7-20-2022

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An ASABE Meeting Presentation
DOI: https://doi.org/10.13031/aim.202200416
Paper Number: 2200416

Effect of bromoform and linseed oil on greenhouse gas emissions from stored beef manure

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Written for presentation at the
2022 ASABE Annual International Meeting
Sponsored by ASABE
Houston, Texas
July 17–20, 2022

ABSTRACT. *Emissions of carbon dioxide, methane, and nitrous oxide – potent greenhouse gases - from stored beef feedlot manure are a significant concern relative to climate change. Research on methane reduction strategies for enteric emissions has identified the application of organic additives, such as bromoform and linseed oil, to ruminant diets as potential solutions for reducing enteric emissions and pathogenic bacteria in excreted manure. The objective of this study was to determine the effect of bromoform and linseed oil on greenhouse gas emissions from beef feedlot manure, and on E. coli concentration in beef cattle manure, during a 5-week storage period. The experiment used a completely randomized block design (CRBD) with 4 replications of 5 treatments: 5.5 g/kg and 11 g/kg of linseed oil, 4.3 g/kg, and 8.6 g/kg of bromoform, and a control receiving no additives. Treatments were added to a 3-liter mix of 50% manure, 50% soil, mixed by hand, and stored in airtight columns (10-cm diameter x 40-cm tall) in a greenhouse maintained at 25 C during the storage period. Gas samples were collected 10 times during the 5-week test period using a 15 ml syringe and were analyzed using gas chromatography to determine concentrations of methane, carbon dioxide and nitrous oxide. A 1-cm diameter core of material was removed from the top 20 cm of each column 4 times during the sampling period to conduct bacterial enumerations. Quantification of E.coli in samples was determined by incubating serial dilutions for 24 hours at 36 C and manually counting colonies. Preliminary results of the study showed that through 5 weeks of observation, 11g/kg linseed oil reduced the average concentration of E. coli ($p < 0.05$) compared to all other treatments. Preliminary results also indicate that bromoform at 8.6g/kg decreased carbon dioxide emissions but neither bromoform concentration had any significant effect on methane or nitrous oxide emissions compared to control. Linseed oil at 11g/kg increased methane emissions compared to control but neither linseed oil concentrations significantly impacted the average flux of carbon dioxide, or nitrous oxide from manure storages when compared to control.*

Keywords. *Animal agriculture, emissions, methane, carbon dioxide, CAFOs, cattle, essential oils*

Introduction

In the last decades, one of the critical concerns worldwide has been climate change, caused by increasing concentrations of greenhouse gases in the earth's atmosphere. It is expected to cause 250.000 deaths per year between 2030 and 2050, with direct health damage of USD 2-4 Billion/year (WHO, 2022). This explains why the United Nations has included combatting climate change and its impacts in their 2030 Agenda for Sustainable Development (United Nations, 2021).

Livestock production

In 2016, just over 30 million beef cattle were finished in animal feeding operations (AFOs) in the United States (USDA-NASS, 2017). By regulatory definition, an AFO is any agricultural livestock or poultry operation that confines any number

of animals for 45 days or more in a 12-month period to an area that cannot sustain vegetation. Much of the beef cattle production in the U.S. occurs in concentrated animal feeding operations (CAFOs), which are AFOs having a capacity for housing 1,000 or more beef cattle. In the United States, the final stage of beef cattle production most frequently is accomplished by moving adult animals to a feedlot – an area of land having no vegetation where animals are grouped within fenced pens – to finish the animal before harvest. Finishing cattle refers to a change of diet that the animals receive at a feedlot that encourages efficient deposition of muscle and fat. The United States Department of Agriculture Natural Resource Conservation Service (USDA-NRCS) estimates that adult beef cattle produce roughly 28 kg of manure per day (Lorimor et al., 2004). As a result, over 150 million tons of manure are produced annually in feedlot systems, most of which is applied to agricultural land as fertilizer. The concentration of manure produced in a feedlot or other confined animal housing system can create environmental concerns. One such concern is the contribution of livestock production to greenhouse gas (GHG) emissions. Indeed, one of the most prominent actors involved in heating the planet is the meat and dairy industry. These two sectors account for 57% of the total GHG emissions from food production (Xu et al., 2021).

