alkaloids. At the time of publishing this report, Elitox is not registered by the Australian Pesticides and Veterinary Medicines Authority.

On farm studies indicated that the toxin binder Elitox® may provide a management tool for producers to reduce clinical staggers in the face of an outbreak of PRGT, improve the welfare of livestock and reduce farmer stress.

The financial impact of PRGT is substantial from what has been previously known and new information on clinical and subclinical findings identified in this project. Strategies to reduce the impact of PRGT include replacing toxic pastures with either safe endophytes perennial ryegrass or other pasture species. Generally, to be cost effective, stocking rates need to increase by at least 4 dse/ha to justify renovation when considering the cost of renovating a pasture is over $250/ha. In view of likely ongoing production losses, it is likely that only an increase in stocking rate of as little as 2 dse/ha would be required in the face of moderate losses due to PRGT. Moreover, the return on investment from introducing new cultivars will be much greater if renovating pastures that have toxic endophytes.

Recommendations arising from this project are;

1. A communication strategy be developed to disseminate the key findings and raise awareness of PRGT across the grazing industry and with key stakeholders.
2. Develop on farm management practices for different classes/breeds of livestock.
3. Develop a tool/application for objective assessment of the economic benefits of implementing different PRGT mitigation strategies on farm for different sheep production systems.
4. Investigate the efficacy of other alkaloid deactivators/binders in mitigating PRGT in grazing livestock

5. Investigate the efficacy of alkaloid deactivators/binders in mitigating any PRGT like effects caused by alkaloids associated with alternative endophytes such as AR37, AR5/Endo 5 and NEA2.
6. Investigate the opportunity for early PRGT detection/warning systems. Early detection before the onset of clinical symptoms will enable better management of stock and reduce losses.

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1. Background

Around six million hectares of land in Australia is sown with perennial ryegrass (*Lolium perenne*) seed, predominantly in Victoria, Tasmania, Western Australia and southern parts of South Australia. Most of these ryegrass pastures are infected by the wild-type (WT) endophyte *Neotyphodium lolii*, a fungus that lives inside the ryegrass plant (Reed *et al* 2004). Standard endophyte produces several classes of alkaloids, particularly when infected plants are stressed. Perennial ryegrass toxicosis (PRGT) is caused by animals ingesting alkaloids associated with WT endophyte in ryegrass. Lolitrem B, a neurotoxin that affects muscle activity, has widespread impact on the respiratory, cardiovascular and digestive systems (McLeay and Smith 1999). It causes tremors and has profound effects on smooth muscle, altering gut motility and severely disrupting digestion. Ergot alkaloids interact with dopamine receptors leading to peripheral vaso-constriction, disruption of thermoregulation and endocrine dysfunction. Reduction of blood circulation to glands, skin and extremities raises blood pressure, temperature and respiration rate causing heat stress, reduced food intake and serum prolactin, and potentially impaired reproductive efficiency.

The effects of PRGT can be devastating, with serious outbreaks causing considerable losses and animal welfare problems (Caple 2005). During the 2002 epidemic, the death of 100,000 sheep during summer and autumn was directly attributed to PRGT and the death of a further 100,000 sheep was indirectly attributed to PRGT. Such outbreaks even if mild are likely to result in considerable productivity losses associated with sub-clinical PRGT but reliable information on this is lacking in Australia. Hot dry conditions are more extreme in Australia than in New Zealand where to date all the animal physiological studies on endophyte-infected perennial ryegrass have been conducted. How the various secondary metabolites interact and possibly create synergistic effects under differing environmental conditions remains unknown and needs to be clarified for free-ranging and confined sheep meat breeds. Animal production experiments in New Zealand have clearly established effects of the endophyte alkaloids result in serious production losses (Thom et al 2012). Apart from the substantial economic cost to the Australian Sheep Industry resulting from clinical, sub-clinical and indirect effects on animal performance, estimated at $100 million dollars annually (Webb Ware 2012, personal communication), there is also a significant negative social impact. Producers can experience stress and poor selfesteem dealing with animal deaths and animal welfare issues which is often compounded by an inability to predict and manage outbreaks of PRGT (Sackett & Francis 2006).

Endophyte in ryegrass provides resistance to insect pests and confers agronomic advantages, and is therefore essential for enhancing the persistence and productivity of ryegrass. Development of novel ryegrass endophytes that do not cause or have reduced animal health problems provides an important option to ensure improved persistence and production of ryegrass. This has been demonstrated in New Zealand (Fletcher 1999; Bluett *et al* 2005) and now needs definitive verification in Australia. In addition, there is potential for utilizing rumen detoxifying agents to provide short term mitigation and/or preventative strategies for managing PRGT (Henry *et al* 2007).

The purpose of this research was to generate reliable information on the sub-clinical and clinical effects of PRGT on productivity and physiology of sheep under controlled environmental conditions and grazing novel endophytes compared with standard endophytes. In addition, this project investigated the efficacy of rumen detoxifying agents in

alleviating PRGT and how this can be used on-farm. This will assist in the development of cost effective ways to mitigate reduced animal productivity and to reduce the risks to animal welfare in sheep enterprises and other livestock production systems. This research also addressed significant knowledge gaps on how consumption of endophyte alkaloids affects nutrient partitioning and stress in sheep in different physiological states and exposed to different environmental temperatures. The latter is especially relevant for Australian conditions and a warming climate.

2. Objectives

This project had three distinct but related streams of research which included:

- Practical farm based research which worked with existing livestock and farm management practices in typical sheep-beef regions
- Well managed and intensively monitored replicated field grazing trials which enabled closer monitoring of livestock and pastures but still under natural environmental conditions
- Intensively managed indoor trials with the ability to closely monitor a large suite of physiological and production parameters in a controlled environment

The objectives of each activity stream were as follows:

2.1 Grazing study

1. Assess the agronomic benefits of novel endophytes (AR1 and AR37) compared with wild type endophyte-infected ryegrass over two seasons.
2. Assess the physiological and productivity responses of young Merino ewes and pregnant and lactating Crossbred ewes grazing the different endophyteinfected ryegrasses over two seasons.
3. Determine whether endophyte alkaloid consumption during pregnancy alters milk production and lamb performance in year 1, and subsequent reproductive and lactation performance of the ewe lambs in year 2.

2.2 Indoor studies

1. Characterize the interaction between known endophyte alkaloid intake and hot environmental conditions in Crossbred ewes and different meat breeds on productivity and physiology.
2. Characterize intake, storage in fat and urinary excretion profiles of endophyte alkaloids in Crossbred ewes under different growth paths.
3. Develop an animal model and experimental approach to investigate the efficacy of currently available alkaloid detoxifying agents on mitigating PRGT.

2.3 On farm studies

1. Assess the production and economic impact of grazing weaner sheep on high risk standard PRG pastures on commercial farms with a significant history of PRGT.
2. Evaluate the efficacy of novel alkaloid detoxifying agents in alleviating PRGT on commercial farms.

3. Methodology

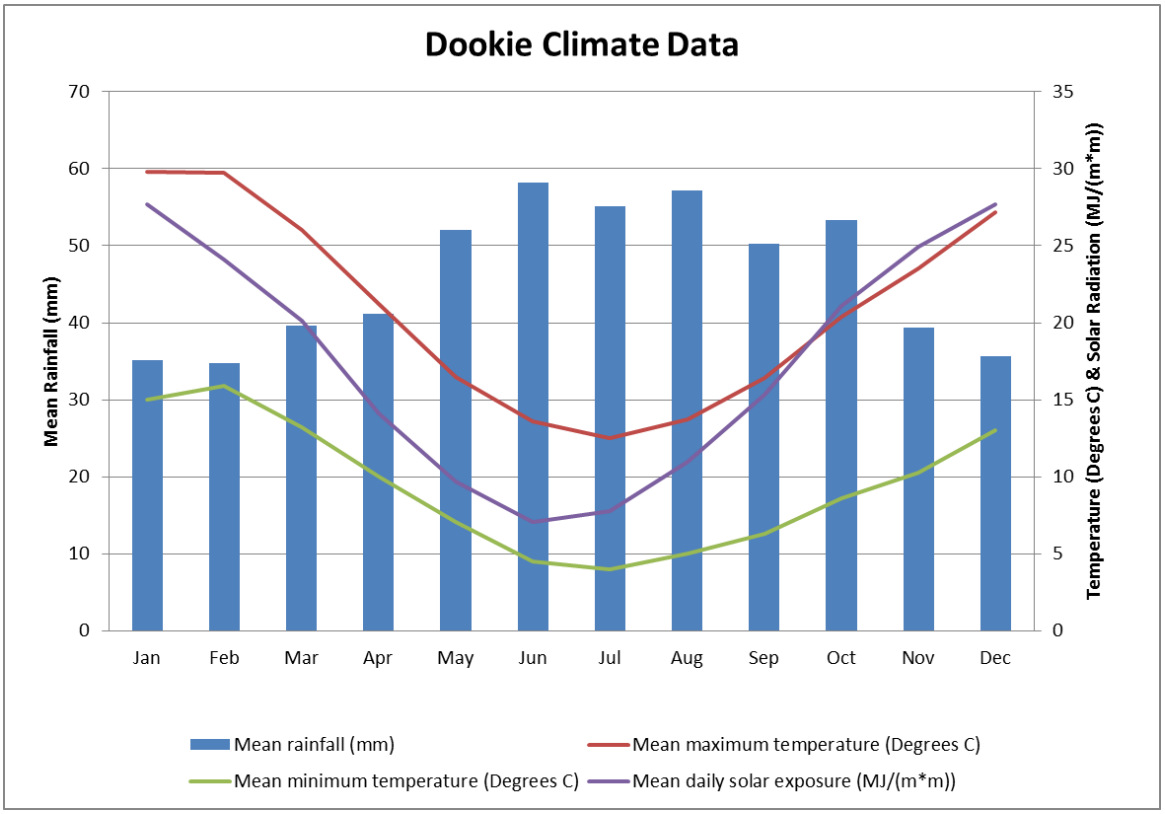
MLA is committed to investing in top quality scientific research, performed by suitably qualified, experienced and registered researchers and organisations. In experiments that involve livestock, MLA acknowledges that such research needs to be done under the auspices of a recognised Animal Care and Ethics Committee (AEC). The responsibility for obtaining AEC approval lies with the researcher. MLA has in the past not specifically asked for evidence that such AEC approval had indeed been obtained.

3.1 Grazing studies

3.1.1 Site description and design

Field based grazing studies were conducted on farm at the Dookie Campus of the University of Melbourne, located in North Central Victoria approximately 28km east of Shepparton and 175 km north of Melbourne. The site has an average annual rainfall of 552mm and is characterized by hot dry summers and cool wet winters (fig 3.1).

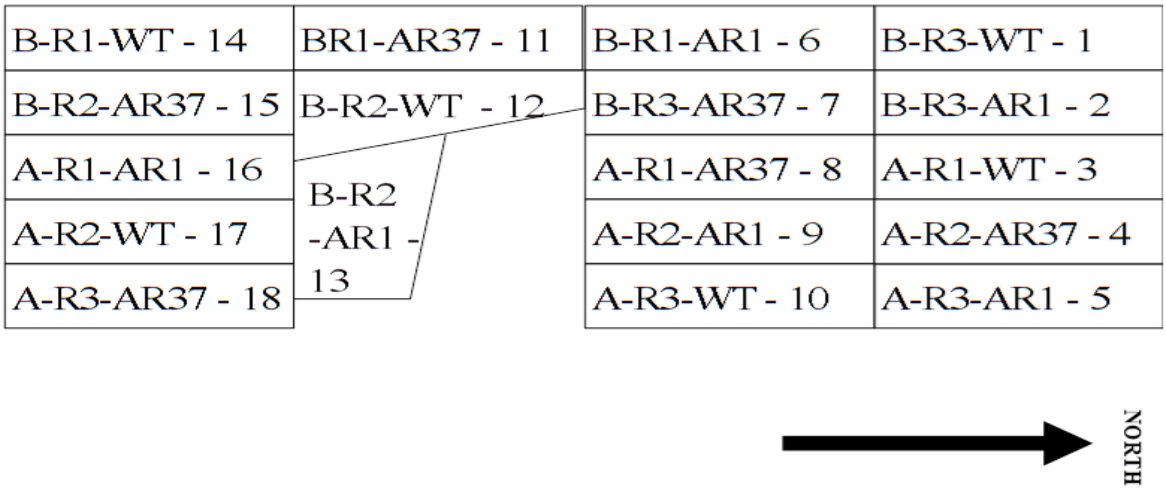
Figure 3.1. Climate data for Dookie campus of the University of Melbourne. Source: Australian Bureau of Meteorology.



The trial layout was designed to investigate the effect of 3 endophyte treatments in two different grazing experiments. The grazing experiments were located adjacent to each other with trial area A utilizing Crossbred ewes and trial area B utilizing Merino ewes (figure 3.2). Trial area A was located on a reasonably flat portion of the site while trial area B was located on a gentle slope (falling from west to east). This design approach was selected in order to limit potential within trial paddock variation and enable better comparison of endophyte treatments in

each trial. It was not the objective of this study to compare different sheep breeds. Each of the two trials had three treatments with three replicates per treatment – totalling 18 paddocks at the site. Treatments were either randomly assigned within each replicate (Group B) or arranged in a row-column lattice design (Group A). The trial layout (figure 3.2) is included below:

Figure 3.2. Dookie PRGT site layout. A and B represent two trial areas and contain different experiments (Cross bred ewes and Merino ewe weaners respectively); R1, R2 and R3 represent replicate 1, 2 and 3 respectively; WT, AR1 and AR37 are the endophyte treatments; the final number in the code represents paddock number.



Each paddock is 1 hectare in size, has its own watering point and sheep handling yards.

3.1.2 Pasture treatments

Pasture treatments comprised perennial ryegrass (*Lolium perenne*) cultivar Samson, infected with either AR1, AR37 or wild type (WT) endophyte (also known as standard endophyte (SE)) and were sown in autumn 2010 in accordance with the design outlined in figure 3.2. Seed was sown at the rate of 20kg/ha using a direct drill with tynes set to place the seed at a depth of 10mm in the soil. Prior to sowing the endophyte infection status of the seed was confirmed to be above a minimum of 75% and then post sowing endophyte infection status of each line was again confirmed to be above the minimum desired level (Table 3.1).

Table 3.1. Infection status of the treatments

	%E prior to shipment to Australia	**%E -field testing of tillers - 24/11/2010**
AR1	**95**	**92**
AR37	**90**	**84**
Wild-type	**80**	**87**

In late winter 2012, it was elected to oversow these treatments with fresh seed at a rate of 15kg/ha of seed in order to top up the plant population. Seed used for this oversowing was of the same cultivar and had the same endophyte as per each of the paddocks oversown. Endophyte infection level of the seed as detected using a seed squash method was; AR1 – 94%, AR37 – 80% and WT – 88%.

3.1.3 Soil nutrients and nutrient management

A representative soil sample was collected on the 5th March 2009 to a depth of 10cm and the nutrient status determined by Nutrient Advantage, a NATA accredited laboratory. pH($CaCl_2$) was 5.5, Colwell Phosphorus (P) 76 mg/kg, Sulphur (S) 62 mg/kg (MCP), Potassium (K) 1.2 meq/100g (Ammonia-acetate), organic carbon 2.7%.

In the year prior to sowing the trial in autumn 2010, 2.5t/ha of Lime was applied to the paddock to lift the pH. In autumn 2010, the pasture seed was sown with 100kg/ha of Di-ammonium Phosphate (DAP) was used and following a hay cut in spring 2010, 100 kg/ha of Urea was applied. In July 2011 130 kg/ha Urea (46% nitrogen (N)) plus 200 kg/ha Superfect (17.6%P, 22%S) was applied.

3.1.4 Experimental procedures

3.1.4.1 Animals and housing

Sheep were housed on one hectare paddocks.

The grazing experiment was undertaken over three years. Over the three years there were three groups of Merinos selected. The first group was grazed between February 2011 and June 2011. The second group was grazed between October 2011 and April 2012 and the third group was grazed between January 2013 and March 2013.

Over the three year grazing experiment there were two groups of Crossbred ewes selected. The first group was grazed between February 2011 and June 2011, between August 2011 and December 2011 and between March 2012 and April 2012. The second group was grazed between January 2013 and March 2013.

Sheep were removed from the pasture at different times during these three periods due to limited pasture availability and staggers occurring (staggers score >3). Stocking rates were adjusted to pasture availability when sheep were returned to the experiment.

3.1.4.2 Animals and housing 2011

Merino ewe weaners (6 months old; 27.58 kg; standard error of the difference (sed) 0.21 kg) were introduced at a stocking rate of 15 sheep per hectare on the 22/2/2011. Sheep were removed from the pasture on the 5/5/2011 due to recording a staggers score above 3. Sheep were returned to the pasture on the 17/5/2011 at a stocking rate of 15 sheep per hectare. Stocking rate was reduced to 12 sheep per hectare on the 2/6/2011 and reduced to 9 sheep per hectare on the 16/6/2011, both times due to decreasing pasture availability. All sheep were

removed from the experiment on the 27/6/2011 due to low pasture availability and staggers scores above 3.

Crossbred ewes (12 months old, 57.8 kg; sed 0.72 kg) were introduced at a stocking rate of 10 sheep per hectare on the 22/2/2011. One ram was introduced to each paddock on the 28/3/2011. Sheep were taken off the pasture on the 2/6/2011 due to low pasture availability. Sheep were returned to the pasture on the 16/6/2011 at a stocking rate of 6 ewes per hectare. Sheep were removed from the pasture on the 27/6/2011, due to low pasture availability. Sheep returned to the pasture on the 16/8/2011 at a stocking rate of 8 ewes per hectare. Sheep lambed on the pasture between August and September. Lambs were weaned on the 6/12/2011 and both ewes and lambs were removed from the pasture on the 21/12/2011.

3.1.4.3 Animals and housing 2011/2012

A new group of Merino ewe weaners (14 months old; 44.6 kg; sed 0.40 kg) were introduced to the pasture on the 26/10/2011 at a stocking rate of 10 sheep per hectare. The stocking rate was lowered to 8 sheep per hectare on the 31/1/2012 due to low pasture availability. Sheep were removed from the pasture on the 6/3/2012 due to low pasture availability. Sheep returned to the pasture on the 14/3/2012 at a stocking rate of 6 ewes per hectare. One ram was introduced to each paddock on the 19/3/2012. Ewes and rams were removed from the experiment on the 6/4/2012 due to low pasture availability.

Crossbred ewes (26 months old; 58.7 kg; sed 0.72 kg) were re-introduced to the pasture on the 14/3/2012 at a stocking rate of 6 ewes per hectare. One ram was introduced per paddock on the 19/3/2012. Sheep were removed from the experiment on the 6/4/2012 due to low pasture availability.

3.1.4.4 Animals and housing 2013

Merino ewes (12 months old; 42.6 kg; sed 0.38 kg) were introduced to the pasture on the 16/1/2013 at a stocking rate of 8 ewes per hectare. The stocking rate was reduced to 6 ewes per hectare on the 12/3/2013 due to low pasture availability. Sheep were removed from the pasture on the 26/3/2013 due to low pasture availability.

Crossbred weaners (6 months old; 33.5 kg; sed 0.38 kg) were introduced to the pasture on the 16/1/2013 at a stocking rate of 9 ewes per hectare. The stocking rate was reduced to 7 ewes per hectare on the 12/3/2013. Sheep were removed from the pasture on the 26/3/2013 due to low pasture availability.

3.1.5 Experimental design

3.1.5.1 Animal health procedures

In all experiments a thorough health check was undertaken before sheep were selected. All sheep were checked to ensure their hooves were disease and injury free and in a healthy state. All teeth and eyes were checked to ensure no abnormalities were present. Additionally all sheep were inspected visually with any ‘unwell’ looking sheep omitted from selection. Prior to the commencement of the experiment all sheep were drenched with active ingredient Levamisole drench.

Sheep were vaccinated for clostridial diseases (5 in 1) prior to entering the grazing trial. Ewes which were in lamb were vaccinated for clostridial diseases (5 in 1), 6 weeks prior to lambing. At marking lambs were vaccinated for clostridial diseases (5 in 1).

3.1.5.2 Allocation of sheep to treatments

Sheep were allocated to treatment paddocks on liveweight using a restricted randomization approach with animals being weighed, blocked into weight groups and then randomly allocated to each paddock. Mean liveweight and variance in liveweight were similar across paddocks.

3.1.6 Animal production measurements

3.1.6.1 Water intake

Sheep had access to a trough located in each paddock. Flow meters were fitted to each trough. Flow meters were read weekly and the difference between weekly readings represented water intake.

3.1.6.2 Liveweight

Sheep were walked individually onto a pair of sheep scales with liveweight displayed in kilograms. Individual liveweight was recorded for each sheep fortnightly. Scales (Trutest XR 3000) were calibrated with a standard weight (20kg) prior to each weighing session.

3.1.6.3 Collection of urine and faeces

Urine and faeces were collected using spot sampling. Urine spot sampling was undertaken using respiratory occlusion. This procedure involved covering the muzzle of the sheep cutting off air flow. The sheep was held in this position for up to twenty seconds and any urine was collected. The normal reflex of the animal is to urinate, however, if urination did not occur the animal was left and the procedure tried again later. To obtain a faecal sample rectal stimulation was undertaken. Urine and faeces were collected monthly during summer and autumn.

3.1.6.4 Dag scoring

Dag scoring was undertaken using the Visual Sheep Scores booklet (Innovation and Australia 2007). Dag scoring was based on a 1-5 scale.

3.1.6.5 Milk yield

Milk yield was measure in the Crossbred ewes during the latter half of 2011. This was undertaken at approximately week 3 (peak lactation) and week 6 post lambing. The 4 hour milk yield method was used. Lambs were separated from the ewes and placed in a separate pen. The ewes were milked after a 0.1 ml (1 IU) intramuscular (IM) injection of oxytocin (Syntocin, Ilium) using a 19 gauge needle to ensure milk ejection. Ewes were machine milked (vacuum pressure: 30 kPa; pulsation rate: 95 - 105/minute) and hand stripped until no more milk could be extracted from the udder. The ewes were kept separate from their lambs and allowed normal access to feed and water. After four hours, the milking procedure was repeated, again preceded by a 0.1 ml IM injection of oxytocin. The time

between the two milkings was accurately recorded. Milk was then weighed and measured in a volumetric flask.

3.1.7 Animal physiological measurements

Rectal temperature and respiration rate were measured in pens located in each paddock. Sheep were moved into the pens one hour prior to undertaking the measurements. Measurements were taken between 1:00PM and 3:00PM.

3.1.7.1 Rectal temperature

Rectal temperature was measured fortnightly.

3.1.7.2 Respiration rate

Respiration rate was measured fortnightly.

3.1.7.3 Jugular venipuncture

Blood sampling was undertaken in the paddock. A 10 ml sample was taken with an 18G needle into a syringe, and transferred into a lithium heparin tube before being placed onto ice. Blood was sampled monthly during summer and autumn.

3.1.7.4 Blood sample preparation

Blood samples were centrifuged (Beckman Coulter, Gladesville, NSW, Australia) at 3000rpm for 10 minutes at 4ºC. After centrifuging, plasma samples were immediately decanted into duplicate sample tubes and stored at -18ºC until analysed.

3.1.8 Faecal drying

Faecal samples of approximately seven grams were weighed while frozen and thawed for approximately one hour. Samples were re-weighed and placed into a drying oven at 100ºC. After 12 hours samples were re-weighed to obtain an initial weight, samples were re-weighed every four hours for 24 hours until a constant weight was reached. Faecal water percentage was calculated using Equation 3.1.

Equation 3.1

Faecal DM% = Post drying weight / Pre drying weight * 100

Faecal water % = 100 – Faecal DM%

3.1.9 Staggers scoring

Sheep were run over a 300 metre distance prior to being yarded. Any sheep which had fallen during the run were scored immediately and not yarded. After being yarded sheep were immediately scored. This was undertaken by the scorer holding their hand underneath the jaw of the sheep and feeling for tremors. Staggers scoring was based on a scale from 0-5 (Keogh 1973).

3.1.10 Pasture measurements

3.1.10.1 Pasture height and mass

Approximately once a week the average pasture height in each paddock was determined using a rising plate meter (RPM) (Jenquip, New Zealand). A minimum of 100 readings were taken in "W" pattern across the paddocks in order to ensure an even distribution of measurements. Pasture height measures from an RPM are a combination of height and density as the measuring plate on the RPM is weighted in order to partially compress the pasture. This enables a more reliable measure of mean pasture height which can then be converted to pasture mass via a calibration equation. A calibration curve specific to the cultivar of perennial ryegrass in this trial was developed and checked periodically. This was achieved by measuring the pasture height using the RPM and then harvesting the pasture below the plate down to ground level and determining the dry mass of this material. This measurement and harvest was undertaken at least 12 times at a single time point, sampling a broad range of pasture heights and levels of biomass. These data were then plotted and a curve fitted using a linear regression approach. The equation developed was then used to convert all RPM measurements to an estimate of pasture mass.

3.1.10.2 Pasture growth

Each paddock was divided into 4 areas and a single grazing exclusion cage (approx. 1.5m x 1.5m in area and 1m height) was randomly allocated within each of the sub areas of the paddock.

Pasture growth was measured inside these grazing exclusion cages by first preparing an area approximately the same size as the cages using either clippers or a rotary hand mower to remove all of the plant material down to 3cm in height. The cages were then securely pegged down over this area and left for approximately one month. At the conclusion of this period the cages were moved and all the material from within a $1m^2$ quadrant placed on the ground was harvested down to a height of 3cm. The material was collected and bagged, if the sample was observed to contain any soil or contaminants then it was washed, otherwise it was placed in an oven dryer and dried at 65^o Celsius until it achieved a stable dry weight. This sample weight was then recorded. Cages were moved once per month on to freshly prepared areas.

3.1.10.3 Pasture composition and feed quality

As a minimum, once per month each paddock was sampled for pasture composition and feed quality. A minimum of 30 "toe cuts" was harvested from each paddock in a W pattern. At each toe cut, all of the plant material in approximately a 10cm x 10cm patch was harvested and collected into one bulked sample per paddock. In the laboratory, each sample was thoroughly mixed and divided into 3 sub samples. One of the sub samples was placed into a freezer for later analysis for feed quality using NIR (NSW DPI Feed Quality Service, Wagga Wagga, NSW). The second sub sample was dissected into dead (ryegrass), green (ryegrass) and weeds (all nonryegrass plants). Each component was dried at 65^o Celsius until stable weights achieved and then weighed in order to determine the

dry matter (DM) proportion of each component. A third sub sample was retained in the freezer.

3.1.10.4 Pasture endophyte infection level

Endophyte infection levels were determined by harvesting a minimum of 100 tillers at random from across the paddock, only taking one tiller per plant and avoiding seed heads. Each tiller was harvested by using a sharp knife or scalpel to cut as close to the growing point as possible – usually this is at or just below ground level. Tillers from each paddock were placed into a labelled ziplock plastic bag, air removed and then either stored on ice until processing or if this was likely to be more than 3-4 days, the tillers were stored in a freezer. In the laboratory each tiller was then cleaned of any dirt and dead material (not washed) and a fresh cut made at the tiller base. This freshly cut tiller end was then lightly pressed on to a Nitro-cellulose membrane (NCM) (blotted). AgResearch (NZ) then processed these NCM sheets using a poly clonal antibody assay to determine the presence of fungal endophyte in the tiller.

3.2 Methodology – Indoor studies

3.2.1 Characterising Impact of Ergovaline on pregnant sheep

3.2.1.1 Experimental procedures

3.2.1.1.1 Animals and housing

Forty-eight Merino ewes, aged 18 months were selected for the experiment based on liveweight and health status. The sheep were previously mated with Poll Dorset rams and were housed in individual pens for the duration of the experiment at the Dookie animal house facility. Sheep initially stayed in group pens for the first seven days to allow for acclimatisation. Ewes were then transferred to undercover indoor-outdoor pens (1.5 x 1.0 metres) where they remained for the duration of pregnancy (152 days). This period was contingent to lambing early or late, depending on the reading that was given when ewes were scanned for pregnancy. The individual pens were bedded with mulch and wood chips and allowed ease of access for measurement of dry matter intake, water intake, live weight and physiology measurements. When ewes were housed individually they were let out to roam freely in a 30.0 x 30.0 metre paddock two days a week with access to *ad libitum* feed and water.

Two groups of sheep were rotated through the individual pens. Group one consisted of 30 ewes and group two consisted of 18 ewes. For the baseline period and until day 21, group two were housed in group pens until pregnancy scanning could be undertaken to determine if sheep were in lamb before moving into the individual pens.

3.2.1.1.2 Animal health procedures

A thorough health check was undertaken before sheep were selected. All sheep were checked to ensure their hooves were disease and injury free and in a healthy state. All teeth were checked to ensure no abnormalities were present along with

a check of all eyes to ensure no infections or abnormalities were present. Additionally all sheep were inspected visually with any 'unwell' looking sheep omitted from selection. Prior to the commencement of the experiment all sheep were drenched with active ingredient Levamisole drench. Sheep were vaccinated prior to entering the grazing trial, and ewes in lamb were vaccinated 6 weeks prior to lambing.

3.2.1.2 Experimental design

There were four treatment diets used in the experiment (Table 3.2). Perennial ryegrass seed containing 15 µg/g Ergovaline was fed to sheep at a moderate dosage of 40 µg/kg live weight (of ergovaline).

Table 3.2 Four different treatment diet groups consisting of varying levels of ergovaline seed.

Treatment diet	Description
Nil	A nil ergovaline diet fed for the entirety of pregnancy.
Nil/Ergovaline (Nil/Ergov)	A nil ergovaline diet fed for the first half of pregnancy (days 1-76), followed by ergovaline for the second half of pregnancy (days 77-152).
Ergovaline (Ergov)	Ergovaline fed for the entirety of pregnancy.
Ergovaline/Nil (Ergov/Nil)	Ergovaline fed for the first half of pregnancy (days 1-76), followed by a nil ergovaline diet fed for the second half of pregnancy (days 77-152).

3.2.1.3 Allocation of sheep to treatments

Sheep were selected to be of similar age and weight within breed (and between breeds where applicable) and randomly allocated to treatments. This allocation method ensured each treatment retained a similar average liveweight.

3.2.1.4 Feeding

Ergovaline seed was calculated in µg/kg LW and was mixed with 200 g of whole barley and given to sheep every morning at 08:00. Sheep were left to eat the seed and barley mix until it was finished or until they stopped eating, at which time seed refusals were collected.

During all stages of the experiment, sheep were given a commercial sheep pellet (13.5 MJ ME/kg DM, crude protein 18%). Split feeding was undertaken with feeding at 08:00 and 16:00. Due to the *ad libitum* feeding regime, extra pellets were weighed, recorded and fed in the afternoon when required.

At parturition, a nil ergovaline diet was given to sheep in all treatment groups where they were fed commercial sheep pellets and water *ad libitum* and free to graze some days of the week in a paddock depending on adequate weather conditions.

3.2.1.5 Production measurements

3.2.1.5.1 Dry matter intake

Individual feed intake was measured by collecting pellet refusals and weighing them daily to determine daily feed intake using a set of scales (@Weigh model QHW-30, @Weigh Pty. Ltd). Dry matter intake (DMI) was calculated using the following equation:

Equation 3.2

Percentage moisture (%) / feed intake (g) = Total moisture in feed

DMI = Feed intake (g) – total moisture in feed

3.2.1.5.2 Water intake

Litre markings were made on each plastic bucket. Before feeding each morning each bucket was read to determine the amount of water consumed in the previous twenty-four hours. Buckets were cleaned out and new water replaced twice weekly.

3.2.1.5.3 Live weight

Sheep were walked individually onto a pair of sheep scales with live weight displayed in kilograms. Individual live weight was recorded for each sheep fortnightly. Scales (Trutest XR 3000) were calibrated with a standard weight (20kg) prior to each weighing session.

3.2.1.5.4 Collection of urine and faeces

Urine and faeces were collected using spot sampling on a monthly basis. Urine spot sampling was undertaken using respiratory occlusion. This procedure involved covering the muzzle of the sheep stopping the breathing of the sheep. A cup was held at the rear of the animal and the sheep was held in this position for up to twenty seconds. The normal reflex of the animal is to urinate, however, if urination did not occur the animal was left and the procedure tried again later. To obtain a faecal sample rectal stimulation was undertaken. This procedure involved the investigator wearing disposable gloves and using a finger to stimulate along the spine of the sheep within the rectum, stimulating a faecal sample. Urine and faeces were sampled monthly.

3.2.1.5.5 Mammary gland measurement

The mammary gland was measured using a tape measure on a monthly basis. This was undertaken by measuring the mammary gland both across and down the mammary gland (vertically and horizontally).

3.2.1.6 Physiological measurements

3.2.1.6.1 Rectal temperature

Rectal temperature was measured fortnightly at weighing with a rectal temperature probe (Vega Technologies Inc, China).

3.2.1.6.2 Respiration rate

Respiration rate was recorded fortnightly at weighing.

3.2.1.6.3 Milk yield

Milk yield was estimated twice during lactation; at approximately week 3 (peak lactation) and week 6 post-lambing. Lambs were separated from ewes whilst the 4 hour milk yield method was used. This method involved injecting ewes intramuscularly with 0.1 ml oxytocin to stimulate milk production, after four minutes the sheep were hand-milked ensuring all milk was stripped. After 4 hours a second injection of 0.1 ml of oxytocin was administered to the sheep, after four minutes sheep were hand stripped. The milk collected was then weighed on a balance and recorded and volume was also recorded.

3.2.1.6.4 Blood sampling

Jugular venepuncture was undertaken with an 18 gauge needle (Terumo Corporation, Tokyo, Japan) with a syringe attached. A 10 ml sample was taken and transferred into a lithium heparin tube and gently mixed to prevent clotting, before being placed onto ice. Blood was sampled monthly.

Blood samples were centrifuged (Beckman Coulter, Gladesville, NSW, Australia) at 3000 rpm for 10 minutes at 4ºC. After centrifuging, plasma samples were immediately decanted into duplicate sample tubes and stored at -18ºC until analysed.

3.2.1.7 Laboratory analysis – faecal samples

Faecal samples of approximately seven grams were weighed while frozen and placed into separate zip lock bags. Samples were then placed into a cool room (4ºC), thawed for approximately one to two hours, placed onto the foil tray and into a drying oven at 100ºC. After 12 hours samples were re-weighed to obtain an initial weight, samples were re-weighed every four hours for 24 hours until a constant weight was reached. Once at a constant weight all samples were weighed. Faecal water percentage was calculated using Equation 3.1 (Section 3.1.8).

3.2.2 Characterising ergovaline threshold in sheep

3.2.2.1 Animals and Housing

Thirty-two Merino ewe weaners were selected from The University of Melbourne, Dookie campus main flock. They were selected based on their health status, age (approximately 8-10 months) and liveweight. Sheep were housed in the animal facility at Dookie College in temperature-controlled rooms, which were kept at

21˚C throughout the entire experiment. A 14h light and 10h dark cycle was used throughout the treatment period of 42 days.

Sheep were housed in group-pens for a seven-day acclimatisation period prior treatment. Sheep were then housed in metabolism crates for 28 days, which consisted of a seven-day acclimatisation period and a 21-day treatment period. Metabolism crates measured 160cm length x 52cm width x 90cm height. Following the treatment period, sheep were housed in group pens for seven days recovery, before returning to the Dookie campus main flock.

3.2.2.2 Experimental design

Four groups of eight sheep (n = 8 over the four replications) were rotated through the two temperature rooms. Four dosages of Ergovaline were used: nil, 5, 15 and 25 µg/kg LW.

Table 3.3 Major periods used throughout the experiment.

Experimental stage	Length of time
Group pens – acclimatisation	Seven days
Metabolism crates – acclimatisation	Seven days
Metabolism crates -treatment period	21 days
Group pens – recovery	Seven days

3.2.2.3 Animal health procedures

All sheep were checked to ensure their hooves were disease and injury free and in a healthy state. All teeth were checked to ensure no abnormalities were present along with a check of all eyes to ensure no infections or abnormalities were present. Additionally all sheep were inspected visually with any 'unwell' looking sheep omitted from selection. Prior to the commencement of the experiment all sheep were drenched with active ingredient Levamisole drench. Sheep were vaccinated with 5 in 1 prior to entering the trial.

3.2.2.4 Allocation of sheep to treatments

Allocation of sheep to treatments was as described in section 3.2.1.3.

3.2.2.5 Feeding

Sheep had *ad libitum* access to water and feed and were fed twice a day, at 08:00 and 16:00. Sheep were fed whole barley, alkaloid infected perennial ryegrass seed and a commercial sheep pellet that contained 13MJ ME/kg DM and 18% crude protein. All sheep had access to barley and the commercial sheep pellet throughout all experimental stages, with the alkaloid seed fed during the treatment period only.

3.2.2.6 Production measurements

3.2.2.6.1 Feed and water intake

Individual feed intake was measured by collecting pellet refusals and weighing them daily to determine daily feed intake using a set of scales (@Weigh model

QHW-30, @Weigh Pty. Ltd). Dry matter intake (DMI) was calculated using equation 3.2 (Section 3.2.1.5.1).

Water intake was measured each day by filling each water bucket to 7L and then each morning recording the amount of water consumed by reading down the lines on the side of the bucket. The buckets were refilled each day to ensure *ad libitum* water was accessible.

3.2.2.6.2 Liveweight

Liveweight was measured at selection, baseline and once weekly throughout the treatment period. The scales (Tru-Test XR-3000) were calibrated using a 20kg weight each time sheep were weighed.

3.2.2.6.3 Urine output

Once weekly and for the last seven days of each replication, urine output was collected. This was achieved by separating the urine and faeces at the bottom of the metabolism crates over 24h periods. After the 24h period, the buckets were collected and urine output was measured in weight (grams), using a balance (Sartorius BP 3100S, made in Germany).

3.2.2.6.4 Faecal collection and faecal moisture measurement

Faecal samples were collected once weekly and for the last seven days of each replication, over 24 hours. These samples were weighed (Sartorius BP 3100S, made in Germany), to give a total faecal output and at this time a representative sample was taken by mixing the overall sample and filling a labelled bag. The remainder of faeces were discarded.

