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IMPACT OF WOOD-SOURCED BIOCHAR ON CARBON AND NITROGEN
CAPTURE IN BEEF FEEDLOT SYSTEMS

by

Jessica L. Sperber

A DISSERTATION

Presented to the Faculty of
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Under the Supervision of Professors Galen E. Erickson and Andrea K. Watson

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IMPACT OF WOOD-SOURCED BIOCHAR ON CARBON AND NITROGEN
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Jessica L. Sperber, Ph. D.

University of Nebraska, 2021

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A feedlot growing and finishing experiment evaluated the effect of including pine-sourced biochar at 0.8 (grower) and 1.0% (finisher) of diet DM on steer performance, carcass characteristics, and greenhouse gas (GHG) production (Exp 1). Two nutrient mass balance experiments were conducted during winter and summer seasons to evaluate the effect of spreading unprocessed red cedar biochar on the feedlot pen surface on manure nutrient capture and cattle performance (Exp 2). In Exp. 1, the inclusion of biochar in the growing diet did not impact steer performance. The inclusion of biochar in the finishing diet significantly reduced intake and gain, resulting in a lighter and leaner carcass compared to control. Emissions of CH₄ and CO₂ were not affected by biochar inclusion in the growing or finishing period. In Exp. 2, the winter phase (December to June) evaluated three treatments (5 pens/treatment, 10 steers/pen): biochar spread to pen surface, hydrated lime spread to pen surface, and negative control. There were no differences in nutrient (N and P) intake, calculated nutrient retention, or excretion. Steer performance and carcass traits were not impacted by pen treatment in winter phase. The summer phase (June to November) evaluated biochar spread to pen surface against negative control (5 pens/treatment, 8 steers/pen). There were no

differences in N and P intake or calculated excretion, however, calculated nutrient retention was significantly greater for steers on biochar-amended pens. Increased nutrient retention by the animal resulted in increased gain, improved feed efficiency, and a heavier hot carcass weight for steers on biochar treatment. In both winter and summer phases, biochar addition to the feedlot pen surface increased N concentration in manure but did not result in increased kg of N or P removed from feedlot pens due to a lesser quantity of manure removed from biochar-amended pens.

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DEDICATION

This dissertation is dedicated to my late Grandpa, Mike Hatala.

Mike Hatala was a respected cattleman, a steward of the land, and a family man. Grandpa instilled a love and passion for agriculture within me and encouraged me to follow my dreams. He taught me lessons about the cattle industry that I could never learn from a textbook, and I am forever blessed to have had his guidance, infinite support, and endless love.

Table of Contents

Introduction	1
CHAPTER I. Review of the Literature	3
Anthropogenic greenhouse gas production	3
Enteric GHG production	5
Methanogenesis	7
Factors that influence ruminal CH ₄ production	10
Roughage vs. Concentrate diet	10
Ruminal rate of passage	13
Lipid inclusion in the diet	13
Ionophores in the diet	15
Defaunation	16
Feed additives to reduce enteric CH ₄ production	17
Biochar	17
Seaweed	23
3-Nitrooxypropanol	26
Manure emission	28
Ammonia (NH ₃)	28
Nutrients on the feedlot pen surface	32
Implications and future research	45
Conclusion	45
LITERATURE CITED	47
CHAPTER II. Evaluation of the effects of pine-sourced biochar on cattle performance and methane and carbon dioxide production from growing and finishing steers	68
Introduction	69
Materials and Methods	71
Results and Discussion	76
LITERATURE CITED	82
CHAPTER III. Evaluation of the effects of wood-sourced biochar as a feedlot pen surface amendment on manure nutrient capture	89
Abstract	89
Introduction	90
Materials and Methods	92
Results and Discussion	98
LITERATURE CITED	106
APPENDIX A: BIOCHAR ON NUTRIENT LOSS FROM CATTLE MANURE	117

List of Tables

Table 2.1. Composition of diet (DM) fed to steers in growing experiment (77 days on feed).....	85
Table 2.2. Composition of diet (DM) fed to steers in finishing experiment (111 days on feed)	86
Table 2.3. Effect of pine-sourced biochar addition at 0.8% of diet DM on performance and gas emissions of growing steers.....	87
Table 2.4. Effect of pine-sourced biochar addition at 1.0% diet DM on performance, carcass characteristics, and gas emissions of finishing steers.....	88
Table 3.1. Composition of diet (DM) fed to steers in WINTER and SUMMER mass balance experiments.....	111
Table 3.2. Performance and carcass characteristics for steers fed the same diet with different pen amendments in WINTER phase.....	112
Table 3.3. Performance and carcass characteristics for steers fed the same diet with different pen amendments in SUMMER phase.....	113
Table 3.4. Monthly precipitation (cm) for WINTER and SUMMER compared to 25-year monthly precipitation average for the Eastern Nebraska Research, Education and Extension Center located near Mead, NE.....	114
Table 3.5. Effect of biochar and lime pen amendments on manure nitrogen (N), phosphorus (P) and organic matter (OM) during WINTER ¹	115
Table 3.6. Effect of biochar as a pen amendment on manure nitrogen (N), phosphorus (P) and organic matter (OM) during SUMMER ¹	116

