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Impact of monensin on methane production and performance of cows fed forage diets

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ABSTRACT

Monensin is an ionophore used to increase the efficiency of feed utilisation when grain-based rations are fed to beef cattle, but it can also lower energy losses to methane. A series of feeding experiments has been undertaken to measure the impact of monensin on methane production by dairy cows fed ryegrass-dominant pastures alone or with white clover, or maize silage. Methane production measured from 15 sets of identical twin cows fed indoors was reduced by 9%, from 16.9 to 15.3 g/kg dry matter (DM; $P < 0.01$), and remained 10% lower ($P < 0.05$) than Controls 72 days after the monensin capsule was given. When pasture was substituted with maize silage at 12, 24 and 36% of DM intake, monensin reduced methane (g/kg DM) by 1, 6.5 and 10.5 % respectively ($P = 0.05$). However, methane emissions were not affected in cows fed pasture with white clover at 15, 30 or 60% of DM intake. Although monensin provides an important opportunity for methane reduction, the data demonstrate a need to understand reasons for variable responses to treatments under differing nutritional regimes. The added benefits of improved feed efficiency and reduced bloat suggest further investigation of monensin for forage fed ruminants is warranted.

Key words: monensin; dairy cows; methane; pasture; white clover; maize silage.

INTRODUCTION

Microbial digestion of pasture by ruminant microflora yields metabolites able to be used for synthesis of milk constituents, as well as carbon dioxide and methane. The types of metabolites arising from fermentation (including acetic, propionic and butyric acids) are affected by diet composition and the microflora, especially bacterial species and the protozoa. Monensin (Rumensin ®) is an ionophore that is able to alter the composition of the microflora, influence the proportions of metabolites available for absorption (Nagaraja *et al*., 1997) and reduce the proportion of feed energy (gross energy; GE) lost to methane (Hegarty, 1999). Methane accounts for 37% of New Zealand's greenhouse gas (GHG) emissions, mainly from ruminant digestion (O'Hara *et al*., 2003); hence investigation of the capacity for monensin to mitigate methane production from dairy cows is justified.

Most studies with cattle involving monensin have been undertaken using grain-based diets (Goodrich *et al*., 1984; Najaraja, 1995) and treatment (typically 200-300 mg/day for adult cattle) increases the proportion of propionate at the expense of acetate. Monensin may also reduce feed intake whilst maintaining or increasing animal performance (Schelling, 1984). Monensin reduces the likelihood of legume bloat in cattle (Bergen & Bates, 1984) and is used in the Australasian dairy industry for this purpose (Lowe *et al*., 1991; Lean & Wade 1997). Monensin reduces methane from anaerobic fermentation (Van Nevel & Demeyer, 1996) and can lower the methane emissions from sheep and cattle fed diets containing concentrates (Johnson & Johnson 1995; Van Nevel & Demeyer, 1996) but there remains debate about the persistence of these effects (Johnson *et al.,* 1994; Fellner, 1997; Mathison *et al*., 1998).

Monensin is toxic to many bacteria (especially Gram-positive) as well as protozoa and fungi (Russell & Strobel, 1989; McGuffey *et al*., 2001; Ipharraguerre & Clark, 2003) and increased cow productivity is unlikely to occur if there is excessive suppression of the microflora. Most evaluations of monensin have involved cattle fed diets containing grain, and in these instances there are clear benefits for the efficiency of feed utilisation. However, benefits of monensin for improving the efficiency of feed utilisation by cattle fed forages without concentrates are equivocal and based on relatively few data (e.g. Goodrich *et al*., 1984; Lean & Wade, 1997). If monensin was able to consistently improve the efficiency of feed utilisation in cattle, then a persistent reduction in the energy loss to methane could be anticipated.

The research presented here evaluates effects of monensin on methane production by dairy cows fed ryegrass-based pasture or pasture with increasing proportions of either white clover or maize silage. Data presented here focus on effects of monensin, with other aspects of maize silage supplementation for dairy cow performance presented by Waugh *et al.* (2005).

