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Anti-methanogenic effects of monensin in dairy and beef cattle: A meta-analysis

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ABSTRACT

Monensin is a widely used feed additive with the potential to minimize methane (CH_4) emissions from cattle. Several studies have investigated the effects of monensin on CH_4, but findings have been inconsistent. The objective of the present study was to conduct meta-analyses to quantitatively summarize the effect of monensin on CH_4 production (g/d) and the percentage of dietary gross energy lost as CH_4 (Y_m) in dairy cows and beef steers. Data from 22 controlled studies were used. Heterogeneity of the monensin effects were estimated using random effect models. Due to significant heterogeneity (>68%) in both dairy and beef studies, the random effect models were then extended to mixed effect models by including fixed effects of DMI, dietary nutrient contents, monensin dose, and length of monensin treatment period. Monensin reduced Y_m from 5.97 to 5.43% and diets with greater neutral detergent fiber contents (g/kg of dry matter) tended to enhance the monensin effect on CH_4 in beef steers. When adjusted for the neutral detergent fiber effect, monensin supplementation [average 32 mg/kg of dry matter intake (DMI)] reduced CH_4 emissions from beef steers by 19 ± 4 g/d. Dietary ether extract content and DMI had a positive and a negative effect on monensin in dairy cows, respectively. When adjusted for these 2 effects in the final mixed-effect model, monensin feeding (average 21 mg/kg of DMI) was associated with a 6 ± 3 g/d reduction in CH_4 emissions in dairy cows. When analyzed across dairy and beef cattle studies, DMI or monensin dose (mg/kg of DMI) tended to decrease or increase the effect of monensin in reducing methane emissions, respectively. Methane mitigation effects of monensin in dairy cows (–12 ± 6 g/d) and beef steers (–14 ± 6 g/d) became similar when adjusted for the monensin dose differences between dairy cow and beef steer studies. When adjusted for DMI differences, monensin reduced Y_m in dairy cows (–0.23 ± 0.14) and beef steers (–0.33 ± 0.16). Monensin treatment period length did not significantly modify the monensin effects in dairy cow or beef steer studies. Overall, monensin had stronger anti-methanogenic effects in beef steers than dairy cows, but the effects in dairy cows could potentially be improved by dietary composition modifications and increasing the monensin dose.

Key words: dairy and beef cattle, meta-analysis, methane, monensin

INTRODUCTION

Methane (CH_4) is a greenhouse gas with a global warming potential 25 times greater than CO_2 over a 100-yr period (IPCC, 2007). Agriculture produces approximately 50% of overall anthropogenic CH_4 emissions globally (IPCC, 2007), and the largest biogenic source of CH_4 is enteric fermentation from ruminants (US EPA, 2006). Besides the environmental concerns, enteric CH_4 production negatively affects energy efficiency in cattle. Up to 11% of gross energy (**GE**) in cattle feed can be lost via eructated CH_4 (Moraes et al., 2012). Two mechanisms primarily control enteric methane production in cattle: (1) the amount of dietary carbohydrates fermented in the rumen and (2) stoichiometry of VFA produced in the rumen, which affects the hydrogen availability for methane production (Johnson and Johnson, 1995; Ellis et al. 2008). Factors influencing one or more of these mechanisms consequently affect methane losses from cattle.

Monensin is a carboxylic polyether ionophore, commonly used to improve efficiency of energy (Byers, 1980) and N utilization (Ruiz et al., 2001) in cattle. Feeding monensin also reduces morbidity and mortality among feedlot cattle by reducing the incidence of acute and subacute ruminal acidosis, bloat, and bovine emphysema (Callaway et al., 2003). The effect of monensin on energy efficiency is related to its ability to selectively

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inhibit gram-positive over gram-negative bacteria that reduce succinate to propionate (McGuffey et al., 2001). Increased propionate to acetate ratios (Rogers and Davis, 1982) and reduced numbers of protozoa-generating hydrogen (Russell, 1987) in the rumen have indicated the potential of using monensin as a CH_4 mitigation strategy in ruminants, particularly in intensive systems (Beauchemin et al., 2008).

Several published studies have investigated the effects of monensin on CH_4 production in cattle, but the results have been inconsistent. For example, Van Vugt et al. (2005) and Odongo et al. (2007) reported significant declines (6.5–12%) in CH_4 emissions from dairy cows fed diets supplemented with monensin, but Grainger et al. (2010) and Waghorn et al. (2008) did not find such an effect. Dry matter intake and the nutrient composition of experimental diets, monensin dose, and length of monensin treatment period may be able to explain most of the between-study variability in the monensin effect (Guan et al., 2006; Beauchemin et al., 2008; Ellis et al., 2012). Meta-analyses compare and combine treatment effects of individual studies (Viechtbauer, 2010) and can also be used to explore between-study variability or heterogeneity of the treatment effects (Duffield et al., 2008). The objective of this study was to conduct meta-analyses to quantitatively summarize the effects of monensin on CH_4 production in dairy cows and beef steers while exploring the factors that significantly explain the heterogeneity.

MATERIALS AND METHODS

Data Sources

Literature searches of the Web of Science (Thomson Reuters Science, New York, NY) and CAB Direct (CAB International, Wallingford, UK) online databases were conducted using the combination of search terms "monensin", "methane", and "cattle", or "cow". The period covered was 1970 to 2011. The search resulted in 123 references related to studies of monensin effects on enteric methane production and rumen fermentation in cattle. All 123 references were scrutinized by reading the abstract of each reference carefully. For inclusion in the database, the studies were required to include a control treatment group that did not receive monensin, to be conducted in vivo using cattle, and include measured CH_4 production as an outcome. Of the 123 references, 82 were related to in vitro studies focusing on the monensin effect in rumen fermentation and 21 were review papers. These were excluded from the database. The remaining 20 papers related to in vivo studies involving dairy and beef cattle and were selected for the database. However, another 4 papers were discarded as they did not contain any measures of sample variance or information helpful in calculating it (i.e., test statistics and *P*-values). Two conference papers were discarded, as they were duplicate publications of the same study. Another paper was removed, as the experiments did not have a control treatment group. The final data set contained 22 studies from the remaining 13 papers. A summary description of the selected studies is given in Table 1.

