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# Dietary inclusion of diallyl disulfide, yucca powder, calcium fumarate, an extruded linseed product, or medium-chain fatty acids does not affect methane production in lactating dairy cows

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## ABSTRACT

Two similar experiments were conducted to assess the effect of diallyl disulfide (DADS), yucca powder (YP), calcium fumarate (CAFU), an extruded linseed product (UNSAT), or a mixture of capric and caprylic acid (MCFA) on methane production, energy balance, and dairy cow performance. In experiment 1, a control diet (CON1) and diets supplemented with 56 mg of DADS/kg of dry matter (DM), 3 g of YP/kg of DM, or 25 g of CAFU/kg of DM were evaluated. In experiment 2, an inert saturated fat source in the control diet (CON2) was exchanged isolipidically for an extruded linseed source (100 g/kg of DM; UNSAT) or a mixture of C8:0 and C10:0 (MCFA; 20.3 g/kg of DM). In experiment 2, a higher inclusion level of DADS (200 mg/kg of DM) was also tested. Both experiments were conducted using 40 lactating Holstein-Friesian dairy cows. Cows were adapted to the diet for 12 d and were subsequently kept in respiration chambers for 5 d to evaluate methane production, diet digestibility, energy balance, and animal performance. Feed intake was restricted to avoid confounding effects of possible differences in ad libitum feed intake on methane production. Feed intake was, on average, 17.5 and 16.6 kg of DM/d in experiments 1 and 2, respectively. None of the additives reduced methane production in vivo. Methane production in experiment 1 was 450, 453, 446, and 423 g/d for CON1 and the diets supplemented with DADS, YP, and CAFU, respectively. In experiment 2, methane production was 371, 394, 388, and 386 g/d for CON2 and the diets supplemented with UNSAT, MCFA, and DADS, respectively. No effects of the additives on energy balance or neutral detergent fiber digestibility were observed. The addition of MCFA increased milk fat content (5.38% vs. 4.82% for control) and fat digestibility (78.5% vs. 59.8% for control), but did not affect milk yield or other milk components. The other products did not affect milk yield or composition. Results from these experiments emphasize the need to confirm methane reductions observed in vitro with in vivo data.
**Key words:** methane, dairy cow, energy balance, feed additives

## INTRODUCTION

The global dairy industry is estimated to contribute 4.0% of global anthropogenic greenhouse gases, with the majority of these gases produced on the dairy farm (FAO, 2010). Enteric methane emissions account for 52% of the total amount of greenhouse gases produced during milk production and processing (FAO, 2010). Dietary strategies can influence the amount of enteric methane produced by dairy cows (Beauchemin et al., 2008; Ellis et al., 2008), and the reduction of enteric methane production has become an important goal in ruminant nutrition research.

Diallyl disulfide (**DADS**), one of the main components of garlic oil, has been shown to decrease methane production in vitro by up to 69% (Busquet et al., 2005b; Macheboeuf et al., 2006). It is thought to act through a direct effect on the enzyme system of the methanogenic archaea, inhibiting their activity (Busquet et al., 2005a). Yucca extract has been shown to decrease the number of rumen protozoa when fed to dairy cows (Lovett et al., 2006) or heifers (Hristov et al., 1999). Some of the rumen methanogens live in close association with the protozoa (Newbold et al., 1995; Hegarty, 1999), and yucca extract has been demonstrated to lower methane production in vitro (Lila et al., 2003). Fumarate is a precursor of propionate in the rumen. Propionogenesis from fumarate consumes hydrogen, thus lowering hydrogen availability for methanogenesis (Wallace et al., 2006), and methane reduction as a consequence of fumarate addition has been demonstrated in vitro (Asanuma et al., 1999). Responses of in vivo methane production to dietary fumarate have been equivocal (Bayaru et al., 2001; Kolver and Aspin, 2006; Wallace et al., 2006; McCourt et al., 2008).The methane-depressing effects of DADS and yucca powder had

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not been confirmed at the time of the implementation of experiment 1. Experiment 1 was designed to test the effect of DADS, yucca powder, and calcium fumarate on methane production by lactating dairy cows. We hypothesized, based on previous in vitro results, that these compounds would lower methanogenesis in the lactating dairy cow.

Addition of fat to ruminant diets is frequently proposed as a strategy to lower methanogenesis (Eugène et al., 2008). However, different fatty acids have different effects on methanogenesis (Czerkawski et al., 1966b; Prins et al., 1972). For the C18 fatty acids, inhibition of methane production appears to increase with the degree of unsaturation (Czerkawski et al., 1966a). Specific medium-chain fatty acids have been found to lower methanogenesis in vitro (Dohme et al., 2001). Ajisaka et al. (2002) observed significant methane reductions when cyclodextrin complexes of caprylic (C8:0) or capric (C10:0) acid were incubated with rumen fluid in vitro, but we are not aware of any in vivo evaluations of these fatty acids. In experiment 2, we exchanged a saturated fat source (containing mainly C16:0) for a fat source containing C8:0 and C10:0 or a source containing extruded linseed (rich in C18:2 and C18:3) to assess the methane-lowering effect of these specific fatty acids. Diets in experiment 2 were isolipidic to avoid effects of dietary fat content on methane production. We hypothesized that methane production would be lower for sources rich in C8:0 and C10:0 or C18:2 and C18:3 compared with the source supplying mainly C16:0.

In both experiments, indirect effects of level of feed intake on methane production were avoided by restricting the amount of feed offered. A reduction of methane emission may lead to increased milk energy output or to an improved energy balance, provided that the extent of fermentation is not affected. To verify this, energy balances were determined in both experiments.

## MATERIALS AND METHODS

### Experimental Procedures

Two completely randomized block experiments (**Exp.**) were conducted, each with 4 treatments and 10 lactating Holstein-Friesian dairy cows per treatment. Cows were blocked on fat- and protein-corrected milk production, parity, and DIM before the experiment (10 blocks of 4 animals in each experiment). Within each block, cows were randomly assigned to 1 of 4 dietary treatments. Cows in Exp. 1 produced 29.8 ± 5.7 kg of milk/d and were 97 ± 70 DIM at the start of the experiment. In Exp. 2, cows produced 27.9 ± 7.0 kg of milk/d and were 167 ± 99 DIM. In Exp. 1, dietary treatments consisted of a control diet (**CON1**) and diets supplemented with 56 mg of DADS/kg of DM (**DADS1**), 3 g/kg of DM yucca powder (**YP**), or 25 g/kg DM calcium fumarate (**CAFU**). In Exp. 2, a rumen-inert fat source (**CON2**) was replaced isolipidically by an extruded linseed product (**UNSAT**; 100 g/kg of DM) or a source containing C8:0 and C10:0 fatty acids (**MCFA**; 20.3 g/kg of DM). In Exp. 2, DADS was again evaluated (**DADS2**) but at an inclusion rate of 200 mg/kg of DM.

