Supplemental fumarate did not influence milksolids or methane production from dairy cows fed high quality pasture

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ABSTRACT

This experiment tested the hypothesis that methane emissions from dairy cows would be reduced, and milk production increased, when sodium fumarate was added to a diet of fresh pasture at a rate of 5% of dry matter (DM) intake. Sixteen ruminally fistulated dairy cows (100 ± 20.7 days in milk; 548 ± 47.8 kg liveweight; mean ± SD) received either a control (water) or fumarate treatment (10.3 ± 1.18 L/cow/day of solution providing 931 ± 126 g sodium fumarate/cow/day) over a 7-day adjustment period while grazing ryegrass pasture, followed by an 8-day experimental period fed pasture in metabolism stalls. During the experimental period cows on both treatments consumed 18.8 ± 1.83 kg DM/day, produced 1.81 ± 0.25 kg milksolids/day, and emitted 350 ± 44.9 g methane/day (5.9 ± 0.71% of gross energy). Total tract DM digestibility was high (78.8 ± 0.84%), and ruminal pH was 6.10 ± 0.15. Fumarate supplementation did not change ruminal fermentation, in vivo digestibility, methane emissions, milk production or composition, or selected plasma metabolites. The opportunity for reducing methane emissions from dairy cows by supplementing with fumarate or breeding high fumarate grasses may be limited for diets of high quality pasture. The limited reports of *in vivo* response to supplemental fumarate suggest that mitigation opportunities may be greater for pasture diets of low digestibility.

Keywords: fumarate, organic acids, pasture, dairy cow, methane

INTRODUCTION

Dicarboxylic organic acids such as malate and fumarate occur naturally in grasses and legumes, and elevated dietary levels have been proposed to reduce methane production by ruminants (Callaway & Martin, 1996). Most *in vitro* studies with high concentrate or mixed diets report a reduction in methane, and the response is dose dependent (Asanuma *et al*., 1999; Carro *et al*., 1999; Carro & Ranilla, 2003; Iwamoto *et al.,* 1999; Lopez *et al*., 1999; Martin & Streeter, 1995).

The mode of methane mitigation is through providing an alternative electron sink for hydrogen when fumarate is reduced to succinate in the succinate-propionate pathway of ruminal bacteria (Martin, 1998). In an *in vitro* comparison of fifteen potential precursors of propionate, Newbold *et al.* (2005) found sodium fumarate to be the most effective compound at reducing methane emissions, capturing as propionate 44% of the hydrogen previously used for methane. The methane response may also be influenced via a stimulatory effect on selected ruminal microbial species (Asanuma *et al.,* 1999; Nisbet & Martin, 1993).

Evidence of a diet-dependent response to malate or fumarate is equivocal, however Garcia-Martinez *et al*. (2005) demonstrated larger reductions in methane production when fumarate was elevated in high forage diets compared to high concentrate diets *in vitro*. Recently Kolver *et al.* (2004) reported a dose-response study in which freeze-dried pasture was fermented in continuous culture. Linear responses in propionate concentrations and methane production were recorded up to 30 mM sodium fumarate (10.5% of DM), with the concentration of propionate increased by 74% and methane production reduced by 54% at the highest dose rate.

Although the methane mitigating properties of malate and fumarate have been demonstrated *in vitro*, only four reported studies have measured methane production *in vivo*, with contrasting results. Bayaru *et al*. (2001) observed a 23% decrease in methane production when fumarate was added at 2% of dry matter (DM) intake to an all-forage (sorghum silage) diet fed to two steers. A smaller methane reduction (12%) was reported by Newbold *et al.* (2002) using sheep fed grass hay and supplemented with 80 g/day of fumaric acid (8% of DM). In contrast, McGinn *et al.* (2004) reported no effect of fumaric acid (1.2% of DM) on methane emissions from steers fed barley silage and concentrate, and Newbold *et al*. (2002) reported a 48% increase in methane emissions per kg DM intake using sheep fed a 50:50 diet of grass hay and concentrate and supplemented with 178 g/day of sodium fumarate (19% of DM).

