EQUINE TRUST SERVICE AGREEMENT
PARTNERSHIP FOR EXCELLENCE

Clinical expression of ryegrass intoxication in New Zealand horses

Program Leader: Prof Joe Mayhew, IVABS, Massey University

Milestones

Milestone	Description	Evidence of Achievement	Completion Date
1.	Signing of contract	Signed copies received by Equine Trust Office	February 2009
2.	Purchase of first of horses and receipt of first report	Receipt of report advising on progress of trial and verifying that the first horses had been purchased and feed transported from ChCh to Manawatu.	March 2009
3.	Completion of data collection	Note from Programme Leader advising Equine Trust of the number of horses challenged	June 2009
4.	Completion of project report	Receipt of final report in Equine Trust Office	November 2009

Budget Payments

The Trust will pay the Total Budget sum inclusive of GST in installments as set out in the table:-

Payment to Provider Date/Month/Year	Payment Amount (GST inclusive)
Completion Milestone 1	$7,953.75
Completion Milestone 2	$7,953.75
Completion Milestone 3	$7,953.75
Completion Milestone 4	$7,953.75
Total	**$31,815.00**

Note: The Trust shall make payment by the 20th of the month following receipt of an appropriate Report and invoice.

Reports on Milestones 4

We exposed seven horses, in two separate groups, by feeding perennial ryegrass seed and hay containing 2 ppm lolitrem B. Data was collected prior to and after two weeks exposure to lolitrem B so the horses acted as their own controls. Tests included video-documented neurological examination, brainstem auditory evoked [BAEP] and magnetic motor evoked [mMEP] potentials, clinical examination, blood sampling and a frusemide challenge.

All horses showed a fine muscular tremor, frequently noticed in the forelimbs but also observed in the neck, flank, hind limbs and the tail-head. The degree of tremor varied between individual horses and also depended on the level of activity - increased during feeding and immediately after lunging. Using an ophthalmoscope it was possible to detect subtle, rapid [~5Hz] tremor of the eyeball in 6/7 horses. Subtle signs of ataxia were observed during handling and when blindfolded motor dysfunction was exaggerated. The mMEPs showed an increase in take-off latency and peak latency [see Figure 1]. During the treatment period heart rate at rest increased significantly ($p = 0.018$) but stayed within normal values. No significant changes were observed in respiration rate, rectal temperature, GIT auscultation, BAEP, CBC or blood biochemistry values.

The frusemide challenge was designed to challenge the renal BK channels with an increase in urine flow and tubular pressure. Blood and urine was collected prior to frusemide administration of 1 mg/kg, IV. Sequential blood and urine samples were then taken at ~ 15, 30, 60 & 120 minutes. Fractional excretions were calculated for K^+ [FEK^+] and Na^+ [$FENa^+$] The graphed results show that the change in FEK^+ during the first 15 minutes after frusemide administration was significantly greater ($p=0.003$) before the horses had been exposed than after two weeks into lolitrem B exposure [Figure 2].

BK channels have been located in the principal cells of the cortical collecting duct and the connecting tubule and have been shown to be involved in flow-mediated potassium secretion (FMKS). The other potassium channel of principal cells, the renal outer medullary potassium (ROMK) channel, is thought to have a greater role in baseline K^+ secretion. The apparent decrease in FMKS after lolitrem B exposure is in keeping with lolitrem B blocking these BK channels. The FMKS was not completely blocked. This may be due to only some of the BK channels being blocked, a compensatory increase of ROMK channel activity (slower activation kinetics of ROMK channels would correspond to the more gradual rise in FEK^+) or compensation by increased aldosterone activity. Aldosterone increases the action of the basolateral Na^+-K^+-ATPase pump and subsequently increases Na^+ reabsorption via apical channels. The more negative luminal charge created by these Na^+ movements may recruit additional BK channels that are not bound by lolitrem B, or alternatively activate ROMK channels. Results of aldosterone analyses are pending.

There also appeared to be a strong trend (p=0.072) for an unexpected decrease in the frusemide-induced $FENa^+$ in a manner similar to the effect lolitrem B blockade of BK channels had on frusemide-induced FEK^+. One explanation for this may be that aldosterone is increased post-exposure, causing an increase in Na^+ reabsorption. Also, blockage of other sub classes of BK channels located in renal blood vessels, glomerular mesangial cells and in the proximal convoluted tubules may disrupt frusemide's delivery to it's site of action in the lumen of the loop of Henle; thereby resulting in a lesser degree of diuresis, naturesis and FMKS.

Another interesting result is the variability of lolitrem B levels in the serum detected by ELISA - these showed a significant increase from non-detectable during the control period to 0.23 - 0.5 ng/ml after exposure. However, the post exposure levels did not correlate with the severity of signs displayed. Could this imply that individual variability in susceptibility to clinical PRGS is due to differences in individual animals' metabolism and excretion of lolitrem B, rather than to its absorption?

