A high dose of monensin does not reduce methane emissions of dairy cows offered pasture supplemented with grain

C. Grainger,* R. Williams,*¹ R. J. Eckard,† and M. C. Hannah*

*Department of Primary Industries, Ellinbank 3821, Victoria, Australia

†Melbourne School of Land and Environment, University of Melbourne, Parkville 3010, Victoria, Australia

ABSTRACT

The primary objective of our research was to determine the effect of a high dose of monensin supplementation on enteric CH₄ emissions of dairy cows offered a ryegrass pasture diet supplemented with grain. An additional objective was to evaluate effects on milk production and rumen function, because a commensurate improvement in milk production could lead to adoption of monensin as a profitable strategy for methane abatement. Two experiments were conducted (grazing and respiratory chambers) and in both experiments monensin (471 mg/d) was topdressed on 4 kg (dry matter)/d of rolled barley grain offered in a feed trough twice daily at milking times. In the grazing experiment, 50 Holstein-Friesian cows were assigned randomly to 1 of 2 groups (control or monensin). Cows grazed together as a single herd on a predominantly ryegrass sward and received monensin over a 12-wk period, during which time measurements of milk production and body weight change were made. The SF₆ tracer technique was used to estimate methane production of 30 of the 50 cows (15 control cows and 15 monensin cows) for 3 consecutive days in wk 3, 5, 8, and 12 of treatment. Samples of rumen fluid were collected per fistula from 8 of the 50 cows (4 per diet) on 2 consecutive days in wk 3, 5, 8, and 12 of treatment and analyzed for volatile fatty acids and ammonia-N. In the metabolic chamber experiment, 10 pairs of lactating dairy cows (control and monensin) were used to determine the effects of monensin on methane emissions, dry matter intake, milk production, and body weight change over a 10-wk period. Methane emissions were measured by placing cows in respiration chambers for 2 d at wk 5 and 10 of treatment. Cows received fresh ryegrass pasture harvested daily. Monensin did not affect methane production in either the grazing experiment (g/d, g/kg of milk) or the chamber experiment (g/d, g/kg of dry matter intake, g/kg of milk). In both experiments, milk

production did not increase with addition of monensin to the diet. Monensin had no effect on body weight changes in either experiment. Monensin did not affect volatile fatty acids or ammonia-N in rumen fluid, but the acetate to propionate ratio tended to decrease. Monensin did not improve milk production of grazing dairy cows and no effect on enteric methane emissions was observed, indicating that monensin cannot be promoted as a viable mitigation strategy for dairy cows grazing ryegrass pasture supplemented with grain.

Key words: dairy cow, monensin, enteric methane, pasture diet

INTRODUCTION

Agriculture was responsible for about 10 to 12% of total anthropogenic emissions of greenhouse gases in 2005 (Smith et al., 2007). Methane (CH₄) is an important greenhouse gas, and enteric CH₄ fermentation from livestock accounted for about 32% of total non-CO₂ emissions from agriculture globally in 2005 (Smith et al., 2007). The most successful CH₄ mitigation strategies will be those that lead to a profitable increase in milk or meat production, as well as achieving a persistent reduction in enteric methane emissions.

The ionophore monensin has been widely used in livestock industries to improve feed conversion efficiency and N metabolism, and is used for bloat relief in dairy cows grazing temperate legume-based pasture (Lowe et al., 1991). Monensin is delivered to the animal as a premix added to the diet or as a slow-release capsule inserted into the rumen. Several reviews of the effect of monensin on milk production of dairy cattle have been published, but perhaps the most thorough is a recent meta-analysis conducted by Duffield et al. (2008b) that used data from 36 papers and 77 trials. They found that monensin use decreased DMI by 0.3 kg, increased milk yield by 0.7 kg, and improved milk production efficiency by 2.5%.

Less evidence exists for the effect of monensin on CH₄ emissions of ruminant livestock. Beauchemin et al. (2008) reviewed the available data and provided evidence that the effect of monensin on CH₄ emissions

Received February 9, 2010.

Accepted July 10, 2010.

¹Corresponding author: richard.williams@dpi.vic.gov.au

may be dose-dependent. Providing a dose of <15 mg of monensin/kg of DMI did not reduce CH₄ emissions, a dose of 20 mg of monensin/kg of DMI had no effect or reduced total CH₄ production, and a dose of 24 to 35 mg/kg of DMI reduced CH₄ emissions by 4 to 10% (g/d) and 3 to 8% (g/kg of DMI). Several experiments have also examined the persistency of reduction in CH₄ emissions resulting from monensin. With total mixed rations, Odongo et al. (2007) reported a 3.6% reduction (g/kg of DMI) in CH₄ for dairy cows fed a diet containing 24 mg of monensin/kg of DMI, which was sustained over a 6-mo period. However, others have shown that reductions in CH₄ emissions due to monensin may not persist beyond several weeks (Johnson and Johnson, 1995; Sauer et al., 1998; Guan et al., 2006).