Limited *in vivo* studies have evaluated the productivity response of ruminants to organic acids supplementation. In general, production responses to malate are variable. Liveweight gain and feed conversion efficiency gains on high-concentrate diets have been reported by Martin *et al*. (1999) in two experiments, and by Sanson & Stallcup (1984), but not by Martin *et al.* (1999) in one experiment when beef cattle were supplemented up to 120 g malate/day. Increased milk persistency and feed conversion efficiencies (Kung *et al.,* 1982; Stallcup, 1979) and increased milk production (Alferez, 1978; Devant & Bach, 2004, Sniffen *et al.,* 2006) have been reported for dairy cows. However no milk production responses were reported by Vicini *et al*. (2003) using dairy cows, or by Salama *et al*. (2002) using lactating dairy goats. Studies where fumarate has been used to supplement diets fed to goats (Isobe & Shibata, 1993), sheep (Newbold *et al*., 2002) and steers (Bayaru *et al*., 2001; McGinn *et al.*, 2004) have not reported productivity, and there have been no reports of fumarate supplementation for dairy cows.

This experiment tested the hypothesis that methane emissions would be reduced, and milk production of dairy cows increased, when sodium fumarate was added to a diet of fresh pasture at a rate of 5% of DM.

MATERIALS AND METHODS

Design and treatments

Sixteen ruminally fistulated Friesian/Friesian cross dairy cows (100 ± 20.7 days in milk; 548 ± 47.8 kg liveweight; mean ± SD) were blocked according to milksolids production and treatments randomly allocated within blocks. Treatments were based on a diet of fresh pasture offered *ad libitum* with either no supplemental fumarate (control) or 900 g supplemental sodium fumarate (98.7% sodium fumarate dibasic anhydrous $C_4H_2O_4Na_2$; Sulkem Company Ltd., Auckland, New Zealand) per cow per day (5% of DM intake).

The design included three sequential periods. The first was a covariate period (d 1-4) during which time cows grazed as one herd and received no treatment. Measurements of milk production and composition, liveweight, body condition score, and rumen and blood metabolites were made and used as covariate data in the analysis of the experimental period.

The second period was an adjustment period (d 5-11) during which time cows grazed pasture at a high pasture allowance (>50 kg DM/cow/day) as one herd, and received treatment. This allowed ruminal and physiological adaptation to the fumarate treatment, during which time no data was collected for analysis.

The third period was the experimental period (d 12-20) in which cows were housed in metabolism stalls. Cows spent the first two days of this period adjusting to the indoor facility followed by a five-day digestibility period. Milk production and composition, liveweight, body condition score, rumen and blood metabolites, and methane emissions data collected within this five-day period are presented as the experimental results. During the experimental period, ryegrass-dominant pasture was cut twice a day and fed at 0900 and 1600 h *ad libitum* to 10% refusals.

The fumarate dose rate of 5% of DM equates to 3.6% fumaric acid of DM intake or 20 mM fumarate, and was based on the *in vitro* dose response results of Kolver *et al*. (2004). Fumarate was administered in solution (90 g/L) with a total of 10 L/cow/day administered three times daily (3.33 L/cow/dose) at 0730, 1200, and 1630 h via the rumen fistula during the adjustment period, or continuously infused into the rumen (10.3 ± 1.18L/cow/day as a solution to provide 931 ± 126g sodium fumarate/cow/day) during the experimental period. Control cows received the equivalent volume of water during the adjustment and experimental periods. The fumarate solution was neutralised with 12.7 ml/L of 50% HCl to reduce solution pH from 9.8 ± 0.3 to 7.0 ± 0.2.

Sample collection and analysis

Pasture was sampled prior to each feed during the experimental period, and dry matter content determined to calculate the fresh weight of pasture to feed to each cow. Refused feed from each cow was weighed and sampled to calculate DM intake. On d 14-18, pasture from each treatment was sampled at each feeding, and a sub-sample dried at 100°C for 24 h and composited over the 5-d digestibility period for determination of chemical composition. A further pasture sub-sample for each treatment was bulked fresh over the 5-d digestibility period for botanical dissection. Individual refusals were sampled twice daily for DM and sub samples dried prior to bulking within treatment for chemical analysis (AOAC, 2000).

Milk weights were recorded daily, with results from d 14 (PM) to 19 (AM) reported as the experimental results. Covariate milk samples were collected on d 2 (PM) and 3 (AM), and experimental period milk samples collected on d 14 (PM) to 19 (AM), and were analysed for SCC and milk composition by infrared procedures (FT120, Foss Electric, Denmark).