3.2.2.6.5 Dry matter digestibility

Faeces were weighed and collected over seven consecutive days during the last week of the experimental period. Feed intake values were corrected for dry matter with values over the same seven day period used for calculation. Faeces from the seven day collection period were dried according to Section 3.2.1.5. Dry matter digestibility was calculated over the entire seven day period using total amount over this time period. Calculation was undertaken using equation 3.2 (Section 3.2.1.5.1).

3.2.2.7 Physiological measurements

Respiration rate, rectal temperature and skin temperature were measured at 08:00, 12:00 and 16:00, three days per week over the treatment period.

An area on the back of the sheep, close to the spine, was chosen for the skin temperature measurement. Parting the wool to the skin, the thermometer (Amcal KJump Health, made in China) was placed on the skin until a stable temperature was reached. Skin temperature for sheep should range from 38.5˚C to 39.5˚C. The same point on the sheep's back was used each time for the skin temperature.

Jugular venous blood samples were collected at each weighing. A 10 ml sample was taken, and the sample was transferred into a lithium heparin tube and gently mixed to prevent clotting, before being placed onto ice.

Blood samples were centrifuged (Beckman Coulter, Gladesville, NSW, Australia) at 3000rpm for 10 minutes at 4ºC. After centrifuging, plasma samples were immediately decanted into duplicate sample tubes and stored at -18ºC until analysed.

3.2.2.8 Laboratory analysis

Faecal samples were dried in accordance with the method outlined in 3.2.1.7.

3.2.3 Intake, storage and excretion profiles of alkaloids under different growth paths

3.2.3.1 Animals and housing

Forty-eight sheep were selected for this experiment. Of the forty-eight, twenty-four were a composite breed and twenty-four were a first cross between Merino and a Poll Dorset. Animals were housed in the Dookie College animal house for a total of 63 days. During this time sheep spent 35 days in undercover individual pens and 28 days in group pens.

3.2.3.2 Experimental design

In this experiment two factors were used, breed and alkaloid. There were two breeds (composite v Crossbred) and four levels of alkaloid (control, lolitrem B, ergovaline and lolitrem B/ergovaline). Thus, the animals were divided into eight treatments (n=6 per treatment) and allocated to treatments as outlined in section 3.2.1.3.

Table 3.4 Treatments used in the current experiment.

Treatment	Alkaloid	Breed	Abbreviation
Treatment 1	Nil	Composite	Nil/Comp
Treatment 2	Nil	Crossbred	Nil/X
Treatment 3	Lolitrem B	Composite	L/Comp
Treatment 4	Lolitrem B	Crossbred	L/X
Treatment 5	Ergovaline	Composite	E/Comp
Treatment 6	Ergovaline	Crossbred	E/X
Treatment 7	Ergo/lol	Composite	EL/Comp
Treatment 8	Ergo/lol	Crossbred	EL/X

The experiment was divided into two stages. In stage one sheep were housed in individual pens for 35 days. This included an acclimatisation period of seven days and treatment period of 28 days where production and physiological measurements were taken.

Following the treatment period, sheep were moved into group pens with automatic feeders where they removed from the alkaloid treatments and placed

onto the nil treatment for 28 days which included 21 days on a negative energy balance diet (0.7 x maintenance) and 7 days recovery.

3.2.3.3 Feeding

Sheep were fed a 1.5x maintenance diet throughout stage one of the experiment. The diet consisted of whole wheat which was introduced gradually during the acclimatisation period, a commercial pellet and perennial ryegrass seed for sheep allocated to the alkaloid treatments. Two different types of perennial ryegrass seed were fed. The first contained 15 µg/g ergovaline and the second contained 8.9 µg/g lolitrem B. The ergovaline was fed at a dose of 25 µg/g /kg LW and the lolitrem B was fed at a dose rate of 35 µg/kg LW.

Sheep were fed the wheat and seed portion of the diet at 08:00 daily, with refusals taken after 20 minutes. This allowed the sheep time to eat the seed and enabled an accurate measurement of actual alkaloid consumption. Following this, half of the pellets were fed. The remaining pellets were fed at 16:00.

During stage two of the experiment sheep were removed from the alkaloid diets and were fed a negative energy balance diet (0.7 x maintenance) to promote the loss of adipose tissue.

3.2.3.4 Production measurements

3.2.3.4.1 Feed intake

During stage one individual feed intake was measured daily. This was undertaken by weighing the orts (leftover feed) daily and subtracting them from the amount given to the sheep. During stage two feed intake was recorded daily from the automated system for individual sheep.

3.2.3.4.2 Water intake

Water intake was measured daily during stage one. Each individual pen had access to a nine litre water bucket with volume markings on it. Buckets were filled to a mark each day and the amount consumed was recorded at the same time the following day. Water intake was not able to be recorded during stage two.

3.2.3.4.3 Liveweight

Sheep were weighed in an electronic weighing crate on day one of the acclimatisation period and one day before they began treatment. They were then weighed weekly for the remainder of stage one. During stage two sheep were weighed twice weekly to ensure they were losing weight.

3.2.3.4.4 Urine output

Urine was collected using respiratory occlusion in which the airways are blocked off for 10 seconds and the sheep then urinates. Urine was collected at baseline, day 14, 28 and 49.

3.2.3.4.5 Faecal collection

Grab samples were collected on day 0, 14, 28 and 49. This was conducted by inserting a gloved finger into the rectum to retrieve faeces.

3.2.3.5 Physiological measurements

Rectal temperature and respiration rate were recorded three times weekly at 10:00 during stage one. During stage two this was twice weekly at 10:00. Jugular venepuncture was undertaken at baseline, day 14, 28 and 49. A 10 ml sample was taken, and the sample was transferred into a lithium heparin tube and gently mixed to prevent clotting, before being placed onto ice. Blood samples were centrifuged (Beckman Coulter, Gladesville, NSW, Australia) at 3000rpm for 10 minutes at 4ºC. After centrifuging, plasma samples were immediately decanted into duplicate sample tubes and stored at -18ºC until analysed.

3.2.3.6 Adipose tissue and muscle biopsies

3.2.3.6.1 Methodology

Adipose tissue and muscle biopsies were undertaken on day 28 (end of stage one) and day 49 (end of stage two). Tissue samples were taken from subcutaneous adipose tissue and muscle located along the backline of the sheep. The procedure was as follows; the biopsy site was scrubbed and soaked with iodine and 70% ethanol (Becton Dickson Company, Franklin Lakes, USA). A local anaesthetic was used along the incision site (Lignocaine Hydrochloride 20mg/ml) (Troy Laboratories Pty. Ltd. Australia). A small incision was made with a no 22 scalpel blade (Kiato Plus, India). Collection of adipose tissue was undertaken by making a small cut with surgical scissors and removing up to two grams of tissue. Adipose tissue samples were then weighed and divided into four 1.5ml Eppendorf tubes (Eppendorf South Pacific Pty. Ltd. North Ryde, NSW, Australia) and transferred onto dry ice immediately before being placed in a -80ºC freezer. A small incision was made in the muscle capsule and a sample of muscle taken using a biopsy punch. The site was then closed with three layers of suturing for the muscle capsule, the subcutaneous dead space (dissolvable suture material was used (Dexon II by Syneture,

Connecticut, USA)) and the skin surface (silk, non-absorbable, Look PBN Medicals, Denmark). Wounds were treated with an antibiotic spray – Centrigen antibacterial wound spray (Virbac, Milperra, Australia) – after the final sutures were applied. Sheep were monitored twice daily to ensure infection did not occur and that the site healed properly. Sutures were removed on the tenth day post-surgery.

3.2.3.7 Dual energy X-Ray absorptiometry scanning (DXA)

Dual energy X-ray absorptiometry (DXA) was used to quantitatively determine body composition in the live animal. DXA scanning was undertaken at baseline, day 28 (end of stage one) and day 39 (end of stage two). The DXA scanning process enables the assessment of whole differences in body composition and changes in body composition over time.

Ewes were scanned using DXA to measure whole body composition at the end of the acclimatisation period (only in the second replication), at the end of the treatment period and at the end of the washout period. All sheep were weighed the day prior to scanning and fasted for a 12 hour period with access to water restricted 12 hours prior to scanning. Sheep scanning was undertaken on the DXA truck, a portable facility which enables the scanning of animals in a variety of locations. Scanning was undertaken at the Dookie animal house facility.

On the day of scanning, sheep were sedated using Xylaxine (20 mg/ml) at 0.1 mg/kg

LW intramuscularly ten minutes before administration of Ketamine (100 mg/ml) at 5mg/kg LW intravenously. Depth of anaesthesia was monitored by eye reflex following induction. For some sheep a top up dose of Ketamine was required if depth of anaesthetic was not sufficient.

Dual energy X-ray absorptiometry scanning uses a hologic QDR 4500A fan beam Xray densitometer. The principle of DXA scanning is based on the differential attenuation of the high and low energy X-ray beams by different components (bone, lean tissue and fat tissue) of the animal's body. Validation of this method has been undertaken with comparisons of DXA values.

Each week prior to scanning the DXA machine was calibrated using two phantoms; the acrylic/aluminium step phantom and the spine phantom. The step phantom is used to calibrate the variation in body composition and depth of tissue, validating the lean/fat composition results. The acrylic portion of this phantom attenuates X-ray beams which are similar to fatty tissue while the aluminium appears leaner. The spine phantom is a simulated lumbar spine of known bone mineral content, which ensures the accurate measurement of bone mineral content.

The whole body was scanned in this experiment using software v826A:3. The QDR4500 software undertakes regional analysis (head, arms, legs and trunk), however, in this experiment the whole body was placed in the left arm region while the head was left in the head region ensuring repeatable measures. DXA scanning measured total tissue mass (TTM), lean tissue mass (LTM), fat tissue mass (FTM) and bone mineral content (ash) (BMC). Raw values were adjusted into chemical composition using the regression equations developed for sheep by (Hunter 2000).

Table 3.5 Regression equations which adjust raw DXA values to chemical composition for sheep for the tissues lean tissue mass (LTM), fat tissue mass (FTM) bone mineral content (BMC) and total tissue mass (TTM) (Hunter 2000).

Tissue	Regression equation
LTM	(0.933 x DXA LTM) + 1.25
FTM	(1.2 x DXA FTM) - 0.067
BMC	(1.08 x DXA BMC) + 0.294
TTM	(1.07 x DXA TBW) – 1.4

Sheep were placed in lateral recumbency with the hind legs extended and the forelegs positioned caudally. Foam blocks were used to hold the forelegs away from the body and to position the head up with an elastic strap used to ensure the head did not move during the scan. After scanning sheep were returned to the holding pen and monitored to ensure safe recovery from the anaesthetic.

3.2.3.8 Faecal moisture content

Faecal sub samples from both stages were dried to determine faecal water percentage. This was undertaken in the lab where wet samples were weighed and then dried at 40°C until the sample reached a constant weight. The dry sample was weighed and faecal moisture percentage was calculated using equation 3.1 (Section 3.1.8).

3.2.4 Physiological and production effects of feeding ergovaline to meat sheep breeds under heated ambient temperature conditions

3.2.4.1 Animals and housing

Twenty-four sheep were selected for this experiment. Of the twenty-four, twelve were a composite breed and twelve were a first cross between Merino and a Poll Dorset breeds. Animals were housed in the Dookie College animal house for a total of 50 days. During this time sheep spent 35 days in undercover individual pens, 8 days in metabolism crates in a climate controlled room and 7 days in a group pen.

3.2.4.2 Experimental design

Two factors were used in this experiment: breed (composite vs. Crossbred) and ergovaline dose (nil vs. ergovaline). Four treatments were used (n=6 per treatment). Sheep were randomly allocated to treatments as per outlined in section 3.2.1.3.

Table 3.6 Treatment groups used in the current experiment.

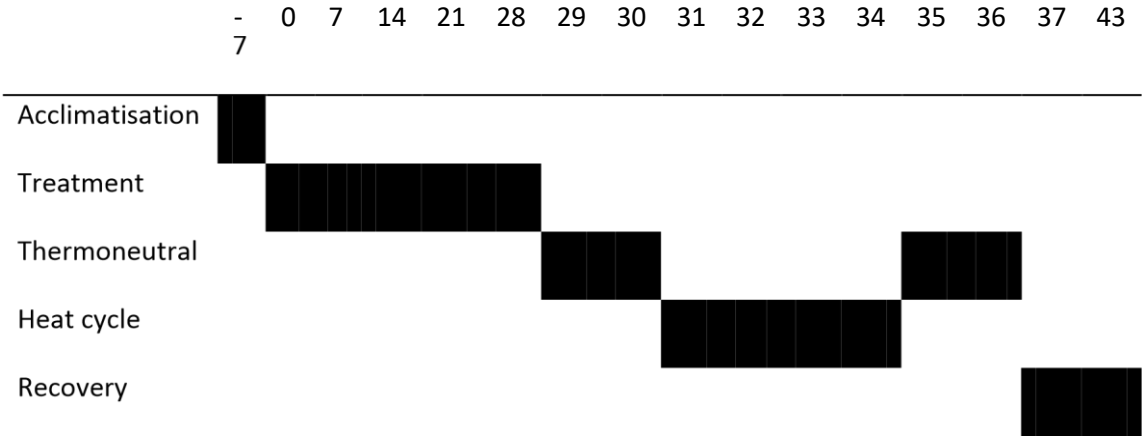
Treatment	Alkaloid	Breed	Abbreviation
Treatment 1	Nil	Composite	Nil/Comp
Treatment 2	Nil	Crossbred	Nil/X-Bred
Treatment 3	Ergovaline	Composite	Ergo/Comp
Treatment 4	Ergovaline	Crossbred	Ergo/X-Bred

This experiment was divided into two stages. In stage one sheep were housed in individual pens for 35 days. This included an acclimatisation period of seven days and treatment period of 28 days where production and physiological measurements were taken.

Following the treatment period, sheep were moved into individual metabolism crates located in a climate controlled room for the second stage of the experiment. Sheep spent two days under thermoneutral conditions followed by four days under heat conditions and two days under thermoneutral conditions. During

thermoneutral conditions, ambient temperature remained at 21°C for the entire day. The heat cycle was set at 36°C between 08:00 and 16:00 and reduced to 21°C between 16:00 and 08:00.

Figure 3.3 Timeline of experiment



Four groups of sheep were rotated through the climate room.

3.2.4.3 Feeding

Sheep were fed a 1.5x maintenance diet throughout the experiment. The diet consisted of whole wheat which was introduced gradually during the acclimatisation period, a commercial pellet and perennial ryegrass seed for sheep allocated to the ergovaline treatments. The perennial ryegrass seed contained 15 µg/g ergovaline and was fed at a dose of 25µg/kg LW.

Sheep were fed the wheat and seed portion of the diet at 08:00 daily, with refusals taken after 20 minutes. This allowed the sheep time to eat the seed and enabled an accurate measurement of actual ergovaline consumption if part of the seed was not eaten. Following this, half of the pellets were fed. The remaining pellets were fed at 16:00.

3.2.4.4 Animal health procedures

All sheep were checked to ensure their hooves were disease and injury free and in a healthy state. All teeth were checked to ensure no abnormalities were present along with a check of all eyes to ensure no infections or abnormalities were present. Additionally all sheep were inspected visually with any 'unwell' looking sheep omitted from selection. Prior to the commencement of the experiment all sheep were drenched with active ingredient Levamisole drench. Sheep were vaccinated with 5 in 1 prior to entering the trial.

3.2.4.5 Allocation of sheep to treatments

Allocation of sheep to treatments was as per outlined in section 3.2.1.3.

3.2.4.6 Production measurements

3.2.4.6.1 Feed intake

Feed intake was measured daily. This was undertaken by weighing the orts (leftover feed) daily and subtracting them from the amount given to the sheep.

3.2.4.6.2 Water intake

Water intake was measured daily. Each individual pen and metabolism crate had access to a nine litre water bucket with volume markings on it. Buckets were filled to a mark each day and the amount consumed was recorded at the same time the following day.

3.2.4.6.3 Liveweight

Sheep were weighed in an electronic weighing crate on day one of the acclimatisation period and one day before they began treatment. They were then weighed weekly for the remainder of stage one. Weight was recorded the day before entry into the metabolism crates and upon exit from the metabolism crates. Scales were calibrated with a 20kg weight prior to weighing.

3.2.4.6.4 Urine output

Urine was collected in two ways. During stage one it was collected using respiratory occlusion in which the airways are blocked off for 10 seconds and the sheep then urinates. Urine was collected at baseline, day 14 and day 28. During stage two, urine was collected by measuring 24 hour output.

3.2.4.6.5 Faecal collection

Faeces were collected using two different methods. Grab samples were collected while sheep were in the individual pens at the beginning of treatment (baseline) day 14 and day 28. This was conducted by inserting a gloved finger into the rectum to retrieve faeces. Faecal collection during stage two of the experiment occurred three times over 24 hour periods (day 31, 34 and 36). Faeces were weighed and sub samples taken for drying.

3.2.4.7 Physiological measurements

Rectal temperature and respiration rate were measured three days per week at 10:00 during stage one. During stage two this was undertaken daily at 08:00, 12:00 and 16:00.

Skin temperature was measured daily during stage two at 08:00, 12:00 and 16:00.

Jugular venepuncture was undertaken with an 18 gauge needle (Terumo Corporation, Tokyo, Japan) in a syringe. A 10 ml sample was taken, and the sample was transferred into a lithium heparin tube and gently mixed to prevent clotting before being placed onto ice. Blood samples were collected on day 31, 34 and 36 during stage two with samples taken at 08:00 and 16:00. During stage one blood samples were collected at baseline, day 14 and day 28.

Blood samples were centrifuged (Beckman Coulter, Gladesville, NSW, Australia) at 3000rpm for 10 minutes at 4ºC. After centrifuging, plasma samples were immediately decanted into duplicate sample tubes and stored at -18ºC until analysed.

3.2.4.8 Faecal moisture content

Faecal sub samples from both stages were dried to determine faecal water percentage. This was undertaken in the lab where wet samples were weighed and then dried at 40°C until the sample reached a constant weight. The dry sample was weighed and equation 3.1 (Section 3.1.8) used to calculate faecal water percentage.

3.2.5 Efficacy of rumen detoxifying agents for mitigating PRGT

3.2.5.1 Animals and housing

Twenty-eight 6-month-old Merino ewe weaners were selected from the main Dookie flock with an approximate initial average weight of 35kg, and transported to the MSLE Animal Parkville facility in two blocks of 14 sheep.

The initial week in the Parkville facility was used as the acclimatisation period and sheep were kept in group pens. A day before the experimental period began, sheep were moved into individual metabolism (MET) crates, which were approximately 1.0 x 0.5m and stood 1.0m off the ground. Feed and water were provided by troughs and buckets connected to the sides of the crates. Ambient temperature was kept constant at 22 with a light/dark cycle of 14/10 hours respectively.

3.2.5.2 Experimental design

Two levels of alkaloid were fed in this experiment: a nil dose and a combined 100µg/kg LW Ergovaline and 80µg/kg LW lolitrem B dose. Elitox was fed at 0, 0.07 and 0.14g/kg LW/day (0, 2 and 4 g/day). Six treatments were used (n=4 per treatment). Sheep were fed the treatment diet for a total of three weeks. Sheep were randomly allocated to treatments as per outlined in section 3.2.1.3.

Table 3.7 Treatment groups used.

Treatment	Alkaloid	Elitox dose (g)
E[1]-	Nil	0
E2+	Nil	2
E4+	Nil	4
A[2]E-	Alkaloid	0
AE2+	Alkaloid	2
AE4+	Alkaloid	4

[1]E: Elitox; [2]A: Alkaloid

Note that the 2g/day dosage rate of Elitox was the recommended dosage and 4g/day was the higher dosage.

3.2.5.3 Animal health procedures

All sheep were checked to ensure their hooves were disease and injury free and in a healthy state. All teeth were checked to ensure no abnormalities were present

along with a check of all eyes to ensure no infections or abnormalities were present. Additionally all sheep were inspected visually with any 'unwell' looking sheep omitted from selection. Prior to the commencement of the experiment all sheep were drenched with active ingredient Levamisole drench. Sheep were vaccinated with 5 in 1 prior to beginning the experiment.

3.2.5.4 Allocation of sheep to treatments

Sheep in each repetition were paired based on similar weights and then randomly assigned to a treatment giving a total of n=4 per treatment.

3.2.5.5 Feeding

Sheep were fed a 1.2 x maintenance diet throughout the experiment. The diet consisted of oaten and lucerne chaff, perennial ryegrass seed and Elitox, the latter two being fed prior to sheep receiving the chaff diet. Sheep were split fed (08:00 and 16:00). Ergovaline was fed at a dose of 100ug/kg LW and lolitrem B was fed at dose of 80ug/kg LW. Elitox was fed at a dose of 0, 0.07 and 0.14g/kg LW/day (0, 2 and 4 g/day).

3.2.5.6 Production measurements

3.2.5.6.1 Feed intake

Feed intake was measured daily. This was undertaken by weighing the orts (leftover feed) daily and subtracting them from the amount given to the sheep.

3.2.5.6.2 Water intake

Water intake was measured twice daily (08:00 and 16:00). Each individual metabolism crate had access to a nine litre water bucket with volume markings on it.

Buckets were filled to a mark each day and the amount consumed was recorded.

3.2.5.6.3 Liveweight

Sheep were weighed in an electronic weighing crate on day one of the acclimatisation period and one day before they began treatment. They were then weighed upon concluding the treatment period. Scales were calibrated with a 20kg weight prior to weighing.

3.2.5.6.4 Urine output

Urine was collected between days 14 and 21 by measuring 24 hour output.

3.2.5.7 Physiological measurements

Rectal temperature, respiration rate and skin temperature was recorded daily at 09:00, 12:00 and 16:00 on weekdays and at 09:00 and 16:00 during weekends.

3.3. Methodology – On farm studies

The objective of the on farm studies was to evaluate the efficacy of Elitox™ Toxin binder (Feedworks) as a detoxifying agent in alleviating the impact of PRGT on the productivity and welfare of sheep grazing on commercial sheep farms. In year one,

one property was selected as a suitable trial site based on previous history of PRGT (Trial 1). This trial property was based at Elaine in central western Victoria and ran 4,000 super fine merino sheep. A suitable paddock with a previous history of rye grass staggers and adequate perennial rye grass was selected for subdivision into six one hectare paddocks with similar pasture composition and topography. Each paddock was supplied with trough water from the property's main water supply. One hundred and twenty super fine Merino weaner sheep (six months old) were randomly selected from a mob of 1,100 weaners and randomly allocated to 6 paddocks (3 treatment and 3 control paddocks) at a stocking rate of 20 weaners per hectare which was typical for the specific paddocks selected to run the trial. All paddocks were supplied with a commercial stock lick, Weather Shield – Sheep™ Propharma in the 3 control paddocks and Weather Shield – Sheep for ryegrass staggers™ Propharma in the 3 treatment paddocks.

Weather Shield – Sheep for ryegrass staggers™ contains 50 g/kg of Elitox Toxin binder. All other components of the licks were the same.

Six month old superfine Merino weaners were selected from a mob of 1,100 and randomly allocated to groups. At this time, weaners were treated with long acting Cydectin™ due to prevailing wet conditions. Weaners were treated with Permasel™ Copper and Selenium pellets as is normal farm practice to prevent known copper and selenium deficiencies that occurs on this property. All weaners were crutched in February as is normal practice on the property. No supplementary feeding was required due to favourable environmental conditions.

On setting up the trial, animal measurements were recorded including bodyweight, fleece weight and fibre diameter, dag score, animal behaviour, including staggers, rectal temperature, and respiration rate where possible, along with pasture assessment (FOO & composition). Perennial ryegrass samples were collected for assessment of endophyte toxin levels. Animal and pasture measurements were repeated throughout the period of the trial at 4-8 week intervals with regular inspection and examination of sheep for evidence of staggers in between the measurements.

Trial 1 was set up in summer 2010-11 and continued to spring 2011 when weaners were shorn and fleeces were weighed.

During 2012, two trials were planned to set up, one in western Victoria on a cattle property near Mortlake running approximately 4,500 angus cattle and the other in south Gippsland on a property running 3,000 crossbred sheep (Merino White Suffolk cross) and Merino sheep. Both properties had a regular and severe history of PRGT. Suitable paddocks were selected on the property in western Victoria to run the trial. However, due to severe drought conditions, the trial site was abandoned for the year due to rye grass having low endophyte levels and lack of available pasture meaning that heavy supplementary feeding was required. A second trial planned to be set up at Yarram in South Gippsland. However, due to high rainfall and very humid conditions the trial site was abandoned due to an outbreak of facial eczema (*Pithomyces chartorum*), which was diagnosed just before sheep were selected for the trial.

A third site was selected in summer 2012 near Baynton in central Victoria that ran 5,000 fine wool Merinos and Coopworth sheep (Trial 2). This property had a previous history of PRGT and environmental conditions were more suitable for running the trial. Due to the late start for the trial, rather than set up a 6 paddock system, a suitable 10 hectare paddock with a dense stand of Victorian Perennial Ryegrass was subdivided into two equal 5 hectare paddocks. One hundred and sixty fine wool Merino weaners were selected to run in the trial and divided at random to run in two groups, control group with access to a lick containing Weather Shield – Sheep™ Propharma and the treatment group had access to Weather Shield – Sheep for ryegrass staggers™ Propharma containing 50 g/kg of Elitox Toxin binder. Sheep in each paddock were rotated weekly with their appropriate lick and observed closely for signs of staggers. Sheep were weighed every 4-6 weeks during the trial period that ran from autumn to early winter. Pasture measurement included collecting samples for endophyte alkaloids, were taken during the trial period.

In 2013, two further trials were run at the sites abandoned the previous year. A third trial was set up at Mortlake (trial 3) in western Victoria, was run during autumn. Two hundred and forty Angus heifers (12 months old) were selected at random to run in the trial and divided at random to run in two groups, a control group with access to a lick containing Weather Shield – Cattle™ Propharma and the treatment group had access to Weather Shield – Cattle for ryegrass staggers™ Propharma containing Elitox toxin binder. Heifers in each paddock were rotated weekly with their appropriate lick and observed closely for signs of staggers. Heifers were weighed every 4-6 weeks during the trial period that ran during autumn. Pasture measurement included collecting samples for endophyte alkaloids, were taken during the trial period.

A fourth trial (trial 4), was run during autumn at Yarram in south Gippsland. One hundred and sixty crossbred weaners (8 months old) were selected at random to run in the trial and divided at random to run in two groups, a control group with access to a lick containing Weather Shield – Sheep™ Propharma and the treatment group had access to Weather Shield – Sheep for ryegrass staggers™ Propharma containing Elitox Toxin binder. Weaners in each paddock were rotated weekly with their appropriate lick and observed closely for signs of staggers. Weaners were weighed every 4-6 weeks during the trial period that ran during autumn. Pasture measurement included collecting samples for endophyte alkaloids were taken during the trial period.

3.4. Methodology – Statistical analysis

3.4.1 Pasture measurements

Statistical analysis was undertaken using Genstat statistical package, 15th Edition (VSN International, UK).To identify differences between endophyte treatments a repeated-measures analysis of variance (ANOVA) was performed with blocking for replicate and where possible using measurements on day 0 as a covariate. Significant differences were determined at the $P<0.05$ level. Data for the different sheep breeds was analysed separately.

3.4.2 Animal measurements

Statistical analysis was undertaken using Genstat statistical package, 13th Edition (VSN International, UK). For all parameters (production and physiological) statistical significance was analysed using general linear model or analysis of variance. For grazing experiments, the model included the fixed effects of alkaloid (WT, AR1, AR37) and time (day or week depending on the parameter), and all possible interactions were measured. Individual sheep were used as a random effect, and the model was blocked for the effects of replicate paddock. Data for the different sheep breeds were analysed separately. For the indoor experiments, the model included the fixed effects of treatments (e.g. alkaloid and/or heat) and time (day or week depending on the parameter) and all possible interactions were measured. Individual sheep were used as a random effect, and the model was blocked for the effects of replicate run where appropriate. Covariates were also included in the analysis using baseline data or day 0 data where appropriate.

3.4.3 On-farm measurements

To evaluate the level of significance, two sample t test was used (control v Elitox) except with non-parametric data where the signed Wilcox test was used. Analysis was performed using Epitools (AusVet Animal Health Services).

4. Results and discussion

4.1 Grazing studies

4.1.1 Agronomic performance of treatments

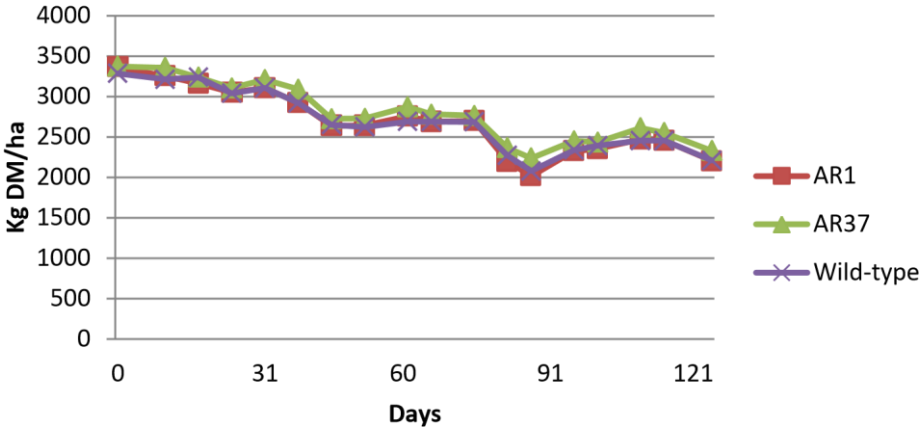
4.1.1.1 Agronomic performance – 2011

Significant endophyte treatment effects on pasture mass and growth occurred very rarely and are discussed as they occur.

4.1.1.1.1 Cross bred ewe trial

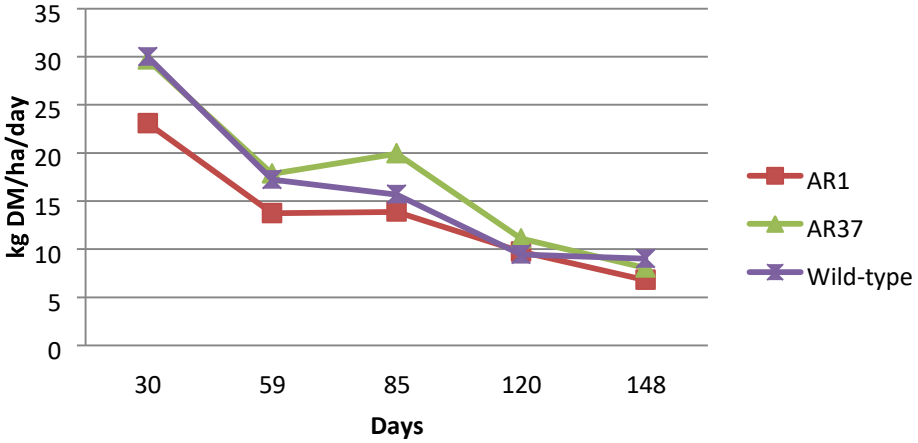
There was no significant difference in the mean pasture mass between the AR1, AR37 and Wild-type (WT) endophyte treatments (Figure 4.1). The AR37 treatment did tend to be slightly higher than the other treatments from 31 days onwards however this was not significant.

Figure 4.1 Mean pasture mass available over time in the AR1, AR37 and Wild-type endophyte treatments grazed by cross bred ewes from February to June 2011. P=0.401, Least Similar Difference (LSD)=248.28.



There were significant effects of endophyte on pasture growth rate and the growth rate x time interaction was also significant (P=0.019). AR1 was significantly lower than AR37 and WT at day 30 and 59 while AR37 significantly greater than WT and AR1 at day 85. Thereafter there were no significant differences between treatments.

Figure 4.2 Mean pasture growth rate (kg DM/ha/day) for AR1, AR37 and Wild-type ryegrass treatments grazed by Crossbred ewes from February to June 2011. P<0.001, LSD=3.098.



There were no significant endophyte effects on the proportion of green and dead plant material in the treatment however these proportions did vary over time.

Table 4.1 Proportion of dead vs green plant material in the cross bred ewe trial over time.

Time	Dead	Green
0	50.1	48.35
31	54.27	45.73
60	54.25	45.75
86	60.74	39.26
121	64.13	35.87
Time P value	<0.001	<0.001
LSD	3.963	4.6

4.1.1.1.2 Merino ewe weaner trial

In the experimental area grazed by merino ewe weaners there were no significant differences between endophyte treatments for either mean pasture mass available or pasture growth rate. This is different from the pasture growth rate results in the Crossbred ewe trial (figure 4.2) however comparison between the two is unwise due to the different stock type, stocking rate and site topography.

Figure 4.3 Mean pasture mass available over time in the AR1, AR37 and Wild-type endophyte treatments grazed by merino ewe weaners from February to June 2011. P=0.984, LSD=667.58

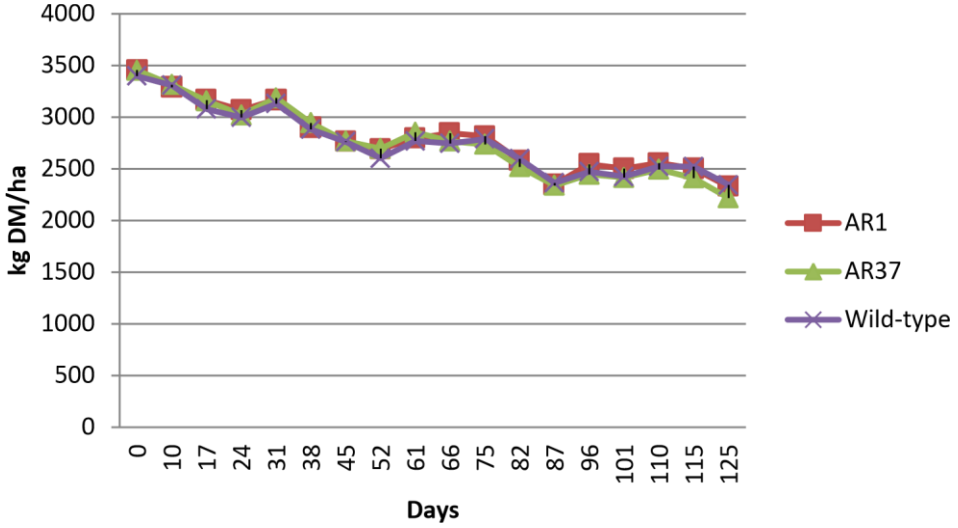
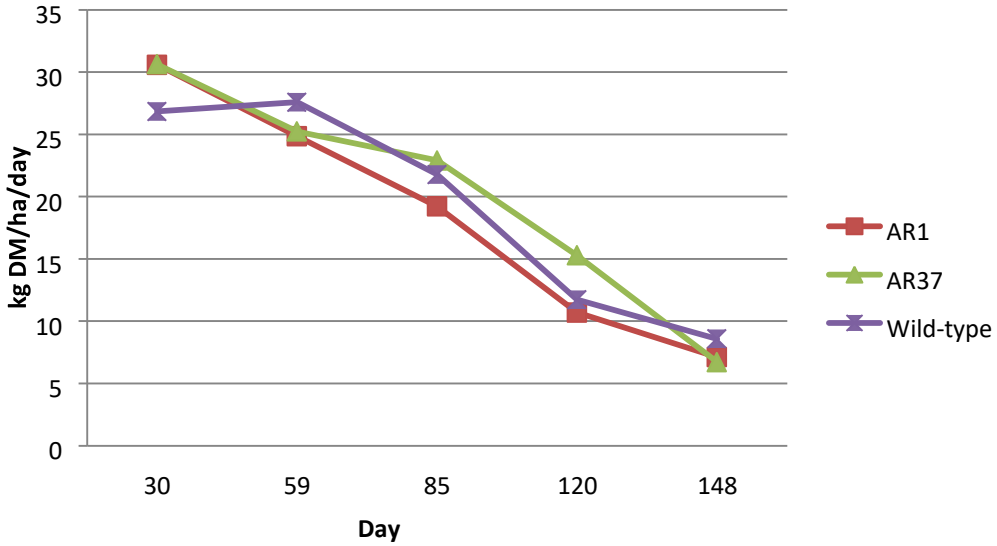


Figure 4.4 Mean pasture growth rate over time for the AR1, AR37 and Wild-type endophyte treatments grazed by merino ewe weaners from February to June 2011. P=0.612, LSD=6.56



There were no significant endophyte effects on the proportion of dead and green material in the trial area however over time the level of dead and green matter did significantly differ.

Table 4.2 Proportion of dead vs green plant material in the merino ewe weaner trial over time.

Time	Dead	Green
0	48.44	48.44
31	48.56	51.44
60	47.67	52.33
86	52.84	47.16
121	63.66	36.34
Time P Value	0.006	<0.001
LSD	6.646	5.078

Discussion – Agronomic performance in 2011

Pasture mass availability for all trials started at well in excess of 3000 kg DM/ha and of its own would not have constrained sheep performance. Over time the pasture availability declined but this was expected given the time of year, likely pasture senescence and loss due to low moisture and high temperatures, reducing pasture growth rate and presence of livestock.

The significant endophyte differences in pasture growth rate in the treatments grazed by first cross ewes is challenging to explain. Insect pests, particularly below ground pests, could provide some explanation for this difference however these were not observed. The relatively small variation in pasture growth rate did not appear to significantly influence the total dry matter available – although it may have contributed to the slight positive trend for AR37 pasture mass. It might have been expected that slightly higher growth rates in the AR37 may have contributed to greater levels of green to dead material however this also did not eventuate.

Although total pasture mass availability appears to be adequate for the duration of this trial, the level of green matter within the pasture did decline significantly and was less than 40% of the total pasture mass by the end of the period. This would correspond to less than 1000kgDM/ha green towards the end of the period and it is well established (Prograze manual) that this level of green feed in June may have been borderline for adequacy for some classes of stock such as rapidly growing lambs and twin bearing ewes in late gestation. Fortunately stock was removed at about this time.

Conclusion

The absence of significant endophyte effects on pasture mass provides some confidence that any animal affects observed are more likely to be due to the endophyte treatment and not large variations in pasture mass availability. Some care needs to be taken with these field observations because the level of variation across each treatment and the limited nature of the sampling means that it can be hard to get statistically significant results for small differences in pasture mass and growth without using many more replicates that the 3 used for this study. The purpose of the agronomic work in this study was focussed on supporting the animal grazing measurements. The absence of significant endophyte treatment effects for pasture mass and green;dead plant material in particular will increase the confidence in any endophyte treatment differences in animal physiology or production measures. If agronomic performance, with no regard for livestock performance, was the focus then a different trial design would have been utilized.