For cows offered pasture-based diets, limited information exists on the effect of monensin on methanogenesis. Van Vugt et al. (2005) offered cows ryegrass-dominant pasture indoors and measured a 12% reduction in CH₄ emissions (g/d) 11 d after dosing started, which persisted for over 2 mo (9.2% CH₄ reduction). In their study, a delivery rate of 320 mg of monensin per day from the slow-release capsule resulted in a calculated dose of 30 to 35 mg of monensin/kg of DMI, based on measured intakes ranging from 9.1 to 10.9 kg of DM/cow per day. Operating at lower doses of 10.8 to 14.5 mg of monensin/kg of DMI, Waghorn et al. (2008) and Grainger et al. (2008) did not find any effect of monensin on CH₄ emissions for cows on pasture-based diets.

The primary objective of our research was to determine the effect of a high dose of monensin supplementation on enteric CH₄ emissions of dairy cows offered a ryegrass pasture diet supplemented with grain. To maximize our chances of detecting any effect of monensin on CH₄ emissions, 2 methods for measuring CH₄ (respiration chamber and SF₆ tracer) were used. An additional objective was to evaluate effects on milk production and rumen function, because a commensurate improvement in milk production could lead to adoption of monensin as a profitable strategy for CH₄ abatement.

MATERIALS AND METHODS

Two experiments were conducted to measure the effects of monensin on milk production and CH₄ emissions of dairy cows. The first experiment (grazing experiment) was conducted with cows grazing pasture, and a tracer gas technique was used to estimate enteric CH₄ emission of cows on pasture. The second experiment (chamber experiment) was conducted in respiratory chambers using a smaller number of cows to obtain precise measurements of CH₄ production and DMI, for cows consuming a diet similar to that con-

sumed by the cows in the grazing experiment. When cows in the chamber experiment were not undergoing CH₄ measurements, they grazed as one group with the cows from the grazing experiment. All cows were from the experimental herd at the Department of Primary Industries–Victoria, Ellinbank Research Centre (38°14' S, 145°56' E), and both experiments were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (www.nhmrc.gov.au).

Grazing Experiment

Fifty Holstein-Friesian cows, including 10 primiparous cows, were balanced for means and variances of age, BW, and calving date as described by Baird (1994), and then assigned randomly to 1 of 2 groups (control or monensin). Four cows in each group were rumen fistulated. In the monensin group, monensin was delivered for a 12-wk period at a dose of 450 mg/cow per day topdressed on the barley grain that cows were offered in a feed trough at milking times. Monensin was preweighed in individual feed sachets (target 225 mg/feed, 2 feeds per day) with 100 g of wheat pollard. Control cows received 100-g sachets of wheat pollard only. The cows were 39 ± 6 DIM when the monensin was first added to the diet. The 2 wk before starting the experiment were considered as a pretreatment period and all 50 cows were grazed on ryegrass pasture as 1 group at a daily allowance of 30 to 35 kg of DM/cow per day and offered rolled barley at 3.6 kg of DM/cow per day. During that time, cows were producing, on average (mean ± SD), 34 ± 6 kg/d of milk, 1.4 ± 0.3 kg/d of milk fat, and 1.1 ± 0.2 kg/d of milk protein, and their BW was 549 ± 60 kg.

Cows were weighed every 2 wk during the experiment and milked twice daily through a common parlor at about 0600 and 1500 h. Milk yield was measured daily for each cow using a DeLaval Alpro milk metering system (DeLaval International, Tumba, Sweden). One day every 2 wk, separate a.m. and p.m. milk samples were taken from each cow using in-line milk meters. Samples were analyzed for concentrations of fat, CP, and lactose using a near-infrared milk analyzer (model 2000, Bentley Instruments, Chaska, MN).

During the 12-wk treatment period, cows grazed a mixture of diploid and tetraploid ryegrass hybrids (*Lolium × boucheanum* syn. *L. hybridum*) at a daily allowance of 30 to 35 kg of DM/cow per day supplemented with 4.0 kg of DM/cow per day of rolled barley (*Hordeum vulgare*) grain. A mineral and vitamin premix was included in the grain at a dose rate of 175 g/cow and this provided (per day) 23 g of calcium, 10 g of magnesium, 1,120 mg of zinc, 315 mg of manganese,

Table 1. Average (mean $\pm$ SD) nutrient composition of pasture offered during wk 1 to 6 and 7 to 12 of treatment and average nutrient composition of grain offered during wk 1 to 12 to control and monensin groups in the grazing and chamber studies

Item	Pasture (Wk 1–6)	Pasture (Wk 7–12)	Grain (Wk 1–12)
Energy, MJ/kg of DM	10.6 $\pm$ 0.3	10.0 $\pm$ 0.4	13.5 $\pm$ 0.1
CP, % of DM	23.1 $\pm$ 2.3	16.9 $\pm$ 2.6	15.3 $\pm$ 2.8
Soluble protein, % of protein	41.2 $\pm$ 2.6	36.1 $\pm$ 2.4	41.9 $\pm$ 8.7
NDF, % of DM	51.1 $\pm$ 3.2	57.2 $\pm$ 2.6	17.7 $\pm$ 2.9
ADF, % of DM	28.9 $\pm$ 1.1	32.3 $\pm$ 1.6	7.6 $\pm$ 1.7
NFC, % of DM	13.6 $\pm$ 1.8	17.4 $\pm$ 2.6	NM ¹
Fat, % of DM	4.4 $\pm$ 0.5	3.4 $\pm$ 0.6	NM
Ash, % of DM	12.5 $\pm$ 1.6	9.5 $\pm$ 1.5	NM
Calcium, % of DM	0.40 $\pm$ 0.10	0.46 $\pm$ 0.07	NM
Phosphorus, % of DM	0.50 $\pm$ 0.04	0.35 $\pm$ 0.04	NM
Magnesium, % of DM	0.27 $\pm$ 0.03	0.28 $\pm$ 0.02	NM
Potassium, % of DM	3.53 $\pm$ 0.31	2.40 $\pm$ 0.95	NM

¹NM = not measured.