Need for short term emission reductions for manure storage

Together manure emissions and enteric fermentation are the largest sources of methane production in the USA, accounting for 36% of the total produced (EPA, 2021; Kumari et al., 2019). Hence, reducing these emissions could represent significant mitigation in the total greenhouse gases produced annually. Nevertheless, less effort has been made to reduce greenhouse gas emissions from manure management from cattle feedlots. Instead, research has focused on how to increase CH4 production and use it as a green energy source (Romero et al., 2020; Abouelenien et al., 2014). While methane collection is a promising opportunity for reducing greenhouse gas emissions from manure storages it must be noted that there will remain occasions for manure production and release for which collection is impossible. For example, wet conditions in outdoor animal housing will temporarily increase methane production, but this effect is not term.

Moreover, the adoption of new storage and treatment technologies has proved slow in the past. Rahelizatovo and Gillespie (2004) found that a lack of knowledge and information was only a small part of the main barriers to adopting manure new technologies among dairy producers in Louisiana. This is due in part to the fact that farmers' relationships with agricultural advisors and researchers have been reported to be a driver for the adoption of manure management best practices (Niles et al 2019). Hou, et al., (2018) concluded, after studying farmers in four important livestock producers' countries in Europe, that stakeholder engagement was essential when developing strategies to increase the adoption of new treatment technologies. Extension programming has a strong potential role to play in bridging this gap, but the establishment of such programs takes time. Therefore, alternative uses for products already on the market may provide a stop-gap measure while methane collection technologies expand.

Application of dietary treatments to manure storage for emission reduction

Together manure emissions and enteric fermentation are the largest sources of methane production in the USA, accounting for 36% of the total produced (EPA, 2021; Kumari et al., 2019). Hence, reducing these emissions could represent significant mitigation in the total greenhouse gases produced annually. Significant work has been done to identify treatments to reduce enteric methane in livestock production. This work, within a highly complex microbiological environment (the rumen), may have promise for similar effects in the different but equally complex microbial communities in manure.

Red seaweed

Inquiry in recent years discovered that supplementing red seaweed (*Asparagopsis taxiformis*), a macroalga distributed in tropical to warm temperate waters, to cows reduces methane emissions by 80% in in vivo studies and up to 99% in vitro studies (Roque et al., 2021; Soliva et al., 2011). These findings could lead to a significant reduction of GHG since most greenhouse gas emissions are in the form of methane (Moraes et al., 2014). This reduction is mainly due to inhibiting methanogenesis by two different active compounds: compounds that mimic CH4 like bromoform or short-chain nitro-compounds like 3-NOP (Dijkstra et al., 2018; Kinley et al., 2020). In detail, bromoform (Halogenated CH4) sequesters the required prosthetic group required for the enzyme methyl-coenzyme M reductase (MCR), which is crucial in the final steps in methanogenesis by bacteria, while the latter inhibits this coenzyme directly (Duin et al., 2016; Kinley et al., 2020; Roque et al., 2021). Nevertheless, no studies have looked at utilizing *A. Taxiformis* or its components for reducing methane emissions from the manure storages.

Essential oil

Essential oils (EO), volatile organic compounds derived from a wide variety of plants, have already being considered for use in the industry as growth promotion and found to have similar health effects as feeding tylosin (Meyer et al., 2009). In one study, essential oils were shown to improve rumen metabolism of proteins by effectively inhibiting select NH3 producing bacteria (McIntosh et al., 2003). EO have also been studied to reduce CH4 enteric emissions from ruminants, with promising results: 91% reduction using garlic oil in vitro studies (Soliva et al., 2011). Similarly, linseed oil has been used in different

studies to reduce enteric fermentation emissions or pathogenic bacteria (Guyader et a, 2016). The use of essential oils to reduce methane emissions is closely linked to the mitigation of methanogenic bacteria since EO have shown repeatedly antimicrobial properties (Mahizan et al., 2019; Brnawi et al., 2019). To our knowledge, EO have not been studied to minimize manure emissions.

Pathogen control

The application of a potentially antimicrobial treatment (such as EO) to reduce manure emissions may have the effect mitigating pathogens as well. Pathogenic bacteria are a chief concern related to manure management (National Research Bicudo and Goyal, 2003). Ruminant animals, like cattle, are major reservoirs of foodborne pathogens like *Escherichia coli* strain O157:H7 (Callaway et al., 2009). This pathogen is common to fresh and stored bovine manure, where it can last for several weeks (Berry & Miller, 2005). Moreover, animal waste and contaminated food or water can cause humans and animals infections (Manyi-Loh et al., 2016). Hence, it is essential to properly manage this kind of waste and reduce its microbial load to avoid a pathogen outbreak. EO have also shown antioxidative and anti-inflammatory effects attributable to binding ion catalysts, decomposing peroxides, and radical scavenging (Tsai et al., 2013). EO have also shown very broad antimicrobial activity (Abers et al., 2021; Chrysargyris et al., 2020), for example mint oils were observed to decrease microbial populations of both gram negative *E.coli* and gram positive *Staphylococcus aureus* in minced meat by more than 50% (Djenane et al., 2012). Moreover, EOs of rosemary, oregano, and linseed have shown to significantly reduce *E. coli* O157:H7 (Díez-Pascual, 2018; Kaithwas et al., 2011).