List of Figures

1.1.Mechanism of enteric CH₄ production during carbohydrate digestion in the rumen (Adapted from Sejian et al., 2013).....	67
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Introduction

The production of greenhouse gases (GHG) and their contribution to climate change are of substantial environmental concern. Carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) are the three significant gases that contribute to global warming and are produced via natural sources (e.g. wetlands) and anthropogenic activity (e.g. fossil fuel combustion). The two largest contributions to anthropogenic GHG production come from the petroleum and natural gas sectors and enteric fermentation from domesticated ruminant livestock (NASEM, 2018). The rumen allows cattle the unique ability to digest and convert plant products (e.g. cellulose), deemed indigestible by humans and monogastric animals, into high-quality protein. Enteric CH₄ production plays a vital role in ruminal fermentation; however, it also represents an energetic loss for the animal (Johnson and Johnson, 1995) and contributes to global warming.

Beef feedlot finishing diets that are typical for the U.S. incorporate high concentrations of N and P in the feed, with approximately 12 and 15% of fed N and P, respectively, being retained by the animal (Kissinger et al., 2007). The remaining N and P are excreted in the manure, where the opportunity for manure N loss via ammonia (NH₃) volatilization from the feedlot pen surface is a risk to the environment through N₂O formation and lowers the value of manure as a fertilizer. Although modern beef production exhibits a 16% decrease in the carbon footprint of beef compared to the 1970s (Capper, 2011), the beef industry has been further challenged to reduce its contribution to GHG emissions.

One proposed method to combat gaseous emissions from ruminal fermentation (CH₄) and manure N loss (NH₃ volatilization) is a product called biochar. Biochar is

produced from burning organic matter (e.g. forestry byproducts) at high temperatures in the absence of oxygen (Hansen et al., 2012), resulting in a carbonized charcoal product. The porous nature and large surface area of biochar has made it a versatile product used in agricultural and environmental applications. When used as a soil amendment, biochar has shown to improve crop yields and soil fertility (Ding et al., 2016). The addition of biochar to livestock manure has resulted in reductions of N₂O emission, potentially due to the sorptive capacity of biochar, reducing the availability of N for N₂O formation (Agyarko-Mintah et al., 2017; Kammann et al., 2015). When biochar is included in the diet of cattle, recent literature has shown reductions in enteric CH₄ production. The literature review presented in Chapter I will outline the climate impact of cattle and manure on GHG emissions and potential mitigation strategies. Chapter II will evaluate the effectiveness of including biochar in the diet of cattle to reduce enteric CH₄ production, and Chapter III will discuss the effectiveness of biochar as a feedlot pen surface amendment on improving manure N capture and reducing N loss via ammonia volatilization.

CHAPTER I. Review of the Literature

Anthropogenic greenhouse gas production

Greenhouse gases (GHGs) are detrimental to the environment as they trap heat in the atmosphere, potentially resulting in climate change. In addition, GHGs are a public safety concern, where an over-concentration of GHGs can result in reduced air quality, leading to pollution and smog and ultimately contributing to respiratory disease. There are three significant gases that contribute to GHG emissions: including carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) (Hristov et al., 2013). The production of GHGs and their impact on climate change is divided into two categories: anthropogenic and natural GHG production. Anthropogenic refers to any GHGs produced by or associated with human activity, while natural refers to the natural cycles and processes of the Earth.

A 2019 overview of the U.S. GHG emissions caused by human activities reported that of these three gases, CO₂ accounted for approximately 80% of all emissions, CH₄ for 10%, N₂O for 7%, and the remaining 3% of U.S. GHG emission came from fluorinated gases (emitted during various industrial processes; USEPA, 2021). Carbon dioxide is the primary GHG emitted through human activity and is predominantly produced through the burning of fossil fuels for energy and transportation. Methane is produced during the manufacturing and transport of natural gas and oil, and various other agricultural practices such as livestock production and manure management (USEPA, 2021). The two largest sources of anthropogenic CH₄ emission in the USA are enteric fermentation from domesticated ruminant animals and the petroleum and natural gas sectors (NASEM, 2018). A GHG inventory of the USA in 2015 reported that of total CH₄ emissions, enteric fermentation from domesticated livestock equated to 25%, or 6.6 Tg, of CH₄ emission,

natural gas equated to 25%, or 6.5 Tg, of CH₄, and manure management and coal mining each contributed an additional 10% of CH₄ emission, or 2.65 and 2.69 Tg, respectively (USEPA, 2021). In addition to anthropogenic activity, CH₄ is emitted by natural sources such as wetlands, which are estimated to contribute 40 to 50% of total global CH₄ emission to the atmosphere each year (Whiting & Chanton, 1993). Enteric fermentation in wildlife (e.g. moose, elk, bison, and deer) is a natural contributor to total CH₄ emissions, contributing to approximately 4.3% of total CH₄ emissions in the USA (NASEM, 2018).