METHODS

Two trials, each consisting of two experiments, were undertaken with two groups of lactating cows fed pasture (Experiments 1 and 2) or pasture plus forage supplements described by Lee *et al*. (2004), (Experiment 3) and Waugh *et al.* (2005); (Experiment 4). Within each group, half the animals were given an intra-ruminal Rumensin® bolus (Anti-Bloat Capsule, Elanco Animal Health, Auckland, New Zealand) with an indicative release rate of 320 mg sodium monensin/day. Methane production was measured from all animals using the SF_6 marker dilution system described by Johnson *et al.* (1994). The trials were conducted in 2003 at the Dexcel research farm in Hamilton, New Zealand.

Trial 1 involved 30 identical twin multiparous cows comprising Friesian and Friesian-Jersey crossbreds, with one of each twin set given a Rumensin® bolus at the commencement of the trial in the seventh month of lactation (March 2003). In Experiment 1, the cows were fed indoors with fresh ryegrass-dominant pasture to enable individual intakes to be recorded (Calan™ gate system), but Experiment 2 was conducted outdoors two months later. Methane production was measured to determine the persistence of the monensin treatment.

Experiment 1 lasted eleven days with methane emissions, feed intakes and milk production measurements taken over the final four days. The cows were then returned to pasture and dried off before the second methane measurements (Experiment 2) 72 days after bolus insertion. These measurements were made when the cows were grazing, so the intakes were estimated using the alkane indigestible marker procedure (Dove & Mayes, 1991).

Trial 2 involved a separate group of 32 multiparous Holstein-Friesian cows in mid-lactation and fed pasture with either white clover or maize silage supplements. Cows were balanced across treatments on the basis of milk solids production, live weight and age. Half of the cows had been given Rumensin® capsules 10 days prior to the first indoor feeding period. Two experiments were carried out using the Calan™ gate feeding system, with a five-week interval separating each indoor trial. Experiment 3 involved feeding pasture with 0, 12, 24, and 36% maize silage whereas Experiment 4 comprised pasture with 0, 15, 30, and 60% fresh white clover. Experiments 3 and 4 (November- December 2003 respectively) were each of 17 days duration, with measurements of pasture and supplement dry matter intake (DMI), methane emissions, and milk production taken over the last four days.

Pasture, feeding and management

The pasture fed in all experiments was leafy, high quality and predominantly perennial ryegrass (*Lolium perenne L.*) cv Bronsyn (New Zealand Agriseeds Limited). Pastures and white clover were harvested using a drum mower, and transported to the feeding facility for weighing and feeding individual cows by 0930 h and 1630 h daily. The maize silage (Experiment 3) was good quality and transported from a nearby stack prior to the morning feed. In Experiment 2 the cows were managed as a single herd and given a new pasture break each day. Pasture mass was about 5000 kg DM/ha with an allowance of 25 kg DM/cow/day.

In Experiments 3 and 4 the proportions of pasture and either maize silage or white clover were calculated daily by rapid (microwave) DM determination of all components, confirmed later by conventional drying. This enabled the proportions of each component to be adjusted daily and achieve predetermined ratios. All cows within each treatment group were offered the same amount of forage at both morning and afternoon feeding times to ensure a daily refusal of about 15% of DM offered.

Cows were milked at 0700 h and 1500 h daily with milk volumes recorded and samples taken at each milking from all cows during the measurement periods. Samples were bulked within 24 h periods (p.m. + a.m.) for analysis of fat, protein, and lactose (Milkoscan, Foss Electric, Denmark).

Intakes

The Calan™ gate system (Experiments 1, 3 and 4) enabled an accurate measurement of DMI for individual cows so that methane production could be expressed in terms of feed eaten. Intakes of individual feed components were determined by measuring feed offered, refused and a visual observation of refusal composition enabled intakes of dietary components to be determined. Feed DM was determined in triplicate at 95°C for 36 hours (48 h for maize silage). Over the final four days of Experiments 1, 3 and 4, samples of refusals were bulked for each cow and sub-samples dried to calculate DMI of individual cows. Chemical composition of daily feed samples during the measurement periods were analysed by near infra red spectroscopy (NIRS) at Feedtech (AgResearch, Palmerston North).