Data Extraction and Calculations

Mean CH_4 production in control and monensin treatment groups was the response variable of primary interest. Additionally, the following variables were considered: (1) ingredient and nutrient composition of experimental diets, including GE, NDF and ADF, NFC, CP, and ether extract (**EE**) contents; (2) measured or estimated (in grazing experiments) DMI; (3) milk production of dairy cows; (4) monensin dose; (5) duration of feeding monensin; and (6) number of animals in treatment and control groups and dispersion estimates (SE or SD) of the CH_4 measurements. Any missing nutrient composition values of the experimental diet were calculated using the ingredient composition and nutritive value tables in NRC (1996, 2001). For studies repeatedly measuring CH_4, only the last CH_4 measurement and the respective treatment durations were used. For studies only reporting the least significant difference and associated *t*-statistics, the standard error of difference (**SED**) was calculated as SED = LSD/*t*. When the number of observations of both control and monensin treatments were similar (which was the case in majority of the studies), the standard error was calculated as: SE = SED/$\sqrt{2}$. If LSD values were not reported, standard errors were calculated using treatment mean difference, *P*-value for the treatment mean difference, and the number of observations.

Methane production was reported in grams per day in the majority of the papers. In some papers, it was reported in liters per animal per day. The liters per day units were converted to grams per day assuming that a mole of CH_4 weighing 16.0 g has a volume of 22.4 L. Besides CH_4 production, we were also interested in the effect of monensin on the percentage of feed GE lost as CH_4 ($\boldsymbol{Y_m}$). Mean Y_m values were available in some papers (Thornton and Owens, 1981; Van Vugt et al., 2005; Waugh et al., 2005); for the others, Y_m was calculated using data on GE content (MJ/kg of DM) in the diet, DMI, and CH_4 production (g/d), along with the fact that combustion of 1 g of CH_4 releases 55.6 kJ of energy. If diet GE content was not reported, it was calculated using Atwater energy equivalents of nutrients (Merrill and Watt, 1973). Standard errors for

Table 1. Data sources and characteristics of studies used in the meta-analyses

Reference	Country	No of studies	n[1]	Duration[2] (d)	Feed (forage to concentrate ratio)	Monensin delivery	Dose[3] (mg/kg of DMI)	CH_4 measurement method
Dairy cows								
Van Vugt et al. (2005)	New Zealand	4	16	42	Pasture plus white clover or maize silage (100:0)	CRC[4]	18–35	SF6[5]
Odongo et al. (2007)	Canada	1	12	180	TMR (60:40)	Premix	24	Hood calorimetry
Waghorn et al. (2008)	New Zealand	1	16	70		CRC	11	SF6
Grainger et al. (2008)	Australia	2	12	79	Pasture with grain (72:28)	CRC	13	SF6, respiratory chamber
Hamilton et al. (2010)	United States	1	9	60	TMR (35:65)	Premix	21	Respiratory chamber
Grainger et al. (2010)	Australia	2	13	77	Pasture with grain (80:20)	Premix	22	SF6, respiratory chamber
Beef steers								
Thornton and Owens (1981)	United States	3	5	15	TMR (20:80, 67:33, 50:50)	Premix	29–36	Respiratory chamber
Wedegaertner and Johnson (1983)	United States	1	6	33	TMR (20:80)	Premix	40	Respiratory chamber
Rumpler et al. (1986)	United States	1	3	23	TMR (20:80)	Premix	28	Respiratory chamber
O'Kelly and Spiers (1992)	Australia	2	5	52	Alfalfa hay (100:0)	Premix	33	Respiratory chamber
McGinn et al. (2004)	Canada	1	4	21	TMR (75:25)	Premix	33	Respiratory chamber
Mwenya et al. (2004)	Japan	1	4	22	TMR (20:80)	Premix	30	Respiratory chamber
Guan et al. (2006)	Canada	2	6	70	TMR (86:14, 31:69)	Premix	33	SF6

[1]Average number of animals per treatment group.
[2]Average number of days of feeding monensin.
[3]Average monensin dose.
[4]CRC = control release capsule.
[5]SF6 = sulfur hexafluoride tracer method.

the calculated Y_m were estimated using the mean difference (**MD**; MD = monensin treatment mean – control treatment mean), *P*-values of the corresponding CH_4 production MD, and the number of observations. As the DMI of each treatment group was not reported, Y_m could not be calculated for the dairy cow grazing experiment in Grainger et al. (2008) and the beef steer trial in Rumpler et al. (1986).

Statistical Analysis

Separate meta-analyses were conducted for quantifying overall antimethanogenic effects of monensin in dairy cows, beef steers, and both dairy cows and beef steers using the metafor package (version 1.6–0) in R (version 2.12.2, R Foundation for Statistical Computing, Vienna, Austria). Moreover, the effects of monensin on DMI and milk production were also analyzed. Functions in the metafor package have been validated by comparing their results with those provided by other software packages, such as metan and metareg in Stata (StataCorp, College Station, TX) and the proc mixed command in SAS (SAS Institute Inc., Cary, NC), for several data sets (Viechtbauer, 2010).

Before beginning with the meta-analyses, effect size estimates and corresponding sampling variances were obtained. The MD and the standardized mean difference (**SMD**; SMD = MD/pooled SD of the 2 groups) are useful effect size measures for continuous response variables such as CH_4 production. Standardized mean difference appropriately weights studies but is hard to interpret rationally because it is in SD units. On the other hand, MD allows effect size interpretation in the original units of the measurements. Also, considering the fact that the functions in the metafor package allow for weighting individual studies for corresponding sample variation (Viechtbauer, 2010), MD was used in meta-analysis models summarizing monensin effect size across all individual studies. Forest and funnel plots were constructed using SMD. The metafor package provides the escalc function for calculating various effect sizes including MD and SMD. It provides arguments for specifying data structure, treatment means, sampling error, sample size, and the preferred effect size measure. Relevant R codes are given in the appendix.

Models

We assumed that

$$y_i = \theta_i + e_i,$$

where y_i = the observed effect size or MD in the ith study; θ_i = corresponding true effect size of the ith

study that is unknown; and e_i = the sampling error [$e_i \sim N$ (0, sampling variance)] assumed to be known and taken as the squared standard error of the effect size. The sampling error remained fixed during estimation and, hence, served to weight the individual studies (Viechtbauer, 2010). Between-study variability (heterogeneity) of the true effects (θ_i) was also assumed to be purely random and this led to random-effect models given by

$$\theta_i = \mu + u_i,$$

where θ_i = true effect size (MD) in the ith study; μ = overall true effect size; and u_i = random deviation from the overall effect size [$u_i \sim N(0, \tau^2)$], which was unknown but estimated from data. The true effects were therefore normally distributed with mean μ and variance τ^2. If $\tau^2 = 0$, it would imply homogeneity among true effects across individual studies so that $\mu = \theta$. Heterogeneity (τ^2) was expressed as a percentage of total variability (τ^2 plus sample variance) yielding I^2 statistics.

