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## Effect of technical cashew nut shell liquid on rumen methane emission and lactation performance of dairy cows

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### ABSTRACT

Technical-grade cashew nut shell liquid (TCNSL) is a by-product of the cashew nut industry in tropical countries, and is known to exhibit a wide range of biological activities, including inhibitory effect against gram-positive bacteria. This study was conducted to investigate the effects of TCNSL (73.3% cardanol, 16.4% cardol, and 3.0% methylcardol) on rumen methane emission, nutrient digestibility, dry matter intake, and milk yield and composition in dairy cows. Eight multiparous Holstein cows were used in a crossover design trial with two 21-d experimental periods. The diet was based on corn silage and alfalfa haylage and was formulated to meet or exceed the energy and metabolizable protein requirements of the cows. Treatments were control (no TCNSL supplementation) or 30 g/cow per day of TCNSL. Rumen carbon dioxide emission was not affected by TCNSL. Treatment had no effect on methane emission (542 vs.  $511 \pm 35.3$  g/cow per day, respectively) and methane emission intensity ( $15.0$  vs.  $13.9 \pm 0.58$  g/kg of energy-corrected milk, respectively) and tended to decrease methane emission per kilogram of dry matter intake ( $20.2$  vs.  $18.6 \pm 1.04$  g/kg, respectively). Dry matter intake (average  $26.9 \pm 1.00$  kg/d), milk yield ( $40.0 \pm 1.73$  kg/d), and milk composition were not different between treatments. The TCNSL had no effect on N losses in urine and feces and total-tract apparent digestibility of nutrients, except digestibility of neutral detergent fiber tended to be increased compared with the control. Plasma urea and glucose concentrations were not affected by TCNSL. Concentration of milk C18:0 tended to be decreased (17%) by TCNSL compared with the control. In this study, TCNSL did not alter absolute methane emission in the rumen, but tended to decrease it by 8%

per kilogram of dry matter intake. The TCNSL had no effect on milk yield and composition in dairy cows.

**Key words:** cashew nut shell liquid, methane, dairy cow

### INTRODUCTION

Research aimed at decreasing enteric methane emissions from ruminant animals has intensified in the last decade, with a variety of mitigation practices available to the livestock industries (Hristov et al., 2013). Cashew nut shell liquid (CNSL) is a dietary supplement that has shown a potential to decrease enteric methane emission in ruminants. Studies with nonlactating dairy cattle reported up to 38% reduction in methane emission (Shinkai et al., 2012). Several earlier and more recent in vitro studies suggested that the methane mitigation effect of CNSL is likely a result of inhibition of methanogenic archaea or specific bacteria contributing methanogenic substrates by the phenol-based constituents of the product (Van Nevel et al., 1971; Danielsson et al., 2014).

Cashew nut shell liquid is a by-product of cashew nut processing and has a variety of industrial uses (Menon et al., 1985; Lubi and Thachil, 2000). Natural CNSL contains mainly 4 constituents, cardanol, cardol, anacardic acid, and 6-methyl cardol (1.2, 11.3, 64.9, and 2.0% by weight, respectively), which are mixtures of constituents differing in side-chain unsaturation (Lubi and Thachil, 2000). The antibacterial properties of anacardic acids are well known, depend on their structure, and may be species-specific (Van Nevel et al., 1971; Kubo et al., 1993). Van Nevel et al. (1971) reported that methane emission tended to decrease in vitro with increasing numbers of double bonds in the alkyl chain of anacardic acid. In view of earlier work (Demeyer and Henderickx, 1967) and results obtained by Gellerman et al. (1969), Van Nevel et al. (1971) hypothesized that anacardic acids probably exert a direct toxic effect on methanogenic archaea.

Technical-grade CNSL (TCNSL) is the most abundant industrial source of the resorcinolic lipid cardol

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and the phenolic lipid cardanol (Kozubek and Tyman, 1999). It contains little or no anacardic acids because of decarboxylation during the roasting process, which converts anacardic acid to cardanol or 2-pentadecadiethyl phenol (Tyman et al., 1978). Thus, the main active constituents of TCNSL are cardanol (63%) and cardol (11%), with up to 23% polymers and other minor materials (Lubi and Thachil, 2000; Voirin et al., 2014). Being phenolic acids (anacardic acid) or lipids (cardanol and cardol), these compounds have been shown to exhibit strong antibacterial, antiprotozoal, and antifungal properties (Stasiuk and Kozubek, 2010). These authors (Stasiuk and Kozubek, 2010) suggested that interactions with biological membranes and DNA structure and cytotoxic activity are responsible for the antimicrobial and antiparasitic activities of phenolic lipids, such as cardanol and cardol from TCNSL or ginkgo fruits. Cardanol has been shown to exhibit antibacterial properties against various species (Boonsai et al., 2014), although its effectiveness is considerably lower than that of anacardic acids (Himejima and Kubo, 1991). Watanabe et al. (2010), for example, reported a 57% decrease with CNSL, but only a numerical 10% reduction in methane emission *in vitro* with heated CNSL (i.e., TCNSL).