Rumen digesta was sampled at 0800, 1200, 1600, and 2000h on two days of the covariate period (d 3 and 4), and bulked across times and days, within cow. During the experimental period, rumen digesta was sampled over two 24-h periods (d 17 and 18). On d 17, sampling occurred every 4 h at 0800, 1200, 1600, 2000, 2400, and 0400h. On d 18, sampling times were advanced by 2 h (1000, 1400, 1800, 2200, 0200, and 0600 h). The pH was determined immediately on strained digesta, samples were centrifuged at 2800 rpm for 10 mins, and acidified (0.6 ml 50% sulphuric acid) or non-acidified 50-ml aliquots of supernatant were stored for subsequent compositing across times and days, within cow, for analysis of volatile fatty acids (VFA; Planye, 1985) and ammonia (Tietz, 1986), respectively.

Blood samples (10 ml) were collected from the coccygeal vessel using heparinised vacutainers at 1200 and 2000 h on d 3 and 4 of the covariate period (approx. 4 hours after initial access to a new break of pasture), and on d 17 and 18 of the experimental period. Samples were centrifuged at 2800 rpm for 10 mins, and plasma collected and frozen for subsequent analysis of glucose and non-esterified fatty acids (NEFA).

Daily urine and faecal output were recorded on d 15 to 19, with sub-samples collected and bulked across days. Urine samples were acidified (1 ml 50% H_2SO_4 per 50-ml aliquot) to pH 3, and energy, nitrogen (N; urine and faeces), and neutral detergent fibre (NDF) and acid detergent fibre (ADF; faeces) content determined (AOAC, 2000).

Methane emissions were measured using the sulphur hexafluoride (SF_6) marker procedure described by Johnson *et al.* (1994). Brass permeation tubes which released SF_6 at a known rate were placed in the rumen via the rumen fistula on d 10. Respired air was sampled continuously above the nose over four 24-h periods from 0900 h on d 15 to 0900 h on d 19 using a fine-bore capillary tube attached to an evacuated yoke.

Statistical Analysis

Data were analyzed using ANOVA in Genstat 5.4.2 and used pre-experimental data as a covariate (except for methane and pasture intake). Analysis excluded data from one cow that was removed from the control treatment after damaging a teat, and methane data from two cows (one from each treatment) due to incomplete gas collection.

RESULTS

Pasture was dominated by ryegrass (60% ryegrass leaf; 21% ryegrass stem; 13% white clover; 1% other grasses; 5% dead material; 0% weeds) and was of high quality (11.8 MJME/kg DM; 78.5% DM in vitro digestibility; 19.8% crude protein CP; 41.3% neutral detergent fibre NDF; 21.5% acid detergent fibre ADF; 17.6% nonstructural carbohydrate NSC; 26.7% non-fibre carbohydrate NFC; 2.9% fat; and 9.3% ash).

No significant treatment differences were detected for methane production (Table 1), milk yield or composition (Table 2), energy balance or apparent total tract digestibility of DM, organic matter (OM), N, NDF, or ADF (Table 3), mean daily ruminal pH and total and individual volatile fatty acid concentrations (Table 4), or plasma concentrations of glucose or NEFA four hours after feeding (Table 4). A small but significant increase in lactose content was detected on the fumarate treatment (Table 2).

TABLE 1: CH_4 production of dairy cows fed pasture with no supplemental fumarate (Control) or pasture supplemented with 931 g sodium fumarate (5% DM) per cow per day (Fumarate).

	Control	Fumarate	SED	P
CH_4 (g/d)	348.6	351.1	24.23	NS
CH_4 (g/kg DM intake)	18.1	18.7	1.15	NS
CH_4 (% of gross energy)	5.7	6.1	0.40	NS
CH_4 (g/kg milksolids)	198.6	190.4	12.59	NS

TABLE 2: Milk yield and composition of dairy cows fed pasture with no supplemental fumarate (Control) or pasture supplemented with 931 g sodium fumarate (5% of DM) per cow per day (Fumarate).

