Enriching for rumen bacteria to degrade the *Pimelea* plant toxin simplexin, in an anaerobic *in vitro* fermenter

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Three species of Australian native plants, *Pimelea trichostachya*, *P. simplex* and *P. elongata*, are endemic to the arid rangelands of Queensland, New South Wales and South Australia and are responsible for *Pimelea* poisoning, also known as St George or Marree disease. Pimelea poisoning occurs in cattle ingesting *Pimelea* plants, with the orthoester simplexin identified as the responsible toxin. There is no effective treatment and economic losses have been estimated at over $50 million during significant *Pimelea* poisoning events. In a previous feeding trial, animals were fed increasing amounts of *Pimelea*, and after initially showing signs of poisoning, the animals appeared to adapt to ingesting *Pimelea*, possibly through rumen microbial degradation of the toxin (Fletcher *et al*., 2014). Kangaroos, forestomach fermenters, often graze pastures containing *Pimelea* with no apparent ill effects. To investigate the degradation effect further, a series of 30 day *in vitro*, anaerobic fermentations were undertaken.

Pimelea plant material was collected from properties in western Queensland, freeze dried and milled through a 3 mm screen. Rumen contents were collected from ruminants (cattle, sheep, goats) by stomach tubing and forestomach contents from culled macropods (Eastern Grey and Red kangaroos) grazing pastures containing *Pimelea* and cryopreserved in glycerol rumen fluid media prior to freezing (-80 °C) (Fletcher and Ouwerkerk, 2018). Anaerobic fermentations were conducted following the method of Klieve *et al.* (2002) with a 3 L fermentation volume, inoculated with cryopreserved rumen/forestomach content, and fed daily either 50:50 Buffel grass (*Cenchrus ciliaris*) hay and *Pimelea* or *Pimelea* alone. Samples were taken for simplexin analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to determine if microbial populations were degrading the simplexin. Bacteria were isolated from Day 30 fermentation fluid using a modified anaerobic media containing a crude ethanol extract of simplexin from *Pimelea* plant material and identified using 16S rRNA gene sequencing.

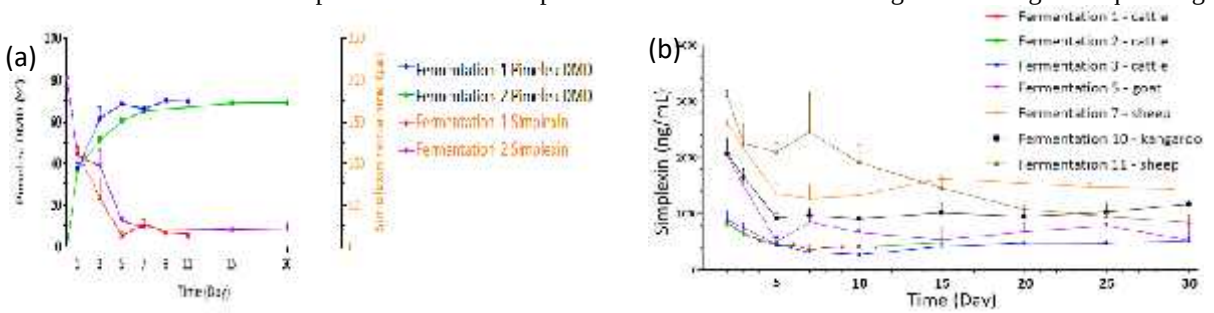

Figure 1. (a) Dry matter disappearance (DMD) of *Pimelea* in cattle rumen fluid started Fermentations 1 and 2 and (b) levels of the toxin simplexin in seven 30 day anaerobic fermentations fed milled *Pimelea* (started with rumen/forestomach fluid from cattle (three), sheep (two), goat and kangaroo).

Analysis of *Pimelea* plant material indicated it contains 30% acid digested fibre and DMD assays showed approximately 70 % of the plant utilised in cattle rumen fluid based fermentations (Figure 1a). The simplexin degradation in the seven fermentations was less clear with the levels of simplexin appearing to decrease and stabilise around Day 5 consistent with the steady-state conditions of addition/removal from the fermenter. From Day 5 onwards, one-way ANOVA with Tukey's multiple comparisons test showed no significant differences ($P>0.05$) between the average simplexin concentration (n=3) at each time point in each of the fermentations (Figure 1b). Using the crude simplexin extract, over 100 isolates representing 23 different bacterial species have been identified from known genera including *Streptococcus, Butyrivibrio, Prevotella, Clostridium, Selenomonas, Succinivibrio, Kandleria, Agathobacter, Pseudobutyrivibrio, Lachnoclostridium* along with a number of as yet unnamed isolates. These isolates are undergoing screening in a simplexin degradation assay. A further purified extract of simplexin will be used to continue isolations from fermentation populations to endeavour to obtain rumen bacteria for use in a rumen probiotic, to prevent *Pimelea* poisoning, in cattle grazing areas with *Pimelea* present.

References

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