

Do Queensland cattle possess rumen bacteria capable of degrading *Leucaena* toxins?

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Leucaena leucocephala is a leguminous fodder tree used by northern Australian producers to provide protein and boost the weight gains of extensively grazing cattle. There is a range of commercial *Leucaena* cultivars available which all contain a toxic non-protein amino acid, mimosine. Many rumen bacteria can degrade mimosine to 3,4-dihydroxypyridine (3,4-DHP), which is also toxic to cattle. To enable cattle to safely gain the full benefits of *Leucaena*, a bacterium, *Synergistes jonesii*, was isolated that could degrade the toxic metabolites 3,4 DHP and 2,3-hydroxypyridine (2,3-DHP) (Allison *et al.* 1992). A fermenter-grown mixed bacterial inoculum, containing *S. jonesii*, has been produced by DAF for over 20 years as an oral drench for cattle to prevent *Leucaena* toxicity and maximise weight gains (Klieve *et al.* 2002).

The necessity for this inoculum has been a contentious topic with speculation that Australian cattle now all possess rumen microbial populations capable of breaking down the three *Leucaena* toxins. The aim of this research is to survey cattle for rumen bacteria able to completely degrade all three of the *Leucaena* toxins, mimosine, 3,4-DHP and 2,3-DHP.

A survey was developed, and animal ethics approval obtained, to sample the rumen of cattle on properties throughout Queensland, in a randomised experimental design with four treatments consisting of different production scenarios with the experimental unit being the property. The treatments included properties where cattle have: (1) never received the DAF inoculum but are grazed on *Leucaena*; (2) received either rumen fluid from the original CSIRO cattle or the fistulated cattle held at Brian Pastures Research Station (pre-1993) and have not received the DAF inoculum and are grazed on *Leucaena*; (3) received the DAF inoculum and are grazing *Leucaena*; or (4) never been exposed to *Leucaena* (naïve).

In total, the survey will visit a minimum of three and maximum of five properties or research stations for each treatment and the primary variable is the concentration of the *Leucaena*-associated toxins mimosine, 3,4-DHP and 2,3-DHP. A mobile laboratory, including a portable incubator and micro-centrifuge, was established to enable crush-side processing and immediate incubation of collected rumen fluid, in toxin degradation assays. Cattle were rumen sampled via a stomach tube to obtain samples of rumen contents. Duplicate 10 mL volumes of the freshly collected rumen fluid were pipetted into pre-gassed Hungate tubes, an aliquot of one of the three purified toxins added and, after mixing, a time 0 (h) subsample was removed and frozen on dry ice. The Hungate tubes were placed into the portable incubator at 39°C, with further samples taken after 48 h and 168 h incubation. The concentration of the three toxins in the degradation assay subsamples were determined using high performance liquid chromatography (HPLC) (Lowry *et al.* 1985).

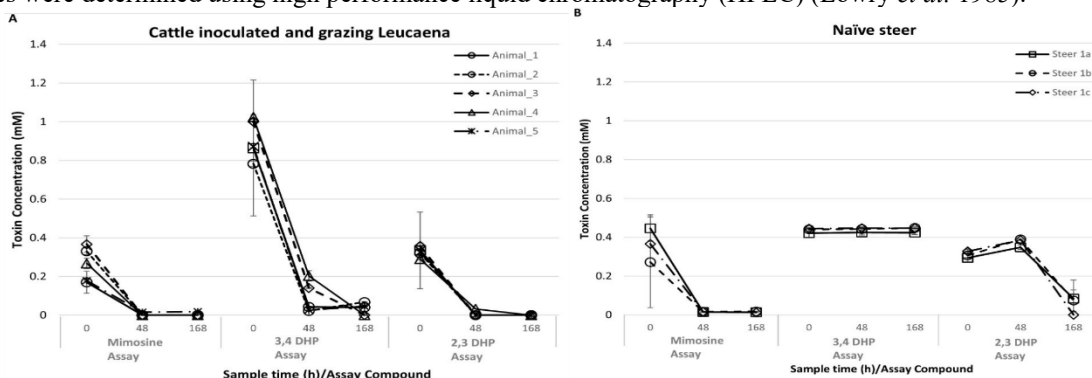


Fig. 1. Degradation assay of mimosine, 3,4-DHP or 2,3-DHP at time 0 (h), 48 h and 168 h samples from (A) Five cattle (received DAF inoculum, grazing *Leucaena*); (B) Naïve steer (never exposed to *Leucaena*); three replicate assays shown.

The mobile laboratory is proving to be operationally successful with initial toxin degradation assays showing that cattle receiving the DAF inoculum and grazing *Leucaena* possessed rumen bacterial populations capable of completely detoxifying all three toxins (Fig.1A). A steer which had never grazed *Leucaena* (naïve) did possess rumen bacterial populations able to degrade mimosine within 48 h and 2,3-DHP by 168 h but the levels of 3,4-DHP remained constant indicating that this compound was not degraded by the rumen bacteria (Fig. 1B). It is anticipated that this on-property survey will provide clarification whether all cattle possess rumen microbial populations capable of breaking down the three *Leucaena* toxins. The results from this study will be used to develop recommendations to industry concerning the use of the DAF inoculum for cattle grazing *Leucaena* pasture systems.

References

- Allison *et al.* (1992) *Systematic and Applied Microbiology* **15**(4), 522–529.
 Klieve *et al.* (2002) *Australian Journal of Agricultural Research* **53**(1), 1–5.
 Lowry *et al.* (1985) *Journal of the Science of Food and Agriculture* **36**, 799–807.

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