Effect of Mootral—a garlic- and citrus-extract-based feed additive—on enteric methane emissions in feedlot cattle

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ABSTRACT: Enteric methane (CH_4) production is the main source of greenhouse gas emissions from livestock globally with beef cattle contributing 5.95% of total global greenhouse gas emissions. Various mitigation strategies have been developed to reduce enteric emissions with limited success. In vitro studies have shown a reduction in CH_4 emissions when using garlic and citrus extracts. However, there is paucity of data regarding in vivo studies investigating the effect of garlic and citrus extracts in cattle. The objective of this study was to quantitatively evaluate the response of Angus × Hereford cross steers consuming the feed additive Mootral, which contains extracts of both garlic and citrus, on CH_4 yield (g/kg dry matter intake [DMI]). Twenty steers were randomly assigned to two treatments: control (no additive) and Mootral supplied at 15 g/d in a completely randomized design with a 2-wk covariate and a 12-wk data collection periods. Enteric CH_4 emissions were measured using the GreenFeed system during the covariate period and experimental weeks 2, 6, 9, and 12. CH_4 yield (g/kg DMI) by steers remained similar in both treatments for weeks 2 to 9. In week 12, there was a significant decrease in CH_4 yield (23.2%) in treatment compared to control steers mainly because the steers were consuming all the pellets containing the additive. However, overall CH_4 yield (g/kg DMI) during the entire experimental period was not significantly different. Carbon dioxide yield (g/kg DMI) and oxygen consumption (g/kg DMI) did not differ between treatments during the entire experimental period. DMI, average daily gain, and feed efficiency also remained similar in control and supplemented steers. The in vivo results showed that Mootral may have a potential to be used as a feed additive to reduce enteric CH_4 production and yield in beef cattle but needs further investigation under various dietary regimen.

Key words: environmental sustainability, greenhouse gas, methane yield, steers

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INTRODUCTION

Global emissions of greenhouse gases (GHG) have risen to unprecedented levels despite a growing number of policies to reduce climate change (International Panel for Climate Change (IPCC), 2014). Anthropogenic sources account for 58% of global GHG emissions (National Academies of Sciences, Engineering, and Medicine, 2018). Methane (CH_4) from enteric fermentation of livestock is the largest contributor with beef cattle contributing 5.95% to global GHG emissions (Gerber et al., 2013).

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There are limited strategies to reduce enteric fermentation that can be widely applied to ruminants. Diet manipulation (i.e., changing substantial amount of diet ingredients) and feed additives constitute two strategies with the greatest potential. Moraes et al. (2014) investigated the potential of diet manipulation to reduce enteric CH_4 emissions and concluded that it is possible to reduce emissions through the reduction of neutral detergent fiber (NDF) levels in the diet. However, Moraes et al. (2015) calculated that the cost of implementing this strategy could be up to 49% greater than diets formulated according to national recommendations (e.g., National Academies of Sciences, Engineering, and Medicine, 2016). Therefore, feed additives may be a better alternative to reduce enteric emissions in a cost-effective manner. Mootral is a feed additive containing allicin obtained from garlic as well as a byproduct of orange processing, referred to citrus extract. In an in vitro study, Busquet et al. (2005) reported that garlic oil (300 mg/L) and allicin (300 mg/L) decreased CH_4 production 73.6% and 19.5%, respectively, compared to control diet consisting of 50:50 forage to concentrate ratio in a 24 h incubation. Further, Ma et al. (2016) investigated the effects of supplementary allicin in sheep diet on CH_4 emissions and reported a 6% decrease compared to control (scaled to metabolic body weight [BW]; L/kg $BW^{0.75}$) when given at a dose of 2 g/d for 42 d. Kim et al. (2012) incubated serum bottles containing 0.3 g of timothy grass and plant extracts (1% of total volume) for 24 h in vitro. The authors reported that citrus extract reduced CH_4 emissions 16.7% and garlic extract 20% compared to control with no plant extracts.

We hypothesize that supplementing diets with a combination of garlic and citrus extracts will result in reduced enteric CH_4 production. The objective of this study was to quantitatively evaluate the response of Angus × Hereford cross steers consuming the feed additive Mootral, which contains extracts of both garlic and citrus, on CH_4 yield (g/kg dry matter intake [DMI])

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee at the University of California, Davis (Protocol No. 20032).

