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Effect of DL-malic acid supplementation on feed intake, methane emissions, and performance of lactating dairy cows at pasture

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ABSTRACT

The objective of this study was to determine the effect of dietary DL-malic acid (MA) supplementation on feed intake, methane (CH_4) emissions, and performance of mid lactation Holstein-Friesian cows at pasture. Twenty-four (6 primiparous and 18 multiparous) mid- to late-lactation cows (206 ± 65 d in milk) grazing a mixed-species grass sward were blocked on parity, days in milk, and pretrial milk yield, and randomly allocated within block to 1 of 2 dietary treatments offered twice daily at milking in 2 equal portions (6 kg/d in total): a control concentrate (0 g/d of MA) and a concentrate supplemented with MA (480 g/d of MA) over a 6-wk period. Cows were allowed a 3-wk acclimation period followed by a 5-d CH_4 measurement period. Enteric CH_4 emissions were estimated using the sulfur hexafluoride tracer gas technique, and herbage intake was measured using the n-alkane technique. Dietary supplementation with MA did not affect voluntary intake of herbage or total dry matter intake, body weight gain, milk yield, fat-corrected milk yield, or daily CH_4 production. These results suggest that there is little benefit to be gained from the dietary supplementation of dairy cows at pasture with MA at least within the inclusion rates used in this study.
Key words: dairy cow, DL-malic acid, methane, intake

INTRODUCTION

Livestock collectively account for about 25% of methane (CH_4) emitted to the atmosphere from human activities, perhaps the most significant emission from a single such activity (Lassey, 2008). Not only is CH_4 a potent greenhouse gas, it is also a significant energy sink to the ruminant animal, accounting for up to 12% of the gross energy (**GE**) consumed (Johnson and Johnson, 1995).

Methane from enteric fermentation is a natural by-product of fermentation and its production serves as the primary electron sink within the rumen (Beauchemin et al., 2008). Therefore, methanogenesis can be seen as the primary means to remove H_2 within the rumen. The ability of dicarboxylic organic acids (**OA**) such as fumaric acid (**FA**) and malic acid (**MA**) to act as inhibitors of methanogenesis is well documented in vitro (Carro and Ranilla, 2003; Newbold et al., 2005) and in vivo (Lila et al., 2004; Wallace et al., 2006; Foley et al., 2009).

Despite several studies reporting reductions in ruminal CH_4 emissions following dietary OA supplementation, there is some variability between studies in the actual magnitude of the reduction. For example, Wallace et al. (2006) reported reductions in CH_4 of up to 75% in lambs offered FA, whereas other studies with beef cattle (Lila et al., 2004; Foley et al., 2009) reported lesser reductions, in the order of 18%, when animals were supplemented with MA compared with unsupplemented controls. In contrast, however, other authors reported no effect of FA supplementation on CH_4 production in beef cattle (Beauchemin and McGinn, 2006) or dairy cows (Kolver and Aspin, 2006; McCourt et al., 2008), nor did these studies report any effect of FA on milk yield. Sniffen et al. (2006) reported a higher milk yield in dairy cows supplemented with 50 g/d of MA and although CH_4 measurement was not considered in that study, increases in animal performance could decrease CH_4 emissions per kilogram of animal product (O'Mara et al., 2008).

Malic acid is naturally present in pasture, albeit at low levels. Muck et al. (1991) measured OA from 2 mixed-sward permanent pastures in Ireland and reported that citric, malic, and palmitic acids were at the highest concentrations with similar ranges (1.5 to 6.7 mg/g of DM). There is, however, a paucity of published literature on the effects of MA supplementation of cattle and particularly dairy cows managed under pasture-based production systems, on performance or enteric CH_4 production. Therefore, the aim of the current study was to examine the effect of MA supplementation of grazing

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Table 1. Ingredient (g/kg as fed) and chemical composition of the herbage and concentrate fed (expressed as g/kg of DM unless otherwise stated)

Variable	CON[1]	MAL[2]	Herbage
Beet pulp molassed	201.1	185.0	—
Maize	157.0	144.4	—
Corn gluten	149.0	137.1	—
Citrus pulp	148.0	136.2	—
Soybean extraction meal	89.0	81.9	—
Corn distillers	73.0	67.2	—
Milk solids	65.0	59.8	—
Pollard	51.0	46.9	—
Wheat	27.0	24.8	—
Calcined magnesite	22.8	21.0	—
Crude palm oil coater	6.0	5.5	—
Limeflour	3.0	2.8	—
Salt	3.0	2.8	—
Minerals[3]	2.5	2.3	—
Mono-dicalcium phosphate	2.2	2.0	—
Cuprotect[4]	0.4	0.4	—
Malic acid	—	80.0	—
DM, g of DM/kg of fresh weight	871.3	874.8	219
CP	143.0	142.7	134
NDF	230.1	238.1	588
ADF	125.4	135.8	321
Ash	91.7	97.8	77
Gross energy, MJ/kg of DM	16.5	16.6	18.2

[1]CON = 0 g dietary inclusion of malic acid.