An I^2 value greater than 50% indicates considerable heterogeneity (Rabiee et al., 2010). Hence, for response variables with $I^2 > 50\%$, the random-effect models were extended to mixed-models including fixed effects of variables having the potential to explain heterogeneity. These analyses are also called meta-regression analyses. The mixed-effect models were given by

$$\theta_i = \beta_0 + \beta_1 x_{1p} + \ldots\ldots + \beta_p x_{ip} + u_i,$$

where β_0 = overall true effect size; x_{ij} = the value of the jth explanatory variable ($j = 1, 2, \ldots, p$) for the ith study; and β_j = change in the true effect size for unit increase in the jth explanatory variable and again $u_i \sim N(0, \tau^2)$. Here, τ^2 denoted the amount of residual heterogeneity, which was not described by the explanatory variables (Viechtbauer, 2010). We used DMI, monensin dose, monensin treatment duration, and dietary NDF, NFC, and EE contents as potential explanatory variables. Values of each explanatory variable were first centered on their means and then regressed individually against MD.

Model Fitting and Model Selection

The meta-analytic models were fitted using the rma function in the metafor package. The observed effect sizes and corresponding sampling variances calculated with the escalc function were respectively supplied via the *yi* and *vi* arguments in the rma function. The random-effect models were then fitted using the REML estimation method to estimate τ^2. Moreover, the rma function estimates the I^2 statistics and tests statistical significance of τ^2 using chi-squared tests (Higgins and Thompson, 2002). The mixed-effect models were constructed by including one or more explanatory variables using the mod argument in the rma function. Effects of the explanatory variables were estimated via weighted least squares with the weights (w) equal to $w = 1/(\text{sample variance} + \text{estimated } \tau^2)$ (Viechtbauer, 2010). The metafor package does not provide functions for model selection. Hence, we first fitted models including individual explanatory variables. Full mixed-effect models carrying all explanatory variables having effects ($P < 0.10$) when fitted individually were then fitted using the maximum likelihood (**ML**) method. Multi-collinearity was considered when selecting variables for the models. For example, monensin dose (mg/kg of DMI) and DMI were not analyzed together as they were highly correlated. Reduced models were formed via stepwise elimination of one variable at a time and fitted again with the ML method. The final mixed-effect models were chosen by testing reduced models versus full models using log-likelihood ratio tests. Furthermore, models with the same number of explanatory variables were compared using log-likelihood value, Akaike information criterion, and Bayesian information criterion given by the rma function. The parameter estimates of the final model were obtained by fitting the model using the REML method. Distinct sets of multivariate mixed-effect models were tested for the monensin effects separately for dairy cows and beef steers, or across both dairy cows and beef steers. When analyzed across dairy cow and beef steer studies, the explanatory variable effects on monensin were controlled for animal group variability by including it as a fixed categorical effect in the models. Publication bias of CH_4 production measures in dairy cow or beef steer studies were assessed using Egger's regression test for funnel plot asymmetry (Viechtbauer, 2010).

RESULTS AND DISCUSSION

As Arnqvist and Wooster (1995) stated, any single study is worth little if not compared and related to other similar studies. Meta-analyses compare and combine findings from many related studies using statistical methods (Viechtbauer, 2010). The meta-analyses in this paper summarized the effects of monensin in both dairy cows and beef steers primarily related to CH_4 production (g/d) and Y_m (%). Control and monensin treatment group means and standardized mean difference estimates of respective variables are presented in Figures 1 and 2 using forest plots. The monensin effects in dairy cows were notably inconsistent, as an almost equal number of studies had positive and nega-

tive monensin effects on methane production (Figures 1A and 2A). Monensin had a more consistent effect on CH_4 mitigation in beef steers than dairy cows (Figures 1B and 2B), but the effect sizes were still variable across studies. Dairy cow diets were supplemented with relatively low monensin doses (average = 21 mg/kg of DM) for a longer period of time compared with high monensin doses in beef steers (average = 32 mg/kg of DM) fed for relatively short periods (Table 1). The beef studies more consistently used monensin in premixes and the respiratory chamber method to measure CH_4. Conversely, a considerable number of dairy studies used control release capsules to deliver monensin and the sulfur hexafluoride tracer method to measure CH_4 (Table 1). Furthermore, nutrient compositions of the experimental diet were notably variable across both dairy and beef studies (Table 2).

Effects of Monensin from Random Effect Models

Meta-analyses using random effect models assume that the studies are a random sample of the entire population of studies so that any inference can be generalized beyond the studies included (Hedges and Vevea, 1998). At an average dose of 21 mg/kg of DM (Table 2), monensin did not significantly affect the amount of CH_4 produced ($P = 0.184$) and Y_m ($P = 0.471$) in dairy cows (Table 3); in contrast, feeding monensin 32 mg/kg of DM, on average, substantially reduced ($P < 0.001$) CH_4 production and Y_m in beef steers by 19 g/d and 0.54 percentage points, respectively. These values correspond to a 15 and 9% decline from the average CH_4 production (131 g/d) and Y_m (5.97%) of steers that did not receive monensin, respectively (Table 3). The CH_4 production decline could partially be explained by the reduced DMI (−0.41 kg; $P = 0.001$) in beef steers. However, the significantly reduced Y_m, which was adjusted for the DMI difference, suggests a potential control of methanogenesis in the rumen by monensin. Sauer et al. (1998) observed significant declines in CH_4 production (17%) and ruminal acetate to propionate ratio (19%) in dairy cows 2 wk after feeding monensin. Monensin also reduced DMI ($P < 0.001$) in dairy cows by 0.48 kg/d, representing a 2.6% decline from the average of cows in control group. Consistently, a meta-analysis by Duffield et al. (2008) revealed a 2.3% DMI decline ($P = 0.001$) among dairy cows for monensin supplementation. Their meta-analysis included 77 trials, only 2 of which were used in the current analyses. Although milk and milk solids yields (kg/d) were unaffected by monensin in the present study, Duffield et al. (2008) reported significant positive effects of monensin in dairy cows. The random effect models further quantified heterogeneity of monensin effects in terms of the τ^2 and I^2 statistics. The effects of monensin were associated with significant ($P < 0.001$) between-study variability or heterogeneity in both dairy cows and beef steers (Table 3). More than 68% of the total variability of the monensin effects was due to heterogeneity in all cases ($I^2 > 68\%$).