Cows in Experiment 2 were dosed twice daily over the eleven-day period with alkane capsules (356 mg C_{32}). Faecal samples were taken at 0800 h and 1500 h during the final 5 days and bulked within cows. Dry matter intakes were calculated from concentrations of C_{31}, C_{32} and C_{33} alkanes in faecal and pasture samples according to Dove and Mayes (1991).

Methane production

Daily methane production was determined using the intra-ruminal SF_6 slow release technique of Johnson *et al*. (1994) described by Woodward *et al.* (2004). Briefly, a brass permeation tube (35 x 15 mm) containing SF_6, which is released at a known rate (about 3 mg/day), was given to each cow (by mouth) about ten days prior to the first methane measurement. These tubes release SF_6 for approximately 300 days, so a single tube enabled measurements during both experiments for each group of cows.

A sample of respired gas was collected adjacent to the nostrils from individual cows over 24 hour periods during the measurement period. These samples were collected into evacuated containers (collection yokes) mounted on the shoulders of the cows to avoid contact with the Calan™ gate feeding facilities. Methane and SF_6 concentrations were measured from each yoke, and background air from the barn or paddock (Experiment 2). Individual cow methane was calculated on the basis of SF_6 rate from the permeation tubes as follows:

$$Q_{CH4} = Q_{SF6} \text{ x } ([CH_4 \text{ yoke}] - [CH_4 \text{ background}]) / ([SF_6 \text{ yoke}] - [SF_6 \text{ background}])$$

Where Q_{SF6} is the calibrated rate of permeation from the SF_6 capsule.

Statistical analysis

Data from all experiments, were analysed separately using residual maximum likelihood (REML) with GenStat 7.1, and presented as means with standard error of difference (SED). In Experiments 1 and 2, twin set was included as a random effect in the analysis. In Experiment 3, pre-treatment milk yields and composition were included in the analysis as a covariate, but not in Experiment 4 as the monensin was already in place at the start of this experiment.

RESULTS

The pasture fed in all experiments comprised 80-88% ryegrass with minimal weeds, 1-12% white clover and 3-14% dead matter. Pasture DM concentrations were lowest in the first two experiments and neutral detergent fibre (NDF) concentrations ranged from 40% to 55% of the DM (Table 1). All pastures contained more than 18% crude protein (CP) in the DM, but low concentrations in maize silage (7.6%) resulted in only 15.8% CP in the DM of the high silage treatment (Waugh *et al.,* 2005). In contrast, the high CP concentration in white clover resulted in dietary CP concentrations up to 24.1% of the DM (Lee *et al.,* 2004). Despite this range, the overall composition of diets fed to cattle in these experiments met cow requirements and the pasture component was typical of New Zealand dairy rations (Holmes *et al*., 2002).

TABLE 1: Composition of pasture fed in Experiments 1-4 and of maize silage and white clover fed in Experiments 3 and 4 respectively. Data are means of samples taken over the 4 day measurement period and are g/100 g DM unless indicated.

Experi-ment	Pasture 1	2	3	4	Maize silage	White clover
DM (%)	13.4	12.4	21.2	19.7	32.5	14.3
CP	22.5	21.3	21.5	18.9	7.6	27.2
NDF	55.1	49.7	39.6	47.8	42.7	27.6
ADF	30.9	27.1	20.6	25.7	25.7	20.8
Sol. CHO	4.1	7.9	14.1	11.6	33.1	12.8
Ash	11.6	10.7	9.6	9.5	4.8	11.2
ME (MJ /kg DM)	9.8	10.6	12.1	11.1	10.6	11.8

Abbreviations: DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; Sol. CHO, soluble carbohydrate; ME, metabolisable energy

