Effects of Litter Amendment in Reducing Ammonia and Greenhouse Gas Emissions from Broiler Litter

by

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Abstract

The United States is the largest poultry producer, consumer, and exporter of poultry products. The broiler industry constitutes 60% of the total poultry industry and produces approximately 9.17 billion broilers annually in the United States. The sector generates about 12.6 million metric tons of broiler litter (BL) annually. One of the major problems faced by poultry producers is the NH_3 production inside the poultry houses. The NH_3 concentration above 25 ppm harm the birds and reduces their productivity. Higher NH_3 emissions also affects the environment. Besides NH_3, BL is also a source of greenhouse gas emissions [GHG (N_2O, CO_2, and CH_4)]. The higher emissions of NH_3 and GHGs have environmental consequences such as global warming and climate change. Therefore, manure management becomes essential in reducing gaseous emissions into the atmosphere. Researchers have identified different methods to reduce NH_3 and GHG emissions from BL. The use of amendments to reduce NH_3 emissions from BL has been investigated previously; however, very few datasets exist that have reported the effectiveness of amendments to reduce GHG emissions from BL. Therefore, the objectives of this study were to evaluate the potential of biochar (B), zeolite (Z), FGD-gypsum (G), and sodium bisulfate (S) at different application rates in reducing NH_3 and GHG emissions from BL.

The BL samples used in the experiments were collected from the commercial poultry farms of Alabama. We conducted two experiments, one for NH_3 emissions and another for GHG emissions. One hundred grams of BL samples were treated with litter amendments at four different rates, kept in mason jars, and incubated at 30 ºC for 40-days. The moisture content of BL was maintained at 40% throughout the experiment. Phosphoric acid traps were used to trap NH_3 produced from BL using a 2.5 cm^3 sponge. The acidified trap was prepared by dipping the

sponge sequentially on 1M H_3PO_4, followed by 1M KOH, DI, and a mixture of 1M H_3PO_4 + Glycerol. The sponge was squeezed after dipping in each solution, and finally, 3 ml of 1M H_3PO_4 + Glycerol was added to the sponge to trap NH_3, which was hung inside the jars. The trapped NH_3 was extracted with 40 mL of 2M KCl solution and analyzed in a flow injection analyzer. A similar experimental setup was developed for GHG (N_2O, CO_2, and CH_4) measurement, where GHGs samples were taken from the headspace of the jars in a pre-evacuated vial using a 10 ml syringe at 0-minute, 60-minute, and 120-minute intervals. The gas samples were analyzed using a Shimadzu Gas Chromatography equipped with a flame ionization detector (FID) and electron capture detector (ECD).

The results showed that 13% and 17% B (w/w) reduced NH_3 emission by 40.7 and 45.6%, respectively, compared to control. Among the different rates of Z, 8% reduced NH_3 by 19.6% and 11% (w/w) reduced by 32.5%. There was no significant difference between the different rates of gypsum however, 15% G reduced NH_3 by 9.1%. Application of S at rates of 2%, 4%, 6%, and 7% (w/w) significantly reduced NH_3 emissions by 90.5, 98.6, 99.7, and 99.8 %, respectively, compared to control. Based on the percent reduction of NH_3 emission compared to control, the efficiency of the amendments can be ranked as follows: S > B > Z > G.

On the contratry, effect of amendments on GHG emissions showed a large variability in results. The application of 13% and 17% B significantly increased cumulative CO_2 and N_2O emissions whereas 5% and 9% B had no effect on GHG emissions compared to control. Zeolite and G application had no significant effect on GHG reduction. Sodium bisulfate at rates of 4, 6, and 7% reduced CO_2 emissions by 13, 29, 32 % and N_2O emissions by 33, 46, 48 %, respectively, compared to control. Among the amendments used, 4, 6, and 7 % of S were found promising for reducing GHG emissions from BL. Further studies with different combinations of amendments will provide additional insights in reducing NH_3 and GHG emissions from BL. These findings contribute to ongoing efforts to identify amendments of choice for poultry producers to minimize emissions from poultry production systems as well as environmental impacts benefiting poultry, human health, and the environment.

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List of Abbreviations

$AlCl_3$: Aluminum Chloride

B: Biochar

BL: Broiler Litter

CH_4: Methane

CO_2: Carbon dioxide

DI: Deionized water

FAO: Food and Agriculture Organization

FGD-G: Flue Gas Desulfurization Gypsum

FGD-G: G

FIA: Flow Injection Analyzer

GDP: Gross Domestic Product

GHG: Greenhouse Gas

GHGs: Greenhouse Gases

GWB: Green Waste Biochar

GWP: Global Warming Potential

H_3PO_4: Phosphoric acid

ICP-OES: Inductively Coupled Plasma Spectroscopy

KCl: Potassium Chloride

KOH: Potassium Hydroxide

N: Nitrogen

$NaHSO_4$: Sodium bisulfate

NH_3: Ammonia

N_2O: Nitrous oxide

PL: Poultry Litter

PLB: Poultry Litter Biochar

Z: Zeolite

CHAPTER 1: LITERATURE REVIEW

1.1 Ammonia (NH_3) and greenhouse gases (GHGs): An overview

United States is the largest producer, consumer, and exporter of poultry products (meat and eggs) (USDA-ERS, 2023). According to John Dunham & Associates (2022), the US meat and poultry industry accounts for $1.02 trillion in total economic output and 5.6% of gross domestic product (GDP). With the increased global population, the demand for poultry products (meat and egg) increased and led to an exponential rise in poultry industries over the last decades. The Food and Agriculture Organization (FAO) projected a 76% increase in global meat consumption and reported a doubling in poultry demand by 2050 (Alexandratos & Bruinsma, 2012). Along with meat, the poultry industry generates millions of tons of broiler litter (BL) every year. The major problem faced by the poultry industry is the manure disposal and contamination issues (Moore Jr. et al., 1995). In addition to this, agricultural sources mainly animal manure and synthetic fertilizers are found as major sources of atmospheric ammonia (NH_3) and are responsible for the increase in particulate matter, low visibility in the atmosphere, and deposition of N into the ecosystem (Behera et al., 2013). Anderson et al. (2020) stated that NH_3 emissions from poultry litter (PL) account for 27% of the total atmospheric NH_3 emissions in the United States. Furthermore, the volatilization losses of NH_3 from broiler production were reported higher in the United States than in Europe because the litter is removed from the houses and replaced with new bedding materials in with each flock of birds in Europe, while in the United States, the litter is cleaned once a year or more and with some parts of the country cleaning out once every 3 to 5 years (Anderson et al., 2020).

Greenhouse gases (GHGs) are an essential component of the earth atmosphere and play a crucial role in regulating the earth temperature by trapping heat from solar radiation

(Easterbrook, 2016; Nema et al., 2012). The solar radiation enters the earth atmosphere as short-wave radiation and is absorbed by the earth surface (Nema et al., 2012). The heated earth surface emits long-wave radiation and is absorbed by certain atmospheric gases. The molecules of gases get energized and emit heat energy in all directions into the atmosphere and are responsible for balancing temperature. The phenomenon of trapping solar radiation energy and raising temperature by certain atmospheric gases is called the greenhouse gas effect and the gases responsible are called greenhouse gases. The greenhouse effect is a necessary phenomenon for life on earth because, without it, the earth surface temperature may reach as low as -18 °C (Harris et al., 2017). The main greenhouse gases are carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O). With increased industrialization and human activities, the concentration of these GHGs exceeds the naturally occurring range and increases the greenhouse effects leading to a rise in the earth temperature and contributing to global warming and climate change (Deng et al., 2019). Even though CO_2, CH_4, and N_2O are naturally found in the atmosphere in gaseous forms, human interventions have modified their levels in the atmosphere (IPCC, 2023). Population growth, industrial growth, and forest degradation have pivotal contributions in increasing atmospheric GHGs (Watts et al., 2011).

Recent studies reported that global CO_2, CH_4, and N_2O emissions had increased by about 5.6%, 4.2%, and 2.7%, respectively, in the year from 2011 - 2020 (NOAA, 2022) and temperature rise. Based on the annual data from NOAA Global Monitoring Lab, the concentration of CO_2 in the atmosphere reached a new record of 419.3 ppm in the year 2023, a serious concern to the sustainability of the earth’s ecosystem. Moreover, the earth temperature rose by 0.06 ºC per decade and is projected to 1.1 - 5.4 ºC warmer by the end of 2100. The ability of a GHG to trap heat in the atmosphere over a specific time is described as Global

Warming Potential (GWP) and is determined by comparing the cumulative radiative energy caused by the immediate release of 1 kg of specific gas to that of 1 kg of a reference gas (Vallero, 2019). The GWP of CO_2 is considered 1 and serves as the reference for comparison where CH_4 and N_2O have GWP values of 25 and 298 times greater than that of CO_2, respectively, over a 100-year timescale (Pachauri and Reisinger, 2007; Watts et al., 2019). Besides these, fluorinated gases such as hydrofluorocarbons, perfluorocarbons, and sulfur hexafluoride have a very high potential for global warming (approximately 25,200 GWP than CO_2 by sulfur hexafluoride) (IPCC, 2021). These gases don't occur naturally and are emitted from industrial processes, and commercial as well as household uses.

The ecology, ecological systems, and socioeconomic systems of the region are seriously threatened by global climate change (Nema et al., 2012). Many observable effects have been noticed with global warming such as changes in temperature and precipitation patterns, rising ocean temperatures, sea levels, acidity, melting glaciers, and variations in the severity, length, and frequency of severe weather (IPCC, 2021). Moreover, in many parts of the United States, harmful ground-level ozone is expected to worsen due to GHG emissions (USEPA, 2023). Therefore, it is essential to address global warming and climate change issues for the benefit of the entire planet.

1.2 Agriculture sector as a source of ammonia and greenhouse gas emissions

The agriculture industry is extremely vulnerable to the effects of climate change, but it is also a significant contributor to NH_3 and GHG emissions into the atmosphere. Over the past century, there has been a significant increase in atmospheric NH_3 levels, endangering human health as well as the quality of the environment (Graaf et al., 2022). Approximately 80% of NH_3 contribution to the atmosphere is from the agricultural sector globally (Behera et al., 2013). The primary source of NH_3 emissions from the agriculture sector includes animal waste from animal husbandry and NH_3 - based fertilizers. In the United States, a total of 4.96 million tons of NH_3 was emitted into the atmosphere in 2023 (Statista, 2023). As NH_3 concentrations are highly variable spatially and temporally it is difficult to reliably measure the change in NH_3 levels in the atmosphere. Similarly, due to the rising demand and need for food production to meet the demand of the expanding population, global agriculture's greenhouse gas (GHG) emissions have increased by more than 20% since 1990 (Tiseo, 2023). The Agricultural sector contributes 593.4 MMT CO_2 Eq. which is 9.4 percent of total U.S. GHG emissions (USEPA, 2024). The manure management contributes to 13.8% of CH_4 and N_2O emissions of the total agricultural sector in the United States. The primary emission sources of GHGs in agriculture are manure spreading, housing facilities, storage facilities, enteric fermentation during animal grazing, and animal waste (Meda et al., 2011). Moreover, anthropogenic activities such as the burning of coal, oil, and gas produce CO_2 and N_2O gases, deforestation increases CO_2 levels, livestock farming produces a significant amount of CH_4, and agricultural nitrogen fertilizers are sources of N_2O emissions (USDA, 2016). The emission levels of CH_4, and N_2O from livestock are influenced by environmental factors (Del Grosso et al., 2008). The gaseous loss of nitrogen from manure and urea-based fertilizers primarily occurs in the form of either NH_3 or N_2O. Similar to ammonia, the majority of N_2O emitted from agriculture is due to nitrogen fertilization, which enhances the availability of substrate for nitrification and denitrification mechanisms (Jarecki et al., 2008). Therefore, managing agricultural practices can contribute significantly to the reduction of NH_3 and GHG, and is important to study the measures to minimize NH_3 and GHG emissions.

More specifically, the poultry industry is a significant contributor of NH_3 and GHG emissions from the agricultural sector. According to USDA-NASS (2023), the USA produced

9.16 billion broilers and that number of broilers can produce approximately 0.3 million M tons of NH_3. In the case of GHG, about 829 million metric tons of CO_2 equivalent is produced from the chicken industry globally (Statista, 2017) and other poultry adds an additional 82 million metric tons of CO_2 equivalent. According to (Watts et al., 2019), the emissions of NH_3 and GHG from broiler houses arise from the management of litter and environmental factors, bird age, litter density, and litter temperature. Overall microbial activity has all been linked to increases or decreases in gaseous emissions (Miles et al., 2016; Watts et al., 2019). Various factors such as the atmospheric concentration of oxygen, the type of organic matter present, and the chemical makeup of manure influence the microbial population. The increased production of NH_3 and GHG is not only a threat to the environment but also impacts poultry productivity and economic loss (Anderson et al., 2021). Even though the poultry industry's contribution to NH_3 and GHG is relatively small when compared to cattle and other sources, reducing NH_3 and GHG emissions from poultry houses can be an additive effort in reducing global warming and environmental protection. The strategies to minimize gaseous emission start with Broiler litter (BL) management.

1.3 Mechanism of NH_3 production from broiler litter

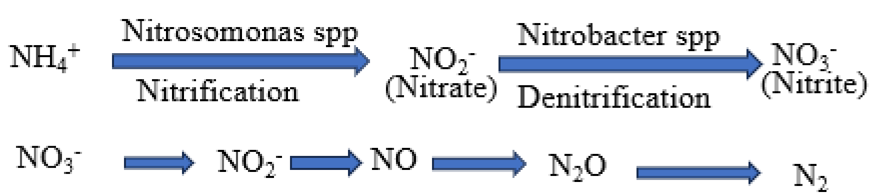
Poultry feeds are a rich source of amino acids and protein (nitrogenous sources). Approximately 1/3 of the N in feed is utilized by poultry in growth and poultry products (meat and eggs) and the excess of the protein and amino acids from the feed of poultry is excreted in manure and have a considerable amount of N in it (Ritz et al., 2004). Therefore, BL is considered a good source of nutrients. The fresh excreta inside the poultry house contains uric acid (in birds) and urea (in animals) which contains 70% of total N and up to 97% of urinary N (Koerkamp, 1994; McCrory & Hobbs, 2001). The urea and uric acid undergo microbial and physio-chemical decomposition by microbes (by the activity of enzyme uricase) in the presence of oxygen and water producing CO_2 and NH_3. *Bacillus pasteurii* is one of the primary uricolytic bacteria that facilitate NH_3 production by the decomposition of uric acid present in a litter (Ritz et al., 2004). The optimum microbial population is observed in pH around 8.5 and therefore accelerates microbial activity producing uricase and urease enzymes. The uricase enzyme helps in the conversion of uric acid into allantoin ($C_4H_6N_4O_3$) and is later converted into glyoxylate ($C_2H0_3^-$) and urea (CH_4N_2O). In the presence of moisture, urease breaks urea into NH_3 and CO_2 where the decomposition of manure by microbes takes place at either aerobic or anaerobic conditions. As NH_3 doesn't have ionic charges that is why it is readily released in gaseous form into the atmosphere.

The primary factors contributing to NH_3 formation are temperature, moisture, pH, and nitrogen content of litter (Moore Jr. et al., 1995; Ritz et al., 2004; Swelum et al., 2021). Temperature, pH, and moisture need to be in a favorable range to facilitate microbial activity which is responsible for enzymatic processes. At high temperatures inside houses, both the microbial activity and NH_3 production increase, where, with a 1 to 2°C increase in temperature has a larger difference in NH_3 produced (Visek, 1968). In general, the pH of poultry manure excreted is between 7.5 to 8.5. Ammonia production is negligible at pH less than 7.0 and accelerates when pH is 8.0 or greater (Carr et al., 1990; Reece et al., 1979). As observed, higher temperature favors microbial activities and NH_3 formation in the litter Li et al. (2013). The most important component in managing poultry houses is the maintenance of litter moisture content of around 20 to 30 which helps in a healthy environment and flock productivity (Linhoss et al., 2019).

1.4 Mechanism of GHG production from poultry manure

Production of methane is accomplished by a process called anaerobic decomposition of organic substances (livestock manure) where (biological oxidation of C in the absence of oxygen). This requires two main groups of anaerobic bacteria called ‘acid formers’ and ‘methane formers/methanogenic archaea’. The acid-forming bacteria convert manure to organic acids and then methane forming bacteria convert organic acids to CH_4 and CO_2. According to Cornejo & Wilkie, (2010) the ideal environment for the production of CH_4 requires an anaerobic (based on water), has a high concentration of nutrients for bacterial development, and warm environment. Furthermore, methane production is best suited in pH between 6 and 8 (around 7), and beyond those ranges stored manure may reduce CH_4 production (Berg et al., 2006).

The various pathways of N_2O formation could describe emissions from the manure, but the potential for N_2O emissions will depend on manure management practice (Broucek, 2020). Nitrous oxide production occurs by processes called nitrification and denitrification (Broucek, 2018). During composting and application of manure, it is subjected to a supply of oxygen from the atmosphere and the nitrifying bacteria converts ammonium to nitrate through the nitrification process in aerobic conditions. Also called nitritation, in which NH_4^+ is oxidized to NO_2, and nitratation, in which NO_2 is further oxidized to NO3, are the two phases of nitrification, which is the aerobic oxidation of NH_4^+ to NO_3^- driven by chemoautotrophic bacteria (Paul, 2007).



In the pathway to nitrification, intermediate compounds such as nitrite are produced, and N_2O is emitted as a byproduct. When there is fluctuation in oxygen level, due to high moisture (waterlogged) or compaction, denitrifying bacteria convert nitrate to nitrogen through the denitrification process. In the anaerobic process, N_2O is an intermediate byproduct. The microbial population and enzyme or nitrifying and denitrifying bacteria, soil aeration, temperature, moisture content, pH, organic matter, C/N ratio, and soil management techniques are some of the elements that influence the production of N_2O (Awale & Chatterjee, 2017; Snyder et al., 2009).

Carbon dioxide gas is produced from both biological and non-biological processes. The biological process involves microbial respiration. At higher temperatures, non-biological processes such as chemical oxidations may produce CO_2 (Rey, 2015). Many factors such as temperature and moisture content, and microbial population have a significant influence on CO_2 production. Research by Wiant (1967) found that respiration rate rapidly increases logarithmically between 20 and 40 degrees Celsius, declines beyond 50 degrees Celsius, and CO_2 production ceases at 10 ºC or below.

1.5 Strategies to reduce ammonia and GHG emissions from manure

The environmental concern, human and animal health, and productivity associated with the higher NH_3 concentration from the poultry industry had set common goals among researchers and producers to reduce NH_3 volatilization. Global warming and climate change due to the effect of GHG emissions drive similar concerns. The most common strategies include ventilation, dietary manipulations, and manure management (Moore et al., 1996; Swelum et al., 2021). Installation of proper ventilation is reported to be useful to reduce gaseous concentration inside the house. However, this method facilitates the direct entry of NH_3 and GHGs into the atmosphere, which is not desirable. The dietary strategies are focused on reducing N content in feeds and optimizing N digestion to reduce N content in excreta and therefore need scientific food formulations and technologies (Cappelaere et al., 2021). Manure management is the most common method to reduce NH_3 and GHG formation and there are numerous amendments that have been used in an attempt to decrease NH_3 and GHG formation and volatilization. Strategies to reduce gaseous production and emissions are mainly focused on the retardation of microbial activities responsible for NH_3 and GHG (Arogo et al 2006). The principal characteristics of such amendments are acidifying agents, hygroscopic, deodorant, and microbial enzyme inhibitors. Many research has been conducted in the past to identify suitable methods to reduce NH_3, and GHG emissions from PL. Most commonly tested amendments are primarily categorized into two categories based on their characteristics: a) amendments inhibiting microbial growth which slow down the enzymatic activity of microbes and retard gaseous formation and b) amendments combine with release gas (such as NH_3) and neutralize it (Carlile, 1984). Acidifying agents have the property of lowering the pH of manure and retard microbial activity reducing emissions of gases.

1.6 Commonly used amendments for NH_3 reduction and their mechanisms

Litter amendments such as acidifiers, chemical absorbents, and chemical/biological inhibitors have become the most widely accepted method for reducing NH_3 emissions from poultry facilities. The primary concern about the rate of amendments can be economical and environmentally sound.

1.6.1 Alum for NH_3 reduction

Alum $[Al_2(SO_4)_3 \cdot 14H_2O]$ is a double-hydrated salt that produces acid compounds and inhibits ammonia production by increasing acidity and lowering pH. Hydrogen ions (H^+) produced from the dissolution of alum react with NH_3 to form nonvolatile ammonium (NH_4^+), which reacts with sulfate to form ammonium sulfate (DeLaune et al., 2004). Ammonia volatilization was reduced up to 99% and N retention was doubled when treated with alum in the

PL (Moore Jr. et al., 1995, 1998; DeLaune et al., 2006). Furthermore, alum has microbial-inhibiting properties. Cook et al. (2008) reported that PL treated with alum reduced the bacterial population by 50% and bacterial urease production by 90% during the four-week study period. In the same experiment, after 16 weeks of alum-treated litter, the fungal population increased by 3 folds compared to non-treated litter. This finding revealed that retreatment of PL with alum would be necessary for long-duration growing poultry (for example, egg-laying hen).

The efficacy of alum to reduce NH_3 differ depending on application rates. Application of 4 and 8 g of alum/100 g of PL resulted in a 77 and 96% reduction in NH_3 volatilization, respectively (Moore et al., 1996). Alum available in liquid form was also found effective in NH_3 loss. Ammonia loss was decreased by 89 and 94%, respectively, when liquid alum was treated at rates of 8.66 g and 17.3 g/100 g of PL (Choi & Moore, 2008). Another relevant research by Moore et al. (1995) studies the effect of alum at different rates on NH_3 volatilization in PL. They had the treatments with control, 100g alum per kg litter, 200 g alum per kg liter, 100 g alum per kg liter + $CaCO_3$, and 200 g alum per kg litter + $CaCO_3$. The nitrogen losses at 42 days of alum treatment with 100 g and 200 g alum in combination with 100 g $CaCO_3$ were found 24 and 57% lower than the control, respectively. Treatment of alum alone applied at rates of 100 g and 200 g per kg litter reduced the cumulative N loss at day 42 by 36 and 99% compared to the control. Additional research by Zhang et al. (2011), also reported a 30% decrease in NH_3 concentration inside broiler houses when treated with alum at a rate of 1 kg/m^2 and maintained lower pH during the 35-day study period.

1.6.2 Biochar for NH_3 reduction

Biochar is a carbon-rich substance produced by burning plant biomass at higher temperatures in the absence of oxygen called pyrolysis (Kalus et al., 2020; Latawiec & Kuboń, n.d.; McCrory & Hobbs, 2001; Ritz et al., 2004). Biochar has some desirable physical, chemical, and structural properties and has been utilized as soil as well as manure amendments. Biochar is black, lightweight, fine-grained, has a greater surface area, and can absorb a larger amount of moisture (Królczyk et al., 2014). Ammonia loss from manures is reduced by biochar because of its adsorption properties and the presence of carboxylic groups (-COOH) formed by bio-oxidation at higher temperatures which binds NH_4^+ and reduces volatilization (Dias et al., 2010; Janczak et al., 2017).

Janczak et al. (2017) studied the relationship between the application rate of biochar in the mixtures and NH_3 emission and found that NH_3 emission decreases with an increase in biochar application rate. Furthermore, they studied the effectiveness of biochar mixture with low C/N composting material in PL (treatments: pine shaving, pine shaving + 5% biochar, and pine shaving + 10% biochar) and reported that pine shavings + 5% biochar and pine shaving + 10% biochar treatments during composting and reported NH_3 reduction by 30% and 44%, respectively. Compelling results were reported by different researchers on biochar effectiveness in reducing NH_3 from manures as well as sewage sludge. Malinska et al. (2014) studied the effect of biochar treatment on composting of sewage sludge mixed with straw in a laboratory and found that the addition of 4% biochar reduced NH_3 volatilization during the first week of composting. Steiner et al. (2010) reported a reduction of 58.0% NH_3 emissions during the composting of PL mixed with 20% biochar.