Funnel plots were used to assess publication bias. Funnel plots in Figure 3A present SMD estimates of CH_4 production on the horizontal axis and the corresponding standard error measures on the vertical axis. A middle vertical line is drawn at the summarized SMD with a confidence interval region (region between the dotted lines; Figure 3) given by ±1.96 SE (Viechtbauer, 2010). The funnel shape occurs as the larger and more precise studies tend to be closer to the expected effect, whereas the smaller, less precise studies are more variable. Funnel plot asymmetry is indicative of publication bias and can be assessed visually or by using a statistical test, such as Egger's regression test. The Egger's regression test revealed nonsignificant funnel plot asymmetry in beef steers ($P = 0.098$; data not presented) indicating an absence of notable publication bias, but the funnel plot of dairy cows was significantly asymmetric ($P = 0.008$; data not presented). Besides publication bias, funnel plot shape can vary due to several other factors, including heterogeneity (Terrin et al., 2005). Therefore, we continued with the dairy cow analysis based on an assumption that explaining heterogeneity with mixed-effect models would improve the funnel plot shape.

Effects of Monensin and Explanatory Variables from Mixed Effect Models

Level of feed intake, type of dietary carbohydrates, and dietary lipid contents generally influence methanogenesis in ruminants (Johnson and Johnson, 1995; Ellis et al., 2007; Beauchemin et al., 2008). Monensin dose (Beauchemin et al., 2008; Ellis et al., 2012), length of monensin treatment period, and dietary forage content (Guan et al., 2006; Odongo et al., 2007) have also been shown to influence effects of monensin on CH_4 production in cattle. We chose DMI (kg/d) of control treatment, basal diet NDF, ADF, NFC, and EE contents (g/kg of DM), monensin dose (mg/kg of DMI), and length of monensin treatment period (d) as potential explanatory variables accounting for the heterogeneity associated with the monensin effects. The random effect models were extended to mixed effect models including the fixed effects of these factors. Before using in the mixed effect models, each explanatory variable was centered on its mean (Table 2). Such a rearrangement allows for interpreting the regression effects in terms of changes in a monensin effect size for a unit change in an explanatory variable from its mean.

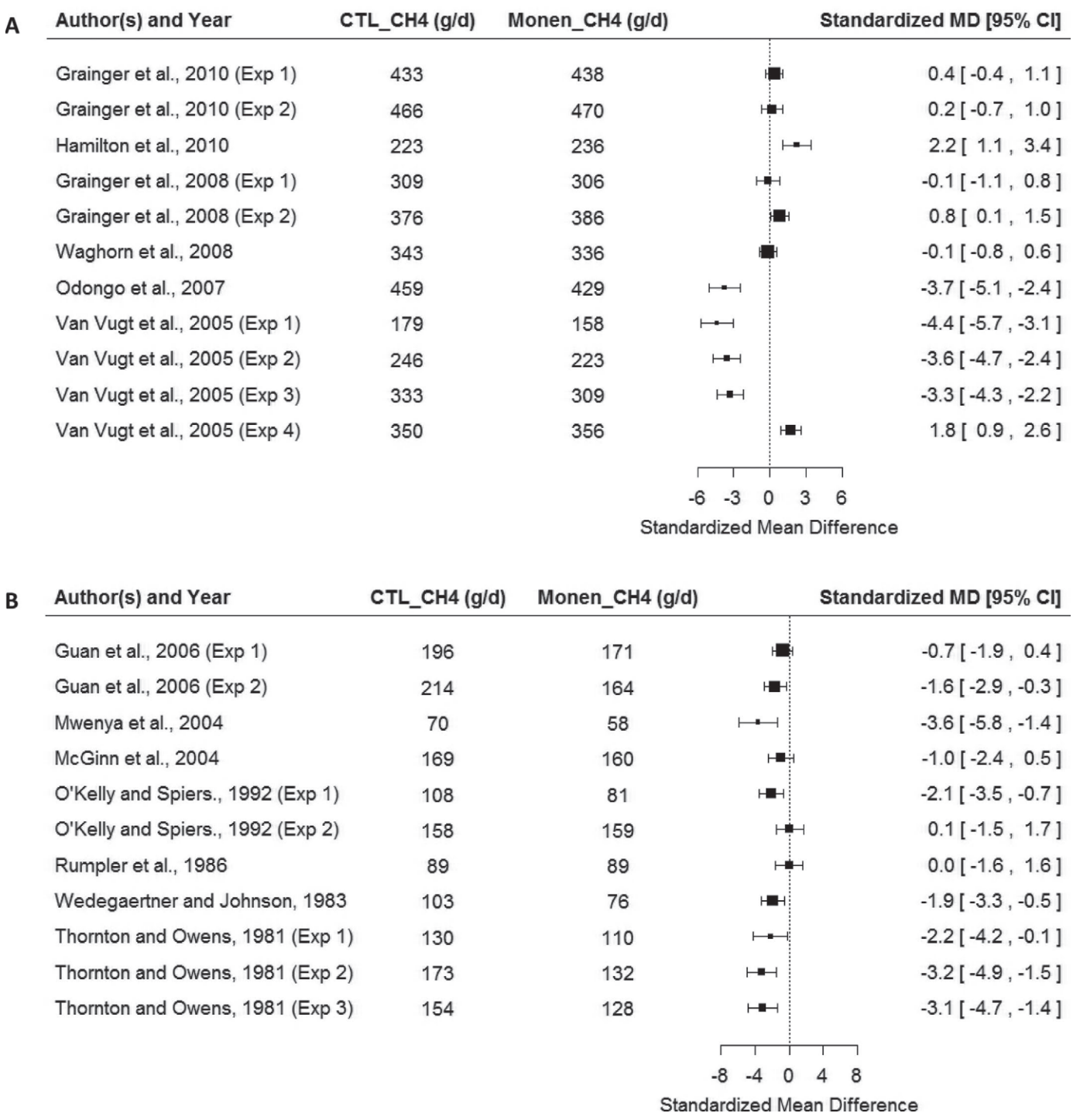

Figure 1. Forest plot showing mean methane production (g/d) in control (CTL_CH4) and monensin treatment (Monen_CH4) groups along with standardized mean difference (MD) and its 95% CI for dairy cow (A) and beef steer (B) studies. The dotted line represents a 0 standardized mean difference.