The monensin slow release capsules given to cows in Trial 1 resulted in a significant reduction in methane compared with control animals in Experiment 1 (P = 0.005) and this reduction persisted two months later in Experiment 2 (P = 0.022; Table 2). Gross methane emissions were reduced by 12% in Experiment 1 and 9.3% in Experiment 2. The reduced methane production was achieved without a decrease in DMI when monensin was given in Experiment 1 (P = 0.76). A small reduction in DMI with monensin treatment may have occurred in Experiment 2 (P = 0.045; Table 2), but recent evaluations of the alkane marker system used to estimate feed intakes of grazing animals (Waghorn *et al*., 2004) have cast doubts on the ability of the technique to detect small differences in intakes. The reduction in methane emissions attributed to monensin occurred despite an increase in the percentage of GE lost to methane by control cows from 5.23 during lactation to 8.00 in dry cows for the respective experiments.

In Experiment 3, the monensin reduced gross methane emission from cows given pasture with maize silage (P = 0.023). However, there were no effects of monensin treatment in Experiment 4 when white clover was fed with pasture (Table 3). Monensin did not affect DMI or milksolids production in either experiment, but the interaction between diet and monensin when maize silage was fed prevented increases in methane/milk solids yields (Waugh *et al.*, 2005) that occurred in the control cows (P = 0.009).

TABLE 2: Intake, milksolids (MS) and methane production of identical twin cattle fed pasture in late lactation (Experiment 1) and two months after monensin (Mon) slow release devices were given (Experiment 2). All data are treatment means expressed on a daily basis unless indicated.

	Control	Mon	SED	P
Experiment 1				
LW (kg)	465.9	456	6.36	0.169
DMI (kg)	10.9	10.8	0.33	0.76
MS (kg)	0.46	0.45	0.03	0.83
CH_4 (g)	179.2	157.6	6.6	0.005
g CH_4/kg DMI	16.9	15.3	0.6	0.009
g CH_4/kg MS	419.9	375	21.2	0.050
CH_4 (% GE)	5.23	4.64	0.19	0.009
Experiment 2				
LW (kg)	523	506	6.1	0.017
DMI (kg)	9.7	9.1	0.25	0.045
CH_4 (g)	245.6	223	8.88	0.022
g CH_4/kg DMI	25.5	24.8	1.03	0.481
CH_4 (% GE)	8.00	7.73	0.32	0.481

Abbreviations: CH_4, methane; DMI, dry matter intake; MS, milk solids; GE, gross energy; LW, live weight

Incremental additions of maize silage to pasture also resulted in a significant increase in methane/kg DMI from 16.3 to 19.0 (P = 0.006) but the cows receiving monensin maintained a relatively constant level of emissions (Figure 1). There were no effects of monensin with additional increments of white clover (Experiment 4) and the cows fed pasture alone in Experiments 3 and 4 did not demonstrate reduced methane emissions with the monensin treatment (Figure 1).

TABLE 3: Monensin (Mon) effects on cow intakes, milksolids, milk composition and methane production when pasture was supplemented with either maize silage (Experiment 3) or white clover (Experiment 4). All data are treatment means expressed on a daily basis unless indicated.

	Control	Mon	SED	P
Maize silage (Experiment 3)				
DMI (kg)	19.0	18.3	0.38	0.065
MS (kg)	1.62	1.59	0.05	0.562
Milk fat (%)	4.06	3.96	0.14	0.487
Milk prot. (%)	3.30	3.25	0.04	0.122
CH_4 (g)	333.0	309	10.1	0.023
g CH_4 /kg DMI	17.54	16.9	Int	Int
g CH_4 /kg MS	209.6	195	Int	Int
CH_4 (% GE)	5.50	5.29	Int	Int
White clover (Experiment 4)				
DMI (kg)	18.4	17.7	0.41	0.071
MS (kg)	1.41	1.42	0.05	0.947
Milk fat (%)	4.35	4.43	0.19	0.700
Milk prot. (%)	3.20	3.17	0.07	0.696
CH_4 (g)	350.2	356	4.7	0.506
g CH_4 /kg DMI	19.2	20.5	0.78	0.109
g CH_4 /kg MS	250.1	255	11.6	0.632
CH_4 (% GE)	6.02	6.41	0.25	0.109

