

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

Michael J Halliday<sup>A</sup>, Hayley E Giles<sup>A</sup>, Scott A Dalzell<sup>B</sup>, Chris S McSweeney<sup>C</sup> and H Max Shelton<sup>A</sup>

<sup>A</sup> School of Agriculture and Food Sciences, The University of Queensland, St Lucia 4072, Australia

<sup>B</sup> Formerly of School of Agriculture and Food Sciences, The University of Queensland, St Lucia 4072, Australia

<sup>C</sup> CSIRO Animal, Food & Health Sciences, Queensland Biosciences Precinct, St Lucia 4067, Australia

Contact email: [m.halliday@uq.edu.au](mailto:m.halliday@uq.edu.au)

**Keywords:** *Leucaena leucocephala*, mimosine, dihydroxypyridine, urine.

## Introduction

*Leucaena leucocephala* (leucaena) is a valuable forage tree legume for tropical animal production that 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 *in vitro* produced inoculum has been 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 sub-clinical 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

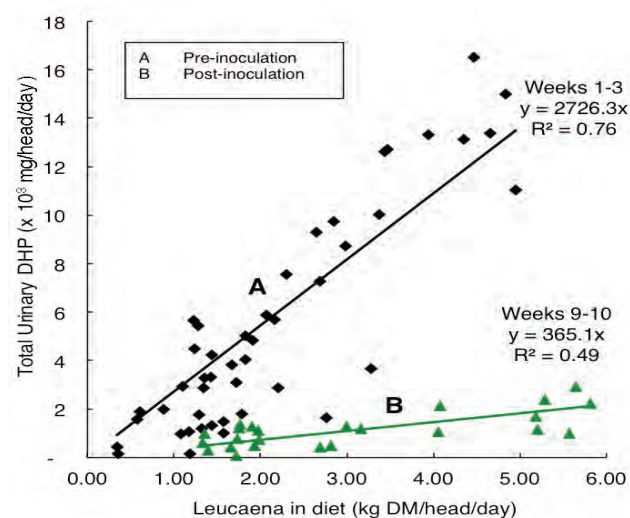
Sixteen mixed breed stall-housed steers, previously naïve to leucaena and *S. jonesii*, weighing 200-300 kg were fed 3 different leucaena/grass diets (25%, 50% and 100% leucaena) offered at 2.5 kg dry matter (DM) / 100 kg live weight over a 6 week period to establish subclinical toxicity (pre-inoculation). 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 leucaena was substituted with lucerne. 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/head/day. Dalzell *et al.* (2012) adopted

a mean herd DHP excretion level above 100 mg/L 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/head/day, thus a mean DHP excretion greater than 350 mg/head/day was regarded as indicative of subclinical toxicity.

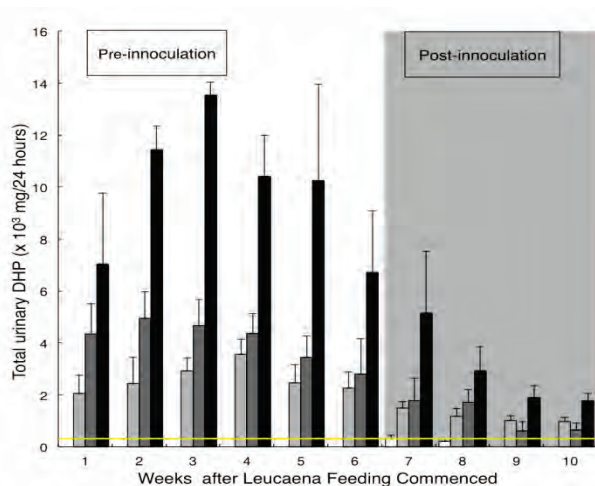
## Results and Discussion

During the first 6 weeks prior to inoculation, total DHP excretion increased with amount of leucaena consumed (Fig. 1). However, total DHP had peaked at 3 weeks and was already declining at 6 weeks when animals were first inoculated (Fig. 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 (Fig. 1 and 2) to low levels albeit still above the threshold level of 350 mg/head/day at higher levels of leucaena feeding. There



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



**Figure 2.** Mean total daily urinary DHP excreted (mg/head/day) over the 10 week period (+ SE) for: □ 25%, ■ 50%, ■ 100%, and □ Control treatments. Yellow line denotes safe threshold level of 350 mg/head/day.

was, however, a sharp decrease in 2,3-DHP to very low levels, while 3,4-DHP remained unchanged from week 6 through to week 10. The control animals ceased excreting DHP within 2 weeks off leucaena.

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 that can be readily produced, stored and administered.

## Acknowledgements

The advice of Del Greenway, Peter Isherwood and Sam Graham from the University of Queensland was greatly appreciated. The assistance of Col Seiler for supply of the leucaena and the help of Les Gardner throughout the trial is also acknowledged.

## References

- Dalzell SA, Burnett DJ, Dowsett JE, Forbes VE, Shelton HM (2012) Prevalence of mimosine and DHP toxicity in cattle grazing *Leucaena leucocephala* pastures in Queensland, Australia. *Animal Production Science* **52**, 365-372.
- Graham SR, Dalzell SA, Nguyen TN, Davis CK, Greenway D, McSweeney CS, Shelton HM (2013) Efficacy, persistence and presence of *Synergistes jonesii* inoculum in cattle grazing leucaena in Queensland: On-farm observations pre- and post-inoculation. *Animal Production Science* **53**, (in press).
- Jones RJ, Hegarty MP (1984) The effect of different proportions of *Leucaena leucocephala* in the diet of cattle on growth, feed-intake, thyroid-function and urinary-excretion of 3-hydroxy-4(1h)-pyridone. *Australian Journal of Agricultural Research* **35**, 317-325.
- Jones RJ, Lowry JB (1984) Australian goats detoxify the goitrogen 3-hydroxy-4(1H) pyridone (DHP) after rumen infusion from an Indonesian goat. *Experientia* **40**, 1435-1436.
- Jones RJ, Megarritty RG (1986) Successful transfer of DHP-degrading bacteria from Hawaiian goats to Australian ruminants to overcome the toxicity of Leucaena. *Australian Veterinary Journal* **63**, 259-262.
- Klieve AV, Ouwerkerk D, Turner A, Robertson R (2002) The production and storage of a fermentor-grown bacterial culture containing *Synergistes jonesii*, for protecting cattle against mimosine and 3-hydroxy-4(1H)-pyridone toxicity from feeding on *Leucaena leucocephala*. *Australian Journal of Agricultural Research* **53**, 1-5.