Methane Production in Dairy Cows. The final mixed effect model for dairy cows included only DMI and dietary EE content, indicating significant independent effects on CH_4 production (Table 4). The intercept of the model expresses the overall mean effect of monensin at mean DMI (18.6 kg/d) and EE content (38 g/kg of DM). When adjusted for the DMI and EE effects, monensin showed a potential ($P = 0.065$) to

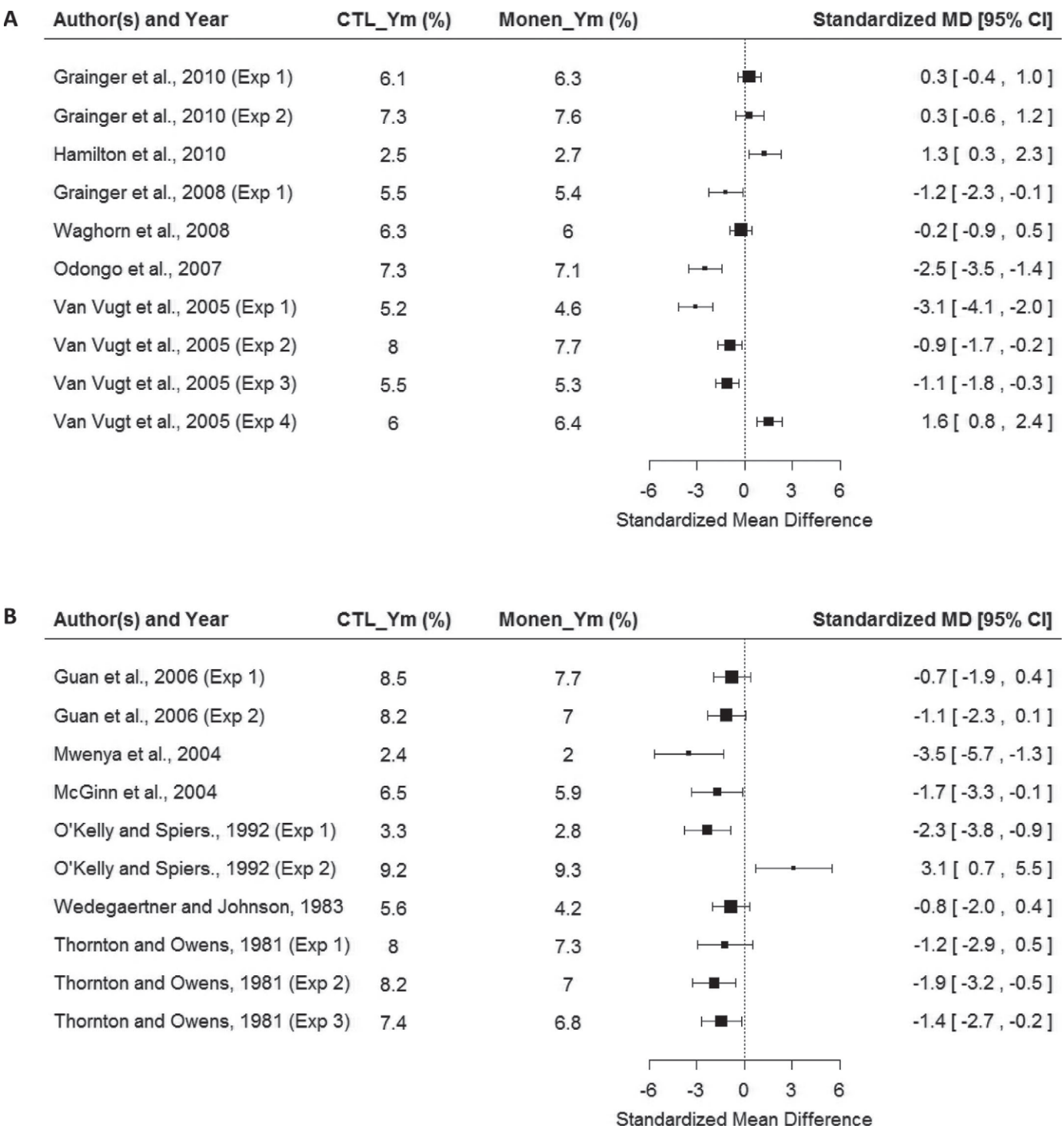

Figure 2. Forest plot showing mean dietary gross energy lost via CH_4 (Y_m, %) in control (CTL_Ym) and monensin treatment (Monen_Ym) groups along with standardized mean difference (MD) and its 95% CI for dairy cow (A) and beef steer (B) studies. The dotted line represents a 0 standardized mean difference.

reduce CH_4 production in dairy cows by 6 g/d. A unit (kg/d) increase in DMI from its mean reduced ($P = 0.020$) potential monensin-induced CH_4 mitigation in dairy cows by 1.4 g/d. In contrast, a unit (g/kg of DM) increase in dietary EE from its mean increased the monensin effect by 4.3 g/d. Addressing these effects reduced heterogeneity ($\tau^2 = 254 \pm 129$ vs. 90.6 ± 58.0) of CH_4 production measures by 64% and thereby

Table 2. Summary statistics for the explanatory variables

Variables	Dairy cows				Beef steers			
	Mean	Median	Minimum	Maximum	Mean	Median	Minimum	Maximum
DMI[1] (kg/d)	18.6	18.4	9.70	28.5	7.2	7.3	5.4	10.5
GE (MJ/kg of DM)	17.5	17.5	17.1	18.2	17.9	17.8	16.5	20.9
NDF (g/kg of DM)	442	461	290	551	379	362	232	605
NFC (g/kg of DM)	224	261	41.0	416	387	371	189	585
Ether extract (g/kg of DM)	38	38	35	43	30	27	20	42
Monensin dose (mg/kg of DMI)	21	20	11	35	32	33	28	40
Duration[2] (d)	72	70	11	180	38	23	15	84

[1]Dry matter intake of the diet supplemented with monensin.
[2]Monensin feeding duration.

improved the funnel plot shape (Figure 3). An Egger's regression test revealed nonsignificant asymmetry ($P = 0.105$) for the new funnel plot shape (Figure 3B).

Methane Production in Beef Steers. None of the explanatory variables except NDF had a tendency to affect the monensin effect on CH_4 production in beef steers, so it was included in the final model (Table 4). Dietary NDF content explained 22% of the monensin effect heterogeneity. Feeding monensin in a diet with average NDF content (379 g/kg of DM) significantly ($P < 0.001$) reduced CH_4 emissions from beef steers by 19 g/d. A unit increase in NDF content from its mean further increased monensin-induced CH_4 mitigation by 0.05 g/d ($P = 0.095$). Nonetheless, the considerable residual heterogeneity (Table 4) in both beef steers ($\tau^2 = 124 \pm 81.9$) and dairy cows ($\tau^2 = 90.6 \pm 58.0$) indicates that some variables other than the ones selected could further explain the variability of monensin effects on CH_4 production.