Methane was originally thought to be a more potent GHG than CO₂ with 21 times the warming potential (Qiao et al., 2014); however, that number has been further refined, as CH₄ has a shorter half-life than originally predicted and only remains in the atmosphere for 10 to 12 years before it is oxidized into CO₂ and H₂O. Agricultural land use, industrial manufacturing, and wastewater treatment are the largest contributors to total N₂O emissions (USEPA, 2021).

The livestock sector contributes approximately 14.5% of total global anthropogenic GHG emissions, originating from enteric fermentation, manure management, feed production, and energy consumption, equating to an estimated 7.1 Gt CO₂ equivalents annually (Gerber et al., 2013). Of this total GHG production from livestock, around 44% is in the form of CH₄ (Gerber et al., 2013).

As an industry, agriculture has been challenged to reduce its contribution to GHG emissions. Moss et al. (2000) suggested that to reduce our GHG footprint, reducing CH₄ emission from agricultural sources, specifically from enteric fermentation, would stabilize atmospheric CH₄ concentration. In comparison to the 1970s, modern beef

production exhibits a 16% decrease in carbon footprint per unit of beef (Capper, 2011). Capper (2011) compared U.S. beef production in 1977 with 2007, reporting that considerably less resources were required to produce 1 billion kg of beef in modern times, with approximately 30% fewer animals, 19% less feedstuffs, 12% less water, and 33% less land. Although strides have been made in increasing beef production efficiency, there is still an opportunity to continue to improve.

Enteric GHG production

Methane is formed through a microbially driven process by highly specialized methanogens that are part of the domain Archae (Qiao et al., 2014) and lack peptidoglycan in the cell wall (Balch et al., 1979). Methanogens are the only known microorganisms capable of producing CH_4 (Hook et al., 2010) and thrive in anaerobic environments that are abundant in organic matter, including swamps, landfills, and sewage treatment plants (Qiao et al., 2014). The reticulorumen of ruminant animals is also an ideal environment for methanogens (Hristov et al., 2013), which utilize hydrogen to reduce CO_2 and other methyl compounds into CH_4 . Once CH_4 is produced in the rumen, a group of microbes known as methanotrophs, which are present in the rumen fluid and found attached to the rumen wall, consume CH_4 and metabolically utilize it as their main source of carbon for energy (Mitsumori et al., 2002; Sahoo et al., 2021).

Methane and CO_2 are produced as waste products of the fermentation process in herbivores, in particular ruminants, where carbohydrates are broken down into simple sugars by various microorganisms to later be absorbed into the bloodstream (Sejian and Saumya, 2011). Methane produced as result of fermentation can occur in the lower gastrointestinal tract, this being more typical for non-ruminants, and in the rumen, which encompasses 89% of total CH_4 emitted from ruminants (Murray et al., 1976). Figure 1.1

depicts the mechanism of enteric CH₄ production in the rumen occurring during the digestion process (Sejian et al., 2013). Upon ingestion of a carbohydrate, cellulolytic bacteria ferment the feedstuff into simple sugars (e.g. glucose), which are then used by the microbes as an energy source for microbial growth and end-product formation. The end products of microbial fermentation of carbohydrates include the three predominant volatile fatty acids (VFA) acetate, propionate, and butyrate, CO₂ and CH₄ (Wanapat et al., 2015; Sejian et al., 2013). Acetate and butyrate act as hydrogen (H₂) providers, while propionate acts as a H₂ consumer (Sejian et al., 2013). In response to the amount of hydrogen pooling and the end-product of digestion, CH₄ is produced in the rumen (Sejian et al., 2013).

Methane formation is the terminal step in carbohydrate fermentation, where the hydrogens must be removed from the rumen to allow the microorganisms to function optimally and to promote complete oxidation of the feed substrate to improve energy recovery (Sharp et al., 1998). If the hydrogen end-product is not removed from the rumen environment, it can hinder specific functions of the rumen microorganisms, and ultimately inhibit metabolism (Sharp et al., 1998). Ruminal CH₄ production limits the increase in partial pressure of H₂, which would otherwise interfere with the ability of microbial enzymes (i.e. NADH dehydrogenase) essential for electron transfer to function properly (Morgavi et al., 2010). The microbes of the rumen ferment feed and the products of the fermentation process provide the animal with its required nutrients, often enabling the ruminant animal to acquire energy by consuming low-value material that many other animals are unable to utilize for energy (Sejian and Saumya, 2011).

Following the process of fermentation, the ruminoreticulum produces large volumes of fermentative gases, predominantly CH_4 and CO_2 , which require a mechanism known as eructation to expel gases (Dougherty, 1968). Of total gas being expelled from the ruminoreticulum, CO_2 and CH_4 comprise approximately 60 and 35%, respectively (Dougherty, 1968). Forward-moving ruminal contractions, beginning at the posterior dorsal sac, push the gases toward the anterior rumen, and further to the esophagus (Weiss, 1953). Eructation expels gas upward through the esophagus and out through the mouth and nose, emitting these gases to the environment (Murray et al., 1976; Wanapat et al., 2015). The eructation reflex is vital for ruminants, as a gaseous buildup in the reticulorumen can lead to death (Dougherty, 1968). In ruminant animals, fermentative gases are expelled predominantly by eructation, with trace amounts being absorbed across the digestive tract to be expelled during defecation (Dougherty, 1968). Murray et al. (1976) reported that of total CH_4 production in small ruminants (sheep), ruminal production accounts for approximately 87% of total CH_4 , and approximately 95% of CH_4 produced in the rumen is eructated. Murray et al. (1976) also highlighted that of the total CH_4 produced in the lower digestive tract, approximately 89% is excreted from the animal via the lungs, and the remaining 11% is excreted through the anus.