A research by Chung et al. (2021) studied the influence of rice husk biochar treatment on NH_3 and other GHGs in in-vessel poultry manure composting and found that the addition of biochar (3%, 5%, and 10%) raised the thermophilic temperature with a significant reduction in NH_3 and CO_2 emissions. Furthermore, treatment of 10% biochar showed the most significant effects on the quality of the compost nutrients. The effect of biochar type on NH_3 emission was studied by Agyarko-Mintah et al. (2017) and two types of biochar green waste biochar (GWB) and poultry litter biochar (PLB) were treated during composting which have mixture of straw and PL at ratio of 1:5 dry matter basis. The biochar was added at 10% (w/w) to straw/ PL and they reported a 51% reduction in N loss and a 60% reduction in NH_3 losses during composting with GWB and retained higher N in manure as compared to control. The PLB was not found as effective as GWB during composting in NH_3 reduction.

1.6.3 Sodium bisulfate for NH_3 reduction

Sodium bisulfate is a hygroscopic nature, granular acid salt, which has been widely used to decrease pH in various agricultural sectors, industrial applications, and food products (Sun et al., 2008; Sweeney et al., 1996, 2000). It has the property of inhibiting bacterial growth levels and is also effective in reducing NH_3 from poultry manure (Sun et al., 2008). Sodium bisulfate helps to reduce pH and moisture content in BL and retard NH_3 formation from litter. Proch et al. (2021) found a decrease in NH_3 emission with the increased level of S treatment.

Li et al. (2013) studied the effectiveness of treating Sodium bisulfate at two rates 183 and 366 g/m^2 applied every weekly basis and found a reduction (14% to 64.5% reduction) in cumulative NH_3 emission rate with 366 g/m^2/week than 183 g/m^2/week (0 to 55.0% reduction on NH_3) treatments in laboratory conditions. Further, the cumulative NH_3 emission was reduced by 51.7% with 244 g/m^2/2-week in a three-flock period under field conditions. Moreover, Sahoo et al. (2017) found that the addition of sodium bisulfate at the rate of 25g/sq.ft. was found more effective in controlling pH, microbial load, and ambiance in broiler houses compared to alum (at the rate of 90g alum /sq.ft.). A study by Pope & Cherry, (2000) found that poultry litter treatment (PLT) treated at a rate of 0.24 kg m^{-2} lowered NH_3 concentrations up to

55.0% over a 2-week study period. Similarly, another research by Li et al. (2006) reported that PLT applied at rates of 0.5 to 1.5 kg m^{-2} reduced NH_3 emission by 74.0 to 92.0% up to 7 days. The NH_3 emission immediately after the application of PLT was dropped up to 72.0% and the overall reduction was 57.0%, however, the effectiveness of PLT after 11 days was reduced (Wood & Heyst, 2016). The result showed that PLT reduced NH_3 emission largely in the initial days and the potential to reduce NH_3 is reduced with an extended period of the experiment. The mechanism of sodium bisulfate in reducing NH_3 emission from manure is mainly governed by its chemical composition and capacity to lower pH. Sodium bisulfate ($NaHSO_4$) is an acidifier, when applied to litter it breaks into sodium, hydrogen, and sulfate ions. The hydrogen ions help to lower the pH and bind ammonium ions thereby preventing the formation of NH_3 (Li et al., 2008; Terzich et al., 1998) as previous research found that the concentration of NH_3 is low when pH is lower than 7 (Blake et al., 2009; Reece et al., 1980). Li et al. (2013) studied the effectiveness of treating $NaHSO_4$ at two rates 183 g m-^{2} and 366 g m^{-2} applied every week and found a reduction in cumulative NH_3 emission ranging between 14.0% to 64.5% and 0 to 55%, respectively, in laboratory conditions. Further, they conducted a field experiment to quantify and delineate the efficacies of frequent litter amendment application with 244 g m^{-2} per 2 weeks in a three-flock period. Additionally, Blake et al. (2009) also reported that the use of PLT as a litter amendment at three different ranges; 24.4, 48.8, and 73.2 kg per 100 m^2 reported the reduction of NH_3 emissions at a higher rate.

1.6.4 Zeolite for NH_3 reduction

Zeolite [(Na_4K_4) (Al_8Si_{40}) $O_{96}\cdot 24H_2O$] are complex chemical compounds that have a three-dimensional linked tetrahedral structure and contain four oxygen atoms each oxygen atom shared by two tetrahedra (Schneider et al., 2017). Due to their typical molecular structure, natural zeolites have been used as ion exchange and adsorbent, pollution abatement, animal nutrition, and in gas separation. More specifically, the greater ion-exchange property of zeolite makes it useful in retaining pollutants and enhancing environmental quality in animal husbandry. Many species of zeolites are found such as Chabazite, Clinoptilolite, Faujasite, Analcime, Heulandite, Laumontite, Mordenite (Schneider et al., 2017). These species differ in characteristics by their chemical formula, void volume, pore size, thermic stability, and ion exchange. In the poultry industry zeolites are used in the diet of laying hens and are found effective to improve egg quality, pH of excreta, and nitrogen digestibility.

Zeolites reduce NH_3 volatilization from BL because of their higher affinity with NH_4^+ ion (Li et al., 2008). Zeolite treatment at low (2.5%), medium (5.0%), and high (10%) doses on laying hen manure reduced NH_3 volatilization by 36, 62, and 92%, respectively in 7 days of litter storage. Moreover, the addition of 12% Z by weight and chopped straw mixture in the air stream trapped greater than 90% of N loss during the composting of pig slurry (Bernal et al., 1993). About 44% reduction in NH_3 emission was reported by Kithome et al. (1999) during composting of PL with surface application of 38% zeolite by weight. Natural Zeolite has a cation-exchange material that has a high affinity for NH_4^+ ions which can adsorb NH_3 (Mumpton & Fishman, 1977). They reported that 35% NH_3 reduction can be done by the addition of 5 kg/m^2 (1024 lb/1000 ft^2) of zeolite to broiler litter. Similarly, Nakaue et al. (1981) reported a reduction of up to 35% NH_3 loss by treatment of 5 kg/m^2 (1024 lb/1000 ft$_2$) of zeolite to BL compared to control.

1.6.5 Gypsum for NH_3 reduction

Gypsum ($CaSO_4 \cdot 2H_2O$) is a sulfate mineral, also called calcium sulfate dihydrate, and is commonly used as a soil amendment (fertilizer) (Klein et al., 1985). Gypsum consists of layers of calcium (Ca^{2+}) and sulfate (SO_4^{-2}) ions and is a good source of both Ca and S nutrients for plants and is also used to treat sodic soil Tubail et al. (2008). It also helps to reduce subsurface acidity, improves deep rooting, and increases yield. Nowadays gypsum has been used to reduce NH_3 losses due to its hygroscopic nature which can absorb excess moisture from litter and retard the rate of NH_3 formation (Tubail et al., 2008). Iowa State University Extension Service, (2004) reported that gypsum amendment in dairy manure storage reduced NH_3 loss by 14%.

Oliveira et al. (2003), conducted an experiment to examine the effect of gypsum on NH_3 volatilization from PL. They studied the dry matter content, pH, and NH_3 volatilization from PL treated with litter additives such as aluminum sulfate, gypsum, and hydrated lime. Their findings showed that gypsum when treated in PL had the lowest NH_3 volatilization during 42 days of study period compared to other treatments. Gypsum lowered the pH of PL from 8.04 to 6.97 that attributed to the reduction of NH_3 formation. Other compelling results from Koenig et al. (2005) describe that gypsum when added at the time of composting PL with wooden chips at the rates of 4% and 12% were capable of reducing NH_3 emission significantly from PL. Ammonia concentration, water content in the litter, and pH were significantly lower in the gypsum-added treatments as compared to shavings treatment at both 21 and 40 days.

Tubail et al. (2008) studies the effect of FGD gypsum by adding different rates of gypsum in dairy manure. The treatments were dairy manure compost, dairy manure compost + 6% gypsum, dairy manure compost +13% gypsum, and dairy manure compost + 23 % gypsum. The effect of gypsum was found to be very significant as 2.0 to 2.4 times less NH_3 was volatilized from the gypsum treatments versus dairy manure without gypsum. The results showed that the mixture of dairy manure with 23 % gypsum was found to be more effective in reducing NH_3 loss from manure. The addition of FGD gypsum to litter decreased pH immediately due to the precipitation of calcium carbonate ($CaCO_3$) from gypsum-derived calcium and litter bicarbonate (Tubail et al., 2008).

1.6.6 Aluminum chloride for NH_3 reduction

Aluminum chloride ($AlCl_3.6H_2O$) is an anhydrous (no water) chemical compound that appears as a white-to-gray powder with a pungent odor. This also showed potential to be used as an amendment to reduce NH_3 emissions from manure. Treatment of aluminum chloride in manure lowers the pH from 7.48 to 6.69 in control manure pits containing 0.75% $AlCl_3$ (Smith et al., 2004). The application of aluminum chloride lowered atmospheric NH_3 concentrations by 97.2% in 42 42-day study (Do et al., 2005). Chemical amendments were added to rice bran at 1.15 kg/m^2 (or 0.287 kg of $CaCO_3$/m^2), whereas potassium permanganate ($KMnO_4$), was added at 0.115 kg/m^2. (Do et al., 2005) reported that the cumulative NH_3 concentrations were reduced by 86.2, 78.7, 75.5, and 69.0% when a litter was treated with aluminum sulfate (alum), alum + $CaCO_3$, aluminum chloride + $CaCO_3$, and $KMnO_4$, respectively, compared to control. Moreover, the addition of a phytase diet and $AlCl_3$ at the rate of 0.75% in poultry feed showed a reduction in NH_3 production compared to the control diet without $AlCl_3$. Smith et al. (2004) which indicates that aluminum chloride can also be used as feed additives to lower NH_3 emission from BL.

1.6.7 Alum Mud Litter Amendments (AMLA) for NH_3 reduction

Alum mud is a residual obtained from alum manufacturing. Mixing alum mud with sulfuric acid, bauxite, and water forms AMLA (Moore, 2016). The mixture of 57% alum mud, 4% bauxite, and 39% sulfuric acid was reported as the most cost-efficient (at least 50% less) than using dry alum (Anderson, 2020). A study by (Moore, 2016) reported a 62 to 73%

reduction in NH_3 loss from different treatments in their study using a different mixture of alum mud which can be used at the industrial level for reduction of NH_3 in poultry houses.

1.6.8 Other amendments for NH_3 reduction

Besides these, some other amendments that have been reported in the literature are paraformaldehyde ($OH(CH_2O)_n H$ (n = 8 - 100), superphosphate, and phosphoric acid (Carlile, 1984b). Paraformaldehyde is used as a disinfectant because of its antimicrobial properties. (Seltzer et al., 1969), studied the effect of paraformaldehyde treatment in litter and its effect on NH_3 production. Measurement was based on atmospheric pH due to NH_3 concentration. Their study revealed that treatment of paraformaldehyde at a rate of 4.5 kg^{-1} 26 sq. m. reduced the atmospheric pH to 7 (equivalent to 5 ppm NH_3) which are not detectable by a human. They reported that after 21 days the NH_3 concentration rapidly increased to 100 ppm and suggested further re-application of treatment. This practice questions the general safety of formaldehyde as such chemicals are not eco-friendly and not desirable due to its carcinogenic nature.

Reece et al. (1979) studies the superphosphate and phosphoric acid effects on NH_3 emission. They have the treatment of 2.5 molar solution phosphoric acid sprayed on litter surface at the rate of 1.7 L/m^2 and mixing with hardwood shavings and superphosphate treatments applied at the rate of 0.5 kg/m^2 and 1.0 kg/m^2 and mixing with litter. They reported that phosphoric acid was found more effective in reducing NH_3 and lowering litter pH than 2 other superphosphate treatments. However, both superphosphate and phosphoric acid were effective up to 17 days and are ineffective afterward and need re-treatments. The use of acetic acid (CH_3COOH) and propionic acids ($C_3H_6O_2$) has shown some positive results. Parkhurst et al., (1974) treated pine sawdust litter with 60% acetic and 40% propionic acid at rates of 1 and 3% (w/w) and litter pH was measured where there was a significant pH drop for 2 weeks and 3 weeks for treatments 1 and 3%, respectively. Furthermore, retreatment was suggested after 6 weeks as there was a rise in litter pH. Several other chemicals such as sorbic acid ($C_6H_8O_2$), gentian violet, and calcium propionate have been treated with PL aiming for a reduction in NH_3 production either by reducing litter pH or by inhibiting microbes' activity (Carlile, 1984).

Researchers have identified several advantages of using amendments besides reducing NH_3 concentrations. Some of the benefits of using acidifying agents are documented in the literature. Acidifying agents, when hydrated, produce hydrogen ions (H^+) that attach to NH_3 to form NH_4^+. As a result of the reaction, the amount of NH_3 emitted from the manure is decreased, thereby preserving the N content of the manure and adding manure value (Moore et al., 2000). The treated litter had reduced pathogens and metals (Moore et al., 1998). Furthermore, reduction in the incidence of ascites (water belly) (Terzich et al., 1998), reduction of respiratory lesions (Terzich et al., 1998) reduction of water-soluble phosphorus concentrations in a litter (Moore et al., 1996) have been documented. The primary goal was to reduce NH_3 emission and improve poultry productivity and a healthy environment which has been achieved with varying levels of success at different rates and time. Although amendments are found to be effective in reducing NH_3 emission from BL and manure there are not sufficient studies about their economic aspects. Moreover, many of the chemical amendments need to be retreated after few weeks as their effectiveness decreased over time, more research is needed to identify the effect of multiple times application of amendments on litter such as increase in metal concentrations to toxicity levels. Therefore, there is still not enough information's to make conclusions on amendments which are environmentally acceptable and at the same time economically viable to the producers and poultry facilities.

1.7 Commonly used amendments for GHGs reduction

1.7.1 Biochar for GHG reduction

Biochar is gaining attention among the scientific community due to its several physiochemical properties and its applicability for broader purposes. The feedstock used to produce biochar as well as the pyrolysis temperature are important in governing the physiochemical characteristics of biochar (Akdeniz, 2019; Yin et al., 2021). The physiochemical properties make biochar different in its capability to reduce GHG emissions.

Biochar mechanisms in reducing N_2O emission during composting of manure are described in different prospectives. Some studies have indicated that biochar reduced the availability of inorganic N (altered mineral N content that can be utilized by nitrifying bacteria and denitrifying bacteria) by capturing NH_4^+ and NO_3^- leading to less activity of those bacteria, lowering N_2O emission (He et al., 2019; Xiao et al., 2017). The functional group on the surface of biochar can absorb N_2O and reduce it to N_2 through biological or abiotic reactions (Harter et al., 2016). Biochar contains (C = O), oxygen-containing functional groups, such as phenolic hydroxyl functional groups, which can provide electrons to promote N_2O reduction whereas, carboxyl, carbonyl, and quinone functional groups can compete for electrons with microorganisms and consequently inhibit the reduction of N_2O to N_2 (Yuan et al., 2021).

In the case of methane, the addition of biochar improves structure and porosity enhancing aeration and lower anaerobic spots that can lower oxidation-reduction potential which retards the activity of methanogens and reduces CH_4 emissions (Sonoki et al., 2013).

Researchers have identified biochar significantly affects CO_2 emissions and results were not consistent between the studies. For example, a study by Wang et al. (2018) added biochar on pig manure which reduced CO_2 emissions by 26.1%. Another study by Mao et al. (2018)

reported that addition of biochar increased CO_2 emissions by 53.2% during pig manure composting. According to Liu et al. (2016) biochar can increase the sequestration of exogenous organic matter during composting, which lowers CO_2 emissions. Alternatively, according to Mao et al. (2018), biochar can improve the habitat for microorganisms, allowing them to be more active and accelerating the degradation of organic matter, which increases CO_2 emissions.

Dang et al. (2024) investigated how gaseous emissions from PL composting were affected by biochar made of wood chips, cornstalks, and rice husks. According to their findings, at a 10% application rate, all biochar treatments decreased CH_4 and N_2O by 18.9 - 27.5% and 35.7 - 52.8%, respectively. Similarly, a study by Chen et al. (2017) examined the impact of biochar on CH_4 emissions during the composting of layer manure. They added ten percent biochar made from coir, cornstalks, bamboo, and wood in layer manure. In comparison to the control, the total CH_4 emission for cornstalk, bamboo, wood, and coir biochar was much lower at 26.1, 15.5, 22.4, and 17.1%, respectively. However, the variability could be associated with pyrolysis temperature as well. The potential of biochar to reduce GHG showed both positive, neutral, and negative responses.

Moreover, the present research findings have identified that the use of biochar in combination with other substances has enhanced its efficacy in reducing GHG emissions than biochar alone (Awasthi et al., 2016; Wang et al., 2018; Wang et al., 2022). Wang et al. (2018) used a 2:1 ratio of swine manure and wheat straw for composting and treated with different rates of biochar, zeolite, Wood Vinegar (WV), and their different combinations. The combination of biochar, zeolite, and WV reduced CO_2 emission by 33.9 - 46.98%, CH_4 by 50.39 - 61.15%, and N_2O by 79.51 - 81.1% compared to control. Similarly, research conducted by Wang et al. (2022) investigated the use of biochar (5%) mixed with different rates of WV (0.5%, 1% and 1.5%) in swine manure. The addition of biochar with different rates of WV decreased CO_2, CH_4, and N_2O emissions by 1.38 - 15.39%, 16.98 - 62.73%, and 4.47 - 19.91%, respectively. Moreover, the addition of WV with biochar reduced cumulative N_2O emission by 0.78% (biochar + 0.5% WV), 19.9% by biochar mixed with 1% WV, and 2.55% by biochar mixed with 1.5% WV compared to treatment with biochar alone. The incorporation of WV could enhance the absorption efficiency of biochar (B), thereby reducing CO_2 emissions. Consequently, the CO_2 released by the B + WV treatments was lower than that released by the biochar treatment alone. The cumulative CO_2 emissions for the treatments B, B + 0.5% WV, B + 1.0% WV, B + 1.5% WV, and B + 2.0% WV decreased by 1.39%, 15.39%, 4.03%, 13.89%, and 8.22%, respectively. Awasthi et al., 2016, studied the effect of biochar amendment, mixing with zeolites, and 1% lime in sewage sludge during composting. They used 12% biochar and mixed with 5 different rates of zeolites 10, 15, 20, and 30%, each treatment with 1% lime on dewatered fresh sewage sludge (DFSS). Results showed that the combination of B + Z significantly reduced CH_4 emissions by 92.85 to 95.34% and reduced N_2O emissions by 95.14 to 97.28% in comparison to the control. Moreover, the treatments with biochar + lime lowered CH_4 by 55.17 - 63.08% and N_2O emissions by 62.24 - 65.53%, when compared to the control. Another study by Yuan et al. (2017) used a mixture of hardwood sawdust + rice hull biochar (produced at low temperature) at a ratio of 2:1:7 (biochar: sawdust: manure) and another treatment with chicken manure + sawdust (3:7 ratio). They reported that biochar + chicken manure compost reduced N_2O emission by 27% compared to control. Similarly, the biochar in chicken manure reduces CO_2 emissions by 35%.

Variations in the characteristics of biochar can impact the direction and magnitude of greenhouse gas emissions. The C: N ratio of biochar, for instance, has no effect on greenhouse gas emissions; nevertheless, feedstock source, temperature during pyrolysis, and pH of the biochar can all have a major impact on soil CO_2, CH_4, and N_2O fluxes following the addition of biochar. He et al. (2017) found that the addition of 3% (dry weight) holm oak biochar has no impact on nitrogen losses or gas emissions from poultry manure compost. The results were not consistent for biochar applied into soils. According to Cong et al. (2018) there can be a wide range in the response of soil CH_4 flux following biochar amendment, leading to both increases and decreases.

1.7.2 Zeolite for GHG reduction

Zeolite has also been gaining widespread attention as an efficient amendment for reducing several environmental pollutions. The principal mechanism of zeolite to reduce GHG emissions in manures is due to its affinity for NH_4^+ - N and increased ammonium content which restricts the activity of methanogens bacterial and reduces CH_4 (Sah et al., 2022). However, zeolite alone has not been investigated well on its effect on reducing GHG from manures. More research is focused on the combination of zeolite with biochar or other substances. Wang et al. (2012) studied the effect of reed straw (10%), zeolite (12%), and superphosphate (5%) on GHG emissions from duck manure during storage for 46 days. Their study showed the cumulative N_2O emissions increased up to 25% as compared to the control. Moreover, the addition of reed straw, zeolite, and superphosphate, alone or in combination, was found effective and significantly lowered the cumulative CO_2 emission by 13 - 32% whereas superphosphate alone was least effective in reducing CO_2 emission.

Another study by Peng et al. (2019) investigated the effect of zeolite, superphosphate, and ferrous sulfate in different combinations on reducing GHG emission. The PL was mixed with zeolite, superphosphate, and ferrous sulfate and subjected to window composting for 46 days, the global warming potential was reduced by 21.9%, 22.8%, and 36.1% by zeolite, ferrous sulfate, and superphosphate, respectively. The zeolite (350 kg), superphosphate (350 kg), zeolite + Ferrous sulfate (350 kg + 210 Kg) FL, or zeolite + superphosphate + ferrous sulfate (140 kg + 210kg + 210 kg) reduced the cumulative CO_2 emission by 7.8%, 27.6%, 12.2%, and 27.5%, respectively compared to control. The cumulative CH_4 emissions were reduced by 56% by zeolite, 50.6% by ferrous sulfate + zeolite, 62.3% by superphosphate, and 73.6% by zeolite + superphosphate + ferrous sulfate. Moreover, the N_2O emission was negligible due to higher temperatures above 60 °C during the composting period. In a study, the use of clinoptilolite (zeolite) as litter amendments were evaluated on the concentrations and emissions of NH_3, N_2O, CO_2, and CH_4 in a breeding hen house under Mediterranean climate conditions where two similar types of poultry houses were selected and one house was control and another with clinoptilolite additives during winter for 20 days (Pereira et al., 2019). The clinoptilolite applications on the litter of a breeding hen house decreased N_2O by 34% and found no effects on CO_2. The CH_4 concentrations were found to be below the detection limit.

In comparison to the control, the co-application of zeolite and biochar decreased NH_3 and CH_4 emissions by an average of 87 and 58%, respectively in organic farming systems. In contrast to the control, CO_2 emissions rose by 104%. Interestingly, a 12% decrease in CO_2 emission was noted in the Z treatment, whereas the increases in CO_2 emission for B1Z, B2Z, and B3Z were 42, 110, and 160%, respectively. 15.7, 15.5, and 11.9 mg kg–1 of soil were the relative amounts of CH_4 emissions from the above-mentioned treatments. Biochar was added, and this increased the CO_2 emission; emission fluxes appear to be directly correlated with the amount of biochar applied. The maximum CO_2 emission was measured at 9 t ha^{-1} of biochar and 5 t ha^{-1} of zeolite, with a 165% increase over control in year 1 and 154% in year 2 (Ali et al., 2022).