Y_m in Dairy Cows or Beef Steers. As with CH_4 production, DMI was positively associated ($P = 0.017$) with the monensin effect on Y_m in dairy cows (Table 4). When individually regressed, dietary NDF content tended ($P = 0.091$; data not presented) to have a negative effect on monensin in dairy cows, but the final mixed-effect model included only the DMI effect, suggesting confounded effects. The DMI alone considerably explained (44%; $P = 0.017$) heterogeneity of monensin effect on Y_m ($\tau^2 = 0.09 \pm 0.05$ vs. 0.05 ± 0.04). None of the selected explanatory variables had a significant effect on the monensin effect on Y_m in beef steers (Table 4).

Explanatory Variable Effects Across Dairy Cows and Beef Steers. With expectation of a more powerful inference on the explanatory variable effects, separate mixed effect model analyses were conducted using data from both dairy and beef trials. The random effect model analysis results revealed that monensin significantly ($P < 0.001$; Table 3) reduced CH_4 emissions across dairy cows and beef steers. The final mixed effect models included only DMI or monensin dose effect. Because of a high correlation between DMI

Table 3. Number of studies used for the analyses (N), control group averages (Average), monensin effect size, and between-study variability estimates from random-effect models[1]

Response variable	N	Average	MD[1]		Heterogeneity[2]		
			Mean ± SE	*P*-value	τ^2 (mean ± SE)	I^2	*P*-value
CH_4 production (g/d)							
Dairy cows	11	338	–7 ± 5	0.184	254 ± 129	97.2%	<0.001
Beef steers	11	131	–19 ± 4	<0.001	158 ± 93	84.2%	<0.001
Both dairy cows and beef steers	22	240	–13 ± 4	<0.001	233 ± 85	95.5%	<0.001
Dietary gross energy lost as CH_4 (Y_m, %)							
Dairy cows	10	6.87	–0.08 ± 0.11	0.471	0.09 ± 0.05	95.0%	<0.001
Beef steers	10	5.97	–0.54 ± 0.14	<0.001	0.12 ± 0.08	88.8%	<0.001
Both dairy cows and beef steers	20	6.35	–0.27 ± 0.09	0.003	0.11 ± 0.05	96.0%	<0.001
DMI (kg/d)							
Dairy cows	10	18.6	–0.48 ± 0.09	<0.001	0.04 ± 0.03	68.8%	0.002
Both dairy cows and beef steers	10	7.24	–0.41 ± 0.13	0.001	0.13 ± 0.08	91.3%	<0.001
Milk production in dairy cows (kg/d)							
Milk yield	10	20.5	0.17 ± 0.22	0.429	0.38 ± 0.22	81.3%	<0.001
Milk solids yield	10	2.58	0.01 ± 0.04	0.729	0.01 ± 0.01	94.8%	<0.001

[1]MD (mean difference) = monensin treatment mean – control group mean.
[2]τ^2 = total amount of heterogeneity; I^2 = heterogeneity as a percentage of total variability.

Table 4. Estimates of overall monensin effect (intercept), effects of explanatory variables, and total heterogeneity estimates (τ^2) from final mixed-effects models

	CH_4 production (g/d)			Dietary gross energy lost as CH_4 (Y_m, %)		
Variable[1]	Mean ± SE	*P*-value	τ^2	Mean ± SE	*P*-value	τ^2
Dairy cows						
Intercept	–6 ± 3	0.065	90.6 ± 58.0	–0.08 ± 0.09	0.383	0.054 ± 0.035
DMI (kg/d)	1.4 ± 0.6	0.020		0.04 ± 0.02	0.017	
Ether extract (g/kg of DM)	–4.3 ± 1.5	0.004				
Beef steers						
Intercept	–19 ± 4	<0.001	124 ± 81.9	ND[2]		
NDF (g/kg of DM)	–0.05 ± 0.03	0.095				
Dairy cows and beef steers						
Model I						
Intercept (beef steers)	–10 ± 6*	0.117	176 ± 52.1	–0.33 ± 0.16***	0.047	0.078 ± 0.037
Intercept (dairy cows)	–16 ± 6	0.010		–0.23 ± 0.14	0.095	0.078 ± 0.037
DMI (kg/d)	1.6 ± 0.7	0.043		0.03 ± 0.02	0.071	
Model II						
Intercept (beef steers)	–14 ± 6**	0.019	185 ± 57.9			
Intercept (dairy cows)	–12 ± 6	0.023				
Monensin (mg/kg of DMI)	–1.1 ± 0.6	0.077				

[1]The explanatory variables centered on the means.

[2]Not determined.

Intercepts were not different: *$P = 0.592$, **$P = 0.880$, ***$P = 0.720$.

and monensin dose across dairy cows and beef steers (r = –0.77; data not presented), they were not assessed together due to multi-collinearity. Basal DMI and monensin dose reduced the heterogeneity of monensin effect on CH_4 production by 25 (τ^2 = 176 vs. 233) and 21% (τ^2 = 185 vs. 233), respectively (Table 4). A unit increase in DMI (kg/d) reduced the monensin effect on CH_4 production by 1.6 g/d ($P = 0.043$), whereas a unit increase in monensin dose (mg/kg of DMI) enhanced it by 1.1 g/d ($P = 0.077$; Table 4). Moreover, when