Methanogenesis

Methanogens produce CH_4 freely through the normal process of feed digestion (Hook et al., 2010) sourced from a handful of rumen substrates, including CO_2 , H_2O , methanol, and acetate (Qiao et al., 2014). Methanogens scavenge CO_2 and H_2 within the rumen that are produced by various other species of fermenting bacteria within the rumen microbiome (Patra et al., 2017). Ruminal production of acetate forms a substrate called formate, which can also be used as a substrate in methanogenesis but is often converted

to H₂ and CO₂ at too rapid of a rate for the methanogens to utilize (Sejian et al., 2013). Methanogenesis utilizes H₂ and CO₂ produced from carbohydrate fermentation, which occurs simultaneously with the production of VFA in the rumen (Hungate et al., 1970). Rumen turnover inhibits the conversion of CO₂ and H₂ from VFAs, so VFAs are not commonly used as a substrate for methanogens, due to the lengthy process of conversion (Hobson and Stewart, 1997). Hydrogen elimination in the rumen is done by the formation of CH₄, where $\text{CO}_2 + 4 \text{H}_2 \rightarrow \text{CH}_4 + 2 \text{H}_2\text{O}$ (Moss et al., 2000).

Rumen methanogen populations are influenced by ruminant species type, where ovine populations vary from bovine (Hook et al., 2010). In addition, diet has also been shown to impact rumen methanogen population (Hook et al., 2010). The most common methanogen found in cattle, *Methanobrevibacter ruminantium*, is able to utilize H₂ and CO₂ as substrates, producing CH₄ as the product (Balch et al., 1979). Most methanogens require hydrogen as an energy source; however, Poulsen et al. (2013) discovered a new group of methylotrophic methanogens that do not require hydrogen, yet also appear to contribute to CH₄ production from ruminants. There are many approaches that aim to reduce CH₄ emission from livestock (described in detail below), and these approaches all focus on reducing H₂ availability for rumen methanogens (Poulsen et al., 2013). Poulsen et al. (2013) observed that when rapeseed oil was supplemented in lactating dairy cows, concentrations of methylotrophic methanogens were significantly reduced. Therefore, targeting methanogens in the rumen that do not require H₂ as their predominant energy source may be a valuable implication for further investigation to reduce ruminal CH₄ production.

The amount of CH₄ produced from rumen methanogens depends on the amount of hydrogen produced as an end-product during the process of carbohydrate fermentation (Palarea-Albaladejo et al., 2017) and, to a lesser extent, amino acid (AA) fermentation in the rumen and hindgut (Hristov et al., 2013). It is estimated that domesticated ruminants, such as cattle, sheep, and goats, produce up to 86 million metric tonnes of CH₄ annually on a global scale (McMichael et al., 2007). Ruminant animals can produce between 250 to 500 L of CH₄ per day, equating to a typical loss of approximately 6% of their ingested energy, ultimately creating an inefficiency in cattle feeding (Johnson and Johnson, 1995). Johnson and Ward (1996) estimated the methane loss of ingested feed energy to be anywhere from 2 to 12% in ruminants, with much of the variation based on diet. The major inefficiencies of anaerobic metabolism are the storage of oxygen released as CO₂ by the animal and the disposal of hydrogen released as CH₄ (Van Soest, 1994).

While methanogens are the predominant source of ruminal CH₄ production, they do not work alone, often creating close relationships with rumen protozoa (Sharp et al., 1998). The association between ruminal methanogens and protozoa is mutualistic, both gaining value from the opportunity for interspecies hydrogen transfer (Wolin, 1974). The most commonly found bovine rumen protozoa that form relationships with methanogens are from the genera *Entodinium*, *Epidinium*, and *Polyplastron* (Sharp et al., 1998). While ruminal fermentation provides energy for methanogens, the protozoa benefit from removal of hydrogen, as lingering hydrogen can also be inhibitory to their metabolic processes (Sharp et al., 1998). Methanogens also interact with ruminal bacteria through interspecies H₂ transfer, mutually benefitting the microbes as the interaction prevents H₂ accumulation and feedback inhibition (Patra et al., 2017).

Factors that influence ruminal CH₄ production

Factors that influence CH₄ production from ruminants include type of carbohydrate in the diet, level of feed intake, feed processing, addition of lipids or ionophores to the diet, and alterations in the microflora of the rumen (Johnson and Johnson, 1995). Johnson and Johnson (1995) described that variation in ruminant CH₄ production was due to the amount of dietary carbohydrate fermented in the reticulorumen and the VFA ratio produced. The ratio of acetate to propionate has a major impact on rumen CH₄ production, where there is a greater CH₄ loss from acetate production than propionate (Johnson and Johnson, 1995).