	Control	Fumarate	SED	P
Milk yield				
Milk (kg/d)	22.8	24.0	1.14	NS
4% FCM[1] (kg/d)	24.2	25.9	1.36	NS
ECM[2] (kg/d)	25.8	27.6	1.33	NS
Milk composition				
Fat (%)	4.45	4.60	0.163	NS
Crude protein (%)	3.26	3.30	0.059	NS
True protein (%)	3.04	3.08	0.057	NS
Casein (%)	2.49	2.52	0.048	NS
Lactose (%)	4.84	4.96	0.032	<0.01
Milk pH	6.51	6.51	0.038	NS
Urea (mmol/l)	5.79	5.41	0.192	NS
Log10 SCC	1.79	1.82	0.098	NS
Milksolids (kg/day)	1.75	1.87	0.088	NS
Milksolids (g/kg DM)	95.1	97.6	5.69	NS

[1]Fat corrected milk
[2]Energy corrected milk

TABLE 3: Energy balance and digestibility coefficients of dairy cows fed pasture with no supplemental fumarate (Control) or pasture supplemented with 931 g sodium fumarate (5% DM) per cow per day (Fumarate).

	Control	Fumarate	SED	P
Intake				
Pasture intake (kg DM/d)	19.1	17.7	0.94	NS
Sodium fumarate intake (g/d)		930.6	38.27	
Total intake (kg DM/d)	19.1	18.6	0.98	NS
Gross energy intake (MJ/day)	334.6	318.0	17.19	NS
Energy balance				
Milk energy (MJ/day)	78.6	78.0	6.37	NS
Faecal energy (MJ/day)	82.6	76.1	5.01	NS
Urine energy (MJ/day)	26.6	23.9	1.51	NS
Methane energy (MJ/day)	19.4	19.5	1.35	NS
Energy balance (MJ/day)	0.0004	0.0004	0.00002	NS
Digestion coefficient				
Dry matter	0.785	0.790	0.004	NS
Organic matter	0.800	0.799	0.004	NS
Crude protein	0.760	0.763	0.006	NS
Neutral detergent fibre	0.813	0.816	0.009	NS
Acid detergent fibre	0.806	0.820	0.009	NS

TABLE 4: Mean daily ruminal pH, concentration of total volatile fatty acids (VFA), molar proportion of individual acids, and plasma concentrations of glucose and non-esterified fatty acids (NEFA) of dairy cows fed pasture with no supplemental fumarate (Control) or pasture supplemented with 931 g sodium fumarate (5% DM) per cow per day (Fumarate).

	Control	Fumarate	SED	P
Ruminal pH	6.10	6.11	0.073	NS
Total VFA (mmol/l)	157.8	159.6	9.65	NS
Individual (mmol/l)				
Acetate	94.9	96.9	6.36	NS
Propionate	30.3	32.3	2.55	NS
Butyrate	22.1	20.3	1.13	NS
Lactate	1.8	1.9	0.07	NS
Plasma				
Glucose (mmol/l)	3.54	3.49	0.05	NS
NEFA (mmol/l)	0.10	0.11	0.06	NS

Cows on both treatments emitted 350 ± 44.9 g methane/day (5.9 ± 0.71% of gross energy; Table 1), produced 1.81 ± 0.25 kg milksolids/day (Table 2), and consumed 18.8 ± 1.83 kg DM/day (Table 3). Cows on both treatments recorded a total tract digestibility of 78.8 ± 0.84 % (Table 3), a mean daily ruminal pH of 6.10 ± 0.15 (Table 4), and a mean total volatile fatty acid concentration of 159 ± 21.8 (Table 4).

DISCUSSION

This appears to be the first reported study to have supplemented dairy cows with fumarate, and the first *in vivo* study of organic acid supplementation to have used fresh pasture as the base diet. Based on our previous dose-response study using continuous culture fermentation and freeze-dried pasture (Kolver *et al.,* 2004), the dose rate used in this *in vivo* study (5% of DM) was expected to reduce methane by 31% and increase ruminal concentrations of propionate by 39%. However this hypothesis was not supported by the current study. The concentration of propionate and methane production was not influenced by treatment, and consequently there were no changes observed (or could be expected) in nutrient digestibility, energy and nitrogen balance, plasma indicators of energy and nitrogen status, or milk production or composition.

This is consistent with the lack of a methane response reported by McGinn *et al*. (2004) who fed steers in calorimeter chambers a diet of barley silage and concentrate supplemented with fumaric acid (80g/d; 1.2% of DM). Montano *et al.* (1999) also calculated no change in methane emissions based on the molar proportions of VFA produced by steers fed a high-grain finishing diet and supplemented with 80 g/d of malic acid. These results contrast with those of Bayaru *et al.* (2001) who reported a 23% reduction in methane emissions from two steers fed sorghum silage and supplemented with sodium fumarate (2% of DM), and Newbold *et al.* (2002) who reported a 12% reduction in methane emissions at the highest dose of fumaric acid tested (8% of DM) with sheep fed grass hay.