### Source of Test Products

Diallyl disulfide (Vetcare PVT, Bangalore, India) in liquid form was applied to a silica carrier (Provimi France, Treize Vents, France) to produce a solid material containing 10% DADS. Yucca powder (Yucca-Plus Powder, Agroin, Ensenada, Mexico) was purchased from Jadis Additiva (Schiedam, the Netherlands). Calcium fumarate was supplied by Kemin Industries Inc. (Herentals, Belgium). The extruded linseed product (Promax 20/20, Provimi France) consisted of 50% extruded linseed, 2% rapeseed, 18% sunflower meal, and 30% wheat bran. The C8/C10 product was produced by applying a mixture of liquid C8:0 and C10:0 (Aveve, Leuven, Belgium) to a silica carrier (Provimi France) to provide a material containing 45% fatty acids and 55% carrier material.

### Housing

The Animal Care and Use Committee of Wageningen University (Wageningen, the Netherlands) approved the experimental protocols of both experiments. Animals were housed in the facilities of Wageningen University and Research Centre. Cows were individually housed in tie-stalls and milked twice daily at 0600 and 1700 h. Animals remained in tie-stalls for 12 d to become accustomed to the diet and restriction in movement. After this period, animals were housed in 1 of 2 identical respiration chambers to determine gaseous exchange, energy balance, and diet digestibility. Because 2 chambers were available, measurements were obtained in 10 periods, staggered in time. Within each period, 2 cows receiving the same treatment were housed in one chamber, and 2 cows receiving a different treatment were housed in the other chamber. Within each chamber, the 2 cows originated from a different block. The experimental unit for data measured in the respiration chambers (e.g., methane production, diet digestibility parameters) therefore consisted of a pair of cows. The respiration chambers have been described in detail by Verstegen et al. (1987). Cows remained in the respiration chambers for a period of 5 d. After completion of the 5-d measurement period, feces, urine, and clean-

ing water were quantitatively collected, weighed, and subsampled for determination of NDF and crude fat. Both NDF and crude fat were assumed to be absent in urine, allowing for calculation of digestibility of these components from analyses of NDF and crude fat in the combined mixture of feces and urine.

### Diets and Feeding

Cows in Exp. 1 were fed a diet consisting of 40% grass silage, 26% corn silage, and 34% concentrates on a DM basis. The concentrates consisted of 30.0% soybean meal, 24.1% wheat, 18.1% corn, 12.1% dried sugar beet pulp, 12.1% rapeseed meal, 1.2% limestone, and 2.4% of a mineral premix. The additives were hand-mixed into the diet at the time of feeding. Because their inclusion rate was low, this did not affect the average chemical composition of the TMR. The chemical compositions of the TMR used in both experiments are shown in Table 1.

Cows in Exp. 2 were fed a TMR containing 41% grass silage, 35% corn silage, 14% concentrates, and 10% of a mixture containing the experimental test products on a DM basis. The concentrates consisted of 52.1% soybean meal, 38.2% wheat, 5.2% limestone, and 4.5% of a mineral premix. The experimental test products were included in a mixture that was hand-mixed into the TMR at the time of feeding. The composition of these mixtures is shown in Table 2.

Animals in both experiments were fed equal portions twice daily during milking. Diets were supplied individually and were supplied ad libitum for the first 8 d in the tie-stalls. From d 8 to 17, feed intake was restricted per block to 95% of the ad libitum feed intake of the animal consuming the lowest amount of feed during d 5 to 8 within a block. In the respiration chambers, orts were collected when present, pooled per cow and period, and frozen pending analyses.

### Sampling and Chemical Analyses

Milk yield was recorded during each milking. During the period in the respiration chambers, 2 representative samples (3 g/kg milk for each sample) were obtained at each milking for each cow. These samples were pooled per cow for the entire period. Milk was analyzed for fat, protein and lactose content according to ISO 9622 (ISO, 1999) and the MUN content was determined employing the pH difference technique (ISO 14637; ISO, 2004). Gross energy content was determined using bomb

**Table 1.** Ingredient, analyzed chemical composition, and calculated fatty acid composition of TMR fed in experiments 1 and 2[1]

| | Exp. 1 | Exp. 2 | | | |
|---|---|---|---|---|---|
| Item | CON1 | CON2 | UNSAT | MCFA | DADS2 |
| Grass silage (% of DM) | 40 | 41 | 41 | 41 | 41 |
| Corn silage (% of DM) | 26 | 35 | 35 | 35 | 35 |
| Concentrates (% of DM) | 34 | 14 | 14 | 14 | 14 |
| Additive mixture (% of DM) | — | 10 | 10 | 10 | 10 |
| DM (g/kg) | 441 | 424 | 430 | 429 | 424 |
| Gross energy (MJ/kg of DM) | 19.3 | 20.2 | 19.9 | 19.6 | 20.2 |
| Crude ash (g/kg of DM) | 76 | 77 | 78 | 94 | 78 |
| CP (g/kg of DM) | 167 | 165 | 163 | 159 | 165 |
| NDF (g/kg of DM) | 415 | 410 | 417 | 403 | 415 |
| Crude fat (g/kg of DM) | 33 | 58 | 55 | 58 | 60 |
| C8:0[2] (g/kg of DM) | 0.0 | 0.0 | 0.0 | 10.1 | 0.0 |
| C10:0 (g/kg of DM) | 0.0 | 0.0 | 0.0 | 10.1 | 0.0 |
| C12:0 (g/kg of DM) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| C14:0 (g/kg of DM) | 0.0 | 0.3 | 0.0 | 0.0 | 0.3 |
| C16:0 (g/kg of DM) | 3.2 | 19.5 | 4.7 | 3.5 | 19.5 |
| C16:1 (g/kg of DM) | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| C18:0 (g/kg of DM) | 0.4 | 1.5 | 1.2 | 0.5 | 1.5 |
| C18:1 (g/kg of DM) | 3.6 | 4.9 | 5.9 | 2.7 | 4.9 |
| C18:2 (g/kg of DM) | 8.0 | 8.1 | 10.2 | 7.5 | 8.1 |
| C18:3 (g/kg of DM) | 5.2 | 8.2 | 17.3 | 7.4 | 8.2 |
| >C20:0 (g/kg of DM) | 0.2 | 0.3 | 0.1 | 0.1 | 0.3 |
| Saturated fatty acids (S; g/kg of DM) | 3.9 | 21.6 | 6.1 | 24.4 | 21.6 |
| Unsaturated fatty acids (U; g/kg of DM) | 17.0 | 21.4 | 33.7 | 17.7 | 21.4 |
| U:S ratio | 4.4 | 1.0 | 5.5 | 0.7 | 1.0 |

[1]CON1 = control diet in Exp. 1; CON2 = control diet in Exp. 2; UNSAT = rumen-inert fat source in CON2 was replaced isolipidically by an extruded linseed product; MCFA = rumen-inert fat source in CON2 was replaced isolipidically by a source containing C8:0 and C10:0 fatty acids; DADS2 = diet supplemented with 200 mg/kg DM of diallyl disulfide.

