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The efficacy of in vitro *Synergistes jonesii* inoculum in preventing DHP toxicity in steers fed leucaena-grass diets

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Introduction

Leucaena leucocephala (leucaena) is a valuable forage tree legume for tropical animal production, which contains the toxin mimosine. The breakdown products of mimosine in ruminants (3,4-DHP and 2,3-DHP) can adversely affect their health and limit weight gains (Jones and Hegarty 1984). The rumen bacterium *Synergistes jonesii*, introduced into Australia in 1983, was shown to completely and rapidly degrade these toxins to safe levels (Jones and Megarrity 1986). Since 1996, an inoculum has been produced in vitro and made commercially available to Australian graziers (Klieve et al. 2002). Accordingly, the issue of leucaena toxicity in Australia was thought to be resolved. However, extensive testing in 2004 found that up to 50% of Queensland cattle herds consuming leucaena were excreting high levels of urinary DHP, suggesting subclinical toxicity remained an issue for graziers (Dalzell et al. 2012). Some of these herds had previously been inoculated with in vitro *S. jonesii*, suggesting the inoculum may not be able to either persist within a herd, or remain effective in degrading DHP.

The aim of this study was to assess the capability of the in vitro *S. jonesii* inoculum to efficiently break down DHP in a controlled feeding trial environment.

Methods

Sixteen mixed breed stall-housed steers, previously naïve to leucaena and S. jonesii and weighing 200−300 kg, were fed 3 different leucaena-grass diets (25, 50 and 100% leucaena) offered at 2.5 kg dry matter (DM)/ 100 kg live weight over a 6-week period to establish subclinical toxicity (pre-inoculation). Animals were then inoculated with the in vitro *S. jonesii* inoculum and continued to consume the same leucaena-grass diet for another 4 weeks (post-inoculation). At inoculation, a control treatment was imposed on half of the animals consuming 50% leucaena, where lucerne (*Medicago sativa*) replaced leucaena. Animal toxicity status was determined by analysis of urinary DHP levels; each animal spent 24 hours in a metabolism crate once per week, where a bulk urine sample was collected. Concentrations of DHP were measured by HPLC (Graham et al. 2013), and urine volume was recorded, allowing total DHP excreted to be expressed in mg/hd/d. Dalzell et al. (2012) adopted a mean herd DHP excretion level above 100 mg/L in urine as indicative of subclinical toxicity. Accordingly, an excretion level of 100 mg/L was used as a mean treatment threshold for toxicity. Mean urine volume was approximately 3.5 L/hd/d, thus a mean DHP excretion greater than 350 mg/hd/d was regarded as indicative of subclinical toxicity.

Results and Discussion

During the 6 weeks prior to inoculation, total DHP excretion increased with amount of leucaena consumed (Figure 1). However, total DHP had peaked at 3 weeks and was already declining at 6 weeks, when animals were first inoculated (Figure 2). This was associated with a decline in the isomer 3,4-DHP, while the isomer 2,3-DHP, which first appeared at week 2, remained static until after inoculation (data not presented).

During the 4 weeks post-inoculation, the rate of total DHP excretion continued to decrease (Figures 1 and 2) to low levels, albeit still above the threshold level of 350 mg/hd/d at higher levels of leucaena feeding. There was, however, a sharp decrease in 2,3-DHP to very low

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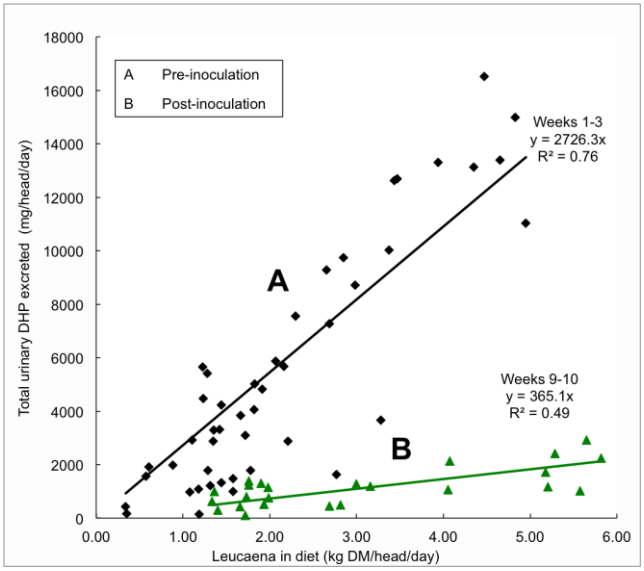

Figure 1. Relationship of total DHP excreted per day (mg/hd/d) with amount of leucaena in diet (kg DM/hd/d).

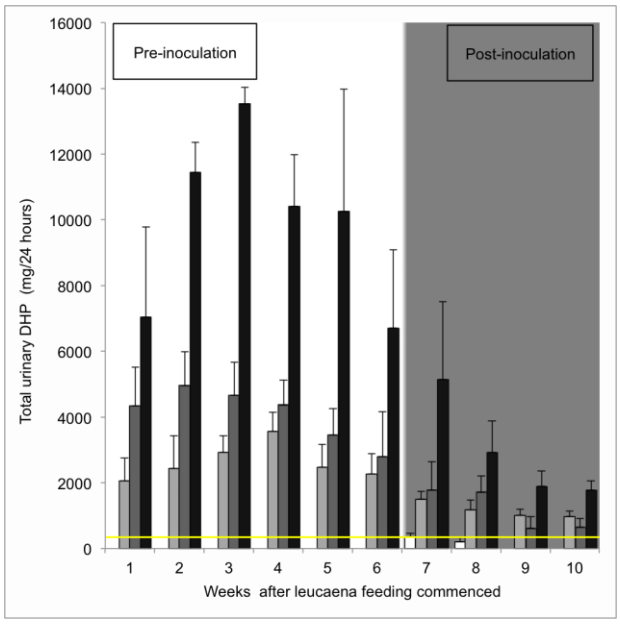

Figure 2. Mean total daily urinary DHP excreted (mg/hd/d) over the 10-week period (+ s.e.) for: ▫ 25%, ▪ 50%, ■ 100%, and □ Control treatments. Yellow line denotes safe threshold level of 350 mg/hd/d.

levels, while 3,4-DHP remained unchanged from week 6 through to week 10. The control animals ceased excreting DHP within 2 weeks of the change from leucaena to lucerne.

The degradation of 3,4-DHP prior to inoculation was presumably due to existing microorganisms capable of degrading 3,4-DHP but not effective at degrading 2,3-DHP. Since these cattle had never previously consumed leucaena or been inoculated with *S. jonesii*, it appears that organisms that can degrade 3,4-DHP to 2,3-DHP may be more widespread than previously thought.

Conclusions

Since previous work demonstrated that in-vivo sources of *S. jonesii* almost completely eliminated urinary DHP excretion within 7-10 days of inoculation (Jones and Lowry 1984), this work has shown that the current in vitro *S. jonesii* inoculum may not be as effective, especially in animals consuming high leucaena diets. The in vitro inoculum appeared to augment degradation of 2,3-DHP by the resident microbial populations more capable of, albeit incomplete, 3,4-DHP degradation. Further research is warranted to seek a more efficient in vitro inoculum, which can be readily produced, stored and administered.

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