4.1.1.1.3 Feed quality 2011 and 2011-2012 trials

During 2011 and 2011-2012 grazing periods for all cross bred and merino ewe trials, there were no significant differences between the endophyte treatments AR1, AR37 and WT for the feed quality parameters of dry matter digestibility (DMD) and crude protein (CP). Time was significant for all feed quality parameters and all trials however the time x endophyte interaction was not.

Figure 4.5 Mean dry matter digestibility of the AR1, AR37 and Wild-type endophyte treatments for the experimental site grazed by cross bred ewes in 2011 and 2012. P=0.828, LSD=6.916

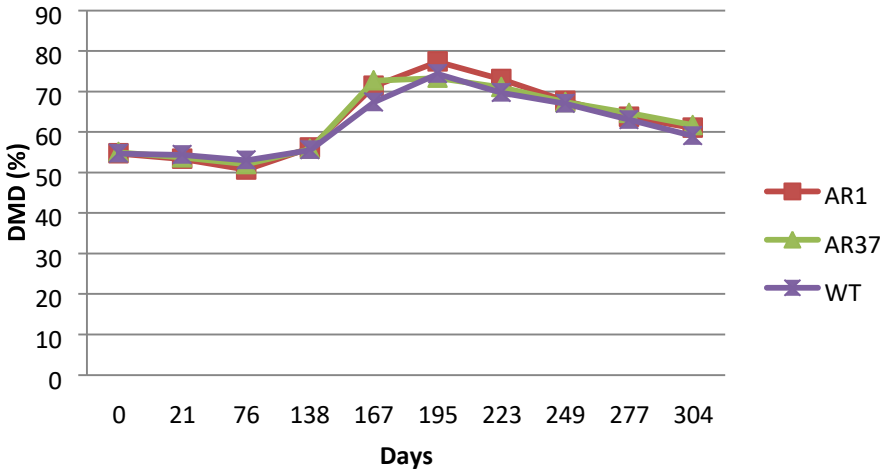


Figure 4.6 Mean crude protein of the AR1, AR37 and Wild-type endophyte treatments for the experimental site grazed by cross bred ewes in 2011 and 2012. P=0.505, LSD=1.5163

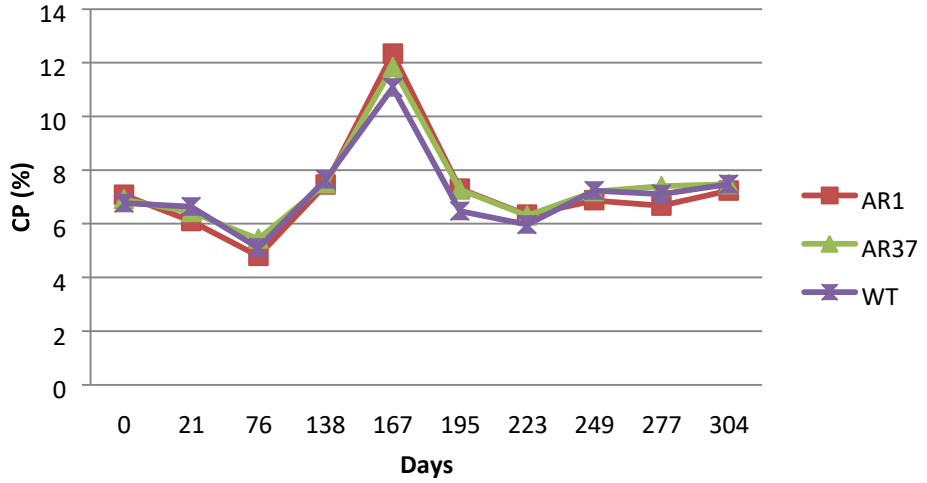


Figure 4.7 Mean dry matter digestibility of the AR1, AR37 and Wild-type endophyte treatments grazed by merino ewe weaners in 2011 and 2012. P=0.932, LSD=5.703

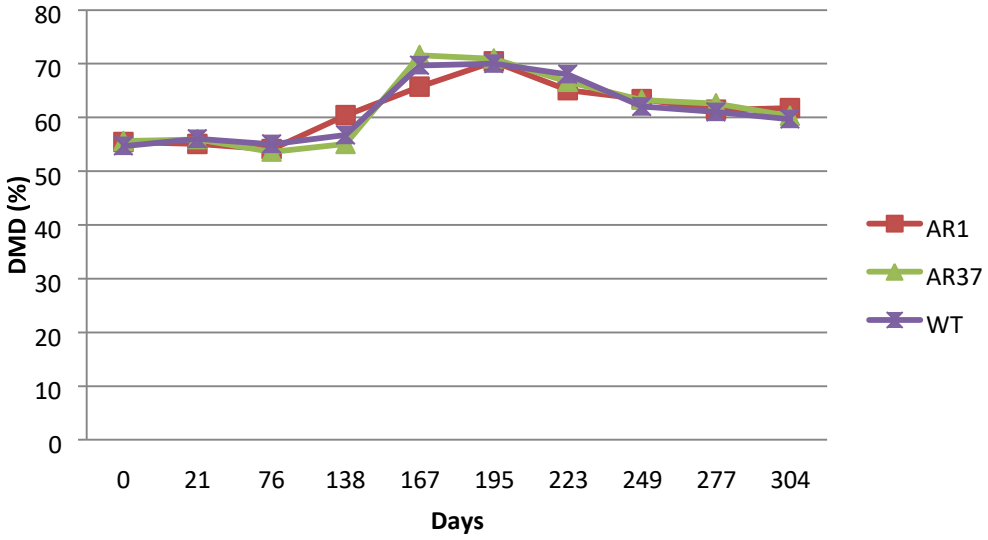
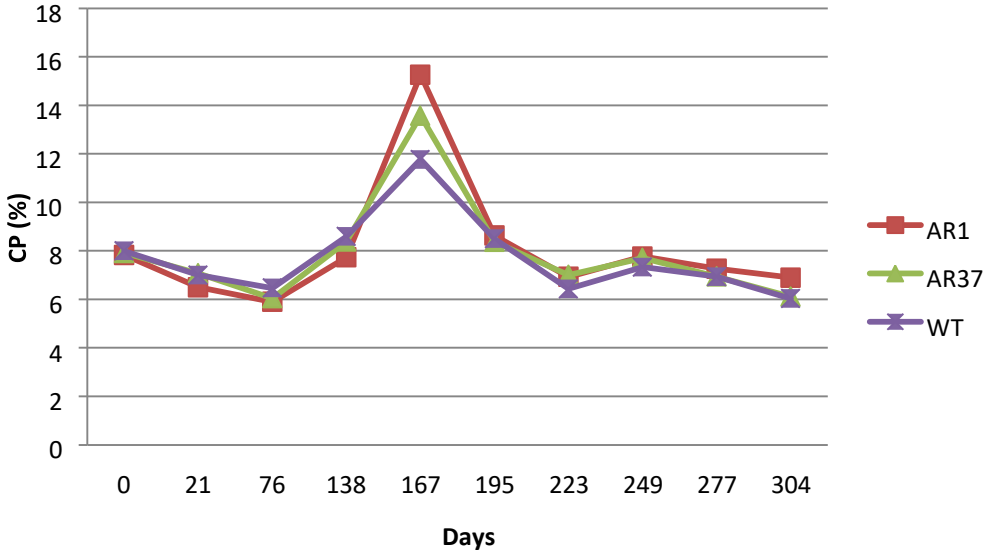


Figure 4.8 Mean crude protein content of the AR1, AR37 and Wild-type endophyte treatments grazed by merino ewe weaners in 2011 and 2012. P=0.272, LSD=1.2131



Discussion – Feed quality

Feed quality results represent the total pasture on offer at the time however sheep are adept at selecting forage of better quality. Although DMD approached 50% and CP dropped to 5-6% during the first ~120 days of the study, sheep have the ability to preferentially select and graze better quality green material in the sward which even towards the end of the period comprised approximately 40% of the mass available. Thus the selected diet is likely to be better than indicated by the feed analysis figures. A similar issue applies to crude protein figures which are acceptable for mature adult stock but low for young growing stock during early autumn and later in spring (e.g. prior to days 76 and after day 223). However, as with DMD, this figure is based on the whole pasture sample. At the time nominated, dry dead forage, which has a naturally lower CP level, was approximately 50% or more of the available forage and livestock would have been able to select greater proportions of green matter with a higher CP level. Despite all of this, the lack of significant endophyte treatment differences suggests that each group of animals was treated the same with respect to feed quality.

Conclusion

In autumn, the feed quality available to mature stock is likely to be sufficient for maintenance or better production levels. Young growing stock however would have been required to actively select the green material in order to ensure adequate nutrition and intake and for much of the experimental period this would have been possible with over 2500 kg DM/ha total being available or approximately 1000 kg DM/ha of green material on offer. The lack of significant agronomic differences between endophyte treatments suggests that the animal groups in this trial had access to the same quantity and quality of feed. This would suggest that any significant livestock differences are as a result of the endophyte treatments.

4.1.1.2 Agronomic performance – 2011-2012

4.1.1.2.1 Lambing and lactation in crossbred ewes (August 2011-December 2011)

There were no significant effects of endophyte on either pasture mass availability or pasture growth rate although there was a slight, consistent positive trend for the AR37 treatment to grow faster than either AR1 or WT treatments. There was no significant interaction between time and endophyte treatment.

Figure 4.9 Mean pasture mass available over time in the AR1, AR37 and Wild-type endophyte treatments grazed by cross bred ewes with lambs at foot from August to December 2011. P=0.962, LSD=187.11

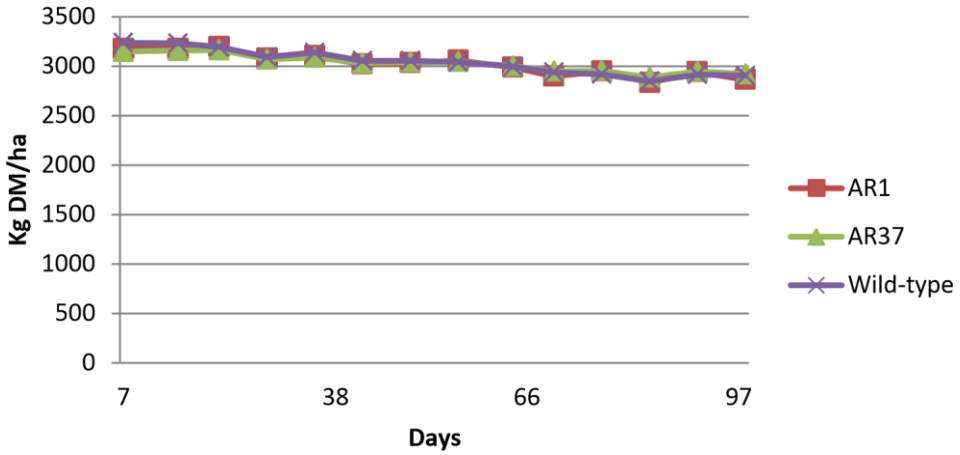
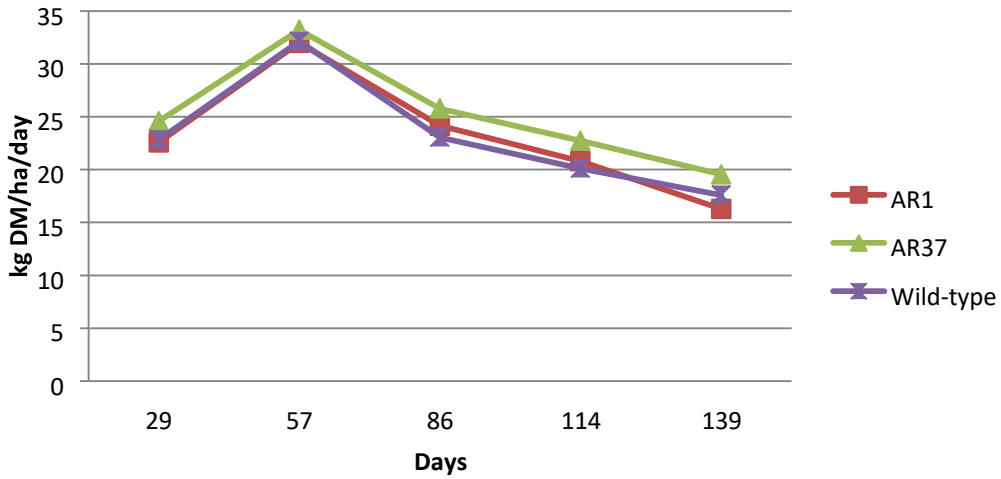


Figure 4.10 Mean pasture growth rate over time in the AR1, AR37 and Wild-type endophyte treatments grazed by cross bred ewes with lambs at foot from August to December 2011. P=0.521, LSD=6.623



There were no significant effects of endophyte treatment on the proportion of green and dead matter in the paddocks however as might have been expected, the proportions did vary significantly with time.

Table 4.3 Proportion of green vs dead matter over time

Time	Green	Dead
29	83.1	16.9
57	78.4	21.6
85	65.4	34.6
111	55.3	44.7
166	23	77
P Value	<0.001	<0.001
LSD	8.73	8.73

Discussion – Agronomic performance

Although pasture growth rate of AR37 was slightly higher than for the other endophytes this was not a significant effect and the total pasture mass in each treatment was similar. Peak pasture growth rates of only 30-35kgDM/ha/day in spring (day 57) are below the expected range of 70-100kgDM/ha/day. Although there was a reasonable mass of pasture on the paddock, the level of dry dead material rapidly increased as the year progressed and the DMD and CP fell to approximately 60% and 7.5% (of the whole pasture sample). This may have effected animal production at the very end of this period, particularly for young growing stock – however there were no significant differences in feed quality between endophyte treatments.

Conclusion

There were no significant endophyte treatment effects on pasture mass, feed quality and dead:green proportions, therefore any differences in animal production and physiology measures may be more likely due to endophyte treatment per se rather than the influence of endophyte on agronomic characteristics.

4.1.1.2.2 Merino ewes October 2011 – March 2012

There was no effect of endophyte on pasture mass availability and pasture growth rate for the trial period however pasture mass did significantly decrease over time and pasture growth rate did vary over time. There was no interaction of time and endophyte treatment.

Figure 4.11 Mean pasture mass available in the AR1, AR37 and Wild-type endophyte treatments grazed by Merino ewe weaners from October 2011 to March 2012. P=0.395, LSD=344.26

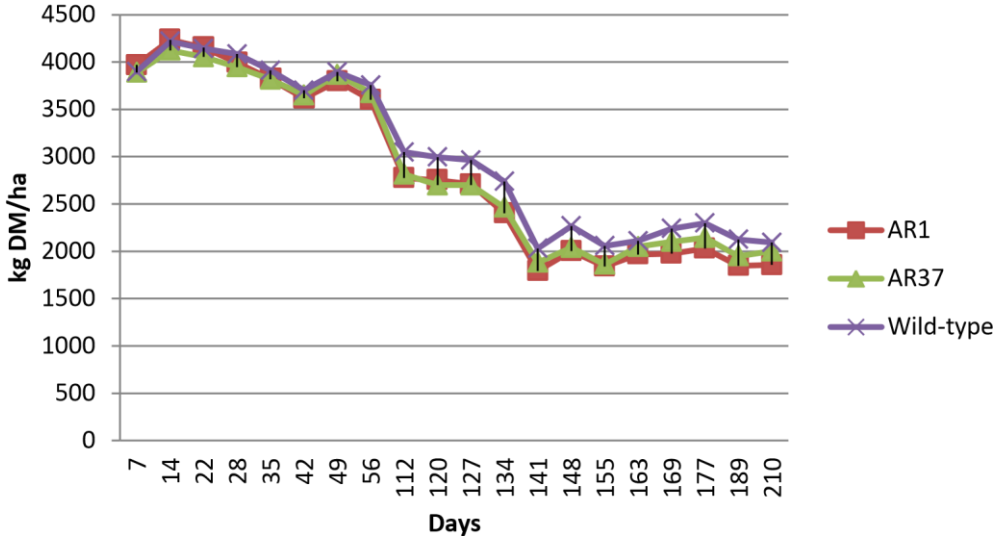
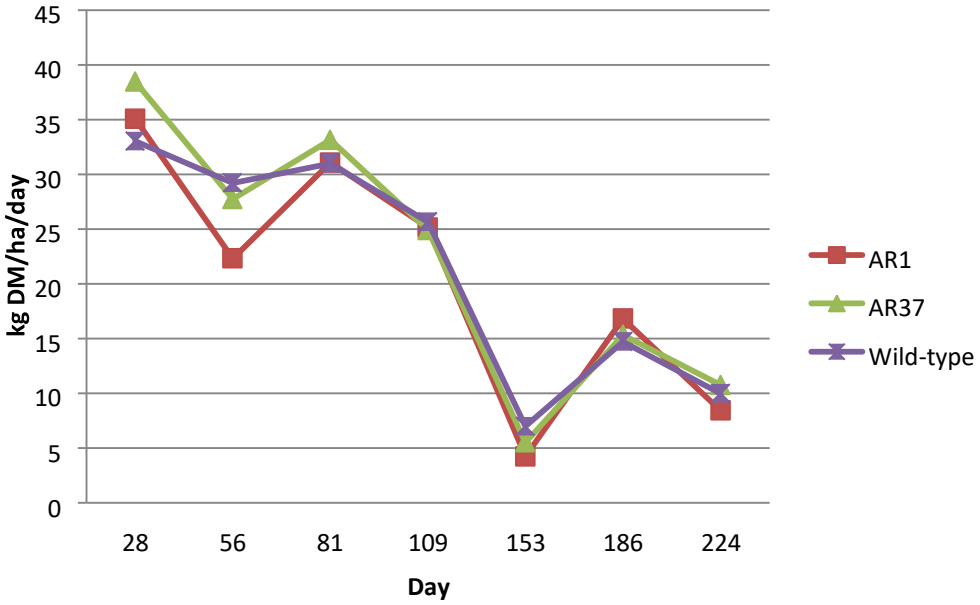


Figure 4.12 Mean pasture growth rate in the AR1, AR37 and Wild-type endophyte treatments grazed by Merino ewe weaners from October 2011 to March 2012. P=0.561, LSD=6.791



There were no effects of endophyte on the proportion of green vs dead material in the trial however the proportions did vary with time.

Table 4.14 Proportion of green vs dead plant material over time

Time	Green	Dead
28	79.8	20.2
54	64.8	35.2
109	37.5	62.5
153	14.3	85.7
186	49.3	50.7
214	76.1	23.9
P Value	<0.001	<0.001
LSD	13.38	13.38

Discussion – Agronomic performance

Although there were no endophyte effects, the pattern of change for pasture mass, pasture growth rate and proportion of green vs dead plant material is within the expected range for the spring-summer-autumn period concerned. Total pasture mass in this period declines due to the combination of grazing, warming conditions, reduced rain, and reduced pasture growth rates. There was a slight trend for the WT endophyte treatment to have higher pasture mass from day 112 onwards, perhaps due to slight, although non-significant, growth rate advantages in the period around day 56 over the other endophyte treatments. The possibility of some toxin mediated minor level of grazing avoidance in the WT endophyte treatment also cannot be discounted.

Conclusion

The absence of significant endophyte treatment results suggest that for the parameters measured, livestock across all treatments were being subject to a similar level of feed quality and feed availability. Any livestock physiology or production measures that are significantly affected by endophyte treatment are possibly more likely to be related to the alkaloid production of that endophyte rather than any influence the endophyte might have had on the plant itself.

4.1.1.2.3 Crossbred ewes March 2012-April 2012

Endophyte treatments were not significantly different for either pasture mass or pasture growth rate during this period. There was no significant interaction between time and endophyte.

Figure 4.13 Mean pasture mass available over time for the AR1, AR37 and Wild-type endophyte treatments grazed by cross bred ewes from March to April 2012. P=0.662, LSD=151.72

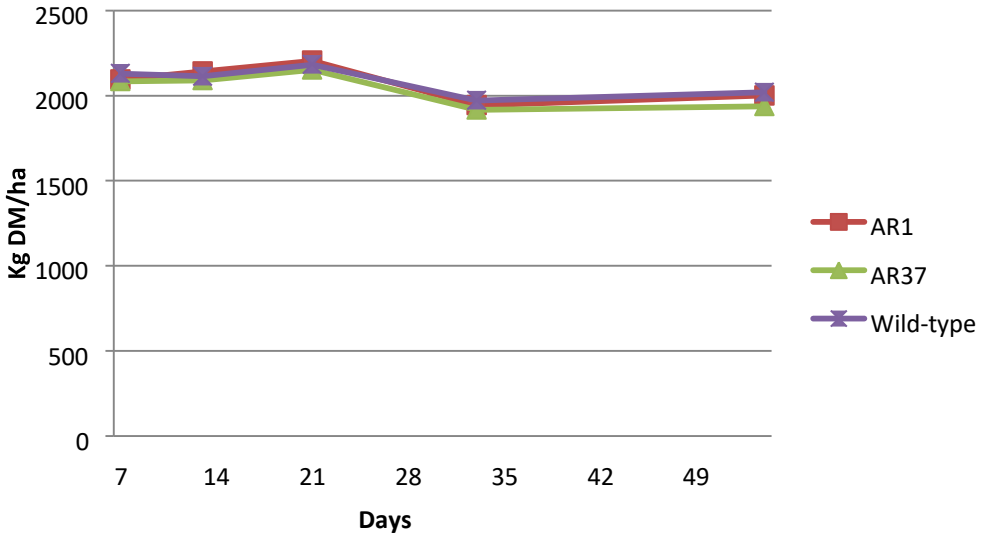
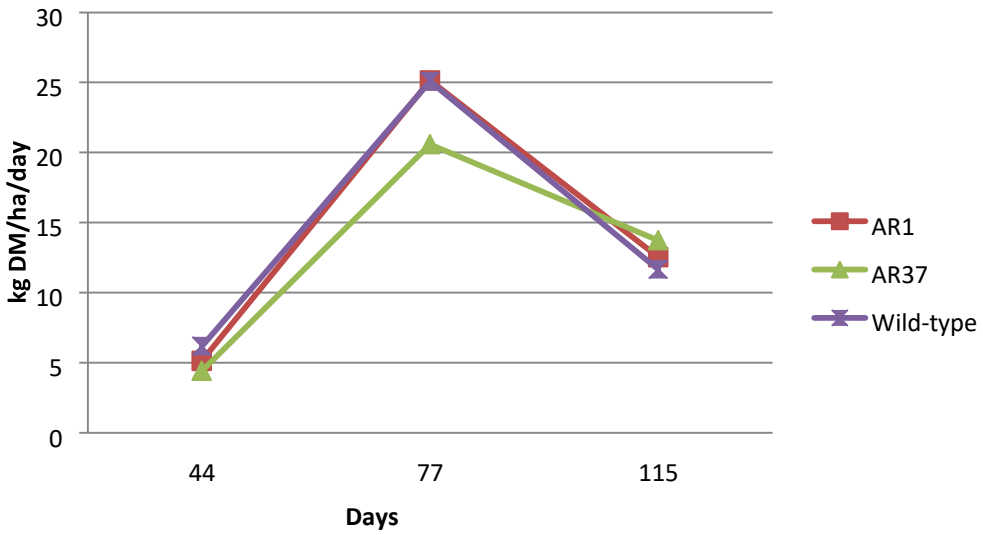


Figure 4.14 Mean pasture growth over time for the AR1, AR37 and Wild-type endophyte treatments grazed by cross bred ewes. Pasture growth data collected from the month before and after the grazing period and has been included in this analysis P=0.441, LSD=3.92.



There was no effect of endophyte on the proportion of green vs dead material in the trial however the proportions did vary over time.

Table 4.5 Proportion of green vs dead plant material over time

Time	Green	Dead
44	45.5	54.5
77	67	33
105	85.4	14.6
P Value	0.018	0.018
LSD	23.99	23.99

Discussion – Agronomic performance

Grazing over this period contributed to a reduction in the level of dry dead material in the paddock and, combined with the low levels of fresh pasture growth, to an increase in the proportion of green pasture material. There was no significant endophyte related effect on the pasture growth and composition during this period.

Conclusion

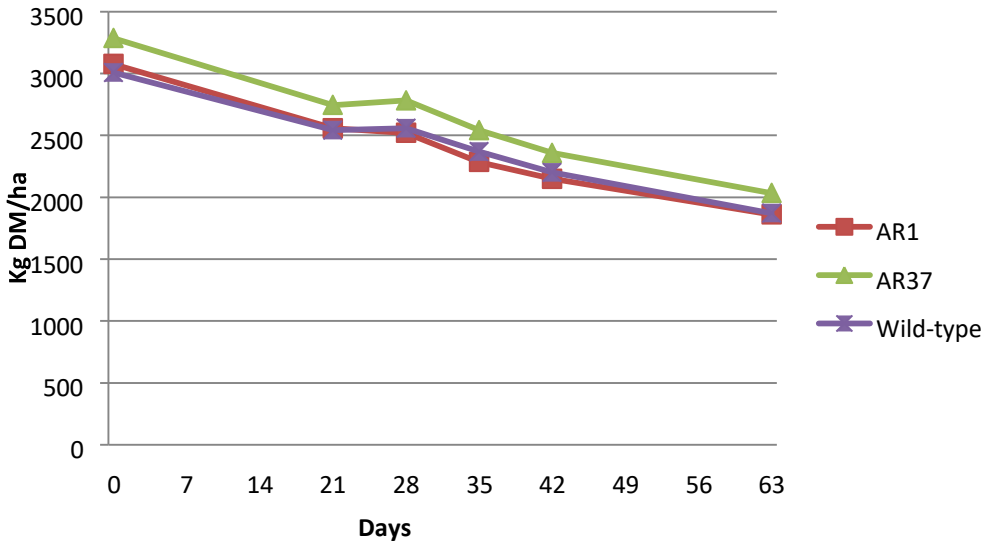
Once again the lack of significant endophyte treatment effects on pasture mass and quality indicators would suggest that each group of animals were exposed to similar conditions and that any endophyte mediated effects on animal performance and physiology might be related to some other factor such as alkaloids.

4.1.1.3 Agronomic performance – 2013

4.1.1.3.1 Cross bred weaners

Although there was a consistent trend for AR37 endophyte treatment to have slightly higher pasture mass than either AR1 or WT, it was not significant and is possibly due to a slightly better pasture mass being present at the beginning of this grazing period. In this instance the absence of pasture mass data just prior to the beginning of this trial, combined with the short period of grazing prevented the use of time 0 as a covariate which may have eliminated this trend. There was no significant interaction of time and endophyte. During this period pasture growth rate in the same area was not significantly affected by endophyte (P=0.863) with AR1, WT and AR37 growing at 13.75, 13.08 and 12.87 kg DM/ha/day respectively.

Figure 4.15 Mean pasture mass available in the AR1, AR37 and Wild-type endophyte treatments grazed by cross bred weaners in March to April 2013. P=0.293, LSD=363.5



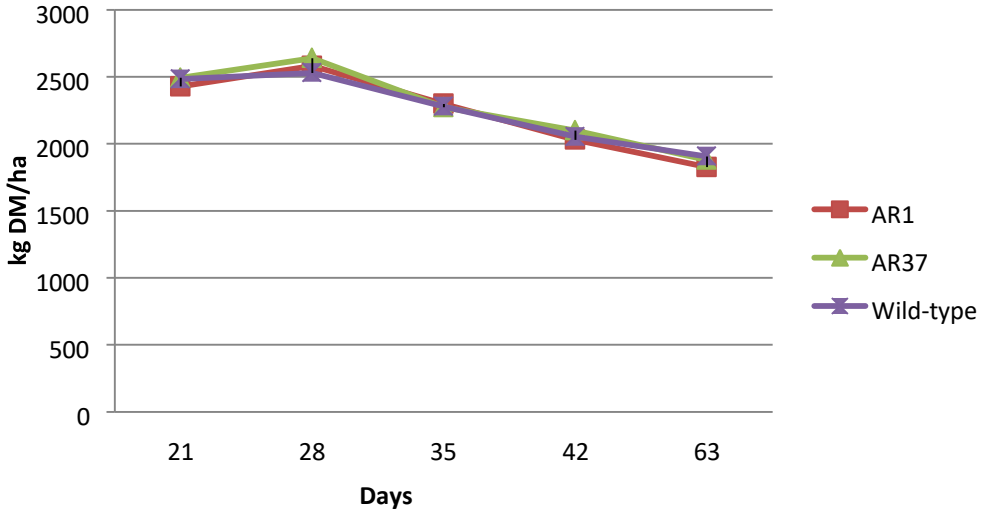
There were no significant endophyte or time effects on the proportion of green vs dead material in the treatments and there was a high proportion of dead material (>95%) for the duration of this period.

4.1.1.3.2 Merino ewes

There were no significant differences between endophyte treatments in the area grazed by Merino ewes for pasture mass availability and pasture growth rate (P=0.952). Pasture mass did significantly decline over time however there was no

significant interaction of time and endophyte treatment. During this period the AR1, WT and AR37 grew at 13.9, 13.33 and 13.25 kg DM/ha/day respectively.

Figure 4.16 Mean pasture mass available in the AR1, AR37 and Wild-type endophyte treatments grazed by merino ewes in March to April 2013. P=0.86, LSD=274.8



There were no significant endophyte or time effects on the proportion of green vs dead material in the treatments and there was a high proportion of dead material (>95%) for the duration of this period.

Discussion – Agronomic performance for 2013 (Merino and Cross bred sheep)

The summer-autumn period of 2013 was hot and dry with low pasture growth rates and a high level of dry dead feed comprising the total pasture mass. A combination of irrigation and sporadic rainfall contributed to the stabilisation and slight improvement in pasture mass at day 28 for both livestock trials however the hot and dry conditions prevailed through into April and drove further decline in pasture mass.

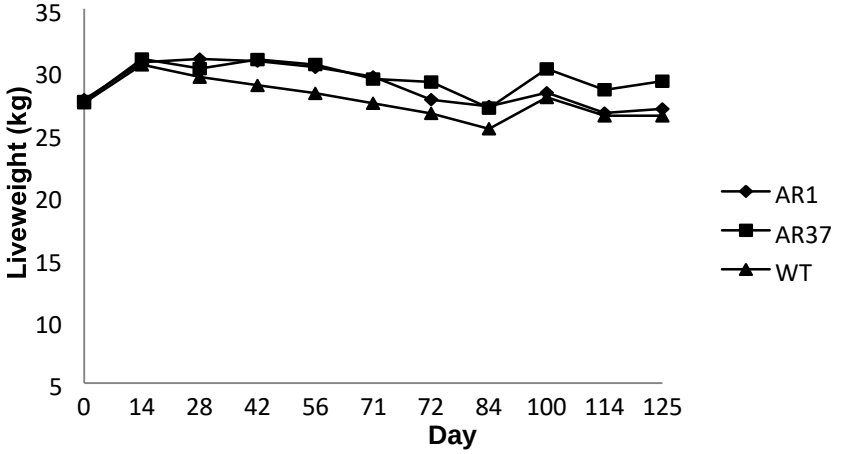
Conclusion – Agronomic performance for 2013 (Merino and Cross bred sheep)

The absence of significant endophyte treatment effects for the agronomic parameters measured suggests that all livestock were exposed to similar feed conditions.

4.1.2 Animal performance in grazing trials

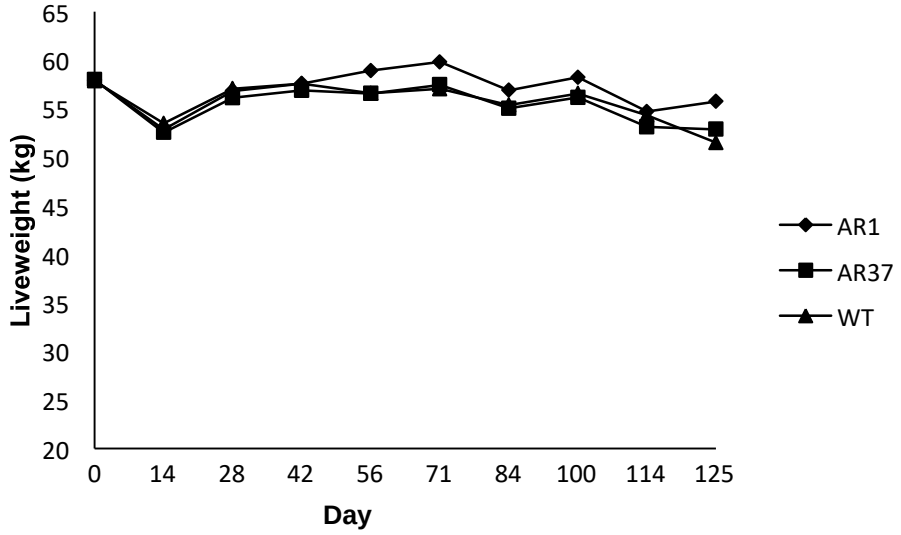
4.1.2.1 Merinos ewe weaners and Crossbreds (February – June 2011)

Figure 4.17 Mean liveweight over time for Merino ewe weaners gazing WT, AR1 and AR37 perennial ryegrass. Pooled sed for Endophyte x Day = 0.83 kg.



There were significant effects of day and endophyte x day for liveweight in the Merino ewe weaners (P<0.001 for both), such that liveweight decreased over time and was significantly lower in the WT group over time (Figure 4.17). Overall, mean liveweight was lower for WT compared with AR1 and AR37 (27.70, 28.79 and 29.42, respectively, P=0.006). There were significant effects of day and endophyte x day (P<0.001, for both) for the Crossbreds, such that liveweight decreased over time and was variable for treatment (Figure 4.18). Overall, endophyte strain did not affect liveweight for Crossbreds (P=0.433).

Figure 4.18 Mean liveweight over time for Crossbred ewes grazing WT, AR1 and AR37 perennial ryegrass. Pooled sed for Endophyte x Day = 1.53.



There were significant effects of day and endophyte x day for rectal temperature in the Merino ewe weaners (P<0.001, P=0.001, respectively), such that rectal temperature decreased over time (Figure 4.19). This decrease was most pronounced in the AR37 and AR1 groups. Overall, mean rectal temperature was higher for WT compared with AR1 and AR37 in the Merinos (40.10, 39.93 and

39.97, respectively, P=0.002). There was a significant effect of day (P<0.001) for respiration rate, such that respiration rate decreased over time (Figure 4.20). There was no endophyte x day effect (P=0.324), for Merinos. Overall, mean respiration rate was higher for WT and AR37 compared with AR1 (107.6, 108.2 and 96.3, respectively, P=0.001), for Merinos.

Figure 4.19 Mean rectal temperature over time for Merino ewe weaners grazing WT, AR1 and AR37 perennial ryegrass. Pooled sed for Endophyte x Day = 0.08.

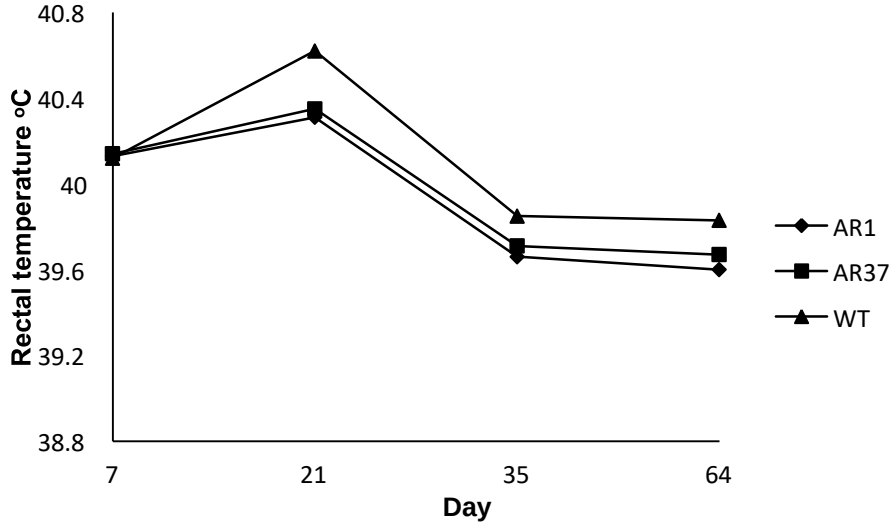


Figure 4.20 Mean respiration rate over time for Merino ewe weaners grazing WT, AR1 and AR37 perennial ryegrass. Pooled sed for Endophyte x Day = 6.2.

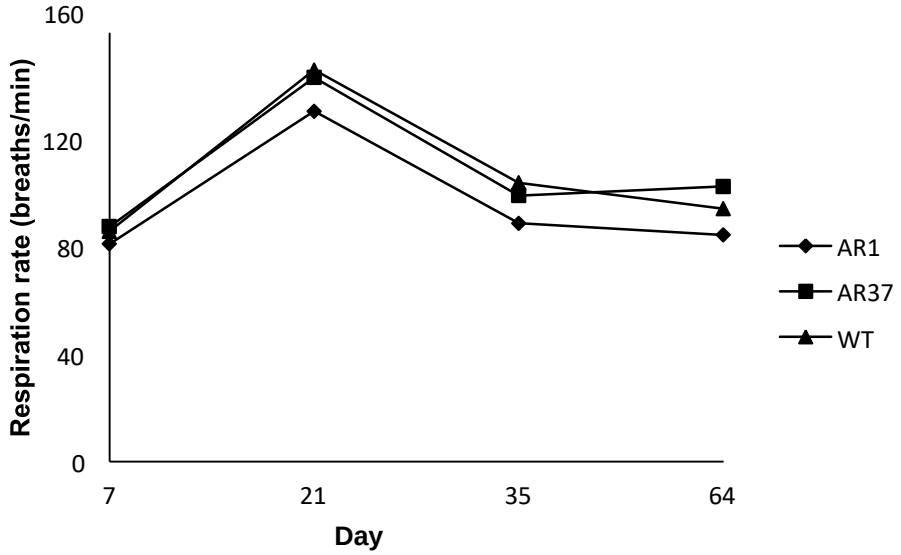


Figure 4.21 Mean rectal temperature over time for Crossbred ewes grazing WT, AR1 and AR37 perennial ryegrass. Pooled sed for Endophyte x Day = 0.08.

