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 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).
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).

**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

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**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 |                           |                             |
| 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              |
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 \( \text{CH}_4 \) yield of steers supplemented with Mootral was 13.3\% lower compared to control steers but it was not significantly different (16.5 \( \pm \) 1.0 g/kg DMI, vs. 14.3 \( \pm \) 1.1 g/kg DMI, for control and supplemented groups, respectively; \( P = 0.16 \)). There were week-to-week variability in \( \text{CH}_4 \) 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. \( \text{CH}_4 \) 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 \( \text{CH}_4 \)-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 \( \text{CH}_4 \) production by altering the archaeal community such that the percentage of \( \text{Methanobacteriaceae} \) was reduced.

![Figure 1. CH4 yield (g CH4/kg DMI) (A), and CO2 yield (g CO2/kg DMI) (B) by steers in the control (solid line) and treatment (broken line) groups during the 12-wk experimental period.](image-url)
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 ($\pm 0.55$ 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 [$\pm 16.3$ kg, $P = 0.59$]; final 557 vs. 569 kg, respectively [$\pm 19.9$ kg, $P = 0.57$]). There was a larger 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 ($\pm 0.10$, $P = 0.80$). 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.

**LITERATURE CITED**

Alemu, A. W., J. Dijkstra, A. Bannink, J. France, and E. Kebreab. 2011. Rumen stoichiometric models and their contribution and challenges in predicting enteric methane production. Anim. Feed Sci. Tech. 166-167:761–778. doi:10.1016/j.anifeedsci.2011.04.054

Beauchemin, K. A., H. Janzen, S. M. Little, T. A. McAllister, and S. M. McGinn. 2010. Life cycle assessment of greenhouse gas emissions from beef production in western Canada: a case study. Agric. Syst. 103:371–379. doi:10.1016/j.agsy.2010.03.008

Busquet, M., S. Calsamiglia, A. Ferret, M. D. Carro, and C. Kamel. 2005. Effect of garlic oil and four of its compounds on rumen microbial fermentation. J. Dairy Sci. 88:4393–4404. doi:10.3168/jds.S0022-0302(05)73126-X

Dijkstra, J., A. Bannink, J. France, E. Kebreab, and S. van Gastelen. 2018. Short communication: antimethanogenic effects of 3-nitrooxypropanol depend on supplementation dose, dietary fiber content, and cattle type. J. Dairy Sci. 101:9041–9047. doi:10.3168/jds.2018-14456

Eger, M., M. Graz, S. Riede, and G. Breves. 2018. Application of MootralTM reduces methane production by altering the archaea community in the rumen simulation technique. Front. Microbiol. 9:2094. doi:10.3389/fmicb.2018.02094

Gerber, P. J., H. Steinfeld, B. Henderson, A. Mottet, C. Opio, J. Dijkman, A. Falucchi, and G. Tempio. 2013. Tackling climate change through livestock—a global assessment of emissions and mitigation opportunities. Food and Agriculture Organization of the United Nations (FAO), Rome.

Hristov, A. N., J. Oh, F. Giallongo, T. W. Frederick, M. T. Harper, H. L. Weeks, A. F. Branco, P. J. Moate, M. H. Deighton, S. R. Williams, et al. 2015. An inhibitor persistently decreased enteric methane emission from dairy cows with no negative effect on milk production. Proc. Natl. Acad. Sci. U. S. A. 112:10663–10668. doi:10.1073/pnas.1504124112

IPCC. 2006. IPCC Guidelines for National Greenhouse Gas Inventories. Institute for Global Environmental Strategies, Hayama, Japan.

IPCC. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the IPCC. IPCC, Geneva, Switzerland.

Kim, E. T., C. H. Kim, K. S. Min, and S. S. Lee. 2012. Effects of plant extracts on microbial population, methane emission and ruminal fermentation characteristics in in vitro. Asian-Australas. J. Anim. Sci. 25:806–811. doi:10.5713/ajas.2011.11447
Ma, T., D. D. Chen, Y. Tu, N. F. Zhang, B. W. Si, K. D. Deng, and Q. Y. Diao. 2016. Effect of supplementation of allicin on methanogenesis and ruminal microbial flora in Dorper crossbred ewes. J. Anim. Sci. Biotech. 7 Article Number: 1. doi: 10.1186/s40104-015-0057-5

Miron, T., A. Rabinkov, D. Mirelman, M. Wilchek, and L. Weiner. 2000. The mode of action of allicin: its ready permeability through phospholipid membranes may contribute to its biological activity. Biochim. Biophys. Acta 1463:20–30. doi:10.1016/s0005-2736(99)00174-1

Moraes, L. E., A. B. Strathe, J. G. Fadel, D. P. Casper, and E. Kebreab. 2014. Prediction of enteric methane emissions from cattle. Glob. Chang. Biol. 20:2140–2148. doi:10.1111/gcb.12471

Moraes, L. E., J. Fadel, A. Castillo, D. Casper, J. Tricarico, and E. Kebreab. 2015. Modeling the trade-off between diet costs and methane emissions: A goal programming approach. J. Dairy Sci. 98:5557–5571.

National Academies of Sciences, Engineering, and Medicine. 2018. Improving characterization of anthropogenic methane emissions in the United States. Natl. Acad. Press, Washington, DC.

National Academies of Sciences, Engineering, and Medicine. 2016. Nutrient requirements of beef cattle: eighth revised edition. Natl. Acad. Press, Washington, DC. doi:10.17226/19014.

Roque, B. M., J. K. Salwen, R. Kinley, and E. Kebreab. 2019. Inclusion of Asparagopsis armata in lactating dairy cows’ diet reduces enteric methane emission by over 50 percent. J. Cleaner Prod. 234:132–138. doi:10.1016/j.jclepro.2019.06.193