[2]Fatty acid profiles were calculated from CVB (2007).

**Table 2.** Ingredient and analyzed chemical composition of mixtures containing the dietary additives for experiment 2; mixtures were added to the TMR at 10% of DM

| | Exp. 2 diet[1] | | | |
|---|---|---|---|---|
| Item | CON2 | UNSAT | MCFA | DADS2 |
| Ground wheat (g/kg of DM) | 250 | | 190 | 248 |
| Mechanically extracted linseed meal (g/kg of DM) | 550 | | 360 | 550 |
| Fractionated palm oil[2] (g/kg of DM) | 200 | | | 200 |
| DADS product[3] (g/kg of DM) | | | | 2 |
| C8/C10 product[4] (g/kg of DM) | | | 450 | |
| Extruded linseed product[5] (g/kg of DM) | | 1,000 | | |
| DM (g/kg) | 899 | 909 | 904 | 897 |
| Gross energy (MJ/kg of DM) | 23.5 | 22.8 | 19.5 | 23.3 |
| Ash (g/kg of DM) | 42 | 52 | 209 | 49 |
| CP (g/kg of DM) | 238 | 209 | 168 | 232 |
| NDF (g/kg of DM) | 232 | 249 | 108 | 282 |
| Crude fat (g/kg of DM) | 239 | 207 | 241 | 255 |

[1]CON2 = control diet; UNSAT = rumen-inert fat source in CON2 was replaced isolipidically by an extruded linseed product; MCFA = rumen-inert fat source in CON2 was replaced isolipidically by a source containing C8:0 and C10:0 fatty acids; DADS2 = diet supplemented with 200 mg/kg DM of diallyl disulfide.

[2]Hyprofat, Provimi B.V., Rotterdam, the Netherlands.

[3]10% diallyldisufide (Vetcare PVT, Bangalore, India), 90% silica (Provimi France, Treize Vents, France).

[4]45% fatty acids (50/50 mixture of C8:0/C10:0; Aveve, Leuven, Belgium), 55% silica (Provimi France).

[5]50% extruded linseed, 2% rapeseed, 18% sunflower meal, 30% wheat bran (Promax 20/20, Provimi France).

calorimetry (IKA-C700, Janke & Kunkel, Heitersheim, Germany) and the N content of milk was determined according to Kjeldahl analysis.

Feed was sampled (±500 g) directly after preparation, before inclusion of the additives. Samples were stored frozen (−20°C) pending further analyses. At the end of the experiment, samples were pooled per period and analyzed for their chemical composition. In Exp. 2, samples (±100 g) of the additive mixtures were taken weekly and stored frozen (−20°C) until analysis. Feces and urine were quantitatively collected over the entire measurement period, weighed, thoroughly mixed, and subsampled for analyses. Prior to analysis, samples of feed and feces were freeze-dried and ground to pass a 1-mm screen. Dry matter, CP, crude fat, sugar, starch, and NDF content of TMR, additive, and manure samples were determined according to the methods described in detail by Abrahamse et al. (2008).

### Statistical Analyses

Data collected during the measurement period only were used for statistical analyses. Daily data were averaged per period before analysis. Data collected for pairs of cows (energy balance traits and diet digestibility) were subjected to ANOVA, with treatment and respiration chamber as fixed factors ($Y_{ij} = \mu_{ij} + \text{respiration chamber}_i + \text{treatment}_j + \varepsilon_{ij}$, in which $Y_{ij}$ = observed response, $\mu_{ij}$ = overall mean, respiration chamber$_i$ = effect of respiration chamber i, treatment$_j$ = effect of treatment j, and $\varepsilon_{ij}$ = residual error). As the 2 cows within a pair originated from a different block, block was not included in the statistical analysis of these traits. Assigning animals to treatments within a block served the purpose of minimizing the reduction in feed intake when feed intake was restricted.

Data collected for individual cows (DMI, milk yield, and milk composition) were subjected to ANOVA, with block, treatment, and respiration chamber as fixed factors ($Y_{ijk} = \mu_{ijk} + \text{block}_i + \text{respiration chamber}_j + \text{treatment}_k + \varepsilon_{ijk}$, in which $Y_{ijk}$ = observed response, $\mu_{ijk}$ = overall mean, block$_i$ = effect of block i, respiration chamber$_j$ = effect of respiration chamber j, treatment$_k$ = effect of treatment k, and $\varepsilon_{ijk}$ = residual error). The effect of chamber was not significant for any of the parameters analyzed in both experiments. When the treatment effect was significant, treatment means were separated by means of Tukey's test. The statistical program Genstat (11th ed., Lawes Agricultural Trust, Rothamsted, UK) was used to analyze the results.

## RESULTS

### Feed Composition and Animal Performance

The chemical compositions of the TMR used in Exp. 1 and Exp. 2 are shown in Table 1. The dietary additives used in Exp. 1 were manually mixed into this TMR. The ingredient and chemical composition of the mixtures, including the dietary additives used in Exp. 2 are shown in Table 2.

Diets in both experiments had a comparable chemical composition, except for the level of crude fat, which was higher for Exp. 2 due to the addition of the fat-rich

mixtures. The shift in fatty acid pattern of the diets was successfully established, with C16:0 being the most important fatty acid in CON2, C18:2 and C18:3 in UNSAT, and C8:0 and C10:0 in the MCFA treatment.

The addition of DADS, YP, or CAFU did not affect animal performance in Exp. 1 (Table 3). In Exp. 2, the addition of MCFA significantly increased milk fat concentration, whereas MUN tended to be lower for UNSAT. Other performance parameters were unaffected by the addition of MCFA, UNSAT, or DADS2 in Exp. 2. In comparison with that in Exp. 1, milk production in Exp.2 was lower for the cows, whereas milk fat and protein concentrations were higher.

### *Methane Production*

Methane production was unaffected by the treatments imposed in these experiments (Table 4). A considerable difference in the level of methane production was observed between Exp. 1 and Exp. 2, when expressed as the absolute amount (g/d) or per unit of DMI or milk production.