Roughage vs. Concentrate diet

Feeding cereal grains to ruminants allows starch-fermenting bacteria to produce propionate, which reduces CH₄ production and ammonia accumulation (by assimilation) in the rumen (Lana et al., 1998; Wanapat et al., 2015). The relationship between dietary concentrate proportion of gross energy intake (GEI) and CH₄ production is curvilinear in nature (Sauvant and Giger-Reverdin, 2007). A review paper by Martin et al. (2020) outlined that, for diets containing 30 to 40% concentrate, CH₄ production remains relatively constant around 6 to 7% of GEI, dropping significantly for diets containing 80 to 90% concentrate, measuring CH₄ at values around 2 to 3% of GEI.

A decrease in ruminal pH has been associated with feeding cereal grains (Slyter, 1976) in addition to increasing ruminal rate of passage (Hook et al., 2010). When measured *in vitro*, once the pH of the rumen drops below 6.0, there is a decrease in CH₄ production from ruminal bacteria (Van Kessel and Russell, 1996), where low ruminal pH can inhibit growth and activity of methanogens (Hegarty, 1999). Feed intake is impacted by level of concentrate in the diet and research has shown that replacing carbohydrates

from forages with energy-dense concentrates reduces feed intake. Dry matter intake (DMI) largely impacts CH₄ production and can be altered by the quality and digestibility of the diet (Blaxter and Clapperton, 1965). In a meta-analysis, Blaxter and Clapperton (1965) demonstrated that CH₄ production increased as a function of increased intake in 48 different experiments performed on sheep. Additionally, Beauchemin and McGinn (2006) evaluated the effects of feeding ad libitum vs. 65% of ad libitum intake high-forage and high-concentrate diets on CH₄ production (g per d), reporting that CH₄ production was greater for ad libitum cattle compared to cattle with restricted intake. Winders et al. (2020) reported similar findings, where CH₄ production was reduced in growing cattle when restricted to 75% of ad libitum intake compared to steers without intake restriction.

In forage-based diets, the neutral detergent fiber (NDF) content of the diet is one of the largest drivers of CH₄ and CO₂ production. Forage quality is described as the extent to which a forage has the potential to produce a desired performance response in the animal, such as weight gain. Forage quality is influenced by palatability, intake, digestibility, and NDF content (Ball et al., 2001). It is well understood that greater enteric emission is associated with forage-based diets compared to concentrate diets; however, the magnitude to which forage quality is associated with CH₄ production has differing results in literature. Cole et al. (2020) studied the relationship between dietary quality and protein supplementation of low-quality, warm-season grass hay and methane emissions of 8 beef steers in a 4 x 4 Latin square design. The treatments were 1) low-quality forage-based diet (7.4% crude protein (CP)); 2) low-quality forage with supplemented cottonseed meal (10% CP); 3) medium-quality forage-based diet (10.5% CP); and 4)

high-quality forage-based diet (13% CP). Protein digestibility increased as quality of the diets increased, yet there was no difference in total daily methane production (Mcal/d) based on the diet. But, CH₄ production per unit of digested organic matter (OM) decreased with increased quality of the diet (Cole et al., 2020). The results from Cole et al. (2020) were similar with Ominski et al. (2006), who found that CH₄ production increased as forage quality of alfalfa-grass silage decreased in growing cattle.

Jennings et al. (2018) looked at the relationship between CH₄ production and excess dietary CP and metabolizable protein (MP) offered to eight steers fed a steam-flaked, corn-based finishing diet at two different CP levels. They fed two dietary treatments in two experiments in a switchback design over three periods: a control at 1x maintenance energy intake (13.8% CP, 9.63% MP) and an excess CP diet at 2x maintenance energy intake (19.5% CP, 14.14% MP). The excess CP diet contained corn gluten meal for increased CP content. Results from their study reported no difference in enteric CH₄ when dietary CP content was increased in high-concentrate diets. These results contradict Shreck et al. (2015) studying dietary CP inclusion in steers fed a low-quality, blue-stem hay (4.6% CP), provided either cottonseed meal (CSM), dried distillers grains with solubles (DDGS) plus urea to meet rumen degradable protein (RDP) provided by the CSM, or no additional protein supplementation (control). Results from their study found that forage intake increased with protein supplementation, CH₄ emission was greatest for CSM, and both CSM and DDGS led to greater CH₄ production than the control. In a low-quality forage-based diet, increasing CP inclusion in the diet increased overall CH₄ production, but decreased CH₄ production as a percentage of gross energy (GE) intake. The contrasting results between Jennings et al. (2018) and Shreck et al.

(2021) suggest that type of diet (concentrate- or roughage-based) and overall digestibility of the diet impact CH₄ production.

