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Producer Research Support

Determining Presence of Toxins in Rye Grass and Fescue Pastures

BOZO Landcare

Members of BOZO Landcare group were interested in determining the presence and level of toxic endophyte alkaloids in pastures in the New England area of New South Wales.

Subclinical levels of alkaloid toxins may reduce weight gains in lambs by up to two kilograms and reproductive rate by at least ten percent.

In the event of finding sufficiently high alkaloid levels, BOZO Landcare group proposed to develop management protocols to minimise negative effects of alkaloids on sheep production and profitability.

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The project

There are a number of non-specific health problems in sheep flocks in the New England region that reduce animal production and profitability. These problems include:

- scouring in weaner and adult sheep (not associated with worm burdens);
- formation of dags with increased incidence of flystrike;
- ill thrift in weaner sheep; and
- lameness and low lamb marking percentages.

Historically, these issues have been thought to be due to sheep worms and or mineral deficiencies, but closer examination has shown that there are many instances where these problems have occurred without worms and in the presence of mineral supplementation.

Over the last few years, the potential of endophyte alkaloid toxins associated with perennial ryegrass and older tall fescue varieties to cause these animal production problems has been identified. The two most abundant alkaloids are ergovaline and lolitrem B. High levels of ergovaline in ingested pasture results in vasoconstriction, increased body temperature, and elevated respiration rate producing symptoms of heat stress in affected animals. Ergovaline also depresses prolactin concentration in blood and therefore affects milk production. In sheep, clinical levels of ergovaline have also been demonstrated to reduce the rumen degradability of feed. Lolitrem B is a class of tremorgic mycotoxin and is the major cause of rye grass staggers. Lolitrem B is the most abundant tremorgen but lolitrems A, C and D also exist but in small quantities. Lolitrem B affects the motility of smooth muscle in the gut and skeletal muscle.

Clinical effects of alkaloid toxicosis were reported by landholders in the New England region of New South Wales at a workshop held at Armidale, NSW in 2002. At the workshop, 60 percent of attendees indicated that their sheep had experienced rye grass staggers in the past. Clinical effects of alkaloids can be severe with reports of thousands of sheep deaths in Victoria, South Australia and Tasmania during 2002 attributed to alkaloid toxins. Clinical thresholds for ergovaline and lolitrem B are suggested in a number of publications, to be 0.4 to 0.8 and 1.8 to 2.0 milligrams per kilogram of dry matter respectively.

Of greater interest to the members of BOZO Landcare are the subclinical effects of endophyte alkaloids. Reports in the literature suggest that subclinical effects include:

- reduced feed intake;
- live weight gain in young sheep and cattle;
- increased dag formation;
- reduced rates of conception; and
- increased lamb mortality and reduced milk production in cattle.

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Key points

- Take a precautionary approach and do not sow perennial rye grass with wild endophyte.
- Avoid grazing rye grass pastures with more than 15 percent rye grass during late summer and autumn with sensitive stock such as weaners.

These effects have been observed in animals grazing endophyte infected rye grass pastures or fed rye grass seed containing ergovaline. Dose response data with sheep suggest that increasing levels of ergovaline is associated with a linear decrease in feed intake and growth. Such effects are less clear with cattle. Dose response data for lolitrem B is difficult to locate but milk production has been reported to be depressed at lolitrem B levels of 0.2 milligrams per kilogram.

Subclinical levels of alkaloid toxins may reduce weight gains in lambs by up to two kilograms and reproductive rate by at least ten percent. The 28 BOZO Landcare group member properties carry a combined 102,000 sheep, including 50,000 ewes. The impact of these subclinical effects on member properties could potentially reduce profitability by approximately $380,000 per annum.

Objectives

1. determine the level of endophyte alkaloids on 12 properties over one year;
2. build awareness of the potential for endophyte alkaloids to cause animal production problems among the 28 group members; and
3. in the event of finding sufficiently high alkaloid levels, develop management protocols to minimise negative effects of alkaloids on sheep production and profitability.

What was done

Twelve properties were selected to cover a range of pasture types and locations. From half of these properties, a paddock was selected from which a sample of perennial rye grass (selected from within the pasture) and a sample of composite pasture was harvested at ground level. The remaining six properties were sampled to the same protocol but older varieties of tall Demeter fescue were sampled rather than perennial rye grass.

Sampling comprised the collection of target plant material at a minimum of 15 randomly chosen locations in the sampling area (20 metres by 20 metres). Plant material was harvested at ground level, soil carefully removed and plant material bulked within paddock. Sampling was repeated in the same paddocks and sampling area in summer, autumn and spring. Winter was not sampled because levels were expected to be near minimum levels. Pasture samples were stored in a cooled container, frozen and sent to FeedTest, Victorian Department of Primary Industry (DPI) for analysis for lolitrem B and ergovaline.

