Combating *Pimelea* poisoning with biodegradable biocomposite-based boluses: an investigation into the slow release of toxins in the rumen environment

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Pimelea poisoning, also known as St George or Marree Disease, was first recorded nearly one century ago and is still costing Australia's beef pastoral industry as much as $50 million AUD annually. Four *Pimelea* forbs were confirmed to be the causative agent and are widely distributed in all states and territories of Australia except Tasmania, including *Pimelea Simplex* subsp. *continua*, *Pimelea simplex* subsp. *simplex*, *Pimelea trichostachya* and *Pimelea elongata*. A series of daphnane- and tigliane-type diterpene esters and orthoesters have been isolated from these species, among which the most abundant toxin, simplexin, was identified to be the major active constituent for *Pimelea* poisoning. Even though comprehensive studies (Fletcher *et al*. 2009) have been conducted to understand this unique syndrome, there is so far no effective prevention or treatment for *Pimelea* poisoning. However, one inspiring finding was obtained from a feeding trial conducted by Fletcher *et al.* (2014), by which prolonged low doses of *Pimelea* plants were able to imbue naïve cattle with the capacity to detoxify or metabolize the toxins. In parallel with the ongoing efforts made to screen for microorganisms and enzymes that can decompose simplexin, we also aim to develop a rumen bolus by embedding the *Pimelea* plant materials in biodegradable polymeric system, which is envisaged to reside in the cattle rumen, releasing toxins in a controlled manner to develop immunity to *Pimelea* poisoning in the host animal.

In this study, different formulations of biocomposites, as candidate materials for the ultimate boluses, were prepared by extruding blends of milled *Pimelea* plant material or an ethanolic crude extract of *Pimelea* into biodegradable poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) polymer or mixtures of PHBV and the porogen icing sugar or starch. In order to accurately monitor the release of trace amounts of simplexin from these biocomposites, an analytical methodology combining solid phase extraction (SPE) and ultra-high performance liquid chromatography hyphenated with a quadrupole Orbitrap mass spectrometer (UHPLC-Q-Orbitrap-MS) was developed, optimized and validated. The concentrations of simplexin in two types of biocomposites that have been exposed to a rumen-fluid fermentation system *in vitro* for 0, 10, 20 and 30 days were determined using the established assay. A cumulative percentage of simplexin released from each biocomposites was back-calculated by comparison of the amount of simplexin remaining in the samples after exposure with that in samples before exposure (Day 0). Although a negligible release was found in biocomposite formulated from milled *Pimelea* plant (3 mm), PHBV and starch (30:35:35), a slow but sustained release was observed from biocomposite formulated with icing sugar instead of starch, as shown in Figure 1.

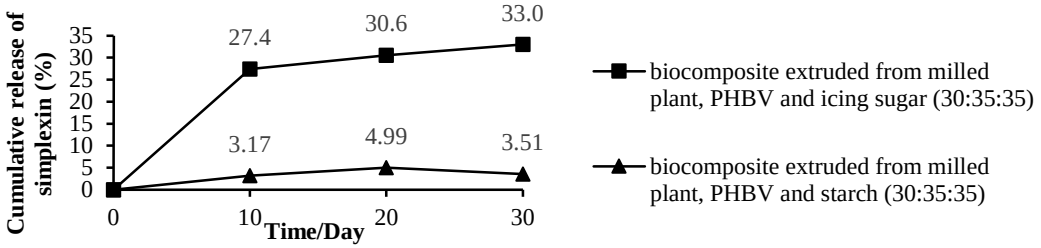

Figure 1. The release profiles of simplexin from two biocomposites after exposure to a rumen-fluid fermentation system *in vitro* for different periods of time.

The results demonstrate that the PHBV-based biocomposite system exhibits a promising slow-release performance in the rumen environment, which can be tailored by incorporating different type and composition of fillers. In our future work, the dominant mechanisms contributing to the release kinetics of simplexin will be evaluated, considering the microorganism's attachment and pore development on the surface of the biocomposite, microcrack propagation at the interface between the fibre and the polymer, enzymatic hydrolysis of PHBV and the diffusion of simplexin.

References

Fletcher MT, Silcock R, Ossedryver SM, Milson J and Chow S (2009) *Understanding Pimelea Poisoning of Cattle*. https://futurebeef.com.au/wp-content/uploads/2011/09/Understanding_pimelea_poisoning_of_cattle.pdf [Accessed 11 February 2020].

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