1.7.3 Gypsum for GHG reduction:

Gypsum is the oldest soil amendment and a significant source of calcium and sulfur that is frequently used for crops and to restore saline-sodic soils that are suffering from salt stress. The effect of gypsum on manure sources is studied relatively less and mostly focused on NH_3 and is limited research on the effectiveness of gypsum on GHG emission from manure. The application of gypsum on wetland rice fields and its influence on CH_4 emission was researched by (Gon & Neue, 1994). They found that gypsum-adjusted plots at a rate of 6.66 tons/ha reduced CH_4 by 55 - 70% compared to unamended soils. Gypsum application and its impact on CH_4 emissions from pig slurry was examined by (Berg & Model, 2008). They discovered that several dosages of gypsum 1, 2, and 3 kg per vessel (75 kg/vessel), reduced CH_4 emissions. About 50% less CH_4 was released when 4% gypsum by mass was added. A study by Watts et al. (2019) stated that the use of flue gas desulfurization (FGD) gypsum has little effect on GHG emissions compared to pine shavings, pine shavings + FGD-Gypsum (50:50 mix) on flocks 4 and 5.

1.7.4 Other amendments for GHG reduction

Some other amendments which were studied previously to reduce GHG emissions include alum (Eugene et al., 2015), sodium bisulfate (PLT) (Ghose & Franchetti, 2016), and AMLA (Anderson et al., 2021). The addition of alum in PL was found effective which significantly reduced CO_2 emission from poultry houses where they measured emission from poultry houses, unlike other studies that measured flux from poultry manure (Eugene et al., 2015). Ghose & Franchetti, (2016) studied the use of sodium bisulfate in GHG emissions in poultry houses and reported 0.0294 kg of CO_2 equivalent per bird in winter and 0.0564 kg of CO_2 equivalent per bird savings in summer and spring and found it advantageous in reducing GHG emissions from poultry houses.

Kavanagh et al. (2021) used additives such as waste products from agriculture, commercially available bacterial additives, and chemical amendments to mitigate GHG from stored cattle slurry in temperate climates. Chemical amendments such as Ferric chloride reduced CH_4 by 6% - 65%, and CO_2 by 6% - 38%, with rates of 1.1%, 0.9%, and 0.38% compared to control. Moreover, ferric chloride can reduce pH with CH_4 up to 98%.

1.8 Need for evaluation of amendments to reduce NH_3 and GHG from broiler litter

Ammonia is an alkaline, water-soluble gas present abundantly in the atmosphere and is also a major reactive form of nitrogen (Huszár et al., 2008). Ammonia gas that escapes into the atmosphere reacts with different forms of Nitrogenous oxides or Sulfur oxides compounds and forms ammonium nitrate and ammonium sulfate particles which are a particulate matter of size less than 2.5 µm (Choi & Moore, 2008), and therefore NH_3 is considered an important pollutant species due to its role as a precursor in fine particulate matter formation. These particulate matters are harmful and cause respiratory problems in all animals and humans. Aerosols of ammonium nitrate and ammonium sulfate produced in the atmosphere by NH_3 gas accounted for up to 70% of fine particulates which results in visual impairment (Choi & Moore, 2008).

An increase in NH_3 concentration inside the poultry houses causes negative impacts on the poultry growth rate, reduces feed efficiency, and reduces egg size (Moore et al., 1996). Ammonia concentration above 25 ppm is detrimental to poultry production and therefore, NH_3 concentration should be below this level inside the poultry houses (Carlile, 1984; Choi & Moore, 2008). Furthermore, respiratory disorders such as cilia paralysis, deciliation (cilia loss), and damage to mucosal necrosis will affect the growth rate in chickens (Swelum et al., 2021). Moreover, prolonged exposure to high NH_3 levels causes inflammation, conjunctivitis, and corneal skin damage and in extreme conditions can cause permanent eye closure. In addition,

NH_3 volatilization is harmful to the environment as it results in acid rain, and lower N/P ratios in litter, also contributes to eutrophication and increases phosphorus runoff into water bodies and odor problems, environmental degradation, and health problems in both animals and humans (Moore Jr. et al., 1995; Smith et al., 2004). Similarly, the environmental consequences of GHGs and its GWP are well understood. The higher concentration of GHG inside the poultry houses also causes several health disorders (nutritional stress, infestations of parasites) as well as suffocations and may cause the deaths of birds. Therefore, both NH_3 and GHG remain a threat to poultry birds, productivity, and environmental sustainability. All these aspects highlight the need to identify practical ways to lower NH_3 and GHG emissions from poultry manures.

Application of amendments into the manures have shown promising results for reduction of NH_3 and GHGs emissions as well as enhance nutrient content of manure. Moreover, amendments are cost-effective, easily accessible, and have lower contamination issues. Therefore, the evaluation of amendments and their efficacy in reducing these gaseous emissions becomes necessary for sustainable poultry production and environment protection. Although previous studies have evaluated the effect of different amendments in reduction of NH_3 and GHG emissions, those study did not provide sufficient information about the use of biochar (B), FGD-gypsum (G), zeolite (Z), and sodium bisulfate (S) and their potential in reducing NH_3 and GHGs from BL. Based on our knowledge there has been no research conducted in past about the use of FGD-G, and S in reduction of GHG from BL. Therefore, our experiment focused on using B, Z, G, and S to study the effect of NH_3 and GHG reduction.

1.9 Objectives

The objectives of this research were to identify the appropriate amendments to reduce NH_3 and GHG from poultry manure. The specific objectives of the study were:

1. To investigate the potential of B, Z, G, and S at different application rates to reduce NH_3 emissions from BL.
2. To investigate the potential of B, Z, G, and S at different application rates to reduce CO_2, CH_4, and N_2O emissions from BL.

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CHAPTER 2: Evaluation of the Efficacy of Amendment Types and Rates in Reducing Ammonia Emissions from Broiler Litter

2.1 Abstract

Ammonia (NH_3) emission from animal manure, such as broiler litter (BL), is one of the major sources of atmospheric NH_3 and impacts poultry birds' health and productivity. Increase in concentration of NH_3 poses a health hazard for birds and animal health inside the houses. Moreover, NH_3 emission compromises the manure nutrient quality and causes atmospheric pollution and environmental consequences. Several amendments have been used to reduce NH_3 emission from BL in the past, however, the most effective amendments and their rates have not been identified. This study aims to evaluate the potential of biochar (B), zeolite (Z), FGD-Gypsum (G), and Sodium bisulfate (S) at different rates in reducing NH_3 emissions from BL. Incubation experiments of 40 days were conducted on jars with 100g of BL treated with amendments (B, Z, G, S) at different rates. The phosphoric acid trap was used to capture NH_3 produced from BL and the trapped NH_3 was then extracted with 2M KCl solution and analyzed in a flow injection analyzer. Results showed that application of 13% B and 17% B reduced cumulative (40 days) NH_3 emission by 40.7 and 45.6%, respectively, compared to control. However, there were no significant differences between 13 and 17% B rates. Zeolite application at 8% and 11% was effective and reduced NH_3 by 19.6 and 32.5%, respectively. There was no significant difference between the different rates of G and were less effective however, 15% G reduced NH_3 by 9.1%. Application of S at rates of 2%, 4%, 6%, and 7% (w/w) significantly reduced NH_3 emissions and reduced by 90.5, 98.6, 99.7, and 99.8 %, respectively. Based on the percent reduction of NH_3 compared to control, the efficiency of the amendments can be ranked

as S > B > Z > G. These findings contribute to an ongoing effort to identify amendments of choice to minimize NH_3 emissions in broiler houses.

2.2 INTRODUCTION

The United States is the largest poultry producer, consumer, and exporter of poultry products (meat and eggs) (USDA-ERS, 2023). The poultry industry in the United States produced about 9.17 billion broilers in 2022 (USDA-NASS, 2022) and generated approximately 12.6 million metric tons of broiler litter (BL) (Qian., 2019). One of the major problems poultry producers face inside the broiler house is ammonia (NH_3) production. An increase in NH_3 concentration inside the poultry houses causes a negative impact on the poultry growth rate, inflammation, watery eyes, respiratory infections, feed efficiency, and egg size (David et al., 2015; Swelum et al., 2021; Yi et al., 2016; Zhou et al., 2020). Moreover, the NH_3 emission during storage reduces the manure nutrient quality and has negative environmental consequences (Kangas and Syri, 2002).

The NH_3 gas that escapes into the atmosphere reacts with NOx or SOx compounds to form ammonium nitrate and ammonium sulfate particles, which are a particulate matter of size < 2.5 μm (Choi and Moore, 2008a). These particulate matters are harmful to humans and animals. Because of this reason, NH_3 is identified as a particulate precursor in maintaining air quality in the livestock and poultry industry (Choi and Moore, 2008b). Anderson et al. (2020) stated that NH_3 emissions from BL accounted for 27% of the total atmospheric NH_3 emissions in the United States. Similarly, Moore et al. (2011) documented that the total estimated NH_3 loss from poultry production amounts to 46 g of NH_3 per broiler. The concentration of NH_3 above 25 ppm is detrimental to poultry production and has environmental consequences. Therefore, NH_3 concentration should be reduced during production inside houses as well as during storage (Bist et al., 2023; Carlile, 1984; Choi and Moore, 2008b; Miles et al., 2004; Rylander and Carvalheiro, 2006).

Numerous amendments have been investigated to decrease NH_3 formation and volatilization losses from BL or other manures. Previous research has reported the use of alum, alum mud, aluminum sulfate, aluminum chloride, phosphoric acid, ferrous sulfate, and potassium permanganate in the poultry house to reduce NH_3 emissions (Moore et al., 1996; Moore Jr., 2016; Do et al., 2005; Choi and Moore, 2008b). The principal characteristics of these amendments are acidifying nature, hygroscopic and deodorant, and microbial enzyme inhibitors (Carlile, 1984; Moore Jr. et al., 1995).

Zeolite (Z) is a chemical compound which has ion adsorption, hygroscopic nature, ion exchange capacity, minute pore cavities, and higher specific surface (Šubová et al., 2021; Burt et al., 2017). These properties make Z a valuable mineral useful for various applications, such as industrial and municipal wastewater treatments (Liu et al., 2021), facilitating the anaerobic digestion of animal manure (Wijesinghe et al., 2018; Ziganshina et al., 2015), and as a feed additive (Papaioannou et al., 2005). Moreover, Z has a higher affinity for NH_4^+ ions and it can be used as an effective amendment for reducing NH_3 emissions from BL, however, the effectiveness of Z on NH_3 reduction in BL has not been well documented. Flue gas desulfurization gypsum (FGD-G) is an industrial by-product produced from coal-fired power plants processed from the flue gas desulfurization process (Koralegedara et al., 2019; Kun and Xiaoping, 2019). The hygroscopic nature of FGD-G absorbs excess moisture from litter (Watts et al., 2019), and retard microbial activity which lowers the rate of NH_3 formation (Tubail et al., 2008). Although few researchers added FGD-G in BL to study the effect on NH_3 emissions

(Oliveria et al., 2003; Koenig et al., 2005; Mishra et al., 2013), the effect of FGD-G on NH_3 reduction from BL and its appropriate application rates warrants further investigation.

Recently, biochar (B) has been widely used as a promising soil and manure amendment and has shown the potential to reduce NH_3 emissions from manure (Janczak et al., 2017; Baral et al., 2023). Biochar is a carbon-rich substance that is produced from plant biomass by the process of pyrolysis in the absence of oxygen (Dias et al., 2010; Janczak et al., 2017). The unique physicochemical characteristics such as high pore volume, high specific surface area (SSA), and oxygen-containing functional groups are advantageous to adsorb NH_3/NH_4^+ in manure and reduce NH_3 formation (Shin et al., 2024). Compelling results were reported by different researchers on the effectiveness of B in reducing NH_3 in manure and sewage (Steiner et al., 2010; Janczak et al., 2017; Malin ska et al., 2014; Chung et al., 2021; Agyarko-Mintah et al., 2017). The beneficial effects of B depend on the properties of B which can vary with the production process and the type of feedstock (Abagandura et al., 2019; Chun et al., 2021), crop, soil type, application rate, pyrolysis temperature (Shrestha et al., 2023; Shin et al., 2024), particle size of B (Yin et al., 2021), climatic conditions. There is still limited information about the effectiveness of B and its application rate in reducing NH_3 emissions from BL.

Sodium bisulfate (S) is a mineral-based acidifier and has been used in the poultry industry for pathogen reduction, NH_3 control, and on-farm pest management (Pope and Cherry, 2000). As it is commercially available and extensively used in the poultry industry, it becomes important to study the effectiveness and appropriate rate of S for NH_3 reduction. Although several research has been conducted in the past to identify a suitable method to reduce NH_3 volatilization from BL (Moore et al., 1996; Moore Jr. & Edwards, 2005), there is limited information available to identify the most effective amendments and their appropriate application rates.

Therefore, our study aims to evaluate the potential of B, Z, G, and S at different application rates to reduce NH_3 emissions from BL. It is hypothesized that the effectiveness of amendment in reducing NH_3 emission will vary with the application rates. We tested this hypothesis by measuring the NH_3 emission in an incubation study for 40 days.

2.3 MATERIALS AND METHODS

2.3.1 Broiler litter and amendments used in the incubation study

The BL used for the experiment was collected from a commercial poultry farm in Alabama. The farm had a grow-out cycle of thirty-three-day and used pine shavings as a bedding material. The BL was collected after two years of bedding material installation. The selected characteristics of amendments used in the study are presented in Table 1. The moisture content of BL was determined by oven drying samples for 6 h at 105 ºC (Wolf et al., 1997).

2.3.2 Laboratory Incubation

The BL was mixed and homogenised prior to the incubation study. One hundred grams of BL was taken in an 8 cm diameter mason jar. Different rates of amendments were evenly spread on the surface of BL inside the jar and the treatments name are presented in Table 2. A completely randomized design with 17 treatments containing different rates of amendments was used in the experiment and replicated twice. All the jars were kept inside the incubator [KB240 UL (E6), Binder Inc] at 30 ºC for 40 days. The initial moisture content in the BL was 30% and moisture level was maintained at 40% (w/w) at the start of experiment to mimic the moisture content in the broiler house. The constant weight was maintained throughout the experiment period by adding water uniformly with a spray bottle. Ammonia emission was captured by acidified sponge cubes (2.5 cm) hung inside the mason jar with thread without touching the sides of the jar. The acidified sponge to trap NH_3 was prepared following the method by Paramasivam et al. (2009). Commercial quality foam material (thickness = 2.5 cm; Jo Ann Fabrics in Savannah, GA) was cut into 2.5 cm side cubes. These sponges were thoroughly washed using deionized water (DI) before proceeding to the acidification stage. The sponges were sequentially soaked in the following solutions: 1M H_3PO_4 followed by 1M KOH, and D. After soaking in each solution, the sponges were squeezed thoroughly to remove the excess solution, and finally soaked in a mixture of 1M H_3PO_4 + Glycerol and squeezed. In the final step, 3 mL of mixture solution (1M H_3PO_4 + Glycerol) was added with a pipette to retain the solution in a sponge to trap NH_3 produced inside the jars. To avoid contamination of acid with the BL, it was hung inside instead of placing it directly above the BL surface.

The acidified sponges were replaced daily (started placing at 8:30 am and taken out starting at 8:30 am the next day) for the first 10 days and after that, the sponges were changed in three-day intervals up to 40 days. The NH_4-N captured in an acidified sponge was extracted individually with 40 mL of 2M KCl solution and kept in a reciprocating shaker for 20 minutes, squeezed, and stored in 50 mL centrifuge tubes. The NH_4-N was analyzed using an autoanalyzer (FIAlab Instruments Inc, Seattle, WA).

2.3.3 Characterization of pre- and post- incubation treatments

The pH of the pre and post incubated BL, treatments was determined using a 1:2 sample/DI ratio (Mitchell & Donald, 1999). Total Nitrogen (TN) and total Carbon (TC) content were analyzed using the thermal combustion method using a CNS Analyzer (Vario Max, Elementar Americas). The pre- and post-incubation BL samples were digested to determine the total digestible elements following Huang and Schulte, (1985) where 0.5 g of dry sample was digested with 5 ml of HNO_3 and 3 mL of H_2O_2 and analyzed in Inductively Coupled Plasma optical emission spectroscopy (ICP-OES).

2.3.4 Re-verification Incubation Experiment

After the completion of the first set of experiments, we performed a re-verification trial on selected amendments. The B at 17%, and S at 7% along with the control was tested in re-verification over 55 days and collected samples at a weekly interval. The sample collection and analysis were performed following the same protocol stated above.

2.3.5 Calculations

Ammonia concentrations were calculated as:

The volume of KCl used for extraction = 0.04 L

$$\text{Mass of } NH_3 \text{ (mg)} = \text{Concentration of ammonia} \times \text{Volume of KCl (0.04 L)} \quad (1)$$

$$\text{Mass of ammonia per kg dry weight of BL} = \frac{\text{mass of NH3 (mg)}}{\text{BL dry weight(g)}} \quad (2)$$

$$\text{Mass of } NH_3 \text{ per kg BL per day} = \frac{\text{mass of NH3 per kg dry weight of BL}}{\text{Time durations (h)}} \times 24 \quad (3)$$

$$\text{Cumulative } NH_3 \text{ emissions} = \sum_{n=1}^{40} (\text{NH3 emissions})n \quad (4)$$

Where, n represents, sampling day from day 1 to 40.

$$\text{Percent reduction} = \frac{\text{Cumulative NH3 emission (Control} - \text{amended BL)}}{\text{Cumulative NH3 emission of Control}} * 100 \quad (5)$$

2.4 Statistical Analysis

Statistical analysis was performed using repeated measurement and Toeplitz was selected based on lower AIC value by the PROC GLIMMIX procedure in SAS 9.4 (SAS Institute), where the treatment, and days were considered fixed effects, and replication was considered as random effects. The mean separation was performed using TUKEY test in SAS. For all analysis, significance was given as $a < 0.05$. The graphs were prepared using Microsoft Excel 365.

2.5 RESULT AND DISCUSSIONS

2.5.1 Characterization of the pre- and post-incubated treatments

The pH of the amendments used in this study is presented in Figure 1a. The differences in pH immediately after the application of amendments (pre-incubation) and after 40 days of incubation (post-incubation) experiment are presented in Figure 1b. The range of pH for all treatments immediately after the application of amendments was between 5.8 and 8.2, whereas the pH after the 40 days of the experiment ranged from 6.7 to 8.2. For pre-incubation, all four rates of S significantly reduced ($P < 0.05$) pH compared to other treatments (Figure 2). Similarly, for post-incubation, the treatments 6% S and 7% S significantly reduced pH compared to other treatments. Among the four rates of biochar, there was no statistical difference in pH between treatments and between pre- and post-incubation. Similar results were observed for zeolite and gypsum. Among all the treatments, pH was significantly different ($P < 0.05$) for 3% Z, 6% Z, 8% Z, 11% G, 2% S, 6% S, and 7% S between pre- and post-incubations. The S application lowered the pH in pre-incubations. However, after 40 days of incubation we observed an increase in pH in post-incubated samples and it could be due to neutralization of acidifying nature of S over time.

The primary factors contributing to NH_3 formation are temperature, moisture, pH, and N content of BL (Moore et al., 1995; Ritz et al., 2004; Swelum et al., 2021). In our study, moisture and temperature were kept constant. The pH was the primary driver governing NH_3 production (Merl & Koren, 2020) and therefore the acidifying nature of S helped lowering the pH significantly in our study. A study by Wood and Heyst, (2016) also reported similar results for PLT (which contains sodium bisulfate), where pH was reduced from 8 to below 5 in BL. The study conducted by Janczak et al. (2017) stated that application of B increased pH in BL during composting and is mainly due to the decomposition of organic matter releasing CO_2 and NH_3. However, treatment with B had no effect on lowering the pH in our study.

The total nutrient content of BL varied depending on the treatments and amendment rates. The TN in pre-incubated samples ranged from 2.8 to 3.4%, whereas for post-incubated samples TN ranged between 2.3 - 3.5% (Table 3). The TC content in pre-incubated treatments ranged from 17.3 to 28.3% and for post incubation TC ranged from 22.4 to 29.4%. According to ANR-FSA9529, University of Arkansas, the TN and TC of BL ranged from 1.0 - 4.4 and 12.2 - 33.0%, respectively. Similarly, Katuwal et al. (2023) reported mean TN and TC in BL from North Carolina were 2.47 and 32.3 %. The total phosphorus (TP) content ranged between 12.3 - 21.5 mg kg^{-1} for pre-incubation and 12.9 - 21.2 mg kg^{-1} for post-incubation. There was no statistical difference in TP between pre- and post-incubation. The total Al content was higher in treatments with Z compared to other treatments as Z itself contains Al. The total Fe content ranged between 0.5 to 1.1 g kg^{-1} for pre- and 0.6 to 1.1 g $kg\text{-}^{1}$ for post-incubation. The total Ca concentration in FGD-G amended litter is higher (up to 86.7 g kg^{-1}) than other treatments as it contains Ca. The total Mg content ranged between 8.6 and 11.4 g kg^{-1} for pre-incubation and 8.7 to 10.9 g kg^{-1} for post-incubation BL samples. The total K ranged between 44.1 to 60.2 g kg^{-1} for pre-incubation and 36.6 to 45.7 g kg^{-1} for post-incubation samples.

2.5.2 Effect of Biochar on NH_3 Emission

The cumulative ammonia emission from BL treated with four different rates of B including control is presented in Figure 2. Over the 40-day incubation period, the average cumulative NH_3 produced from control was 1.42 mg kg^{-1} which is the highest of all the treatments. The cumulative NH_3 emissions from 5% B was 1.2 mg kg^{-1}, 9% B was 1.0 mg kg^{-1}, 13% B was 0.8 mg kg^{-1}, 17% B was 0.8 mg kg^{-1}. There were significant differences *($P < 0.05$)* in cumulative NH_3 emission between treatments on each sampling day over the 40 days. Higher rates of B (13% B and 17% B) significantly reduced NH_3 emissions compared to control; however, there was no significant difference in the percent reduction between the two rates (Figure 7a). With respect to control, for the overall experimental period, 5% B, 9% B, 13% B and 17% B (w/w) reduced NH_3 emissions by 13.5, 27.8, 40.7, and 45.6 %, respectively (Figure 6a).

Janczak et.al. (2017) conducted an experiment to evaluate the effectiveness of wood B (0%, 5% and 10% wet weight) to reduce NH_3 emissions with the addition of wheat straw during composting poultry manure. They reported that addition of 5% and 10% B with pine shaving in composting of manure reduced NH_3 emission by 30% and 44%, respectively compared to control. Furthermore, Malinska et al. (2014) studied the effect of B addition on composting of sewage sludge (SS) mixed with straw and they found that the addition of 4% B (by wet weight) + SS + woodchips reduced NH_3 volatilization during the first week of composting. Steiner et al. (2010) researched to reduce nitrogen loss using biochar during composting using poultry litter (PL) + 0% BC (BC0), PL + 5% BC (BC5), and PL + 20% (BC20), (by weight) and observed a reduction of NH_3 by 60.35% from BC5 and 64% from BC20, which indicates that higher rates of B application have higher efficacy in reducing NH_3 emissions compared to control. Similar observations match our findings and show that higher application rates of biochar with 13% B and 17% B reduced higher ammonia emissions i.e 40.7 and 45.7%, respectively.

A study by Alarefee et al. (2023) used rice husk B in composting PL with a C/N ratio of 20, 25, and 30, which reduced NH_3 emission by 78.4, 94.6, and 97.3%, respectively as compared to control. Their NH_3 reduction percentages were very high in comparison to our study. The findings suggested that the higher rates of B are effective in reducing NH_3 emissions from BL. Ammonia loss from manures is reduced by B because of its adsorption properties and the presence of carboxylic groups (-COOH) formed by bio-oxidation at higher temperatures which binds NH_4^+ and reduces volatilization (Dias et al., 2010). The properties of B and its potential to reduce NH_3 emission from manures differ largely in literature and depend on the feedstock used to prepare B as well as the process involved in manufacturing (Shrestha et al., 2023).