Study Design, Animals, and Diets

The study consisted of 20 Angus × Hereford cross steers that were blocked by initial BW, to reduce weight variability, then randomly allocated to one of the following treatments: control (no additive) and Mootral supplied at a dose of 15 g/d for the duration of the trial. The steers were individually housed and were approximately 12 months in age with an average BW of 419 ± 16 kg at the beginning of the trial. The experiment followed a completely randomized design, with a 2-wk covariate and a 12-wk data collection periods. Mootral was first pressed in to an alfalfa pellet then delivered to each of the treatment animals along with their daily total mixed ration (TMR). The control group received "blank" pellets with their daily TMR to ensure Mootral was the only difference between the two treatments. Details on the Mootral supplement formulation has been published by Eger et al. (2018).

Daily intake was calculated as the TMR and alfalfa pellets offered subtracted by individual feed refusal weights. Steers were fed 105% of the previous day's intake twice daily at 0600 and 1800 hours. Steers were fed TMR (Table 1) formulated to meet or exceed their growth requirement according to the National Academies of Sciences, Engineering, and Medicine (2016) recommendations. Two feedlot diets, a starter and finisher diet were formulated (Table 1). The starter diet was used as a backgrounding diet to acclimate the animals to a low forage TMR whereas the finisher diet was used as a typical low forage, feedlot diet. Steers were offered water ad libitum.

Sample Collection and Analysis

CH_4, carbon dioxide (CO_2), and oxygen gas emissions from cows were measured using the GreenFeed system (C-Lock, Inc, Rapid City, SD). Gas emissions using the GreenFeed system were measured during the covariate period and experimental weeks 2, 6, 9, and 12. During each measurement period, gas emission data were collected during three consecutive days as follows: starting at 0700, 1300, and 1900 hours (sampling d 1); 0100, 1000, and 1600 hours (sampling d 2); and 2200 and 0400 hours (sampling d 3). Breath gas samples were collected for at least 5 min followed by a 2-min background gas sample collection. The GreenFeed unit was calibrated weekly with a standard gas mixture containing (mol %): CO_2, 0.98, CH_4, 0.151, and the balance being nitrogen gas (Air Liquide America Specialty Gases, Rancho Cucamonga, CA). Recovery rates for both CO_2 and CH_4 observed in this study were between +/− 1% of the known quantities of gas that was released. Alfalfa pellets were used as bait feed and was offered at each sampling event and was kept below 5% of the total DMI during each sampling period. Alfalfa pellets consumed at the GreenFeed machine contained no Mootral additive, regardless of treatment. The composition of alfalfa pellets is shown in Table 2.

BW was measured once a week to monitor growth rate and the average daily gain (ADG) was calculated from the BW measurements. TMR was sampled once a week and analyzed for dry matter, acid detergent fiber, NDF, lignin, crude fat, total digestible nutrient and mineral content (Cumberland Valley Analytical Services, Waynesboro, PA).

Table 1. Formulation of starter and finisher rations

Ingredients	Starter (% of dry matter)	Finisher (% of dry matter)
Forage		
Alfalfa hay	15.0	6.00
Wheat hay	12.0	4.00
Dry distillers grain	20.0	5.00
Concentrate		
Rolled corn grain	42.0	
Flaked corn		74.0
Molasses	8.00	5.00
Fat	1.50	3.00
Urea	0.35	0.50
Beef trace salt[1]	0.32	1.00
Magnesium Oxide		0.20
Limestone	0.82	0.70
Phosphate monosodium		0.10
Potassium chloride		0.40

[1]Beef Trace Salt sourced from AL Gilbert.

Table 2. Chemical composition of starter diet, finisher diet, and alfalfa pellets (% of dry matter)

Nutrients	Starter TMR	Finisher TMR	Alfalfa pellets
% Dry matter			
Crude protein	14.1	13.5	21.9
Acid detergent fiber	18.8	9.5	30.2
NDF	29.1	17.4	42.1
Lignin	3.41	1.93	7.02
Crude fat	5.29	6.94	2.17
Total digestible nutrients	76.7	85.3	60.9
Ash	6.02	4.78	10.4
Ca	0.49	0.48	1.13
Phosphorus	0.34	0.27	0.3
Magnesium	0.27	0.22	0.59
Potassium	1.44	0.92	2.1
Sodium	0.15	0.36	0.16
Parts per million			
Iron	119	124	1319
Manganese	47	56	55
Zinc	49	59	25
Copper	7	7	12