210 mg of copper, 4.2 mg of iodine, 10.5 mg of cobalt, 4.2 mg of selenium, 192 mg of Tylan (Elanco Animal Health, West Ryde, New South Wales, Australia), 9,100 IU of vitamin A, 4,200 IU of vitamin D₃, and 140 mg of vitamin E. Representative samples of feeds offered were collected each week, oven-dried, ground through a 0.5-mm sieve, and then analyzed by near-infrared spectroscopy by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Analytical composition of feeds is shown in Table 1.

Methane Measurements

The SF₆ tracer technique was used to estimate CH₄ production in 30 of the 50 cows (15 control cows and 15 monensin cows) in the grazing experiment. This technique involves placing a permeation tube containing ultra-pure SF₆ into the rumen before starting the experiment. The permeation tubes used in the present experiment were manufactured in June 2008 by the National Institute of Water and Atmospheric Research, New Zealand, and were filled with about 2.4 g of SF₆. The release rate of SF₆ was predetermined over a 10-wk period by weighing each permeation tube weekly; the average release of SF₆ was 5.1 $\pm$ 0.5 mg/d.

The permeation tubes containing SF₆ were placed in the rumen of the cows by mouth 1 wk before the first measurements of CH₄ were performed. Methane emissions were measured for 3 consecutive days in wk 2 of the covariate period and wk 3, 5, 8, and 12 of treatment. During each day of measurement, an evacuated, stainless steel canister (304 stainless steel tube, 75 mm diameter, 200 mm long) was placed on a harness fitted to the head of each cow. The canisters were used to draw a sample of air (initial flow rate 0.6 mL/min) from around the nasal cavity, consisting of respired and

eructated gases. Each canister (1 per cow per day) was flushed and vacuum-tested before use. Gas samples were analyzed using a method similar to Grainger et al. (2007). Briefly, a gas chromatograph (Varian CP-3800, Varian Analytical Instruments, Walnut Creek, CA) fitted with 2 columns using ultra-high-purity nitrogen (Nitrogen5.0, Coregas, Thomastown, Victoria, Australia) as the carrier gas and both columns at 80°C. Methane separation was done on an Alltech Porapak-Q 80-100 mesh column (3.6 m $\times$ 3 mm stainless steel, Grace Davison Discovery Sciences, Deerfield, IL) using a flow rate of 45 mL/min. Detection was by a flame ionization detector operating at 250°C. Separation of SF₆ was done using an 80-100 mesh Alltech molecular sieve (1.8 m $\times$ 3 mm stainless steel, Grace Davison Discovery Sciences) using a flow rate of 30 mL/min. Detection was by an electron capture detector operating at 300°C. At the end of each day, the gas chromatograph was baked out overnight using a column temperature of 200°C. Samples of ambient air were collected in canisters located east and west of the grazing cows to estimate and correct for background SF₆ and CH₄ concentrations.

The CH₄ emission (Q_{CH₄}; g/d) for each cow was calculated using the SF₆ and CH₄ mixing ratio (μ mol/mol) sampled by the canisters on the cows (C_{SF₆} and C_{CH₄}, respectively) and those used for background (C^b_{SF₆} and C^b_{CH₄}, respectively), and the predetermined SF₆ release rate (Q_{SF₆}; g/d) from the permeation tubes (Equation 1), where MW is the molecular weights of the gases:

$$Q_{CH_4} = \frac{C_{CH_4} - C_{CH_4}^b}{C_{SF_6} - C_{SF_6}^b} Q_{SF_6} \frac{MW_{CH_4}}{MW_{SF_6}} \quad [1]$$

Further details of the SF₆ tracer technique and analysis of the concentration of CH₄ and SF₆ in the canisters as

used in this study were reported previously (Grainger et al., 2007).

Ruminal Fermentation

Samples of rumen fluid were collected per fistula from 4 locations in the rumen (in the fiber mat, below the fiber mat, anterior to the fiber mat, and midway down the posterior end of the fiber mat), approximately 4 h after grain feeding in the morning, from 8 cows (4 per diet) on 2 consecutive days in wk 2 of the covariate, and wk 3, 5, 8, and 12 of treatment. The rumen fluid samples were analyzed for VFA and ammonia-N. For VFA, 4 mL of rumen fluid was pipetted into a tube containing 1 mL of 25% metaphosphoric acid and frozen for subsequent analysis, based on the method of Erwin et al. (1961), on a gas chromatograph (Varian CP-3800), using a polar column (Alltech Capillary Column, stationary phase: AT-1000, film thickness: 0.20 μ m, length 25 mm, ID: 0.25 mm) and a flame-ionization detector. For ammonia-N, 10 mL of rumen fluid was pipetted into a tube containing 10 mL of 0.1 M HCl and frozen for subsequent analysis. Before analysis, 10 mL of the preserved sample was further diluted with 20 mL of 0.1 M HCl and then centrifuged at $1,780 \times g$ for 15 min. Supernatant was collected and the pH adjusted with concentrated NaOH to pH >12.5. Ammonia concentration was measured with an ion selective electrode (uni-PROBE Ammonia ion selective electrode, part number 121700, TPS Pty. Ltd., Springwood, Queensland, Australia) previously calibrated to standard solutions of 1 and 25 ppm NH_3 .

