Animal Feed Science and Technology 209 (2015) 51–59

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Dose–response to eugenol supplementation to dairy cow diets: Methane production, N excretion, ruminal fermentation, nutrient digestibility, milk production, and milk fatty acid profile

C. Benchaar*, F. Hassanat, H.V. Petit

Agriculture and Agri-Food Canada, Dairy and Swine Research and Development Centre, 2000 College Street, Sherbrooke, Quebec, Canada J1M 0C8

ARTICLE INFO

Article history:
Received 5 March 2015
Received in revised form 28 July 2015
Accepted 29 July 2015

Keywords:
Essential oils
Enteric methane
N excretion
Milk production
Milk fatty acid profile
Dairy cow

ABSTRACT

The objective of this study was to evaluate the effects of increasing dietary concentrations of eugenol on enteric CH_4 production, N excretion, ruminal fermentation characteristics, nutrient digestibility, milk production, and milk fatty acid (FA) composition. For this purpose, eight ruminally cannulated multiparous lactating cows were used in a replicated 4×4 Latin square design (28-d periods). Cows were fed a total mixed ration without (0 mg/kg dry matter [DM]) or with (25, 50, 75 mg/kg DM) eugenol supplementation. Enteric methane production was measured using the sulfur hexafluoride (SF_6) tracer gas technique. Dry matter intake, N excretion, and apparent total-tract digestibility of DM, organic matter, acid detergent fiber, neutral detergent fiber, and gross energy (GE) were not changed by increasing dietary doses of eugenol. Likewise, total volatile fatty acid (VFA) concentration was unaffected while of the individual VFA, only the molar proportion of branched-chain VFA decreased linearly with increasing eugenol levels. Diet supplementation with increasing doses of eugenol had no effect on ruminal ammonia concentration, but tended ($P = 0.08$) to increase linearly total protozoa number. Milk production and milk concentrations of protein, lactose, and urea nitrogen were unchanged, whereas milk fat yield was quadratically affected by increasing dietary levels of eugenol (1.38, 1.32, 1.31, and 1.33 kg/d for 0, 25, 50 and 75 mg/kg eugenol, respectively). Diet supplementation with eugenol had minor effects on milk FA profile. Enteric CH_4 emissions (g/d) and CH_4 energy losses (as a proportion of GE intake) were not changed by including incremental levels of eugenol in the diet. Results from this study show no effects of supplementing dairy cow diets with eugenol (up to 75 mg/kg) on enteric CH_4 emissions, N excretion, and milk performance. We concluded that at the dosage levels assessed in the current study (up to 75 mg/kg), eugenol cannot be promoted as an effective dietary approach to mitigate enteric CH_4 emissions, decrease N excretion, and enhance feed efficiency in dairy cows.

Abbreviations: ADF, acid detergent fiber; BCVFA, branched-chain volatile fatty acid; BW, body weight; CP, crude protein; DM, dry matter; DMI, dry matter intake; FCM, fat-corrected milk; FA, fatty acid; GE, gross energy; SF_6, sulfur hexafluoride; MUN, milk urea nitrogen; aNDF, neutral detergent fiber with residual ash; OM, organic matter; TMR, total mixed ration; VFA, volatile fatty acid.

* Corresponding author.
E-mail address: chaouki.benchaar@agr.gc.ca (C. Benchaar).

http://dx.doi.org/10.1016/j.anifeedsci.2015.07.027
0377-8401/

1. Introduction

In recent years, because of their antimicrobial properties, essential oils have been investigated for their potential to mitigate enteric CH_4 production from ruminants (Benchaar and Greathead, 2011). One potential essential oil of interest is eugenol, which is a phenolic compound mainly present in clove bud (850–900 g/kg; Màthé, 2009) and cinnamon (760 g/kg; Fraser et al., 2007) oils. Information from animal studies with eugenol is limited and to our knowledge only 2 studies (Yang et al., 2010; Benchaar et al., 2012) have investigated the effects of diet supplementation with eugenol on feed intake, digestion, ruminal fermentation characteristics and animal performance. Several *in vitro* studies have evaluated the effects of eugenol (or oils rich in eugenol) on rumen fermentation (Busquet et al., 2005; Cardozo et al., 2005; Busquet et al., 2006; Castillejos et al., 2006; Benchaar et al., 2007; Lourenço et al., 2008), but very few of them have specifically assessed the inhibitory effect of eugenol on ruminal methanogenesis. Fraser et al. (2007) reported a decrease in CH_4 production when cinnamon oil (containing 760 g/kg eugenol) was supplied at the concentration of 500 mg/l in continuous-culture systems. Chaves et al. (2008) examined the effects of cinnamon oil on ruminal methanogenesis *in vitro*. When added in batch cultures at the concentration of 250 mg/l, cinnamon oil markedly inhibited the specific methanogenic activity of ruminal fluid and CH_4 production. Despite this demonstrated *in vitro* antimethangenic property of eugenol, no *in vivo* studies have been conducted to confirm the potential of eugenol as an effective feed additive to mitigate enteric CH_4 emissions from ruminants.