Ruminal rate of passage

Feed processing methods performed on roughage sources, such as grinding and pelleting, have been shown to decrease ruminal CH₄ production, while improving animal performance (Blaxter, 1989). Passage rate directly impacts ruminal CH₄ (Hook et al., 2010). When feed spends less time in the rumen (fast passage rate) a reduction in CH₄ production is expected due to a reduction in ruminal digestion (Moss et al., 2000). Kennedy and Milligan (1978) noted a 30% decline in CH₄ production when passage rate increased. Passage rate is intrinsically influenced by plant stage of maturity, plant leaf-to-stem ratio, and species forage type (Poppi et al., 2001; Kuoppala et al., 2009). Krizsan et al. (2010) performed a meta-analysis on ruminal passage rate, concluding that passage rate of concentrate particles is greater than that of forage particles, and therefore, a diet greater in concentrate has reduced CH₄ production. Johnson and Johnson (1995) noted that carbohydrates from the cell wall yield greater CH₄ production than cell solubles, attributing this difference to the greater acetate-to-propionate ratio produced in the rumen during the fermentation process. Johnson and Johnson (1995) also noted the importance of passage rate through the rumen in determining CH₄ concentration, suggesting that 25 to 28% of variation in CH₄ production is due to particulate and fluid rate of passage through the gut.

Lipid inclusion in the diet

Addition of lipids to the diet have been shown to reduce the production of enteric CH₄ in ruminants (Hook et al., 2010). Dietary lipid additions include fatty acid and oil supplementation and are thought to provoke an antagonistic effect on CH₄ production

(Hook et al., 2010). When lipid content is increased in the diet, methanogenesis decreases, likely due to the inhibition of protozoa, increased production of propionic acid, and cellular process of biohydrogenation (Johnson and Johnson, 1995). Biohydrogenation is the process of saturating unsaturated fatty acids (Johnson and Johnson, 1995). Unsaturated fatty acids inhibit methanogens by acting as hydrogen acceptors (Johnson and Johnson, 1995) and by interrupting membrane transport by directly binding to the cell membrane (Dohme et al., 2001). Beauchemin et al. (2008) reported a 5.6% reduction in CH₄ (g/kg of DMI) from cattle and sheep for every percentage unit of lipid supplemented in the diet.

One way to introduce lipids to the diet is through the addition of oil or oilseed supplements, more specifically, through supplementation of unsaturated fatty acids. In a meta-analysis comparing saturated fatty acids to unsaturated fatty acids and their impact on CH₄ production, Patra (2013) showed no effect of saturated fats on CH₄ production and a significant reduction in CH₄ production (g/d) for both mono- and poly-unsaturated fats. Similarly, Sauer et al. (1998) found that CH₄ was slightly reduced in Holstein cows that had been supplemented with approximately 600 g of soybean oil (unsaturated fat) daily when compared to cows that had no fat supplementation. Hales et al. (2017) looked at the impact of feeding corn oil at 0, 2, 4, and 6% of dietary DM, displacing dry-rolled corn (DRC), on overall CH₄ production. Results from their study found that CH₄ production (g/d) was reduced linearly as inclusion of corn oil increased, with the 6% corn oil treatment reducing CH₄ production by 34%. Similarly, Winders et al. (2020) saw a 15% reduction in CH₄ production when corn oil was included at 3% of the diet compared to a negative control diet, suggesting that either the lipids have a toxic effect on certain

groups of bacteria, and/or biohydrogenation may act as a hydrogen sink (Beauchemin et al., 2007).

Ionophores in the diet

The addition of ionophores, which are antimicrobials included in the diet of livestock to improve production efficiency, has also been shown to reduce enteric CH₄ production (Hristov et al., 2013). The most widely used and studied ionophore included in the diet of beef cattle is monensin, which acts as a rumen modifier and actively reduces CH₄ emissions (Hristov et al., 2013). Monensin causes a shift toward propionate production by selecting for gram-negative microorganisms (Bergen and Bates, 1984). Due to this mechanism of action, it is hypothesized that monensin causes inhibitory growth of bacteria and protozoa in the rumen, which then become a substrate for methanogenesis, instead of directly affecting CH₄ production by inhibiting methanogens (Bergen and Bates, 1984; Van Nevel and Demeyer, 1977). Appuhamy et al. (2013) completed a meta-analysis on 22 controlled studies where monensin (given at 32 mg/kg DM) was included in the diet of both beef and dairy cattle. Results from their meta-analysis suggest there was a greater anti-methanogenic influence of monensin when included in the diet of beef steers compared to dairy cows (Appuhamy et al., 2013). These differences were likely due to diet, where monensin had a greater impact on concentrate-based diets fed to beef steers, in comparison to forage-based diets fed to dairy cows (Appuhamy et al., 2013) because of the greater acetate-to-propionate shift associated with concentrate diets. When monensin is included in the diet, reductions in CH₄ have varied from minor decreases to large reductions of up to 25% (Johnson and Johnson, 1995). The incorporation of monensin in the diet may only reduce CH₄ production in the short-term. Guan et al. (2006) evaluated the short- and long-term effects

of including monensin in the diet of beef steers. Treatments included low- vs. high-concentrate diets, with steers receiving either monensin or lasalosis ionophores. Ionophore inclusion decreased CH₄ production (L/kg of DMI) by 27% in the steers fed the high-concentrate diet (first 2 weeks) and 30% in steers fed the low-concentrate diet (first 4 weeks). For the low- and high-concentrate diets, original CH₄ production levels returned after 3 and 6 weeks, respectively, suggesting that ruminal microbes may adapt to ionophores over time.