### *Energy Balance and Digestibility*

In Exp.1, energy retention was negative for all treatments and unaffected by treatment (Table 5). In Exp. 2, energy retention was also unaffected by treatment, but was approximately zero for all treatments. Cows consumed similar amounts of ME in both experiments, but those in Exp. 2 generated less energy as milk, heat, and methane. Digestibility of NDF and fat did not differ between treatments in Exp. 1. In Exp. 2, NDF digestibility was unaffected by treatment, but fat digestibility was higher with MCFA than with all other treatments.

## DISCUSSION

### *DADS*

To our knowledge, this work is the first evaluation of in vivo effects of dietary DADS on methane emission and animal performance in dairy cows. Garlic oil is known to possess antimicrobial properties and has been shown to decrease methane production in vitro (Chaves et al., 2008; García-González et al., 2008). The main component of garlic oil, DADS, is also known to reduce methane emissions in vitro (Busquet et al., 2005b), but this has not yet been confirmed in vivo. Diallyl disulfide has been hypothesized to directly inhibit the enzyme 3-hydroxy-2-methyl-glutaryl coenzyme A in human cholesterol synthesis (Gebhardt and Beck, 1996). Archaea have membrane lipids that contain isoprenoid units, the synthesis of which uses the same precursors

**Table 3.** Dry matter intake, milk production, and milk composition of dairy cows fed diets containing diallyl disulfide, yucca powder, calcium fumarate, a product containing extruded linseed, or a mixture of C8:0/C10:0 fatty acids[1] (n = 10/treatment)

| | Experiment 1 | | | | | | Experiment 2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | CON1 | DADS1 | YP | CAFU | SEM | *P*-value | CON2 | UNSAT | MCFA | DADS2 | SEM | *P*-value |
| DMI (kg/d) | 17.7 | 17.9 | 17.3 | 16.7 | 0.83 | 0.490 | 16.5 | 16.9 | 16.7 | 16.8 | 0.21 | 0.766 |
| Milk (kg/d) | 30.3 | 29.5 | 29.8 | 28.7 | 1.21 | 0.822 | 24.4 | 25.4 | 22.3 | 24.8 | 1.01 | 0.244 |
| Fat (%) | 3.98 | 4.00 | 3.96 | 3.95 | 0.174 | 0.996 | 4.82[a] | 4.47[a] | 5.38[b] | 4.52[a] | 0.155 | <0.001 |
| Protein (%) | 3.16 | 3.24 | 3.28 | 3.17 | 0.071 | 0.594 | 3.41 | 3.33 | 3.59 | 3.40 | 0.078 | 0.172 |
| Lactose (%) | 4.72 | 4.65 | 4.73 | 4.69 | 0.051 | 0.696 | 4.59 | 4.62 | 4.58 | 4.54 | 0.045 | 0.617 |
| MUN (mg/dL) | 13.6 | 12.6 | 12.9 | 12.4 | 0.43 | 0.252 | 11.6 | 12.7 | 10.7 | 11.7 | 0.50 | 0.083 |
| SCC (×1,000 cells/mL) | 93 | 160 | 143 | 126 | 57.9 | 0.864 | 332 | 272 | 91 | 271 | 128.4 | 0.519 |

[a,b]Data with different superscripts in the same row within experiment differ significantly ($P < 0.05$).

[1]CON1 = control diet in Exp. 1; DADS1 = diet supplemented with 56 mg/kg DM of diallyl disulfide; YP = diet supplemented with 3 g/kg of DM yucca powder; CAFU = diet supplemented with 25 g/kg DM of calcium fumarate; CON2 = control diet in Exp. 2; UNSAT = rumen-inert fat source in CON2 was replaced isolipidically by an extruded linseed product; MCFA = rumen-inert fat source in CON2 was replaced isolipidically by a source containing C8:0 and C10:0 fatty acids; DADS2 = diet supplemented with 200 mg/kg DM of diallyl disulfide.

**Table 4.** Methane production of dairy cows fed control diets or diets containing diallyl disulfide, yucca powder, calcium fumarate, a product containing extruded linseed, or a mixture of C8:0/C10:0 fatty acids (n = 5/treatment)[1]

| | Experiment 1 | | | | | | Experiment 2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | CON1 | DADS1 | YP | CAFU | SEM | *P*-value | CON2 | UNSAT | MCFA | DADS2 | SEM | *P*-value |
| $CH_4$ (g/cow per day) | 450 | 453 | 446 | 423 | 12.9 | 0.378 | 371 | 394 | 388 | 386 | 26.1 | 0.945 |
| $CH_4$ (g/kg of DMI) | 25.5 | 25.4 | 25.6 | 25.1 | 0.41 | 0.872 | 23.2 | 23.2 | 23.2 | 22.9 | 1.22 | 0.870 |
| $CH_4$ (g/kg of milk) | 15.0 | 15.4 | 15.0 | 14.8 | 0.70 | 0.941 | 15.8 | 16.0 | 18.2 | 15.5 | 1.83 | 0.731 |
| $CH_4$ (% of gross energy intake) | 7.3 | 7.3 | 7.4 | 7.4 | 0.11 | 0.916 | 6.3 | 6.4 | 6.6 | 6.4 | 0.15 | 0.619 |

[1]CON1 = control diet in Exp. 1; DADS1 = diet supplemented with 56 mg/kg DM of diallyl disulfide; YP = diet supplemented with 3 g/kg of DM yucca powder; CAFU = diet supplemented with 25 g/kg DM of calcium fumarate; CON2 = control diet in Exp. 2; UNSAT = rumen-inert fat source in CON2 was replaced isolipidically by an extruded linseed product; MCFA = rumen-inert fat source in CON2 was replaced isolipidically by a source containing C8:0 and C10:0 fatty acids; DADS2 = diet supplemented with 200 mg/kg DM of diallyl disulfide.

**Table 5.** Energy balance of dairy cows fed control diets or diets containing diallyl disulfide, yucca powder, calcium fumarate, a product containing extruded linseed or a mixture of C8:0/C10:0 fatty acids (n = 5/treatment)[1]