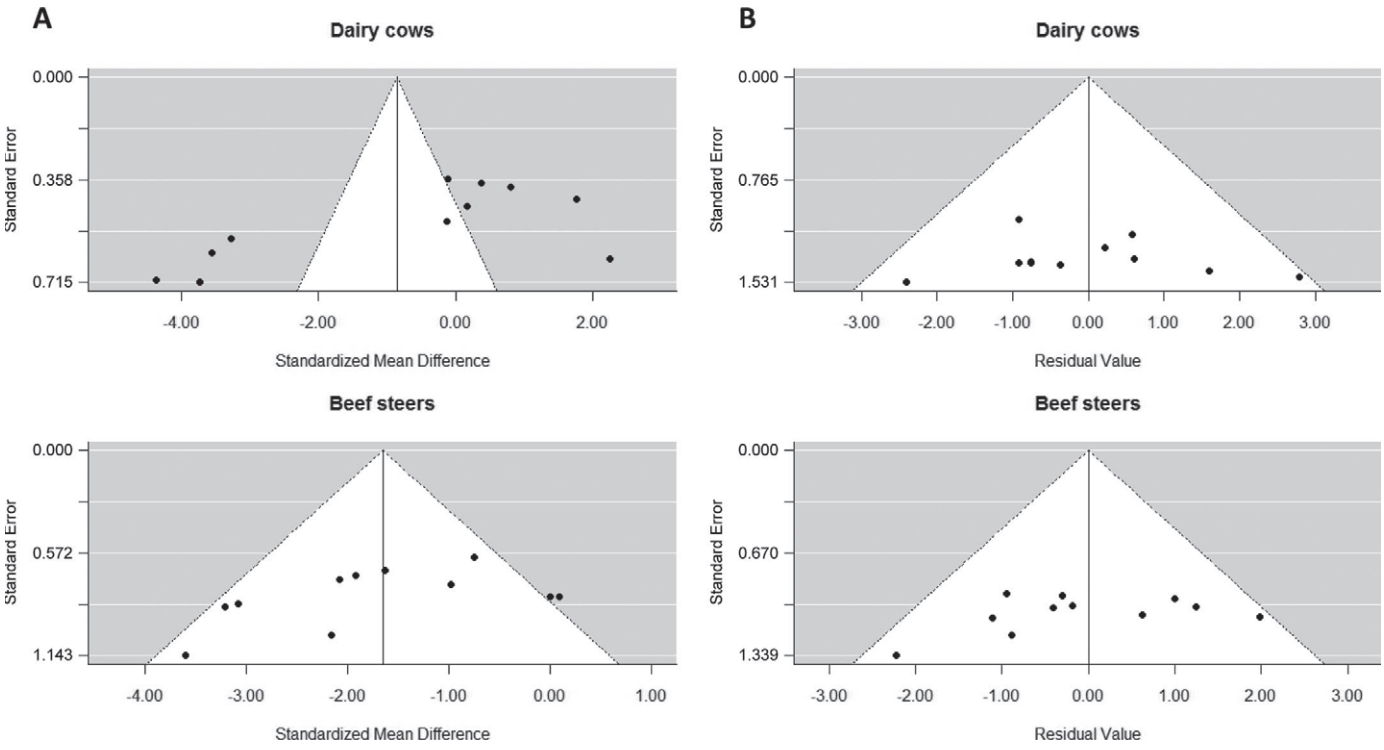

Figure 3. Funnel plots for monensin effect on CH_4 production (g/d) in dairy cows and beef steers from random-effect models (A) and mixed-effect models (B).

adjusted for basal DMI or monensin dose variability, effect sizes of monensin on CH_4 production in dairy cows and beef steers were not significantly different from each other. Similarly, when adjusted for basal DMI differences, monensin effect sizes on Y_m in dairy cows and beef steers were similar to each other. After adjusting for the dose differences, monensin feeding was associated with 14 g/d ($P = 0.019$) and 12 g/d ($P = 0.023$) of CH_4 production reductions in beef steers and dairy cows respectively (Table 4). These results indicate that the differential monensin effects observed between dairy cows and beef steers were partly due to monensin dose differences between the animal groups. A unit increase in monensin dose (mg/kg of DMI) showed a potential ($P = 0.077$) to increase monensin-induced CH_4 mitigation by 1.1 g/d across dairy cows and beef steers. Moreover, the negative relationships between monensin dose and DMI (Table 5) suggest that the negative effect of increasing DMI on monensin effect could be related to an inadequate monensin supply (mg/d) to animals compared with their DMI. This inadequacy was more notable in dairy cows than beef steers. Average DMI of a dairy cow was almost 3 times greater than a beef steer (18.6 vs. 7.2 kg/d, respectively), but the cows were supplemented with less monensin compared with supplementation to beef steers (21 vs. 32 mg/kg of DMI, respectively).

Dietary ingredient and nutrient composition also appeared to modify the monensin effects on CH_4. Dietary NDF content tended to enhance ($P = 0.095$) the CH_4 mitigation effects of monensin in beef steers. Consistently, Thornton and Owens (1981) demonstrated greater CH_4 mitigation by monensin in steers fed high-forage diets compared with those fed low-forage diets. Increasing dietary EE content increased the CH_4 production (g/d) alleviation by monensin in dairy cows. Adding lipid to the diet generally reduced enteric methane emissions (Beauchemin et al., 2008). The mechanism through which lipid could specifically enhance monensin is not clear. Clary et al. (1993) and Mathew et al. (2011), who tested effects of dietary lipid on monensin, did not find any significant change in acetic to propionic ratios or protozoan numbers in the rumen in response to addition of lipid over monensin. However, drawing a sensible conclusion about the interaction between dietary lipid and monensin is difficult because half of the dairy cow studies were based on fresh forages (Table 1) and about half of the forage EE comprises undegradable cuticular waxes.

Persistency is an important requirement for any dietary strategy to be successful in mitigating CH_4 emissions from ruminants (van Zijderveld et al., 2011). Findings related to the persistency of CH_4 mitigation by monensin were inconsistent. Rumpler et al. (1986), Sauer et al. (1998), and Guan et al. (2006) showed that CH_4 mitigation effects of monensin in cattle were short lived and would not last more than 30 d. Conversely, Van Vugt et al. (2005), O'Kelly and Spiers (1992), and Odongo et al. (2007) found significantly reduced CH_4 production from feeding monensin, even after 50 d. Our results did not find a significant effect of monensin feeding duration (data not shown) on CH_4 emissions in dairy cows ($P = 0.678$), beef steers ($P = 0.646$), or across both dairy cows and beef steers ($P = 0.693$). The CH_4 mitigation effects of monensin in cattle therefore appeared to be fairly independent of how long monensin had been fed within the range included in the study (Table 2). Moreover, we tested the effects of CH_4 measuring method (SF6 vs. chambers) on the monensin effect across dairy and beef studies and again did not find significant effects ($P = 0.228$; data not presented).

