

Changes in rumen protozoal community by condensed tannin fractions of different molecular weights from a *Leucaena leucocephala* hybrid in vitro

ABSTRACT

Aims: To evaluate the effects of condensed tannins (CTs) fractions of differing molecular weights (MWs) from a *Leucaena leucocephala* hybrid-Rendang on the rumen protozoal community in vitro.

Methods and Results: The effects of unfractionated CTs (F0) and CT fractions of different MWs (F1 > F2 > F3 > F4 > F5) on protozoal population and community were evaluated in vitro using rumen microbes and ground guinea grass as the substrate. Higher-MW CT fractions F1 and F2 significantly ($P < 0.05$) decrease the number of ciliate protozoa. The real-time PCR analysis showed that the total protozoa was significantly ($P < 0.05$) lower in F0 and all CTs with fractions F1 and F2 having the lowest value. High-throughput sequencing of the partial 18S rRNA gene showed that the genus *Entodinium* significantly ($P < 0.05$) decreased with increasing MWs of CT, whereas *Anoplodinium*-*Diplodinium* were significantly ($P < 0.05$) increased. Inclusion of the highest MW CT fraction F1 decreased the relative abundance of the minor genera such as *Eudiplodinium* and *Polyplastron* compared to the control and CT fractions F2-F5.

Conclusion: CTs of differing MWs could reduce and alter the rumen protozoa population in vitro. This effect was more pronounced for higher-MW CTs.

Significance and Impact of the Study: The high MW CTs should be considered as a feed supplement in the ruminant diet to reduce the protozoal population which are known to be associated with methanogens as a means to mitigate methane production in the rumen.

Keyword: 18S rRNA gene sequence; Condensed tannin fractions; Illumina Miseq; *Leucaena leucocephala*; Molecular weight; Protozoa diversity