| | Experiment 1 | | | | | | Experiment 2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | CON1 | DADS1 | YP | CAFU | SEM | *P*-value | CON2 | UNSAT | MCFA | DADS2 | SEM | *P*-value |
| Metabolic weight (kg/cow) | 120 | 121 | 120 | 121 | 2.4 | 0.989 | 120 | 121 | 122 | 122 | 2.7 | 0.942 |
| Gross energy (GE) intake (kJ/kg$^{0.75}$ per day) | 2,837 | 2,858 | 2,780 | 2,648 | 72.9 | 0.206 | 2,726 | 2,788 | 2,670 | 2,709 | 107.0 | 0.992 |
| ME intake (kJ/kg$^{0.75}$ per day) | 1,705 | 1,699 | 1,662 | 1,577 | 49.4 | 0.278 | 1,654 | 1,681 | 1,645 | 1,630 | 69.5 | 0.960 |
| ME:GE ratio (%) | 60.1 | 59.4 | 59.8 | 59.5 | 0.40 | 0.753 | 60.7 | 60.3 | 61.6 | 60.0 | 0.65 | 0.429 |
| Methane production (kJ/kg$^{0.75}$ per day) | 208 | 208 | 206 | 195 | 4.7 | 0.192 | 171 | 180 | 176 | 175 | 8.9 | 0.923 |
| Heat production (kJ/kg$^{0.75}$ per day) | 1,057 | 1,048 | 1,044 | 1,027 | 20.1 | 0.726 | 949 | 945 | 978 | 928 | 31.1 | 0.793 |
| Energy in milk (kJ/kg$^{0.75}$ per day) | 788 | 761 | 766 | 739 | 25.8 | 0.680 | 694 | 681 | 666 | 674 | 44.6 | 0.970 |
| Energy retention total (kJ/kg$^{0.75}$ per day) | −140 | −109 | −147 | −189 | 29.6 | 0.331 | 12 | 55 | 2 | 28 | 48.7 | 0.866 |
| Energy retention protein (kJ/kg$^{0.75}$ per day) | −2 | −2 | −3 | −5 | 0.81 | 0.381 | 37 | 39 | 42 | 33 | 15.1 | 0.980 |
| Energy retention fat (kJ/kg$^{0.75}$ per day) | −142 | −104 | −149 | −197 | 27.9 | 0.238 | −22 | 17 | −40 | −2 | 32.0 | 0.811 |
| NDF digestibility (%) | 69.6 | 70.0 | 67.6 | 64.8 | 1.77 | 0.194 | 69.2 | 69.2 | 69.3 | 69.9 | 1.61 | 0.985 |
| Crude fat digestibility (%) | 59.4 | 60.7 | 58.6 | 53.3 | 2.24 | 0.149 | 59.8[a] | 66.7[a] | 78.5[b] | 60.6[a] | 1.41 | <0.001 |

[a,b]Data with different superscripts in the same row within experiment differ significantly ($P < 0.05$).

[1]CON1 = control diet in Exp. 1; DADS1 = diet supplemented with 56 mg/kg DM of diallyl disulfide; YP = diet supplemented with 3 g/kg of DM yucca powder; CAFU = diet supplemented with 25 g/kg DM of calcium fumarate; CON2 = control diet in Exp. 2; UNSAT = rumen-inert fat source in CON2 was replaced isolipidically by an extruded linseed product; MCFA = rumen-inert fat source in CON2 was replaced isolipidically by a source containing C8:0 and C10:0 fatty acids; DADS2 = diet supplemented with 200 mg/kg DM of diallyl disulfide.

as human cholesterol synthesis. It has been demonstrated previously that cholesterol-lowering compounds that inhibit 3-hydroxy-2-methyl-glutaryl coenzyme A, lovastatin, and mevastatin can inhibit the growth of rumen methanogens and methane production in vitro (Miller and Wolin, 2001).

In the in vitro study of Busquet et al. (2005b), a significant methane decrease was observed at a concentration of 300 mg of DADS/L in a batch culture system. In the experiment of Kamel et al. (2008), 3 levels of DADS (0.5, 5, and 10 mg of DADS/L) were investigated for their methane-suppressing activities in vitro. None of the doses used had a suppressing effect. Apparently, the lowest effective dose of DADS for methane reduction lies in the range of 10 to 300 mg of DADS/L when tested in in vitro batch culture systems.

The level of DADS employed in the study of Busquet et al. (2005b) corresponds to a level of 30,000 mg/kg of substrate. In the study of Kamel et al. (2008), the levels corresponded to 50, 500, and 1,000 mg of DADS/kg of substrate, respectively. In our studies, DADS was fed at levels of 56 mg/kg of DM and 200 mg/kg of DM in Exp. 1 and Exp. 2, respectively. This is equivalent to 1.0 or 3.3 g/cow per day, respectively. The dose level in Exp. 1 was selected to prevent the occurrence of garlic taint in milk. After completion of the first experiment, in which no milk taint was observed, a higher dose of DADS was selected for Exp. 2. However, a clear and distinctive garlic taint was detected by the technical staff and the authors in the milk from the cows on the DADS2 treatment. The doses used in our experiments were clearly lower than the effective dose employed in the in vitro study of Busquet et al. (2005b) and this may explain the lack of effect on methane emissions in the in vivo studies. However, results from the in vivo experiments demonstrate that the applicability of higher doses of DADS is limited due to the occurrence of garlic taint in milk.

Diallyl disulfide did not affect milk production or composition at the inclusion levels tested in these experiments. When garlic essential oils were included in the diet of lactating dairy cows (5 g/cow per day), total VFA concentrations were increased but animal performance was not affected (Yang et al., 2007). Diallyl disulfide is one of the main components of garlic oil and might be expected to exert similar results on rumen fermentation. However, no effects on milk production were observed in these experiments.

### *YP*

Dose-dependent decreases in methane production have been observed in vitro when yucca saponins were added to the incubation medium (Lila et al., 2003). This effect may be explained by the symbiotic relationship between methanogens and protozoa in the rumen. Saponins have been shown to have strong detergent properties (Cheeke, 2000) and to reduce the number of rumen protozoa by disrupting their cell membrane. Hegarty (1999) proposed that 37% of rumen methanogenesis originated from methanogens living in a methanogen-protozoan symbiotic relationship and Newbold et al. (1995) demonstrated that elimination of protozoa diminished methane production by 9 to 25% in vitro. Elimination of protozoa thus has the potential to lower methanogenesis. In the experiment of Lovett et al. (2006), using steers, a significant decrease in protozoa numbers was observed in response to yucca extract (1.2 and 2.6 g/kg of DM, respectively). We used a higher dose (3 g/kg of DM) of YP in an attempt to obtain this defaunating effect and the consequent reduction in methane production.

However, we observed no effect of YP on methane production. Thus, our findings of a lack of an effect of yucca on methane emissions confirm the findings of Holtshausen et al. (2009), who reported no differences in the number of protozoa when yucca powder was included at 10 g/kg of DM. The effectiveness of different forms of yucca products might differ; in the study of Lovett et al. (2006) yucca extract was used, which is likely to contain a higher concentration of saponins than the yucca powder used in the current study (Cheeke, 2000).

In a meta-analysis, Eugène et al. (2004) concluded that too few data are available in the literature to draw sound conclusions concerning the effects of defaunation on dairy cow performance. In the current experiment, feeding yucca powder did not affect milk production or milk composition, supporting the findings of other researchers (Valdez et al., 1986; Wilson et al., 1998; Lovett et al., 2006; Holtshausen et al., 2009).