Abbreviations: DMI, dry matter intake; MS, milk solids; GE, gross energy; prot., protein; Int – there was a diet by monensin treatment interaction

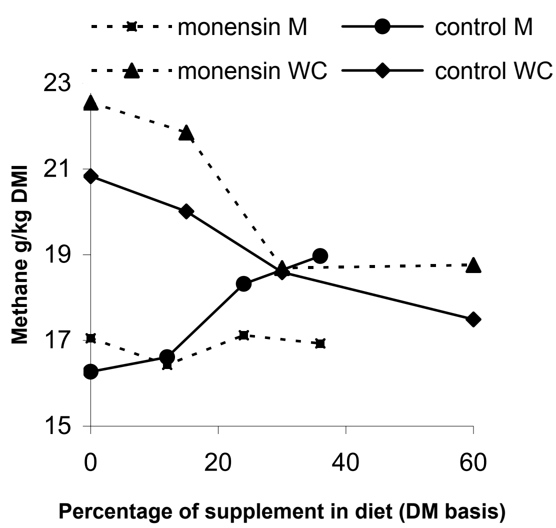
DISCUSSION

The principal findings from these experiments were that monensin can reduce methane production (per unit DMI) from rumen digestion of pasture-based diets and this reduction may persist for at least two months. However effects of monensin on methanogenesis were inconsistent and affected by diet as well as other undefined factors. Providing monensin alters the microflora and products of digestion, methane production will be reduced, but this did occur with some of the experiments reported here.

In all assessments of methane mitigation, accurate measurement of cow DMI will be central to successful determination of monensin effects. Treatment effects were small in the current study and without accurate DMI measurements, results could otherwise have been interpreted as a response to reductions in intake.

When maize silage was fed with pasture, monensin prevented the increase in methane emissions/kg DMI that occurred in control cows. Maize silage fed at 12, 24 and 36% of DMI would have increased dietary readily fermentable carbohydrate concentrations from about 14 to 16, 19 and 21% respectively. The increased proportion of propionate, characteristic of grain-based diets, would have been facilitated by monensin effects on the microflora, resulting in a reduced methane production. Cows given maize silage may have received additional benefits from the monensin treatment through reduced rumen proteolysis (Ruiz *et al*., 2001) and improved amino acid nutrition, despite a lower dietary CP concentration as the proportion of maize silage in the diet increased. These are important benefits for maize silage supplementation of pasture, because incremental additions of maize silage did not increase methane emissions/kg milksolids.

FIGURE 1: Methane production expressed in terms of dietary dry matter intake (DMI) from cows fed pasture with increasing proportions of either white clover (WC) or maize silage (M) with or without monensin.



The main question arising from these measurements concerns the failure of monensin to affect methane emissions from cows fed white clover with pasture. There are two potential reasons for a lack of response; either the monensin was inactive or the dietary composition did not enable an increased propionate production in response to suppression of Gram-positive bacteria. The effectiveness of monensin can be compromised by high intra-ruminal potassium concentrations (Dawson & Boling, 1987) that may have exceeded 3% of the DM in both white clover and pastures, compared with < 1.5% in maize silage (Holmes *et al*., 2002). However methane production was suppressed when the cows were fed pasture in Experiments 1 and 2, which does not support a potassium inhibition. The daily administration of detergent for bloat prevention to cows given white clover may also have compromised the activity of the lipid-soluble monensin, but there do not appear to be experimental data to either support or refute this possibility.

The results presented here do suggest potential benefits for lowering methane production when monensin is given to lactating cows fed pasture or pasture-dominant diets, but findings were inconsistent. In this respect, the results are similar to most published information concerning monensin effects on cattle fed forage diets — that benefits to performance are inconsistent. It is most important that future research includes accurate measurement of intake and rumen parameters (volatile fatty acid proportions, microbial growth and nitrogen kinetics) to identify underlying causes of the variable effects on methanogenesis. It is pointless continuing to measure effects of treatments, knowing outcomes will be variable, without attempting to understand the physiological bases for the responses.

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