2.5.3 Effect of Zeolite on NH_3 Emission

The cumulative ammonia emission from BL treated with Z at four different rates is shown in Figure 3. The cumulative NH_3 emissions from different rates of Z were 1.3, 1.3, 1.1, 1.0 mg kg^{-1} from 3% Z, 6% Z, 8% Z, and 11% Z, respectively (Supplementary Table 1). Higher rates, 11% Z and 8% Z have higher efficacy in reducing NH_3 by 32.5 and 19.6% as compared to control, whereas the lower rates 3% Z and 6% Z reduced NH_3 emissions by 5.8 and 7.0%, respectively (Figure 6a). There was a significant difference ($P < 0.01$) in cumulative NH_3 emission between treatments with respect to control. However, there was no significant difference between the 9% Z and 11% Z treatments.

A study by Li et al. (2008) used 2.5, 5.0, and 10.0 % Z (by weight) in hen manure and reported NH_3 volatilization decreased by 36, 62, and 92%, respectively within seven days from laying hen manure storage compared to control. Furthermore, in the same experiment with same application rate over 14 days the NH_3 emission was reduced by 20, 50, and 77% compared to control (no zeolite). Wang et al. (2024) conducted an experiment with chicken manure mixed with straw at a ratio 5:2 (chicken manure: straw by wet weight) with the three application rates of Z i.e. 0% (control), 5%, and 10%. They reported that the addition of 5% and 10% Z had a reduction of NH_3 by 15% and 28%, respectively, compared to the control during composting of chicken manure. Three different rates of zeolite, low (183 g/m^2), medium (366 g/m^2), and high (732 g/m^2) were applied to PL for a three-week experimental period. The result showed a reduction in NH_3 emissions by 28.0%, 39.0%, and 62.0% from low, medium, and high rates, respectively, compared to control (Lin, 2014).

Research conducted by Awasthi et al. (2016) indicated that when Z is combined with 1% lime, it can lower pH and significantly decrease NH_3 emissions. In addition to this, Z (88 g by weight) when mixed with chopped straw and pig slurry reduced greater than 90% of N loss during composting of pig slurry (Bernal et al., 1993). In a study conducted by Wang et al. (2012), the effects of various amendments on gas emissions from duck manure found that the addition of 10% Z (dry weight basis) reduced cumulative NH_3 emissions by 36.0% in a 46-day study period compared to the control. The differences in results could be mainly due to differences in manure types, moisture levels, and application rates. The mechanism by which Z works is due its hygroscopic nature, microbial inhibiting properties, and chemical three-dimensional tetrahedral structure with four oxygen atoms shared by two tetrahedra (Schneider et al., 2017). The shared oxygen has a partial negative charge and has a higher affinity with NH_4^+ ions minimizing the formation of NH_3 (Baur & Fischer, 2023; Li et al., 2008), and can be used as an amendment to reduce NH_3 emission from manure (Kithome et al., 1999).

2.5.4 Effect of FGD-Gypsum on NH_3 Emission

The trend of cumulative NH_3 emission from four different rates of G along with control is presented in Figure 4. Application of G at 6%, 11%, 15%, and 19% (w/w) reduced NH_3 emission by 3.3, 1.1, 9.1, and 6.4%, respectively (Figure 6a), with respect to control, but there was no significant difference in cumulative NH_3 emission between the different rates of gypsum and control for all sampling days. Cumulative NH_3 emissions from 6% G was 1.4 mg kg^{-1}, 11% G was 1.4 mg kg^{-1}, 15% G was 1.3 mg kg^{-1} and 19% G was 1.3 mg kg^{-1} (Supplementary table 1). The efficacy of G in NH_3 emissions has shown mixed results in previous findings. A study by Oliveira et al. (2003) examined the effect of FGD-G and other amendments on NH_3 volatilization from BL and found that FGD-G treated BL had the lowest NH_3 emission (the quantity of emitted ammonia (11.3 mg/kg) during 42 days of the study period compared to other treatments. The addition of gypsum to dairy manure compost at a dry weight rate (w/w) of 0, 6, 13, and 23%, showed that 13% gypsum was found most effective in reducing NH_3 by 12.3% compared to the control (Tubail et al., 2008). Similarly, Iowa State University Extension Service, (2004) reported that gypsum amendment in dairy manure storage reduced ammonia loss by 14.0%. A laboratory study was conducted by Mishra et al. (2013) by applying FGD-G on the soil where broiler litter (BL) + 0 FGD-G g^{-1} and BL + 0.25 g FGD-G g^{-1} litter applied on the soil surface at a constant temperature of 25 ºC throughout the 63 days of the experiment period. The results from this study reported that FGD-G has no significant effect on NH_3 emission compared to the control. However, the study by Burt et al. (2017) used 0, 20%, and 40% of FGD-G in 10 g of BL based on BL wet weight in an incubation experiment for 21 days and found a noticeable reduction in NH_3 emission between 22.0 to 28.0 % compared to control. Although there was no significant reduction in NH_3 using G in many of the findings, the differences were due to the different compositions of mined gypsum and FGD-G. The addition of gypsum to litter forms precipitations of calcium carbonates by reacting gypsum-derived calcium and litter bicarbonates and decreasing pH immediately which can retard NH_3 formation (Burt et al., 2021).

Additionally, the hygroscopic nature can absorb moisture from manure and retards microbial activity associated with nitrogen mineralization, thus potentially lowering NH_3 emissions (Mishra et al., 2013; Oliveira et al., 2003). Furthermore, the use of FGD-G as an amendment needs more research to understand its potential characteristics to reduce NH_3.

2.5.5 Effect of Sodium bisulfate on NH_3 Emission

The trends of cumulative ammonia emission from the four different rates of S and control are presented in Figure 5. For the 40-day experiment, the cumulative NH_3 emissions for 2% S, 4% S, 6% S, and 7% S were 0.13, 0.02, 0.01, and 0.003 mg kg^{-1}, respectively (Supplementary Table 1). We find that all four rates of S significantly reduced NH_3 emissions and were effective in reducing NH_3 emissions as compared to control and other treatments. Higher rates had higher efficacy in reducing NH_3 loss, whereas 2%, 4%, 6%, and 7% (w/w) of S reduced NH_3 emissions by 90.6, 98.7, 99.7, and 99.8 %, respectively (Figure 6a). This indicated that even the lower rate of S has the potential to reduce NH_3 emissions above 90%. The lower rate 2% S showed the potential to reduce NH_3 initially for up to a week and afterward, the emission started increasing whereas higher rates have the potential to inhibit NH_3 production throughout the study period. There was a significant difference ($P < 0.05$) in cumulative NH_3 emission between treatments compared to control. However, there was no statistical difference between 6% S and 7% S (w/w). A study by Pope and Cherry, et al. (2000) found that S treated at a rate of 0.24 kg m^{-2} lowered NH_3 concentrations by up to 55% over a 2-week study period. Similarly, another research by Hong Li et al. (2006) reported that S applied at rates of 0.5 to 1.5 kg m^{-2} reduced NH_3 emission by 74.0 to 92.0% during 7 days. The NH_3 emission immediately after the application of S dropped up to 72.0% and the overall reduction was 57.0%, however, the effectiveness of S after 11 days was reduced (Wood and Heyst, 2016). This indicates that S reduced NH_3 emission largely in the initial days and the potential to reduce NH_3 is reduced with an extended period of the experiment.

The mechanism of S in reducing NH_3 emission from manure is mainly governed by its chemical composition and capacity to lower pH. Sodium bisulfate, which is an acidifier, when applied to litter it breaks into sodium, hydrogen, and sulfate ions. The hydrogen ions help to lower the pH and bind ammonium ions thereby preventing the formation of NH_3 (Li et al., 2008; Terzich et al., 1998) as previous research found that the concentration of NH_3 is low when pH is lower than 7 (Blake et al., 2009; Reece et al., 1980). Li et al. (2013) studied the effectiveness of treating S at two rates 183 g m^{-2} and 366 g m^{-2} applied on BL on a weekly basis and found a reduction in cumulative NH_3 emission ranging between 14% to 64.5% and 0 to 55%, respectively, in laboratory conditions. Further, they conducted a field experiment to quantify and delineate the efficacies of frequent litter amendment application with 244 g m^{-2} per 2-week in a three-flock period. The cumulative NH_3 emission was reduced by 51.7% in a field setup. Sahoo et al. (2017) found, that the addition of S at the rate of 25 g per sq. ft. was found more effective in lowering pH, microbial load, and ambiance in broiler houses compared to alum (at the rate of 90 g alum per sq.ft.). Additionally, Blake et al. (2009) also reported that the use of S as a litter amendment at three different ranges; 24.4, 48.8, and 73.2 kg per 100 m^2 reported the reduction of NH_3 emissions at a higher rate. Fairchild et al., (2006) experimented with six commercial broiler houses during colder months, applying S at rates of 50, 100, and 150 lbs. per 1000 ft^2. Their findings indicated that the broiler house treated with 100 lbs. per 1000 ft^2 experienced a 50% reduction in ammonia concentration compared to the house treated with 50 lbs. per 1000 ft^2. A study by Lin. (2014) experimented on mitigating NH_3 emissions from BL in an emission vessel with the application of S at different rates from 0, 183 (low), 366 (mid), and 732 g/m^2 (high) and found that all rates of S reduced NH_3 emission more than 90% on the first day for all the moisture levels (20%, 30%, and 40%) and from different application rates. Similarly, higher application rates of S (732 g/m^2) at three different moisture levels reduced cumulative NH_3 by more than 95.0% compared to control.

Overall, our study showed different efficacies of amendments in reducing NH_3 emissions from BL however, some of the previous research provided significant findings when different amendments were combined and their synergistic effects on NH_3 reduction. The research conducted by Wang et al. (2017) studied the effects of B, Z, and their combination on nitrogen conservation and organic matter transformation during pig manure (PM) composting. They mixed PM with wheat straw (WS) at a ratio of 2:1 on a dry weight basis. They reported that among the three treatments: PM + WS + 10% B, PM + WS + 10% Z, and PM + WS + 10% (B + Z) decreased ammonia emissions by 35.8%, 31.1% and 63.4% respectively with respect to control. This indicates that the combination of amendments, B + Z enhanced the potential to reduce NH_3 emissions. Moreover, Febrisiantosa et al. (2018) had a similar finding where B alone reduced NH_3 emission by 22.2% whereas B combined with FGD-G reduced NH_3 emission by 58.9% at the time of composting of livestock waste. Therefore, future studies with different combinations of amendments are needed to develop and identify an effective combination of amendments to reduce NH_3 emissions.

2.5.6 Re-verification trial

We performed another set of experiments with selected amendments in similar settings to develop more confidence and draw conclusions from our 1st incubation study. Comparison between the 1st and re-verification trials for those selected amendments showed no significant difference between the treatments from the 1st and re-verification trials for 17% B but showed significant difference for 7% S. However, the percent reduction by 7% S was 97.8% which is very close to the value from 1st experiment (99.8%) (Figure 6b). The validation experiment showed similar results to those of the 1st experiment and, therefore, provided us with additional evidence to support our results for the potential use of amendments to reduce NH_3 emissions from BL.

2.6 CONCLUSIONS

This study revealed notable differences in the efficacy of amendments and their rates in NH_3 emission from BL. We observed a general pattern of decreased NH_3 emission with the increased rates of amendments. Among the B, Z, G, and S, all four rates of S demonstrated the highest potential for reducing NH_3 emissions above 90% compared to control, followed by B, Z, and G. Although G reduced NH_3 emissions up to 9%, there was no statistically significant difference in cumulative NH_3 emission compared to control, indicating its ineffectiveness in reducing NH_3 emissions with respect to other amendments. This study has provided valuable insights into the selection of amendments and their rates for future investigations on amendments. These findings contribute to ongoing efforts to identify amendments of choice for poultry producers to minimize losses in poultry production systems and environmental impacts benefiting poultry, human health, and the enviornment. However, more investigation and implementation of findings at various scales are needed to address several issues associated with NH_3 emissions from manure. Further testing of amendments should be carried out so that the actual efficacy of the amendments in reducing NH_3 emissions could be helpful for poultry producers.

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Tables

Table 1: Characteristics of amendments used in this study.

Parameters	Biochar	Zeolite	FGD-Gypsum	Sodium bisulfate
TC (%)	22.8	0.2	0.3	0.1
TN (%)	0.1	0.1	0.1	0.1
TP (g kg^{-1})	2.6	0.2	0.2	0.2
TMg (g kg^{-1})	4.2	1.6	1.4	0.2
TK(g kg^{-1})	5.7	22.4	0.2	ND
TAl (g kg^{-1})	7.1	26.8	ND	ND
TCa (g kg^{-1})	35.9	9.4	200.8	0.2

Note: ND, Not determined; TC, Total Carbon; TN, Total Nitrogen; TP, Total Phosphorus, TMg, Total Magnesium; TK, Total Potassium; TAl, Total Aluminum; TCa, Total Calcium.

Table 2: Different types of amendments and their rates (w/w) used in the incubation study to reduce NH_3 emission from broiler litter.

Treatment Description	Amendment Type	Treatment Name
100 g BL (Unamended litter)	Broiler litter	BL (Control)
100g BL + 5 g Biochar	Biochar	5% B
100 g BL + 10 g Biochar		9% B
100 g BL + 15 g Biochar		13% B
100 g BL + 20 g Biochar		17% B
100 g BL + 3 g Zeolite	Zeolite	3% Z
100 g BL + 6 g Zeolite		6% Z
100 g BL + 9 g Zeolite		8% Z
100 g BL + 12 g Zeolite		11% Z
100 g BL + 6 g FGD-Gypsum	FGD-Gypsum	6% G
100 g BL + 12 g FGD-Gypsum		11% G
100 g BL + 18 g FGD-Gypsum		15% G
100 g BL + 24 g FGD-Gypsum		19% G
100 g BL + 2 g Sodium bisulfate	Sodium bisulfate	2% S
100 g BL + 4 g Sodium bisulfate		4% S
100 g BL + 6 g Sodium bisulfate		6% S
100 g BL + 8 g Sodium bisulfate		7% S

Table 3: General chemical characteristics of BL treated with different amendments after 40 days of incubation studies.

Treatments	TN		TC		TP		TAl	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
	----------%-----------				gkg^{-1}			
BL	3.4 a-e	3.3 a	27.1 a-c	27.7 a-c	20.3 ba	20.9 ba	2.3 d-f	2.4 ed
BL+ 5% B	3.3 a-e	3.2 ab	27.0 a-c	28.8 a-c	19.5 a-d	20.1 ba	2.4 d-f	2.5 c-e
BL + 9% B	3.4c-f	3.0 ba	27.3 ab	29.4 ab	18.8 a-d	20.4 ba	2.53 d-f	2.9 b-e
BL + 13% B	3.18 d-g, A	3.02 a-c, B	28.3 a	28.4 a-c	18.6 a-d	19.8 a-c	2.6 d-f	2.9 b-e
BL + 17% B	3.24 e-g, A	2.9 ab, B	28.2 a	28.4 a-c	18.0 a-d	19.2 a-c	2.94 d	3.1 b-e
BL + 3% Z	3.4 a-e	3.1 ba	27.3 ab	27.2 a-c	19.9 ba	21.2 a	2.72 d-f	3.1 b-e
BL + 6% Z	3.1b-e	3.1 a-c	24.5 a-f	28.4 a-c	21.5 a	18.9 a-c	3.81 bc	3.6 a-c
BL + 8% Z	3.1e-g	2.9 a-c	24.1 a-f	25.7 a-c	19.9 ba	19.3 a-c	4.12 ba	4.0 ab
BL + 11% Z	3.1 e-g	2.9 a-c	22.7 d-f	24.0 a-c	18.5 a-d	18.38 a-c	4.92 a	4.6 a
BL + 6% G	3.1 e-g	3.0 a-c	23.0 c-f, B	29.4 ab, A	19.5 a-d	20.1 ba	2.36 d-f	2.4 de
BL + 11% G	2.8 e-g	2.9 b-d	24.8 a-f	30.6 a	18.6 a-d	18.5 a-c	2.05 ef	2.2 e
BL + 15% G	2.9 gh	2.6 a-d	21.9 e-g	22.7 bc	17.5 b-d	16.8 b-d	2.07 ef	2.1 e
BL + 19% G	2.8 hi	2.3 a-d	18.1 gh	22.4 c	16.0 c-e	17.2 a-d	2.02 ef	2.1 e
BL + 2% S	3.1 a-d,A	3.4 a-c, B	25.8 a-e, B	28.0 a-c, A	20.1 ba	20.3 ba	2.2 d-f	2.4 ed
BL + 4% S	3.1 a-c	3.4 a-c	26.1 a-e, A	22.9 bc, B	20.0 ba	19.3 a-c	2.27 d-f	2.3 ed
BL + 6% S	3.1 ba	3.4 a-c	25.71 a-e	26.5 a-c	19 a-d	19.8 a-c	1.91 ef	2.3 ed
BL + 7% S	3.1 a, B	3.5 a-c, A	23.3 b-f, B	27.8 a-c, A	20.9 ba	18.5 a-c	2.55 d-f	2.2 e

Treatments	TFe		TCa		TMg		TK	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
	gkg^{-1}							
BL	0.7 b-e	0.6 a	39.0 d	36.9 d	10.2 a-c	10.7 a	54.1 a	45.7 a
BL+ 5% B	0.6 ed	0.6 a	38.2 d	37.9 d	9.9 a-c	10.3 ba	53.1 a	43.9 a
BL + 9% B	0.7 b-e	0.8 a	37.5 d	40.3 d	9.9 a-c	10.9 a	51.0 a	44.6 a
BL + 13% B	0.7 c-e	0.8 a	38.8 d	36.9 d	9.8 a-c	10.5 a	55.2 a	43.4 a
BL + 17% B	0.7 b-e	0.8 a	39.6 d	39.3 d	9.7 a-c	10.3 ba	51.0 a	41.9 a
BL + 3% Z	0.6 ed	0.7 a	37.4 d	40.1 d	10.5 a-c	10.4 a	52.3 a	46.1 a
BL + 6% Z	1.1 a-c	1.0 a	39.5 d	38.0 d	11.4 a	10.2 a-c	60.2 a	43.8 a

BL + 8% Z	0.9 a-e	0.9 a	38.6 d	37.7 d	10.7 ba	10.1 a-c	53.2 a	43.7 a
BL + 11% Z	1.0 a-d	1.1 a	37.46 d	35.7 d	10.4 a-c	9.6 a-d	54.5 a	43.0 a
BL + 6% G	0.7 b-e	0.8 a	54.4 b-d	52.1 b-d	9.9 a-c	10.5 a	51.3 a	43.6 a
BL + 11% G	0.6 ed	0.7 a	63.0 a-d	63.2 a-c	9.7 a-c	9.6 a-d	50.0 a	40.2 a
BL + 15% G	0.6 ed	0.8 a	84.6 ba	78.1 d	9.4 a-c	9.0 b-d	47.1 a	37.1 ba
BL + 19% G	0.8 b-e	0.7 a	86.7 a	77.6 d	8.6 b-d	8.7 dc	44.1 a	36.6 ba
BL + 2% S	0.5 e	0.8 a	36.8 d	36.4 d	10.3 a-c	10.3 ba	55.2 a	43.4 ba
BL + 4% S	0.6 c-e	0.8 a	38.6 d	34.0 d	10.5 a-c	9.7 a-c	58.6 a	41.9 a
BL + 6% S	0.6 c-e	0.8 a	35.9 d	35.7 d	9.6 a-c	10.1 a-c	50.4 a	43.6 a
BL + 7% S	1.1 ba, A	0.7 a, B	39.4 d	33.6 d	10.6 ba, A	9.5 a-d, B	58.1 a	40.3 ab

Mean values followed by the same letter represent no significant differences at $P < 0.05$. Lowercase represents the mean comparison between the treatments for the pre- (immediately after the application of amendments) and post (after the incubation), uppercase represents the mean comparison between the pre and post-experiment. Abbreviations: TN, total nitrogen; TC, total carbon; TP,total phosphorus; TAl, aluminum; TFe, total iron; TCa, total calcium; TMg, total magnesium; TK, total potassium.

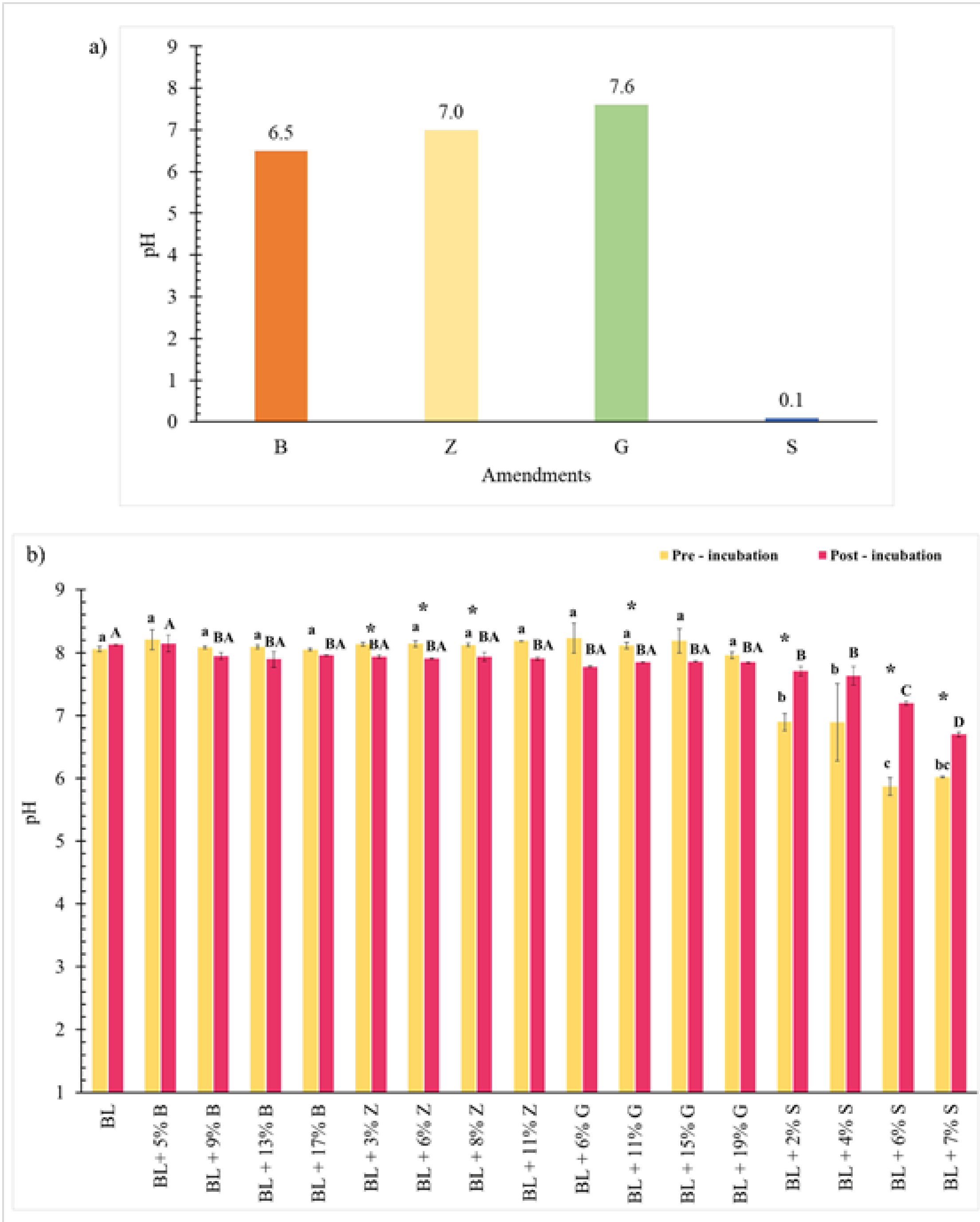

Figure 1: The pH of the amendments and treatments used in this study. a) pH of amendments b) comparison of pH of BL treated with amendments between pre- and post-incubation experiments. Means with the same letter represent no significant difference, *represents that there is a significant difference between pre- and post-incubation, the bar represents a standard error, and all mean comparisons were at $P < 0.05$. The upper case represents the mean comparison of pH for all the treatments of post-incubation, and the lower case represents the mean comparison of pH among all pre-incubation treatments.