The meta-analysis of Eugène et al. (2004) demonstrated that defaunation increased the efficiency of microbial protein synthesis and the flow of microbial protein to the duodenum. Consequently, defaunation would be expected to be especially effective in enhancing animal performance when diets are limiting in MP. In the current experiment, diets were formulated to meet or exceed requirements for MP of the dairy cows, which may explain the lack of response of production parameters.

### *CAFU*

The use of fumarate in methane mitigation has been researched extensively both in vitro (Asanuma et al., 1999; García-Martínez et al., 2005) and in vivo. The results of in vivo experiments have been variable, with

some reports of decreased methane production (Bayaru et al., 2001; Wallace et al., 2006) and others reporting no effect (McGinn et al., 2004; Beauchemin and McGinn, 2006; Kolver and Aspin, 2006; McCourt et al., 2008). Methane reductions through fumarate feeding are hypothesized to originate from the consumption of hydrogen in the conversion of fumarate to propionate. However, if the considerable amount of Ca-fumarate (420 g/cow per day) fed in this experiment had been fully converted to propionate, this would have decreased methane emissions by only 11 g/d (2.6%). The actual, nonsignificant decrease in methane production observed in this experiment (−5.8%) was greater than the potential reduction. Moreover, Ungerfeld et al. (2007) demonstrated, by meta-analysis of in vitro data, that fumarate is often not fully converted to propionate, but also to acetate, generating hydrogen. This almost entirely offset the hydrogen used in propionogenesis. The large quantity of fumarate that would be required to achieve substantial reductions in methane production, together with its costs and poor palatability, precludes the use of this substance as a methane inhibitor.

In this experiment, the addition of fumarate to dairy cow diets did not affect milk yield from dairy cows, supporting the findings from previous research (Kolver and Aspin, 2006; McCourt et al., 2008). Milk composition was also unaffected in both other studies, except for the lactose content in the study of Kolver and Aspin (2006), which was higher for the fumarate-fed cows.

### *Increasing the Unsaturated Fatty Acid Content of the Diet*

Dietary unsaturated fat may affect methane production in several ways: indirectly, through decreased DMI or dilution of fermentable OM; through direct toxic effects on the rumen microflora; or by consumption of hydrogen during biohydrogenation (Martin et al., 2010). In Exp. 2 we ruled out indirect effects of fat addition by providing equal amounts of fat in each treatment and by restricting DMI. In this way, any effects on methane emissions could only have come from a direct effect of the increased dietary content of unsaturated fatty acids on the rumen microflora or by the hydrogen sink function of the unsaturated fatty acids supplied by the product containing the extruded linseed. We hypothesized that the increased content of dietary unsaturated fatty acids would lower methanogenesis due to specific effects of these fatty acids on methanogenesis observed in earlier research (Czerkawski et al., 1966b; Prins et al., 1972).

Products containing extruded linseed, a source rich in C18:2 and C18:3, have been demonstrated to reduce methane production when added to dairy cow rations (Martin et al., 2008), but this reduction appeared to originate mainly from a reduction in DMI and NDF digestibility: methane production expressed per unit of digested NDF was unaffected. In the current experiment, no methane-lowering effect was observed when fractionated palm oil was isolipidically exchanged for a product containing extruded linseed. In this experiment, apparent total-tract digestibility of NDF was unaffected by supplementation with the product containing extruded linseed. The methane-suppressing effects of C18:2 and C18:3 observed in earlier research may be due to a more general toxic effect on the rumen microbes, rather than a specific toxic effect on the rumen methanogens alone (Maia et al., 2007).

In our experiment, cows consumed approximately 850 g of DM/d of the extruded linseed product, which contained 20.7% crude fat (352 g of linseed oil/d). Linseed oil consists mainly of C18:2 and C18:3 fatty acids and if all the double bonds in this molecule were hydrogenated in the rumen, this would reduce methane emissions by approximately 6 g/d or 1.6% (Martin et al., 2010).

It is thus likely that our approach would reveal the direct effect of unsaturated fatty acids on the rumen microflora and consequently methane production. The fact that no differences in methane production were observed may mean that the mechanism of methane reduction by products containing extruded linseed is due mainly to indirect effects (e.g., reduced NDF digestibility, reduced DMI, dilution of fermentable OM) rather than a direct toxic effect on the rumen methanogens. Eugene et al. (2008) concluded that the methane reduction observed as a consequence of fat or oil consumption was mainly due to a reduction in DMI, which may originate from a reduced NDF digestibility.

Fat-rich feed materials such as extruded linseeds can be utilized to enhance the dietary energy content of dairy cow diets and stimulate milk production. Indeed, enhancing dietary energy content by including linseed oil increased milk production (Bu et al., 2007). However, in the experiment of Martin et al. (2008), the addition of extruded linseed significantly lowered DMI and lowered milk production despite an increase in dietary energy content. The inclusion of extruded linseed lowered rumen digestibility of OM and in particular NDF in that experiment. It is generally recommended not to exceed crude fat levels of 6.5% DM (NRC, 2001). The addition of extruded linseed to the diet did not affect dairy cow performance in our study. Feeding the extruded linseed product tended to decrease MUN contents; this may have been a consequence of the lower CP content of the mixture containing the extruded linseed.

### Capric and Caprylic Acids

Caprylic acid and capric acid were demonstrated to lower methanogenesis in vitro (Ajisaka et al., 2002). These authors added these fatty acids to 2 different matrices (α-cyclodextrin or β-cyclodextrin) to produce a solid feed material, similar to the procedure followed in the current in vivo experiment. A reduction of 60% in methane production was observed when 40 mg of capric acid on the β-cyclodextrin carrier was added to 60 mL of medium (0.7 g/L or 139 g/kg of substrate) and a nonsignificant 40% reduction in methane production observed when 20 mg of capric acid was added. This observation was later confirmed for capric acid by Goel et al. (2009) who found methane reductions of 45 and 88%, respectively, when 20 or 30 mg of capric acid was added to 50 mL of incubation medium with 0.5 g of substrate (40 or 60 g/kg of substrate, respectively). Dohme et al. (2001) observed no reduction in methane production when C8:0 or C10:0 were added to a Rusitec system at 0.6 g/L or 50 g/kg DM substrate.

In the current experiment, which is the first to investigate in vivo effects of these fatty acids on methanogenesis, cows on the MCFA treatment consumed 16.7 kg DM containing 45 g of product/kg of DM. This product contained 45% fatty acids, so the amount of C8:0 or C10:0 consumed was 169 g/cow per day or 10 g/kg of DM of each fatty acid. However, when concentrations of C8 or C10 are expressed in relation to the substrate supplied to the in vitro system (40 to 139 g of fatty acids/kg of substrate), concentrations provided in the in vitro systems were higher than those in the in vivo experiment.