Objective

Significant work has been done to illuminate the contribution of livestock and manure management to risks of GHG emission and into the mechanisms of enteric methane development and reduction. However, despite understanding the variety of strategies that are available and often in use in the livestock industry to control GHG and pathogens, less research has been done to determine what impacts such approaches may have on emissions from manure storages, especially for short term applications. Specifically, the objectives of this research were the following:

1. Quantify the effect of linseed oil and bromoform, at two different concentrations, on the GHG emissions from short-term manure storage when compared to no treatment control.
2. Quantify the effect of linseed oil and bromoform, at two different concentrations, on the concentration of the indicator organism *E.coli* in short-term manure storage.

Materials and Methods

Material Collection

Freshly excreted bovine manure was collected from the Eastern Nebraska Research, Extension & Education Center (ENREC) of the University of Nebraska-Lincoln (UNL) near Ithaca, Nebraska. To simulate the soil and manure mix on the surface of a feedlot agricultural soil was collected from the top 15 cm of profile from row crop field near Julian, NE, the type of soil used in this study was a silty clay loam. Following collection, the soil had been allowed to air dry in the laboratory, thus water was added to the soil to return the soil to 40% water-filled pore space (WFPS), based on the gravimetric moisture content of a sample of the soil. A baseline sample of manure and soil used in this study was characterized at a commercial laboratory (Ward Laboratory, Inc., Kearney, NE) (Table 1).

Experimental Set Up

The experiment was conducted using columns fabricated from Schedule 40 (Sch. 40) polyvinyl chloride (PVC) with an inside diameter of 10.2 cm (4 in.) and a total length of 50.8 cm (20 in.), based on description in Hidayat et al. (2021). Based on previous work by Miller and Berry (2005), equal parts of the fresh manure and wet agricultural soil were mixed by hand in 2 to 3-liter batches, gradations on mixing buckets were used to maintain equal manure and soil volumes (Choice Food Service Co, Layton, UT). Treatment additives were included in each batch of manure as it was mixed. The treatments were 11.7 mL/kg (L1) and 23.5 mL/kg (L2) of linseed oil (Spectrum Chemical MFG, New Brunswick, NJ), 1.5 mL/kg (B1) and 3.1 mL/kg (B2) of Bromoform (TCI America, Portland, OR), and no amendment control (C). These five treatments with four replicates (20 total columns) were assessed in a completely randomized block design. The mixture of manure, soil, and treatments was hand packed into columns on the first day of sampling. The total depth of soil within the columns was 40 cm to allow sufficient headspace (475 cm^3) for greenhouse gas sampling. All columns were held in a greenhouse at the University of Nebraska maintained at 20-25°C during the experiment. The experiment began on October 26 of 2021 and ran until November 30 for a total of 35 days (5 weeks).

Gas sampling and analysis

Greenhouse gas (GHG) emissions from the top of the columns were measured over the course of study. Collection chambers were created from PVC caps that were fitted over each column. Each cap had a 1.25 cm hole drilled into it which was fitted with a threaded brass hose bar equipped with a rubber cap and an O ring to prevent air leakage. The rubber cap allowed for a syringe to be used for gas collection. In five of the caps a second 1.25 cm hole was drilled and fit with a liquid-in-glass thermometer (Thermco, La Port, In), the five caps were each placed on one of the treatment types to monitor the temperature in the headspace at the time of sampling. The total headspace of the cap was 475 cm^3 and the headspace of each column was determined by lining the manure surface with plastic and measuring the volume of water that could be held. On the day the treatments were applied, and columns established (Day 0) gas flux was measured from the columns. After the caps were applied a 30-mL syringe was used to remove 15 mL of gas from the rubber cap and replaced to thoroughly mix headspace gases. Then, 25 mL of gas were sampled and placed in an evacuated glass bottle. Headspace gases were sampled three times after capping to calculate gas flux: 0, 10, and 20 minutes (Hidayat et al., 2021; Roscioli et al., 2015). On all subsequent sampling days (1, 2, 4, 6, 8, 11, 14, 19, 24, 29, and 35) the flux measurement was replaced by a single collected sample of the cumulative concentration of gases released during the preceding experimental period. Columns were left capped between sampling days, then 25 mL of gas was sampled and placed in an evacuated glass bottle. After sampling, columns were opened to let air flush the headspace for 45 min. Headspace gas samples were analyzed for CO2, CH4, and N2O using an 8610C gas chromatograph (SRI Instruments, Torrance, CA) equipped with helium ionization and thermal conductivity detectors. The instrument was configured as specified by (Miller & Berry, 2005).