In vitro supplementation of eugenol lowered ammonia concentration (Busquet et al., 2006; Castillejos et al., 2006), an indication of an inhibition of ruminal protein degradation. This suggests that eugenol may be a potential alternative for improving N utilization in ruminants and thereby, decreasing N excretion into the environment. Dijkstra et al. (2011) examined the interrelationship between N excretion and enteric CH_4 emissions in ruminants. The authors concluded that the interrelationship between N excretion and enteric CH_4 production is complex and needs to be understood to ensure that the reduction of one pollutant (*e.g.*, CH_4) does not lead to higher emission of other pollutants (*e.g.*, N).

Given the reported *in vitro* anti-methanogenic and anti-proteolytic activities of eugenol, we hypothesized that supplementation of lactating dairy cows with eugenol may help to mitigate enteric CH_4 emissions and reduce N excretion. Thus, the main objective of this study was to determine the effects of feeding increasing doses of eugenol to dairy cows on enteric CH_4 production, N excretion, nutrient digestibility, ruminal fermentation characteristics, protozoa counts, milk production, milk composition, and fatty acid (FA) profile.

2. Materials and methods

2.1. Cows, experimental design, and treatments

Eight multiparous lactating Holstein cows fitted with ruminal cannulas (10 cm, Bar Diamond Inc., Parma, ID, USA) were used in a replicated 4×4 Latin square (28-d periods) balanced for residual effect (Cochran and Cox, 1957). The cows averaged (mean ± SD) 81 ± 13 days in milk at the start of the experiment with an average body weight (BW) of 658 ± 69.1 kg and 42 ± 5.7 kg/d of milk yield. Cows were housed in individual tie stalls and had free access to water during the experiment. They were fed for *ad libitum* intake (50 g orts/kg, on an as-fed basis) a total mixed ration ([TMR]; Table 1) not supplemented (0 mg/kg dry matter [DM]), or supplemented (25, 50 or 75 mg/kg DM) with eugenol (4-allyl-2-methoxyphenol, $C_{10}H_{12}O_2$, purity > 0.99; Phodé S.A., Albi, France). The feed additive was premixed with ground corn (*i.e.*, carrier) and incorporated in the TMR to achieve the appropriate feeding rates. Adaptation to experimental treatments was from day 1 to 18, sampling of milk, feces and urine and CH_4 production measurements from day 19 to 25, and ruminal sampling on day 27. Cows were cared for in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

2.2. Intake, apparent total-tract digestibility, milk production, and N balance

Measurements of feed intake, apparent total-tract digestibility and N balance were described in details by Benchaar et al. (2013). Briefly, the diets were offered in equal amounts twice daily (9:00 and 19:00 h) and feed intake was determined daily by weighing feeds offered to and refused by the cows. Samples of the TMR, feed ingredients, and orts were collected daily and stored at −20 °C. Samples were composited by cow within period, freeze-dried, ground to pass a 1-mm screen using a Wiley mill (standard model 4; Arthur M. Thomas, Philadelphia, PA, USA) and analyzed for dry matter (DM), organic matter (OM), total N, neutral detergent fiber (aNDF), acid detergent fiber (ADF), starch, ether extract, gross energy (GE), and FA profile.

Cows were milked twice daily at 07:30 and 19:30 h in their stalls and milk production was recorded at each milking. Milk samples were collected from each cow at each milking, stored at +4 °C with a preservative (2-bromo-2-nitropropan-1,3-diol), and then sent to a commercial laboratory (Valacta Dairy Production Center of Expertise Quebec-Atlantic, Ste-Anne-de-Bellevue, QC, Canada) for analysis of milk components (fat, protein, lactose, MUN, and SCC). Milk FA composition was determined on samples pooled on milk yield basis and frozen without preservative at −80 °C until analyzed.

Total collection of feces and urine was performed by fitting cows with harnesses and tubes allowing the collection of feces and urine separately. Feces were collected daily in preweighed plastic-lined plywood box. They were weighed, mixed thoroughly, and a representative sample (20 g/kg) was collected, stored at −20 °C, freeze-dried, and ground to pass a 1-mm screen using a Wiley mill for later analysis of DM, OM, total N, aNDF, ADF, and GE. Total urine was collected daily into