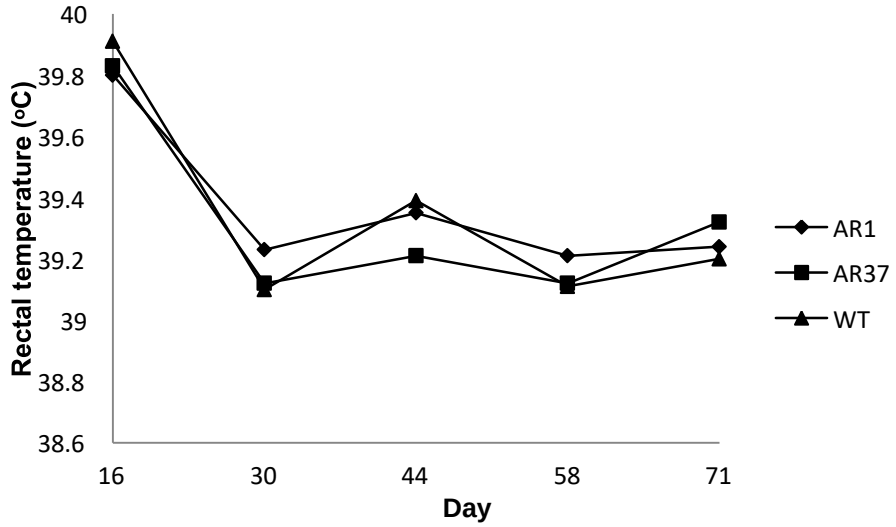
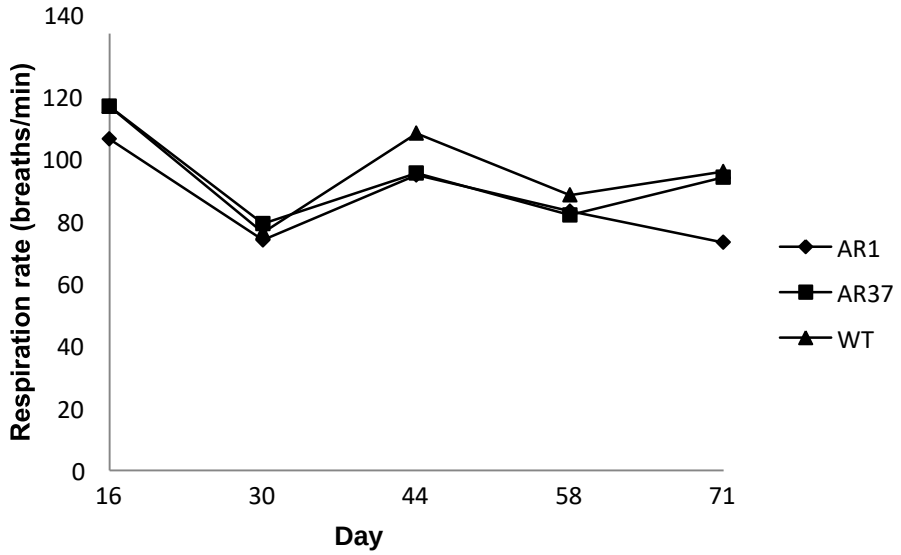


Figure 4.22 Mean respiration rate over time for Crossbred ewes grazing WT, AR1 and AR37 perennial ryegrass. Pooled sed for Endophyte x Day = 6.3.



There were significant effects of day and endophyte x day for rectal temperature in the Crossbreds (P<0.001 and 0.003, respectively), such that rectal temperature decreased over time, but was variable for treatment groups. Overall, there was no endophyte effect (P=0.755). There was a significant endophyte effect on respiration rate, such that the WT and AR37 groups had higher respiration rate compared with the AR1 group (97, 93 and 86, respectively). There were significant day and day x endophyte effects (P<0.001 and 0.028, respectively) for respiration rate, such that respiration rate was variable over time for all treatment groups.

Figure 4.23 Mean water intake (per paddock) over time for Merino ewe weaners grazing WT, AR1 and AR37 perennial ryegrass. Pooled sed for Endophyte x Day = 15.2.

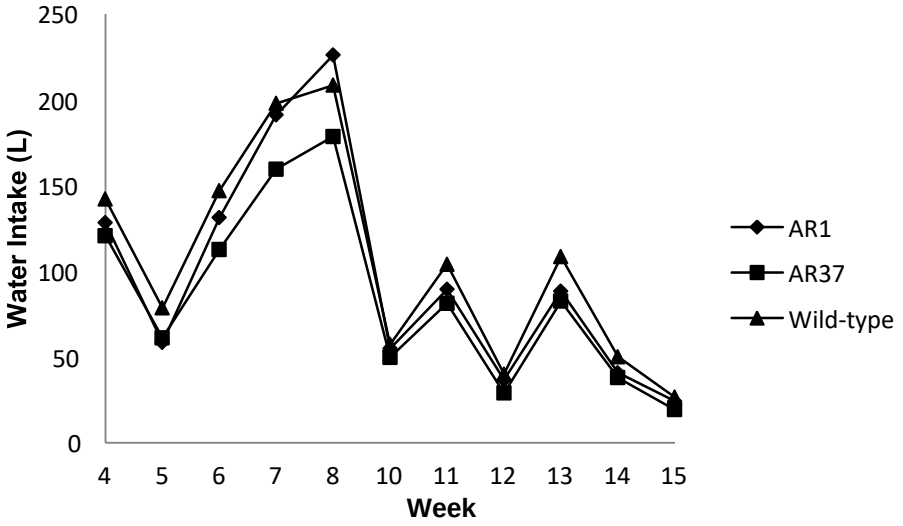
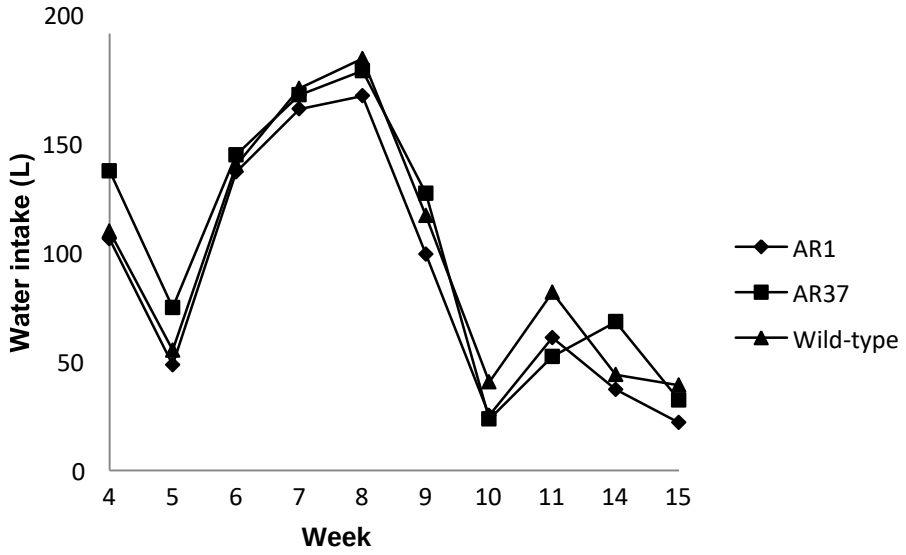


Figure 4.24 Mean water intake (per paddock) over time for Crossbred ewes grazing WT, AR1 and AR37 perennial ryegrass. Pooled sed for Endophyte x Day = 26.7.



Water intake did vary over time for the Merino ewe weaners (Figure 4.23) and Crossbred ewes (Figure 4.24), presumably in line with ambient temperature and humidity (data not presented). However there was no endophyte x time interaction (P=0.316 and P=0.857) for the Merino ewe weaners and Crossbred ewes respectively. There was no endophyte effect on water intake for either stock type (Table 4.6). There was also no effect of endophyte on wool growth rate (Table 4.6) and no interaction between endophyte and time (P=0.458).

Table 4.6 Mean water intake (per paddock) for Merino ewe weaners and Crossbreds and wool growth for Merino ewe weaners grazing WT, AR1 and AR37 perennial ryegrass.

Endophyte	AR1	AR37	WT	SED	P-value
Water Intake Merinos (L)	97.1	84.6	105.4	10.7	0.229
Water intake Crossbred (L)	87.1	101.3	98.8	21.0	0.778
Wool growth (g/cm^2)	0.072	0.068	0.068	0.003	0.469

Data on the incidence of staggers are presented in Table 4.7. As expected no staggers was observed in either Crossbred ewe or Merino ewe weaner sheep grazing AR1. The proportion of Cross bred ewes exhibiting staggers was generally lower in AR37 compared to WT although eventually did achieve similar levels at the end of the trial. The proportion of Merino ewe weaners exhibiting staggers was initially lower in AR37 compared to WT however quickly achieved and maintained the same level. Comparisons between breeds in this instance are not possible due to the sheep breeds location in different trials with different management.

Table 4.7 Number of Crossbred ewes and Merino ewes in each treatment group with staggers scores greater than or equal to 1

Day	Crossbred Ewes			Day	Merino Ewes		
	WT	AR1	AR37		WT	AR1	AR37
16	0/30	0/30	0/30	7	0/45	0/45	0/45
35	0/30	0/30	0/30	35	30/45	0/45	1/45
44	30/30	0/30	4/30	44	45/45	0/45	45/45
54	24/30	0/30	13/30	54	45/45	0/45	45/45
72	27/30	0/30	11/30	72*			
102	29/30	0/30	28/30	102*			

*, Merino ewes were removed from grazing treatments intermittently during this period because of severity of staggers. Therefore, scores recorded on these days have not been included.

Table 4.8 Mean dag scores for Crossbred ewes and Merino ewe weaners in each treatment group.

Day	Crossbred Ewes			Merino Ewes		
	WT	AR1	AR37	WT	AR1	AR37
28	1.8	1.0	1.3	1.8	1.3	1.3
42	1.9	1.0	1.3	1.9	1.2	1.3
56	2.2	1.0	1.3	2.1	1.3	1.3
71	2.5	1.0	1.5	2.5	2.0	1.5
125	2.1	1.0	1.4	2.0	1.4	1.3

Dag scores recorded at baseline (day 0) for the Merinos and Crossbreds were all one (1-5 scoring table). There were no endophyte or endophyte x day effects for dag score in the Crossbred ewes (P=0.293 and P=0.487, respectively). There was a significant day effect for the Crossbreds (P=0.033), such that dag score increased over time. For the Merinos, there were significant endophyte and day effects (P<0.001, for both), such that dag score increased over time, especially in the WT treatment. There was no endophyte x day interaction (P=0.107).

Discussion

Production response

Overall, Merino ewe weaner liveweight was lower in the WT group compared to the AR1 and AR37 groups. All sheep lost weight over time however this was most significant in the WT group. Overall, liveweight was not significantly altered by endophyte type in the Crossbreds however the AR1 treatment did tend to have slightly higher weights than the AR37 and WT treatments.

Previous studies undertaken in New Zealand have found that sheep grazing WT infected pasture versus nil pasture had depressed growth rates (Fletcher and Barrell 1984; Fletcher *et al.*, 1996; Fletcher *et al.*, 1999). Previous grazing trials have been undertaken in New Zealand using Coopworth sheep, comparing AR1, WT, nil and AR37 pastures (Fletcher and Sutherland 2009a). These studies were undertaken on a short-term and long-term basis and found liveweight was lower in the WT groups, however, unless staggers score increased above 2 in the AR37 group, liveweight did not change (Fletcher and Sutherland 2009b). Liveweight was also significantly higher in the AR1 group compared to the WT group, but not different from AR37 (Fletcher and Sutherland 2009b), this finding is echoed in the current study.

In the current experiment, staggers was most severe in the WT treatment compared to the AR37 treatment for both breeds. Both breeds were also affected by staggers in the AR37 treatment, initially at a lower incidence than WT, however by the conclusion of the grazing period, number of ewes with staggers in the WT treatment was similar to the AR37 treatment. Severe staggers is associated with reduced growth rates due to an animal's inability to graze (Fletcher and Sutherland 2009b). In the current study it is not surprising that liveweight was lowest in the WT treatment (Merinos) due to the early onset and prolonged nature of staggers. In the Merinos, staggers occurred in the AR37 treatment nine days after staggers was detected in the WT treatment.

In the Crossbreds it took more time for the AR37 group to be affected compared to the WT group. Liveweight may not have been depressed in the WT and AR37 treatments (Crossbreds) due to the mature age and large size of the Crossbred ewes buffering changes in intake and reducing short term fluctuation in growth rate.

Water intake was not affected by endophyte strain in either breed of sheep. Water intake fluctuated over time, in accordance with fluctuations in climatic conditions (in particular ambient temperature, data not presented). No other studies have measured water intake in outdoor grazing studies. Indoor studies have found variable responses with depressions in water intake when feeding

lambs endophyte infected tall fescue (TF) feed (De Lorme *et al.*, 2007) and no response in steers fed TF hay (Matthews *et al.*, 2005). Unpublished work has found an increase in water intake in sheep fed infected PRG seed (Henry *et al.*,). Variability in climatic conditions may have masked any differences in water intake. Investigation into potential effects on water intake is valid, given that it is thought many sheep deaths are directly related to sheep falling into dams due to misadventure (Reed 2005).

Dag scores were significantly higher in the WT treatment compared to the AR1 and AR37 treatments in the Merinos. Moreover, dag score increased over time in the WT treatment. In the Crossbreds dag score increased over time for all treatment groups, however, did not change due to endophyte type. Dag score has previously been found to increase in sheep grazing WT versus nil endophyte PRG pasture (Pownall *et al.*, 1993; Fletcher *et al.*, 1999). Previous grazing studies which compared WT, AR1, AR37 and nil found dag scores to be similar in the AR1, AR37 and nil treatments while the WT treatment had a significantly higher dag score (Fletcher and Sutherland 2009b). The alkaloid thought to be associated with increased dagging in sheep is lolitrem B (McLeay *et al.*, 1999), lolitrem B is not present in AR37, however, AR37 does produce a similar alkaloid which causes tremors and staggers. It has been suggested that ergovaline may have a role in increasing faecal water and therefore dagging (Fletcher *et al.*, 1999). Therefore, it may be likely that a combination of alkaloids is responsible for increasing faecal water in sheep and therefore, it is likely that dags will not be observed in AR37 pastures. Increased dagging in sheep can be detrimental to animal health causing an increase in fly strike (Fletcher *et al.*, 1999) and deaths indirectly attributed to PRGT.

Wool growth was only measured in the Merinos and was not affected by endophyte strain. Very few studies have measured wool growth in sheep grazing infected PRG pastures. Anecdotal information suggests that livestock surviving outbreaks of PRGT have poor wool growth and strength (Cummins 2005). A study conducted in New Zealand found wool growth to decrease in sheep grazing infected pasture versus sheep on non-infected pasture (Eerens *et al.*, 1998). A longer period of time may be needed to ascertain this.

Physiological response

Overall, rectal temperature and respiration rate increased in the WT treatment compared to the AR37 and AR1 treatments in Merinos. Rectal temperature was not affected in the Crossbreds, however, respiration rate increased significantly in the WT treatment compared to the AR1 and AR37 treatments. AR37 and AR1 do not produce ergovaline, the causal alkaloid in heat stress, therefore, it is not surprising that heat load did not increase significantly in these endophyte strains. Previous grazing studies comparing AR37, AR1, WT and nil endophyte strains are in agreement with these findings (Fletcher and Sutherland 2009b).

Conclusion

Although the two breeds were run in different trials, the results from the current study suggest that the Merino ewe weaners were less able to cope with PRG

alkaloids as compared to the Crossbred ewes. This may have been due to the younger age of the Merino ewes and/or possibly an increased sensitivity of Merino's to PRG alkaloids. It is also possible that there were differences between the trial sites which at this stage cannot be ruled out. Merinos had decreased liveweight, increased dag score and increased rectal temperature and respiration rate in the WT treatment compared to the AR37 and AR1 treatments. Crossbreds had some changes in liveweight such that the WT and AR37 treatments were lower over time compared to the AR1 treatment and respiration rate increased significantly in the WT treatment. Staggers occurred in both breeds and occurred quickly and most severely in the Merinos compared to the Crossbreds. Staggers on the WT treatment occurred first and most severely, however, by the conclusion of the grazing study staggers in the AR37 treatment was as severe as in the WT treatment.

4.1.2.2 Lambing and lactation in crossbred ewes (August 2011-December 2011)

Sheep were removed from the experiment in June 2011 and were re-introduced in August 2011 for lambing.

There were significant effects of endophyte and day and endophyte x day for liveweight (P=0.025, P<0.001 and P<0.001, respectively). Overall, mean liveweight was higher for the AR37 treatment compared to both the AR1 and WT treatments, which were similar (58.7, 60.0 and 64.3 for WT, AR1 and AR37, sed 2.2). Liveweight in the AR1 and WT treatments decreased over time, but was maintained in the AR37 treatment (Figure 4.25).

Figure 4.25 Mean liveweight over time for Crossbreds gazing WT, AR1 and AR37 perennial ryegrass. Pooled sed for Endophyte x Day = 2.5 kg.

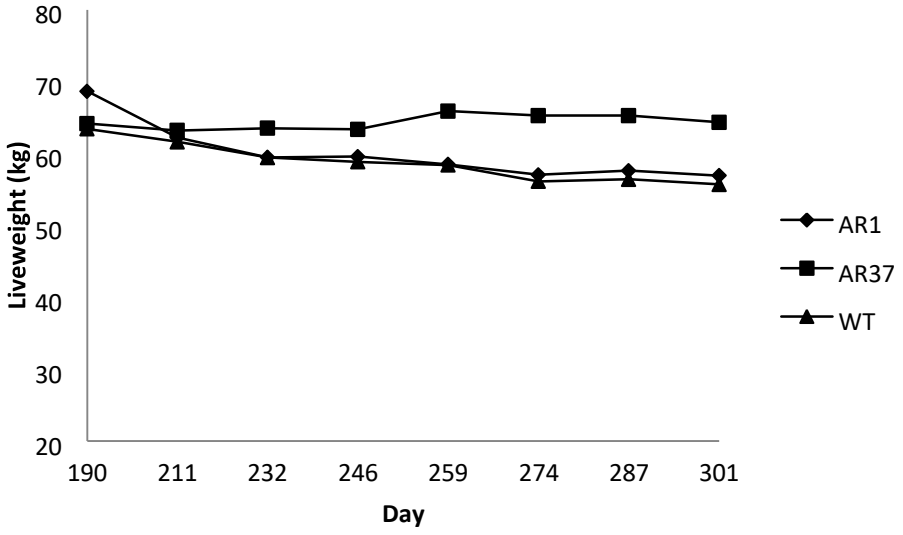
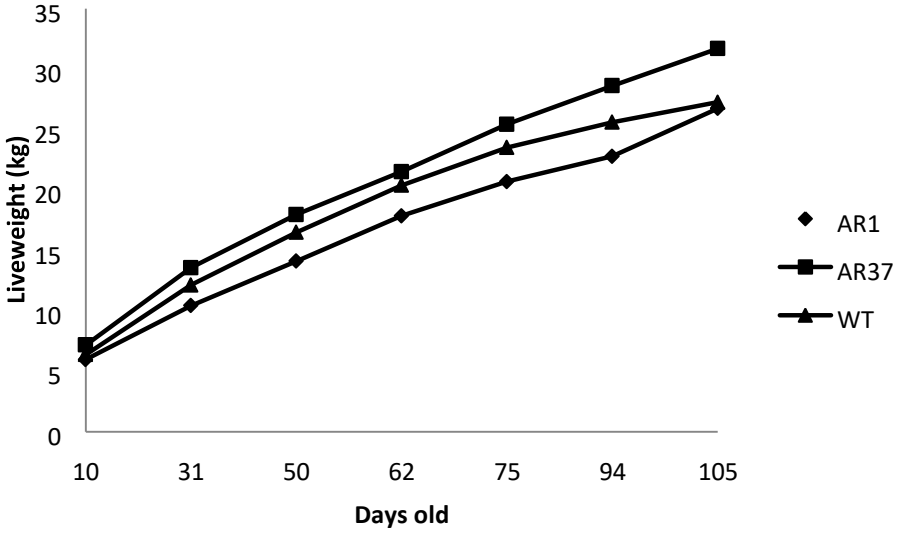
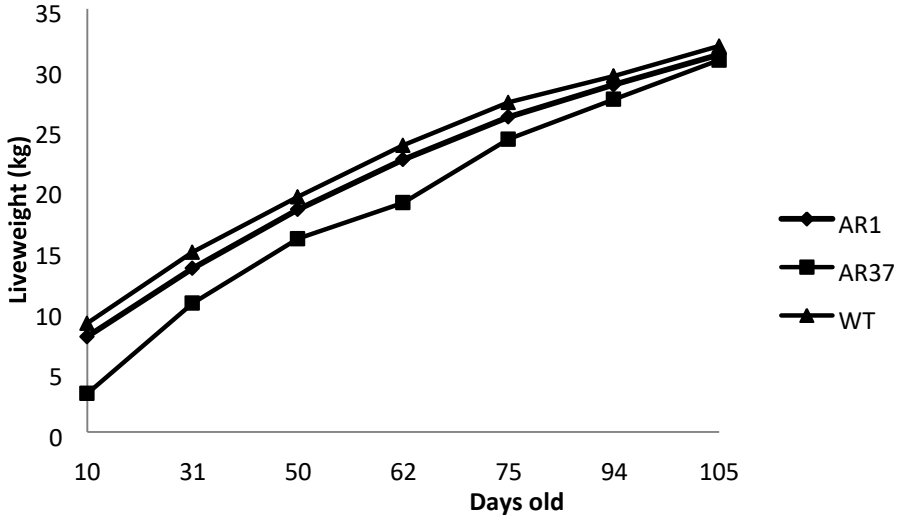


Figure 4.26 Mean liveweight over time for female Crossbred lambs born on and grazing WT, AR1 and AR37 perennial ryegrass. Pooled sed for Endophyte x day = 1.9.



There was a significant effect of endophyte on female lamb liveweight such that the AR37 and WT groups had higher mean liveweight compared to the AR1 group (20.04, 19.30 and 16.88, respectively; P<0.001; sed 0.90; Figure 4.26). There was a significant day effect (P<0.001) such that liveweight increased over time. There was no endophyte x day effect (P=0.636).

Figure 4.27 Mean liveweight over time for male Crossbred lambs born on and grazing WT, AR1 and AR37 perennial ryegrass. Pooled sed for Endophyte x day = 3.8.



There were no significant endophyte or endophyte x day effects for male lambs (P=0.733 and P=0.906, respectively; Figure 4.27). There was a significant day effect such that liveweight increased over time (P<0.001).

Table 4.9 Mean water intake (per paddock) for Crossbred ewes and lambs grazing WT, AR1 and AR37 perennial ryegrass.

Endophyte	AR1	AR37	WT	sed	P-value
Water Intake (L)	230.8	197.5	230.6	27.3	0.536

There was a significant week effect for water intake such that water intake increased over time (P<0.001), likely related to growth of lambs and the trial period extending from winter into the warmer spring months (Figure 3.1). There was no week x endophyte effect for water intake (P=0.148).

Table 4.10 Mean rectal temperature and respiration rate for all Crossbred ewes over a 43 day period between August and December 2011. Ewes were grazing WT, AR1 and AR37 perennial ryegrass.

Endophyte	AR1	AR37	WT	sed	P-value
Rectal Temp (^{0}C)	39.30	39.22	39.24	0.10	0.747
Resp.Rate (breaths.min^{-1})	93.5	112.4	112.9	5.1	<0.001

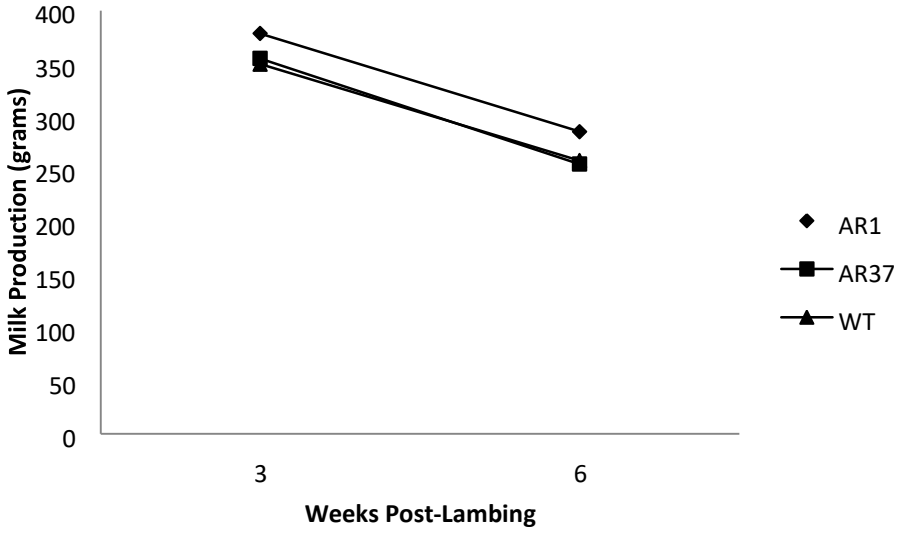
There were no day or endophyte x day effects for rectal temperature (P=0.310 and P=0.352, respectively). There was a significant endophyte effect for respiration rate, such that respiration rate was higher for the AR37 and WT groups compared with AR1 (Table 4.10). There was a significant day and endophyte x day effect (P<0.001 and P=0.010, respectively) for respiration rate such that respiration rate increased over time and the AR1 group was lower over time compared with AR37 and WT.
Dag score remained at one for all sheep between August and December.

Table 4.11 Number of Crossbred ewes in each treatment group with staggers scores greater than or equal to 1.

Day	AR1	AR37	WT
270	0/24	0/24	2/24
274	0/24	0/24	0/24
287	0/24	0/24	0/24

Data on the incidence of staggers are presented in Table 4.11. For Crossbred ewes staggers occurred only in the WT grazing treatment. As expected no staggers was observed in sheep grazing AR1. Two lambs were given a staggers score of two on day 270 (their mother was given a score of 4 on the same day).

Figure 4.27 Four hour milk production in Crossbred ewes grazing AR1, AR37 and WT perennial ryegrass. Pooled sed = 55.



There was no effect of endophyte on milk production (P=0.643). Milk production decreased over time (P<0.001) for all endophyte groups. There was no endophyte x day effect (P=0.992).

Discussion

The current study was undertaken over winter and spring. Ewe liveweight was significantly lower in the WT treatment compared to the AR37 treatment, but was similar to the AR1 treatment. These results suggest even though staggers was not detected liveweight can still decrease. Liveweight was mildly affected in the previous study. The same ewes were placed back onto the pasture for the current study, possibly explaining this difference. Alkaloid concentration was presumably low at this time of year (Reed *et al* 2011b) however, effects were still observed, possibly due to these animals being more sensitive to alkaloid intake due to their previous exposure. A previous study which compared AR37, WT, AR1 and nil endophyte pasture found WT growth rates to be significantly worse compared to all other treatments in sheep grazing during winter (Fletcher and Sutherland 2009b), suggesting that even when alkaloid concentration is low in pasture, animal production can still decrease.

Lamb liveweight was analysed for female and male lambs separately. There was no effect of endophyte strain on male lamb liveweight due to the large variability observed. Female lamb liveweight was significantly higher in the WT and AR37 groups compared with the AR1 treatment. Performance of suckling lambs has been investigated previously in New Zealand over two seasons, comparing PRG endophyte-infected versus non-infected pasture (Watson *et al.*, 1999). This study found no difference in lamb liveweight in the first year, however, in the second year the lambs suckling ewes on infected pasture had significantly lower liveweight compared to those on non-infected pastures (Watson *et al.*, 1999), however, lamb numbers were small leading to high variability. Authors of this study suggested the difference was due to lower milk production in ewes grazing infected pasture. In the current study, milk production was numerically lower in the AR37 and WT pastures, however this was not significant. Milk production decreased over time as expected. The lamb liveweight results suggest that milk production in the WT and AR37 treatments was in plentiful supply

as is indicated in the results. No other studies have measured milk production in ewes grazing endophyte infected pastures.

Water intake was not affected in the current study. There was high variability observed over time and therefore no strong effects were observed. As discussed in the previous study, water intake is often variable with findings from previous studies varying from decreased water intake (De Lorme *et al.*, 2007), increased water intake (Henry *et al.*, unpublished) and no change (Matthews *et al.*, 2005).

Dag score did not change in the current study. The reason for this is likely due to the low concentration of alkaloids found in pasture during winter and spring (Reed *et al* 2011b). Dag score was not affected in Crossbreds as discussed in the previous section, therefore along with low alkaloid levels, this finding is not surprising. Additionally, dag scores are often associated with considerable individual and season variation (Fletcher *et al.*, 1999).

Physiological response

Rectal temperature was not affected by endophyte strain, as observed in the previous study, suggesting ergovaline levels were not high enough in the WT treatment to cause an increase. Respiration rate increased in the WT and AR37 treatments compared to the AR1 treatment. This indicates heat load increased, causing an increase in respiration rate. It is surprising that this was observed in the AR37 treatment because AR37 does not contain the ergot alkaloids. This may indicate that other classes of alkaloid may be present and causing additional effects, however, this was not found in the previous grazing study. Previous studies comparing AR37, WT, AR1 and nil endophyte pasture found no effect of grazing AR37 on respiration rate (Fletcher and Sutherland 2009b).

Conclusion

Results from the current study indicate that when sheep lamb on endophyte infected pasture during winter and spring there can be some changes in liveweight, however, these changes were probably carried over from the previous grazing season as the same animals were used. Lamb liveweights were not depressed in the AR37 and WT treatments as may have been expected and this is likely due to the low levels of alkaloid presumable in the pasture, and low sheep numbers. Moreover, milk production was not affected. Sheep increased respiration rate in response to grazing WT and AR37 pasture, possibly indicating that other alkaloids may be causing a heat stress response in sheep in the AR37 pasture due to there being no ergovaline present. The results show that during winter and spring lactation and lamb liveweight were not affected, however, further investigation using more sheep during summer and autumn is warranted to determine if milk production and ewe and lamb animal production are altered.

4.1.3 2011-2012 Grazing study

4.1.3.1 Merino ewes October 2011 – March 2012

Liveweight is presented in Figure 4.28. There was no difference between endophyte treatments overall (50.0, 50.2 and 49.1 for AR1, AR37 and WT; P=0.168; sed 0.64). Liveweight did vary over time, likely as a result of changes in feed availability and feed quality (Figure 4.11, Table 4.14). There was no endophyte x day interaction (P=0.143).

Figure 4.28 Mean liveweight over time for Merino ewes grazing WT, AR1 and AR37 perennial ryegrass. Pooled sed for Endophyte x day = 0.74

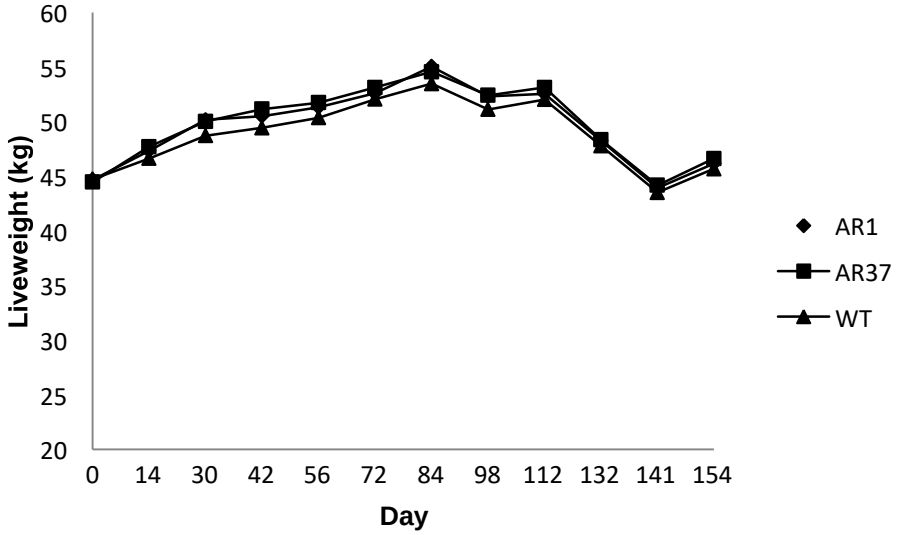


Table 4.12 Mean water intake (per paddock) and wool growth for Merino ewes grazing AR1, AR37 and WT perennial ryegrass.

Endophyte	AR1	AR37	WT	sed	P-value
Water intake (L)	209.3	221.2	224.3	16.0	0.638
Wool growth (g/cm^2)	0.082	0.080	0.088	0.006	0.451

There was no endophyte or endophyte x day effect for water intake (P=0.638 and P=0.934, respectively). There was a significant day effect such that water intake was variable over time (P<0.001). Wool growth was not affected by endophyte strain (Table 4.12).

Table 4.13 Number of Merino ewes in each treatment group with staggers scores greater than or equal to 1.

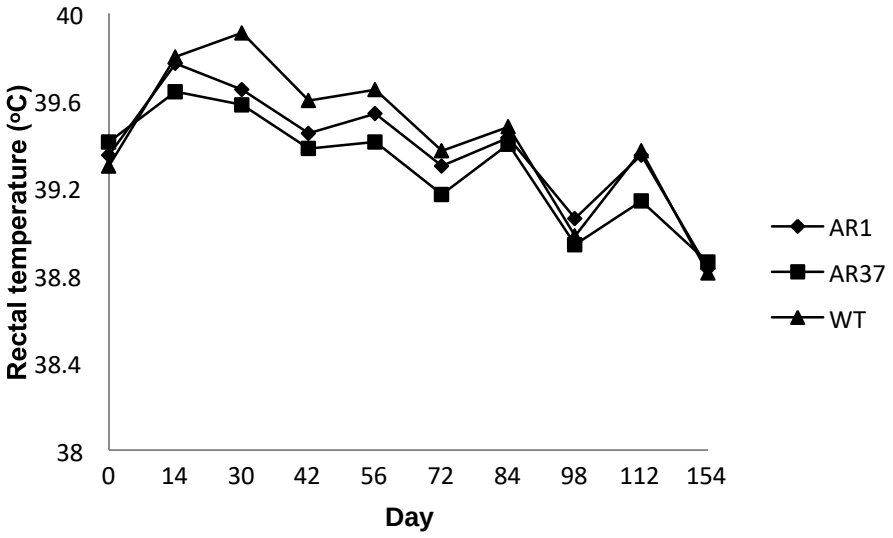
Day	AR1	AR37	WT
22	0/30	0/30	0/30
42	0/30	0/30	0/30
56	0/30	0/30	1/30
72	0/30	0/30	0/30
84	0/30	2/30	0/30
98	0/30	2/30	0/30
112	0/30	3/30	0/30
145	0/30	0/30	0/30

Data on the incidence of staggers are presented in Table 4.13. For Merino ewes staggers occurred earliest in the WT grazing treatment compared with the AR37 grazing treatment, however, the AR37 treatment persisted for longer. As expected no staggers were observed in sheep grazing AR1. Dag score did not vary over the treatment period for endophyte type (Table 4.14).

Table 4.14 Mean dag scores for Merino ewes grazing AR1, AR37 and WT perennial ryegrass.

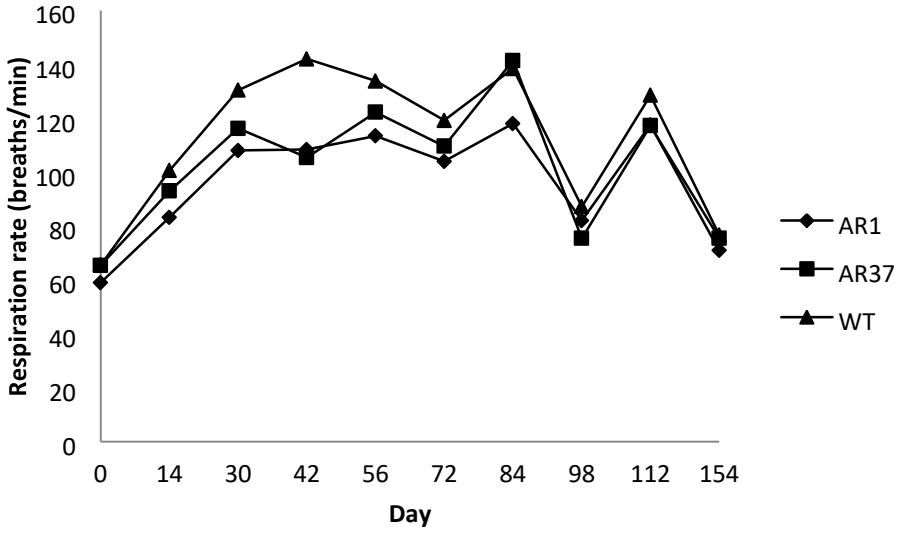
Day	AR1	AR37	WT
42	1.0	1.03	1.13
56	1.0	1.0	1.0
72	1.0	1.0	1.0
84	1.0	1.0	1.0
154	1.0	1.05	1.1

Figure 4.29 Mean rectal temperature over time for Merino ewes grazing AR1, AR37 and WT perennial ryegrass. Pooled sed for Endophyte x day = 0.09.



There was a significant endophyte effect for rectal temperature (P=0.006), such that WT treatment had higher rectal temperature compared with AR37 and AR1 (39.44, 39.28 and 39.38, respectively; sed 0.05; Figure 4.29). There was a significant day and endophyte x day interaction (P<0.001 and P=0.001, respectively; Figure 4.29), such that rectal temperature decreased over time but was also variable within treatment groups.

Figure 4.30 Mean respiration rate over time for Merino ewes grazing AR1, AR37 and WT perennial ryegrass. Pooled sed for Endophyte x day = 8.6.



There was a significant endophyte effect for respiration rate (P<0.001), such that the WT treatment had higher respiration rate compared with AR37 and AR1 (117.4, 106.2 and 100.6, respectively; sed 4.47). There was a significant day and endophyte x day interaction (P<0.001, for both; Figure 4.30), such that respiration rate was variable over time and variable within treatment groups.

Discussion

A new group of Merinos were used for this experiment. Liveweight, water intake, wool growth and dag score were not altered by endophyte strain. It is likely that the level of alkaloid found in the pasture was low, explaining the dulled effects. Staggers were observed in WT and AR37 treatments. Staggers occurred in the WT treatment first but was more prevalent in the AR37 treatment. Staggers scores were mild with no sheep removed from the treatment site, once again indicating that alkaloid levels would have been low.