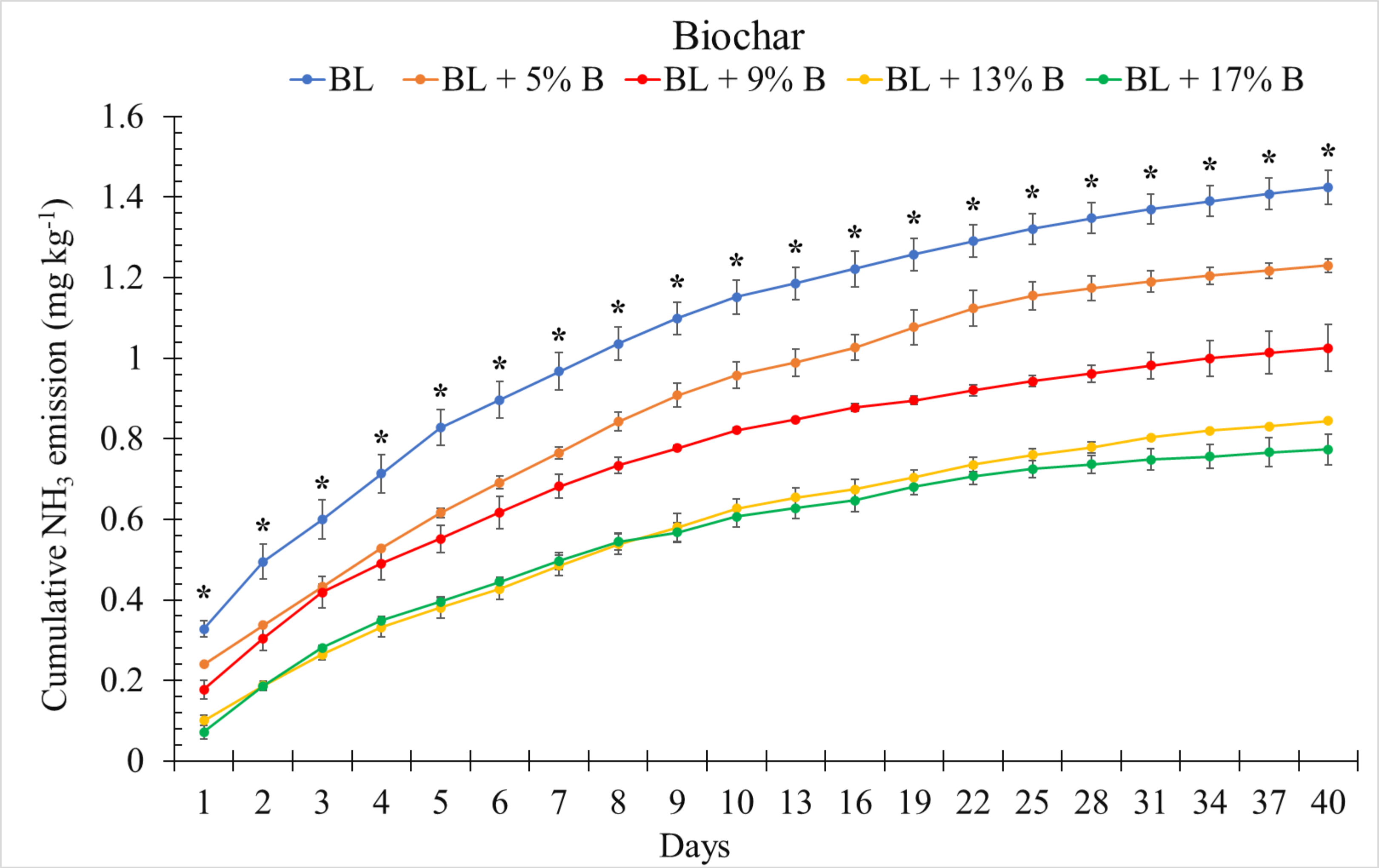

Figure 2: Cumulative ammonia emissions from BL treated with biochar (w/w) over 40 days of incubation studies. * indicates significant difference in a given day at $\alpha = 0.05$, bar represents standard error of mean. The mean comparision is between four rates of biochar and control.

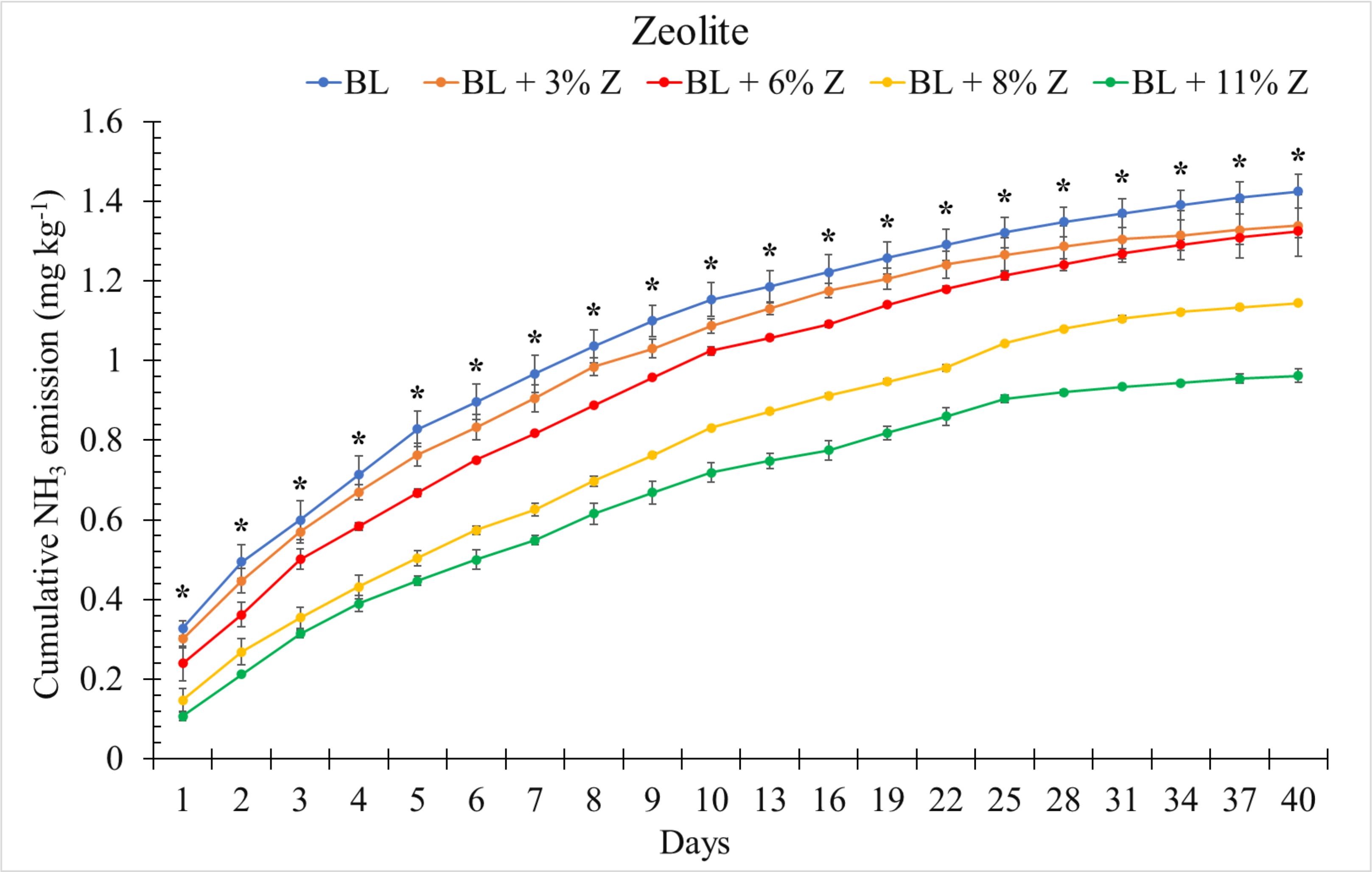

Figure 3: Cumulative ammonia emissions from BL treated with zeolite (w/w) over 40 days of incubation studies. * indicates ssignificancet difference in a given dayat $\alpha = 0.05$, bar represents standard error of mean. The mean comparision is between four rates of zeolite and control.

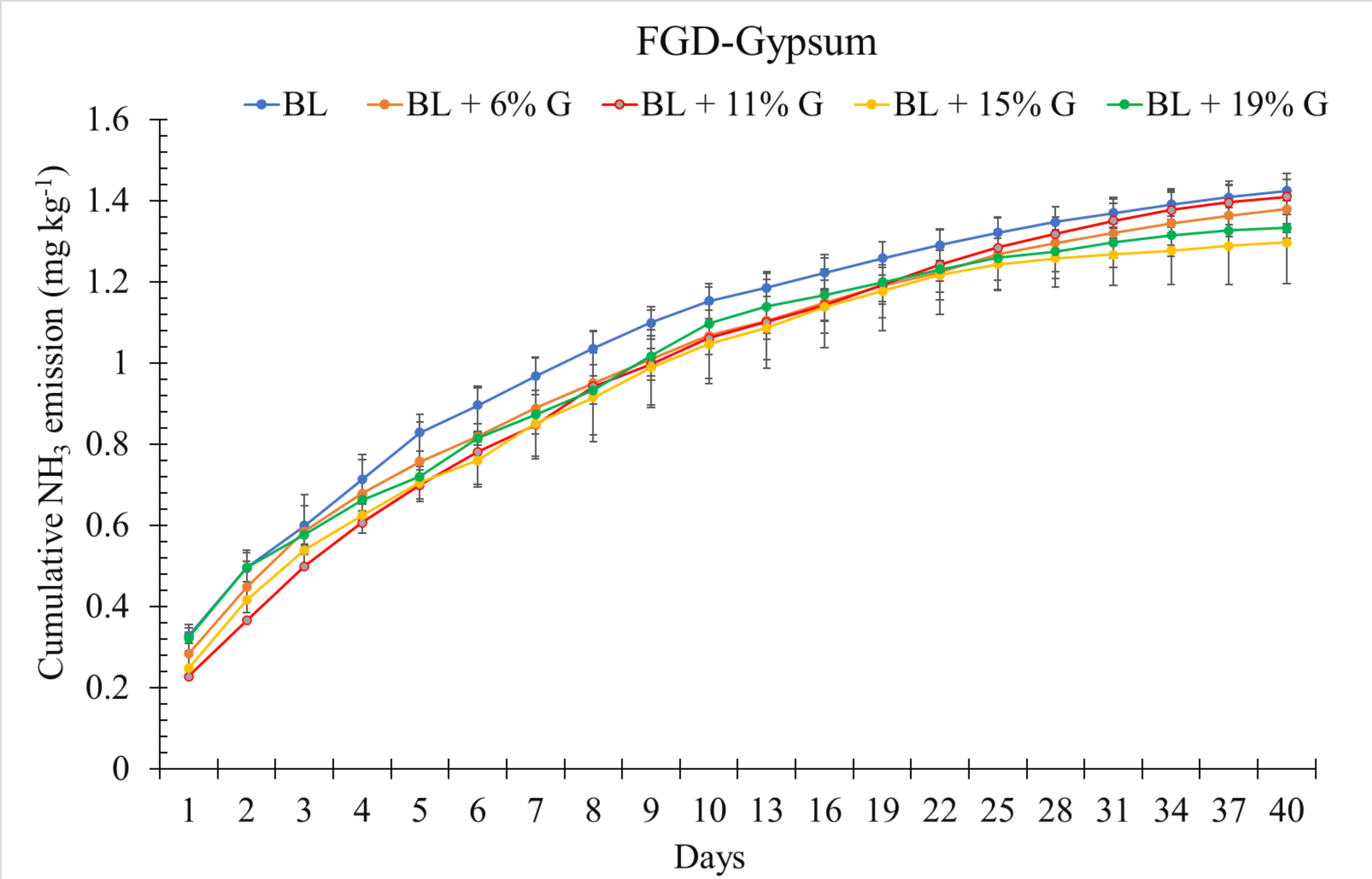

Figure 4: Cumulative ammonia emissions from BL treated with FGD-gypsum (w/w) over 40 days of incubation studies. * indicates significant difference in a given day at $\alpha = 0.05$, bar represents standard error of mean. The mean comparision is between four rates of FGD-Gypsum and control.

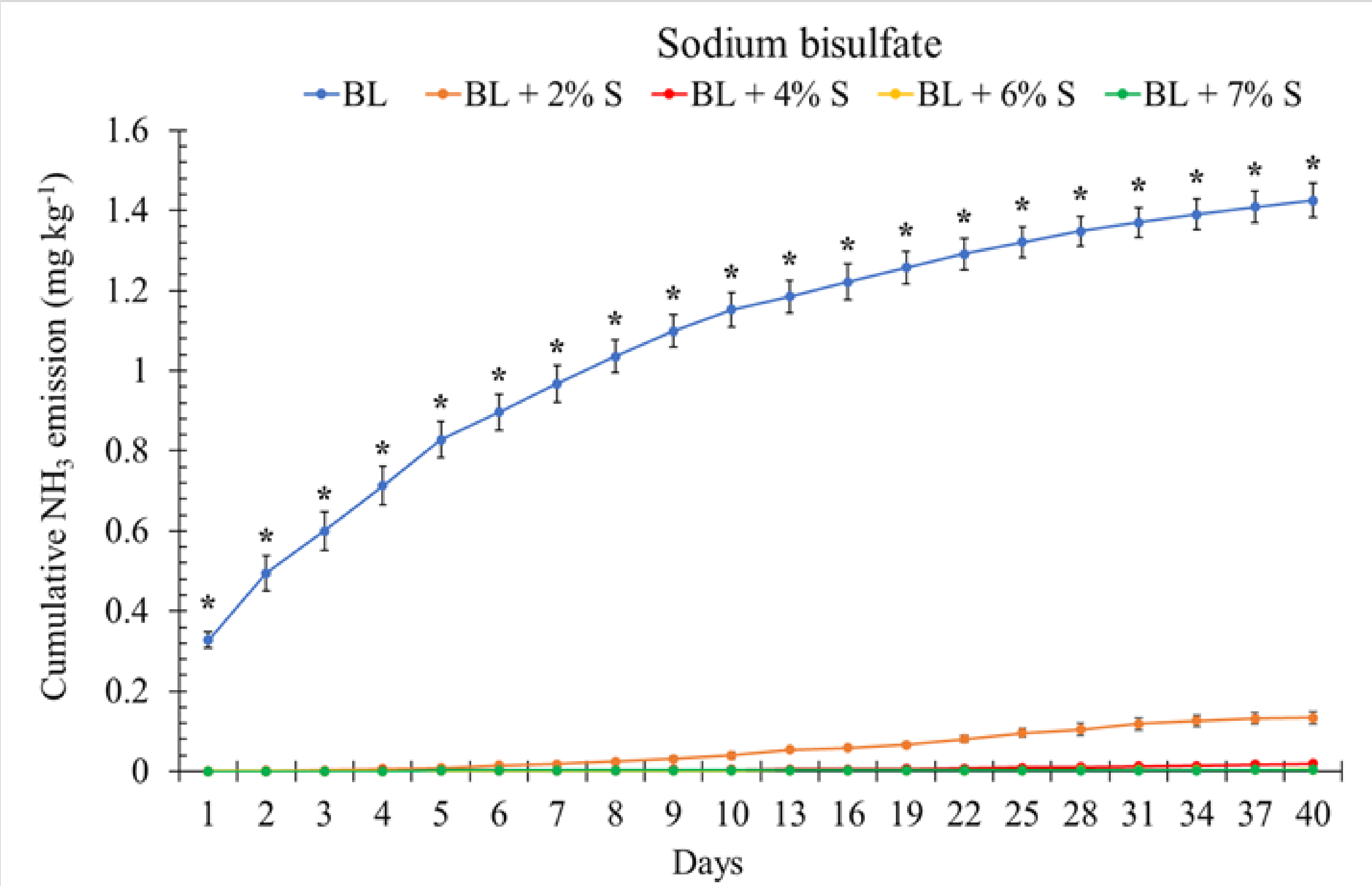

Figure 5: Cumulative ammonia emissions from BL treated with sodium bisulfate (w/w) over 40 days of incubation studies. * indicates significant difference in a given day at α = 0.05bar represents standard error. The mean comparision is between four rates of sodium bisulfate and control.

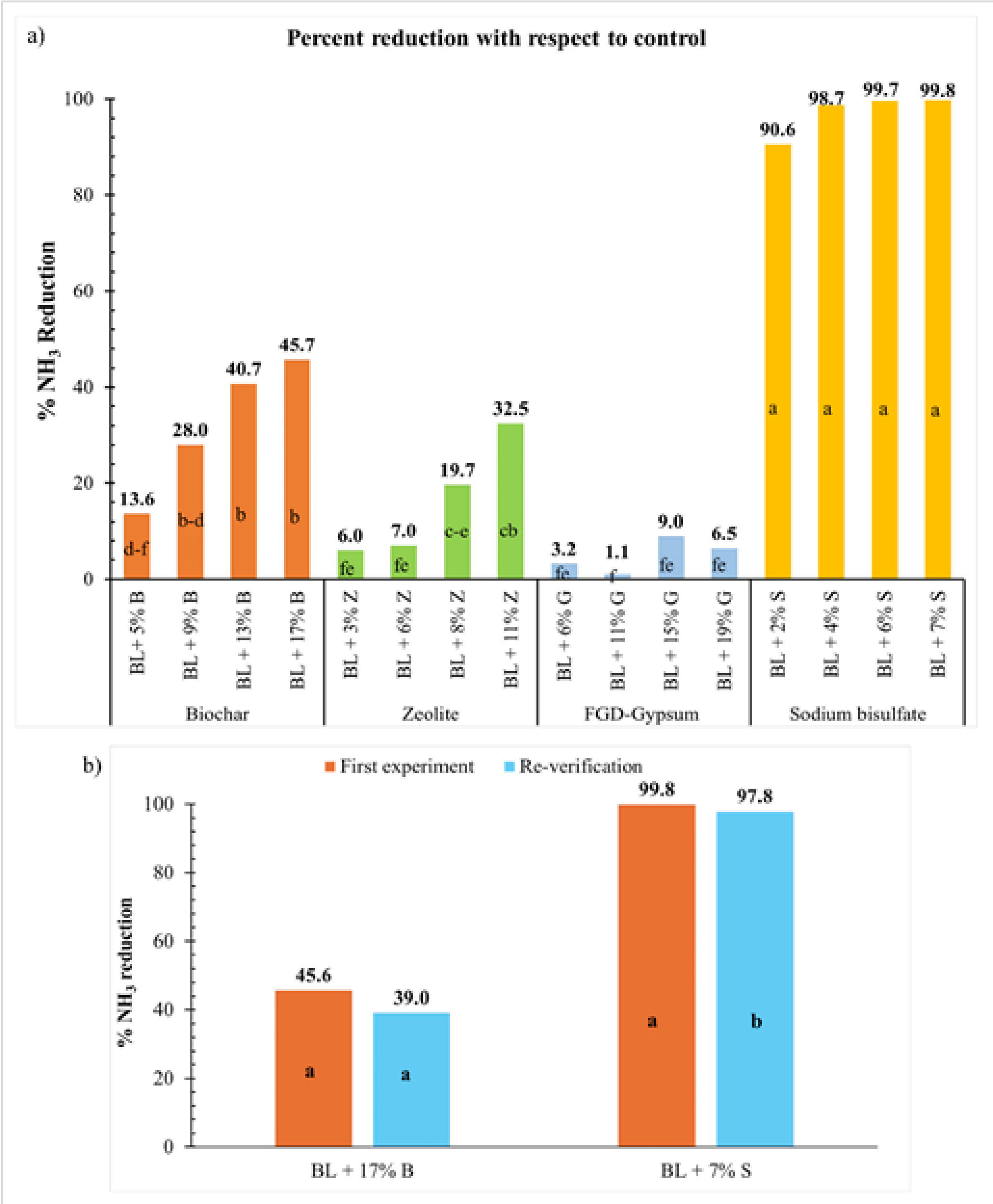

Figure 6: Percent reduction of the cumulative ammonia emissions with respect to control for a) 21 treatments over 40-day experiment period; b) comparison between first experiment and reverification experiment for selected amendments with respect to control. Means with the same letter represents no significant difference, all mean comparison were at $P < 0.05$.

Supplementary table:

Supplementary Table 1: Cumulative emissions of NH_3 for 40 days of incubation experiment.

Treatments	Total emissions for 40 days (mg kg^{-1})
BL	1.4
BL+ 5% B	1.2
BL + 9% B	1.0
BL + 13% B	0.8
BL + 17% B	0.8
BL + 3% Z	1.3
BL + 6% Z	1.3
BL + 8% Z	1.1
BL + 11% Z	1.0
BL + 6% G	1.4
BL + 11% G	1.4
BL + 15% G	1.3
BL + 19% G	1.3
BL + 2% S	0.1
BL + 4% S	0.02
BL + 6% S	0.01
BL + 7% S	0.003

CHAPTER 3: Evaluation of the Effects of Litter Amendments to Reduce Greenhouse Gas Emissions from Broiler Litter

3.1 Abstract

Broiler litter (BL) is one of the byproducts of the poultry industry and contributes to greenhouse gases (N_2O, CO_2, and CH_4) emissions. Managing and reducing GHG emissions from BL is essential to mitigate global warming and climate change. A practical litter management approach can help minimize poultry industry GHG emissions. The addition of amendments in litter has been identified as a promising and sustainable tool for reducing gaseous emissions; however, the results from previous studies were not consistent with or informative enough on amendments and their rates. Therefore, the objective of our study was to investigate the rate effect of biochar (B), Zeolite (Z), FGD-gypsum (G), and Sodium bisulfate (S), on N_2O, CO_2, and CH_4 emissions from BL. Different rates of amendments were applied to 100 g of BL kept in jars and incubated at 30 °C. The gas samples were collected from the headspace of the jars and analyzed by gas chromatography. The results showed that 13% B and 17% B significantly increased cumulative CO_2 and N_2O emissions, whereas 5% B and 9% B did not affect GHG emissions compared to control. Zeolite and G application had no significant effect on GHG reduction. Sodium bisulfate at 4%, 6%, and 7% reduced CO_2 and N_2O emissions compared to control. Among the treatments used, 4, 6, and 7 % of S were found to be a promising amendment to reduce GHG emissions from BL. Additional research in commercial poultry houses should be conducted to better understand the effects of amendments and their potential to minimize GHG emissions.

3.2 INTRODUCTION

Greenhouse gases (GHGs) are essential components of the earth's atmosphere, which play a vital role in regulating the earth's temperature by trapping heat from solar radiation (Nema et al., 2012). They are crucial for sustaining life on Earth, as the absence of GHGs might result in surface temperatures dropping as low as -18 °C (Harris et al., 2017). With increased industrialization and human interventions in the ecosystem, the concentration of GHGs has exceeded the naturally occurring range and increased the greenhouse effect, contributing to global warming and climate change (Deng et al., 2019; Easterbrook, 2016). According to USEPA (2024), agricultural activities are major sources of GHG emissions and contribute 593.4 MMT CO2 equivalent, representing 10% of the overall GHG emissions in the United States. The primary GHG emitted by agricultural activities include carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O). Among these, CH4 has a global warming potential (GWP) that is 25 times more than CO_2, and N_2O has a GWP 298 times higher than CO_2 (IPCC, 2021). In recent decades, a significant increase in GHG emissions has impacted the earth's climate. Specifically, CO_2, CH_4, and N_2O emissions have increased by approximately 5.6%, 4.2%, and 2.7% respectively, between 2011 and 2020 (NOAA, 2022). These findings are alarming and pose a major threat to environmental sustainability.