[2]MAL = 480 g dietary inclusion of malic acid.

[3]Minerals = vitamin A, 6,000 IU/kg; vitamin D_3, 2,000 IU/kg; magnesium, 9 g/kg; copper, 52 mg/kg; selenium, 0.65 mg/kg; and iodine, 10 mg/kg.

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dairy cows on feed intake, milk yield and composition, and enteric CH_4.

MATERIALS AND METHODS

Animals, Experimental Design, and Treatments

This experiment was conducted at the University College Dublin Lyons Research Farm from June 19 to August 1, 2005. Twenty-four Holstein-Friesian cows with a mean milk yield of 23.9 kg/d (±4.3) and a BW of 628.1 kg (±36.5) were used for this experiment; 18 of the cows were multiparous. Animals were in mid to late lactation (206 ± 65 DIM) at the start of the experiment. Pre-experimental milk yields, calving date, and parity were used to block the experimental animals to 1 of 2 experimental diets (n = 12/diet). In this study, to minimize palatability issues and feed sorting, the concentrate portion of the diet was pelleted. Two separate concentrates were prepared and pelleted (Table 1). One of the concentrates contained no MA (**CON**), whereas the other concentrate contained 480 g/d MA on a DM basis (**MAL**). Ingredient and chemical compositions of concentrates and herbage are shown in Table 1. Both concentrates contained a vitamin and mineral supplement (Table 1) and cows were offered either CON or MAL individually twice daily at milking in 2 equal portions (6 kg/d in total). The chemical nature of the supplemental MA used was DL-malic acid (Bartek Ingredients Inc., Stoney Creek, Ontario, Canada). Cows were fed the experimental diets on an individual basis for 3 wk before CH_4 emissions were measured in the fourth week, using the sulfur hexafluoride ($\mathbf{SF_6}$) tracer gas technique. Daily herbage intake was measured during the same period using the n-alkane technique, as described by Dillon and Stakelum (1989).

Grassland Management and Pre-Experimental Preparation

The composition of the experimental sward used was approximately 40% perennial ryegrass (*Lolium perenne*), 40% rough stalk meadow grass (*Poa trivialis*), 10% annual meadow grass (*Poa annua*), and 10% white clover (*Trifolium repens*). The experiment required a total of 24 single-day grazing plots managed to provide a constant pregrazing herbage mass. Postgrazing, each subpaddock received a total of 50 kg of N/ha.

Animal Grazing Management

At pasture both experimental groups were managed as one group. Strip-grazing of paddocks was accom-

plished through the use of a temporary electric fence. Fresh pasture was offered after each milking. Pasture herbage mass was determined with a rising plate meter (Jenquip, Feilding, New Zealand) and pastures were grazed to achieve a constant postgrazing residual stubble height of 6 cm. The daily herbage allowance was divided between daytime and nighttime, with a 45 and 55% allowance, respectively.

Herbage and Concentrate Sampling

Approximately 1.5 kg (fresh weight) of pregrazing herbage was sampled from each grazing plot immediately before access by the experimental animals, at a height of 4 cm above ground, for subsequent chemical analysis. Samples to represent grass actually grazed were taken by following an animal to two individual grazing sites within the paddock. At each site a sample of grass immediately neighboring the grazed grass site was taken, with the cut height replicating that harvested by the animal. In total, grazing behavior of 5 animals/treatment per day was recorded over 4 individual times (0630, 1030, 1630, and 1930 h). Concentrates were sampled directly from the feeding point with 8 feed points sampled at random twice weekly. The concentrate was then bulked per treatment across feed points and subsampled for analysis.

Animal Measurements

Individual daily herbage intake was determined by using the n-alkane procedure of Mayes et al. (1986), with modifications as described by Dillon and Stakelum (1989). The n-alkane pellets were administered to all cows twice daily before both the morning and evening milking with a paper pellet (Carl Roth GmbH, Karlesruhe, Germany) containing 500 mg of dotriacontane (C32-alkane). Dosing lasted for a period of 12 d, which commenced on d 18 of the trial. Fecal grab samples (approximately 100 g) were taken twice daily over a 6-d period commencing on d 24. Enteric CH_4 emissions were measured for a total of 5 d starting on d 22 of the trial, using the SF_6 tracer gas technique described by Johnson et al. (1994).