Chamber Experiment

The experiment was conducted during spring and early summer 2008 using 10 pairs of multiparous lactating dairy cows. The 20 cows were balanced for mean and variance of age, BW, and DIM as described by Baird (1994) and then randomly allocated to 1 of 2 treatment groups (control and monensin, 10 animals each). Each monensin animal was paired with a control animal of similar BW. These pairs were arranged to form a 10-pair by 2-chamber, row-column design with equal numbers of treatments and controls in each pair and each calorimeter. Finally, the pair order and calorimeter were randomized.

Only 2 respiration chambers were available, so each pair of cows began the experiment 3 d after the preceding pair, such that each pair was on treatment for the same number of days when CH_4 measurements were made. In the monensin group, monensin was delivered for a 10-wk period at the same dose and manner as for the grazing experiment. On average, cows were 51 ± 5

DIM when monensin was topdressed to their diet, but this ranged from 31 to 71 d because of the staggered start. During the 2 wk before starting the experiment, all 20 cows were grazed as 1 group with the 50 cows from the grazing experiment and were offered a pasture allowance of 30 to 35 kg of DM/cow per day and rolled barley at 3.6 kg of DM/cow per day. During that time, cows were producing, on average (mean $\pm$ SD), 36 ± 5 kg/d of milk, 1.5 ± 0.3 kg/d of milk fat, and 1.1 ± 0.2 kg/d of milk protein, and their BW was 574 ± 60 kg. Cows had been previously trained to accept halters and headstall restraint. Cows were moved from the main herd in pairs to a metabolism facility for 1 d, and each cow was fitted with a body harness and collection apparatus to enable containment of urine and feces. This adaptation period facilitated a smooth transition into the 2 chambers where CH_4 and DMI were measured over 2 d. Each pair of cows was moved to the metabolism stalls on d 12 of their pretreatment period, and on d 1 of both wk 5 and 10 of their treatment.

Details of the operation of the chambers have been reported previously (Grainger et al., 2007) and are only briefly presented here. Within the chambers, the volume of air (41.5 m^3) was independently recycled back into each chamber after passing through filters and conditioners that maintained the air at 55% relative humidity and 17°C. A small portion of this recycled air was continuously removed and replaced with fresh air drawn in through an inlet remote from the chambers. A sample of the fresh inlet and exhausted air was pumped through a gas analyzer (Xentra 4100C1, Servomex, East Sussex, UK) and the CH_4 mixing ratio ($\mu\text{mol/mol}$), exhaust air flow rate, and relative humidity and temperature of the exhaust air were recorded every 10 s. In each 12-min period, the air stream at the common inlet and 2 exhaust ducts (chambers 1 and 2) was sampled for 4 min each. A check on the gas analyzer was conducted every week on a day the chambers were empty. After the experiment, the accuracy of the entire system was checked by releasing a known quantity of CH_4 into each chamber and then comparing against the measured chamber emission, where the latter was simply a mass balance of exhaust minus intake amounts. Calibration consisted of injecting CH_4 at a controlled rate while running the regular data collection process. Methane3.5 (Coregas) was injected at 0.350 L/min. Flow rate was controlled by a Sierra mass flow controller (model C100L.DD.2.0V1.SV1.PV2.V1.SO.CO with viton seals and seat, Sierra Instruments, Monterey, CA). Methane was injected, via a tube through the outside wall about 1.8 m above floor level, into chamber 1 and then chamber 2 for approximately 4 h each. The recovery of CH_4 was 100% for chamber 1 and 96% for chamber 2.

Cows were weighed before they entered the metabolism stalls and after they exited the chambers. They were milked twice daily using a portable milking apparatus. Fresh ryegrass-based pasture, which was harvested daily from the same paddock being grazed by the main herd, was provided twice daily to ensure *ad libitum* intake. During the experiment, cows also received 4.0 kg of DM/cow per day of rolled barley with a mineral and vitamin premix included as per the grazing experiment. All feed offered and refused was weighed daily, samples of feed and refusals were dried to determine DM content, and total daily DMI was calculated per cow. Representative samples of the pasture and grain that had been collected for analytical composition in the grazing experiment were used for the chamber experiment as all cows were offered the same feed (Table 1).

Statistical Analyses

The following variables were analyzed in the chamber experiment: CH₄ (g/d, g/kg of DMI, g/kg of milk) and DMI. Milk data are daily milk yield with a half-day lag from DMI, and DMI is total DMI (pasture + supplement). The analysis was performed using a linear mixed model (GenStat, 2009). The fixed effects in the mixed model included factorial effects for treatment × week, as well as a main effect of chamber, age, initial BW, and DIM, for which the allocation of animals to treatments had been balanced. The random effects of the mixed model included effects for each paired run of the chambers, cow, cow × week (that specifies a split-split repeated measures structure over weeks), and for cow × day within weeks (that specifies a split-split plot repeated-measures structure over days within weeks). All cows were specified as controls during the 2 pretreatment weeks.