Soil samples (zero to ten centimetres) were collected at the first sampling, and sent to Pivotest Laboratories for a standard soil analysis.

An initial BOZO Landcare group meeting was held in October 2003 to discuss the project methodology and select test sites. A final meeting was held during August 2004 to discuss results and implications for animal management. Two field days (one at Walcha and another at Glen Innes), organised by the New England branch of the NSW Grasslands Society, were held during November 2003. These field days included a presentation about this project and a discussion about the role of endophytes in pastures.

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Producer Research Support

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What happened?

The sampling dates for perennial rye grass, Demeter fescue and composite pasture samples are provided in *Table 1.*

Table 1. Sampling dates for perennial ryegrass, Demeter fescue and composite pasture.

	Perennial ryegrass sites (date)	Demeter fescue sites (date)
Sample 1	27th November 2003	18th November 2003
Sample 2	10th February 2004	19th March 2004
Sample 3	16th May 2004	27th May 2004

Levels of ergovaline and lolitrem B were lowest at the Demeter fescue sites in northern New England. Only one sample, of the total of fourteen, of Demeter fescue exceeded the clinical threshold for ergovaline. One composite pasture sample had lolitrem B and ergovaline levels where subclinical effects on animal production may have been expected.

Biologically significant levels of ergovaline and lolitrem B were recorded from the sites in southern New England. Increasing alkaloid level in ryegrass samples from spring to autumn was observed at most properties. Statistical analysis indicated that on average, lolitrem B levels in ryegrass increased significantly (P=0.008) from November to February and then to May. The change in ergovaline levels in ryegrass with time was also significant (P=0.022) with lowest levels at all properties recorded during February. On average, ergovaline levels were numerically highest in May but these levels did not differ significantly from those recorded in the November sampling.

There was a suggestion from the data that lolitrem B levels were greatest in recently sown pastures, but one other site – sown to perennial rye grass in 1989 – had a similar average lolitrem B level. Supporting the lack of certainty between pasture regime and lolitrem B level was the observation that one recently sown site was ranked fifth of seven properties for average lolitrem B level. There was no apparent relationship between pasture regime and ergovaline level in rye grass.

Lolitrem B levels in rye grass exceeded the clinical threshold of 1.8 parts per million (for sheep and cattle) in three of the 21 samples (14 percent). Ten samples (48 percent) recorded lolitrem B levels of greater than one part per million and less than 1.8 parts per million. Eight samples (38 percent) recorded levels less than one part per million. Assuming a clinical threshold of 0.8 parts per million for ergovaline level in sheep and cattle, no rye grass samples were observed to exceed this value. Six of the 21 rye grass samples (29 percent) equalled or exceeded an ergovaline level of 0.4 parts per million.

There was a statistically significant (P=0.005) relationship between lolitrem B and ergovaline level in rye grass with increasing lolitrem level associated with an increase in ergovaline. Among the small data set there were few correlations between average lolitrem B and ergovaline level and soil chemistry. Progress reports highlighted a negative relationship between soil potassium and lolitrem B but this was not apparent across all sampling events. Soil sulphate concentration (KCl 40) was associated with average lolitrem B and average ergovaline level in rye grass samples. The nature of the relationship (but not statistically significant) was such that higher soil sulphate concentration was associated with higher lolitrem B and ergovaline levels. This association was not confounded with pasture type.

Lolitrem B and ergovaline concentrations in composite pasture samples were very low at all sampling times with the exception of one site where rye grass contributed significantly to herbage mass.

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Discussion

Evidence from the literature suggested that lolitrem B and ergovaline levels (alkaloids) would be at a low point for the year at sample one (November) due to lower levels of moisture and heat stress, but would rise in subsequent samples. The increase in lolitrem B and ergovaline levels over summer and autumn was associated with increase stress (heat, moisture, frost). This trend was not observed uniformly across all sites. Samples collected from northern New England had very low levels of alkaloid. Samples collected from southern New England did show a seasonality of alkaloid concentration, with highest levels of lolitrem B and ergovaline being recorded from the final sample in May 2004. This sample was collected when moisture stress was at the highest level during the collection period and after some early frosts.

Next Steps

Although the results of this project are inconclusive, they do warrant further investigation. MLA endorsed a background paper prepared by Stephen Page that recommends the development of a workshop to address the widespread losses (deaths and production) caused by perennial rye grass toxicity.

MLA also recommends

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