J. Dairy Sci. 100:1–11
https://doi.org/10.3168/jds.2016-11644
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Tea saponin reduced methanogenesis in vitro but increased methane yield in lactating dairy cows

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ABSTRACT

The effect of tea saponin supplementation in the ruminant diet on methane emissions, rumen fermentation, and digestive processes is still under debate. The objective of this study was to assess the effect of this plant extract on methanogenesis, total-tract digestibility, and lactating performances of dairy cows. The work included 2 independent and successive experiments. First, the effect of 7 tea saponin doses (from 0 to 0.50 g/L) on methane emissions and protozoa concentrations was tested in 2 repeated in vitro batch culture incubations using bovine rumen contents as inoculum and a cereal mixture as substrate. After 18 h of incubation, total gas production and composition as well as rumen fermentation parameters and protozoa concentration were analyzed. Increasing dosage of the plant extract reduced methane production and protozoa concentration, with a maximum reduction of 29% for CH_4 (mL/g of substrate) and 51% for protozoa (10^5/mL). Tea saponin did not affect volatile fatty acids concentration, but marginally decreased total gas production by 5% at the highest dose. Second, a 2-period crossover design experiment was carried out with 8 lactating dairy cows fed a basal diet (54% corn silage, 6% hay, and 40% pelleted concentrates on a dry matter basis) without (control) or with 0.52% tea saponin (TSP). Each experimental period lasted 5 wk. Animals were fed ad libitum during the first 3 wk of the period (wk 1, 2, and 3) and restricted (95% of ad libitum intake) during the last 2 wk (wk 4 and 5). Intake and milk production were recorded daily. Methane emissions were quantified using open chambers (2 d, wk 4). Total-tract digestibility and nitrogen balance were determined from total feces and urine collected separately (5 d, wk 5). Rumen fermentation parameters and protozoa concentration were analyzed from samples taken after morning feeding (1 d, wk 5). Milk production, dry matter intake, and feed efficiency were reduced with TSP (−18, −12, and −8%, respectively). As daily methane production (g/d) was not affected, methane emissions (g/kg of dry matter intake) increased by 14% with TSP. Total-tract digestibility and nitrogen balance were similar between diets, except for acid detergent fiber digestibility, which tended to be improved with TSP (+4 percentage units). Rumen fermentation parameters and protozoa concentration were relatively unchanged by diets. Under the conditions of this experiment, tea saponin is not efficient to reduce methane emissions from dairy cows.
Key words: dairy cow, methane, rumen fermentation, tea saponin

INTRODUCTION

Saponins have an inhibitory action toward protozoa by affecting cell membrane integrity (Goel and Makkar, 2012). This biological property has been used to implement dietary CH_4 mitigation strategies in ruminants, as protozoa are known to be positively correlated with methanogenesis (Guyader et al., 2014). For instance, Wang et al. (2012) reviewed the possible antimethanogenic potential of tea saponin. Using rumen inoculum from sheep fed 60% forage for in vitro experiments, Hu et al. (2005b) and Guo et al. (2008) reported a CH_4 reduction of 14 and 8% (mL/g of substrate, DM basis), respectively, when using 0.24 g/L of pure tea saponin powder mixed with a substrate containing 50% hay. However, the CH_4-mitigating effect of this plant extract in vivo remains controversial. Yuan et al. (2007), Mao et al. (2010), and Zhou et al. (2011) reported a significant decrease of CH_4 emissions (g/kg of DMI; −9, −27, and −11%, respectively) and protozoa concentrations (−42% on average, expressed as a percent of bacterial 16S rDNA) in sheep fed 60% forage mixed with 0.50% tea saponin powder in DM. In contrast, a similar dosage of tea saponin included in pelleted concentrates did not reduce methanogenesis in nonlactating dairy cows fed 50% forage (Guyader et al., 2015a). A lower dosage (0.37% tea saponin powder in DM, mixed with diet) also failed to reduce CH_4 emissions in steers fed

Received June 21, 2016.
Accepted November 22, 2016.
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85% (DM basis) concentrate diet (Ramírez-Restrepo et al., 2016). Such a discrepancy could be related to differences in feed retention times within the rumen as influenced by the level of intake and composition of basal diet (Kumar et al., 2009; Martin et al., 2010), or in the quality of the plant extract among experiments (Li and Powers, 2012). The effect of tea saponin supplementation on CH_4 production and protozoa population of lactating dairy cows has never been tested.