The analysis of data measured using the SF₆ tracer was performed similarly using a linear mixed model with factorial fixed effects for monensin treatment × week, and regression terms for age, age², initial BW and DIM. The random effects were specified as date (measurement day within weeks), cow, cow × week, and cow × date. Milk production data for both experiments were reduced to cow × 2-wk averages before analysis. The analysis was performed using a linear mixed model (GenStat, 2009). The fixed effects were specified with covariates for age, age², BW, and DIM, and factorial effects of treatment (control and monensin) by 2-wk period (grazing experiment: 2-wk periods 1 to 6; and chamber experiment: 2-wk periods 0 to 5). Milk yields in the pretreatment period were measured immediately postcalving and were highly variable; therefore, these

were excluded from the analysis. A first-order autoregressive correlations structure was used for repeated measurements.

The BW data for both experiments were analyzed using a random coefficients mixed model. This compares the slope of BW versus time for each treatment by regarding the mean and slope for each animal as realizations from their joint distributions. Analyses were restricted to treatment periods: wk 0 to 10 for the chamber experiment, and wk 0 to 12 for the grazing experiment.

Rumen fluid analyses (VFA and NH₃-N) were analyzed by ANOVA with factorial treatment structure treatment × week, and factorial blocking structure cow × date. All cows were specified as controls during the pretreatment week.

Distributional assumptions of normality and constant variance were checked using graphs of residuals versus fitted values, histograms, and quantile plots.

RESULTS

Methane Emissions and DMI

Problems were encountered in the data from the grazing experiment when using the SF₆ technique to measure CH₄. After analysis by gas chromatograph of samples from the covariate period and wk 3, the gas chromatograph needed repairs that were not completed for 5 mo, which meant that gas samples for wk 5, 8, and 12 had to be stored for this period before they could be analyzed. Because of a limited supply of stainless steel containers in which the samples were originally collected, subsamples from wk 5 and 8 had to be transferred from the stainless steel collection containers into foil sachets of approximately 400 mL (made from 273-mm-wide Rewind-Code 1, Wests Packaging Services P/L, Carrum Downs, Victoria, Australia) for storage until they could be analyzed. Samples from wk 12 remained in the stainless steel containers in which they were originally collected until analysis. When analyzed, the samples stored in foil sachets were only 36% (wk 5) and 77% (wk 8) of the average of the values for CH₄ (g/kg of DMI) measured in the covariate and wk 3 periods. Samples stored in the stainless containers (wk 12) had similar values (102%) to those in the covariate and wk 3 periods and were retained in the statistical analysis. However, samples that did not store well in the foil sachets (wk 5 and 8) were discarded from the experiment.

The interaction term treatment × week was not significant ($P > 0.05$) for any of the CH₄ (grazing and chamber experiments) or DMI (chamber experiment)

Table 2. Effects of adding monensin (471 mg/cow per day) to a pasture and barley grain diet on methane emissions, measured using the SF₆ tracer technique, and DMI of lactating dairy cows measured over 3 consecutive days during wk 3 and 12 of treatment (grazing experiment; n = 15 per treatment)

Item	Control	Monensin	SED ¹	P-value ²
Methane, g/d				
Wk 3	520	534		
Wk 12	433	438		
Mean	476	486	13.1	0.78
Methane, g/kg of milk				
Wk 3	15.8	15.4		
Wk 12	15.2	15.2		
Mean	15.5	15.3	0.46	0.32
Estimated DMI, ³ kg/d				
Wk 3	25.7	25.7		
Wk 12	23.3	22.7		
Mean	25.1	23.7	NA ⁴	
Calculated methane, g/kg of DMI				
Wk 3	20.2	20.8		
Wk 12	18.6	19.3		
Mean	19.4	20.1	NA	

¹SED = standard error of differences.²The interaction term treatment × week was not significant for any of the variables so the *P*-values presented are for the main effect and were determined by using a one-tailed test.³DMI was estimated using energy algorithms from the Australian Feeding Standards (Standing Committee on Agriculture, 1990) based on BW, BW change, and milk production data over the 35-d treatment period for each treatment group.⁴NA = not applicable because DMI was estimated.

variables, so main effects of treatment are reported. Monensin had no effect on CH₄ production in the grazing experiment (g/d, g/kg milk; Table 2) or in the chamber experiment (g/d, g/kg of DMI, g/kg of milk; Table 3). In the chamber experiment, the DMI of the monensin group was 2% lower than that of the control, but this was not significant (*P* > 0.05).

Milk Production, BW Change, and Rumen Fluid Parameters

The interaction term treatment × week was not significant (*P* > 0.05) for any of the milk production variables so main effects of treatment are reported (Table 4). In both the grazing and chamber experiments,

Table 3. Effects of adding monensin (471 mg/cow per day) to a pasture and barley grain diet on methane emissions and DMI of lactating dairy cows measured over 2 d in chambers during wk 5 and 10 of treatment (chamber experiment; n = 10 per treatment)

Item	Control	Monensin	SED ¹	P-value ²
Methane, g/d				
Wk 5	429	435		
Wk 10	466	470		
Average	448	453	23.8	0.42
Methane, g/kg of DMI				
Wk 5	22.0	21.6		
Wk 10	22.5	23.7		
Average	22.2	22.6	0.87	0.67
Methane, g/kg of milk				
Wk 5	13.2	13.0		
Wk 10	16.5	16.2		
Average	14.9	14.6	0.80	0.36
DMI, kg/d				
Wk 5	20.0	20.2		
Wk 10	20.9	20.0		
Average	20.5	20.1	0.85	0.67

¹SED = standard error of differences.²The interaction term treatment × week was not significant for any of the variables so the *P*-values presented are for the main effect of monensin and were determined by using a one-tailed test.

