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Effect of tea (*Camellia sinensis*) seed saponins on *in vitro* rumen fermentation, methane production and true digestibility at different forage to concentrate ratios

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ABSTRACT

The present study was conducted to evaluate the effect of tea (*Camellia sinensis*) seed saponins (TSS) on *in vitro* rumen fermentation with different forage to concentrate ratios in the *in vitro* gas production technique. Experimental treatments were a low forage diet (forage: concentrate = 30:70), a medium forage diet (forage: concentrate = 50:50) and a high forage diet (forage: concentrate = 70:30). TSS was added at levels of 0.0%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% and 1.0% of substrate. Protozoal count, ammonia-N production and methane production decreased linearly up to the dose level of 0.8% in all the substrates. The maximum reduction obtained was 54.6%, 57.2% and 60.6% for protozoal counts; 29%, 33% and 36% for methane production; and 36.6%, 36.6% and 33.8% for ammonia-N production at low, medium and high forage diets, respectively. Net 24 h gas production, short chain fatty acid production, metabolizable energy value increased and *in vitro* true dry matter and organic matter digestibility significantly decreased due to addition of saponins. Results suggest that TSS has the potential to reduce methane production and positively impact rumen fermentation across different forage to concentrate ratios.

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KEYWORDS
Forage to concentrate ratio; *in vitro* digestibility; methane; tea seed saponin

1. Introduction

Excessive production of greenhouse gas (GHG) has been cited by many scientists world over as responsible for climate change. The adverse effects of climate change are enormous. The livestock sector is one of the important contributors of GHG. GHG emission from the global livestock sector was 7.1 gigatonnes CO_2-equivalent per annum, representing 14.5% of human-induced GHG emissions for the year 2005 (FAO 2013). The global cattle population is responsible for 65% of the GHG emissions from livestock. The rapid increase in demand for animal products in developing nations may result in higher emission shares and volumes over time. Methane production during enteric fermentation from ruminants is one of the important emission sources and 2.7 gigatonnes CO_2-equivalent per annum GHG emission was produced in the year 2005 (FAO 2013). Average loss of gross energy in the form of methane is reported to be 7.89% of intake for dry cows (Wilkerson et al. 1995). So there is an urgent need to reduce methane emission from ruminants.

Recent research suggests that plant secondary metabolites such as saponins may have potential to reduce methane emission, and improve N utilization by defaunating action in rumen (Wina et al. 2005; Gurbuz & Davies 2010). Jayanegara et al. (2014) observed in a meta-analysis study that despite large structural diversity, addition of saponin significantly reduced *in vitro* methane emission. They also reported that higher levels of saponin in the diet did not negatively influence digestibility. There are reports which suggest that forage to concentrate ratio is one of the primary factors which affect the effect of saponin supplementation (Goel et al. 2008; Gurbuz 2009; Manatbay et al. 2014) on rumen fermentation and animal production.

In view of this, the present experiment was conducted to test the effect of saponin extracts of tea (*Camellia sinensis)* seed on rumen fermentation and methane production by using the *in vitro* gas production technique (IVGPT) with three different forage to concentrate ratios and also to get the best level of saponin that should be added to improve rumen fermentation.

2. Materials and methods

The experimental protocols were reviewed and approved by the Institute Research Committee and Animal Ethics Committee.

2.1. Extraction of saponin from tea seeds

Saponin was extracted from tea seeds as per the method of Joshi et al. (2013). Dried and powered tea seeds were defatted with hexane in a percolator at room temperature. The defatted tea seed residue was suspended in 70% aqueous methanol. The methanolic extract was concentrated and dried into powder containing saponins. The powder was chromatographed on Diaion HP-20 eluting with $H_2O \rightarrow CH_3OH \rightarrow CHCl_3$ to get H_2O, CH_3OH and $CHCl_3$ fractions. The methanolic fraction was dried

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