The poultry industry is a quantitatively important agro-industry in the USA and worldwide. Within the agricultural livestock sector, the poultry industry is one of the significant sources of GHG, followed by cattle and swine (IPCC, 2021). With the increased global population, the demand for poultry products (meat and egg) has increased, leading to an exponential rise in poultry production in the last decades. The Food and Agriculture Organization (FAO) projected a 76% increase in global meat consumption and reported a doubling in poultry demand by 2050 (Alexandratos & Bruinsma, 2012). Broiler production occupies a significant portion of the poultry sector and accounts for approximately 60% of the total acreage (NASS, 2023). Broiler litter (BL), a by-product of the broiler industry, is a major source of ammonia and GHG emissions. Innovative litter management strategies can play an important role in reducing ammonia and GHGs emissions (Dangal et al., 2020; Zisis et al., 2023). Litter amendments have been found to be an economical and eco-friendly practice for broiler litter management.

Several amendments such as biochar (B), zeolite (Z), FGD-gypsum (G), Sodium bisulfete (S) or alum, and others have been found useful in reducing ammonia emissions from BL. However, limited studies have examined the effects of those amendments on GHG emissions from BL. The impact of amendments so far investigated has shown mixed types of responses, where some have reported increase, decrease, and no significant effect under different experimental settings from laboratory incubation studies to fields (Shrestha et al., 2023; Shakoor et al., 2021; Spokas & Reicosky, 2009; Troy et al., 2013; Wang et al., 2024; Watts et al., 2019; Yin et al., 2021).

Biochar is a carbon-rich substance produced by pyrolysis by burning plant biomass or organic materials at high temperatures in anaerobic conditions (Geng et al., 2024; Kalus et al., 2020). Zeolites are complex chemical compounds with a three-dimensional, tetrahedral structure. They contain four oxygen atoms, and each atom is shared by two tetrahedra (Schneider et al., 2017). Due to their typical molecular structure, natural Z has been used as an ion exchange and adsorbent, for pollution abatement, animal nutrition, and gas separation. The FGD-G is an industrial byproduct obtained from combustion gases at coal-fired power plants and has beneficial chemical and physical characteristics for use as litter amendment (Chen et al., 2015;

Koralegedara et al., 2019; Kun & Xiaoping, 2019). Sodium bisulfate is a commercially available acidifier, which can break into sodium, hydrogen, and sulfate ions when applied to the PL (Li et al., 2008) and can lower the pH of litter. Based on commercially and readily accessible amendments and previous findings, we selected B, Z, G, and S as candidate amendments in our study. In addition, most studies on GHG emissions have focused on one or two GHGs (such as CO_2, CH_4, or N_2O), leading to insufficient evaluations of the net benefits of amendments for reducing emissions. Therefore, our study aimed to investigate the rate effect of B, G, Z, and S on CO_2, CH_4, and N_2O emissions from BL during a 40-day study period.

3.3 MATERIAL AND METHODS

3.3.1 Broiler litter and amendments used in the incubation study

The BL used for the experiment was collected from commercial broiler farms in Alabama and has pine shaving as a bedding material. The BL was collected from a poultry house that produces chickens on a thirty-three-day flocks cycle. The BL was a two-year-old litter. The information about the characteristics of amendments used in this experiment is provided in Table 4. The treatments used in the study i.e., BL + amendments: B, G, Z, and S, with their four different rates, are presented in Table 5.

3.3.2 Laboratory Incubation

The incubation experiment was performed at Auburn University, Alabama. Broiler litter was homogenized before using them for incubation. The moisture was maintained at 40% throughout the experiment to mimic the moisture content of BL in broiler houses. One hundred grams of BL was taken in an 8 cm diameter mason jar and different rates of amendments (Table 4) were evenly spread on the surface of BL inside the jar. The experiment was conducted with a completely randomized design comprised of 17 treatments. All the jars were kept inside the incubator [KB240 UL (E6), Binder Inc] at 30 ºC for 40 days.

3.3.3 Characterization of amendments

The pH of pre- and post-incubated treatments was determined using a 1:2 (sample: Deionized water) ratio (Mitchell & Donald, 1999). Total nitrogen (TN) and total carbon (TC) content were analyzed using the thermal combustion method using a CNS Analyzer (Vario Max, Elementar Americas). The amendments were digested to determine the total digestible elements following Campbell & Plank, (1992); Huang & Schulte, (1985); where 0.5 g of dry sample was digested with 5ml of HNO_3 and 3 mL of H_2O_2 and analyzed in Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

3.3.4 Gas sampling and analysis

The gas sampling process comprised of sealing the empty glass vials (5ml) with the rubber stopper placed on top of each vial and capped and crimped to ensure they were properly closed and secured for sampling. The vials were evacuated using air and helium for two minutes and ten seconds. Evacuation of vials removed the residual gases or contaminants from the vials and ensured accurate sampling. The gas (CO_2, CH_4, N_2O) samples from the headspace of the jars were collected in a pre-evacuated vial using a 10 ml syringe immediately after the application of amendments at 0-minute, 60-minute and 120-minute intervals. The gas samples were collected on days 1, 2, 3, 6, 9, 12,16, 20, 24, 28, 32, 36, and 40. The syringe was flushed three to five times to ensure that the jars' gases were well-mixed and homogenized. Flushing the syringe enhances the precision and reliability of the gas sampling process. This procedure was repeated during each sampling. The gas samples were analyzed using a Shimadzu Gas Chromatography equipped with a flame ionization detector (FID) and electron capture detector (ECD).

3.3.5 Calculations:

Gas emission rate was calculated as:

$$E = P \times V \times \frac{\Delta C}{\Delta t} \times \frac{1}{RT} \times \frac{M}{m} \quad .... (1)$$

where E stands for emission rate (μg g^{-1} h^{-1}), P refers to standard atmospheric pressure (Pa), V is the headspace volume (m^3), c is the concentration of CH_4 (ppm) CO_2 (ppm), and N_2O (ppm), t is the time between two samples (h), R (m^3 Kpa $mol^{-1}k^{-1}$) refers to the universal gas constant, T stands for absolute air temperature (K), M is N_2O molecular mass (g mol^{-1}) and m dry weight of BL (g) (Deng et al., 2016).

GWP was calculated using the following equations:

CO_2 eq = CO_2e = ∑ (Ei × GWPi) …. (2)

Where, CO_2 eq = CO_2 equivalent, Ei = Total cumulative emission of gases (CH_4, CO_2, CH_4, N_2O)

GWP = Global Warming Potential of the CO_2, CH_4, N_2O

GWP = [Cumulative CO_2 × 1] + [Cumulative CH_4 × 25] + [Cumulative N_2O × 298] ….(3)

% contribution on GWP (CO_2 or CH_4 or N_2O) = [(Cumulative CO_2 or CH_4 or N_2O) / GWP] × 100 ….(4)

3.4 Statistical Analysis

Statistical analysis was performed using repeated measurement, and Toeplitz was selected based on lower AIC value by the PROC GLIMMIX procedure in SAS 9.4 (SAS Institute). We measured gas samples for three different time interval 0-minute, 60-minute and 120-minute, where the intervals of 0 and 60 minute were considered as replication 1 and the intervals between 0 and 120 minutes were considered as replication 2. The treatment rate, and days were considered fixed effects, and replication was considered as random effects. The ANOVA was performed to see the interaction between fixed effects (day and treatment rate). Treatment means were separated using the TUKEY test in SAS. We used the linear interpolations method in R studio to estimate the emissions between non sampling days. For all analyses, significance was given as a < 0.05. The graphs were prepared using Microsoft Excel 365.

3.5 RESULTS AND DISCUSSIONS

3.5.1 pH of broiler litter treated with amendments

The differences in pH of the BL immediately after the application of amendments (pre-incubation) and after 40 days of incubation (post-incubation) are presented in Table 6. The range of pH for all treatments immediately after the application of amendments was between 5.8 and 8.2, whereas the pH after the 40 days of the experiment ranged from 6.70 to 8.15. For pre-incubation, all four rates of S significantly reduced ($P < 0.05$) pH from 8.1 (control) to 5.9 (6% S) compared to other treatments. Similarly, for post-incubation, BL treated with 6% S and 7% S significantly reduced pH compared to other treatments. Among all the treatments of B, there was no statistical difference in pH between treatments and pre- (range: 8.1 - 8.2) and post-incubation (range: 7.9 - 8.2) . Similar results were observed for Z and G between treatments. Among the 21 treatments, pH was significantly lower for 3% Z, 6% Z, 8% Z, 11% G, 2% S, 6% S, and 7% S between pre- and post-incubations. Application of S lowered the pH immediately in BL. However, after 40 days of incubation, the effectiveness of S in lowering pH was neutralized, resulting in an increase in pH in post-incubated samples.

3.5.2 Effect of amendments on CO_2 emission

The daily CO_2 emission from BL treated with four different rates of B along with control are presented in Figure 7a. For the 40 days of incubation, the daily CO_2 emissions ranged from 0.07 g kg^{-1} day^{-1} to 7.27 g kg^{-1} day^{-1}. The ANOVA showed significant differences in daily CO_2 emissions between the treatment rates, day, and their interactions (Table 7). We observed higher CO_2 emissions from 17%, and 13% B treated BL. On sampling day 2, CO_2 emissions from 5% B and 17% B were higher compared to other treatments. With 5% B application, the daily trends in CO_2 emissions showed larger variation throughout the experiment. With 13% B and 17% B, CO_2 emissions peaked on day 12 and day 24 up to 7.27 g kg^{-1} day^{-1} and slightly decreased. Among the treatments, control had the lowest emission throughout the study period. The cumulative CO_2 emissions over the 40 days were 27.84, 55.26, 51.83, 105.18, and 96.16 g kg^{-1} for control, 5%, 9%, 13% and 17% B, respectively. There was no significant difference in cumulative CO_2 emissions between treatments from day 2 to day 9; however, there was a significant difference *($P < 0.05$)* between treatments for other days (Supplementary Figure 1a). There was no statistical difference between the rates 5% B and 9% B; similarly, there was no difference between 13% and 17% B treatments throughout the experiment period. The effect of B treatment on animal manures showed mixed types of results in previous studies. A study by Wang et al. (2018) added B on swine manure during composting, and reported a 26.1% reduction in CO_2 emission. In contrast, a study by Mao et al. (2018) found that adding B on swine manure increased CO_2 emissions by 53.2%. Liu et al. (2017) reported that biochar derived from bamboo can increase the sequestration of exogenous organic matter during poultry manure composting, which lowers CO_2 emissions. Mao et al. (2018) found that biochar can improve the habitat for microorganisms, allowing them to be more active which accelerates the degradation of organic matter and increases CO_2 emissions and might be the similar mechanism that increased CO_2 emissions in our study. Increased CO_2 emissions from biochar application were also observed by Saarnio et al., 2013; Spokas, 2013; Tang et al., 2021 in field and laboratory conditions. The CO_2 emissions from B are also affected by the feedstock that is used during pyrolysis. A study by Shakoor et al. (2021) identified that B from lignocellulosic materials contributed to higher emissions of CO_2 as whereas B derived from herbaceous feedstock had no effect on CO_2 emissions, and manure-derived B showed the greatest mitigation potential for CO_2 emissions.

The daily CO_2 emissions from BL treated with four different rates of Z are presented in Figure 7b. The CO_2 emissions of different rates of Z ranged from 0.07 g kg^{-1} day^{-1} - 2.76 g kg^{-1} day^{-1}. The treatment rates, day, and their interaction effects were significant for the daily CO_2 emissions (Table 7). The daily emissions of CO_2 from different rates of Z do not show a consistent pattern. All the rates of Z, including control, higher CO_2 emissions up to 40 days compared to day 1. Application of 3% Z had higher emission and reached a peak on day 6 (1.67 g kg^{-1} day^{-1}) and day 16 (2.76 g kg^{-1} day^{-1}), after that it decreased up to day 21, again gained peak on day 24 and decreased gradually on later stages. The BL treated with 6% Z and 11% Z showed fluctuations throughout the experimental period. The cumulative CO_2 emission, from 3% Z, was 42.71 g kg^{-1}, 6% Z was 26.67 g kg^{-1}, 8% Z was 28.35 g kg^{-1}, and 11% Z was 28.03 g kg^{-1}. There was a significant difference *($P < 0.05$)* in CO_2 emissions between the treatments from day 2 to day 13 and no significant differences were observed for other days; however, the cumulative CO_2 emissions with 3% Z were distinctly higher compared to other treatments (Supplementary Figure 1b). The results indicated that zeolite was not effective in reducing CO_2 emissions from BL. However, few studies conducted in the past reported reduction of CO_2 when Z was treated alone or in combination with other substances. Peng et al. (2019) investigated the effect of Z, superphosphate, and ferrous sulfate in different combinations on reducing GHG emissions during composting of PL for 46 days. The treatment of zeolite (350 kg per 7-ton chicken litter) alone reduced the cumulative CO_2 emission by 7.8%. Similarly, Wang et al. (2012) studied the effect of Z (12%) and other substances (10% reed straw, 5% superphosphate dry weight basis) on GHG emissions from duck manure during storage for 46 days. The addition of reed straw, Z, and superphosphate, alone or in combination, was effective and decreased the cumulative CO_2 emission by 13 - 32% compared to control. Moreover, the use of clinoptilolite (zeolite) as litter amendments was studied in a breeding hen house under Mediterranean climate conditions where two similar types of poultry houses were selected; one house was control treatment while the another had clinoptilolite additives during winter for 20 days (Pereira et al., 2019). The clinoptilolite applications on the litter of a breeding hen house had no effects on CO_2 emissions. The differences in results could be due to properties of Z where the ion exchange and adsorption properties can lead to the removal of the specific nutrient substrate (nitrogen) and compounds necessary for microbes and alter the microbial abundances. This can influence CO_2 emissions and also influenced by bedding materials, moisture content and litter pH.

The daily trends of CO_2 emissions from BL treated with different rates of G are presented in Figure 7c. The daily CO_2 emissions ranged from 0.24 to 4.04 g kg^{-1} day^{-1}. The effect of treatment rates and days was significant for daily CO_2 emissions from G, however their interaction was not significant (Table 7). The CO_2 emissions from all the treatments fluctuated throughout the experiment period. The CO_2 emission from 15% G peaked on day 28 (4.04 g kg^{-1} day^{-1}) and was the highest emission observed. The cumulative CO_2 emissions over the 40 days were 27.84 g kg^{-1} for control, 31.79 g kg^{-1} for 6% G, 30.79 g kg^{-1} for 11% G, 52.73 g kg^{-1} for 15% G, 40.59 g kg^{-1} for 19% G. The cumulative CO_2 emission showed that with the addition of

G, there was an increase in CO_2 emissions compared to the control and the treatment with 15% G had the higher cumulative CO_2 (Supplementary Figure 1c). However, there was no statistical difference in cumulative CO_2 emissions for day 40. Although there were no statistical differences in CO_2 emissions between G treatments in our study, G at 15 and 19% had increased CO_2 emissions by 89 and 46%, respectively (Table 9). The potential use of G in broiler houses as a bedding materials has been studied by Watts et al., (2017); however, relatively little research focused on the effect of G on GHG emissions. A study by Watts et al. (2019) investigated the use of FGD-G in broiler houses and reported no effect on CO_2 emissions.

The daily emissions of CO_2 for four different rates of S ranged from 0.31 - 8.39 g kg^{-1} day^{-1} (Figure 7d). We observed a significant effect of treatment rates, days, and their interactions with daily CO_2 emissions from BL treated with S (Table 7). The CO_2 emissions were higher on the first sampling day and decreased sharply on day 2. The daily emissions rate for all different rates of S, including control decreased CO_2 emissions up to 20 days, and after that, it slightly increased up to 24 days and started decreasing constantly after day 28. The cumulative CO_2 emissions were 30.26 g kg^{-1} from 2% S, 24.12 g kg^{-1} from 4% S, 19.86 g kg^{-1} from 6% S, 19.04 g kg^{-1} from 7% S. Compared to control, S at rates of 4%, 6%, and 7% reduced CO_2 emissions whereas CO_2 emissions were higher for 2% S. There was a significant difference *($P < 0.05$)* in cumulative CO_2 emissions up to day 15 and no significant difference between treatments afterward (Supplementary Figure 1d). This indicates that higher application rates of S can potentially inhibit CO_2 production throughout the study period. The effect of S on CO_2 emissions has not been investigated yet, even if S has been commercially used in poultry production systems to inhibit microbes and reduce ammonia emissions. Our findings showed that S helps significantly to reduce CO_2 emissions in comparison to other amendments. The S addition lowers the pH of BL, creates a less favorable environment for microbial activity, and may influence gaseous emissions; however, firther research should be conducted to understand its actual mechanism and processes.

3.5.3 Effect of amendments on CH_4 emission

The daily trends of CH_4 emission from BL treated with B are presented in Figure 8a. The daily CH_4 emissions showed higher variability ranging from positive to negative emissions. The daily emissions varied between -0.16 mg kg^{-1} day^{-1} and 0.42 mg kg^{-1} day^{-1}. The treatment rates, day, and interaction effects significantly differed for daily CH_4 emissions (Table 7). The net CH_4 emissions were low, and treatment characteristics varied as a source and sink throughout the experiment. The cumulative CH_4 emissions were 0.54 mg kg^{-1} from control, -0.92 mg kg^{-1} from 5% B, 1.48 mg kg^{-1} from 9% B, 1.48 mg kg^{-1} from 13% B, and 1.14 mg kg^{-1} from 17% B. Biochar at rates 5% acts as a sink whereas the other rates of B act as a net source and had higher emissions compared to control. There was a significant difference $(P < 0.05)$ in cumulative CH_4 emissions between treatments up to day 14, and afterward, we observed no significant differences (Supplementary Figure 2a). Among the treatments of B, 5% B had negative CH_4 emissions whereas 13% B and 17% B always acted as a source throughout the study. The emissions from control increased only after 14 days of the experiment and started acting as a source. Similar to CO_2, results from previous research showed larger variability in CH_4 emissions from B treatments. Dang et al. (2024) reported that different types of B (wood chip, corn stalk, rice husk) at a rate of 10% (by dry wt. PL) during composting of poultry manure decreased CH_4 by 18.9 to 27.5% compared to control. The production of CH_4 is accomplished by a process called anaerobic decomposition of organic substances (biological oxidation of carbon (C) in the absence of oxygen) (Jones et al., 2014). This requires two main groups of anaerobic bacteria called ‘acid formers’ and ‘methane formers/methanogenic archaea’. The acid-forming bacteria convert manures to organic acids and then methane-forming bacteria convert organic acids to methane and carbon dioxide. According to Cornejo and Wilkie. (2010), the ideal environment for the production of CH_4 requires anaerobic conditions, a high concentration of nutrients for bacterial development, and a warm environment. Furthermore, CH_4 production is best suited in pH between 6 and 8 (around 7), and beyond those ranges, stored manure may reduce CH_4 production (Berg et al., 2006). Therefore, the addition of biochar also showed mixed results in their findings. Biochar influences CH_4 emissions by modulating the activity of specific microorganisms, particularly methanotroph communities, and by altering various properties, including aggregation, porosity, moisture, temperature, pH, and nitrogen content (He et al., 2021; Troy et al., 2013; Zhao et al., 2021). These changes impact the movement of CH_4 and its subsequent oxidation by methanotrophs. In contrast, some findings reported that the labile C component in B that can decompose and can becomes a predominant substrate for methanogens and enhances CH_4 emissions (Knoblauch et al., 2011). The conflicting results on CH_4 emissions may be due to variable responses of methanogenic bacterial activity and methanotrophic activity due to B characteristics.

The daily trends of CH_4 emission from BL treated with Z are presented in Figure 8b and showed higher variability ranging from positive to negative values -0.11 mg kg^{-1} day^{-1} to 0.27 mg kg^{-1} day^{-1}. There was significant difference in daily CH_4 emissions for treatment rates, days, and their interaction (Table 7). The net CH_4 emissions were very low, and the treatments acted as a sink in the early stage of the experiment, acted as a source in the mid stage of the experiment, and again acted as a sink at a later stage, except for 3% Z and 9% Z. The cumulative average emission from 3% Z was 0.65 mg kg^{-1}, 6% Z was 0.012 mg kg^{-1}, 8% Z was 0.34 mg kg^{-1}, and

11% Z was 0.19 mg kg^{-1}. Zeolites at 3% acts as a sink from day 3 to day 35, 6% act up to day 19, 8% act as a sink up to day 17, 11% Z act as a sink up to day 21, whereas on the other days different rates of Z act as a net source and had lower emissions compared to control except 3% Z. There was no significant difference in cumulative CH_4 emissions for all the treatments rates of Z (Supplementary Figure 2b). This indicated no significant effect of different rates of Z application on CH_4 emission from BL. A study by Wang et al. (2012) used Z (12%) mixed with 10% reed straw and 5% superphosphate on GHG emissions from duck manure during storage for 46 days. Their study showed that the cumulative CH_4 emissions increased compared to the control. Another study by Peng et al. (2019) investigated the effect of Z, superphosphate, and ferrous sulfate in different combinations during poultry litter composting for 46 days. The cumulative CH_4 emissions were reduced by 56% by Z, 50.6% by ferrous sulfate + Z, 62.3% by superphosphate, and 73.6% by Z + superphosphate + ferrous sulfate. Awasthi et al. (2016) studied the role of biochar amendment, mixing with zeolites, 1% lime, and sewage sludge during composting and found that the combination of B + Z considerably boosted the activity of enzymes and reduced CH_4 by 92.8 - 95.3% compared to control. The study by Wang et al. (2017) found that Z treatment showed a reduction of CH_4 emission up to 88.45% compared to the control. The contrasting results in our study and previous studies might be due to the differences in experimental setup where the earlier studies were performed during the composting process that differs the physiochemical processes involved, whereas our study was under laboratory incubation.

The daily trends of CH_4 emission from BL treated with G are presented in Figure 8c. There were fluctuations in the daily CH_4 emissions, with values ranging from positive to negative. The daily emissions for treatments with G application varied between -0.15 mg kg^{-1} day^{-1} to 0.22 mg

kg^{-1} day^{-1}. We observed significant differences in treatment rates, days, and their interactions with daily CH_4 emissions. Similar to Z, the CH_4 emission was negative during the initial stage, positive in the mid stage, and negative in the later stage of the experiment. The cumulative CH_4 emissions were 0.29 mg kg^{-1} from 6% G, 0.76 mg kg^{-1} from 11% G, -0.04 mg kg^{-1} from 15% G, and 0.53 mg $kg^{-1}$from 19% G. The treatment with 15% G act as sink whereas other treatments including control were the source of CH_4 (Supplementary Figure 2c). In comparison to the control, the treatments with 6% G and 15% G had lower emissions. There was no significant difference between the treatments for all days except days 1, 2, 6, 7, 8, 9, 10, and 11 (Supplementary Figure 2c) and no significant effect on CH_4 emissions from BL. Similar results were observed by Watts et al. (2019) where they investigated the use of FGD-G and its impact on GHG emissions from broiler houses and found no effect. In contrast Berg and Model (2008) studied the impact of G application on CH_4 emissions from swine slurry under different setup and reported a reduction in CH_4 emissions. This indicated that G effectiveness on reducing CH_4 emissions differs by type of manure and its compositions as well.