*In vivo* studies with lactating animals investigating the methane-mitigating effect of CNSL or TCNSL are scarce. Most of the data reported to date are from *in vitro* experiments or with nonproducing animals. To the best of our knowledge, in only one study was TCNSL tested in lactating dairy cows producing 25 to 26 kg milk/d (Coutinho et al., 2014). Thus, a need exists to study the effect of CNSL, and particularly TCNSL (which is more available commercially), on rumen methane emission and animal productivity *in vivo* in trials with high-producing animals. The hypothesis of the current experiment was that TCNSL would decrease methane emission and have no negative effects on feed intake and milk production in lactating dairy cows. The objectives of the trial were to investigate the effect of supplementation of a corn silage-based diet with 30 g/cow per day of TCNSL on methane and carbon dioxide emission in the rumen, nutrient digestibility, urinary and fecal N losses, milk FA profile, DMI, and milk production and composition in high-producing dairy cows.

## MATERIALS AND METHODS

Animals involved in this experiment were cared for according to the guidelines of the Pennsylvania State University Animal Care and Use Committee. The committee reviewed and approved the experiment and all procedures carried out in the study.

## Animals and Experimental Design

The experiment was a crossover design with 2 periods and was conducted at the tiestall facility of the Pennsylvania State University's Dairy Center. Each experimental period lasted 21 d, with the first 14 d for adaptation and the last 7 d for sampling and data collection. The experiment was conducted with 8 multiparous Holstein dairy cows grouped in 2 treatment groups and averaging,  $2.6 \pm 0.32$  lactations,  $142 \pm 5.1$  DIM,  $40.4 \pm 1.71$  kg/d of milk yield, and  $669 \pm 47.8$  kg of BW at the beginning of the trial. The basal TMR (Table 1) was formulated to meet or exceed the  $NE_L$  and MP requirements of the cows based on their milk production and DMI at the beginning of the trial. Treatments were control (basal TMR) and TCNSL fed at 30 g/cow per day. The TCNSL was provided by Palmer International, Inc. (Skippack, PA) and contained cardanol (73.3%), cardol (16.4%), and 2-methylcardol (3.0%). In a previous study with lactating cows, we used TCNSL application rates of up to 7 g/cow per day (at 20 to 22 kg/d of DMI) and did not observe any negative effects on production, DMI, or total-tract nutrient digestibility (Coutinho et al., 2014). Based on these data and the application rates of CNSL used by Shinkai et al. (2012; 20 and 37 g/cow per day), the 30-g/d dose was arbitrarily chosen for the current study. The 30 g/d of TCNSL dose was close to the dose of 4 g/100 kg of BW per day suggested for CNSL by Shinkai et al. (2012). Cows were fed once daily at approximately 0800 h and feed was offered *ad libitum* to achieve approximately 10% refusals. Cows were milked twice daily at approximately 0600 and 1800 h. The TCNSL was fed top-dressed and mixed with approximately 2 kg of the freshly delivered TMR before cows returned from the morning milking. Feed was pushed up to the cows 4 to 6 times daily. Cows received recombinant bST (Posilac, Elanco Co., Greenfield, IN; 500 mg/cow *i.m.*) on d 1 and 10 of each experimental period.

## Sampling and Measurements

Feed delivered and refusal weights were recorded daily. Forages were sampled weekly and dried in a microwave oven to determine DM. Weekly changes were made in the TMR if changes occurred in forage DM. Individual forage and concentrate ingredient samples were collected once weekly and samples of the TMR and refusals were collected twice weekly. Individual forage and TMR samples were composited (equal weight basis) by experimental period, dried at 65°C in a forced-air oven for 48 h, and ground in a Wiley Mill (Thomas Scientific, Swedesboro, NJ) through a 1-mm sieve for further analyses.

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