Rates of Urinary Toxin Excretion in Unprotected Steers Fed *Leucaena leucocephala*

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Introduction

Leucaena (*Leucaena leucocephala*) is a productive, nutritious, leguminous forage tree with high capacity for ruminant live weight gain. The plant does however contain the non-protein amino acid mimosine which is degraded within the rumen to 3-hydroxy-4(1H)-pyridone (3,4-DHP) with potential to cause adverse effects on animal health and production. Stock can be protected via rumen inoculation with the bacterium *Synergistes jonesii*, which is capable of degrading the toxin. However surveys have demonstrated sub-clinical toxicity is persisting in Queensland herds (Dalzell *et al*. 2012).

Currently, testing for toxicity involves analysis of urine samples using high performance liquid chromatography (HPLC). A colorimetric urine test protocol has also been developed with the aim of providing a robust and reliable means for routinely testing herds (Graham *et al*. 2013). A significant problem affecting interpretation of the results from either method is the high variation in the concentrations of toxins excreted among animals on similar diets and by individual animals over time (Dalzell *et al*. 2012). Factors such as feed intake, water consumption, urine volume, as well as timing of sampling may be the cause of this variation. This research investigated the effect of sample timing by measuring the time taken for mimosine and its breakdown products, to present in the urine following the introduction of leucaena to the ration of cattle naïve to the plant.

Methods

Seven naïve, stall-housed Charolais x Santa Gertrudis steers of average weight 328 kg were fed an initial ration of barley chaff at a daily dry matter intake of 2.5 kg/100 kg body weight (BW) for 10 days. The animals were then placed on a 60:40 leucaena cv. Tarramba and barley chaff diet. Leucaena feeding continued for 3 days before animals were returned to a barley chaff only diet for a further 7 days. Water was provided *ad libitum*. Leucaena leaves were hand-harvested during active summer growth, so as to target seasonally high leaf mimosine levels (Masafu 2006) and then dried. Animals were fed routinely at 9 am each morning. Intakes were high with minimal refusals consisting only of low palatability leucaena stalks.

Sampling frequency was every 2.5 hours for the first 24 hours of leucaena feeding, every 4 hours for days 2-5, then every 6 hours for the remainder of the feeding period. Urine samples were preserved in a 1:19 HCl-urine solution and subsequently analysed using HPLC for concentrations of mimosine, 3,4-DHP and 2,3-DHP (Dalzell *et al*. 2012).

Results and Discussion

Mimosine and 3,4-DHP were detected in urine approximately 9 hours after the commencement of leucaena feeding. Mean mimosine concentrations peaked at 11.6 µg/mL 35 hours into the leucaena feeding period and remained low, but persisted for 67 hours until cessation of the leucaena feeding (Fig. 1). No mimosine was detected thereafter. The low levels of mimosine excretion indicated that the majority of mimosine was degraded to 3,4-DHP. Accordingly, urine 3,4-DHP concentrations continued to increase throughout the leucaena feeding period, reaching a mean peak of 316.2 µg/mL at 67 hours. Following cessation of leucaena feeding, mean 3,4-DHP concentrations fell slowly to low levels <20 µg/mL within 58 hours of the last leucaena ration (Fig. 2). Very low concentrations of 2,3-DHP were detected (<15 µg/ml) from 6 to 28 hours after commencement of leucaena feeding but none thereafter (data not presented).

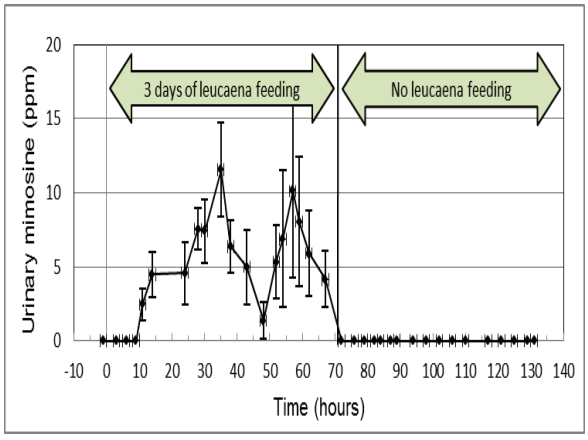

Figure 1. Mean excretion of mimosine ±SE for periods during and post leucaena.

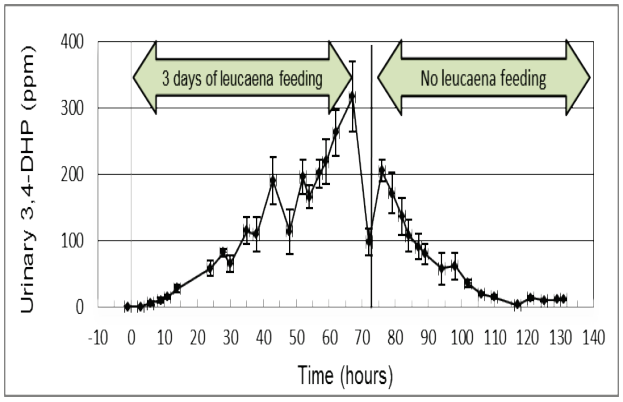

Figure 2. Mean excretion of 3,4-DHP ±SE for periods during and post leucaena feeding.

The data demonstrated significant variation, both among animals and temporally across the sampling period despite similar leucaena intake, perhaps related to differences in water consumption and urine volume. Data for both mimosine and 3,4-DHP excretion appeared to demonstrate diurnal patterns of excretion (Fig. 1 and 2). The patterns were likely an effect of varying patterns of leucaena intake (largely occurring from 9 am to 12 noon each day), rather than fluctuating rates of digestive processes or kidney glomerular activity. It was also observed that the experimental animals consumed their daily rations at different rates.

Conclusions

Given the potential for lost production from sub-clinical toxicity, it is important that methodology for testing of urinary DHP is robust and reliable. It is clear from this and other work (Giles *et al*. 2013; Graham *et al*. 2013) that testing of multiple samples will be necessary to obtain a reliable assessment of toxicity status of animals. Our experimental findings indicate that urine testing for presence of DHP should occur only during periods when there are high levels of leucaena in diet for at least 3 days prior to sampling and should occur preferably within 5 hours after removal of animals from leucaena feeding. Fasting overnight may lead to reduced urinary DHP concentrations and a possible false assessment of toxicity status of the herd.

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