The daily CH_4 emissions from the BL treated with S ranged from negative to positive (-0.13 to 0.22 mg kg^{-1} day^{-1}) over the study period (Figure 8 d). There was a significant effect of treatment rates, days, and their interactions for daily CH_4 emissions from BL treated with S (Table 7). The daily emission from different treatment rates didn't show a definite pattern and showed alternate source and sink behaviors. All the treatments for CH_4 emissions were positive on day 1^{st} except control, and from day 2 to day 7 all the treatments, including control, acted as a sink. Furthermore, 6% S and 7% S act as a source from day 8 to 12, then act as a sink until day 22; again, it starts to act as a source up to day 28; after that, it behave as a sink until day 39. Control acted as a source from day seven and had higher CH_4 emissions on day 16, which is 0.13

mg kg^{-1}, and afterward, the emissions decreased up to day 27 and started to act as a sink until day 39. Moreover, 4% S showed an inconsistent trend throughout the experiment then, the emissioons was negative upto day 11, acts as a source from day 12 to day 19, then acts as a sink up to day 20, and acts as a source until day 26, which again started to increase CH_4 emissions and reached a peak (0.17 mg kg^{-1} day^{-1}) on day 36 and decreased. Similar trends were observed for 2% S, which peaked on day 36 (0.22 mg kg^{-1} day^{-1}). The cumulative CH_4 emissions from both 2% S and 4% S was 0.57 mg kg^{-1}, 6% S was -0.35 mg kg^{-1} (sink) and 7% S was -0.26 mg kg^{-1} (sink) (supplementary Figure 2d). There was a significant difference between *($P < 0.05$)* the treatments for cumulative CH_4 emissions on day 1 and afterward, no statistical differences were observed between the treatment rates.

3.5.4 Effect of amendments on N_2O emission

The daily N_2O emissions from BL treated with four different rates of B are presented in Figure 9a. The daily emissions for all treatments ranged from 0.15 mg kg^{-1} - 2.57 mg kg^{-1}. The ANOVA showed significant effects of treatment rates, days, and their interactions for daily N_2O emissions (Table 7). Higher N_2O emissions were observed for higher rates of biochar compared to control. The daily emissions showed a more significant fluctuation in N_2O emissions for the BL treated with 13% B and 17% B. In contrast, control, 5%, and 9% B had higher emissions during the initial sampling days and gradually decreased over time. The daily emissions of N_2O, with 9% B, sharply decreased on the second day, and then the emissions rate increased for up to six days, showing decreasing trends afterward. The cumulative N_2O emissions from control were 18.62 mg kg^{-1}, 26.76 mg kg^{-1} from 5% B, 19.45 mg kg^{-1} from 9% B, 46.41 mg kg^{-1} from 13% B, and 51.16 mg kg^{-1} from 17% B. There was no significant difference in cumulative N_2O emissions between all treatments for the first and second sampling days, and afterward, there were no significant differences between control, 5% B, and 9% B (Supplementary Figure 3a). However, the cumulative N_2O emission was significantly higher ($P < 0.05$) for 13% B and 17% B compared to other treatments. The results indicated that the B application was ineffective in reducing N_2O emissions from BL, and the treatment rate of < 9% B significantly increased N_2O emissions. The results from previous findings were also not consistent for N_2O emissions. For example, Dang et al. (2024) performed composting of poultry manure with varying types of B (wood chips, cornstalks, and rice husk applied at 10% dry wt.) and found that B application reduced N_2O by 35.7 to 52.8 %. A review article by Shrestha et al. (2023) pointed that 27 out of 37 published articles reported a reduction in N_2O emissions with increasing B application rate. The mode of action of B in reducing N_2O emission during composting of manure or application in soils are described in different prospectives**.** Some studies have indicated that B reduced the availability of inorganic N (altered mineral N content that can be utilized by nitrifying bacteria and denitrifying bacteria) by capturing NH_4^+ and NO_3^- leading to less activity of those bacteria, lowering N_2O emission (Harter et al., 2016; L. He et al., 2019; Xiao et al., 2017). The functional group on the surface of B can also absorb N_2O and reduce it to N_2 through biological or abiotic reactions (Harter et al., 2016). Biochar contains (C = O) oxygen-containing functional groups, such as phenolic, hydroxyl groups, which can provide electrons to promote N_2O reduction, whereas carboxyl, carbonyl, and quinone functional groups can compete for electrons with microorganisms and consequently inhibit the reduction of N_2O to N_2 (Yuan et al., 2021). Although most findings reported that adding B reduced N_2O emissions, our results showed a significant increase in N_2O emissions with increasing application rate (> 9%) of B. Few research have showed similar results to our study where their findings showed no effect or increase in N_2O emissions with biochar application (Shen et al., 2014; Spokas & Reicosky, 2009; Sun et al., 2020). However, those results were from soils and field application. We hypothesize that the significant contributing factor for increased N_2O emission could be due to the additional N input from BL that supplied more substrates for nitrifying and denitrifying bacteria (Shen et al., 2014). Nitrous oxide production occurs by nitrification and denitrification processes (Zainudin et al., 2020). During composting and application of manure, atmospheric oxygen is supplied, and the nitrifying bacteria converts ammonium to nitrate through the nitrification process in aerobic conditions. In addition, there are two phases of nitrification called nitritation, in which NH_4^+ is oxidized to NO_2, and nitratation, which involves NO_2 to NO_3. The aerobic oxidation of NH_4^+ to NO_3^- is driven by chemoautotrophic bacteria (Paul and Musgrave, 2007). When oxygen level fluctuates due to moisture or compaction, denitrifying bacteria convert nitrate to nitrogen through the denitrification process, and N_2O is released as an intermediate byproduct. The rates of denitrification and the proportions of N_2O, NO, and N_2 produced by this process are influenced by intricate interactions between physio-chemical properties and environmental factors, including temperature, pH, moisture status, and the availability of oxygen (O_2), nitrogen (N_2), and labile carbon (C) (Case et al., 2015). Therefore, the mechanisms to explain how B influences N_2O emissions from manures are uncertain and subtle.

The daily N_2O emissions from BL treated with four different rates of Z are presented in Figure 9b. The daily N_2O emissions from all zeolite treatments ranged from 0.11 mg kg^{-1} day^{-1} to 2.01 mg kg^{-1} day^{-1}. There were significant differences in daily N_2O emissions between the treatments rates and days whereas their interaction was not significant (Table 7). Throughout the trial, there was fluctuating pattern in the daily N_2O emissions. On the first day of sampling, N_2O emission was higher for all the treatments; control was highest compared to other treatments. Furthermore, BL treated with 3% Z reached a peak on day 9 and day 16 and afterward showed a decreasing trend. Similarly, 6% Z had higher emissions on the first day, decreased up to day 6, gained a peak on day 16, and decreased in the later stage of the experiment. For the treatment with 8% Z, emissions decreased up to day 16, reached on day 24, decreased up to day 28, and slightly increased up to 40 days. Similarly, 11% Z, N_2O emissions decreased up to day 20, reached a peak on day 28, decreased and increased again up to day 36, and gradually decreased after onwards. The cumulative N_2O produced by control was 18.62 mg kg^{-1}, 3% Z was 24.81 mg kg^{-1}, 6% Z was 19.68 mg kg^{-1}, 8% Z was 19.71 mg kg^{-1}, 11% Z was 19.18 mg kg^{-1}. There was no significant difference in cumulative N_2O emissions between all treatment rates of Z for all 40 days (Supplementary Figure 3b), which indicates that there was no significant effect of different rates of Z application on N_2O emissions from BL. However, previous studies have some contrasting results. A study by Wang et al. (2024) used zeolite (10%) during PL composting and found that N_2O emission reduced by 55% compared to control. Another study that used clinoptilolite (zeolite) as litter amendments under Mediterranean climate conditions inside a breeding hen house decreased N_2O emissions by 34% compared to control (Pereira et al., 2019). The properties of Z, which had a higher affinity for NH_4^+ ions, reduce ammonium nitrogen availability and may contribute to a reduction of N_2O production during nitrification. However, the process might be influenced by environmental factors.

The daily N_2O emissions from BL treated with four different rates of G, including control, are presented in Figure 9c. The daily emissions for all treatments of G ranged from -0.05 mg kg^{-1} day^{-1} to 2.57 mg kg^{-1} day^{-1}. The treatment rates, days and interactions effect were significant for daily N_2O emissions for G (Table 7). The daily emission pattern of N_2O from BL treated with G was higher in the initial days and reduced later in the experiment. The cumulative N_2O produced was 18.6, 19.9, 20.4, 23.3, and 21.5 mg kg^{-1} from control, 6% G, 11% G, 15% G, and 19% G, respectively. There was no statistical difference in N_2O emissions from BL treated with G for all 40 days of the experiment period (Supplementary Figure 3c). The results for G application and its effect on N_2O emissions from BL had no significant effect in our study and is consistent with Watts et al. (2019), who reported no effect of G on GHG emissions inside broiler houses.

The daily N_2O emission from BL treated using four different rates of S and control are shown in Figure 9d. The daily emissions for four S treatments ranged from -0.01 to 3.64 mg kg^{-1} day^{-1}. There were significant differences in treatment rate, day, and their interactions for daily N_2O emissions for the overall experiment period (Table 7). The N_2O emission was higher for all treatments on the first day and decreased sharply on day 2. Control had lower emissions on day 3; however, the emissions of N_2O from control increased slowly up to day 6 and started to decrease gradually up to day 9 and steady afterward. Broiler litter treated with lower to higher rates of S showed a decreasing trend during the whole experimental period. Higher rates of S resulted in lower emissions compared to lower rates. Over 40 days of incubation, the mean cumulative N_2O emissions from control, 2% S, 4% S, 6% S, and 7% S were 18.6, 13.62, 12.45, 10.05, and 9.76 mg kg^{-1}, respectively. All the rates of S had shown potential to reduce N_2O emissions compared to control, and higher application rates of 6% S and 7% S had higher N_2O reduction. There was no significant difference in cumulative N_2O emissions between treatments for day 1 and days 4 - 26, and treatments were significantly different *($P < 0.05$)* on other days (Supplementary Figure 3d).

The mean comparison of daily gaseous emission between the 17 treatments under study are presented in Table 8. The mean daily CO_2 emissions was highest for 13% and 17% B and was significantly higher than other treatments and lowest for 6% and 7% S. The mean daily CH_4

emissions were greater for 9% and 13% B however no significant differences between the treatments except for 5% B and 6% S. Similar to CO_2, the mean daily N_2O emission was significantly higher for 13% and 17% B treatments and lowest for 6% S and 7% S. This indicated that the mean daily gaseous emissions were also lowest for 6% S and 7% S whereas highest for 13% B and 17% B.

Oveall, the effect of each amendment and their rates varied largely between the studies and among different gases. The factors attributing to gaseous emissions depend on the properties of amendments, the manure characteristics, and the environment. The specific mechanism for an increase or decrease or no effect for specific gases is difficult to justify as the mechanism involves complex physical, chemical, and biological processes interacting over time. Some of the research findings in literatres have identified that the use of amendment in combination with other substances has enhanced its efficacy in reducing GHG emissions than an amendment alone (Awasthi et al., 2016; Wang et al., 2018; Wang et al., 2022). For example, research conducted by Wang et al. (2022) investigated the use of B (5%) mixed with different rates of wood vinegar (WV) (0.5%, 1%, and 1.5%) in swine manure. The addition of B with different rates of WV decreased CO_2, CH_4, and N_2O emissions by 1.38 - 15.39%, 16.98 - 62.73%, and 4.47 - 19.91%, respectively, compared to the control. The incorporation of WV could enhance the absorption efficiency of B, thereby reducing GHG emissions. Another study by Yuan et al. (2017) used a mixture of hardwood sawdust + rice hull B (produced at low temperature) at a ratio of 2:1:7 (B: sawdust: manure) and another treatment with chicken manure + sawdust (3:7 ratio). They reported that B + chicken manure compost reduced N_2O emissions by 27% and CO_2 emissions by 35% compared to the control. A combination of amendments was used in previous research (Awasthi et al., 2016). They studied the role of B mixed with Z during the composting of sewage sludge and found that the combination of B + Z reduced N_2O between 95.1 to 97.3% compared to control.

Moreover, there are insufficient datasets for amendments and poor understanding of the mechanism by which those amendments reduce GHG emissions. Further studies that combine two or three amendments in different ratios can provide information about their synergistic effects and identify a suitable combination for GHG reductions. As the effect of amendments on GHG emissions from BL or other manure sources (from literature) have demonstrated large ambiguity, and further investigation from physical, chemical, and biological perspectives is suggested.

3.5.5 Percent increase and percent decrease of GHG emissions from BL

The percent increase and decrease of cumulative emissions from the BL treated with different rates of amendments for 40 days are presented in Table 9. All the rates of biochar increased CO_2, CH_4, and N_2O emissions compared to control. Among the four rates of Z, 6% Z reduced CO_2 by 4.2%, and CH_4 by 98% whereas other rates increased emissions compared to control. The Z application increased N_2O emissions up to 33%. Gypsum application increased the emission of CO_2 and N_2O compared to control. The application of 6% G and 19% G decreased CH_4 emissions by 45% and 1.8%, respectively. The S application reduced CO_2 emission by 13% to 31%, however, 2% S increased CO_2 emission by 8% compared to control. The 6% and 7% S reduced CH_4 emission by 164% and 149%, whereas lower rates 2% S, increased CH_4 emission compared to control. All rates of S reduced N_2O emissions up to 47%.

3.5.6 Global Warming Potential

The ability of a gas to trap heat in the atmosphere over a specific time is described as Global Warming Potential (GWP). It is determined by comparing the cumulative radiative energy caused by the immediate release of 1 kg of specific gas to that of 1 kg of a reference gas (IPCC, 2021). The GWP of CO_2 is considered one and serves as the reference for comparison whereas CH_4 and N_2O have GWP values of 25 and 298 times greater than that of CO_2, respectively, over a 100-year timescale (Pachauri et al., 2014; Watts et al., 2019). The GWP for each treatment and the percentage contributions of each gas to the total GWP are presented in Table 10. The highest GWP was obtained for 13% B, and the lowest was observed for 7% S. Comparison between the three gases showed the overall contributions to GWP were higher from CO_2, ranging from 81 to 90% across all the treatments, whereas CH_4 contribution was lowest and ranged from -0.05 to 0.06%. The N_2O contribution to GWP ranged between 9 to 18%. Treatment B had an average of 17.99, 0.02, and 11.99% contribution to GWP from CO_2, CH_4, N_2O, respectively. For Z treatments, average contributions from CO_2, CH_4, and N_2O, to GWP were 83.26, 0.02, 16.72%, respectively. For FGD-G, the contributions of N_2O < CO_2 and CH_4 to GWP were 14.36, 85.62, 0.02 %, respectively. For BL treated with S, the contributions to GWP were 12.88% by N_2O, 87.11% by CO_2 and, 0.01 % by CH_4. Since the emissions of CO_2 and N_2O increased significantly in B treatment, their contributions to total GWP were higher. Among the three gases, CH_4 was found to be the most minor contributor to GWP from BL, and our findings were consistent with Watts et al. (2020), who reported < 0.01% contributions of CH_4 to total GWP in control and G treatments. Based on these results, as CH_4 is a lesser contributor to total GWP from BL, future research should focus on reducing CO_2 and N_2O gases from BL to reduce GWP from the poultry production.

3.6 CONCLUSIONS

This study investigated the rate effect of amendment (B, Z, G, and S) addition to BL on GHG emissions. The effect of amendments and their rates on daily and cumulative emissions varied largely for individual gases. Adding B at higher rates (13 and 17%) increased the N_2O, CO_2, and CH_4 emissions, whereas lower rates (5 and 9%) had no effect compared to control. Zeolite and G did not help reduce GHG emissions compared to the control. Among the four amendments, 7% S reduced CO_2, CH_4, and N_2O by 31.6, 149.6, and 47.6 %, respectively, from BL. As CH_4 had fewer contributions to GWP from BL in this study, 4% S, 6% S, and 7% S, were found as a potential amendment to minimize GWP from the broiler industry. This study provided valuable insights into the selection of amendments and their rates for future investigations on amendments. To confirm our laboratory findings, future investigation can be carried in commercial poultry houses.

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Tables:

Table 4: Characteristics of the amendments used in the incubation study.

Parameters	Biochar	Zeolite	Gypsum	Sodium bisulfate
pH	6.50	7.00	7.60	0.10
TC (%)	22.80	0.22	0.32	0.05
TN (%)	0.11	0.06	0.13	0.10
TP (g kg^{-1})	2.61	0.23	0.23	0.18
TMg (g kg^{-1})	4.17	1.62	1.44	0.21
TK (g kg^{-1})	5.73	22.4	0.18	0.00
TAl (g kg^{-1})	7.05	26.78	0.04	0.04
TCa (g kg^{-1})	35.93	9.43	200.8	0.24

Note:TC, Total Carbon; TN, Total Nitrogen; TP, Total Phosphorus; TMg, Total Magnesium; TK, Total Potassium; Tal, Total Aluminum; TCa, Total Calcium

Table 5: Different types of amendments, their description, and application rates evaluated for greenhouse gas emission in this study.

Treatment Description	Amendment Type	Treatment name
100 g BL (Unamended litter)	Broiler litter	BL (control)
100 g BL + 5g Biochar	Biochar	5% B
100 g BL + 10g Biochar		9% B
100 g BL + 15g Biochar		13% B
100 g BL + 20g Biochar		17% B
100 g BL + 3g Zeolite	Zeolite	3% Z
100 g BL + 6g Zeolite		6% Z
100 g BL + 9g Zeolite		8% Z
100 g BL + 12g Zeolite		11% Z
100 g BL + 6g FGD-Gypsum	FGD-Gypsum	6% G
100 g BL + 12g FGD-Gypsum		11% G
100 g BL + 18g FGD-Gypsum		15% G
100 g BL + 24g FGD-Gypsum		19% G
100 g BL + 2g Sodium bisulfate	Sodium bisulfate	2% S
100 g BL + 4g Sodium bisulfate		4% S
100 g BL + 6g Sodium bisulfate		6% S
100 g BL + 7g Sodium bisulfate		7% S

Table 6: Comparison of pH for broiler litter (BL) treated with amendments between pre- and post-incubation experiments.

Treatments	Pre-incubation	Post-incubation
BL	8.1 a	8.1 a
BL+ 5% B	8.2 a	8.2 a
BL + 9% B	8.1 a	8.0 ba
BL + 13% B	8.1 a	7.9 ba
BL + 17% B	8.1 a	8.0 ba
BL + 3% Z	8.1 a	7.9 ba, *
BL + 6% Z	8.1 a	7.9 ba, *
BL + 8% Z	8.1 a	7.9 ba, *
BL + 11% Z	8.2 a	7.9 ba
BL + 6% G	8.2 a	7.8 ba
BL + 11% G	8.1 a	7.9 ba, *
BL + 15% G	8.2 a	7.9 ba
BL + 19% G	8.0 a	7.9 ba
BL + 2% S	6.9 bc	7.7 b, *
BL + 4% S	6.9 bc	7.6 b
BL + 6% S	5.9 cd	7.2 c, *
BL + 7% S	6.0 d	6.7 d, *

Note: Means with the same letter represent no significant difference among treatments; all mean comparisons were at $P < 0.05$. The letter represents the mean comparison between the treatments of pre-incubation and post-incubation. *Represents a significant difference between pre and post-incubation.

Table 7: Probability (P) values derived from analysis of variance (ANOVA) for the effects of treatment rates (grouped by amendments including control), days and their interactions on CO_2, CH_4, and N_2O emissions.

Amendments	Rate	Day	Rate x Day
		CO_2	
B	< 0.0001	< 0.0001	< 0.0001
Z	< 0.0001	< 0.0001	0.0065
G	< 0.0001	0.0011	0.2248
S	< 0.0001	< 0.0001	< 0.0001
		CH_4	
B	< 0.0001	< 0.0001	< 0.0001
Z	0.0393	<0.0001	<0.0001
G	0.0037	<0.0001	<0.0001
S	<0.0001	<0.0001	<0.0001
		N_2O	
B	< 0.0001	< 0.0001	< 0.0001
Z	0.0003	< 0.0001	0.1196
G	< 0.0001	< 0.0001	0.0229
S	< 0.0001	< 0.0001	< 0.0001

Note: B, Biochar; Z, Zeolite; G, FGD-Gypsum; S, Sodium bisulfate

Table 8: Mean comparisons of daily GHG emissions for each treatments for the 40 days of incubation experiments. Letters after the mean represent a significant difference between treatments for individual gases at *P* < 0.05.

Treatment	CO_2 (g kg^{-1} day^{-1})	CH_4 (mg kg^{-1} day^{-1})	N_2O (mg kg^{-1} day^{-1})
BL	0.70 ed	0.014 a-c	0.47 b-d
BL+ 5% B	1.38 b	-0.023 c	0.67 b
BL + 9% B	1.30 b-d	0.037 a	0.49 b-d
BL + 13% B	2.63 a	0.037 a	1.16 a
BL + 17% B	2.56 a	0.028 ba	1.28 a
BL + 3% Z	1.07 b-e	0.016 a-c	0.62 bc
BL + 6% Z	0.67 e	0.0003 a-c	0.49 b-d
BL + 8% Z	0.71 ed	0.009 a-c	0.49 b-d
BL + 11% Z	0.70 ed	0.005 a-c	0.48 b-d
BL + 6% G	0.79 b-e	0.007 a-c	0.5 b-d
BL + 11% G	0.77 c-e	0.019 a-c	0.51 b-d
BL + 15% G	1.32 bc	-0.001 a-c	0.58 bc
BL + 19% G	1.02 b-e	0.013 a-c	0.54 b-d
BL + 2% S	0.76 c-e	0.014 a-c	0.34 cd
BL + 4% S	0.60 e	0.014 a-c	0.31 cd
BL + 6% S	0.50 e	-0.009 bc	0.25 d
BL + 7% S	0.48 e	-0.007 a-c	0.24 d

Table 9: Percent reduction and percent increase in GHG emission from BL treated with amendments B: Biochar, Z: Zeolite, G: FGD-Gypsum, S: Sodium bisulfate. Positive value represent percent increase and negative value represents percent decrease with respect to control (untreated BL).

Treatment	CO_2	CH_4	N_2O
BL+ 5% B	+98.5	-269.5	+43.7
BL + 9% B	+86.1	+173.1	+4.5
BL + 13% B	+277.7	+172.8	+149.3
BL + 17% B	+245.3	+109.8	+174.8
BL + 3% Z	+53.4	+20.9	+33.2
BL + 6% Z	-4.2	-97.9	+5.7
BL + 8% Z	+1.8	-37.1	+5.8
BL + 11% Z	+0.7	-63.9	+3.0
BL + 6% G	+14.2	-45.6	+6.8
BL + 11% G	+10.6	+40.3	+9.4
BL + 15% G	+89.4	+106.5	+24.9
BL + 19% G	+45.8	-1.8	+15.6
BL + 2% S	+8.7	+5.5	-26.8
BL + 4% S	-13.4	+5.4	-33.1
BL + 6% S	-28.7	-164.3	-46.0
BL + 7% S	-31.6	-149.6	-47.6

Table 10: Cumulative CO_2, N_2O, and CH_4 emissions during 40 days study period and their global warming potential (GWP) for all treatments and their percentage contributions to total GWP.