A permeation tube containing SF_6, an inert gas tracer, was placed into the rumen of each animal approximately 2 wk before CH_4 measurements commenced. The permeation tubes were manufactured at University College Dublin and were filled with in excess of 1 g of SF_6 per bolus. The average release rate was $1{,}201 \pm 110$ ng/min, which was predetermined over the preceding 11-wk period by weighing each permeation tube at the same time point once weekly.

A halter fitted with a capillary tube was placed on each animal's head and connected to an evacuated sampling canister designed to half fill over a 24-h period. As the vacuum in the sampling canister slowly dissipated, a steady sample of the air around the mouth and nose of the animal was collected. After collection of a sample, the canister was pressurized with nitrogen, and CH_4 and SF_6 concentrations were determined by gas chromatography. This technique eliminates the need to restrain or enclose animals, thus allowing the animal to move about and graze. It is however, necessary to train the animals to wear the halter and collection canister.

Measurements for milk yield were taken daily at 0530 and 1530 h for each animal using flow meters (Dairymaster Milk Manager Farming Systems, Co. Kerry, Ireland). Milk from each animal was sampled on one a.m. and p.m. milking once per week; samples were then composited for each animal on a proportional basis for analysis.

During the simultaneous determination of CH_4 and feed intake, all animals were held within a large holding pen. In an attempt to avoid damage to equipment, the collection canister valves were closed, the canister removed, and the time recorded for each animal before entering the crush gate. Upon entering the crush, alkane boluses were administered and a fecal grab sample was taken. This took place at approximately 0500 h (before morning milking) or 1500 h (before evening milking). Depending on the time, either a new collection canister was put on the animal and the time recorded (morning), or the same canister was returned to the animal (evening). This continued until the processing of the total experimental group was complete, and then all animals were returned to pasture.

Laboratory Analysis

Herbage and concentrate samples collected were composited on a weekly basis. Nitrogen, ammonia-N, and GE contents were determined on the fresh samples of the pasture. A subsample of the composite herbage samples as well as the concentrate samples were dried at 55°C for 72 h and subsequently milled through a 1-mm screen using a Christy and Norris hammer mill (Christy Turner, Suffolk, UK) and analyzed for ash, NDF, ADF, acid detergent lignin (**ADL**), and ether extract content, and the CP content of the concentrates was also determined on the dried samples. The NDF, ADF, and ADL concentrations were determined using the Fibertec system (Tecator, Hoganas, Sweden) according to the methods of Van Soest (1973) and Van Soest et al. (1991). Fiber analysis was carried out in-

dividually for NDF and ADF; however, the residue for ADF was used for analysis of ADL. No sodium sulfite or amylase was used. Ether extract was measured using a Soxtec instrument (Tecator) according to the method of AOAC (1970), and GE of the concentrate samples was determined using a Parr 1201 oxygen bomb calorimeter (Parr, Moline, IL), whereas the GE of the pasture was measured using the method of Porter (1992). The CP content was determined as N × 6.25 using a Leco FP 528 instrument (Leco Instruments UK, Cheshire, UK) according to the method of Dumas (AOAC, 1990). Ash content was determined by incineration of 5 g of sample in a muffle furnace at 600°C for 6 h.

Gas concentrations of CH_4 and SF_6 within the collection canisters were determined by gas chromatography (Varian 3800, Varian, Mulgrave, Australia). The gas chromatograph was calibrated daily using 3 National Institute of Standards and Technology certified standards (Scott Marin Inc., Riverside, CA) of CH_4 and SF_6 with internal verifications for both gases run every 12 injections. Each collection canister was analyzed in duplicate, with sample injected (50°C) via a 1-mL sample loop. Once injected, the sample was then split (approximate ratio 1:2) for the determination of CH_4 and SF_6 (Johnson et al., 1994), thus enabling the simultaneous determination of CH_4 using a flame-ionization detector (250°C) and a 3.18 mm × 1.22 m stainless steel Porapak N column 80-100 mesh (Varian), and SF_6 concentration using an electron capture detector (300°C) with a 3.18 mm × 1.83 m stainless steel column packed with a molecular sieve (5A) 40 to 60 mesh (Varian). The oven temperature was maintained at 50°C throughout the analysis.