Table 4. Effects of adding monensin (471 mg/cow per day) to a pasture and barley grain diet on milk production, milk composition, and BW change over a 12-wk treatment period for cows in grazing (n = 25 per treatment) and a 10-wk period in the chamber experiments (n = 10 per treatment)

Item	Grazing experiment				Chamber experiment			
	Control	Monensin	SED ¹	P-value ²	Control	Monensin	SED	P-value ²
Milk yield, kg/d	31.9	32.8	1.00	0.21	32.5	33.3	1.02	0.18
Fat, %	4.07	4.05	0.125	0.43	4.08	4.06	0.129	0.57
Fat yield, kg/d	1.288	1.334	0.0354	0.11	1.310	1.356	0.0379	0.12
Protein, %	3.16	3.13	0.041	0.16	3.15	3.12	0.044	0.76
Protein yield, kg/d	1.007	1.027	0.0274	0.30	1.020	1.037	0.0273	0.26
Lactose, %	5.02	5.05	0.031	0.25	5.04	5.06	0.031	0.25
Lactose yield, kg/d	1.603	1.658	0.0509	0.17	1.633	1.687	0.0514	0.15
Fat + protein, kg/d	2.295	2.361	0.0611	0.16	2.330	2.393	0.0613	0.15
BW change, kg/d	0.29	0.32	0.051	0.26	0.22	0.13	0.120	0.78

¹SED = standard error of differences.

²The interaction term treatment × week was not significant for any of the variables for both the grazing and chamber experiments, so the *P*-values presented are for the main effect of monensin and were determined by using a one-tailed test.

monensin increased yields of milk (2.5 to 2.8%), fat yield (3.5 to 3.6%), protein yield (1.7 to 2.0%), lactose yield (3.3 to 3.4%), and fat plus protein (**FP**; 2.7 to 2.9%), but none of these increases were significant ($P > 0.05$). Fat, protein, and lactose percentages in the milk were similar for control and monensin groups in both experiments. Body weight change was not affected by addition of monensin to the diet in either experiment.

No significant ($P > 0.05$) interaction terms (treatment × week) were found for any of the rumen fluid variables, so analyses of the main effects are presented (Table 5). Monensin had no effect on rumen ammonia-N and rumen VFA (acetate, propionate, butyrate, or acetate:propionate ratio).

DISCUSSION

The measured dose that the cows received was 471 mg of monensin/cow per day, which was slightly above the intended dose of 450 mg/cow per day. This makes it just outside the dose range of 350 to 450 mg/cow per day recommended for high-production, high-feed-intake herds (Elanco, 2009). In our study, monensin

had no effect ($P > 0.05$) on milk yield or FP yield. In a previous grazing study at our center, 60 cows were used to assess the effect of monensin on milk production (Grainger et al., 2008). They measured a significant 4.8% increase in FP production, and based on the LSD ($P = 0.05$) in that study, a difference in FP of 3.4% had a 50% power of detection. In the current grazing study, 50 cows were used and a difference of 4.5% in FP could have been detected with 50% power. This would have been sufficient to detect the 4.8% increase in FP production achieved in the earlier study, but not the 2.7 to 2.9% increase in FP in the current experiment. It is acknowledged that the experimental variance in the current study was higher than expected based on previous work at our center.

In our study, topdressing of monensin did not, however, result in a decrease in CH₄ emissions, and this was consistent with both methods (chamber or SF₆ tracer) used to measure emissions. Monensin also showed no tendency to decrease CH₄ emissions. The experimental design was sensitive and achieved a 50% power of detection, based on the LSD values, for differences of 6.7% (chamber method) or 4.6% (SF₆ tracer method).

Table 5. Effects of adding monensin (471 mg/cow per day) to a pasture and barley grain diet on rumen fluid ammonia-N and volatile fatty acids (VFA) (n = 4 per diet); rumen fluid was taken at 4 h after feeding

Item	Control	Monensin	SED ¹	P-value ²
Ammonia-N, mg/L	202	218	32.4	0.32
Rumen VFA				
Acetate, mmol/L	55.5	57.9	10.67	0.41
Propionate, mmol/L	19.3	23.3	4.14	0.15
Butyrate, mmol/L	11.0	11.4	2.15	0.43
Acetate:propionate ratio	2.86	2.62	0.175	0.09

¹SED = standard error of differences.

²The interaction term treatment × week was not significant for any of the variables so the *P*-values presented are for the main effect of monensin and were determined by using a one-tailed test.