Statistical Analysis

Statistical analysis was performed using the open-source R statistical software (version 3.1.1; The R Foundation for Statistical Computing, Vienna, Austria.). Statistical analysis was completed using the linear mixed-effects models (lme) procedure, with the steer serving as the experimental unit. GreenFeed emission data were averaged per steer and gas measurement period and the averaged data used in the statistical analysis. Weekly DMI, growth, and gas emission data were analyzed as repeated measure with a rational quadratic spatial correlation structure. The statistical model included treatment, week, and treatment × week interactions, and the covariate term, with the error term assumed to be normally distributed with mean = 0 and constant variance. Individual animal was used as random effect, whereas all other factors were considered fixed. Statistical significance was established when $P \leq 0.05$ and a trend at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Although there are several feed additives that have shown potential to reduce CH_4 emissions (e.g., Dijkstra et al. 2018; Roque et al. 2019), currently none are available that can be applied in a commercial setting. This section presents the results of the gas emission analysis and impact on productivity due to supplementation with Mootral.

Gas Production

Enteric CH_4 yield by steers in the control and Mootral-supplemented groups are shown in Fig. 1A. There was no statistical differences in baseline CH_4 yield between the control and supplemented groups. During the subsequent weeks 2, 6 and 9, although the CH_4 yield from supplemented steers remained below the control, the two treatments were not statistically different (week 2 control = 18.0 ± 1.4 g/kg DMI, treatment = 17.5 ± 1.5 g/kg DMI; week 6 control = 10.3 ± 1.4 g/kg DMI, treatment = 9.5 ± 1.5 g/kg DMI and week 9 control =18.2 ± 1.4 g/kg DMI, treatment = 15.6 ± 1.5 g/kg DMI). CH_4 yield was significantly different between control and treatment in week 12 (19.4 ± 1.4 g/kg DMI vs. 14.9 ± 1.5 g/kg DMI, respectively). The differences in CH_4 yield between treatment and

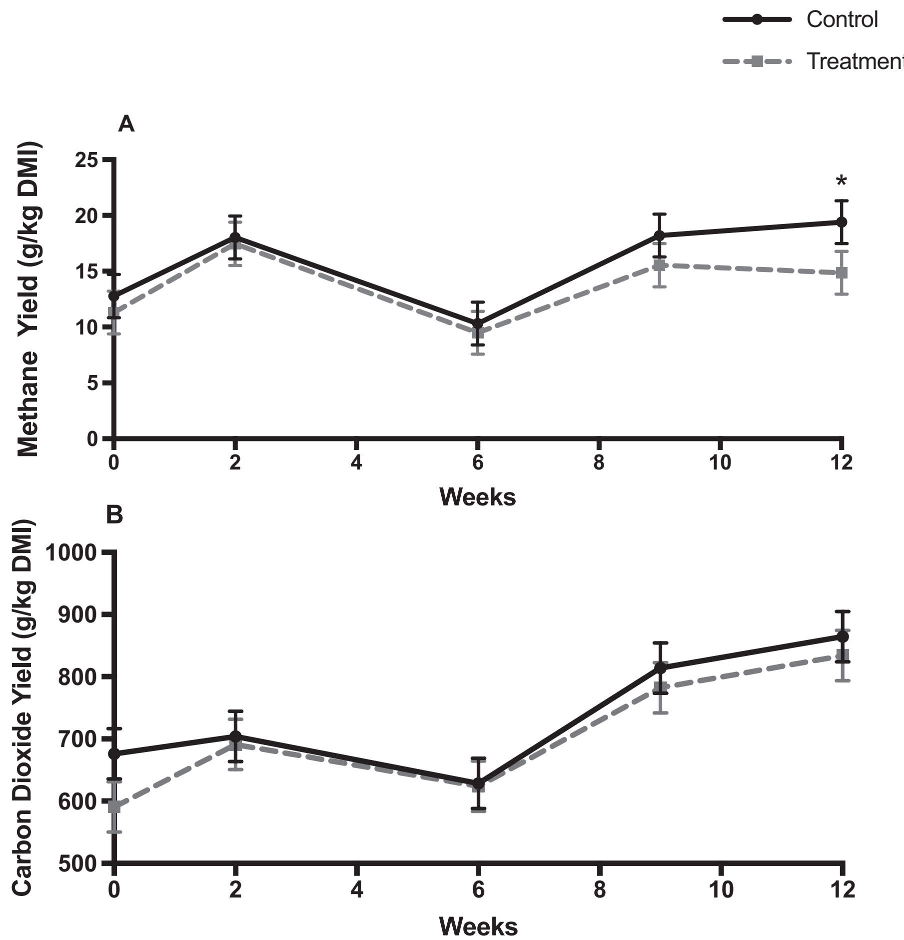

Figure 1. CH_4 yield (g CH_4/kg DMI) (A), and CO_2 yield (g CO_2/kg DMI) (B) by steers in the control (solid line) and treatment (broken line) groups during the 12-wk experimental period.