Rectal temperature and respiration rate increased significantly in the WT treatment compared to the AR1 and AR37 treatments and they were both variable over time, influenced by climatic conditions. These results suggest that the level of ergovaline was high enough to increase heat load in sheep. As discussed in previous section, increases in rectal temperature and respiration rate have been found in sheep grazing WT pasture compared to AR1 and AR37 pastures (Fletcher and Sutherland 2009b), due to the presence of ergovaline in WT.

Conclusion

The results from the current study suggest that alkaloid level was low in the pasture with very few changes in production parameters measured. Mild staggers was observed in the WT and AR37 groups and staggers was most prevalent in the AR37 treatment. Heat load in sheep increased, resulting in increased rectal temperature and respiration rate, however, these effects did not result in depressed animal production.

4.1.3.2 Crossbred ewes March 2012-April 2012

Crossbred ewes were returned to the pasture after lamb weaning in December. Liveweight was not affected by endophyte (P=0.499) or endophyte x day (P=0.811). Liveweight increased over time (P=0.001).

Figure 4.31 Mean liveweight over time in Crossbred ewes grazing AR1, AR37 and WT perennial ryegrass. Pooled sed for Endophyte x day = 1.9.

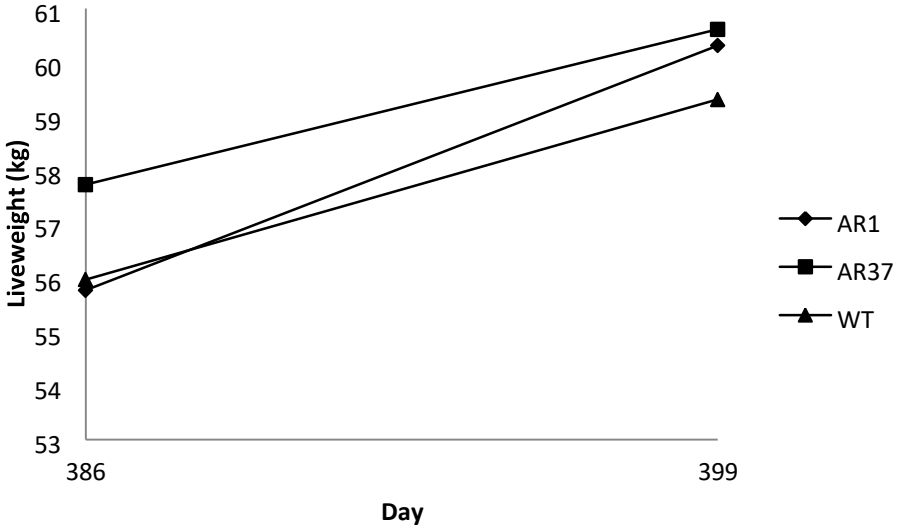


Table 4.15 Mean water intake (per paddock), rectal temperature, respiration rate and dag score measured on day 399 of grazing for Crossbred ewes grazing AR1, AR37 and WT perennial ryegrass.

Endophyte	AR1	AR37	WT	sed	P-value
Water intake (L)	50.7	81.6	48.8	33.6	0.580
Rectal Temp (^{0}C)	38.96	38.98	38.99	0.09	0.934
Resp.Rate (breaths.min^{-1})	77.6	88.3	100.0	10.1	0.098
Dag score	1.33	1.38	1.66	0.17	0.139

Water intake was not affected by endophyte, day or endophyte x day (P=0.580, P=0.631 and P=0.220, respectively). Rectal temperature was not altered due to endophyte. There was a strong tendency for respiration rate to increase in the WT group compared to the AR1 group (Table 4.15). There was a weak tendency for dag score to increase in the WT group compared with AR1 and AR37 (Table 4.15). Staggers scoring was undertaken on day 399 and no staggers or tremors were detected in any groups.

Discussion

Crossbred ewes were returned to the pasture for a short amount of time due to decreased availability of pasture. During this time no major effects were observed on liveweight, water intake, or rectal temperature. Dag score tended to increase in the WT treatment, as finding reported in previous studies in this report and elsewhere (Fletcher *et al.*, 1999). There was a strong tendency for respiration rate to increase in the WT treatment compared to the AR1 and AR37 treatments. These results reflect what was observed in the previous grazing experiment in Merinos.

4.1.4 2013 Grazing study

4.1.4.1 Merino ewes and crossbreds weaners January 2013 – April 2013

Overall, liveweight in Merino ewes was lowest in the WT treatment compared with AR37. The WT and AR1 treatments were similar (43.25, 43.67 and 44.90 for WT, AR1 and AR37, respectively; P=0.052; sed 0.67). There was a significant day effect (P<0.001) such that liveweight increased over time indicating that feed availability was adequate (Figure 4.16) and quality not restrictive. There was a significant endophyte x day effect (P=0.041; Figure 4.32) such that the AR37 group maintained the highest liveweight over time.

Figure 4.32 Mean liveweight over time in Merino ewes grazing AR1, AR37 and WT perennial ryegrass. Pooled sed for Endophyte x day = 0.77.

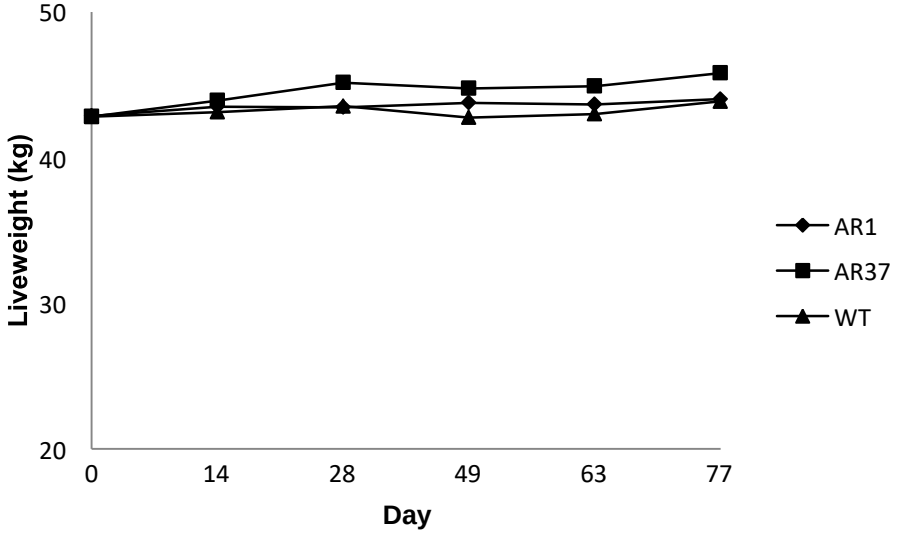
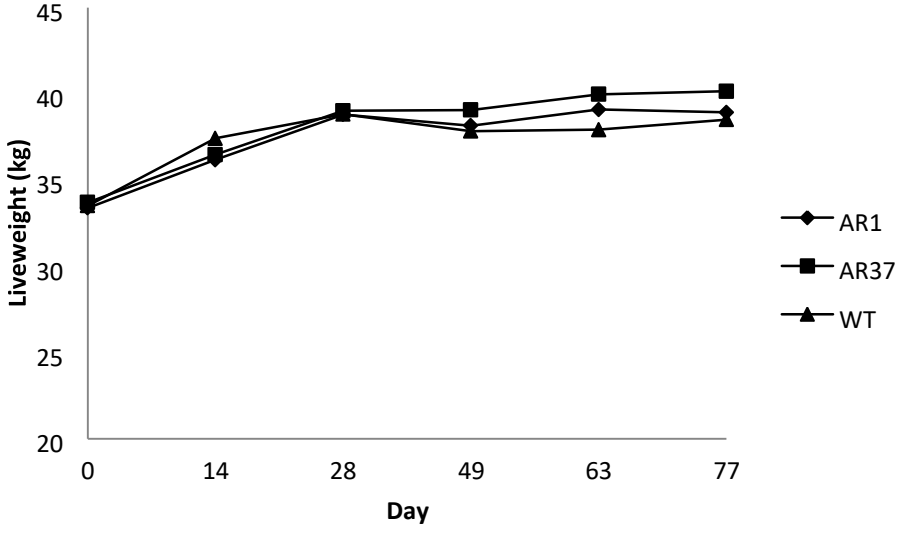


Figure 4.33 Mean liveweight over time in Crossbred weaners grazing AR1, AR37 and WT perennial ryegrass. Pooled sed for Endophyte x day = 1.06.



There was no effect of endophyte on liveweight in the Crossbred ewes (P=0.734; Figure 4.33). There were significant day and endophyte x day effects (P<0.001, for both), such that liveweight increase over time and was lower in the WT treatment over time.

Table 4.16 Mean water intake (per paddock), rectal temperature, respiration rate and dag score measured over 69 days of grazing AR1, AR37 and WT perennial ryegrass in Merino ewes.

Endophyte	AR1	AR37	WT	sed	P-value
Water intake (L)	235.7	285.1	267.6	19.1	0.102
Rectal Temp (^{0}C)	39.04	39.13	39.09	0.05	0.344
Resp.Rate (breaths.min^{-1})	80.8	86.4	91.1	3.3	0.021
Dag score	1.2	1.3	1.2	0.07	0.277

Rectal temperature was not affected by endophyte in the Merino ewes (Table 4.16), however, it decreased over time in all treatment groups (P<0.001). There was no endophyte x day interaction (P=0.433). Respiration rate increased in the WT treatment compared with AR1, while AR37 was higher than AR1, and WT and AR37 were similar (P=0.021; Table 4.16). There was a significant day effect such that respiration rate decreased over time in all treatment groups (P<0.001). There was a significant endophyte x day interaction (P=0.043), such that respiration rate was variable in treatment groups over time. There was a tendency for water intake to increase in the WT and AR37 treatment compared with AR1 (Table 4.16) for Merinos. Water intake was variable across time (P<0.001). There was no endophyte x day interaction (P=0.316). Dag score was not affected by endophyte (Table 4.16). There was a significant day effect such that dag score increased over time for all treatment groups (P<0.001) and there was a significant endophyte x day interaction such that dag score increased in the AR37 treatment over time compared with AR1 and WT (P<0.001).

Table 4.17 Mean water intake (per paddock), rectal temperature, respiration rate and dag score measured over 69 days of grazing AR1, AR37 and WT perennial ryegrass in Crossbred weaners.

Endophyte	AR1	AR37	WT	sed	P-value
Water intake (L)	207.9	193.2	203.2	41.0	0.936
Rectal Temp (^{0}C)	39.13	39.06	39.21	0.05	0.027
Resp.Rate (breaths.min^{-1})	94.9	93.3	100.2	6.4	0.605
Dag score	1.1	1.1	1.05	0.05	0.435

Rectal temperature increased in the WT treatment compared with AR37, and was similar in the WT and AR1 treatments in the Crossbred weaners (Table 4.17). Overall, rectal temperature decreased over time (P<0.001) and there was a significant endophyte x day effect (P=0.021) such that rectal temperature was variable within treatment group and over time. Respiration rate was not affected by endophyte (P=0.605) or endophyte x day (P=0.516). There was a significant day effect such that respiration rate was variable over time (P<0.001). Water intake was not affected by endophyte (Table 4.17) and there was no endophyte x day interaction (P=0.655) (Crossbreds). There was a significant day effect such that water intake decreased over time. Dag score was not affected by endophyte (Table 4.17) and there was no endophyte x day interaction (P=0.467). Dag score was variable over time (P=0.001).

Table 4.18 Number of Crossbred weaners and Merino ewes in each treatment group with staggers scores greater than or equal to 1.

Day	Crossbred Ewe			Merino Ewes		
	WT	AR1	AR37	WT	AR1	AR37
14	3/27	0/24	2/27	1/24	0/24	0/24
28	0/27	0/24	0/27	0/24	0/24	0/24
42	0/27	0/24	0/27	0/24	0/24	0/24
55	0/27	0/24	0/27	0/24	0/24	0/24
69	0/27	0/24	0/27	0/24	0/24	0/24

Data on the incidence of staggers are presented in Table 4.18. For both breeds, staggers occurred in the first fortnight of grazing was not detected at any other time. As expected no staggers was observed in sheep grazing AR1.

Discussion

Liveweight decreased significantly in the WT treatment overall in Merinos. Staggers was mild in the Merinos and was only detected in the first week of the treatment period in the WT and AR37 treatments. Therefore, the mechanism for decreasing liveweight in the WT treatment may not be directly related to staggers occurring. In similar studies, decreased liveweight has often only been observed when staggers occurs (Fletcher and Sutherland 2009b). For the Crossbreds, liveweight was at times lowest in the WT treatment; however, there was no overall effect on liveweight due to endophyte strain. Staggers was mild and only occurred during the first week of treatment for the Crossbreds in the WT and AR37 treatments, with no further detection occurring throughout the treatment period. Merinos in this experiment were 12 months older than the Crossbreds, but were still more severely affected; suggesting that Merinos may be more susceptible to PRG alkaloids than Crossbreds, regardless of age. Although both groups of sheep were run alongside each other in separate trial areas, they had access to similar and adequate pasture mass and quality.

There was a weak tendency for water intake to increase in the AR37 and WT treatments compared with the AR1 treatment in Merinos. Additionally, respiration rate increased in these treatment groups, indicating heat load increased in these animals. Sheep may have increased water intake and respiration rate to alleviate heat load. As previously discussed water intake is often variable when measured, however, this indicates that water intake may in fact increase due to alkaloid consumption and may be worth further exploration. Water intake was not affected in the Crossbred ewes.

Dag score increased over time in the AR37 treatment in Merinos, however, was not affected in the Crossbred ewes. Respiration rate was not affected in the Crossbreds and rectal temperature was not altered in the Merinos. However, rectal temperature increased in the WT treatment but was similar to AR1 in the Crossbreds. This indicates that heat load may have increased in these treatments, however, due to the low amount of variation differences between treatments groups was low. The variation in results and lack of differences is likely due to two factors. Variation is likely due to individual sheep variation in response to

alkaloid intake and the lack of difference is likely due to the use of mature stock (Merino's), high pasture availability and short term exposure.

Conclusion

Results from the current experiment were variable and no large differences observed. There were some differences in liveweight, however, staggers was mild in both breeds and cannot explain differences in liveweight, indicating another mechanism may be responsible for decreased liveweights. The Crossbreds were more tolerant to alkaloid intake despite their younger age compared to the Merinos. This indicates that Merinos are more sensitive to alkaloid consumption, a finding reflected in the previous grazing experiments in this report. Water intake increased along with respiration rate in Merinos suggesting heat load increased in these animals. A combination of individual sheep variation in response to alkaloid intake, use of mature animals, high initial pasture availability and perhaps shorter exposure period are likely to explain the results observed.

4.2. Indoor studies

4.2.1 Characterising impact of ergovaline on pregnant sheep

Table 4.19 shows ergovaline intake in each treatment group during each of the two stages of the experiment. Experimental diets were switched during week 7. Lambs were born at week 16.

Table 4.19 Ergovaline intake (µg/kg LW), in each treatment group.

Experimental stage	Nil	NilErgov	ErgovNil	Ergov
Week 1-7	0.0	0.0	16.2	14.5
Week 7-16	0.0	14.5	0.0	18.9

There was a significant treatment effect overall such that all groups fed endophyte had lower mean DMI compared to the control group (1604, 1448, 1426 and 1458 for control, control ergovaline, ergovaline and ergovaline control; $P<0.001$; sed 17.4). There was a significant week effect, such that overall DMI decreased over time. ($P<0.001$) and there was a significant endophyte x week effect, such that there was variability over time for treatment groups, however, a large decrease in DMI was observed at week 7 for the nil ergovaline group.

Figure 4.34 Mean dry matter intake over time in each treatment group. Pooled sed for treatment x week = 116.3.

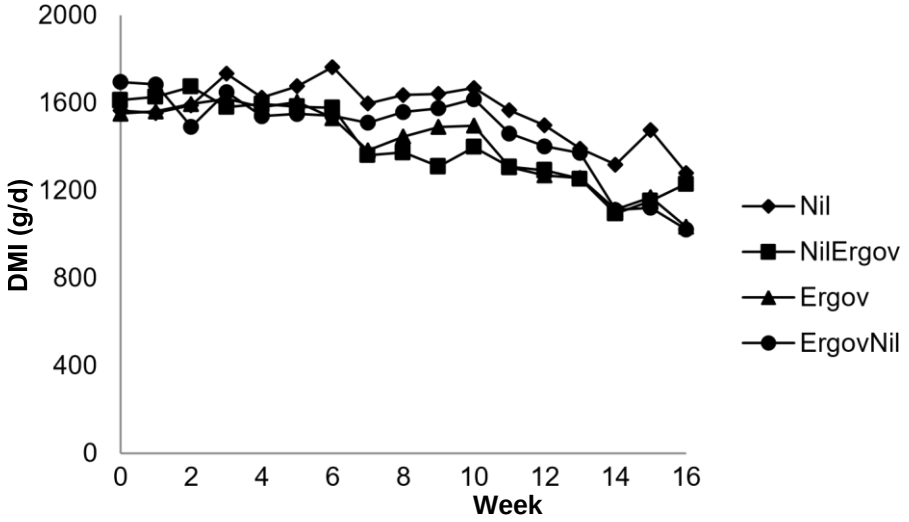
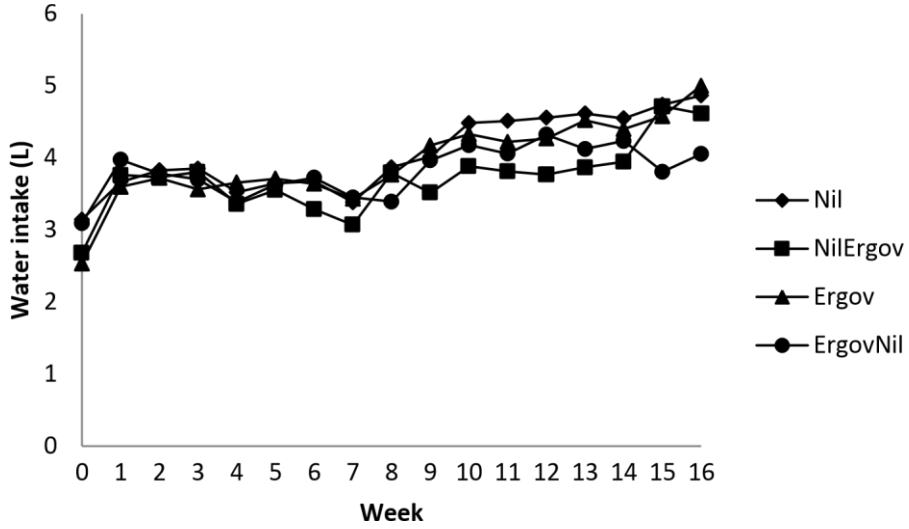


Figure 4.35 Mean water intake over time for each treatment group. Pooled sed for treatment x week = 0.50.

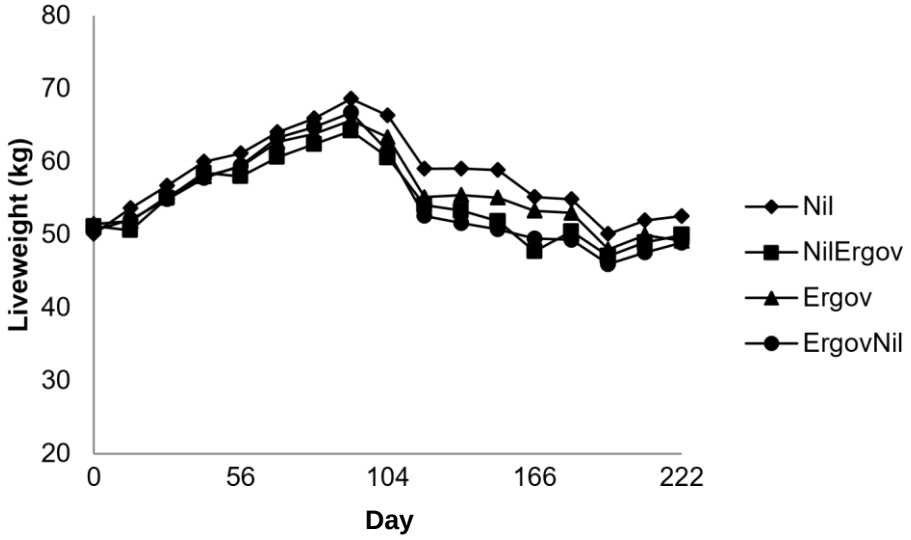


There was no treatment effect for water intake (P=0.812). There was a significant week effect (P<0.001), such that water intake increased over time in all treatment groups. This may be related to changes in ewe liveweight, lactation and lamb growth over the experimental period but unlikely to be related to ambient conditions due to the trial being conducted indoors during winter and early spring. There was a significant treatment x week effect (P<0.001), such that there was variability within each treatment group over time.

There was no treatment effect for liveweight (P=0.363). There was a significant day effect such that liveweight varied across time (P<0.001; Figure 4.35). There was no interaction between treatment x day (P=0.167). When the analysis was broken into stages of the experiment (pregnancy versus lactation), there was a significant treatment effect (P<0.001) over pregnancy such that the nil/endophyte treatment had significantly lower liveweight compared with the nil group. All other treatments were similar. Liveweight during pregnancy increased significantly (P<0.001), however, there was no treatment x day interaction (P=0.667). When liveweight was analysed during lactation there was no treatment effect (P=0.365). Liveweight decreased during lactation (P<0.001), however, there

was a weak tendency for a treatment x day effect (P=0.110), such that sheep previously on switched diets lost weight at a quicker rate compared to sheep previously on ergovaline and nil diets (Figure 4.35).

Figure 4.36 Mean liveweight over time in each treatment group during the pregnancy and lactation phases. Pooled sed for treatment x week = 2.90.



There were no treatment effects for rectal temperature or respiration rate (Table 4.20). There were significant day effects for rectal temperature and respiration rate (P=0.053 and <0.001, respectively), such that both measurements increased over time. There was no treatment x day interaction for rectal temperature (P=0.944). There was a strong tendency for a significant treatment x day interaction for respiration rate (P=0.097), such that the ergovaline treatment increased at a faster rate over time compared with the other treatments.

Overall, faecal water was not affected by treatment (Table 4.20). There was a significant day effect (P=0.055) such that faecal water decreased over time. There was a significant treatment x day effect (P=0.029), such that all treatment groups were variable over time.

Table 4.20 Mean rectal temperature and respiration rate, averaged over the pregnancy phase of the experiment, for each treatment group.

	Nil	NilErgov	ErgovNil	Ergov	sed	P-value
Rectal Temp (^{0}C)	39.63	39.29	39.59	39.34	0.38	0.738
Resp.Rate (breaths.min^{-1})	83	80	86	84	6.5	0.804
Faecal water (%)	67.4	68.4	67.6	69.3	1.4	0.567

Table 4.21 Milk yield, mammary gland width and vertical height of ewes from all treatments estimated at day 21 and 42 post-lambing.

	Nil	NilErgov	Ergov	ErgovNil	sed	P-value	
						Trt	Day
Milk yield (ml/4h)							
Day 21	187.3	207.1	216.7	233.3	32.6	0.541	<0.001
Day 42	48.6	165.5	145.3	176.4			
Mammary width (mm)							
Day 21	28.9	29.3	30.4	31.4	1.45	0.208	0.056
Day 42	28.5	27.4	30.0	29.6			
Mammary length (mm)							
Day 21	18.7	18.2	20.5	21.8	1.58	0.157	0.595
Day 42	18.7	18.6	20.4	20.3			

Estimated 4h milk yields for all ewes at day 21 and 42 post-lambing are presented in Table 4.21. As expected, milk yield decreased significantly from day 21 to day 42 ($P<0.001$) and this was associated with a small reduction in the width of the mammary gland ($P=0.056$) but no significant change in vertical length. There was no difference in milk yield or dimensions among treatments at both sampling times. However, these data have to be treated with caution as there was variation in the number of twins versus singles between treatments which could partly account for this. In addition, all ewes were on a nil endophyte diet post parturition, and the level of ergovaline ingestion was only low to moderate during pregnancy. The milk yield data was also analysed taking single and twin lambs into account (Table 4.22). Results were similar to data in Table 4.21. Results were more pronounced for single ewes compared with twin ewes (Table 4.22).

Table 4.2.2 Milk yield, mammary gland width and vertical height of ewes from all treatments estimated at day 21 and 42 post-lambing, analysed for ewes with single lambs and twin lambs.

	Nil	NilErgov	Ergov	ErgovNil	sed	P-value	
						Trt	Day
Single lambs							
Milk yield (ml/4h)							
Day 21	200.7	218.9	221.7	225.0	56.6	0.881	0.003
Day 42	51.9	168.1	131.1	182.9			
Mammary width (mm)							
Day 21	29.7	29.7	32.6	31.7	2.0	0.032	0.017
Day 42	28.5	27.1	29.3	32.0			
Mammary length (mm)							
Day 21	19.6	18.5	20.0	20.4	1.8	0.250	0.092
Day 42	18.5	17.6	21.7	19.2			
Twin lambs							
Milk yield (ml/4h)							
Day 21	160.0	171.7	214.2	245.0	75.9	0.699	0.034
Day 42	140.0	155.0	175.0	163.8			
Mammary width (mm)							
Day 21	27.0	28.0	29.3	31.0	3.2	0.825	0.488
Day 42	28.6	28.3	30.1	24.2			
Mammary length (mm)							
Day 21	16.6	17.3	20.8	23.8	4.4	0.514	0.618
Day 42	19.3	21.3	20.1	22.0			

Mean liveweight for lambs at birth and up to day 126 (weaning) are presented in Table 4.23. Statistical analysis was not undertaken due to the small number of lambs involved in this study. Means were derived from single lambs and twin lambs separately. The lambs in the ergovaline treatment seemed to have lower liveweights compared to the other groups, however this was most pronounced in single lambs only. Caution must be taken when interpreting these data due to the low number of lambs.

Table 4.2.3 Mean liveweight (kg) of lambs at birth until weaning, analysed as single and twin lambs.

	Nil	NilErgov	Ergov	ErgovNil	sed
Single lambs					
Birth	4.9	4.7	4.3	5.3	0.53
14	7.8	8.7	6.0	11.1	3.9
28	11.3	11.8	11.7	11.5	
42	14.0	14.8	14.0	15.0	
56	16.7	17.2	15.3	18.1	
70	19.2	20.3	17.1	19.6	
84	19.6	22.6	17.5	21.4	
98	22.6	25.3	20.2	23.2	
112	24.5	27.8	22.4	26.0	
126	27.1	29.3	19.3	27.5	
Twin lambs					
Birth	3.1	2.7	3.5	3.1	0.53
14	4.2	6.8	5.0	7.3	3.9
28	7.0	10.0	8.0	8.5	
42	9.4	12.5	10.3	10.6	
56	12.2	16.0	13.3	14.1	
70	15.4	18.0	15.2	14.2	
84	17.2	19.6	17.1	16.0	
98	20.1	22.8	18.8	17.4	
112	22.8	23.4	21.4	19.5	
126	24.6	26.0	22.0	22.0	

Discussion

Production response

Actual ergovaline intake was lower than expected, suggesting there was some individual sheep variability in consumption of infected seed. All groups fed ergovaline during the experimental period had lower DMI compared to the nil group. In particular, the nil/ergovaline group decreased DMI when introduced to the seed diet during week 7. The group whom received ergovaline through the entire treatment period had lower DMI throughout the entire treatment period and the ergovaline/nil group did not increase DMI once removed from the seed diet. This suggests that the introduction of ergovaline into the diet had a profound effect in reducing DMI. Most interesting is the ergovaline/nil

group did not recover after being removed from the ergovaline diet indicating feed aversion may have developed.

Additionally, effects on DMI became most pronounced by week 6-7. There have been no studies which have introduced alkaloid into the diet at different stages of an experiment and not during different stages of pregnancy. However, studies have found DMI to decrease when feeding TF infected feed to sheep and cattle (Hemken *et al.*, 1981; Waghorn *et al.*, 1994). Implications of reduce DMI often results in reduced liveweight and growth. In the current study all sheep increased liveweight over time due to pregnancy. The nil/ergovaline group had the lowest liveweight across the pregnancy phase of the experiment which reflects DMI in this group. During the lactation phase of the experiment all groups which had been fed ergovaline throughout pregnancy lost weight at the quickest rate, however, this was most pronounced in groups on switched diets (nil/ergovaline, ergovaline/nil). These sheep may have developed a feed aversion and therefore may not have consumed as much feed as other treatment groups throughout lactation. Feed intake was not measured throughout lactation.

Lamb liveweights were not statistically analysed due to the small numbers used. The results were split into single and twin lambs. The ergovaline treatment seemed to have lower liveweights throughout the measurement period for both single and twin lambs and the ergovaline/nil treatment seemed to have lower liveweight in twin lambs, suggesting that feeding ergovaline throughout the entirety of pregnancy may reduce lamb growth rate, compared to feeding ergovaline for either half of pregnancy or feeding a nil diet. There have been no studies which have investigated the effect of PRG alkaloid on sheep during pregnancy and lactation, however, one study investigated the effect of feeding endophyte-infected versus non-infected pasture to ewes with suckling lambs (Watson *et al.*, 1999). This study used low numbers of sheep and found no difference in lamb growth rate in the first year, but found a significant decrease in growth rate in the second year in lamb suckling ewes on infected pasture (Watson *et al.*, 1999). The authors of the study suggested that decreased milk production could be to blame. It is likely that alkaloid level in the pasture may also help to explain differences in the study by Watson *et al.*, (1999). Moreover, if the current study was repeated a higher concentration of alkaloid ergovaline could be fed, along with the addition of the indole-diterpenes.

Milk production analysis was separated into single and twin lambs. Milk production was not altered due to ergovaline intake for single or twin ewes. Milk production decreased over time as expected, for single and twin ewes and this was associated with a reduction mammary width, however, there was no reduction in mammary vertical length. The reason for no response in milk production and mammary gland measurements may be due to the level of ergovaline fed. If a higher level was able to be fed there may have been pronounced effects. Moreover, ergovaline feeding ceased at parturition and therefore sheep did not have access to ergovaline throughout lactation. Therefore, the effects of ergovaline may have diminished over time. Ergovaline is known to cause a decrease in plasma prolactin levels (Fiorito *et al.*, 1991; Bernard *et al.*, 1993; Aldrich *et al.*, 1993a). Prolactin is extremely important for many functions within animals; however, it is critical in establishing lactation. Prolactin increases during pregnancy so levels are much higher than normal. Therefore, a decrease in prolactin throughout pregnancy due to ergovaline intake could have severe consequences for ruminants. Additionally, sheep which graze WT PRG pasture are exposed to a range of difference alkaloids. Ergovaline may have a role in altering animal production

and physiology; however, this effect may be more severe when a range of alkaloids are fed, due to alkaloid interactions. Therefore, further study should focus on feeding alkaloids singly and in combination to elucidate potential effects and the role of each alkaloid class.

With respect to the potential for pasture alkaloids to affect pregnancy, there has been one study to investigate the effect of feeding TF alkaloids to mares during the establishment and maintenance of early pregnancy (Arns *et al.*, 1997). There were no adverse effects found during the establishment and maintenance of pregnancy however, critically, horses are not ruminants and thus it was important to undertake this work in ruminants.

Physiological response

Rectal temperature and respiration rate were not altered due to ergovaline intake. This experiment was undertaken during late autumn/winter in outdoor pens exposed to natural climatic conditions. There may not have been any response in rectal temperature and respiration rate due to the low level of ergovaline fed and due to the absence of heated conditions. This suggests heat load in sheep did not increase due to ergovaline consumption. When analysed over time, the ergovaline treatment was found to increase respiration rate at a faster rate compared to all other treatments. This suggests sheep fed ergovaline for a longer period of time are likely to respond differently compared to those fed for a shorter period of time. Previous studies have found ergovaline to cause increases in rectal temperature and respiration rate, however, these studies have largely been undertaken feeding infected tall fescue (Rhodes *et al.*, 1991; Osborn *et al.*, 1992; Waghorn *et al.*, 1994). There have been no studies which have measured physiological response to ergovaline intake in pregnant ruminants.

Conclusion

The results from the current study demonstrate that when a low amount of ergovaline is fed during different stages of pregnancy, DMI reduces and is affected differently depending on when ergovaline is introduced. Interestingly, DMI did not recover when sheep were fed the ergovaline/nil treatment and those sheep fed the nil/ergovaline treatment displayed a clear reduction in DMI upon introduction to the seed diet. Further, during pregnancy those sheep on switched diets lost weight at a quicker rate, indicating feed aversion may be occurring and carried over into the lactation phase. Lamb growth rate seemed to be lower in the ergovaline and ergovaline/nil treatments, however, this data was not statistically analysed due to the low numbers of lambs used. Milk production and mammary gland size was not reduced due to ergovaline intake as hypothesised. Therefore, feeding a higher concentration of ergovaline in the diet and a combination of different alkaloids may be warranted to elucidate effects. Rectal temperature and respiration rate were not affected during the treatment period and once again a higher dose of alkaloids may be warranted to simulate doses found in the field during summer/autumn. Further, ergovaline only was fed during this experiment. Further studies feeding ergovaline and lolitrem B singly and in combination may be warranted to further develop understanding towards the effects of PRG alkaloid in sheep during pregnancy. Additionally, feeding a range of alkaloids during pregnancy may more clearly represent field conditions.

4.2.2 Characterising ergovaline threshold in sheep

Dry matter intake was not affected by the alkaloid levels fed in this experiment (Table 4.24). There was a significant week effect such that DMI increased over time. Liveweight and ADG reflected DMI, in that there was no treatment effect, however, liveweight increased over time (Table 4.24). There was no interaction between treatment and week (Table 4.24). Actual ergovaline intake was different between treatment groups as expected (Table 4.24). The 25 level treatment did not consume all seed throughout the experiment (Table 4.24).

Water intake, urine output and faecal output were not affected by the alkaloid levels fed in this experiment (Table 4.25), however, increased over time ($P<0.001$). Faecal moisture was not affected by treatment or time (Table 4.25). There was a weak tendency for dry matter digestibility to increase in the alkaloid level 15 treatment compared to the nil and 5 treatments (Table 4.25).

Table 4.24 Mean dry matter intake over time for each treatment group.

	Alkaloid level					P-value		
	Nil	5	15	25	SED	TRT	Week	TRTxWeek
DMI (g/d)								
W1	1295	1364	1137	1230	131	0.498	<0.001	0.814
W2	1379	1469	1299	1345				
W3	1442	1555	1370	1450				
Ergovaline intake (µ	/kg LW)							
W1	0.0	4.6	15.0	21.7	1.9	<0.001	0.053	0.026
W2	0.0	4.6	14.8	19.5				
W3	0.0	5.0	15.0	20.6				
Liveweight (kg)								
Baseline	37.5	37.9	37.9	38.5	1.4	0.913		
W1	38.9	38.4	38.1	38.0	0.75	0.584	<0.001	0.982
W2	39.8	39.6	39.4	38.9				
W3	41.0	40.9	40.4	40.2				
ADG (g/d)								
W1	130	62	23	5	86	0.741	0.029	0.874
W2	134	179	191	127				
W3	175	188	143	191				

Table 4.25 Mean urine output, faecal output and faecal water over time in each treatment group.

	Alkaloid level					P-value		
	Nil	5	15	25	SED	TRT	Week	TRTxWeek
Water intake (L/d)								
W1	3.1	3.3	2.9	3.0	0.3	0.871	<0.001	0.994
W2	3.5	3.5	3.3	3.3				
W3	3.6	3.8	3.6	3.6				
Urine output (L/d)								
W1	0.57	0.31	0.61	0.47	0.27	0.599	<0.001	0.275
W2	0.55	0.57	0.73	0.55				
W3	0.57	0.60	0.93	0.64				
Faecal output (g/d)								
W1	1431	1902	1289	1344	276	0.302	<0.001	0.596
W2	1496	1928	1573	1622				
W3	1731	2004	1532	1680				
Faecal water (%)								
W1	59.3	65.6	59.6	62.5	4.0	0.157	0.171	0.280
W2	60.2	65.2	58.2	61.6				
W3	60.8	64.6	55.2	60.8				
DMD (%)								
W3	54.6	54.5	59.0	56.4	2.0	0.143		

Table 4.26 Mean rectal temperature, respiration rate and skin temperature over time in each treatment group.

	Alkaloid level					P-value		
	Nil	5	15	25	SED	TRT	Week	TRTxTime
Rectal temperature (°C)					0.13			
08:00	38.94	39.22	39.27	39.29		0.054	<0.001	0.199
12:00	39.15	39.45	39.42	39.43				
16:00	39.16	39.37	39.37	39.38				
Respiration rate (brea ths/min)								
08:00	65	77	84	74	10	0.332	<0.001	0.806
12:00	82	93	95	86				
16:00	83	101	103	91				
Skin temperature (°) 37.24								
08:00		37.39	37.41	37.29	0.31	0.922	<0.001	0.913
12:00	37.32	37.51	37.57	37.48				
16:00	37.38	37.54	37.55	37.49				

Figure 4.36 Mean rectal temperature over time for each treatment group. Pooled standard error of the difference = 0.17.

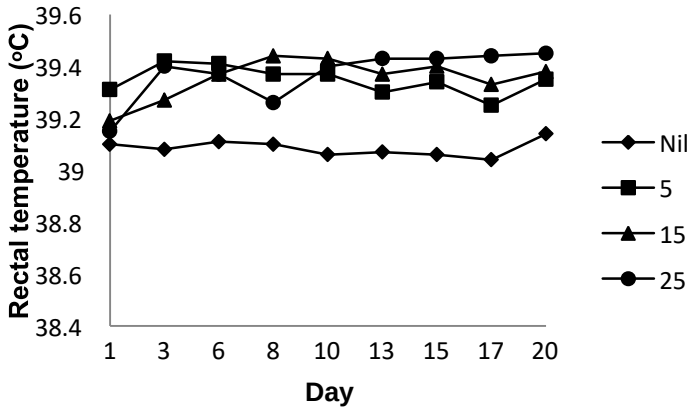


Figure 4.37 Mean respiration rate over time for each treatment group. Pooled standard error of the difference = 17.3.