In contrast to our results showing an effect of monensin on Y_m, which varies with diet composition and DMI, the IPCC (2007) tier 2 approach uses a fixed Y_m (e.g., 6.5% of GE in dairy cows) in current inventories of enteric CH_4 emissions. The IPCC tier 2 model does not have the capacity to fully describe changes in dietary composition and is limited in usefulness when estimating the effects of various nutritional strategies on CH_4 emissions (Ellis et al., 2010). Given the significant effects of monensin on Y_m and the modifying effect of various dietary characteristics on methane

Table 5. Correlation coefficients for relationships between DMI, gross energy (GE), NDF, NFC, and ether extract (EE) contents in diets, monensin dose, and length of monensin treatment period (Length) in dairy cows (above the diagonal) and beef steers (below the diagonal)

Item	DMI	GE	NDF	NFC	EE	Monensin dose	Length
DMI		−0.29	−0.72	0.86	−0.22	−0.44	0.23
GE	0.18		−0.32	−0.16	0.46	−0.16	−0.03
NDF	0.04	−0.41		−0.73	−0.27	0.12	−0.39
NFC	−0.13	0.27	−0.96		−0.08	0.33	0.57
EE	−0.23	0.04	−0.65	0.73		0.39	0.41
Monensin dose	−0.41	−0.03	−0.28	0.22	0.31		−0.04
Length	0.31	−0.06	−0.04	−0.06	−0.09	0.23	

emissions, approaches other than IPCC tier 2 are required. Mechanistic models allow prediction of CH_4 emissions in response to dietary changes that are more credible than empirical approaches, including the IPCC tier 2 method (e.g., Alemu et al., 2011). Recently, Ellis et al. (2012) developed equations to estimate the monensin dose-dependent change in VFA profile, and hence enteric CH_4 production, in high-grain-fed beef cattle, and showed that monensin increased propionate and decreased acetate and butyrate molar proportions. Such equations combined with mechanistic models may help to better predict the CH_4 mitigating effect of monensin in various dietary situations.

CONCLUSIONS

In summary, monensin reduced DMI in both dairy cows and beef steers but did not affect milk yield or milk solids yield in dairy cows. Monensin significantly reduced (−19 g/d) CH_4 emissions in beef steers but the effect (−6 g/d) was marginal in dairy cows. Dry matter intake and dietary nutrient composition appeared to modify monensin effect on CH_4. When adjusted for the differences in DMI or monensin dose between dairy cows and beef steer studies, monensin had similar and significant CH_4 mitigation effects in both dairy cows and beef steers. Monensin supplemented at a higher rate (mg/cow per day), proportional to DMI, can potentially reduce CH_4 emissions from dairy cows.

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Appendix

The R code used for the meta-analysis of the monensin effect on CH_4 production in dairy cows is given below. Comments and code explanations are given by #.

```
#calculations of the explanatory variables centered on
#the means within the original data set namely "d"
d <- within(d, {
                cDMI  <- DMI_C - mean(DMI_C)
                cMonensin <- Monensin_DMI -
                  mean(Monensin_DMI)
                cDuration <- Duration -
                  mean(Duration)
                cEE <-  EE - mean(EE)
                cNDF <- NDF - mean(NDF)
                cNFC <- NFC - mean(NFC)
                cGE <- GE - mean(GE)
                              })
summary(d)
#loading the metafor package
library(metafor)
#Compute the effect measure, raw mean difference (MD)
#by supplying control and treatment means(CH4_C
#and CH4_M), corresponding SE (SE_C and SE_M),
#and sample size (N_C and N_M). This will create a
#new data set namely "dat".
dat <- escalc(m1i=CH4_M,  sd1i=SE_M, n1i=N_M,
              m2i=CH4_C,  sd2i=SE_C, n2i=N_C,
              measure="MD", data=d,
               append=TRUE)
#Compute standardized MD (SMD) for the forest plots
dat2 <- escalc(m1i=CH4_M,  sd1i=SE_M,
   n1i=N_M,
              m2i=CH4_C,  sd2i=SE_C, n2i=N_C,
              measure="SMD", data=d,
                append=TRUE)
#creating a forest plot using the SMD (using the
#"dat2" data)
x11(11, 7)
forest(dat2$yi, dat2$vi, refline=0,cex = 1.2,
       ,slab = dat2$Reference,
       xlim = c(-60, 25), at=c(-6, -3, 0, 3,
         6), digits=1,
       ilab= cbind(round(dat2$CH4_C),
         round(dat2$CH4_M)),
       ilab.xpos = c(-30, -15) )
op <- par(cex = 1.2, font = 2)
text(c(-30, -15), 12.5, c("CTL_CH4 (g/d)",
   "Monen_CH4 (g/d)"))
text(-60, 12.5, "Author(s) and Year", pos = 4)
text(25, 12.5, "Standardized MD [95% CI]",
   pos = 2)
par(op)
#fit random effects model (using MD measures in the
#"dat" data)
fm <- rma(yi, vi, data=dat)
summary(fm)
#constructing the funnel plot
funnel(fm, main="Dairy cows")
#conducting Egger's regression test for publication bias
regtest(fm, predictor="sei")
#fit the mixed-effect models including individual
#centered-explanatory variables
fm01 <- rma(yi, vi, data=dat, mod= ~ cDMI)
   summary(fm01)
fm02 <- rma(yi, vi, data=dat, mod= ~ cMonen
   sin)
summary(fm02)
fm03 <- rma(yi, vi, data=dat, mod= ~
   cDuration)
summary(fm03)
fm04 <- rma(yi, vi, data=dat, mod= ~ cEE)
summary(fm04)
fm05 <- rma(yi, vi, data=dat, mod= ~ cNDF)
summary(fm05)
fm07 <- rma(yi, vi, data=dat, mod= ~ cNFC)
summary(fm07)
fm09 <- rma(yi, vi, data=dat, mod= ~ cGE)
summary(fm09)
#Obtaining summary statistics of the mixed-effect
#models need to be compared
fmL1 <- rma(yi, vi, data=dat, method="ML",
   mod= ~ cDMI + cEE+cMonensin)
summary(fmL1)
fmL2 <- rma(yi, vi, data=dat, method="ML",
   mod= ~ cMonensin + cDMI )
summary(fmL2)
fmL3 <- rma(yi, vi, data=dat, method="ML",
   mod= ~ cDMI + cEE)
summary(fmL3)
fmL4 <- rma(yi, vi, data=dat, method="ML",
   mod= ~ cMonensin + cEE )
summary(fmL4)
#model comparison with log likelihood ratio tests
anova(fmL1, fmL2)
anova(fmL1, fmL3)
anova(fmL1, fmL4)
# Final model fitting
fm3REML <- rma(yi, vi, data=dat,
method="REML", mod= ~ cDMI + cEE)
summary(fm3REML)
#funnel plot construction with the final model
funnel(fm3REML, main="Dairy cows")
```