Defaunation

Alterations to the microflora of the rumen have been described to reduce CH₄ emissions from ruminants (Johnson and Johnson, 1994). The alteration technique is referred to as defaunation, where protozoa are removed from the rumen and studied for their role in CH₄ production and rumen function (Hook et al., 2010). As described earlier, the relationship between rumen protozoa and methanogens provides the methanogens with the hydrogen molecules that they need to reduce CO₂ into CH₄ (Machmuller et al., 2003). The methanogens associated with the ciliate protozoa have been shown to be responsible for 9 to 37% of ruminal CH₄ production (Machmuller et al., 2003; Newbold et al., 1995). To decrease CH₄ production within the rumen, treatments that decrease the protozoal population associated with the methanogen population must be reduced (Hook et al., 2010). These treatments include, but are not limited to, copper sulphate, acids, lipids, and tannins (Hobson and Stewart, 1997).

Increasing the concentrate-to-forage ratio of the diet, improving forage digestibility, and adding dietary lipids and ionophores have all been shown to impact enteric CH₄ production. Although these management strategies may be effective in

reducing enteric methane production, they may not offer the scale of GHG reduction required to reduce the beef industry's overall impact on emissions and climate change.

Feed additives to reduce enteric CH₄ production

There has been a plethora of enteric CH₄ abatement strategies proposed and researched throughout the literature (Hook et al., 2010). In addition to the factors that influence CH₄ production mentioned above, recent literature has been focused on the addition of feed additives as CH₄ abatement strategies. While modifications made to the diet have shown to be effective short-term methods for mitigating CH₄ emissions from cattle, there is a critical need for long-term mitigation strategies (Palarea-Albaladejo et al., 2017). Biochar, seaweed, and 3-Nitrooxypropanol (NOP) are three products that have been studied heavily within the last decade to determine their impact on enteric CH₄ production.

Biochar

Biochar is a carbon-rich substance produced during pyrolysis of organic matter (Kammann et al., 2017). Biochar has long been used as a soil amendment to improve nutrient capture, decrease ammonia volatilization, and decrease organic matter loss (Schmidt et al., 2019). Prior to the introduction of biochar (wood-sourced) into livestock feeding in the early 2010s, charcoal was considered a remedy for indigestion and poisoning in domestic animals (Decker and Corby, 1971). Because biochar has a high adsorption capacity due to its large and highly porous internal surface area (Thies and Rillig, 2009), feeding biochar may adsorb a variety of different toxins, metabolites, and pathogens in the gastrointestinal tract (Schmidt et al., 2019). Furthermore, toxins can be removed from the bloodstream when biochar is introduced into the animal's body, where

the surface area properties of the biochar interact with tissue permeability of major organs (Pond, 1986) and positively impact animal health.

In addition to adsorption capacity, biochar has a high potential for redox activity. Various biochars act as electron acceptors and store and mediate electrons during biochemical reactions (Sun et al., 2017). In general, biochars that are produced at low temperature (highest temperature treatment (HTT) 400-450°C) are suggested to act like geobatteries and high-temperature biochars (HTT >600°C) act like electrical conductors (Yu et al., 2015). When microbial decomposition of organic feedstuffs occurs in the GI tract (specifically the rumen), the microbes require a terminal electron acceptor to help reduce the concentration of accumulated electrons (Schmidt et al., 2019). Biochar is a sufficient electron mediator due to its redox potential, and when consumed by the animal along with high-energy feedstuffs, biochar may improve the efficiency of a multitude of redox reactions, resulting in the potential for improved feed intake efficiency (Liu et al., 2011).

Biochar has been utilized in research trials as a livestock feed additive for cattle, goats, pigs, and poultry. When fed to livestock, many of the effects of biochar are based on one or more of the following mechanisms: adsorption within the rumen or into the bloodstream, adsorption followed by a chemical reaction, selective colonization of biochar with bacteria, and binding of biochar to rumen substrates (Schmidt et al., 2019). Over the last decade, regular feeding of biochar at inclusion rates ranging from 0.2 to 3% of diet DM have been studied to determine the impact of biochar on enteric CH₄ production, feed conversion, body weight gain, and meat quality. Improved performance was found when feeding either wood-sourced biochar (Choi et al., 2012) or bamboo-

sourced biochar to pigs (Chu et al., 2013). Chu et al. (2013) compared bamboo-sourced charcoal at 0.3 (T1) and 0.6% (T2) of the basal diet DM to a control and found that average daily gain (ADG) increased by 14.5 and 8.2% for T1 and T2, respectively, and feed efficiency was improved by 14.9 and 11.7% for T1 and T2, respectively, compared to the control. Similarly, Choi et al. (2012) combined stevia (natural sweetener) and charcoal supplementation in varying treatment levels and found ADG was greatest in the treatments that included 0.3% stevia and a combination of 0.3% stevia and 0.3% charcoal.

The use of biochar as a feed additive has been examined both *in vitro* and *in vivo*; however, there is less research on the effects of biochar when included in the diet of beef cattle. Furthermore, the results from recent literature in this area are varying.