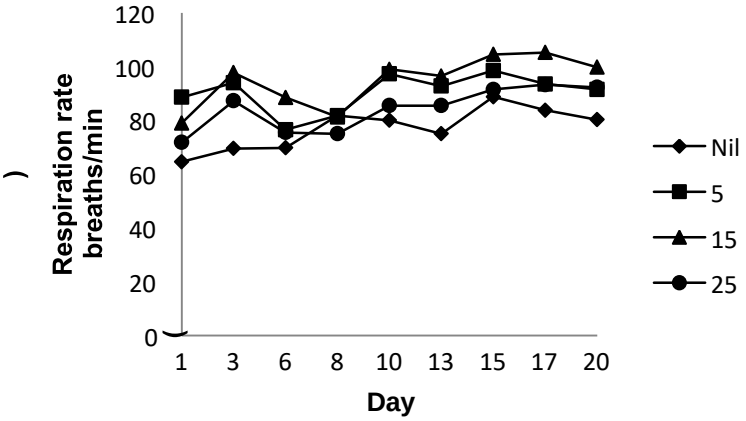
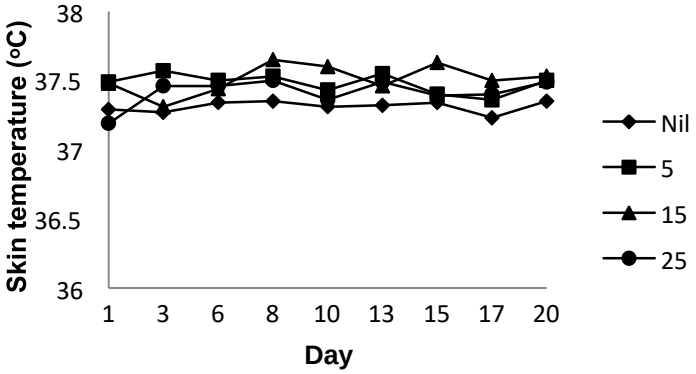


Figure 4.38 Mean skin temperature over time for each treatment group. Pooled standard error of the difference = 0.38.



Rectal temperature increased significantly in alkaloid level 5, 15 and 25 groups compared with the nil treatment at 08:00 and 12:00 (Table 4.26). Respiration rate increased in the level 15 treatment compared with the nil treatment at 16:00. Skin temperature was not affected by the fed alkaloid levels. Rectal temperature, respiration rate and skin temperature significantly increased over time, however there were no treatment x time effects (Table 4.26).

Rectal temperatures tended to increase from day 1 to day 3 and then remain fairly constant with (P=0.078; Figure 4.36). There was a significant treatment x day interaction (P<0.001), such that the alkaloid groups were variable over day, while the nil group remained stable. Respiration rate increased over day (P<0.001). There was no treatment x day interaction (P=0.792). Skin temperature was not affected by day (P=0.398), and there was no treatment x day interaction (P=0.632).

Discussion

Production effects

There were no significant effects on most of the production parameters measured. DMI, liveweight, average daily gain, urine output, faecal output and faecal moisture were not affected by the alkaloid doses administered in this experiment. Some previous studies have found no effect on DMI when residual pasture has been measured in a grazing study (Fletcher *et al.*, 1999). The likely reason for these results is due to the low doses of alkaloid fed, thermoneutral conditions used and short-term nature of the study. These results show that when ergovaline dose is low in pasture (not unlike during winter and spring), animal productivity is not affected. Caution must be taken, however, as ergovaline was fed singly, without the combination of the indole-diterpenes. Further investigation into the effect of below threshold doses of multiple alkaloids fed singly and in combination is warranted to determine effects which may be occurring during winter and spring on WT pastures.

There was a weak tendency for dry matter digestibility percentage to increase in the level 15 treatment compared to the nil and level 5 treatments. There have been no published reports of DMD measured in PRGT studies. Unpublished work (Henry *et al.*,) has found DMD to decrease in sheep fed WT infected PRG seed. Some tall fescue toxicosis (TFT) studies which have fed cattle and sheep infected tall fescue (TF) seed have found DMD to decrease due to ergovaline intake (Hannah *et al.*, 1990; Aldrich *et al.*, 1993a). However, some reports suggest lowering in DMD occurred under TN conditions when feeding cattle infected TF material (Aldrich *et al.*, 1993b) resulting in a disagreement between studies. There may be a number of factors responsible for change in DMD including a direct alkaloid effect on gastrointestinal function, difference in level of DMI and ruminal flow kinetics (Matthews *et al.*, 2005). Increased DMD indicates that digestibility in sheep fed a below threshold levels of alkaloid may in fact improve digestibility of feed.

Physiological response

Rectal temperature increased in all of the alkaloid groups compared to the nil treatment. This indicates that even at below clinical threshold doses of ergovaline, heat load increases. Previous research has found that rectal temperature in sheep and cattle increases in response to high levels of standard (WT) PRG and endophyte infected tall fescue (Hemken *et al.*, 1981; Hannah *et al.*, 1990; Fletcher *et al.*, 1999). This is the first study to feed low levels of ergovaline only PRG seed to sheep. No dose dependent relationship could be demonstrated in this study, probably because the doses were too low. This study indicates that sheep may need to consume only a very small amount of ergovaline in order to elicit a response in increased rectal temperature. Additionally this was found in thermoneutral conditions, suggesting that sheep grazing infected pastures during winter and spring may increase rectal temperature. Moreover, if heated conditions had been applied the effect may have been greater. The implications of increased rectal temperature can result in sheep

using more energy to maintain a normal core temperature rather than using this energy for growth. Over a longer period of time losses in production may be observed. Clearly more research is required to determine the longer term (3-6 months) implications of feeding low levels of alkaloids to sheep. This study fed ergovaline only, lolitrem B has also been implicated in increasing rectal temperature (McLeay and Smith 1999). Therefore, investigation into the effect of feeding low doses of ergovaline and lolitrem B singly and in combination is warranted.

Respiration rate and skin temperature were not affected in the current study. This finding suggests that the increase in rectal temperature did not increase heat load sufficiently to induce an increase in respiration rate and skin temperature in an attempt to dissipate excess heat. Previous studies have found that respiration rate usually increases when rectal temperature increases (Fletcher *et al.*, 1999), however, differences between animals fed nil endophyte versus endophyte have been most prevalent when heated climatic conditions have been used (Osborn *et al.*, 1992). Skin temperature has not been measured routinely in PRGT studies. TFT studies have found skin temperature to increase (Carr and Jacobson 1969; Browning and Leite-Browning 1997) and decrease (Rhodes *et al.*, 1991; Browning 2004). Differences in mode of administration of alkaloids, alkaloid concentration, alkaloid class, environmental temperature and animal species may explain differences. Sheep do not rely solely on the transfer of heat through the skin surface to cool down, with respiratory pathways more important, compared to cattle. Therefore, when fed a low dose of ergovaline the lack of response in skin temperature and respiration rate may indicate heat load is not in excess.

Conclusion

Results from the current study show when feeding low doses of ergovaline to sheep under thermoneutral conditions, animal production is not altered in terms of DMI, liveweight, average daily gain (ADG), urine output, faecal output and faecal moisture. DMD tended to increase in the level 15 treatment suggesting that digestibility increased in this treatment. The levels of ergovaline fed in this experiment were not unlike levels observed in the field during winter and spring and suggest that animal production may not be altered in the field. However, caution must be taken because ergovaline only was fed and there are a range of alkaloids, including lolitrem B, found in pastures. Rectal temperature increased in all ergovaline groups, and there was no dose response observed. This suggests that even a low dose of ergovaline can elicit a response and increase heat load in sheep, even under thermoneutral conditions. This increase did not result in an increase in respiration rate and skin temperature, suggesting that heat load did not increase enough. The implication of increased rectal temperature over the long term is not clear, animal production could decrease over a longer period of time, however the magnitude of increase in rectal temperature was not great, and therefore, this is not likely. It is more likely that a combination of higher ambient temperatures during spring and early summer (due to changing climatic conditions) and increased rectal temperature due to ergovaline intake, may adversely affect animal productivity and physiology in the field. Therefore, further investigation into the interaction between low doses of alkaloids fed singly and in combination under heated conditions is warranted to fully determine possible effects occurring in the field during winter/spring and even early summer.

4.2.3 Intake, storage and excretion profiles of alkaloids under different growth paths

Dry matter intake was not affected by alkaloid or breed. There was no alkaloid x breed interaction (P=0.906). Dry matter intake increased over time. Water intake was not affected by alkaloid or breed. There was no alkaloid x breed interaction (P=0.718). Water intake varied across time. There was a small amount of variation in actual ergovaline and lolitrem B intake between treatment groups. Sheep did not always consume the amount of seed fed. These measurements are all summarised in Table 4.27.

Table 4.82 Mean dry matter intake, water intake, actual ergovaline intake and lolitrem B intake during weeks 1-4 for each alkaloid and breed treatment. (Nil= Nil Ergovaline, Comp = Composite sheep breed, E = Ergovaline, L=lolitrem B, EL=Ergovaline = lolitrem B, X=Crossbred sheep, ALK=alkaloid, BRD=breed, Wk=week)

	Alkaloid type/Sheep breed									P-Value			
	Nil/Comp	Nil/X	E/Comp	E/X	L/Comp	L/X	EL/Comp	EL/X	SED	ALK	BRD	ALKxBRD	Wk
DMI (g/d)													
W1	973	884	893	887	907	891	866	879	88	0.785	0.198	0.906	<0.001
W2	1198	1032	1114	1082	1111	1072	1132	1067					
W3	1225	1132	1160	1128	1110	1043	1182	1139					
W4	1244	1159	1200	1144	1136	1075	1193	1141					
Water intak (L/d)													
W1	1.9	1.7	2.2	2.0	2.2	1.7	1.7	2.1	0.3	0.482	0.347	0.718	<0.001
W2	2.3	1.9	2.5	2.4	2.2	1.8	2.3	2.2					
W3	2.0	2.1	2.5	2.3	2.0	1.8	2.2	2.2					
W4	2.2	2.0	2.1	1.9	2.1	1.7	2.2	2.3					
Ergovaline intake (µg/kg LW)													
Overall	0.0	0.0	24.2	25.0	0.0	0.0	21.1	23.2	1.2	<0.001	0.237	0.578	
Lolitrem B intake (µg/kg LW)													
Overall	0.0	0.0	0.0	0.0	28.0	33.2	29.3	32.2	2.7	<0.001	0.152	0.489	

Table 4.28 Liveweight during stage one and two and faecal moisture by time for each alkaloid and breed treatment. (Nil= Nil Ergovaline, Comp = Composite sheep breed, E = Ergovaline, L=lolitrem B, EL=Ergovaline = lolitrem B, X=Crossbred sheep, ALK=alkaloid, BRD=breed)

	Alkaloid type/Sheep breed									P-Value			
	Nil/Comp	Nil/X	E/Comp	E/X	L/Comp	L/X	EL/Comp	EL/X	SED	ALK	BRD	ALKxBRD	Day
Liveweight (kg) St		ıge one (	.5x mainte	nance + a	lkaloid do	se)							
0	37.2	34.9	36.5	34.3	37.2	35.2	37.2	34.7	2.0	0.941	0.029	0.998	
7	37.0	33.4	35.5	32.8	36.4	35.2	36.3	33.4	2.0	0.835	0.011	0.854	<0.001
14	38.5	34.3	36.5	34.3	37.4	35.6	38.4	35.1					
21	40.2	36.0	38.2	35.6	38.7	37.4	39.4	36.4					
28	40.6	36.7	38.7	36.2	38.8	37.3	39.9	37.2					
Liveweight (kg) Stage two (0			.7x mainte	ıance wit	ı Nil alkal	›ids) 36.9							
35	39.2	33.8	36.2	35.1	37.1		37.6	35.7	2.0	0.558	0.036	0.380	<0.001
42	37.6	32.2	34.9	34.1	36.7	35.8	36.1	35.3					
49	36.4	32.0	34.0	33.1	36.8	35.6	34.8	34.2					
Faecal moisture (%)													
14	60.7	61.3	66.3	66.9	70.9	61.5	72.4	66.3	4.0	0.389	0.151	0.217	<0.001
28	62.0	62.7	69.0	65.5	67.6	59.8	68.8	70.8					
49	56.6	61.6	59.5	59.2	59.9	56.4	53.7	49.0					

Table 4.29 Rectal temperature and respiration rate during weeks 1-4 for each alkaloid and breed treatment. (Nil= Nil Ergovaline, Comp = Composite sheep breed, E = Ergovaline, L=lolitrem B, EL=Ergovaline = lolitrem B, X=Crossbred sheep, ALK=alkaloid, BRD=breed)

	Alkaloid type/Sheep breed									P-Value			
	Nil/Comp	Nil/X	E/Comp	E/X	L/Comp	L/X	EL/Comp	EL/X	SED	TRT	BRD	ALKxBRD	Day
Rectal temperature (°C) Stage one													
W1	38.87	38.89	38.98	39.04	38.96	39.04	38.95	39.12	0.14	0.085	0.302	0.752	<0.001
W2	38.99	38.98	39.18	39.06	39.02	39.03	39.08	39.34					
W3	38.88	39.06	38.94	39.08	38.94	38.84	39.06	39.18					
W4	38.83	39.00	38.96	39.09	38.89	38.79	39.08	39.02					
Rectal temperature (°C) Stage two													
Overall	38.87	38.89	38.96	38.99	38.98	39.03	38.84	39.06	0.15	0.755	0.343	0.742	
Respiration rate (°C) Stage one													
W1	39	43	48	52	56	42	45	49	5	0.618	0.264	0.672	<0.001
W2	41	37	42	36	42	37	43	39					
W3	41	42	36	36	43	42	46	45					
W4	39	39	38	35	40	33	38	40					
Respiration rate (°C) Stage two													
Overall	32	35	33	35	34	33	37	34	4	0.734	0.897	0.829	

Liveweight was analysed over each stage of the experiment. During the baseline period there was a breed difference, such that the composite breed was heavier compared with the Crossbred breed. During stage one and two there were no alkaloid differences detected (Table 4.28). During stage one liveweight increased over time (Table 4.28) and during stage two liveweight decreased over time (Table 4.28). There were no further interactions detected at either stage of the experiment.

Faecal moisture was not affected by alkaloid or breed (Table 4.28). There was no alkaloid x breed interaction (P=0.217). Faecal moisture decreased over time (Table 4.28). At day 14 the lolitrem B composite treatment had significantly higher faecal moisture compared to both of the nil treatments. No interactions were detected.

There was a strong tendency for rectal temperature to be higher in the ergovaline/lolitrem B group compared to all other groups (Table 4.29) during phase one. There was no breed effect (Table 4.29) and no alkaloid x breed interaction (P=0.752). There was a significant day effect such that rectal temperature varied over time (Table 4.29). No further interactions were detected during stage one. During stage two, rectal temperature was not affected by treatment or breed (Table 4.29). There were no interactions detected.

During stage one respiration rate was not affected by treatment or breed (Table 4.29). Respiration rate decreased significantly over time (Table 4.29). Additionally there was a strong tendency for a alkaloid x week interaction (P=0.078), such that groups which ingested alkaloids were more variable compared with the nil treatment. During stage two there were no significant effects.

Some DXA values were affected by breed (Table 4.30). Fat percentage, total fat and total lean tissue increased between day 0 and 29 and decreased between day 29 and 51 resulting in a significant day effect (Table 4.30).

Developing increased understanding, awareness and potential mitigation strategies for perennial ryegrass toxicosis in sheep production systems

Table 4.30 Mean ash, fat percentage, total fat and lean tissue in each treatment group across time. (Nil= Nil Ergovaline, Comp = Composite sheep breed, E = Ergovaline, L=lolitrem B, EL=Ergovaline = lolitrem B, X=Crossbred sheep, ALK=alkaloid, BRD=breed)

	Alkaloid type/Sheep breed									P-Value			
	Nil/Comp	Nil/X	E/Comp	E/X	L/Comp	L/X	EL/Comp	EL/X	SED	ALK	BRD	ALKxBRD	Day
Ash (g)													
0	928.2	864.4	927.6	811.8	918.6	898.2	927.0	814.9	58.5	0.733	0.011	0.622	
29	933.0	917.9	933.7	840.0	939.2	954.8	977.4	917.8	70.7	0.478	0.311	0.905	0.454
51	938.9	907.4	912.6	868.6	978.8	961.5	969.0	934.6					
Fat (%)													
0	21.4	20.0	22.7	20.4	22.9	20.0	21.7	21.0	1.2	0.761	0.006	0.662	
29	22.9	20.4	23.0	22.4	24.2	21.7	23.1	22.1	1.2	0.499	0.015	0.637	<0.001
51	21.6	19.4	22.3	21.2	22.8	20.4	21.4	21.0					
Fat (g)													
0	8021	6708	7984	6871	8308	7032	7874	6858	577	0.859	<0.001	0.981	
29	9553	7710	8978	8182	9318	8228	9379	8193	590.7	0.876	<0.001	0.534	<0.001
51	8144	6290	7685	6962	8275	7061	7648	7087					
Lean (g)													
0	25511	23310	23570	23171	24441	24279	24726	22453	1231	0.532	0.047	0.475	
29	27991	25941	26108	24645	25786	25976	27164	25393	1542	0.614	0.084	0.666	<0.001
51	25566	22392	23253	22459	24368	24146	24365	23208					

Discussion

Production response

DMI was not affected by alkaloid treatment at the dosages used in this study, or breed. Diet allowance increased after week one of the experiment period due to sheep losing weight. The level of alkaloid fed in the experiment was at threshold level. One of the challenges in feeding PRG seed to sheep is variability in consumption. Therefore, the doses chosen were based on optimizing seed intake. Additionally, sheep were kept in an area exposed to natural ambient temperature and experimentation was undertaken over late autumn/winter. The lack of interaction with high ambient temperature may explain why no decrease in DMI was observed. A previous study which investigated the effect of ergovaline on cattle, found DMI decreased due to ergovaline intake, and this decrease was exacerbated when heat was applied (Osborn *et al.,* 1992).

Water intake was not affected by alkaloid intake at the dosages used in this study, or breed. The low dose of ergovaline and lolitrem B may be responsible for the lack of response, additionally the time of year in which the study was undertaken indicates that heat may be required to produce more severe PRGT effects. Unpublished work (Henry *et al.,*), has found water intake to increase when a higher dose of WT PRG seed was fed to Merinos under heated conditions. In TFT studies there have been no clear alkaloid effects on water intake. Studies which have fed cattle infected TF seed and hay have found no effect on water intake (Aldrich *et al.,* 1993a; Matthews *et al.,* 2005) when feeding an alkaloid concentration of 1, 170 ppb and tiller infection of 87.5%, respectively. Therefore, alkaloid dose and ambient temperature conditions may be important in increasing water intake.

There was a small amount of variability in actual ergovaline and lolitrem B intake. The amount of seed fed to the lolitrem/ergovaline combination treatment was large and therefore a decrease in intake was seen at times.

Liveweight was significantly higher in the composite breed compared with the

Crossbreds. This was evident at baseline and throughout the treatment period. During stage one all sheep put weight on due to the 1.5 x maintenance diet fed. During stage two all sheep lost weight due to the 0.7 x maintenance diet fed. There were no further effects on liveweight in accordance with DMI. This suggests that the dose of alkaloids may have been too low for effects to be observed.

Numerically, the alkaloid groups had higher faecal moisture compared to the nil treatments and at times (day 28), the combined lolitrem B/ergovaline treatment had the highest faecal moisture, suggesting feeding a combination of alkaloids may increase faecal moisture higher than when they are fed singly. There have been no previous studies which have had fed individual alkaloids to ruminants. Therefore, this is the first time such a comparison of alkaloid classes has been undertaken. Previous grazing studies have only reported dag score and have not measured faecal water directly (Fletcher 1999). These studies have found faecal water to have high variability in faecal dag scores and have attributed this to individual variation (Fletcher 1999). Additionally lolitrem B has been thought to be solely responsible for increasing faecal moisture and dagging (McLeay *et al.,* 1999), however, ergovaline may have a role also (Fletcher *et al.,* 1999), as suggested in the current study.

Physiological response

There was a strong tendency for rectal temperature to increase in the ergovaline/lolitrem B combined treatment compared to all other groups during stage one of the experiment. This indicates that the combination of alkaloids will produce a more severe effect compared to when alkaloids are fed separately. Previously it has been suggested that ergovaline causes heat stress and therefore is responsible for increases in core body temperature (Hemken *et al.,* 1981; Oliver 1997; Al-Haidary *et al.,* 2001). There have been no previous studies which have compared the physiological response of feeding alkaloids separately and in combination, however it has been previously suggested that effects are worsened when a range of alkaloids are present (Gadberry *et al.,* 1997). Heart rate, respiration rate and body temperature have been found to increase due to lolitrem B alone when injected in a purified form (McLeay and Smith 1999) and due to the ergot alkaloids (Rhodes *et al.,* 1991; Al-Haidary *et al.,* 2001).

Respiration rate was not affected in the current study during stage one of the experiment, this is likely due to the low dose of alkaloid fed and the environmental conditions, and suggest that even through there was a tendency for rectal temperature to increase, this increase was not large enough to increase respiration rate to dissipate the heat produced.

Rectal temperature and respiration rate were not different during the second stage of the experiment. During stage two of the experiment sheep were fed a 0.7 x maintenance diet to induce weight loss. It was hypothesised that sheep whom have previously been fed PRG alkaloids will stored alkaloids in the adipose tissue and during times of negative energy balance, may mobilise the stored alkaloids, producing further PRGT effects. The low level of alkaloid fed and the short time frame in which it was fed may explain why no further effects were observed. Investigation into the life-time effects of PRG alkaloid consumption may elucidate whether animals that have had exposure to PRG alkaloids over a long period of time, have carry-over effects. This is particularly relevant under Australian conditions where animals frequently enter negative energy balance over summer and autumn when alkaloid levels are highest and when environmental conditions are most harsh. Previous work has found no such carry-over effects to occur (Henry *et al.,* unpublished), however sheep were only fed alkaloid infected feed for a period of 6 weeks prior to negative energy balance. No other studies have investigated this issue.

There were no effects on body composition. Fat % and fat (grams) increased between day 0 and day 29, and decreased between day 29 and day 51 as expected. Differences in all parameters due to breed were due to the higher starting weight of the composite breed compared to the Crossbreds. Additionally, the results show that the composite breed was leaner with more fat content. There have been very few studies to measure body composition in sheep fed PRG alkaloids and thus far no differences have been observed (Henry *et al.,* unpublished).

Conclusion

The results from the current study demonstrate when a low level of ergovaline and lolitrem B are fed to sheep separately and in combination, effects can vary. When lolitrem B and ergovaline were fed together rectal temperature and faecal moisture were found to increase. DMI, liveweight and body composition were not altered in the current study which was likely due to the low alkaloid doses chosen and mild climatic conditions. Moreover,

there were no carry-over alkaloid effects during the wash-out period, however, this is not surprising as rectal temperature and respiration rate were not greatly affected during stage one. Further study investigating life-time effects of alkaloids following seasonal cycles is warranted as carry-over effects may not have occurred due to the relative short feeding time and the mild climatic conditions experienced. In the field if negative energy balance occurs it is usually due to harsh environmental conditions. These conditions may therefore predispose sheep to severe outbreaks of PRGT.

4.2.4 Physiological and production effects of feeding ergovaline to meat sheep breeds under heated ambient temperature conditions

DMI was analysed during weeks 1-4 and week 5 separately. At the dosage fed, there were no alkaloid, breed or alkaloid x breed effects during week 1-4 (Table 4.31). During week 5 there was no alkaloid or breed effect (Table 4.31). There was an alkaloid x breed effect ($P<0.001$), such that the ergovaline Crossbred group had significantly lower DMI compared to all other groups. When DMI was analysed over week 1-5, there were no alkaloid, breed or alkaloid x breed effects ($P=0.809$, $P=0.760$, $P=768$, respectively), however there was a time effect ($P<0.001$), such that DMI increased during weeks 1-4, and decreased during week 5.

When water intake was analysed during weeks 1-4, there were no alkaloid, breed or alkaloid x breed effects (Table 4.31). When analysed at week 5 there was a significant alkaloid effect (Nil 3.0 versus Ergovaline 3.5; $P<0.001$; sed 0.16), such that the ergovaline treatment consumed more water and there was a significant breed effect (Composite 3.6 versus Crossbred 2.8; $P=0.034$; sed 0.26), such that the composite breed consumed more water. There was a significant alkaloid x breed effect (Table 4.31), such that the ergovaline composite treatment consumed significantly more water compared with all other treatments. When water intake was analysed over week 1-5 there were no alkaloid, breed or alkaloid x breed effects ($P=0.194$, $P=0.612$ and $P=0.700$, respectively).

Actual ergovaline intake was constant throughout the treatment period (Table 4.31).

There were no alkaloid, breed or alkaloid x breed effects on liveweight (Table 4.32). Overall, liveweight increased significantly over the first four weeks and decreased over the last week of treatment (Table 4.32). Average daily gain was variable and not affected by alkaloid, breed or alkaloid x breed (Table 4.32). Average daily gain was variable across time resulting in a significant time effect (Table 4.32).

Table 4.90 Mean dry matter intake, water intake and ergovaline intake, during phase one (week 1-4) and phase two (week 5), for each alkaloid and breed treatment.

	Alkaloid level/Sheep breed					P-value			
	Nil/Comp	Nil/X-Bred	Ergo/Comp	Ergo/X-Bred	SED	ALK	Breed	ALKxBreed	Week
DMI (g/d)									
W1	1095	1003	1024	1075	67.5	0.777	0.980	0.524	<0.001
W2	1203	1178	1171	1174					
W3	1225	1223	1193	1203					
W4	1230	1222	1192	1240					
W5	1180	1211	1238	1101	48.0	0.443	0.565	<0.001	
Water intake (L ′d) 2.2									
W1		1.8	2.0	2.3	0.35	0.265	0.714	0.823	<0.001
W2	1.8	2.2	2.5	2.3					
W3	2.2	2.2	2.7	2.4					
W4	2.3	2.2	2.6	2.6					
W5	3.0	3.0	4.2	2.7	0.33	<0.001	0.034	<0.001	
Ergovaline intake (µg/kg LW)									
W1	0.0	0.0	25.0	25.0	0.01	<0.001	0.336	0.336	0.142
W2	0.0	0.0	25.0	24.9					
W3	0.0	0.0	25.0	25.0					
W4	0.0	0.0	24.9	24.9					
W5	0.0	0.0	25.0	25.0					

Table 4.32 Liveweight and average daily gain for each treatment group.

	Alkaloid level/Sheep breed					P-value			
	Nil/Comp	Nil/X-Bred	Ergo/Comp	Ergo/X-Bred	SED	ALK	Breed	ALKxBreed	Week/Day
Liveweight (kg)									
7	36.7	36.1	35.7	35.8	2.0	0.682	0.814	0.957	<0.001
14	37.6	37.3	37.1	36.8					
21	38.0	37.5	37.3	37.3					
28	39.5	39.1	38.8	38.5					
37	38.7	38.6	38.4	37.6					
ADG (g/d)									
W1	1517	1000	-17	1117	748	0.338	0.392	0.421	<0.001
W2	850	1267	1350	1017					
W3	433	167	-100	983					
W4	1467	1567	1483	1233					
W5	-783	-483	-350	-933					

Table 4.33 Mean urine output, faecal output and faecal moisture collected through grab samples in phase one of the experiment and through 24 hour samples during phase two of the experiment, in each treatment group.

	Alkaloid level/Sheep breed					P-value			
	Nil/Comp	Nil/X-Bred	Ergo/Comp	Ergo/X-Bred	SED	ALK	Breed	ALKxBreed	Week/Day
Urine output (L/d)									
31	226.7	310.8	584.9	281.7	330.5	0.167	0.319	0.130	0.004
34	268.3	374.2	1259.2	385.8					
36	279.2	500.8	1163.3	411.7					
Faecal output (;/d)									
31	1037	1113	1073	1183	155	0.353	0.866	0.513	0.226
34	1030	1168	1344	1181					
36	1048	1099	1210	1105					
Faecal moisture (%) Grab samples									
14	63.2	60.4	63.2	60.0	3.3	0.412	0.182	0.428	0.645
28	62.4	56.0	62.8	63.3					
Faecal moisture (%) 24 hour samples									
31	55.3	52.4	60.1	57.2	3.9	0.058	0.321	0.482	0.103
34	51.0	51.1	60.2	53.4					
36	52.0	52.6	58.0	55.0					

At the alkaloid dosage fed, urine output was not affected by alkaloid, breed or alkaloid x breed (Table 4.33). However, there was a strong tendency for a treatment x day effect, such that the ergovaline treatment had higher urine output compared with the nil treatment. Further, there was a strong tendency for an alkaloid x breed x day effect (P=0.090) such that the ergovaline composite group had higher urine output on days 34 and 39, compared with all other groups. Faecal output was not affected by alkaloid or breed and there was no alkaloid x breed interaction (Table 4.33).

Faecal moisture was analysed when grab samples were taken in the individual pen stage of the experiment and when 24 hour samples were taken during the met cage stage of the experiment. During stage one of the experiment there were no differences in faecal moisture (Table 4.33). During stage two of the experiment there was a strong tendency for an alkaloid effect (Table 4.33), such that the ergovaline treatment had higher faecal moisture compared with the nil treatment. No other differences were found during stage two of the experiment.

Table 4.34 Mean rectal temperature, respiration rate and skin temperature over time measured during stage one of the experiment (individual pens) for each treatment group.

	Alkaloid level/Sheep breed					P-value			
	Nil/Comp	Nil/X-Bred	Ergo/Comp	Ergo/X-Bred	SED	ALK	Breed	ALKxBreed	Week/Day
Rectal tempera ture (°C)									
W1	39.28	39.26	38.92	39.26	0.14	0.815	0.153	0.932	0.001
W2	38.90	39.11	39.04	39.11					
W3	38.94	39.10	38.96	39.11					
W4	38.87	38.92	39.03	38.95					
Respiration rate (breaths/min)									
W1	52	57	49	47	7	0.968	0.316	0.843	0.088
W2	45	49	46	45					
W3	44	46	48	55					
W4	44	45	38	48					

Figure 4.39 Mean rectal temperature over time during stage two of the experimental period for each group. Pooled standard error of the difference = 0.09.

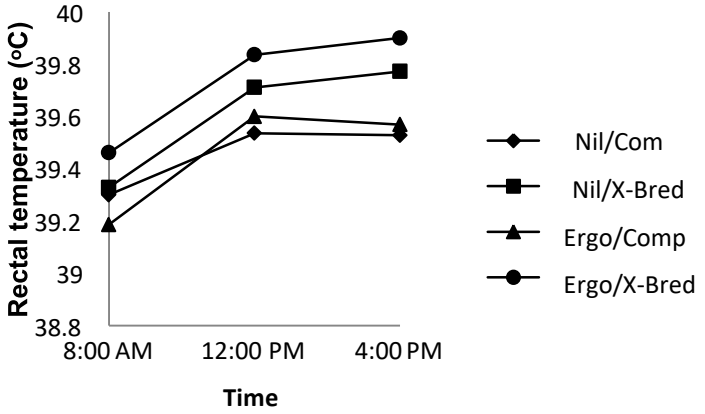


Figure 4.40 Mean respiration rate over time during stage two of the experimental period for each group. Pooled standard error of the difference = 13.

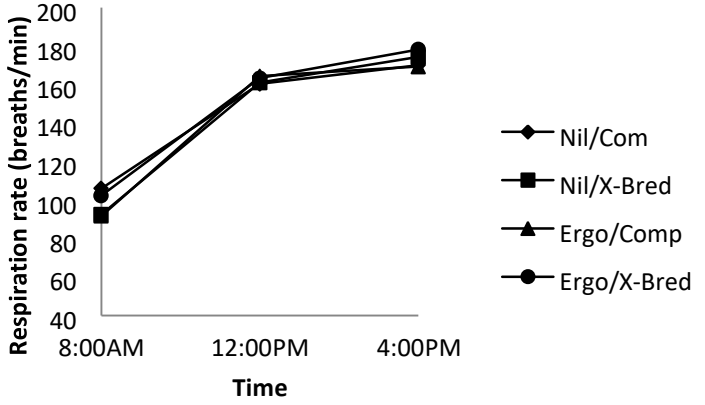
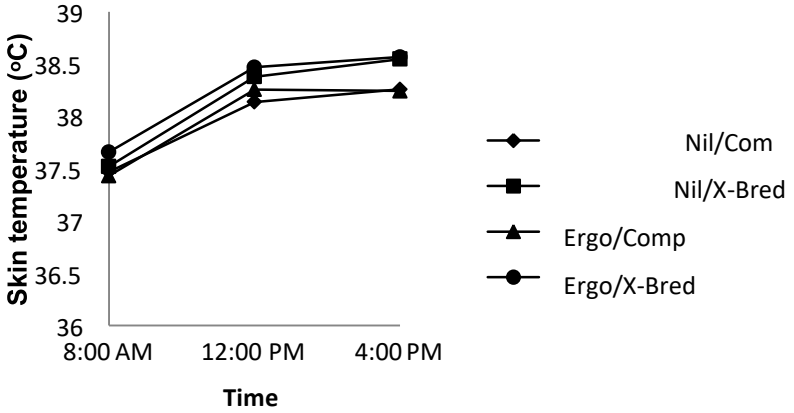


Figure 4.41 Mean skin temperature over time during stage two of the experimental period for each group. Pooled standard error of the difference = 0.16.



At the dosage fed, rectal temperature and respiration rate were not affected by alkaloid, breed or alkaloid x breed during stage one of the experiment (Table 4.34). Rectal temperature and respiration rate decreased significantly over day (Table 4.34). When analysed over stage two of the experiment, rectal temperature was not affected by alkaloid (P=0.326). There was a significant breed effect (P=0.003), such that the Crossbred sheep had higher rectal temperature compared with the composite sheep. There was no alkaloid x breed interaction (P=0.306). Rectal temperature increased across time (P<0.001; Figure 4.39). There were no further interactions detected.

Respiration rate was not affected by this dosage of alkaloid, breed or alkaloid x breed during stage two (P=0.919, P=0.839 and P=0.585, respectively). Respiration rate increased significantly over time (P<0.001; Figure 4.40). There were no further interactions detected.

Skin temperature was not affected by alkaloid at the chosen dosage (P=0.562). There was a significant breed effect such that Crossbred sheep had a higher temperature compared with the composite sheep (P=0.020). There was no alkaloid x breed effect (P=0.730). Skin temperature increased across time (P<0.001; Figure 4.41). There were no further interactions detected.

Discussion

Production effects

Dry matter intake was not affected during the first four weeks of the treatment period while sheep were housed in individual pens, however, once moved into the heat room DMI decreased significantly in the ergovaline Crossbred treatment compared to all other treatments and DMI decreased significantly during week 5 for all treatments. This reduction in DMI during the heat stress period is presumably due to sheep minimising the heat increment of feeding and the subsequent whole body heat load (Beede and Collier 1986). The reduction in DMI in the ergovaline Crossbred sheep may indicate the Crossbred's ability to cope with the heat challenge. This effect was not found in the composite breed which includes a range of different breeds. Merinos have been found to be sensitive to heat stress when consuming PRG alkaloids (Henry *et al.,* unpublished). The Crossbred ewes were a Merino (F) x poll dorset (M) and therefore a larger proportion of their genetic make-up was Merino, possibly explaining why this difference occurred. The grazing studies discussed in this report, found Merinos to be more sensitive to PRG alkaloid consumption compared with Crossbred sheep. There have been no studies to investigate the breed differences in sheep consuming PRG alkaloids. There has been limited research investigating the effect of TF alkaloids on *Bos indicus* v *Bos taurus* cattle in tall fescue work (Browning 2000), however this research has focused on the acute sensitivity of cattle to ergotamine challenges and found no difference between breeds.

Water intake was not changed during weeks 1-4. However, during week 5 water intake increased in the alkaloid treatment compared with the nil treatment and increased in the composite breed, mostly due to the ergovaline composite breed increasing water intake. Increased water intake due to alkaloid intake has not been widely reported previously. However, in unpublished work undertaken by Henry (*et al.,*), water intake was found to increase due to alkaloid consumption. The implications for this for management of sheep exposed to PRG alkaloids in the field warrants further consideration (Dixon *et al.,* 1999). In TFT studies there have been no clear alkaloid effects on water intake. The current study fed

ergovaline only seed, and studies which have fed cattle infected TF seed and hay have found no effect on water intake (Aldrich *et al.,* 1993a; Matthews *et al.,* 2005).

As a result of high water intake in the ergovaline composite treatment, urine output was numerically increased in this group. No further significant effects were found for urine output due to high error. Very few studies have investigated urine output change due to ergovaline intake and those that have, have found no effect on urine output (Matthews *et al.,* 2005). Increased urine output due to heat exposure have been reported (30-32ºC 8 hours per day), and this increase was thought to be related to increased water intake in sheep (Dixon *et al.,* 1999).

The increase in water intake in the composite breed may reflect their ability to cope with the heat stress conditions, compared to the Crossbred sheep which were found to not increase water intake in response to the heated conditions and decreased DMI significantly.

Faecal output was not affected by any treatments imposed. Faecal water percentage was not altered during stage one, however during stage two there was a strong tendency for increased faecal water in the alkaloid treatment. This increase in faecal water may be a reflection of increased water intake during this stage of the experiment. The alkaloid diet contained ergovaline only and lolitrem B has long been the causal alkaloid involved with increased faecal water (Fletcher *et al.,* 1999). Therefore, the current study indicates that increased faecal water may not be due to lolitrem B only. Sheep consuming ergovaline are likely to increase water intake, in particular during heated weather conditions. This increase in water intake may increase faecal water, and along with the consumption of lolitrem B, the combination of alkaloids consumed (especially in WT pasture) may be worsening effects.

Physiological effects

Rectal temperature and respiration rate were not altered during the first stage of the experiment. This is probably a reflection of the lower ambient temperature experienced during this stage of the experiment (late autumn/winter outdoor conditions). Additionally this may be due to lower dose of ergovaline fed in this experiment which was kept at threshold level. Previous grazing studies have found small increases in rectal temperature and respiration rate during summer and autumn (Fletcher *et al.,* 1999). Controlled, unpublished, studies have shown increases in rectal temperature, respiration rate and skin temperature (Henry *et al.,*), however, a higher dose of alkaloid (WT) was fed during those experiments and ambient temperature was increased.