Bacterial Enumeration

Six times during the study period (sampling day 0, 8, 14, 21, 28, 38) all columns were sampled for concentrations of generic *E.coli.* Individual 10 cm long, 15 mm diameter tubes were constructed from LevGo SmartSpatula® disposable Spatulas (LevGo, Berkeley, CA) for each column on each sampling day, this tube was inserted into the column of manure to retrieve a sample of roughly 10 g. The surface of the column was arranged to close the opening created after sampling. In the lab, manure was removed from the tubes and homogenized by hand before a 1g subsample was removed to begin a serial dilution. Six dilutions (10-1, 10-2, 10-3, 10-4, 10-5, and 10-6) were created for each sample. Then, 0.100-ml of each dilution was transferred to a CHROMagar *E. coli* plate (DRG International, Inc., Springfield, NJ). Dilution material was spread evenly over the plate using an L-shaped spreader (Andwin Scientific, Simi Valley, CA). Spread plates were incubated overnight at 37°C. Following incubation, blue colonies on the plates were enumerated as *E. coli*.

Statistical Analysis

The statistical analysis for this paper was generated using SAS/STAT software, Version 9.4 of the SAS System for Windows. Copyright © 2016[1]. All the gas measurements during the study period were converted to flux measures and the cumulative emission during the testing period was determined using an area under the curve approach and these values were compared using a generalized linear mixed model (GLIMMIX) procedure to analyze treatment effects for the total GHG emission, and average *E. coli* concentrations. All means were separated using a least square means comparison at the 0.05 level. Where differences existed a post-hoc Tukey-Kramer test was used to conduct pairwise comparisons.

Results

Gas emissions

For carbon dioxide emissions neither of the concentrations of linseed oil had a significant effect when compared to control, however, the higher concentration of bromoform lowered emissions when compared to control (Figure 1). For methane emissions, the bromoform treatments were not statistically different than control (all were very close to zero), however, both concentrations of linseed oil significantly increased overall methane emissions compared to control (Figure 2). Nitrous oxide emissions were close to zero for all treatments and no significant differences were observed.

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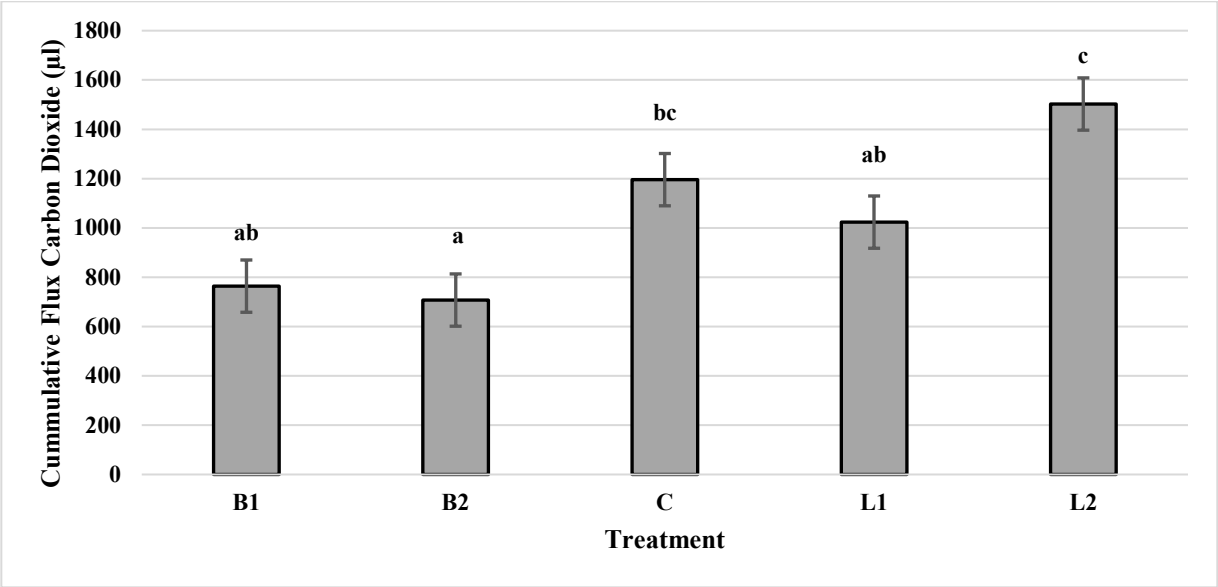

