Development of a mixed microbial drench for detoxification of three Leucaena cultivars

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The adoption of *Leucaena leucocephala* in Queensland, as a high protein, leguminous fodder shrub, has been hindered by insect infestation, with psyllids thriving on Leucaena planted in high humidity regions. A psyllid-resistant cultivar of Leucaena has therefore been developed (Redlands). Nonetheless, all Leucaena cultivars contain the non-protein amino acid, mimosine, which in the rumen of cattle can be degraded by many different bacteria to the toxic metabolite 3-hydroxy-4-(1H)-pyridone (3,4-DHP). For over 20 years, a mixed microbial drench containing *Synergistes jonesii* has been produced by DAF to degrade mimosine, 3, 4-DHP and its degradation product 2,3-dihydroxypyridine (2,3-DHP), to reduce any toxic side-effects of feeding Leucaena to cattle (Klieve *et al*. 2002). This drench is produced in an *in-vitro* fermentation system supplied with leaf material from the Leucaena cultivar, Cunningham. Previous research found replacing the Cunningham leaf with either psyllid-resistant Redlands or psyllid-tolerant Wondergraze leaf, negatively impacted the mixed bacterial populations' ability to degrade 3,4-DHP (Ouwerkerk *et al*. 2019). This study aimed to test how supplying leaf material from a combination of three cultivars, Cunningham, Redlands and Wondergraze of Leucaena (TriMix) to the fermentation system, would affect *S. jonesii* populations, the ability of the mixed microbial populations to degrade mimosine, 3,4-DHP and 2,3 DHP and if these microbial populations would grow and retain activity, in fermentations supplied leaf from each single Leucaena cultivar.

The first fermentation (TriMix) was initially inoculated with three cryopreserved microbial starters of fermenter fluid harvested from the final day (day 30) of three respective, single-cultivar fermentations. The TriMix fermentation was supplied daily with a three-cultivar leaf combination and ran for 30 days. Daily subsamples of fermenter fluid were collected and stored at –20°C for further analysis. On the final day of the Trimix fermentation, fermenter fluid was harvested and cryopreserved as future starter material. Three further fermentations were then conducted, each supplied on the first day of fermentation with cryopreserved microbial starter from the initial TriMix fermentation. Each fermentation was supplied with leaf material from a single Leucaena cultivar and ran for a 30-day duration, with sub-samples of fermenter fluid collected daily. All daily subsamples of fermenter fluid were analysed for (a) toxin breakdown, determined by degradation assays and HPLC; and (b) daily *S. jonesii* numbers, determined by quantitative PCR (Fig. 1).

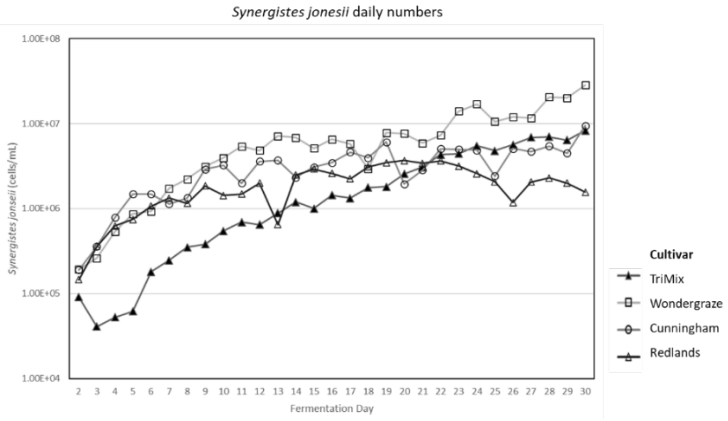

Fig. 1. Daily *S. jonesii* population numbers (cells/mL) determined by qPCR, for four fermentations: TriMix (fed leaf from all three cultivars); and three subsequent fermentations (fed leaf from a single cultivar, either Wondergraze, Cunningham or Redlands).

While the TriMix fermentation had an initial reduction in *S. jonesii* numbers, by 15 days of fermentation the *S. jonesii* numbers had increased to concentrations >10^6 cells/mL (Fig. 1). In all subsequent fermentations inoculated with the TriMix starter and supplied with leaf from a single cultivar, this initial decline in *S. jonesii* numbers did not occur, instead there was a rapid increase in *S. jonesii* numbers from the first day of fermentation (Fig. 1). In addition, all four fermentations were able to effectively degrade mimosine, 3,4-DHP and 2,3-DHP in toxin degradation assays undertaken every 5 days, from day 10 of the fermentation. This study showed that the microbial populations of the fermenter system could adapt to the nutritional and chemical composition of three different Leucaena cultivars and that a mixed microbial drench could be developed to provide similar numbers of *S. jonesii* to the original formulation, to be used to facilitate toxin breakdown in cattle grazing psyllid-resistant Leucaena cultivars.

References

Klieve A *et al*. (2002) *Australian Journal of Agricultural Research* **53**(1), 1–5.
Ouwerkerk D *et al.* (2019) *Proceedings* **36**(1), 96.

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