During stage two of the experiment there were no significant effects of alkaloid on rectal temperature, respiration rate or skin temperature. This is likely due to the low level of ergovaline fed and the short time in which sheep were exposed to the heated conditions (4 days). Previous studies have found increases in rectal temperature and respiration rate when feeding PRG alkaloids (Henry *et al.,* unpublished; (Fletcher *et al.,* 1999) and tall fescue alkaloids (Osborn *et al.,* 1992). However, there have been studies which have fed TF alkaloids to ruminants and found no effect on rectal temperature and respiration rate (Browning and Leite-Browning 1997; Browning 2000; Hill *et al.,* 2000). The authors of these studies fed lower concentrations of ergovaline under thermoneutral conditions (22ºC), explaining why no effects were observed. In general, the Crossbred sheep increased rectal temperature and skin temperature compared to the composite sheep. This indicates the Crossbred sheep were unable to cope with the heated conditions. Additionally the Crossbred sheep decreased DMI, did not increase water intake in response to the heat and increased physiological parameters suggesting this breed is not as well suited to heated

conditions compared to the composite breed. There have some studies which have compared the physiology of Omani to Merino sheep and found Omani sheep to be more heat tolerant compared to Merino sheep (Srikandakumar *et al.,* 2003). This is not surprising due to the origins of Omani sheep. However, there have been no studies which have compared meat breeds of sheep commonly used in Australia. The likely reason for the inability of Crossbreds to withstand heat stress as successfully as the composites is due to increased Merinos genetics. The grazing studies reported in this report found that Merinos were more sensitive to PRG alkaloids compared to Crossbreds. Moreover, studies have found that sheep can be selected for sensitivity or resistance to PRG alkaloids, indicating that genetics play a role in the severity of PRGT (Morris and Amyes 2007).

Conclusion

The results from the current study demonstrate feeding a low concentration of ergovaline under thermoneutral conditions does not produce measurable effects associated with ergovaline intake. The Crossbred ergovaline treatment decreased DMI during week 5 which resulted in decreased liveweight gain. The Crossbred treatment were also unable to cope with the heat exposure as well as the composite treatment, increasing rectal temperature and skin temperature, not altering water intake and decreasing DMI, indicating selection of meat sheep breed may be more important than first thought in minimising the effects of alkaloid consumption on animal physiology and production response. This difference in breed may be due to the higher proportion of Merino genetics found in the Crossbreds. Additionally the current study found a decrease in faecal water percentage in sheep consuming ergovaline feed, likely due to increased water consumption. This indicates that lolitrem B may not be the only causal alkaloid increasing faecal water and ergovaline may have an important role. Animal physiology was not altered due to ergovaline intake, this was likely due to the low dose of ergovaline fed, therefore, a higher dose may need to be used in future studies.

4.2.5 Efficacy of rumen detoxifying agents for mitigating PRGT

Dry matter intake was significantly lower in the alkaloid treatment compared with the nil treatment (1059.4 and 1025.4, for Nil and Alkaloid; sed 14.7; P=0.033). There were no Elitox or alkaloid x Elitox effects on DMI (Table 4.35). There was a significant Elitox x day effect (P=0.047), such that all treatments were variable, especially during the first 7 days, however, the 2 and 4 gram Elitox groups had the highest DMI compared with the 0 gram group. There was a strong tendency for a significant treatment x Elitox x day effect (P=0.087; Figure 4.42), such that the alkaloid group fed no Elitox had the lowest DMI compared to all other groups over time.

Initial liveweight was similar between nil and alkaloid groups, however, there was a strong tendency for liveweight to be higher in the 4 gram Elitox group compared with the 0 and 2 gram Elitox groups. Final liveweight was not affected by alkaloid, however, it was significantly higher in the 4 gram Elitox group compared with the 0 and 2 gram groups. Additionally there was an interaction between alkaloid x Elitox, such that liveweight was significantly higher in the alkaloid 4 gram treatment. Weight change was significantly lower in the alkaloid treatment compared with the nil treatment (1.0 v 2.8, respectively, sed 0.51, P=0.003). Further there was a significant alkaloid x Elitox interaction, such that the 0 gram alkaloid treatment lost weight over the treatment period compared to all other treatments (Table 4.35).

Water intake and urine output were not affected by alkaloid or Elitox, and there was no interaction between alkaloid x Elitox (Table 4.35). There was a strong tendency for water intake to vary over day for all treatments (P=0.071). There were no interactions for water intake. Urine output did not vary over time and there were no interactions.

Rectal temperature significantly increased in the alkaloid treatment compared with the nil treatment (Table 4.36). There was a tendency for rectal temperature to be lower in the 2 gram Elitox treatment compared with the 0 and 4 gram treatments, which were similar (Table 4.36). There was no interaction between alkaloid x Elitox. There were no main effects of alkaloid, Elitox or alkaloid x Elitox for respiration rate (Table 4.36). Skin temperature was significantly higher in the alkaloid treatment compared with the nil treatment (Table 4.36). There were no Elitox or alkaloid x Elitox effects detected (Table 4.36).

Table 4.35 Mean overall dry matter intake, initial liveweight, final liveweight, water intake and urine output recorded between days 14-21, for each treatment group.

Elitox (g/d)	Nil Alkaloid			Alkaloid			SED	P-value		
	0	2	4	0	2	4		Alk	Eli	AlkxEli
DMI (g/d)	1060	1059	1059	990	1022	1063	25	0.033	0.158	0.151
Initial liveweight (kg)	34.8	32.3	35.4	32.8	33.8	36.6	1.7	0.822	0.079	0.330
Final liveweight (kg)	38.0	35.4	37.7	32.4	34.9	39.1	1.8	0.161	0.032	0.036
Weight change (kg)	3.2	3.0	2.2	-0.4	1.1	2.5	0.8	0.003	0.292	0.020
Water intake (L/d)	3.9	2.5	2.9	4.6	3.3	3.9	1.0	0.166	0.203	0.978
Urine output (L/d)	1.9	0.78	0.78	1.3	0.64	1.25	0.65	0.846	0.154	0.544

Table 4.36 Mean rectal temperature, respiration rate and skin temperature over the entire treatment period, for each treatment group.

	Nil Alkaloid			Alkaloid			SED	P-value		
Elitox (g/d)	0	2	4	0	2	4		Alk	Eli	AlkxEli
Rectal temp (◦C)	39.21	39.14	39.33	39.75	39.33	39.50	0.15	0.004	0.102	0.196
Resp rate (b/min)	25	27	28	33	30	31	5	0.124	0.966	0.784
Skin temp (◦C)	38.05	38.00	38.10	38.52	38.11	38.25	0.17	0.024	0.191	0.308

Figure 4.42 Dry matter intake over time for each treatment group. Pooled standard error of the difference for alkaloid x Elitox x day = 46.4.

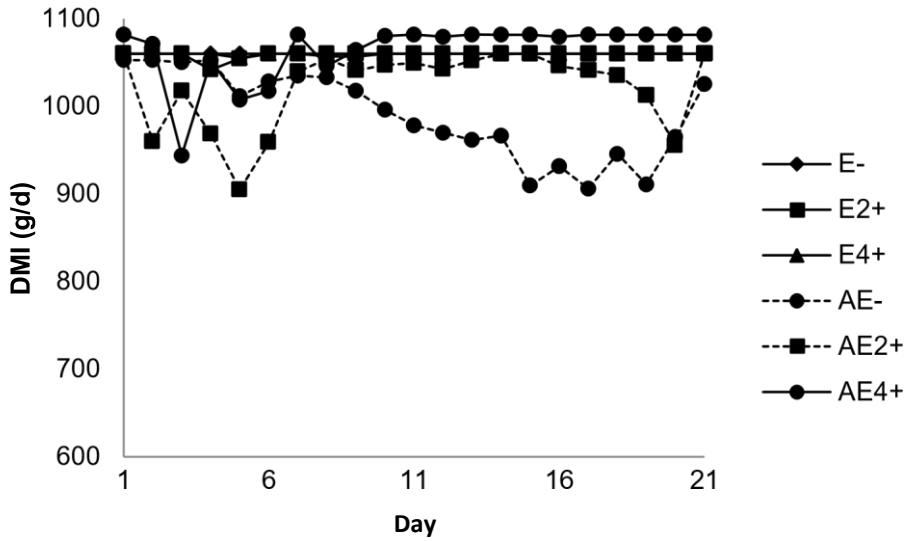


Figure 4.43 Mean rectal temperature over day for each alkaloid and Elitox treatment group. Pooled standard error of the difference for alkaloid x Elitox x day = 0.18.

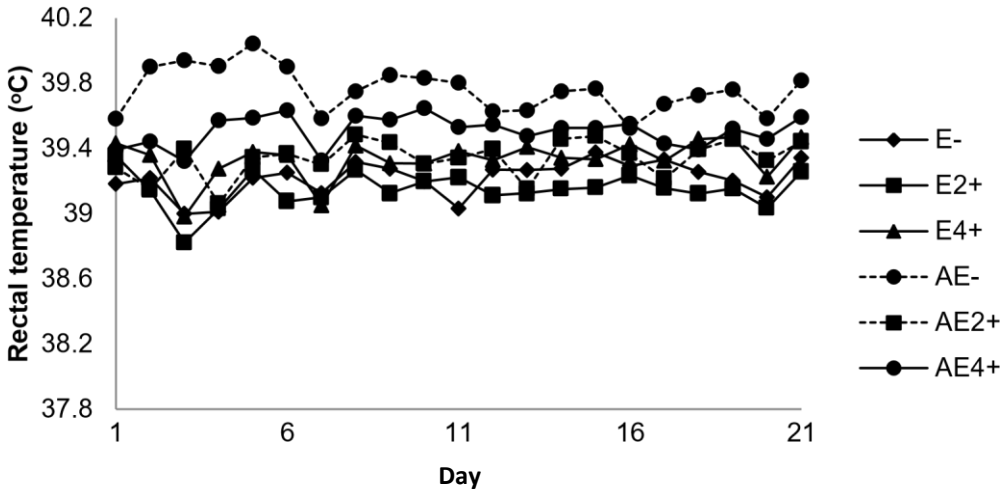


Figure 4.44 Mean respiration rate over day for each alkaloid and Elitox treatment group. Pooled standard error of the difference for alkaloid x Elitox x day = 6.0.

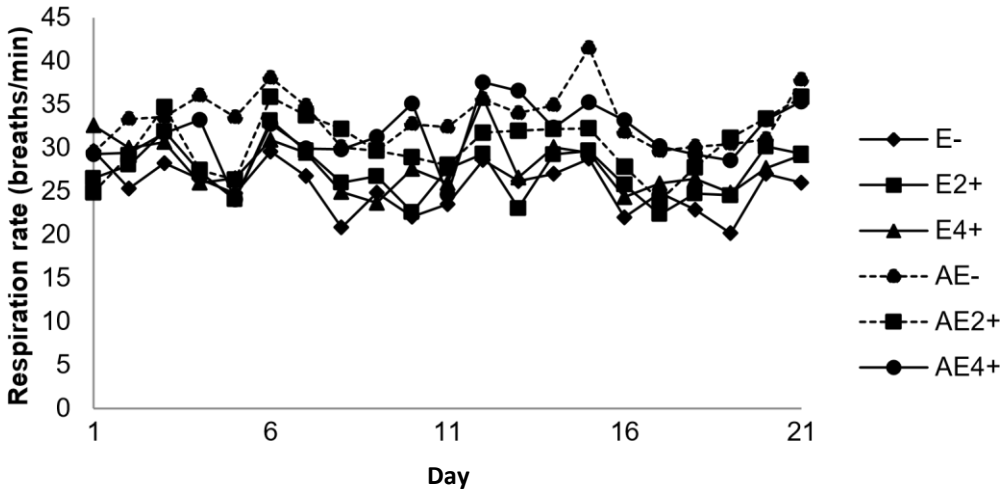
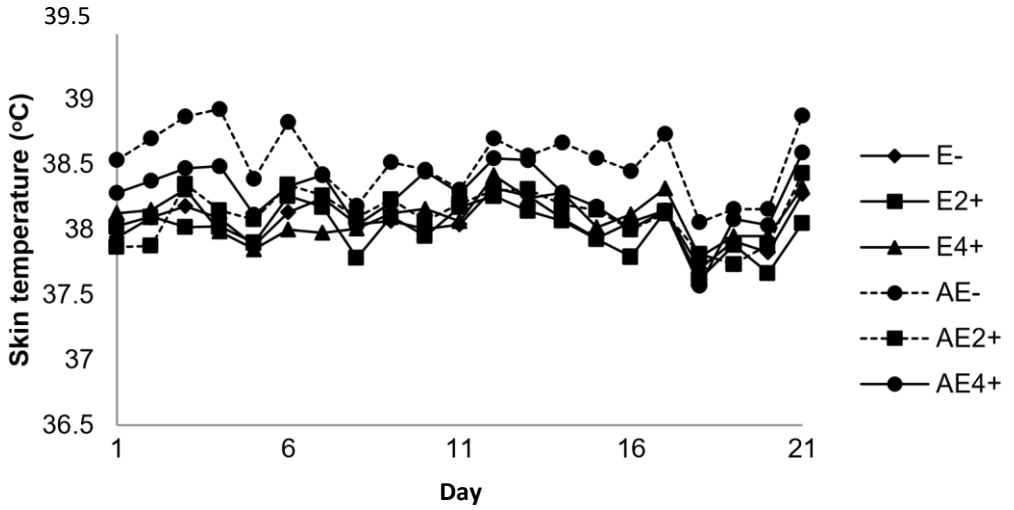


Figure 4.45 Mean skin temperature over day for each alkaloid and Elitox treatment group. Pooled standard error of the difference for alkaloid x Elitox x day = 0.23.



There was a significant day effect for rectal temperature (P<0.001), such that there was variability over time. There was a significant alkaloid x day interaction (P<0.001), such that the alkaloid treatment had higher rectal temperature throughout the treatment period compared with the nil treatment. There was a significant Elitox x day interaction (P=0.011), such that there was significant variability over time, and the 2 gram dose group had the lowest rectal temperature, along with the 4 gram dose group at times, however, the 0 gram and 4 gram dose groups were similar most of the time. There was a significant alkaloid x Elitox x day interaction (P=0.010), such that the alkaloid 0 gram Elitox treatment had significantly higher rectal temperature compared to all other treatment groups over time.

When analysed over day respiration rate was variable (P<0.001). There was a significant Elitox x day interaction (P=0.048), such that there was variability over time.

There was a significant alkaloid x day interaction (P=0.004) such that the alkaloid treatment had higher respiration rate over the entire treatment period compared with the nil treatment. There was no alkaloid x Elitox x day interaction detected (P=0.968).

There was a significant day effect for skin temperature (P<0.001), such that there was variability over time. There were no alkaloid x day (P=0.160) or Elitox x day (P=0.702) interactions. There was no alkaloid x Elitox x day interaction (P=0.294).

Discussion

Production response

Dry matter intake was significantly reduced in the alkaloid treatment, overall, compared with the nil treatment. In particular, DMI decreased during the first week of treatment in the alkaloid groups. The 2 gram and 4 gram Elitox alkaloid sheep recovered DMI after the first week, however, DMI remained low and variable in the 0 gram Elitox alkaloid sheep throughout the remainder of the treatment period. The increase in DMI in the 2 gram and 4 gram Elitox groups, after the initial decrease, and reduced DMI in the 0 gram group suggests Elitox can improve DMI in sheep consuming perennial ryegrass alkaloids. This may be

occurring due to Elitox binding alkaloids in the rumen, preventing adverse effects from occurring. Improved DMI due to Elitox, may benefit animal productivity by increasing average daily gain, fertility, reducing faecal dagging and the occurrence of fly strike.

The mechanism responsible for reduced DMI in animals consuming perennial ryegrass alkaloids is not clear. Higher physiological mechanisms such as the suppression of liver enzymes causing aminoacidemia resulting in increased tryptophan concentration may be occurring (Oliver *et al.,* 2000b). These increased concentrations act on the central nervous system, resulting in increased serotonin concentrations, the serotonin break down production of 5-hydroxyindolacetic acid (5IAA) and appetite suppression (Oliver *et al.,* 2000b). It has also been suggested that DMI may decreased due to bad taste (Munday-Finch and Garthwaite 1999) or smell (Layton *et al.,* 2004), however, in the current study Elitox improved DMI over time, therefore, if alkaloids have a bad taste or smell, increased DMI would not have been expected. Moreover, the author suggests negative feedback could be occurring in which the animal feels 'unwell' and associates this feeling with alkaloid consumption resulting in reduced DMI.

Conclusions regarding the effect of alkaloid consumption on DMI have been varied due to the grazing approach used in previous studies, which often precludes accurate measurement of DMI. DMI has not been affected by alkaloid presence in previous grazing studies (Fletcher *et al.,* 1999). Some reductions in DMI have been found in tall fescue toxicosis studies (ergot alkaloid effects only) with cattle, both when grazing (Bluett *et al.,* 1999b; Matthews *et al.,* 2005) and when fed infected seed (Wax *et al.,* 2007; Aiken *et al.,* 2009). However, the differences in research conditions (grazing versus indoor controlled studies, tall fescue versus perennial ryegrass, animal species, alkaloid concentration fed and environmental conditions) can influence results and they factors must be taken into consideration when comparing results. There has been little study into the effectiveness of alkaloid rumen binders in improving DMI. One study fed perennial ryegrass alkaloids and Elitox in a pellet and found variable results with the addition of Elitox decreasing DMI at times, thought to be due to palatability issues (Henry *et al.,* 2007). No other studies have measured DMI when feeding alkaloid binders.

Due to a strong tendency for the 4 gram Elitox to have higher liveweight initially, when final liveweight was recorded the 4 gram Elitox group had the highest liveweight. Change in liveweight indicated that in the presence of alkaloids, the Elitox treatments resulted in greater gains. Further, the 0 gram Elitox alkaloid treatment exposed to alkaloids lost weight throughout the treatment period, reflecting DMI. The 2 gram Elitox alkaloid treatment did not gain as much weight as the 4 gram Elitox alkaloid treatment or the nil treatments. This finding suggests that inclusion of Elitox in the diet of sheep consuming perennial ryegrass alkaloids may improve animal productivity, especially when fed a higher dose.

Previous studies investigating effect on growth have found reductions in ADG to occur when animals ingest alkaloid infected material (Fletcher and Barrell 1984; Bluett *et al.,* 1999b; Matthews *et al.,* 2005). However, these studies have been undertaken using a grazing approach and at times have reported small liveweight decreases of up to 10 g per day during the summer and autumn periods due to higher alkaloid concentrations compared to 3-4 g per day losses during spring when alkaloid concentration as lower (Fletcher 1999), however, the alkaloid concentration ingested was not reported. These differences in ADG are extremely low and hard to measure, questioning the accuracy of these results.

Previous studies have investigated the effectiveness of Mycofix®, mycotoxin binder in alpacas (Reed *et al.,* 2010) and lambs (Reed *et al.,* 2011a) under grazing conditions. The study using lambs found a tendency for improved growth rate of lambs fed Mycofix® and greater use of shaded areas of the paddock compared to the non-treated lambs (Reed *et al.,* 2011a). The study using alpacas found no effect on average daily gain, however, neurotoxic signs may have been reduced in those alpacas fed the binder (Reed *et al.,* 2010). It is clear that mycotoxin binders may be beneficial in reducing the effects of PRGT, however, results from previous studies have been variable and unclear at times, suggesting more research is needed to elucidate effects and potential benefits for livestock producers. Caution must be taken when comparing studies using different toxin binding products.

Water intake and urine output were not affected by alkaloid or Elitox in this study. There have been very few studies which have investigated water intake and urine output in sheep ingesting PRG alkaloids. Moreover most TFT studies have found no effect on urine output (Matthews *et al.,* 2005) which is consistent with the variable effects reported for water intake in these studies. Some previous work has found an increase in water intake and urine output in sheep fed PRG alkaloids under heated ambient temperature conditions (Henry *et al.,* unpublished). Therefore, the interaction between heated ambient temperature and alkaloid intake may be required to affect water balance in sheep.

Physiological response

Rectal temperature increased in sheep fed alkaloid versus the nil alkaloid diet (0.3ºC), however, respiration rate was not affected. When the 0 gram alkaloid group was analysed over time, this group had significantly higher rectal temperature compared to all other groups. This finding indicates that the consumption of Elitox at either 2 or 4 grams mitigates the heat stress effects of PRG alkaloids. Skin temperature increased in sheep fed alkaloid versus nil alkaloid diet (0.25ºC), however, Elitox did not alleviate this effect. The increase in skin temperature suggests peripheral blood flow was not altered as suggested previously in TFT studies (Osborn *et al.,* 1992). Skin temperature has been found to increase due to alkaloid ingestion in previous PRGT studies (Henry *et al.,* unpublished), questioning whether vasoconstriction occurs during PRGT. This finding suggests that sheep are able to adequately reduce heat load by dissipating heat through heat exchange through the skin. Moreover, unexpectedly respiration rate did not increase due to alkaloid consumption, suggesting even though core body temperature increased, this did not induce an increased respiration rate to dissipate heat, presumably due to sufficient heat loss through the skin.

Grazing based studies have measured the effect of infected PRG pasture on rectal temperature and respiration rate, indicating small increases occur during summer and autumn (Fletcher *et al.,* 1999), the current study provides more information when alkaloid dose is known and when an alkaloid binder is being fed.

Conclusion

The results from the current study demonstrate that feeding a moderate level of PRG alkaloids can decrease DMI significantly. This effect was countered by the consumption of Elitox, suggesting that Elitox may be binding alkaloids in the rumen, reducing alkaloid absorption and/or modifying gut function, therefore, dulling PRGT effects. Change in liveweight was lowest for the 0 gram Elitox alkaloid group due to decreased DMI in this group. This finding suggests that Elitox may play an important role in increasing the

production response in sheep fed PRG alkaloids, therefore mitigating adverse effects. Rectal temperature and skin temperature increased due to alkaloid consumption, and the 0 gram alkaloid Elitox group seemed to have higher temperatures over time, further demonstrating the benefits of Elitox in mitigating PRGT effects. Further investigation over a longer period of time is warranted to further elucidate the beneficial effects of feeding Elitox to sheep, in particular investigation into whether Elitox is able to reduce the severity of staggers and tremors in sheep. Elitox may be a useful tool for producers experiencing PRGT in mitigating effects in the short-term by maintaining animal production and reducing heat stress in sheep, while pasture renovation is undertaken over the longer term.

4.3On farm studies

4.3.1 2010-2011 On farm study

Trial 1 Elaine Central Western Victoria

Due to extremely wet climatic conditions during the spring and summer of 2010, some difficulty was experienced in identifying suitable trial sites to undertake on farm studies. Whilst wet conditions in summer often precede conditions suitable for the development of PRGT, the extreme rainfall resulted in overgrowth of bent grass (*Agrostis capillaries*) diluting the amount of perennial ryegrass present in pastures at potential trial sites. A suitable trial site was selected and set up in February 2011 at Elaine in central west Victoria.

Climate

Following setting up the trial site in February, extreme rainfall was recorded including one individual rain event of 136 mm in February. Rainfall at the closest BOM rainfall site highlights that above average rainfall was received between November and February preceding the start of the trial with below average rainfall over autumn and winter. Maximum temperate were below the long term average from January through autumn.

Table 4.37 Rainfall and percentile compared with long term at local BOM recording station 2010-11

	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Rainfall mm (Meredith Dara)	115	41	129	179	35	45	53	43	46	17	37	107
Approximate Percentile	90th	44th	96th	97th	43rd	45th	47th	40th	38th	15th	14th	86th
Average maximum temperature ^{0}C	20.3	23.8	25.6	23.1	20.9	18.8	13.8	13.3	12.3	15.0	16.8	18.6
Long term average ^{0}C	20.9	23.3	25.6	25.3	22.7	19.1	15.4	12.8	12.2	13.4	15.4	18.0

Pasture endophyte alkaloid levels

As a consequence of significant summer rainfall and cool temperatures with daily temperatures 1-2 0C lower than normal between February and May, luxuriant pasture growth occurred and pastures remained green with little pasture senescence occurring. The proportion of perennial rye grass reduced to about 10-15% compared with 35% in early summer when the trial site was selected due to over growth of bent grass and less desirable species. Table 4.38 below summarises endophyte alkaloid levels measured between February and the completion of the trial in October 2011.

Table 4.38 Average endophyte alkaloid levels of perennial ryegrass in trial 1

	Ergovaline mg/kg (toxic level >0.8-1.2)	Lolitrem B mg/kg (toxic level >1.8-2.0)	Ergotamine Ergocryptine Ergocryptine Ergosine
23/02/2011	0.46	1.23	Not tested
15/04/2011	0.36	1.95	Not detected
28/04/2011	0.16	1.01	Not detected
26/05/2011	0.17	0.81	Not detected
2/06/2011	<0.1	0.5	Not detected
24/08/2011	0.18	<0.1	Not detected
13/10/2011	0.13	0.13	Not detected

Initial ergovaline levels were moderate at the start of the trial and reduced at subsequent measurements whereas lolitrem B levels increased to be just above the toxic threshold in

April then dropped to low levels for the remainder of the trial period with the onset of cool moist weather.

Outbreaks of PRGT in South Eastern Australia have been associated with dominance of PRG with a combination of wet spring and summer followed by dry autumn conditions with above average maximum temperatures. Extreme outbreaks are usually associated high rainfall in spring-summer (>350 mm) that prolonged the period of high pasture growth rate and increased spring-summer growth and dry conditions in March and high average maximum temperatures in March (~23ºC) and April (~20ºC). Whilst the rainfall conditions were met in the spring and summer of 2010-2011, and rainfall was slightly below average in autumn, the maximum temperature was well below average and not conducive the development of PRGT.

Results

Weaners were examined weekly and more detailed measurements were recorded every 4- 8 weeks from the start of the trial in late February-March to October when lambs were shorn with 12 months wool.

Ingestion of Weather Shield – Sheep™ and Weather Shield – Sheep for ryegrass staggers™

Weaners quickly became accustomed to consuming Weather Shield which was located in troughs close to watering points. Weaners ingested about 15 grams per day of Weather Shield – Sheep for ryegrass staggers in the treatment groups and about 20 grams per day of Weather Shield – Sheep in the control groups. During autumn consumption rates were higher at about 25 grams per day for both groups but dropped in winter when conditions were wet and it was noticeable that ingestion dropped off more in the Weather Shield with Elitox toxin binder more over winter. These consumption estimates are approximate due to wastage around the troughs.

Clinical signs of PRGT

No clinical signs of PRGT were observed in any group during the trial. No temperature (Elitox 40.16^0Cand control 40.21^0C p = 0.374) or respiration rate (p = 0.183) differences were observed in sheep during autumn in treatment or control groups; although temperatures were slightly elevated and respiratory rates were elevated in both groups, this is possibly due to handling and mustering when measurements were taken.

Bodyweight observations

Bodyweights were measured during the trial period from March through to October. Table 4.39 below summarises the bodyweights and bodyweight changes of weaners during the trial (kg).

Table 4.39 Bodyweights and bodyweight changes of sheep during the trial 1 (kg)

Paddock	Treatment	BW March	BW April	BW change March April	BW June	BW change March June	BW August	BW change March August	BW October	BW change March October
1	Elitox	22.95	23.75	0.80	24.35	1.40	24.87	1.66	30.36	7.47
3	Elitox	23.23	24.75	1.53	26.63	3.40	26.28	2.72	31.39	8.16
5	Elitox	23.66	25.13	1.47	26.03	2.37	27.79	4.13	33.63	9.97
Total Elitox Group		**23.27**	**24.53**	**1.26**	**25.66**	**2.39**	**26.31**	**2.84**	**31.83**	**8.45**
2	Control	23.60	25.05	1.45	26.85	3.25	26.72	2.92	31.22	7.42
4	Control	22.70	24.50	1.80	26.25	3.55	28.25	5.28	33.92	10.94
6	Control	22.40	24.26	1.86	26.80	4.40	26.18	3.87	31.55	9.24
Total control Group		**22.90**	**24.60**	**1.70**	**26.63**	**3.73**	**27.04**	**4.02**	**32.22**	**9.20**
Difference between control and Elitox group				**p = 0.163**		**p = 0.001**		**p = 0.011**		**p = 0.198**

There were no significant differences in body weight and weight change between groups during the trial period though there was an advantage in favour of the control group during the March to June and the march to august period. It was interesting to note that plots 1, 2 and 3 visually had slightly inferior pasture composition with more overburden of rank feed (even though compared with plots 4, 5 and 6 measurements of pasture availability (Table 4.42) and pasture composition (Table 4.43) show little difference in pasture availability and quality between treatment groups. This could possibly explain the weight advantage for the control group.

Environmental conditions were unfavourable for the development of PRGT once the trial was set up meant there was unlikely to be any benefit of Elitox treatment compared with the control group.

Dags and worm egg counts

Worm egg counts (WEC's) were zero through autumn until July and were not substantially different between groups of sheep, through the trial. WEC's rose rapidly in mid-July which coincided with the cessation of activity of the long acting anthelmintic drench dosed before the start of the trial. All groups were drenched with Oxfendazole which was 100% effective on the property based on the most recent Worm Egg Count Reduction Trial and confirmed when worm egg counts were taken 10 days post drenching returned to 0 epg. Worm egg counts rose again in August about 5 weeks post drenching and were re-drenched again in late August. There were no differences in worm egg counts between groups during the trial period. Worm egg counts were high in winter which was not surprising given the extremely wet summer that is likely to have increased survival of worm larvae on pastures as well as reducing the efficacy of the summer drench program.

Weaners were crutched in mid-February just before the start of the trial so all weaners recorded a zero dag score on a scale of 1-5 and were still zero in April. Dag scores rose in winter though with the numbers, the differences were not significant, even though the Elitox group had slightly higher dag scores. Weaners were crutched again in September in preparation for shearing so no further measurements were recorded.

Table 4.40 Dag score results during trial 1

Paddock	Treatment	Dag score March	Dag score April	Dag Score June	Dag Score August
1	Elitox	0	0	0.70	1.78
3	Elitox	0	0	0.45	1.17
5	Elitox	0	0	1.05	1.92
Total Elitox Group		**0**	**0**	**0.73**	**1.62**
2	Control	0	0	0.25	0.44
4	Control	0	0	0.80	1.44
6	Control	0	0	0.55	1.32
Total control Group		**0**	**0**	**0.53**	**1.06**
Difference between control and Elitox group		**ns**	**ns**	**p = 0.327**	**p = 0.094**

Fleece weight measurements

All sheep in the trial had greasy fleece weight and fibre diameter measurements recorded at shearing. There was no significant difference in Greasy fleece weight or fibre diameter recorded between groups. These results are consistent with body weight results.

Table 4.41 Summary of fleece measurements of groups in trial 1

Paddock	Treatment	Greasy Fleece Weight (kg)	Mean Fibre Diameter (Micron)	Coefficient of Variation FD (%)
1	Elitox	1.77	14.85	20.50
3	Elitox	1.90	14.63	20.30
5	Elitox	1.92	14.72	21.23
Total Elitox Group		**1.86**	**14.73**	**20.69**
2	Control	2.02	15.12	22.24
4	Control	1.92	14.98	20.68
6	Control	1.95	14.84	20.05
Total control Group		**1.96**	**14.98**	**21.01**
Difference between control and Elitox group		**p = 0.362**	**p = 0.171**	**p = 624**

Pasture measurements

Pasture availability was exceptional during the trial due to extreme summer rainfall with pasture growth and availability continuing to be well above average in autumn. There were no substantial differences in the amount of pasture available between groups as is listed in Table 4.42 below.

Table 4.42 Pasture available (kg DM/ha) on plots in trial 1

		March		**June**		**August**		**October**	
Pasture Availability		Green	Dead	Green	Dead	Green	Dead	Green	Dead
1	Elitox	1650	2200	1100	1700	900	1500	1150	1400
3	Elitox	1600	2200	1050	1800	800	1700	1200	1300
5	Elitox	1700	1900	1200	1650	900	1600	1300	1450
2	Control	1600	2100	1000	1700	800	1600	950	1400
4	Control	1800	2000	1150	1600	900	1650	1200	1400
6	Control	1700	2200	1050	1800	850	1750	1100	1500

Whilst there was abundant pasture during the trial, pasture quality was relatively poor. When the trial site was selected, the proportion of perennial rye grass was estimated to be about 35%. Due to exceptional summer rain there was an enormous growth of bent grass and other less desirable species so apart from the proportion of perennial rye grass diminishing, the endophyte levels were lower than desirable hence no clinical or sub clinical PRGT was observed or measured. Table 4.43 summarises pasture composition of the plots in trial 1.

Table 4.43 Pasture composition of trial plots in trial 1.

Pasture Composition March		**Perennial Rye Grass**	**Bent**	**Sweet vernal, Silver grass, Fog**	**Sub Clover**	**other**
1	Elitox	30%	33%	31%	7%	0%
3	Elitox	10%	37%	51%	3%	0%
5	Elitox	27%	37%	27%	3%	6%
2	Control	17%	47%	33%	3%	0%
4	Control	27%	27%	36%	7%	3%
6	Control	13%	30%	53%	3%	0%

Table 4.44 Pasture composition at the end of the trial

Pasture Composition October		Perennial Rye Grass	Bent	Sweet vernal, Silver grass, Fog	Sub Clover	Other
1	Elitox	27%	27%	30%	9%	6%
3	Elitox	12%	27%	53%	8%	0%
5	Elitox	30%	28%	23%	18%	3%
2	Control	13%	23%	50%	7%	6%
4	Control	29%	24%	43%	12%	3%
6	Control	16%	22%	56%	6%	0%

Summary

Whilst climatic conditions were potentially suitable for the development PRGT when the trial site was selected, an extremely wet and cool climatic period between January and late Autumn resulted in conditions unfavourable for the development of PRGT. Whilst endophyte alkaloid measurements approached toxic levels at the start of the trial there was no clinical evidence of PRGT. As a consequence the efficacy of Elitox toxin binder in Weather Shield™ for sheep could not be could not be determined in this trial.

4.3.2 2011-2012 On farm study

During 2012, two trials were proposed, one in South Gippsland near Yarram on a property that regularly experienced severe PRGT in merino sheep and one in central western Victoria near Mortlake that regularly has PRGT in yearling cattle grazing Victorian perennial rye grass. Both trials were abandoned in 2012 for different reasons. The trial in western Victoria was abandoned due to near drought conditions were significant supplementary feeding was necessary diminishing the

likelihood of PRGT occurring. The trial near Yarram was abandoned due to wet conditions (even though severe PRGT was occurring) causing an outbreak of severe facial eczema. An alternative site was identified and a modified trial was set up in late summer near Lancefield (Trial 2) in North Central Victoria.

Trial 2 Baynton North central Victoria

Climate and pasture

The trial was subdivided in late summer 2012. After above average rainfall in 2011, the summer was relatively dry until late February/March when a one off rainfall event resulted in significant pasture growth in March. Subsequently, April and May were relatively dry. Temperatures were about average to slightly below average during late summer and autumn though temperatures were in the range regarded as high risk for inducing PRGT.

Table 4.45 Rainfall and percentile compared with long term at local BOM recording station (Baynton) 2011-12.

	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Rainfall Baynton	86	41	47	65	102	16	42	93
Approximate Percentile	80th	42th	66th	82nd	96th	20th	40th	75th
Average maximum temperature ^{0}C 11-12	24.7	26.8	30.0	28.5	23.1	21.2	16.0	13.1
Long term average ^{0}C	23.5	26.3	29.2	28.7	25.0	20.4	16.0	12.7

Green pasture availability increased from about 300 kg DM/ha in February to 1600 kg DM/ha in March then gradually declined to 800 kg DM/ha by June. The amount of dead dry matter reduced from about 2000 kg/ha in February to 1100 kg DM/ha in June 2012. Victorian perennial rye grass contributed over 75% of the pasture sward throughout the trial period.
Pasture samples were collected and measured ergovaline and Lolitrem B levels. Table 4.46 summarises the alkaloid levels measured in ryegrass samples collected during the trial period.

Table 4.46 Endophyte alkaloid levels measured in perennial ryegrass samples collected between March and June 2012.

	Ergovaline mg/kg (toxic level >0.8-1.2)	Lolitrem B mg/kg (toxic level >1.8-2.0)	Ergotamine Ergocryptine Ergocryptine Ergosine
16/03/2012	0.39	1.2	Not tested
16/04/2012	0.23	1.3	Not detected
26/04/2012	0.69	1.87	Not detected
7/06/2012	0.38	0.92	Not detected
28/06/2012	0.32	0.57	Not detected

Animal measurements

Clinical signs of PRGT

Clinical rye grass staggers was observed in merino weaners grazing the paddock in early January before the trial was set up. With heavy late summer/early autumn rain staggers stopped but re-emerged in mid-May for about one month and stopped in late June when the trial finished. The number of sheep with staggers was higher in the control group throughout this period and the severity of staggers was more severe in the control group with 23% of sheep observed with staggers had a staggers score of 4/5 in the control when the number of sheep with staggers peaked in late May to early June and only one sheep (8%) had a staggers score of 4/5 in the Elitox group at this time. The number of weaners with staggers was assessed in the paddocks rather than handling sheep over the period when staggers was evident between mid-May to mid-June so it is likely the numbers observed with staggers is an under-estimate as weaners with mild staggers could have been missed. No other behavioural signs or differences between groups were observed over this period, in terms of evidence of sheep seeking shelter or standing around water, presumably because temperatures were cool by mid-May and Ergovaline levels were only marginally toxic during this

period. Lolitrem B levels peaked in late April and this coincided with the observation that staggers occurred in May. Table 4.47 summarises the staggers observations during the trial.

Table 4.47 Staggers score >1 (scale 0-5)

	March	April	Early May	Mid May	Early June	Mid June
Control	0/81	0/81	0/81	13/81	22/81	2/81
Treatment Elitox	0/81	0/81	0/81	3/81	12/81	2/81
	ns	ns	ns	p = 0.002	p =0.002	ns

Table 4.48 Average rectal temperature observations

	March	April	June
Control	40.0	39.9 se = 0.05	40.3 se = 0.044
Treatment	40.1	40.0 se = 0.073	40.1 se = 0.048
		p = 0.073	p = 0.026

Small temperature differences were observed during the trial between the control and Elitox treatment group with a significant difference favouring the Elitox group in June.