The addition of C8:0 and C10:0 increased milk fat content, but did not affect milk yield or milk protein content. Fat digestibility was higher on the MCFA treatment than for the other treatments, providing a possible explanation for the higher milk fat contents.

### Difference in Methane Production Between Experiments

A considerable difference in the overall level of methane production was observed between experiments (443 g/d for Exp. 1 and 385 g/d for Exp.2), although the dietary composition was broadly similar in both experiments. The crude fat content of the TMR used in Exp. 2 was clearly higher than that for Exp. 1 (58 g/kg of DM vs. 33 g/kg of DM for Exp. 2 and Exp. 1, respectively). Eugène et al. (2008) conducted a meta-analysis and provided an equation to predict methane production from DMI and daily lipid intake. Use of this equation results in predicted methane productions of 328 and 299 g/d for Exp. 1 and Exp. 2, respectively. Although the absolute level of methane production observed in both experiments was higher than predicted, the difference in methane production predicted from the model is similar to the observed difference in our experiments (29 g/d for the prediction equation vs. 22 g/d observed between Exp. 1 and 2), providing a likely explanation for the difference in methane emission between experiments.

The test products had no effect on methane production in either experiment, whereas their efficacy had previously been demonstrated in vitro. These findings emphasize that results observed in vitro should be confirmed in vivo (Flachowsky and Lebzien, 2009). It also shows that in vitro experiments showing significant methane reductions often use concentrations of the active ingredient, expressed in grams per kilogram of substrate, that are not practical to use in vivo.

## CONCLUSIONS

Addition of diallyl disulfide, yucca powder, calcium fumarate, a product containing extruded linseed, or a mixture of capric and caprylic acids to dairy cow diets did not affect enteric methane emissions or energy balance in concentrations that have practical applications. Fat digestibility and milk fat content were elevated by the addition of caprylic and capric acids to the diet.

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## REFERENCES

Abrahamse, P. A., J. Dijkstra, B. Vlaeminck, and S. Tamminga. 2008. Frequent allocation of rotationally grazed dairy cows changes grazing behavior and improves productivity. J. Dairy Sci. 91:2033–2045.

Ajisaka, N., N. Mohammed, K. Hara, K. Mikuni, K. Hara, H. Hashimoto, T. Kumata, S. Kanda, and H. Itabashi. 2002. Effects of medium-chain fatty acid-cyclodextrin complexes on ruminal methane production in vitro. Anim. Sci. J. 73:479–484.

Asanuma, N., M. Iwamoto, and T. Hino. 1999. Effect of the addition of fumarate on methane production by ruminal microorganisms in vitro. J. Dairy Sci. 82:780–787.

Bayaru, E., S. Kanda, T. Kamada, H. Itabashi, N. Andoh, T. Nishida, M. Ishida, T. Itoii, K. Nagara, and Y. Isobe. 2001. Effect of fumaric acid on methane production, rumen fermentation and digestibility of cattle fed roughage alone. Anim. Sci. J. 72:139–146.

Beauchemin, K. A., M. Kreuzer, F. O'Mara, and T. A. McAllister. 2008. Nutritional management for enteric methane abatement: A review. Aust. J. Exp. Agric. 48:21–27.

Beauchemin, K. A., and S. M. McGinn. 2006. Methane emissions from beef cattle: Effects of fumaric acid, essential oil, and canola oil. J. Anim. Sci. 84:1489–1496.

Bu, D. P., J. Q. Wang, T. R. Dhiman, and S. J. Liu. 2007. Effectiveness of oils rich in linoleic and linolenic acids to enhance conjugated linoleic acid in milk from dairy cows. J. Dairy Sci. 90:998–1007.

Busquet, M., S. Calsamiglia, A. Ferret, P. W. Cardozo, and C. Kamel. 2005a. Effects of cinnamaldehyde and garlic oil on rumen microbial fermentation in a dual flow continuous culture. J. Dairy Sci. 88:2508–2516.

Busquet, M., S. Calsamiglia, A. Ferret, M. D. Carro, and C. Kamel. 2005b. Effect of garlic oil and four of its compounds on rumen microbial fermentation. J. Dairy Sci. 88:4393–4404.

Chaves, A. V., M. L. He, W. Z. Yang, A. N. Hristov, T. A. McAllister, and C. Benchaar. 2008. Effects of essential oils on proteolytic, deaminative and methanogenic activities of mixed ruminal bacteria. Can. J. Anim. Sci. 88:117–122.

Cheeke, P. R. 2000. Actual and potential applications of *Yucca schidigera* and *Quillaja saponaria* saponins in human and animal nutrition. J. Anim. Sci. 77:1–10.

CVB. 2007. CVB feed tables 2007: Chemical Compositions and Nutritional Values of Feedstuffs. Productschap Diervoeder, The Hague, the Netherlands.

Czerkawski, J. W., K. L. Blaxter, and F. W. Wainman. 1966a. The effect of linseed oil and of linseed oil fatty acids incorporated in the diet on the metabolism of sheep. Br. J. Nutr. 20:485–494.

Czerkawski, J. W., K. L. Blaxter, and F. W. Wainman. 1966b. The metabolism of oleic, linoleic and linolenic acids by sheep with reference to their effects on methane production. Br. J. Nutr. 20:349–362.

Dohme, F., A. Machmuller, A. Wasserfallen, and M. Kreuzer. 2001. Ruminal methanogenesis as influenced by individual fatty acids supplemented to complete ruminant diets. Lett. Appl. Microbiol. 32:47–51.

Ellis, J. L., J. Dijkstra, E. Kebreab, A. Bannink, N. E. Odongo, B. W. McBride, and J. France. 2008. Aspects of rumen microbiology central to mechanistic modelling of methane production in cattle. J. Agric. Sci. 146:213–233.

Eugène, M., H. Archimède, and D. Sauvant. 2004. Quantitative meta-analysis on the effects of defaunation of the rumen on growth, intake and digestion in ruminants. Livest. Prod. Sci. 85:81–97.

Eugene, M., D. Masse, J. Chiquette, and C. Benchaar. 2008. Short communication: Meta-analysis on the effects of lipid supplementation on methane production in lactating dairy cows. Can. J. Anim. Sci. 88:331–334.

FAO. 2010. Greenhouse Gas Emissions from the Dairy Sector. A Life Cycle Assessment. Food and Agriculture Organization, Rome, Italy.

Flachowsky, G., and P. Lebzien. 2009. Comments on in vitro studies with methane inhibitors. Anim. Feed Sci. Technol. 151:337–339.