Statistical Analysis

Data were checked for adherence to a normal distribution before conducting statistical analysis (PROC UNIVARIATE, version 9.1, 2002; SAS Institute Inc., Cary, NC) analyzed using mixed models ANOVA (PROC MIXED, version 9.1, 2002; SAS Institute Inc.) with terms included for the fixed effects of treatment, block, and their interaction as appropriate. Animal within treatment was considered as a random effect. Where repeated measures within animal observations were available (i.e., feed intake, milk yield and composition, and daily CH_4 emissions) a repeated measures ANOVA was conducted (PROC MIXED). The PDIFF statement of SAS and the Tukey test were applied as appropriate to evaluate pairwise comparisons of treatment means.

RESULTS AND DISCUSSION

Feed Intake

Following a preliminary experiment, the inclusion of 480 g/d dietary MA was chosen as most appropriate, as feed intake was reduced above this level. Malic acid is an expensive feed ingredient that could add significantly to the feed cost even at low inclusion rates (O'Mara et al., 2008). Our overall dietary inclusion rate of 2.6% of diet DM is within the range of MA concentrations found in some forage varieties (Callaway et al., 1997), and these varieties potentially offer a low-cost means of supplementing animals with this OA. In the current study there was no effect of MAL on DMI and chemical composition of the herbage and concentrate (Tables 1 and 2). No reduction in DMI following dietary supplementation with MA is in agreement with earlier literature where sodium fumarate (Kolver and Aspin, 2006), MA (Sniffen et al., 2006), and FA (McCourt et al., 2008) were offered. This is, however, a lower inclusion rate than that employed in other studies (Wallace et al., 2006; Molano et al., 2008; Foley et al., 2009), which have reported reductions in DMI following supplementation of sheep or cattle diets with OA. For example, Wallace et al. (2006) and Molano et al. (2008) supplemented wethers with FA up to 10% of DMI, whereas Foley et al. (2009) offered MA as high as 7.5% DMI to finishing beef heifers.

Ruminal Methane Emissions

Numerous in vitro studies have demonstrated the ability of MA and FA to reduce CH_4 emissions (Lila et al., 2004; Mohammed et al., 2004; Newbold et al., 2005). Fumarate and malate are key intermediates in the succinate-propionate pathway. An increase in propionate, leading to a decrease in the acetate:propionate ratio, appears to be a major determinant in reducing enteric CH_4 emissions. Newbold et al. (2005) examined the ability of 15 potential propionate precursors to decrease CH_4 production using an in vitro rumen culture system and found sodium malate to increase propionate by 51%, which in turn led to a 4% reduction in CH_4. Similarly, Lila et al. (2004) examined the effects of β-cyclodextrin diallyl maleate (**CD-M**) in vitro at various concentrations (0 to 7.5 g/L). Total gas and VFA production increased from 21.6 to 36.5 mL/bottle and 65.0 to 72.4 mmol/L, respectively, as the concentration of CD-M increased from 0 to 7.5 g/L. It was concluded that CH_4 production linearly decreased from 5.94 to 1.41 mL/incubation as concentration of CD-M

Table 2. Effect of dietary supplementation of malic acid on mean ± SEM feed intake and methane emission

Variable	CON[1]	MAL[2]	SED[3]	*P*-value
Herbage DMI, kg	13.5	13.7	0.24	0.63
Concentrate DMI, kg	5.2	5.3	0.02	0.93
Total DMI, kg	18.7	18.9	0.34	0.63
CH_4, g/d	374.5	369.3	18.34	0.84
CH_4, g/kg of total DMI	19.5	19.5	0.45	0.94
CH_4, g/kg of milk	19.4	19.3	0.99	0.96
CH_4, g/kg of milksolids	323.0	292.5	41.43	0.46

[1]CON = 0 g dietary inclusion of malic acid.
[2]MAL = 480 g dietary inclusion of malic acid.
[3]Standard error of the difference.

increased from 0 to 7.5 g/L, a reduction of 14 to 76%. In that study, increased dietary CD-M caused a linear decrease in the proportion of acetate and a quadratic increase in propionate and butyrate.

Despite reductions in CH_4 emissions reported in vitro, in the current study there was no effect of treatment on ruminal CH_4 production when expressed in terms of grams per cow per day, grams per kilogram of DMI, or grams per kilogram of product (Table 2). Several studies have failed to replicate in vitro success following dietary inclusion of MA in vivo. Sniffen et al. (2006) reported no effect of 0, 50, or 100 g of supplemental MA per cow per day on total VFA, propionic acid, butyric acid, ratio of acetic:propionic acid, or pH in vitro with a diet of 35% corn silage, 17% alfalfa-grass silage, and 48% supplement concentrate. Although CH_4 production was not measured, any change was unlikely at such a low rate of inclusion. Carro et al. (2006) suggested that inconsistent responses between in vivo and in vitro studies could be related to the different experimental conditions found between the two systems. Differences in dose or supplementation rate are possibly other factors leading to variation in the ruminal fermentation response to OA. Carro et al. (2006) suggested that it is possible that greater dietary inclusion rates of malate would be necessary to detect significant effects on in vivo VFA production.