Figure 1: Average cumulative emission of carbon dioxide during the storage period. Treatments are C=control, L1=linseed oil at lower concentration, L2=linseed oil at higher concentration, B1=bromoform at lower concentration, B2=bromoform at higher concentration. Error bars show standard error, letters indicate significant differences at the α=0.05 level.

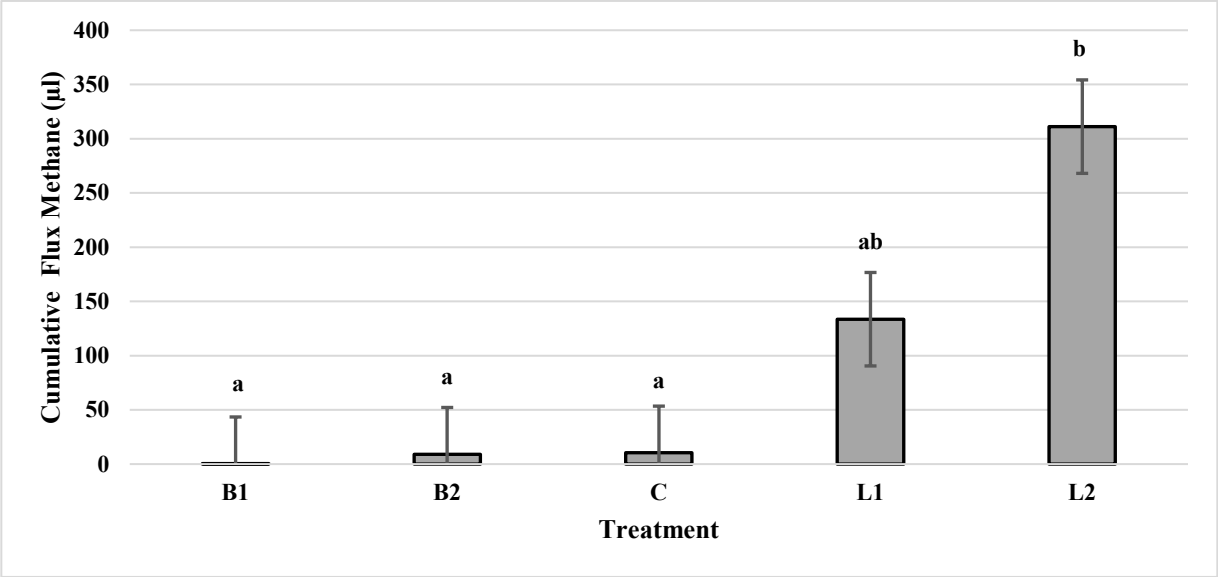

Figure 2: Average cumulative emission of methane during the storage period. Treatments are C=control, L1=linseed oil at lower concentration, L2=linseed oil at higher concentration, B1=bromoform at lower concentration, B2=bromoform at higher concentration. Error bars show standard error, letters indicate significant differences at the α=0.05 level.

Bacterial concentration

Bacterial populations decreased as the material dried but there were no observed significant interactions of time and treatment. However, there were significant differences based on treatment type. The higher concentration of linseed oil decreased the average *E.coli* concentration observed during the study period, but none of the other treatments were different from control (Figure 3).