Beauchemin et al. (2008) reviewed data for the effect of monensin on CH₄ emissions and concluded that the effect may be dose-dependent. In our study, the lack of effect of monensin on CH₄ emissions agrees with other recently published studies with cows consuming pasture (Waghorn et al., 2008) and pasture plus grain (Grainger et al., 2008). However, in those studies, the monensin dose was low (11 to 13 mg/kg of DMI), whereas in our study the dose was much higher (20 to 23 mg/kg of DMI) and just above the recommended dose rate of 450 mg/cow per day (Elanco, 2009). The method of delivery of monensin to the cow was also different, as Waghorn et al. (2008) and Grainger et al. (2007) used a controlled-release capsule, whereas our experiment provided monensin topdressed on the grain fed to the cows twice daily at milking time. Our experiment and the other 2 experiments cited above do not support the contention by Beauchemin et al. (2008) that the effect of monensin on CH₄ emissions may be dose dependent, at least for pasture-based diets. This assumes that the method of delivery of monensin (controlled-release capsule or topdressing) is not having any effect on CH₄ emissions. The findings of the 3 experiments cited above are consistent with those from an earlier experiment in which controlled-release capsules releasing 250 mg/d of monensin into the rumen of lactating cows grazing white clover-perennial ryegrass pasture had no significant effect on the rate of CH₄ production or on the ratio of CH₄:CO₂ in fermentation gas (Moate et al., 1997).

In contrast, Van Vugt et al. (2005) found that for 30 lactating cows in their seventh month of lactation offered cut pasture fed indoors, CH₄ emissions (g/kg of DMI) significantly decreased by 9.5% after insertion of a controlled-release monensin capsule. Methane emissions were measured using the SF₆ tracer technique. The release rate of the monensin capsule in the study of Van Vugt et al. (2005) was not measured, but was assumed to be 320 mg/d as per the manufacturer's specifications. With a measured DMI of 10.8 kg/d, the monensin addition to the diet would have been 30 mg/kg of DMI in that study. Fat plus protein yield (0.46 kg/d), as well as DMI, was low and not affected by monensin.

Once again, a key difference between the experiment of Van Vugt et al. (2005) and our experiment was the method of delivery of monensin to the cow. Van Vugt et al. (2005) used a controlled-release capsule, whereas in our experiment the monensin was topdressed on the grain fed to cows twice daily at milking time. Duffield et al. (2008b) hypothesized that sudden concentrated delivery of monensin via topdressing a grain supplement is different from monensin consistently delivered via a controlled-release capsule. It affected metabolic

outcomes (Duffield et al., 2008a) and enhanced protein yield response, possibly via effects on rumen bacteria and availability of specific substrates such as starches, sugars, and peptides; this is an area requiring further research (Duffield et al., 2008b). The method of delivery could also affect CH₄ emissions, but it might be expected that 2 sudden concentrated doses per day would have a greater impact on archaea methanogens, and hence reduce CH₄ emissions more than a controlled release of monensin via a controlled-release capsule. However, delivery of monensin at a high dose via the controlled-release capsule reduced CH₄ emissions (Van Vugt et al., 2005), but topdressing of monensin at a high dose in our experiment did not affect CH₄ emissions.

Monensin increases the availability of glucose precursors by decreasing acetate to propionate ratio in the rumen (Schelling, 1984). Our VFA data tended to support this with a decrease of 8.4% in the acetate to propionate ratio ($P = 0.09$).

Johnson and Johnson (1995), reviewing ionophore addition to forage and grain-based diets, concluded that any decrease in CH₄ is short-lived, that CH₄ returns to normal levels after 2 wk, and that this was due to the ability of the ruminal microflora to adapt to feed additives. They further commented that the reduction in CH₄ production was likely related to the reduction in DMI and not to a direct effect on methanogenesis. In a more recent review by Beauchemin et al. (2008), when monensin was added (24 to 33 mg/kg of DMI) to forage and grain-based diets for beef and dairy cattle, it reduced CH₄ in the short-term (2 to 4 wk), but this effect did not persist. These results were in agreement with the earlier conclusion by Johnson and Johnson (1995). An exception was the study by Odongo et al. (2007), who measured a lower CH₄ production over a 6-mo period with dairy cattle offered a TMR diet, illustrating that adaption to ionophores does not always occur.

In their meta-analysis of monensin experiments, Duffield et al. (2008b) detected a reduction in DMI of 2.3% when monensin was added to the diet or directly into the rumen via a controlled-release capsule. In our experiment, the decrease in DMI was 2%, but it was not significant. Assuming a fixed value for CH₄ (g/kg of DMI), a 2.3% reduction in DMI would result in a 2.3% reduction in CH₄ (g/d). Such a small effect would be difficult to detect because the sensitivity of detection of treatment differences due to monensin addition was about 5% in our experiment and it would be difficult to improve on this level of detection.

Tedeschi et al. (2003) proposed that, even in the case of microbial adaption, ionophores are an effective CH₄ mitigation practice because of their potential to improve feed conversion efficiency and consequently to reduce

CH₄ per kilogram of meat or milk. In our experiment we measured only small (1.3 to 2.0%), nonsignificant reductions in CH₄ (g/kg of milk) and so our data, for pasture diets supplemented with grain, do not support the premise of Tedeschi et al. (2003) that ionophores are an effective CH₄ mitigation practice.