The lack of effect of MA supplementation on CH_4 production in the current study is in agreement with other published reports in which dairy cows were fed OA at pasture. For example, Kolver and Aspin (2006) failed to observe any effect on CH_4 emissions from grazing dairy cows supplemented with fumarate at a rate of 5% DMI (equivalent to 3.6% fumaric acid) in early lactation. These authors stated that the opportunity for reducing CH_4 emissions from dairy cows by supplementing with fumarate or through breeding high-fumarate grasses may be limited. Similarly, McCourt et al. (2008) recently supplemented first-lactation Holstein dairy cows with up to 1.4 kg (>11% DMI) of encapsulated fumaric acid and reported no effect on CH_4 emissions. In agreement, Molano et al. (2008) offered FA at a rate of up to 10% DMI to wether lambs, in a diet containing dried ground Lucerne, and failed to establish any effect on CH_4 emissions.

In contrast to the findings of Molano et al. (2008), Wallace et al. (2006) offered similar levels (10% DMI) of encapsulated fumaric acid to wethers and achieved reductions in CH_4 emissions of up to 75% per kg of DMI. However, a greater proportion of the diet consisted of concentrates in that study (Wallace et al., 2006) compared with the other studies mentioned above. Foley et al. (2009) offered MA at up to 7.5% of DMI to finishing beef heifers and reported reductions in CH_4 of up to 9% per kg of DMI, but again the concentrate portion of the diet was greater than in the current study.

Taking the preceding discussion into consideration, it appears that reductions in CH_4 emissions in vivo are more probable in diets with a higher concentrate:forage ratio. In contrast, Carro and Ranilla (2003) suggested an inverse effect in vitro, as there was evidence that fumarate could be more effective in decreasing CH_4 production with forage-based diets. This further highlights the lack of consistency between in vivo and in vitro studies. As higher dietary inclusion rates reduce the acetate:propionate ratio (Moss et al., 2000), and this in turn has been associated with lower CH_4 emissions, it is possible that OA inclusion in high-concentrate rations may further augment the reduction in ruminal acetate:propionate ratio. This may provide some explanation toward the apparent improved inhibiting effects of dietary OA on methanogenesis under high-concentrate regimens.

Animal Performance

An increase in animal performance can lower the CH_4 emissions per kilogram of animal product (O'Mara et al., 2008). There was no treatment × week of measurement interaction and no effect of treatment for milk yield, protein and fat concentrations, or BW gain (Table 3). This is in agreement with earlier studies

Table 3. Effect of diet on milk yield and composition

Variable	CON[1]	MAL[2]	SED[3]	*P*-value
Milk yield, kg/d	20.55	20.00	0.439	0.396
CP, g/kg	27.57	29.17	0.699	0.134
Milk fat, g/kg	33.89	34.15	1.016	0.859
Milksolids, g/kg of DMI	67.61	67.98	6.73	0.956
BW gain, kg	19.93	19.42	0.317	0.107

[1]CON = 0 g dietary inclusion of malic acid.
[2]MAL = 480 g dietary inclusion of malic acid.
[3]Standard error of the difference.

involving dairy cows (Vicini et al., 2003; Kolver and Aspin, 2006; McCourt et al., 2008) and goats (Salama et al., 2002) in which supplementation of OA had no effect on performance.

Kung et al. (1982), on the other hand, reported some positive effects of MA supplementation in dairy cows fed corn and corn silage and recorded greater feed conversion efficiency to milk in animals fed a high MA (140 g/d) diet. These animals also had a greater persistency of lactation compared with the average of the 3 other dietary groups (0, 70, and 105 g/d). Similarly, Sniffen et al. (2006) reported that cows fed 50 g of supplemental MA per day increased milk yield with minimal effect on milk composition. As with ruminal CH_4 emissions, effects of OA supplementation on milk production variables appear to be dependent on the composition of the basal diet, with more favorable results being found with diets containing greater levels of concentrate.

In conclusion, there was no evidence from our study of an effect of MA supplementation on ruminal CH_4 emissions or any milk production variable recorded. Potentially greater levels of MA than those employed here may be necessary to detect significant effects on CH_4 emissions under pasture-based regimens. These decisions need to be made in light of associated increases in feed costs.

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