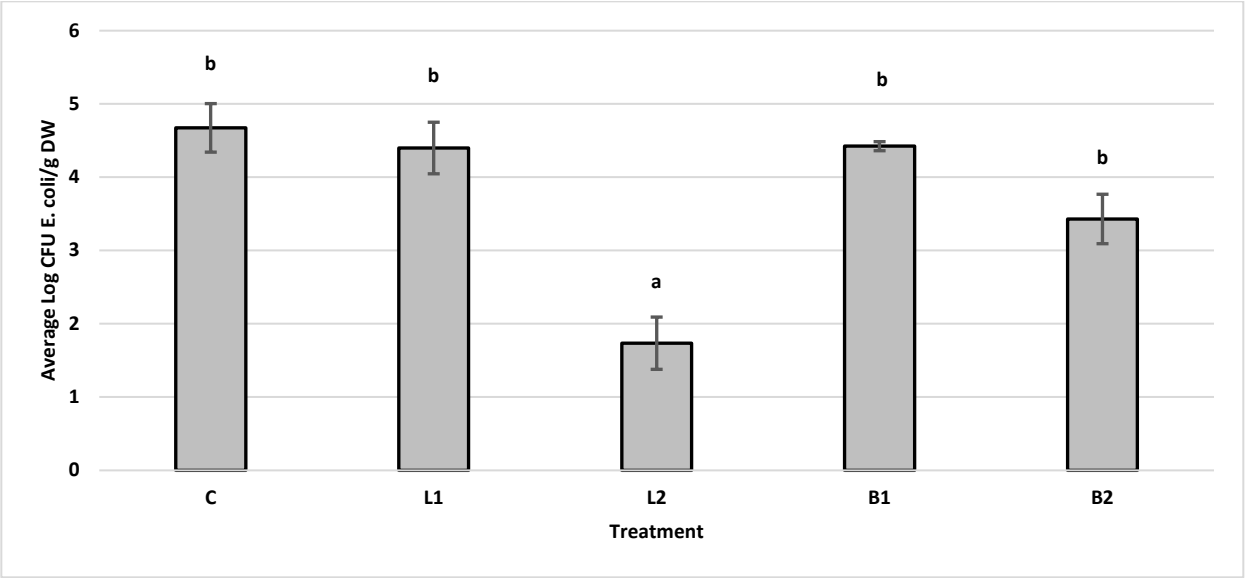

Figure 3: Average concentration of *E.coli* during the storage period. Treatments are C=control, L1=linseed oil at lower concentration, L2=linseed oil at higher concentration, B1=bromoform at lower concentration, B2=bromoform at higher concentration. Error bars show standard error, letters indicate significant differences at the α=0.05 level.

Discussion

In contrast with the results shown in this study, diverse researchers have found that giving cattle linseed oil as a feed supplement reduces methane gas emissions from enteric fermentation (Guyader et al., 2016; Benchaar et al., 2015; Kliem et al., 2018). According to Lyons et al. (2017), linseed oil reduces 19% of *Methanobrevibacter* at the genus level, where high emitters present 2.54X more of these archaea (Wallace et al., 2015). Nevertheless, Tapio et al. (2017) suggest that *Methanosphaera* spp. proportion in the total archaea population also plays a crucial role in methanogenesis, especially in beef cattle. Linseed oil has been found to increase by 65.3% the count of *Methanosphaera* spp. when applied to ruminant diets (Lyons et al., 2017). Hence, the increase in methane production presented in this study could be related to the fact that the proportion of *Methanosphaera* spp. was affected by the application of linseed oil by at least 2.44X compared to the other archaea, as established by Wallace et al. (2015). However, to confirm this, a metagenomic study should be conducted. Likewise, the mechanism behind the mitigation of methane emissions has not yet been established using linseed oil. It has been suggested that the oil might have a toxic effect on the bacteria that produce H_2, a compound that is essential in the methanogenesis pathway (Yons et al., 2017). Hence, future efforts should be focused on further exploration of the microbial population dynamics to explore how linseed oil is effectively increasing methanogenesis in manure storages.

On the other hand, as shown above, linseed oil treatment reduced *E.coli* bacteria. Similar results have been reported by Petropoulos et al. (2021) and Diez-Pascual (2018). This antibacterial property can be related to the high content of α-linolenic acid (71.9 % of the total fatty acids) because this fatty acid is well known for its antimicrobial properties and disruptive membrane behavior (Petropoulos et al., 2021; Yoon et al., 2018; Casillas-Vargas et al., 2021).

Conclusions

The results of this research indicate the addition of bromoform with a concentration of 3.1 mL/kg can decrease carbon dioxide emissions from manure storage but has no significant impact on methane or nitrous oxide emissions when compared to control. Linseed oil, incorporated at a concentration of 23.5 mL/kg into feedlot manure increased methane emissions compared to control but also reduced the bacterial concentrations of the pathogenic indicator bacteria *E.coli*. Future research may explore how the microbial community interactions are driving the increased emissions observed with the addition of linseed oil and confirm the emission reduction potential of bromoform in a field environment.

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