Treatments	Total CO_2 emission	Total N_2O emission (mg kg^{-1})	Total CH_4 emission	CO_2 + N_2O + CH_4 GWP (mg kg^{-1})	Percent contribution to GWP		
					% CO_2	% N_2O	% CH_4
BL	27848.42	18.62	0.54	33411.32	83.35	16.62	0.04
BL+ 5% B	55266.36	26.76	-0.92	63218.06	87.42	12.62	-0.04
BL + 9% B	51839.45	19.45	1.48	57673.16	89.88	10.05	0.06
BL + 13% B	105181.11	46.41	1.48	119051.22	88.35	11.62	0.03
BL + 17% B	96167.76	51.16	1.14	111443.24	86.29	13.68	0.03
BL + 3% Z	42708.47	24.81	0.66	50118.82	85.21	14.75	0.03
BL + 6% Z	26670.63	19.68	0.01	32537.63	81.97	18.03	0.001
BL + 8% Z	28353.39	19.71	0.34	34235.71	82.82	17.16	0.02
BL + 11% Z	28039.16	19.18	0.20	33761.13	83.05	16.93	0.01
BL + 6% G	31791.97	19.89	0.30	37727.65	84.27	15.71	0.02
BL + 11% G	30791.44	20.38	0.76	36883.85	83.48	16.46	0.05
BL + 15% G	52733.89	23.26	-0.04	59664.91	88.38	11.61	0.001
BL + 19% G	40599.40	21.52	0.53	47027.89	86.33	13.64	0.03
BL + 2% S	30261.84	13.62	0.57	34336.11	88.13	11.82	0.04
BL + 4% S	24117.34	12.45	0.57	27843.17	86.62	13.33	0.05
BL + 6% S	19866.57	10.05	-0.35	22854.25	86.93	13.11	-0.04
BL + 7% S	19040.08	9.76	-0.27	21943.12	86.77	13.26	-0.03

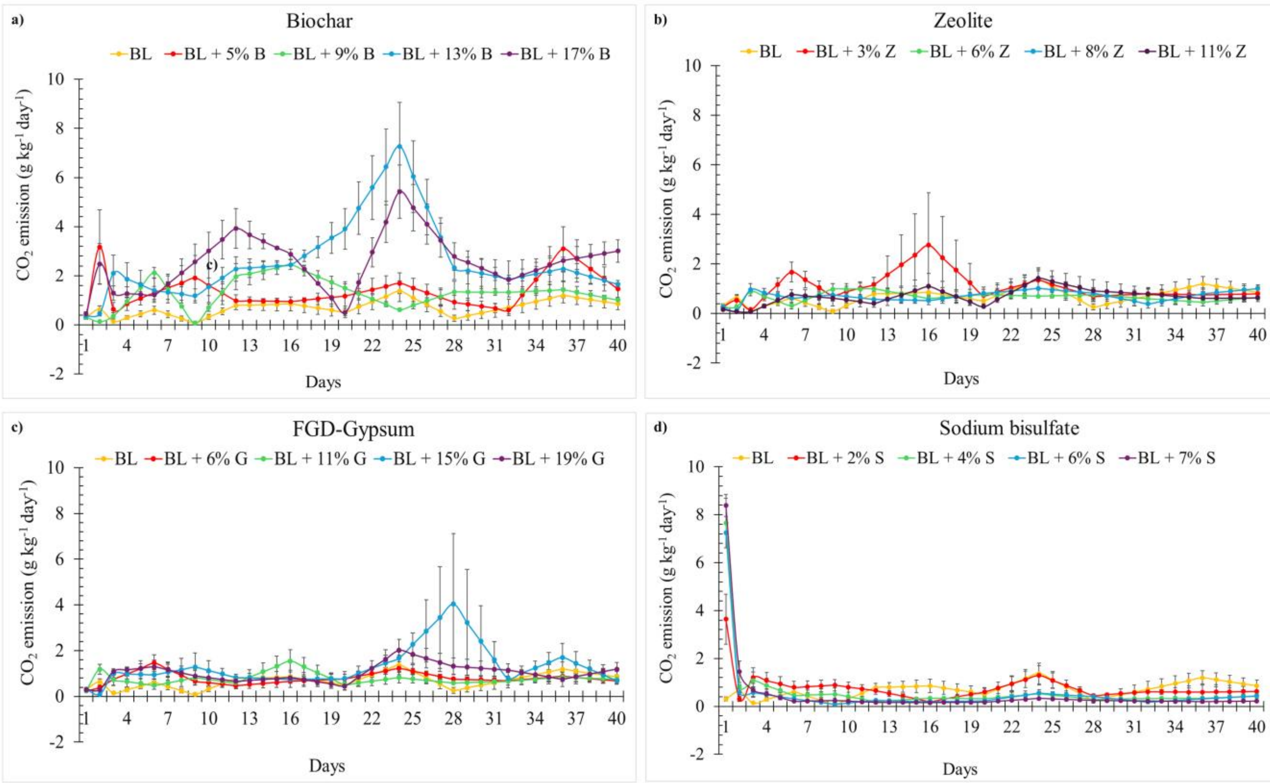


Figure 7: The daily CO_2 emissions from BL treated with different rates of amendments, over 40 days of incubation studies. a) biochar b) zeolite c) FGD-Gypsum d) sodium bisulfate. Bar represents standard error of mean (N = 2).

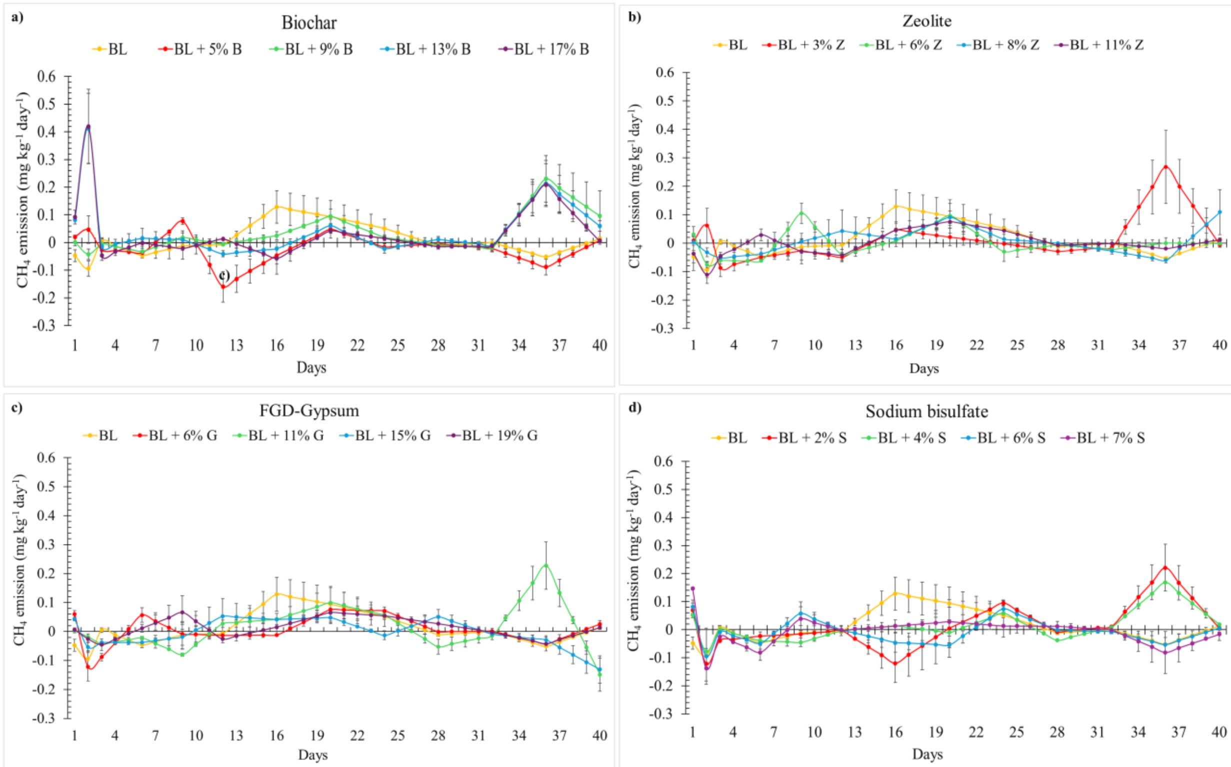


Figure 8: The daily CH_4 emissions from BL treated with different rates of amendments over 40 days of incubation studies a) biochar b) Zeolite c) FGD-Gypsum d) Sodium bisulfate. Bar represents standard error of mean (N = 2).

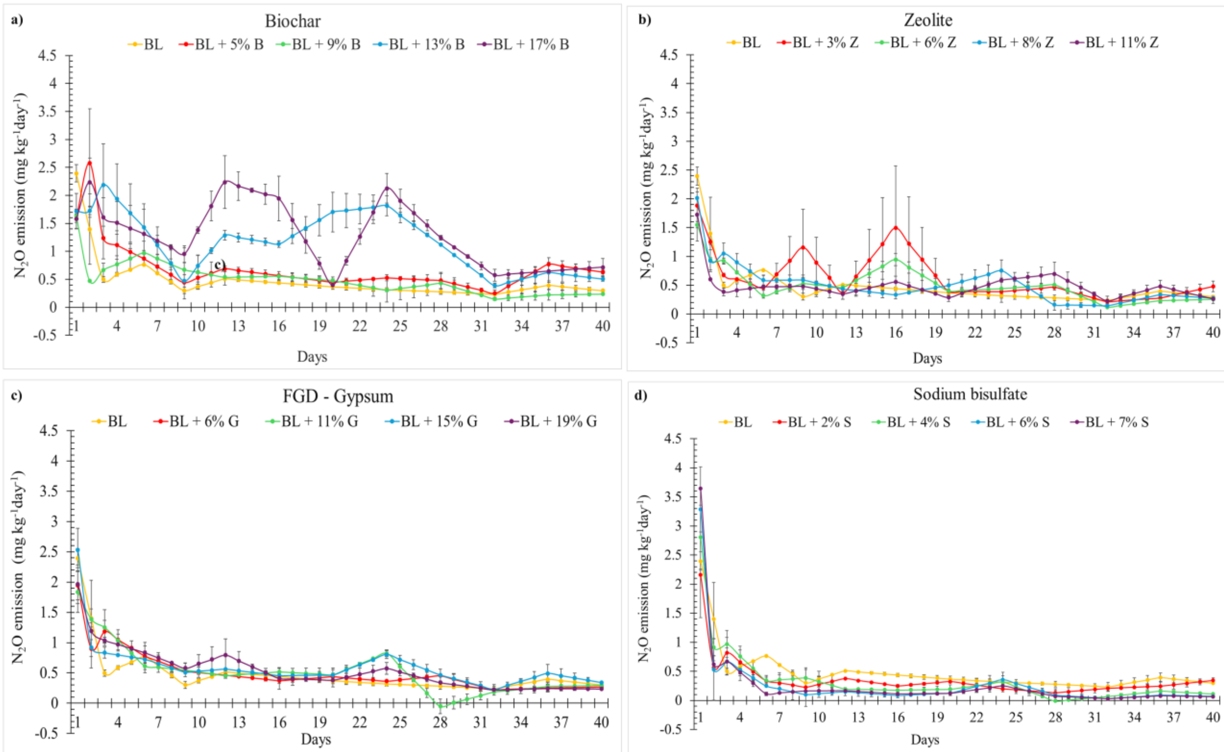
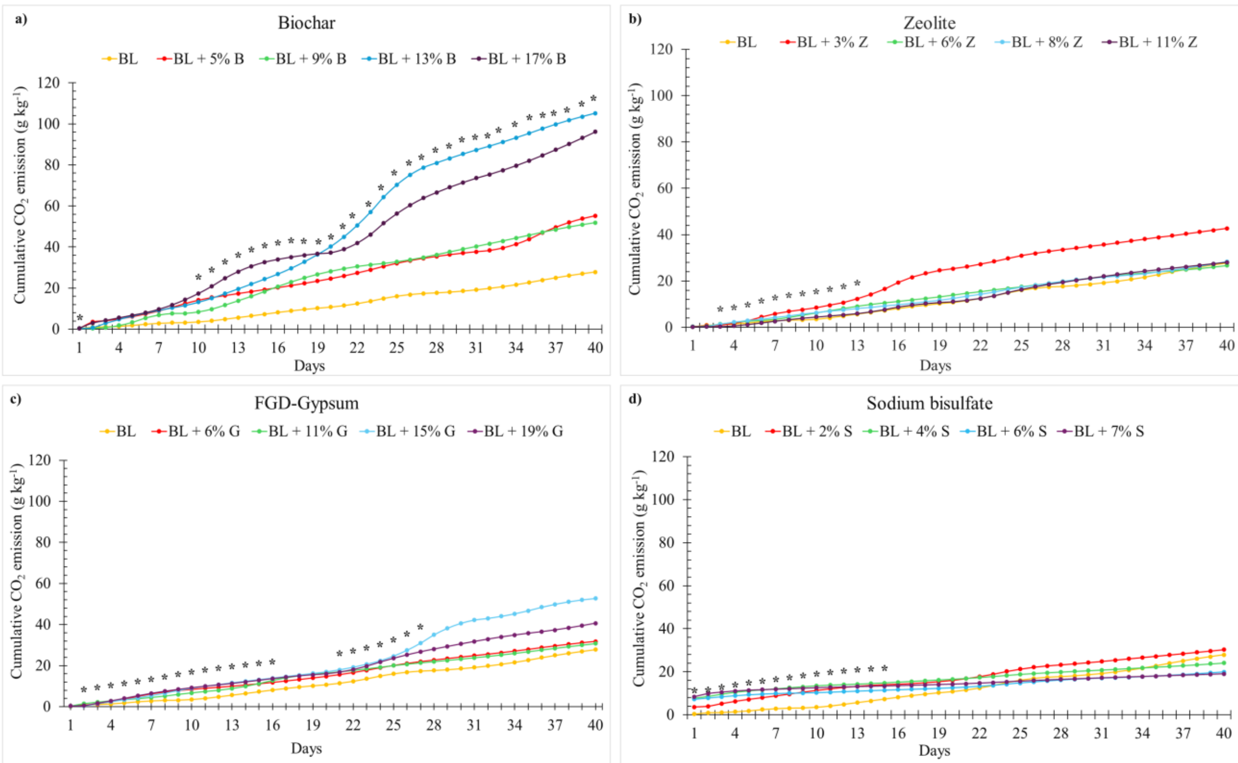
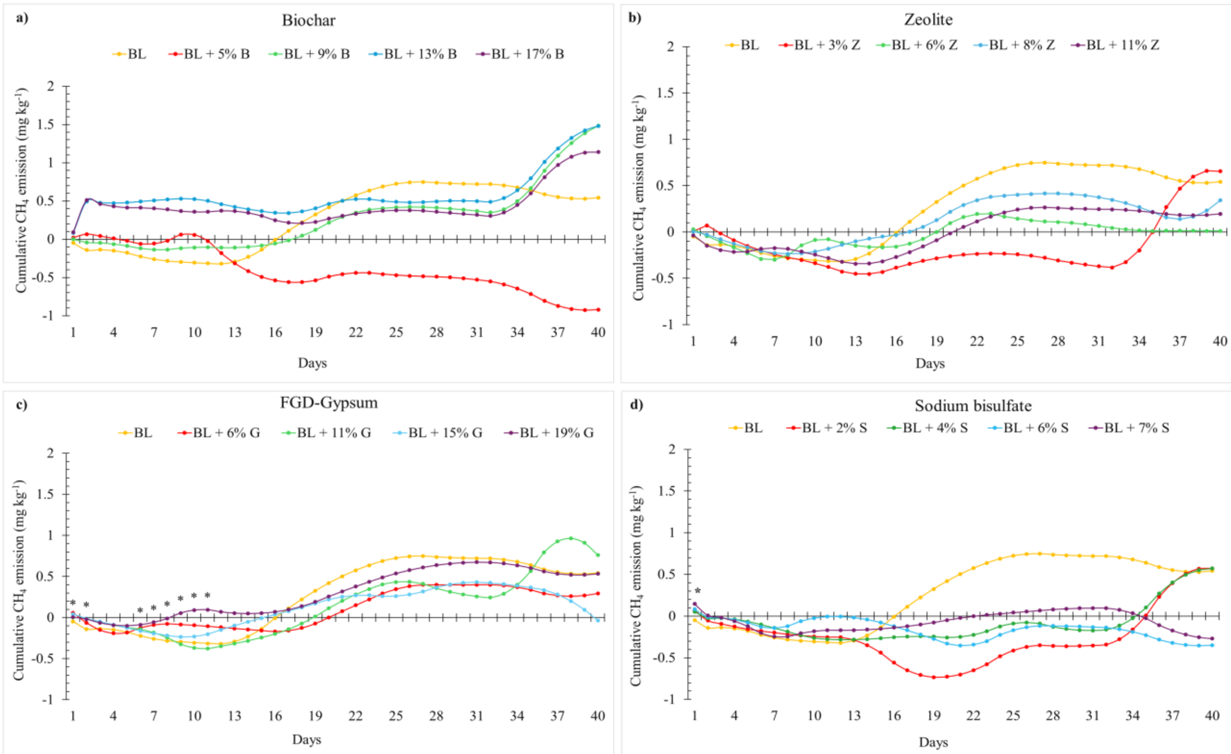


Figure 9: The daily N_2O emissions from BL treated with different rates of amendments, over 40 days of incubation studies. a) biochar b) Zeolite c) FGD-Gypsum d) Sodium bisulfate. Bar represents standard error of mean (N = 2).

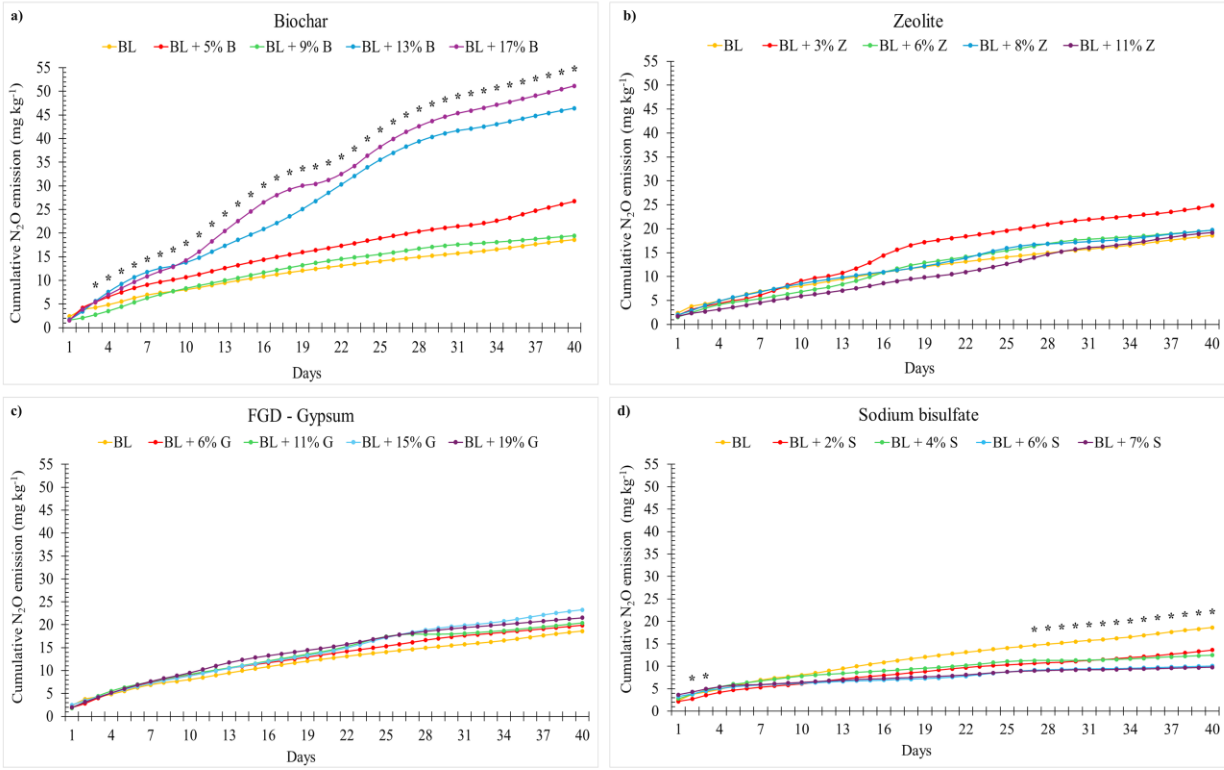
Supplementary Figures



Supplementary Figure 1: The cumulative CO_2 emissions from broiler litter (BL) treated with different rates of amendments over 40 days of incubation studies a) biochar b) Zeolite c) FGD-Gypsum d) Sodium bisulfate. * represents significant difference between treatments on a given day at $\alpha = 0.05$.



Supplementary Figure 2: The cumulative CH_4 emissions from broiler litter (BL) treated with different rates of amendments over 40 days of incubation studies a) biochar b) Zeolite c) FGD-Gypsum d) Sodium bisulfate. * represents significant difference between treatments on a given day at $\alpha = 0.05$.



Supplementary Figure 3: The cumulative N_2O emissions from broiler litter (BL) treated with different rates of amendments over 40 days of incubation studies a) biochar b) Zeolite c) FGD-Gypsum d) Sodium bisulfate. * represents significant difference between treatments on a given day at $\alpha = 0.05$.

4. Summary and Future Recommendations

The goal of this study was to evaluate the potential use of amendments B, Z, G, and S at four different rates in reducing NH_3 and GHG emissions from BL. Two set of incubation experiments were conducted, one for NH_3 and another for GHG. One hundred grams of BL samples were treated with litter amendments at four different rates, kept in mason jars, and incubated at 30 ºC, at 40% moisture for 40 days. Phosphoric acid (H_3PO_4) traps were used to trap NH_3 produced from BL whereas GHGs samples were taken from the headspace of the jars in a pre-evacuated vial using a 10 ml syringe at 0-minute, 60-minute, and 120-minute intervals. The trapped NH_3 was extracted with 2 M KCl solution and analyzed in Flow injection analyzer and GHG was measured using gas chromatography. The results of NH_3 experiment showed that 13% and 17% B (w/w) reduced NH_3 emission by 40.7 and 45.6%, respectively, compared to control. Among the different rates of Z, 8% reduced NH_3 by 19.6% and 11% (w/w) reduced by 32.5%. There was no significant difference between the different rates of G however, 15% G reduced NH_3 by 9.1%. Application of S at rates of 2%, 4%, 6%, and 7% (w/w) significantly reduced NH_3 emissions by 90.5, 98.6, 99.7, and 99.8 %, respectively, compared to control. Based on the percent reduction of ammonia emission compared to control, the efficiency of the amendments can be ranked as follows: S > B > Z > G. The effects of amendments vary largely for the GHG (CO_2, CH_4 and N_2O) emissions from BL. Treatment of 13% and 17% B significantly increased cumulative CO_2 and N_2O emissions whereas 5% and 9% B had no effect on GHG emissions compared to control. Zeolite and G application had no significant effect on GHG reduction. Sodium bisulfate at rates of 4, 6, and 7% reduced CO_2 emissions by 13, 29, 32 % and N_2O emissions by 33, 46, 48 %, respectively, compared to control. Among the amendments used, 4, 6, and 7 % of S were found promising for reducing GHG emissions from BL. These results were helpful to understand the efficacy of amendments and their rates on NH_3 and GHG emissions and provide fundaments for future research.

Future recommendations

1. This incubation study performed at constant temperature and moisture provided valuable insights into the selection of amendments and their rates for future. To confirm our laboratory findings, further testing of these amendments in poultry houses could yield more convincing information on NH_3 and GHG emissions.
2. In our study, higher application rates of B, Z, and S, reduced NH_3 emission. It is suggested that multiple application of amendments over different time intervals can be tested further.
3. Investigating different combinations of amendments (mixing 2 or 3 amendments at varying rates) can provide insights for the synergistic effects of amendments in reducing NH_3 and GHG emissions.
4. Biochar treatments did not distinctly reduce GHG emissions. Future research should explore biochar from different feedstocks, pyrolysis temperatures, and production processes.
5. Methane had the least impact on global warming potential (GWP) from BL. Research should focus on minimizing CO_2 and N_2O emissions from BL.