Bodyweight observations

There was no apparent benefit in terms of weight grain of the Elitox group throughout the trial. Table 4.49-4.51 below summarises body weight, weight change and weaner growth rates throughout the trial. No May measurements were recorded due to clinical staggers occurring.

Table 4.49 average bodyweights of weaners (kg) during the trial provided access to Elitox compared with control

	March	April	June
Control	22.6	27.5	29.8
Treatment Elitox	22.3	26.7	28.4

Weaners in both groups grew well consistent with available pasture during the trial period, with initial growth rate increases very good

Table 4.50 Weight changes (kg) during the trial provided access to Elitox compared with control

	March- April	April-June	March - June
Control	4.9	2.3	7.28
Treatment Elitox	4.4	1.6	6.01
			P<0.001

Table 4.51 Average growth rates g/day of weaners during the trial provided access to Elitox compared with control

	March- April	April-June
Control	189	33
Treatment Elitox	171	23

Weaner growth rates were initially very high due to high pasture availability but reduced during autumn due to reducing pasture availability. There was no apparent weight gain benefit of providing Elitox in a lick observed during this trial, even though clinical staggers was observed late in the trial period. In fact, there was a significant difference in weight gain favouring the control group that persisted for the trial. The reason for the difference is uncertain gain weaners were grazing the same pasture but swapped every week.
No differences in worm egg counts or dag formation was observed between groups during the trial.

Environmental and pasture conditions were suitable for the development of PRGT during the trial period. Pasture endophyte alkaloid levels exceeded toxic levels for a period from April 2012 during Trial 2. Whilst there were no production differences recorded in favour of the Elitox treatment group during the trial, the lower level of staggers observed in the treatment group would make a significant difference to the management of sheep during a staggers outbreak if sheep could not be removed to safe pastures as is often the case in commercial farming situations.

4.3.3 2012-2013 On farm study

Trial 3 Mortlake Western Victoria

A large 5,000 hectare beef cattle property near Mortlake in western Victoria was selected with a significant history of PRGT causing significant animal health and management issues, especially in weaned calves. The initial trial was intended to start in 2012 but due to very dry conditions the trial was postponed until 2013.

Climate and pasture

After a dry and warm finish to the spring and early summer in 2012, it was decided to set up the trial even though conditions were dry in early summer as some December rain induced some pasture growth. Conditions remained very dry in summer and autumn with well above average temperatures. Table 4.52 summarises rainfall and temperature conditions during the trial.

Table 4.52 Rainfall and percentile compared with long term at local BOM recording station (Baynton) 2011-12.

	Nov	Dec	Jan	Feb	Mar	Apr	May
Rainfall mm Mortlake	26.0	45.0	1.6	22.6	16.6	11.6	60.8
Approximate Percentile	21st	60th	0	27th	25th	0	65th
Average maximum temperature ^{0}C 12-13	21.9	24.5	28.5	29.3	27.8	21.1	17.2
Long term average ^{0}C	20.8	23.2	25.7	26.3	23.5	19.9	16.1

The trial was set up in February. At the start of the trial pasture availability was about 1,500 dry matter per hectare with less than 50 kg/ha green feed. The green pick was mostly Victorian perennial ryegrass. Pasture availability steadily reduced to about 600 kg DM per hectare in May at the time of the opening autumn rains. The composition of the pasture was consistently about 60% Victorian perennial rye grass, 20% Australian phalaris with the remaining pasture annual grasses. Due to limited pasture availability, heifers were supplemented with about 15-20kg of barley and cereal hay to maintain liveweight.
Pasture samples were collected and measured ergovaline and Lolitrem B levels. Table 4.53 summarises the alkaloid levels measured in ryegrass samples collected during the trial period.

Whilst Ergovaline levels were consistently in toxic levels, the total amount of dry matter ingested from pasture by the weaner heifers was small due to limited pasture availability.

Table 4.53 Endophyte alkaloid levels measured in perennial ryegrass samples collected between February and March 2013.

	Ergovaline mg/kg (toxic level >0.4-0.8)	Lolitrem B mg/kg (toxic level >1.8-2.0)	Ergotamine mg/kg	Ergocryptine mg/kg	Ergocornine mg/kg	Ergosine mg/kg
25/02/2013	0.8	1.40	Not tested	Not tested	Not tested	Not tested
28/03/2013	0.62	0.66	0.19	<0.1	not detected	0.17
20/04/2013	0.72	0.51	not detected	0.85	0.84	0.42

Animal measurements

Clinical signs of PRGT

During the trial period there was no evidence of staggers or differences in behaviour in the treatment and control groups. Temperature measurements whilst high in the high end of the normal range were similar during the trial period with no differences between the Elitox and control group.

Bodyweight observations

Due to the very dry conditions only two measurements were recorded at the start of the trial in February and at the time of the autumn break in May. Table 4.54 below summarises average weights of the two groups and weight changes. There was no significant difference between groups measured during the trial period though the trend was for the Elitox group to have a higher weight gain.

Table 4.54 Average weights and weight changes (kg) between treatment (Elitox) and control heifers during the trial period

	February	May	Weight change
Control	262.1	271.8	9.7 (se = 1.699)
Treatment Elitox	260.7	272.5	11.7 (se = 1.826)
			p = 0.411

Whilst conditions were potentially suitable for the development of PRGT at the start of the trial, rapid deterioration in climatic conditions resulted in significant supplementary feeding and whilst pastures were potentially toxic (ergovaline), no clinical PRGT was observed nor any production differences observed between the Elitox treatment group and the control nil treatment group. This was presumably due to the limited amount of pasture ingested in comparison with supplementary feed.

Trial 4 Yarram South Gippsland

Climate and pasture

Rainfall on the property from late spring was slightly below average, but December, February and March rain resulted in rain resulted in some pasture growth. The area selected for the trial was not grazed over summer as the area required repairs of the watering system and paddock subdivision.

Table 4.55 Rainfall and percentile compared with long term at local BOM recording station (Yarram airport) 2012-13.

	Nov	Dec	Jan	Feb	Mar	Apr	May
Rainfall mm Yarram	27.4	55.6	*22.4*	47.0	*45.2*	*32.4*	*37.2*
Approximate Percentile	25th	59th	20th	66th	64th	32nd	40th
Average maximum temperature ^{0}C 12-13	21.6	24.1	*26.1*	25.5	*26.0*	*20.1*	*17.9*
Long term average ^{0}C	22.4	22.9	26.0	24.3	23.4	20.3	16.9

In March at the start of the trial, pastures availability was have about 1,100 kg green DM/ha plus 1,400 kg dry DM/ha, of which 90% was Victorian Perennial Rye Grass (cv Victorian, Ellet), the remaining pasture included annual grass barley grass and silver grass, capeweed and sub clover. Green pasture availability remained at a similar level during the trial, though the amount of dead dry matter reduced to about 1,000 kg DM/ha

Pasture samples were collected and measured ergovaline and Lolitrem B levels. Table 4.56 summarises the alkaloid levels measured in ryegrass samples collected during the trial period. Ergovaline levels were consistently at moderate levels during the trial, and Lolitrem B levels were just below levels considered to be toxic for sheep.

Table 4.56 Endophyte alkaloid levels measured in perennial ryegrass samples collected between February and April 2013.

	Ergovaline mg/kg (toxic level >0.8-1.2)	Lolitrem B mg/kg (toxic level >1.8-2.0)	Ergotamine mg/kg	Ergocryptine mg/kg	Ergocornine mg/kg	Ergosine mg/kg
9/02/2013	0.38	1.19	not detected	not detected	not detected	not detected
24/03/2013	0.41	1.1	not detected	not detected	not detected	not detected
11/4/2013	0.43	1.32	not detected	not detected	not detected	not detected

Animal measurements

Clinical signs of PRGT

During the trial, only a few sheep were observed with staggers and only when sheep were handled. When sheep were moved between paddocks no staggers or abnormal behaviour was observed. At the final recording in May 5% of the untreated control sheep showed evidence of staggers and 2% of the sheep with access to Elitox in their lick. Table 4.57 below summarises the staggers score of sheep in the trial in May at the final measurement

Table 4.57 Staggers score of sheep (0-5 scale)

	Score 0	Score 1	Score 2	Score 3	Score 4	Score 5
Treatment Elitox group	92	2	0	0	0	0
Control	86	1	3	1	0	0

Whilst the number of sheep with clinical staggers was very low, there was a slight trend in favour of the control sheep to have more staggers (5%) compared with the sheep with access to Elitox (2% with staggers). Using a Wilcoxon signed rank test the difference in staggers between the Elitox and control group was not significant (p = 0.0975 2-tailed). p=0.0295 Control staggers > Elitox group staggers. p = 0.9705 Elitox staggers > Control group staggers.

Table 4.58 Average rectal temperature observations

	March	May
Control	39.6	39.7 se=0.098
Treatment Elitox	39.6	39.5 se=0.157
		p = 0.277 ns

There was not a significant difference in temperature observed during the trial between the control and Elitox treatment group.

Bodyweight observations

The sheep grew very well over the period of the trial with the control sheep gaining 6.6 kg and the Elitox treatment group gaining 7.0 kg per head. A summary of bodyweights is outlined below in Table 4.59.

Table 4.59 Average weights (kg) and weight changes between treatment (Elitox) and control weaners during the trial period.

	March	May	Weight change
Control	37.5	44.1	6.6 se=0.228
Treatment Elitox	37.3	44.2	7.0 se=0.253
			p = 0.309 ns

No differences in worm egg counts or dag formation was observed between groups during the trial with the average dag score (scale 0-5) commencing at dag score 0.4 and increasing to 1 over the duration of the trial in the control group and ranging from dag score 0.3 at the start of the trial and increasing to 1 during the trial.

Discussion and conclusions

During the on farm trials run on 4 farms over 3 years there were few differences noted between the sheep and cattle grazed on potentially toxic perennial rye grass pasture given access to a stock lick with and without Elitox toxin binder (Feedworks) Weather Shield – Sheep/cattle or for Weather Shield – Sheep/cattle ryegrass staggers™ respectively.

Climatic conditions at selected trial sites, in all years, either became too wet, promoting fresh pasture growth low in toxins, or too dry resulting in insufficient toxic PRG pasture, after the trials

commenced. This is despite the sites being initially selected based on a significant history of PRGT and with pastures initially containing a high proportion of Perennial rye grass with wild type endophytes producing alkaloids. When provided access to licks, stock consumed the licks, though it was not possible to measure if all sheep and cattle consumed the licks at recommended quantities. Productivity differences including bodyweight, fleece weight or dag score of sheep were not substantial even where clinical staggers was observed for a short period in late autumn between control and Elitox groups in these trials.

In one trial (Trial 2) at Baynton substantial clinical differences in staggers developed between the control and Elitox group with the staggers more severe and affecting more sheep in the control group. Whilst this result was only observed in one trial, Elitox toxin binder may offer a very useful management tool to minimise the management impact of PRGT where staggers occurs. In major staggers outbreaks the direct management impact of PRGT can be very large, with extra costs including labour, supplementary feeding, additional fencing and water infrastructure costing up to $2.50/dse. The cost benefit of using the products will depend on the cost and the amount of time during the year that stock need to be supplemented. If products such as Elitox can minimise these management costs, producers will have an important tool to minimise the financial impact of PRGT. Further field investigations when livestock are grazing toxic endophyte alkaloids would be valuable to determine how effectively Elitox can minimise the production losses due to PRGT including weight loss, mortalities, dags in sheep and fertility. Whilst in the long term, changing pasture cultivars and species to either perennial rye grass with safe endophytes or other species will potentially eliminate PRGT, given such large areas of Australia have established perennial ryegrass with wild type endophytes, the need for reducing the impact of PRGT with products such as Elitox in the long term will be critically important to managing the financial and production impact will be critical for livestock producers.

On farm strategies to reduce the financial impact of PRGT

Based on previous estimates of the cost of PRGT is substantial. Sackett et al AHW.089 (2006) estimated that the total cost of PRGT was about $72.3M. In this report he cost of a severe outbreak was estimated to be $12.15 per head for merino sheep flock which when allowing for the frequency of PRGT outbreaks, results in an annual average cost of $4.78 per head. The cost of a severe outbreak in a prime lamb flock is $20.86 per head which equates to an annual average cost of $4.90 per head. The cost of an outbreak in beef herds which tend to be less severely than sheep flocks is estimated to be $4.98 per head which translates to an annual average cost of $1.00 per head for cattle.

In light of the current research, the potential cost of PRGT is likely to be greater due to subclinical effects of PRGT in three important areas including:

- Milk production 13% lower in sheep – this is similar to estimates recorded in the New Zealand dairy industry. Lower milk production will result in lower lamb (or calf) growth resulting in lambs sold as suckers lighter bodyweight and replacement weaners lighter thus more vulnerable to weaner illthrift and mortality in summer
  - o Estimated weight of lambs 2.4 kg lower
- Adult sheep with consistently lower bodyweight when grazing toxic pastures even when clinical staggers is not apparent. This has several potential effects including:
  - o Lower bodyweight of adult sheep so lower sale value (~2 kg)
  - o Lifetime wool impact of ewes consistently in lower body condition estimated to be 0.1 kg clean and 0.3 micron broader.

o Lower conception rate in ewes due to lower body condition of ewes likely to be an additional 2-4%

It is likely that subclinical production losses are more significant on properties that already have a high risk of PRGT. Using a gross margin analysis, using 2012 commodity prices and incorporating subclinical production penalties the cost of PRGT the cost of PRGT is likely to be substantially more than previous estimates. Table 4.60 below summarises the risk ($/head) for different sheep classes and different relative risk of PRGT including ongoing subclinical losses with high PRGT risk flocks.

Table 4.60 Estimated cost of PRGT for different classes of sheep with different risk of PRGT ($/head)

	High risk	Medium risk	Low risk	Average $/head
Merino	**-$9.25**	**-$2.21**	**-$0.09**	**-$4.22**
Dual purpose	**-$12.01**	**-$3.45**	**-$0.21**	**-$7.18**
Prime lamb	**-$16.70**	**-$4.63**	**-$0.27**	**-$9.59**
Across breed				**-$5.33**

On farm strategies to minimise the potential financial costs of PRGT include:

- Replacing toxic pastures with either perennial rye grass with safe endophytes such as AR37 or alternative pasture species
- Managing an outbreak with toxin binding compounds such as Elitox or providing Elitox as a longer term strategy
- Removing sheep from toxic pastures during high risk times or managing sheep intensively during an outbreak

Replacing toxic pastures with either perennial rye grass with safe endophytes such as AR37 or alternative pasture species

The logical long term solution to eliminate the risk of PRGT in high risk wild endophyte perennial ryegrass pastures is to change pasture species from toxic pasture to either a safe endophyte such as AR37, Endo 5 or AR1, that either have minimal or no effect on animal performance whilst protecting against insect attack and ensuring long term plant persistence. An added potential benefit of changing to either a cultivar of perennial rye grass with a safe endophyte or different pasture species such as phalaris, fescue or cocksfoot may be improved plant productivity and animal performance.

The risk associated with changing cultivar or pasture species is that the new pasture may fail to establish, the new pasture is outcompeted with residual wild type perennial ryegrass, the new

pasture has an alternative toxic element such as phalaris toxicity or staggers or the new pasture is less productive or of lower quality or both. All risks are possible but can be minimised with careful management and selection of appropriate replacement pasture.

The financial success of the changeover to a safe pasture species will depend on the:

- current enterprise profitability
- current financial cost of PRGT
- the cost of replacing the existing toxic pasture
- the persistence of the replacement pasture
- the improved productivity of the replacement pasture

To examine the financial benefit of replacing the toxic pasture to a safe pasture a model was set up to compare the base cost of livestock grazing PRGT with new pasture. Sensitivity analyses were conducted comparing the cost of renovation, increase in carrying capacity with replacing pasture, financial impact of PRGT and profitability of enterprise.

The base assumptions for the analysis were

- Base gross margin - $28/dse (long term sheep enterprise in SWMFP DEPI Vic)
- Capital value of stock $50/dse
- Stocking rate on toxic wild type perennial ryegrass pasture - 14 dse/ha
- Stocking rate in year 1 - 50% of base
- Analysis runs for 10 years
- Cost of renovation - $300/ha (includes herbicide $ insecticide, seed, sowing & spraying costs, fertiliser) range $250-$400/ha – note that a cleanup strategy to reduce the seed bank of perennial ryegrass with wild type endophyte may take up to 0-2 years in some situations
- Fertiliser application 0.8 kg/dse
- Stocking rate increase range – 0 to 8 dse/ha
- Cost of PRGT on toxic pasture $0 - $8/dse per annum
- Discount rate - 6%

The net present value $/ha of the base pasture was $2,278/ha. The net present value with a discount of between $0 to $8/dse is listed in table 4.61 below.

Table 4.61 net present value $/dse of base pasture with varying discount of PRGT applied.

PRGT discount $/dse	$0	$2	$4	$6	$8
NPV $/ha	**$2278**	**$2072**	**$1866**	**$1660**	**$1454**

When considering the investment in replacing the pasture, the net present value of the investment depends on the increase in stocking rate and the cost of the replacement pasture.

Table 4.62 change in stocking rate dse/ha compared with base pasture (14 dse/ha) v's cost of renovation $/ha

Change dse/ha	-2	0	2	4	6	8
$250/ha	$1,419	$1,746	$2,072	$2,398	$2,724	$3,050
$300/ha	$1,369	$1,696	$2,022	$2,348	$2,674	$3,000
$350/ha	$1,319	$1,646	$1,972	$2,298	$2,624	$2,950
$400/ha	$1,269	$1,596	$1,922	$2,248	$2,574	$2,900

Table 4.62 shows the large range in return on investment with different cost of investment and consequent increases in stocking rate. In the resulting pasture carries 2 dse/ha less stock than the original pasture that carried 14 dse/ha, (unlikely unless poor cultivar selected or pasture renovation fails) and the renovation cost was $250/ha, the net present value would only be similar if the base pasture had severe PRGT with an annual cost of $8/dse (worst case scenario). In this case the base pastures NPV is $1454/ha and the renovated pasture costing $250/ha and the resulting stocking rate is 2 dse/ha less the NPV in $1419/ha.
If a more typical situation where the annual cost of PRGT costs $4/dse the NPV of the base pasture is $1866/ha, the return on investment from changing the pasture would be better than doing nothing if the increase in stocking rate is only 2 dse/ha at any cost of renovation listed.

If there is not penalty due to PRGT (NPV in base pasture $2278/ha) the stocking rate must increase by at least 4 dse/ha as long as renovation costs less than $350/ha.

If the penalty caused by PRGT is large, the required increase in stocking rate from renovation is relatively small to achieve a satisfactory return on investment. The bigger the penalty due to PRGT, the smaller the increase in stocking rate required. Producers with experience of severe PRGT are starting to adopt more extensive renovation programs to reduce their exposure to PRGT where a high proportion of their pastures have perennial ryegrass infested with wild type endophyte.

Managing an outbreak with toxin binding compounds such as Elitox or providing Elitox as a longer term strategy

Whilst the on-farm studies during this project did not measure substantial production benefits of supplementing stock primarily due to seasonal conditions unfavourable for the development of PRGT there were substantial benefits observed with regard to minimising the impact of staggers in sheep where there was a 2-3 fold reduction in the level of staggers observed. Controlled studies have noted substantial differences in sheep behaviour, feed intake and cardinal signs (Temperature

and respiration rate) indicate that there are likely production benefits to be gained by supplementation with Elitox toxin binder. Considering the staggers benefit alone, products such as Elitox offers substantial management flexibility if supplementing at risk sheep during high risk period. This include being able to graze larger areas of the farm, enable handling sheep at critical times such as drenching or fly control whilst reducing potential losses due to staggers. The alternative may be to either destock sheep from at risk pastures the cost of destocking and either feedlotting or heavy paddock feeding may be up to $4/dse/month during high risk periods. The cost of Elitox is likely to be substantially less than this.

The alternative of living with PRGT in medium to high risk situations, especially where ongoing subclinical losses are evident is likely to be a high risk, low profit option.

5. Success in achieving objectives

5.1Grazing study

1. *Assess the agronomic benefits of novel endophytes (AR1 and AR37) compared with wild type endophyte-infected ryegrass over two seasons.*

AR1, AR37 and WT endophyte infected perennial ryegrass has been assessed under two different types of sheep (Merino and first cross) over a total of 7 grazing periods. In the vast majority of instances there was no significant difference between the endophyte treatments with respect to pasture growth rate, pasture mass while being grazed, pasture quality (DMD and CP) and proportion of dead and green matter in the sward. On the occasions where there were significant differences between endophyte treatments, the effect was small, transient and did not affect the total amount of pasture mass in the treatment area. This project has established quite clearly that, with reasonable grazing management neither the use of AR1 nor AR37 provided a dis-benefit compared to WT ryegrass with respect to total pasture mass and pasture growth rate. This agronomic assessment of these endophytes necessarily occurred in an animal trial context and was not specifically set up specifically to test just the agronomy. Other trial designs are better suited to this kind of work however it is worth noting that a recent review of trials specifically designed to investigate endophyte effects, Hume and Sewell (in print) found that endophyte infection never caused a statistically significantly negative result and was often was associated with a significant positive effect in an agronomic parameter. In this project although there were no observed agronomic advantages to the novel endophytes AR1 and AR37 over WT, there were significant animal production and welfare benefits that should make these options highly attractive to produces.

2. *Assess the physiological and productivity responses of young Merino ewes and pregnant and lactating Crossbred ewes grazing the different endophyteinfected ryegrasses over two seasons.*

Working with Merino and cross bred ewes, the project spanned three summerautumn periods – a time of year known as a high PRGT risk period, and also ran a cross bred ewe and lamb grazing period during one spring-summer period. In total there were seven distinct grazing periods over which the productivity and physiological responses of sheep were assessed and on that basis the project has significantly exceeded the requirements of this project and this particular objective. Across the duration of the project the following summarized significant results occurred (not necessarily in all trials):

- WT endophyte resulted in lower live weights – with either AR37 or AR1 having better live weights, depending on the trial
- Rectal temperatures and respiration rates were generally higher in the WT treatment although not always for both parameters at the same time.
  Sometimes AR37 also had elevated respiration rates.

- When severe staggers occurred, they occurred most rapidly and were most severe in WT, developed less rapidly and were less severe in AR37 and were completely absent from AR1
- WT increased dags score in merino ewes

3. Determine whether endophyte alkaloid consumption during pregnancy alters milk production and lamb performance in year 1, and subsequent reproductive and lactation performance of the ewe lambs in year 2.

This study commenced in 2011 and preliminary results found a trend for better milk production in ewes grazing AR1 compared to AR37 or WT however it was not significant. The first cross ewes and the female cross bred lambs birthed and raised on AR37 finished the grazing period with higher live weights than WT or AR1 however male lamb live weights were not significantly different.

Unfortunately we were unable to generate sufficient numbers of female lambs from each treatment to allow future robust trials so an alternative approach was taken to investigating ewe and lamb development in a more controlled setting. A long term controlled feeding pen study was undertaken in 2012 and is reported on in the indoor studies section of this report. In short however this study found that exposure of pregnant ewes to alkaloids such as ergovaline resulted in a depressed DMI which stayed depressed for those group of ewes during pregnancy even once the ergovaline had been removed from the diet. The study also showed that exposure of pregnant ewes to ergovaline also tended to result in lower LWT during pregnancy. There were insufficient ewe lambs, and insufficient time available to progress to a second level of investigation once these lambs had matured.

5.2 Indoor studies

4. Characterize the interaction between known endophyte alkaloid intake and hot environmental conditions in Crossbred ewes and different meat breeds on productivity and physiology.

This objective was addressed by the conduct of a controlled indoor study using cross bred sheep compared with meat composite sheep. Past work has suggested that ergot alkaloids in particular might be significant in impairing a sheep's ability to tolerate heat loads and so this study specifically used an ergovaline only source of toxin with no lolitrem B present. This study successfully compared cross bred sheep with composite breed sheep and found that cross bred sheep, when exposed to ergovaline and a heat load, had reduced DMI but no change in water intake while the composite sheep exposed to ergovaline and a heat load had an increased water intake but no change to their DMI. There were clear breed related effects, possibly related to the merino component of the cross bred sheep, and the effect of breed may be worth considering further when developing management strategies for reducing sub clinical effects of PRGT over summer.

5. Characterize intake, storage in fat and urinary excretion profiles of endophyte alkaloids in Crossbred ewes under different growth paths.

A series of indoor trials was conducted in order to address this objective. These studies included:

- 4.2.2 Characterising Ergovaline threshold in sheep. This study involved a range of low ergovaline doses, within the range of what might be found in pastures during winter and spring. Even at the highest of these doses, during the cooler months of the year (i.e. no heat load) there was no significant effect of alkaloid dose production responses of the sheep. There was however significant responses to ergovaline for rectal temperature at all dosage levels but not for respiration rate or skin temperatures.
- 4.2.3 Intake, storage and excretion profiles of alkaloids under different paths. This was one of the first studies to ever use separate sources of both ergovaline and lolitrem B to achieve specific, controlled doses of known alkaloid delivered to two different breeds of sheep. A wide range of production and physiological responses were measured as well as body composition using DXA.

6. Develop an animal model and experimental approach to investigate the efficacy of currently available rumen detoxifying agents on mitigating PRGT.

A model for testing rumen detoxifying products was developed and tested using seed with a known concentration of alkaloids plus the agent Elitox (4.2.5). This experiment clearly demonstrated that feeding a moderate level of alkaloids resulted in a decreased DMI and that the addition of Elitox to the diet was able to counter this. Two doses of Elitox were used and both were effective however the higher dose did appear to be more effective with sheep gaining more weight. Elitox was also able to reduce the rectal temperature of animals exposed to alkaloids. The study was effective in demonstrating both the effect alkaloids can have on sheep and the ability of the Elitox toxin binder to counter this. This study demonstrated that this type of approach was a viable and effective method for testing this type of toxin binder.

5.3 On farm studies

7. Assess the production and economic impact of grazing weaner sheep on high risk standard PRG pastures on commercial farms with a significant history of PRGT.

8. Evaluate the efficacy of novel rumen detoxifying agents in alleviating PRGT on commercial farms.

In order to meet these two objectives trials were run as follows:

- Elaine – super fine merino weaners (2011)
- Baynton – merino weaners (2012)
- Mortlake – beef cattle (2013)
- Yarram – cross bred ewe weaners

These trials demonstrated the challenging nature of undertaking on farm field experiments into PRGT. Although the trials were set up in conditions that appeared to favour the development of PRGT, weather conditions over the trial period either resulted in excellent pasture growth and no PRGT being detected or, a rapid cessation of pasture growth occurred due to hot, dry conditions and there was insufficient pasture available for livestock. At all sites the Elitox treatment was offered however PRGT did not develop and at no site did Elitox result in any improvement in any production or physiological measure – although when ryegrass staggers occurred (1 site, short term effect) it did reduce the incidence of ryegrass staggers. Elitox was found to be relatively easy to administer however there remains some uncertainty about the individual animal doses given the ad-lib and

voluntary nature of administration. Indeed, at one site there was a depression in total animal growth for the Elitox treatment, possibly due to some kind of substitution and/or reduction in total feed intake as a result of Elitox ingestion.

In addition to the on farm trials, results from the replicated trials at Dookie combined with and information and experiences of John Webb Ware's producer clients were used to produce an up to date estimate of the PRGT cost to individual farm businesses as well as the broader industry. This work clearly highlights a strong case for addressing this issue on farm through both short term management via the use of Elitox, and interestingly, through pasture renovation with improved, safe endophyte options.

6. Impact on meat and livestock industry

6.1 Impact on meat and livestock industry – now

This is the first research to occur in Australia looking at the impact of novel endophyte infected perennial ryegrass compared to standard or WT type endophyte, under sheep grazing. The research clearly demonstrated that WT ryegrass results in production losses and alters physiological parameters. This research should provide producers with confidence that the novel endophyte options tested are a viable alternative with fewer, and in the case of AR1, none of the problems associated with WT. A high proportion of the Australian sheep flock contains either pure merino (wool) sheep or cross bred sheep with a merino background, thus it was particularly important that this work included merino sheep under our hot Australian conditions. Differences between breeds of sheep with respect to response to alkaloids, particularly in hot conditions, were demonstrated – merino's appearing to be particularly susceptible. As a result of this work and its subsequent publication and dissemination, producers should:

- Be aware that WT ryegrass does have significant impact and cost within their businesses via clinical and subclinical effects.
- Be confident in the improvement in animal production, health and welfare outcomes possible through the simple use of alternatives to WT ryegrass such as the novel endophyte options AR1 and AR37 that were tested.
- Be highly wary and avoid the continued use of WT infected ryegrass seed in future pasture sowings
- Be aware of the potential for amelioration of PRGT, at least on a short term basis, though the use of toxin binding feed additives such as Elitox. At the very least this will improve producers to manage livestock in high risk PRGT areas (i.e. stock movement, crutching, drenching), potentially increasing feed available (due to their ability to use paddocks that are known to be problem areas) over summer and set up improved production and outcomes in following months and year.
- Become aware that in medium to high PRGT risk areas, there is a positive financial return from renovating pastures in order to eliminate WT endophyte – even if no improvement in stocking rate is factored in due to the concurrent likely use of improved plant genetics.
- Become aware that they should perhaps pay even more attention to some of these issues if they have merino sheep or crosses containing merino sheep genetics.

Although the tools, and now the evidence exist for producers to address PRGT, change will be slow due to low pasture renovation rates and a lack of awareness and confidence in the alternatives.

A PRGT workshop run in July 2012 which included presentations on this project as well as by other researchers, highlighted that within a small group of experienced people, awareness and appreciation of PRGT was growing. The challenge remains for this awareness and appreciation to become more mainstream and accepted across the whole producer community.

In addition, this research, along with other PRGT projects conducted over the same period, have been important in raising awareness of the PRGT problem with researchers and as a result a number of new research projects have been initiated to develop further and better PRGT solutions.

6.2 Impact on meat and livestock industry – in five years' time

A recent revision of the Holmes and Sackett estimate of PRGT cost to the sheepbeef industry by John Webb Ware (MacKinnon Project) saw the cost placed at approximately $100m p.a. With continued dissemination to producers information about the impact and cost of PRGT to their livestock and farm businesses, plus concurrently an increased awareness of the short term amelioration options and longer term pasture options, the cost to the red-meat industry should start to diminish. Feed additives appear to be a useful stop-gap measure for producers while they set about the longer term task of replacing their WT ryegrass pasture with novel endophyte infected safe ryegrass pastures or alternative safe species. The annual pasture renovation rate is somewhat less than 2% therefore complete change across the entire feedbase is unlikely to occur in the next 5 or 10 years. However, producers will be able to start creating safe zones for grazing during high risk periods and the demonstrated success of these areas in reducing PRGT, especially ryegrass staggers, will give producers greater confidence to continue the renovation program. Financial modelling suggests that renovating pastures in medium to high PRGT risk areas, purely in order to eliminate the WT endophyte and without any improvement in feed distribution profile, quality or yield, is a worthwhile proposition. However, most producers with significant PRGT problems are likely to have a high proportion of their farm sown to old pasture genetics (e.g. Victorian) and well managed, new, modern plant germplasm with safe endophytes will not only alleviate the PRGT problem but should also allow significant increases in stocking rates. The case for change is compelling and needs to be promulgated widely.

7. 7 Conclusions and recommendations

7.1 Grazing Studies - conclusions

Agronomic benefits of novel endophytes (AR1 and AR37) were compared with wild type endophyte-infected perennial ryegrass over two seasons. The purpose of the agronomic work in this project was to aid in the interpretation of the animal grazing measurements. Over all seasons (2011-2013), there was no significant effect of endophyte type on pasture mass, only occasional significant effects on pasture growth and no significant effects on pasture quality and the availability of green versus dead material. Most measures however did vary through time, as would be expected, but there was no significant interaction between endophyte and time. The lack of significant differences suggests that the different classes of sheep grazing the different endophyte treatments had access to the same quantity and quality of feed at any given time and would support the hypothesis that any differences in livestock performance were due to endophyte treatments.

In autumn 2011, Merino ewe weaners grazing wild type endophtye pasture had decreased liveweight, increased dag score and increased rectal temperature and respiration rate compared to the AR37 and AR1 treatments. In crossbred ewes, these effects were also evident but the magnitude

of the effect was reduced. This suggests that young Merino ewes are more sensitive to the adverse impact of PRG alkaloid ingestion. Staggers was observed in both breeds but the onset was quicker and the symptoms more severe in the Merino ewe weaners compared to the Crossbreds. For both animal groups, staggers on the WT treatment occurred first. By the conclusion of the grazing study in autumn staggers in the AR37 treatment was as severe as observed in the WT treatment.

Crossbred ewes were mated during autumn and continued to graze the pasture entophyte treatments until they lambed during winter and spring. There were some changes in liveweight, however, these changes were probably carried over from the previous grazing season as the same animals were used. There was no significant difference in milk production or lamb live weights during this time but sheep numbers were relatively low. Interestingly, respiration rate was elevated in those sheep grazing WT and AR37 pasture.

Older Merino ewes were selected for the grazing experiment from Oct 2011 to Mar 2012.The results from the current study indicate that alkaloid levels were low in the pasture and there were few changes in production parameters measured. Mild staggers was observed in the WT and AR37 groups and staggers was most prevalent in the AR37 treatment. During autumn 2012, Crossbred ewes were returned to the pasture for only a short amount of time due to decreased availability of pasture. There were no major differences in productivity measurements during this period. Both Merino and crossbred ewes grazed the treatments during summer/autumn 2013. There were some differences in live weight. Staggers was mild in both breeds grazing WT and AR37 pastures. Crossbreds appeared to be more tolerant to alkaloid intake despite their younger age compared to the Merinos. This indicates that Merinos are more sensitive to alkaloid consumption, a finding reflected in the previous grazing experiments in this report.

7.2 Indoor Studies - conclusions

We investigated feeding a low level of ergovaline-only feed to ewes during different stages of pregnancy. Feed intake was reduced during the periods when ergovaline was offered. Moreover, feed intake did not recover when sheep were fed the ergovaline and then the nil treatment. Those sheep on switched diets also lost weight at a quicker rate. Lamb growth rate tended to be lower in the ergovaline and ergovaline/nil treatments; however, this data was not statistically analysed due to the low numbers of lambs used. Milk production and mammary gland size was not reduced due to ergovaline intake as hypothesized.

We investigated the threshold at which feeding very low levels of ergovaline to sheep would alter production and/or physiological parameters under thermoneutral conditions. The levels of ergovaline fed in this experiment were not unlike levels observed in the field during winter and spring. Animal production was not affected by the levels of ergovaline offered. Rectal temperature increased in all ergovaline treatments, and there was no dose response observed. This suggests that even a low dose of ergovaline can increase heat load in sheep, even under thermoneutral conditions, but is not likely to adversely impact productivity.

Feeding a low level of ergovaline and lolitrem B, individually and in combination, to growing crossbred and composite breed sheep for several weeks under thermoneutral conditions did not adversely affect animal productivity. However, rectal temperature and faecal moisture were found to increase when ergovaline and lolitrem B were fed in combination. Moreover, there were no carry-over effects on production and/or physiological parameters following prior alkaloid ingestion, during a wash-out period. These data support previous observations that at low levels of ingestion,

alkaloid intake is not likely to have a severe impact on short term production, either in positive or negative energy balance, at least under thermoneutral conditions.

Feeding ergovaline-only feed to growing crossbred and composite breed sheep under thermoneutral and hot environmental conditions resulted in different production and physiological responses. Crossbred ewes were unable to cope with the heat exposure as well as the composite breed ewes, with increased rectal temperature and skin temperature, no change in water intake and decreased DMI. These data indicate that meat sheep breeds may be more important in minimizing the effects of alkaloid consumption on animal physiology and production responses. This difference in breed may be due to the higher proportion of Merino genetics found in the Crossbreds.

Feeding a moderate level of PRG alkaloids decreased feed intake in Merino ewes and this was countered to some extent by the consumption of a commercial alkaloid deactivator, Elitox®. This suggests that Elitox may play an important role in increasing the production response in sheep fed PRG alkaloids. These data suggest that inactivation of the alkaloids mitigates some of the adverse physiological responses associated with alkaloid ingestion in sheep and may improve weight gain, most likely by reducing alkaloid absorption and/or modifying activity in the rumen.

7.3 On farm studies - conclusions

In 2011, climatic conditions were unfavourable for the development of PRGT. Whilst endophyte alkaloid measurements approached toxic levels at the start of the trial there was no clinical evidence of PRGT. As a consequence the efficacy of Elitox toxin binder in Weather Shield™ for sheep could not be could not be determined in this trial. In the following seasons (2012 and 2013), there were variable results again because of unfavorable weather conditions. However, at one site, Baynton, substantial clinical differences in staggers developed between the control and Elitox group with the staggers more severe and affecting more sheep in the control group. Whilst this result was only observed in one trial, Elitox toxin binder may offer a very useful management tool to minimize the impact of PRGT where staggers occurs.

7.4 Recommendations

The following recommendations are made:

1. Develop communication strategies to disseminate the key findings of the project and increase awareness of PRGT in the grazing industries and with other key stakeholders.

2. Develop on farm management practices for different classes/breeds of livestock. For instance, older and heavy sheep are more likely to tolerate PRG alkaloid ingestion in comparison to younger stock, which will require careful management.

3. Develop a tool/application for objective assessment of the economic benefits of implementing different PRGT mitigations strategies on farm for different sheep production systems.

4. In light of the evidence from both on farm and controlled studies that the ingestion of an alkaloid deactivator, may provide an important management tool for producers to reduce clinical staggers in the face of an outbreak of PRGT, it is recommended that further work is undertaken to develop efficient delivery systems of alkaloid binders to livestock on farm.

5. Further work is required to investigate the efficacy of other alkaloid deactivators/binders in mitigating PRGT in grazing livestock

6. Further work is required to investigate the efficacy of alkaloid deactivators/binders in mitigating any PRGT like effects caused by alkaloids associated with alternative endophytes such as AR37, AR5/Endo 5 and NEA2.

7. There is an opportunity to investigate the opportunity for early PRGT detection systems using a similar model as developed for the testing of alkaloid deactivators/binders and the study of low doses of alkaloids. Early detection before the onset of clinical symptoms will enable better management of stock and reduce losses.

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