control were greater in the last two sampling periods compared to the beginning weeks of the experiment. Visual observation led us to speculate that some of the differences may be attributed to sorting of the feed as some pellets were not consumed. This could be attributed to individual animal preference, or lack thereof, for alfalfa pellets. As the experiment progressed, the steers consumed the daily doses as recommended. Over the course of the trial, the mean enteric CH_4 yield of steers supplemented with Mootral was 13.3% lower compared to control steers but it was not significantly different (16.5 ± 1.0 g/kg DMI, vs. 14.3 ± 1.1 g/kg DMI, for control and supplemented groups, respectively; $P = 0.16$). There were week-to-week variability in CH_4 yield, however, even after removing week 6 from the analysis, which were lower compared to other weeks, the results were not affected.

Although Ma et al. (2016) did not use the same formulation as in this study, allicin was the main ingredient in both studies. The authors used similar concentrations of allicin when scaled to BW and observed reduced CH4 production by over 160 mL/kg $BW^{0.75}$. Acetate production was also reduced while butyrate and iso-butyrate productions were increased. CH_4 production has been positively associated with greater molar proportions of acetate and negatively associated with increased propionate and butyrate production (Alemu et al. 2011). Proportions of acetate, butyrate, and propionate determine the quantity of hydrogen available in the rumen for utilization by CH_4-producing microbes. Pathways resulting in propionate production contribute the least to the quantity of hydrogen, whereas pathways resulting in acetate contribute the most.

The mode of action of allicin is thought to be through reductions in protozoa and methanogenic archaea populations in the rumen (Ma et al. 2016). Miron et al. (2000) showed that allicin is highly permeable through membranes and may contribute to its biological activity. Furthermore, Eger et al. (2018) reported that mixture of garlic and citrus compounds appeared to reduce CH_4 production by altering the archaeal community such that the percentage of *Methanobacteriaceae* was reduced without exhibiting negative side effects on rumen fermentation.

Compared to stockers, feedlot cattle produce less enteric CH_4 (6.5% vs. 3% Gross Energy intake; IPCC, 2006), mainly because the diet contains highly digestible carbohydrates, which changes the molar proportions of volatile fatty acid production resulting in a greater propionate to acetate ratio. Beauchemin et al. (2010) estimated that 53% of the enteric CH_4 from beef production comes from the cow-calf herd whereas only 10% is attributed to the feedlot cattle. Therefore, feed additives including Mootral have a greater potential to reduce CH_4 production when given to cattle fed forage based diets rather than concentrate diets.

CO_2 production throughout the experiment (Fig. 1B) was similar between the two groups and was not significantly different ($P = 0.48$). Similarly, Hristov et al. (2015) using a feed additive to reduce CH_4 emissions also reported that CO_2 production were not different between control and treatment. The mean oxygen consumption between the two experimental group was also not significantly different ($P = 0.48$; data not shown).

BW Changes

DMI of steers in the control group and treatment groups were also similar; 9.94 vs. 9.47 kg/d respectively (± 0.55 kg, $P = 0.46$). The initial and final BW measurements show that there was no differences in the production efficiency between control and Mootral-supplemented steers (initial 419 vs. 428 kg, respectively [± 16.3 kg, $P = 0.59$]; final 557 vs. 569 kg, respectively [± 19.9 kg, $P = 0.57$]). There was a large day-to-day variability in BW; therefore, a larger number of animals compared to those used in the experiment would have been required to accurately estimate differences in BW due to treatment, if any. ADG was calculated as differences in BW between two consecutive measurements periods. There was no statistical difference in ADG between control and Mootral treatment; 1.65 vs. 1.67 kg/d respectively (± 0.10, $P = 0.80$). Hence, no significant differences were observed between the feed conversion efficiencies of between the treatments ($P = 0.96$).

In conclusion, steers fed a diet with Mootral added at a rate of 15 g/d had a 23% reduction in CH_4 yield after 12 weeks of supplementation. Despite the average CH_4 production over the entire experimental period not being different between the two dietary treatments, the steers receiving the Mootral treatment had lower CH_4 yields than the steers receiving the control treatment over time with no effect on DMI, ADG, and feed conversion efficiency.

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Conflict of interest statement. None declared.

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