An analysis of the current experiment and studies that have supplemented fumarate *in vivo* (Bayaru *et al.,* 2001; McGinn *et al.,* 2004; Newbold *et al.,* 2002;) or *in vitro* (Asanuma *et al*., 1999; Callaway & Martin, 1996; Iwamoto *et al.,* 1999; Lopez *et al*., 1999; Kolver *et al*., 2004) reveals that the greatest reduction in methane emissions in response to fumarate has been obtained from fermentation of diets that generate high levels of methane emissions. This analysis showed that fumarate reduced *in vivo* methane emissions by 3.5% for every gram of methane emitted per kg DM intake in the control diet (R^2=0.59), and by 1% for every mM of methane per litre of gas produced *in vitro* (R^2=0.11). Diets

that resulted in high methane emissions tended to be high forage diets of low digestibility, and diets resulting in low methane emissions tended to have a high content of grain or starch. The methane emissions from digestion of fresh pasture in the current study were low (5.9% of gross energy; 18.4 g/kg DM intake) and are consistent with values reported when high quality spring pasture has been fed to dairy cows (Waghorn *et al*., 2001). The low methane emissions also reflect the high total tract digestibility values observed for DM, OM, N, NDF, and ADF in this study. It may be possible that there is a methane threshold below which addition of fumarate does not further reduce methane emissions. This may indicate that the mode of action of fumarate is dependent on diet, with production responses on low digestible forage diets resulting from increased propionate and reduced methane, and production responses on high grain diets resulting from improved lactate utilization, buffering of ruminal pH, and microbial efficiency. This diet-dependent response has been recently demonstrated *in vitro* by Garcia-Martinez *et al.* (2005) who reported a greater reduction in methane in response to disodium fumarate on high forage diets compared to high concentrate diets. Fresh, high quality pasture may fall between these two modes of fumarate action, having neither the high methane emissions to mitigate, nor the lactate-induced sub-optimal ruminal pH to ameliorate. Consequently a reduction in methane emissions and improvement in productivity may not be expected.

Fresh pasture is also expected to have high basal levels of organic acids, which may have reduced the effectiveness of supplemental fumarate. It is known that organic acid content is higher in legumes than grasses, higher in immature than mature forages, and higher in fresh forage than in hay or pelleted forage (Callaway *et al.,* 1997). It is possible that the response to supplemental organic acids is dependent on the concentration of organic acids in the base diet. Salama *et al*. (2002), working with dairy goats, attributed a lack of a production response to malic acid on the relatively high concentration of malic acid in the base diet (lucerne). The fresh, immature nature of the pasture, and the inclusion of 13% white clover in the pasture mix of the current study, would indicate that organic acid concentrations would be expected to be higher than most base diets used in other studies.

The greater diversity of ruminal microbial species *in vivo* may also explain the differences between *in vitro* and *in vivo* responses to fumarate. Somewhat surprisingly, propionate concentrations in this *in vivo* study did not increase with fumarate supplementation, although this was consistent with the results of McGinn *et al.* (2004). While fumarate can be converted into acetate as well as propionate (Demeyer & Henderickx, 1967), neither acetate nor any of the other VFA concentrations changed with treatment. This may reflect metabolism and interactions between mixed specie populations that are not replicated *in vitro*. The inconsistent responses between *in vitro* and *in vivo* emphasise the importance of evaluating laboratory observations in animals under conventional feeding systems.

The higher sodium intake of cows fed fumarate (1.4% of DM) may have also influenced results. Cows fed sodium fumarate drank twice the volume of water and excreted twice the volume of urine compared to control cows.

The dose rate used in this study was the highest reported *in vivo*, but was lower than the highest dose used *in vitro* by Kolver *et al*. (2004) at which the greatest reduction in methane in response to fumarate was observed.

In conclusion, feeding sodium fumarate at 5% of DM to dairy cows fed fresh, high quality pasture, did not change ruminal fermentation and digestibility, methane emissions, milk production or composition, or selected plasma metabolites. The opportunity for reducing methane emissions from dairy cows by supplementing with fumarate or breeding high-fumarate grasses may be limited for high quality pasture diets. The limited reports of *in vivo* response to supplemental fumarate suggest that mitigation opportunities may be greater for pasture diets of low digestibility.

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