Aside from its potential CH_4-mitigating effect, tea saponin may have a beneficial effect on fiber digestibility. Indeed, whereas other sources of saponins, such as *Quillaja saponaria* or *Yucca schidigera*, have no effect on diet digestibility (Lila et al., 2005; Pen et al., 2007; Holtshausen et al., 2009), tea saponin improved in vitro OM digestibility (21%; Wei et al., 2012) and numerically increased NDF digestibility in nonlactating dairy cows (2 percentage units; Guyader et al., 2015a).

The objective of the current study was to clarify the effect of tea saponin on methanogenesis and protozoa concentrations as well as its effect on diet digestibility of cattle. A dose-response in vitro experiment was designed to evaluate the efficacy of the commercial tea saponin extract to decrease CH_4 production and protozoa concentrations. An in vivo experiment was then conducted to test the effect of tea saponin supplementation on diet digestibility, milk production, and methane emission of lactating dairy cows.

MATERIALS AND METHODS

In both experiments, a tea saponin extract containing 689 g of pure tea saponin/kg of DM was used (Choisun Tea Sci-Tech Co. Ltd., Hangzhou, Zhejiang, China).

In Vitro Experiment

Batch Culture Incubations. Two repeated batch culture incubations were conducted to assess the effectiveness of a tea saponin extract to reduce CH_4 production and protozoa concentrations. Incubations were carried out in 120-mL bottles containing 400 mg of substrate, 15 mL of rumen fluid as inoculum, and 25 mL of an anaerobic buffer solution (Goering and VanSoest, 1970). On a DM basis, the ground substrate (1-mm screen) was composed of wheat (50.5%), corn (30.0%), dehydrated beet pulp (8.2%), calcium carbonate (3.4%), urea (2.4%), beet molasses (2.0%), binder (2.0%), and mineral-vitamin mix (1.5%). The OM, CP, starch, NDF, and ADF content of the substrate were 92.2, 18.1, 50.8, 12.8, and 4.6% of DM, respectively. Seven concentrations of pure tea saponin were tested (0, 0.04, 0.08, 0.12, 0.15, 0.30, and 0.50 mg/mL), with dosages selected according to previous in vitro experiments with tea saponin (Hu et al., 2005a; Guo et al., 2008; Arhab et al., 2014). Within each incubation, every treatment was assayed in duplicate.

Rumen fluid inoculum was prepared from strained (polyester monofilament fabric, 250 μm pore size) and pooled whole ruminal contents collected manually before feeding from 3 cannulated nonlactating dairy cows receiving (DM basis): 38% corn silage, 32% natural grass hay, and 30% concentrate (30% dehydrated beet pulp, 23% wheat, 20% barley, 15% rapeseed meal, 7.8% soybean meal, 1.5% cane molasses, 1.0% dicalcium phosphate, 0.6% salt, 0.5% magnesium carbonate, 0.5% premix, 0.05% fungicide, and 0.03% aroma). Animals were adapted to their diets during 3 wk before the first sampling. Inoculum was mixed with the substrate and the anaerobic buffer under a CO_2 stream into prewarmed (39°C) incubation bottles. The bottles were then sealed with a butyl rubber stopper and incubated anaerobically at 39°C for 18 h.

Sampling and Gas Measurement. At the end of the incubation, gas production in each bottle was measured with a pressure transducer. Gas samples were collected with 10-mL syringes for CH_4 and H_2 concentration analysis by GC with a thermal conductivity detector (Micro GC 3000, Agilent Technologies, France; Morgavi et al., 2013). Bottles were then opened and pH was immediately measured on the total mixed content with a portable pH meter (CG840, electrode Ag/AgCl, Schott Geräte, Hofheim, Germany). Samples from total mixed content were taken for VFA analysis [0.8 mL of medium in 0.5 mL of a 0.5 *M* HCl solution containing 2% (wt/vol) metaphosphoric acid and 0.4% (wt/vol) crotonic acid] and protozoa counting [2 mL of medium in 2 mL of a 0.06% (wt/vol) methyl green-formalin solution]. Samples for VFA analysis were kept at 4°C for 2 h before being centrifuged at 16,500 × *g* for 10 min at 4°C. Supernatant was collected and stored at −20°C before VFA analysis by GC with a flame ionization detector (Morgavi et al., 2008). Samples for protozoa counting were stored at room temperature and in the dark until counting by microscopy (Ogimoto and Imai, 1981), and concentrations were $\log_{10}$-transformed before statistical analysis.

In Vivo Experiment

The experiment was conducted at the INRA's Saint-Genès-Champanelle research center in France from January to May 2014. All procedures involving animals were performed in accordance with the French Ministry of Agriculture and the European guidelines (European Parliament and Council of the European Union, 2010) for regulations of animal research and experimentation (approval number 01784.01).

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