CONCLUSIONS

Monensin topdressed on grain twice daily at a high dose of 471 mg/d for cows offered ryegrass pasture supplemented with grain did not improve milk production of dairy cows, in line with expectations based on previous studies. The dose of monensin used was slightly higher than the highest recommended dose (471 vs. 450 mg/cow per day) for high-production, high-feed-intake herds, but no short- or long-term reductions in enteric CH₄ emissions were noted, per day, per kilogram of DMI, or per kilogram of milk. We conclude that monensin cannot be promoted as a viable CH₄ mitigation strategy for dairy cows grazing pasture supplemented with grain.

ACKNOWLEDGMENTS

We thank P. Moate (Department of Primary Industries, Ellinbank, Victoria, Australia) for constructive comments on the manuscript. This research was funded by The Department of Primary Industries–Victoria and the Ecologically Sustainable Action Statement fund.

REFERENCES

- Baird, D. B. 1994. The design of experiments with covariates. PhD Thesis. University of Otago, New Zealand.
- 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.
- Duffield, T. F., A. R. Rabiee, and I. J. Lean. 2008a. A meta-analysis of the impact of monensin in lactating dairy cattle. Part 1. Metabolic effects. *J. Dairy Sci.* 91:1334–1346.
- Duffield, T. F., A. R. Rabiee, and I. J. Lean. 2008b. A meta-analysis of the impact of monensin in lactating dairy cattle. Part 2. Production effects. *J. Dairy Sci.* 91:1347–1360.
- Elanco. 2009. http://www.elanco.com.au/dairy_cattle/rumensin_100_premix/index.html Accessed Dec. 4, 2009.
- Erwin, E. S., G. J. Marco, and E. M. Emery. 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. *J. Dairy Sci.* 44:1768–1771.
- GenStat. 2009. GenStat. 12th ed. VSN International Ltd., Hemel Hempstead, UK.
- Grainger, C., M. J. Auldist, T. Clarke, K. A. Beauchemin, S. M. McGinn, M. C. Hannah, R. J. Eckard, and L. B. Lowe. 2008. Use of monensin controlled-release capsules to reduce methane emissions and improve milk production of dairy cows offered pasture supplemented with grain. *J. Dairy Sci.* 91:1159–1165.
- Grainger, C., T. Clarke, S. M. McGinn, M. J. Auldist, K. A. Beauchemin, M. C. Hannah, G. C. Waghorn, H. Clark, and R. J. Eckard. 2007. Methane emissions from dairy cows measured using the sulfur hexafluoride (SF₆) tracer and chamber techniques. *J. Dairy Sci.* 90:2755–2766.
- Guan, H., K. M. Wittenberg, K. H. Ominski, and D. O. Krause. 2006. Efficacy of ionophores in cattle diets for mitigation of enteric methane. *J. Anim. Sci.* 84:1896–1906.
- Johnson, K. A., and D. E. Johnson. 1995. Methane emissions from cattle. *J. Anim. Sci.* 73:2483–2492.
- Lowe, L. B., G. J. Ball, V. R. Carruthers, R. C. Dobos, G. A. Lynch, P. J. Moate, P. R. Poole, and S. C. Valentine. 1991. Monensin controlled-release capsule for control of bloat in pastured dairy cows. *Aust. Vet. J.* 68:17–20.
- Moate, P. J., T. Clarke, L. H. Davis, and R. H. Laby. 1997. Rumen gases and bloat in grazing dairy cows. *J. Agric. Sci. (Camb.)* 129:459–469.
- Odongo, N. E., R. Bagg, G. Vessie, P. Dick, M. M. Or-Rashid, S. E. Hook, J. T. Gray, E. Kebeab, J. France, and B. W. McBride. 2007. Long-term effects of feeding monensin on methane production in lactating dairy cows. *J. Dairy Sci.* 90:1781–1788.
- Sauer, F. D., V. Fellner, R. Kinsman, J. K. G. Kramer, H. A. Jackson, A. J. Lee, and S. Chen. 1998. Methane output and lactation response in Holstein cattle with monensin or unsaturated fat added to the diet. *J. Anim. Sci.* 76:906–914.
- Schelling, G. T. 1984. Monensin mode of action in the rumen. *J. Anim. Sci.* 58:1518–1527.
- Smith, P., D. Martino, Z. Cai, D. Gwary, H. Janzen, P. Kumar, B. McCarl, S. Ogle, F. O'Mara, C. Rice, B. Scholes, and O. Sirotenko. 2007. Agriculture. In *Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. B. Metz, O. R. Davidson, P. R. Bosch, R. Dave, and L. A. Meyer, ed. Cambridge University Press, Cambridge, UK.
- Standing Committee on Agriculture. 1990. *Feeding Standards for Australian Livestock: Ruminants*. CSIRO, Clayton South, Victoria, Australia.
- Tedeschi, L. O., D. G. Fox, and T. P. Tytlutski. 2003. Potential environmental benefits of ionophores in ruminant diets. *J. Environ. Qual.* 32:1591–1602.
- Van Vugt, S. J., G. C. Waghorn, D. A. Clark, and S. L. Woodward. 2005. Impact of monensin on methane production and performance of cows fed forage diets. *Proc. N.Z. Soc. Anim. Prod.* 65:362–366.
- Waghorn, G. C., H. Clark, V. Taufa, and A. Cavanagh. 2008. Monensin controlled-release capsules for methane mitigation in pasture-fed dairy cows. *Aust. J. Exp. Agric.* 48:65–68.