All horses appeared to recover completely following cessation of feeding the test diets.

We now have a clearer appreciation of the clinical signs and variability of perennial ryegrass intoxication in horses and may be closer to defining changes in electrolyte handling by affected horses that may be diagnostically useful in future.

Figure 1: Examples of right extensor carpi radialis mMEP latencies for Horse #1.

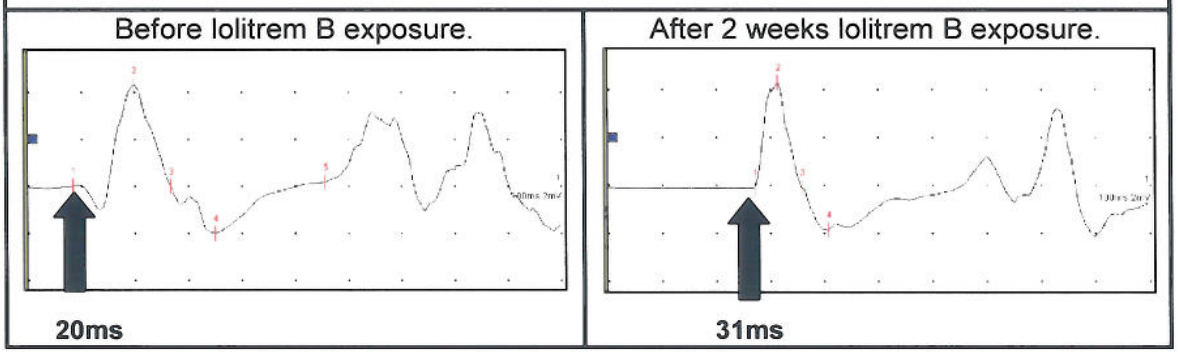
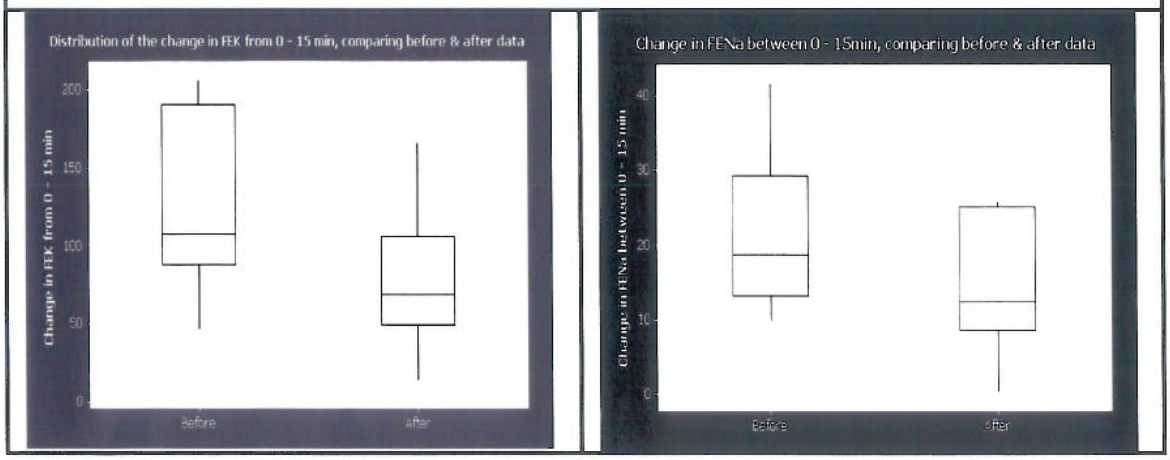


Figure 2: Early changes in FEx induced by frusemide before and after exposure to lolitrem B.



Ryegrass hay and grain was purchased from SI and arrived on farm at Feilding in May. Horses were purchased in May and June and exposed to control fodder in 2 groups of 3 and of 4 for at least 2 weeks before being fed high endophyte ryegrass hay and grain. They were monitored closely according to protocols for cardinal signs, neurologic signs, urinary and cerebrospinal fluid [CSF] throughput of electrolytes and urinary electrolyte responsiveness to diuretic challenge, and electrophysiologic latency and amplitude sensory evoked potentials induced by non-noxious auditory [BAEP] and magnetic [mMEP] stimuli. Clinical signs were recorded manually and by video and consisted of muscle fasciculation, limb tremor, eyeball tremor, heightened awareness to familiar surroundings and stimuli and vestibular ataxia when blindfolded. The serum content of divalent cations Ca and Mg tended to rise and mMEP latencies tended to increase with exposure to the mycotoxins. All data is being collated and analysed for submission as MSc dissertation and as papers for publication in NZVJ.