García-González, R., S. López, M. Fernández, R. Bodas, and J. S. González. 2008. Screening the activity of plants and spices for decreasing ruminal methane production in vitro. Anim. Feed Sci. Technol. 147:36–52.

García-Martínez, R., M. J. Ranilla, M. L. Tejido, and M. D. Carro. 2005. Effects of disodium fumarate on in vitro rumen microbial growth, methane production and fermentation of diets differing in their forage:concentrate ratio. Br. J. Nutr. 94:71–77.

Gebhardt, R., and H. Beck. 1996. Differential inhibitory effects of garlic-derived organosulfur compounds on cholesterol biosynthesis in primary rat hepatocyte cultures. Lipids 31:1269–1276.

Goel, G., K. Arvidsson, B. Vlaeminck, G. Bruggeman, K. Deschepper, and V. Fievez. 2009. Effects of capric acid on rumen methanogenesis and biohydrogenation of linoleic and α-linolenic acid. Animal 3:810–816.

Hegarty, R. S. 1999. Reducing rumen methane emissions through elimination of rumen protozoa. Aust. J. Agric. Res. 50:1321–1328.

Holtshausen, L., A. V. Chaves, K. A. Beauchemin, S. M. McGinn, T. A. McAllister, P. R. Cheeke, and C. Benchaar. 2009. Feeding saponin-containing *Yucca schidigera* and *Quillaja saponaria* to decrease enteric methane production in dairy cows. J. Dairy Sci. 92:2809–2821.

Hristov, A. N., T. A. McAllister, F. H. Van Herk, K. J. Cheng, C. J. Newbold, and P. R. Cheeke. 1999. Effect of *Yucca schidigera* on ruminal fermentation and nutrient digestion in heifers. J. Anim. Sci. 77:2554–2563.

ISO. 1999. Whole milk. Determination of milk fat, protein and lactose content—Guidance on the operation of mid-infrared instruments. International Standardisation Organisation, Geneva, Switzerland.

ISO. 2004. Milk. Determination of urea content—Enzymatic method using difference in pH (reference method). International Standardisation Organisation, Geneva, Switzerland.

Kamel, C., H. M. R. Greathead, M. L. Tejido, M. J. Ranilla, and M. D. Carro. 2008. Effects of allicin and diallyl disulfide on in vitro rumen fermentation of a mixed diet. Anim. Feed Sci. Technol. 145:351–363.

Kolver, A. S., and P. W. Aspin. 2006. Supplemental fumarate did not influence milksolids or methane production from dairy cows fed high quality pasture. Proc. N.Z. Soc. Anim. Prod. 66:409–415.

Lila, Z. A., N. Mohammed, S. Kanda, T. Kamada, and H. Itabashi. 2003. Effect of sarsaponin on ruminal fermentation with particular reference to methane production in vitro. J. Dairy Sci. 86:3330–3336.

Lovett, D. K., L. Stack, S. Lovell, J. Callan, B. Flynn, M. Hawkins, and F. P. O'Mara. 2006. Effect of feeding *Yucca schidigera* extract on performance of lactating dairy cows and ruminal fermentation parameters in steers. Livest. Sci. 102:23–32.

Macheboeuf, D., B. Lassalas, M. J. Ranilla, M. D. Carro, and D. P. Morgavi. 2006. Dose-response effect of diallyl disulfide on ruminal fermentation and methane production in vitro. Reprod. Nutr. Dev. 46:S103.

Maia, M. R. G., L. C. Chaudhary, L. Figueres, and R. J. Wallace. 2007. Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. Antonie van Leeuwenhoek 91:303–314.

Martin, C., D. P. Morgavi, and M. Doreau. 2010. Methane mitigation in ruminants: From microbe to the farm scale. Animal 4:351–365.

Martin, C., J. Rouel, J. P. Jouany, M. Doreau, and Y. Chilliard. 2008. Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil. J. Anim. Sci. 86:2642–2650.

McCourt, A. R. Y. T., S. Mayne, and R. J. Wallace. 2008. Effect of dietary inclusion of encapsulated fumaric acid on methane production from grazing dairy cows. Page 64 in Proc. Br. Soc. Anim. Sci. British Society of Animal Science, Scarborough, UK.

McGinn, S. M., K. A. Beauchemin, T. Coates, and D. Colombatto. 2004. Methane emissions from beef cattle: Effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. J. Anim. Sci. 82:3346–3356.

Miller, T. L., and M. J. Wolin. 2001. Inhibition of growth of methane-producing bacteria of the ruminant forestomach by hydroxymethylglutaryl-CoA reductase inhibitors. J. Dairy Sci. 84:1445–1448.

Newbold, C. J., B. Lassalas, and J. P. Jouany. 1995. The importance of methanogens associated with ciliate protozoa in ruminal methane production in vitro. Lett. Appl. Microbiol. 21:230–234.

NRC. 2001. Nutrient Requirements of Cattle. 7th rev. ed. National Academy Press, Washington, DC.

Prins, R. A., C. Van Nevel, and D. Demeyer. 1972. Pure culture studies of inhibitors for methanogenic bacteria. Antonie van Leeuwenhoek 38:281–287.

Ungerfeld, E. M., R. A. Kohn, R. J. Wallace, and C. J. Newbold. 2007. A meta-analysis of fumarate effects on methane production in ruminal batch cultures. J. Anim. Sci. 85:2556–2563.

Valdez, F. R., L. J. Bush, A. L. Goetsch, and F. N. Owens. 1986. Effect of steroidal sapogenins on ruminal fermentation and on production of lactating dairy cows. J. Dairy Sci. 69:1568–1575.

Verstegen, M. W. A., W. Van der Hel, H. A. Brandsma, A. M. Henken, and A. M. Bransen. 1987. The Wageningen respiration unit for animal production research: A description of the equipment and its possibilities. Pages 21–48 in Energy Metabolism in Farm Animals:

Effects of Housing, Stress and Disease. Martinus Nijhoff Publishers, Dordrecht, the Netherlands.
Wallace, R. J., T. A. Wood, A. Rowe, J. Price, D. R. Yanez, S. P. Williams, and C. J. Newbold. 2006. Encapsulated fumaric acid as a means of decreasing ruminal methane emissions. Int. Congr. Ser. 1293:148–151.
Wilson, R. C., T. R. Overton, and J. H. Clark. 1998. Effects of *Yucca schidigera* extract and soluble protein on performance of cows and concentrations of urea nitrogen in plasma and milk. J. Dairy Sci. 81:1022–1027.
Yang, W. Z., C. Benchaar, B. N. Ametaj, A. V. Chaves, M. L. He, and T. A. McAllister. 2007. Effects of garlic and juniper berry essential oils on ruminal fermentation and on the site and extent of digestion in lactating cows. J. Dairy Sci. 90:5671–5681.