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Methane production, fermentation characteristics, and microbial profiles in the rumen of tropical cattle fed tea seed saponin supplementation

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ABSTRACT

Belmont Red Composite rumen-cannulated steers ($n = 8$, 364 ± 8.4 kg liveweight, LW; least squares means ± s.e.m.) were used to assess effects of feeding tea seed (*Camellia sinensis* L.) saponin (TSS) supplementation on performance, methanogenesis, fermentation pattern and rumen microbial communities. The expectation was to use TSS to potentially modulate the rumen microbial population and decrease enteric methane (CH_4) production. The steers were fed twice a day with a basal diet (BD) that contained a mixture of 0.15 Rhodes grass (*Chloris gayana*) hay plus 0.85 of a commercial concentrate before CH_4 emissions were measured in open-circuit respiratory chambers for 48 h. Steers were then adapted progressively to doses of 20 and 30 g/day of TSS for 10 and 6 days, respectively before new CH_4 measurements were recorded. Final placement in chambers was conducted after 13 days of TSS removal (BDP). Rumen fluid samples from each steer were collected for the treatments BD, BD + 20 g TSS, BD + 30 g TSS and BDP. Growth performance and CH_4 emissions were not affected by the addition of TSS, but compared to the BD and TSS diets, daily CH_4 emissions (g) and yield (g CH_4/kg DMI) were lower ($P < 0.05$) by 18 and 22%, respectively, after TSS treatment. Concentrations of total volatile fatty acids, acetate and propionate were not affected by TSS treatment, as were total rumen bacteria and methanogens numbers. Relative to the BD and BDP, butyrate concentration was higher ($P < 0.05$) in TSS treated animals, resulting in a reduced ratio of acetate to butyric acid ($P < 0.05$). In comparison with BD control, the relative abundance of *Fibrobacter succinogenes* increased by 2 fold ($P < 0.001$) in the other three dietary groups. However, compared to the BD and BD + 20 g of TSS, the abundance of *R. albus* increased by 100 fold ($P < 0.01$) in the BD + 30 g of TSS and BDP diets, while the abundance of *R. flavefaciens* was 100 fold lower in TSS supplemented and

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BDP groups than in BD control group ($P < 0.001$). Thermoplasmatales-related RCC archaea and protozoa counts increased linearly with 20 and 30 g of TSS addition, but returned to BD control levels after the TSS supplement was withdrawn. It was concluded that TSS supplementation changed rumen microbial community in cattle, but was not inhibitory to methane production, which was inconsistent with published *in vitro* results and small ruminant trials where TSS caused a dose-dependent reduction in CH_4 emissions.

1. Introduction

Methane (CH_4) is the third most abundant greenhouse gas (GHG) in the atmosphere, behind water vapour and carbon dioxide (CO_2), and CH_4 has been responsible for about 20% of the global radiative forcing since 1750 (IPCC, 2001). Over the past 250 years, CH_4 emissions have increased in 149% and possess higher global warming potential (i.e. 23 times) and longer atmospheric lifetime (8.4 years) than CO_2 (Thorpe, 2009). It has been suggested that ruminant enteric CH_4 accounts for a quarter of anthropogenic emissions (Lassey, 2008).

Apart from environmental issues, CH_4 also represents a significant feed energy loss to the host animal from 2 to 12% of gross energy intake (Johnson and Johnson, 1995). Thus, numerous efforts are underway to manipulate rumen fermentation and the rumen microbial ecosystem to reduce CH_4 emissions and improve feed efficiency in ruminants. Among these options, saponins or saponin like substances have showed potential to modulate rumen fermentation patterns and mitigate CH_4 production in *in vitro* ruminal fermentation (Patra et al., 2006; Pen et al., 2006; Goel et al., 2008), but not in some *in vivo* experiments (Pen et al., 2007; Holtshausen et al., 2009).

Recent studies provide evidence that tea seed (*Camellia sinensis* L.) saponin (TSS) may have potential to be used as a antimethanogenic agent due to the antiprotozoal effect *in vitro* (Hu et al., 2005) and in small ruminants (Mao et al., 2010; Zhou et al., 2011). However, there is little published information on the CH_4-suppressing effects of TSS supplementation in cattle, and the inclusion level of plant-derived saponins for different ruminants with variable diets should be tested to achieve the optimum response. The objective of this study was to evaluate the responses of methane production, rumen fermentation and rumen microbial community structure to the increasing doses of TSS in a basal diet. Quantitative real-time PCR was used to monitor the relative changes in the cellulolytic bacteria and methanogens (*Methanobrevibacter* spp. and Rumen Cluster C), and the abundance of total protozoa.

2. Materials and methods

2.1. Experimental design

The experiment was conducted between 11th February 2013 (late summer) and 25th May 2013 (late autumn; 103 days) at Lansdown Research Station, Woodstock near Townsville, QLD (lat. 19° 39′ 30″S, long. 146° 50′ 17″E). Animal care was provided according with the approved CSIRO Animal Ethic Committee protocol No A12/2012.

Eight Belmont Red Composite (Africander (African Sanga) x Brahman (*Bos indicus*) x Hereford-Shorthorn (3/4 *B. taurus*) steers [364 ± 8.4 kg LW; least squares means ± standard error of means (s.e.m.)] fitted with a permanent rumen cannula were used to assess the desired range of TSS (Zhejiang Oriental Tea Technology Co., Ltd., Changshan, Zhejiang, China) supplementation in a conventional-finishing feedlot basal diet [BD; mixture of 0.15 Rhodes grass (*Chloris gayana*) hay and 0.85 of a commercial mixed high-grain diet (Coleman Stock Feeds Pty. Ltd., Charters Towers, QLD, Australia)]. The effects of the natural supplementation were assessed in terms of dry matter intake (DMI), LW, CH_4 emissions measured in open-circuit respiration chambers, rumen fermentation and rumen microbial ecology.

All steers were accustomed to the respiration chambers during previous weeks of the CH_4 measurements and allowed to exercise daily in the cattle yard in the early morning to reduce stress and facilitate cleaning of pens. Fresh water was provided *ad libitum*. Internal and external parasites were controlled from day 0 using Dectomax Pour-on (Doramecting, 10 mg/50 kg; Pfizer, São Paulo, Brazil). At the end of the experiment, the steers grazed on pasture close to the cattle yard over 7 days to observe any adverse clinical symptoms.

2.2. Feeding and supplementation

Management of feeding and supplementation in this study followed the methodologies outlined by Ramírez-Restrepo et al. (2014, 2016) for the evaluation of natural bioactive compounds on cattle. Grazing rumen-cannulated steers were accustomed to the BD in the cattle yard for 10 days, whilst a 56 day period was used in the animal house for diet adaptation and preliminary measurements on the control BD (i.e. the last 8 days). Supplementation up to 20 g/d of TSS was administered for 10 days and steers remained on that level of supplementation for 3 days. During the following 6 days steers were supplemented up to 30 g TSS/day lasting for 4 days in the upper dosage. This was followed by 13 days suspension of the supplement

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