Biochar utilized in agriculture is predominantly sourced from forest wood waste, but many other products such as nuts, rice, coffee, corn stover, and animal manure have also been utilized. Therefore, it is important that biochar utilized in agriculture for both research and commercial application be properly characterized. When biochar is included in the diet of ruminants, the nutritive value, *in vitro* digestibility, volatile fatty acid (VFA) production, and gas production (McFarlane et al., 2017) are all important measures to consider when characterizing biochar. In a 2 x 3 factorial experiment with orchard grass as the basal diet, McFarlane et al. (2017) compared two different biochar processing sizes, either fine-ground or coarse, sourced from 1) Chestnut Oak, 2) Yellow Poplar, or 3) White Pine included at 0.81% of basal dietary DM. Results from this study revealed that total gas production was greater for finely ground compared to coarse biochar and was not influenced by biochar type. Additionally, VFA production was not influenced by

biochar size or type (McFarlane et al., 2017). Crude protein digestibility exhibited a type by size interaction, where CP digestibility was lower for finely ground Chestnut Oak and White Pine biochar compared with coarsely ground biochar from these sources, while no difference was exhibited in fine vs. coarse biochar for Yellow Poplar (McFarlane et al., 2017).

When biochar was added *in vitro* to ruminal fluid at concentrations of 0.5 and 1%, Leng et al. (2012) reported a reduction in methane production of 10 and 12.7%, respectively. Similarly, Winders et al. (2019) found that when compared to a control treatment with no biochar inclusion, biochar inclusion at 0.8% of the diet numerically reduced methane production (measured as g/kg DMI) by 9.5% in steers fed a grower diet and 18.4% in steers fed a finisher diet. Biochar utilized in Winders et al. (2019) had a C content of 85%, bulk density of 88.10 kg/m³, and surface area of 323 m²/g.

Terry et al. (2019) demonstrated minimal numerical changes in performance when 8 ruminally cannulated heifers were supplemented with 0, 0.5, 1, or 2% enhanced pine biochar on a basal diet of barley silage and grain in a 4 x 4 Latin square. Ruminal fermentation, apparent total tract digestibility, methane emissions, rumen microbiome, and fecal microbiome were analyzed. Results from this study found ammonia N concentration and protozoa counts responded quadratically with biochar inclusion, where ammonia N and protozoa were decreased when biochar was included at 0.5 and 1% of diet DM compared to the control (0%) and 2% biochar. There was no impact by enhanced pine biochar on total tract digestibility, N balance, or CH₄ production compared to negative control.

Teoh et al. (2019) utilized the *in vitro* rumen simulation technique (RUSITEC) to investigate the effect of supplementing either 400 or 800 mg per day of hardwood-sourced biochar on CH₄ production over a 15-d period. The biochar utilized in their study was a mineral-activated biocarbon sourced from hardwood sawdust with additives of bentonite, zeolite, urea, and other mineral compounds, was 10% total carbon, and underwent slow pyrolysis at 650°C. Results from their study found that biochar supplementation at 800 mg/d had a tendency to reduce the percentage of CH₄ released during fermentation compared to the 400 mg/d treatment (Teoh et al., 2019). In addition to a reduction in methane, the 800 mg/d treatment decreased the abundance of one Methanomethylphilaceae OTU and one *Lactobacillus* spp OTU, suggesting that when biochar is included at higher concentrations, a reduction in enteric methane production may occur from reducing the abundance of the two aforementioned rumen microbiota.

Leng et al. (2012) included rice hull biochar at 0.6% of diet DM in a basal diet of cassava root chips and fresh cassava foliage and reported a 20% improvement in live-weight gain in local “Yellow” cattle (n=12) that had biochar added to their diet. They also captured one sample per head of eructated CH₄ at the end of the experiment using Gasmeter equipment, reporting a 24% reduction in CH₄ production for biochar-supplemented cattle. The increase in weight gain that Leng et al. (2012) reported has not been consistently found across biochar-feeding research. Terry et al. (2019) reported minimal changes in performance of cattle fed enhanced biochar. The difference in body weight (BW) gain improvement found in the literature may be due to production and technology differences, type of cattle, and/or digestibility of the diet (feed processing).

Terry et al. (2020) utilized enhanced pine biochar fed at 0.5, 1, and 2% of dietary DM in a high-forage diet and a high-grain diet to determine the impact of biochar on growth performance, carcass quality, and feeding behavior of 160 steers. They reported no significant impact of enhanced biochar on DMI, feed efficiency, ADG, or final BW for the backgrounding phase or the finisher phase. However, total weight gain and overall ADG tended to decrease with 2% biochar inclusion in the diet. There was no impact of biochar inclusion in the diet on carcass characteristics including hot carcass weight (HCW), 12th rib fat, ribeye area (REA), or marbling; however, there was a significant increase in lean meat yield for 2% biochar treatment compared to all other treatments. This improvement in lean meat yield (lower assigned yield grade) with 2% biochar treatment was likely due to the trend of reduced HCW in the 2% biochar treatment. Terry et al. (2020) reported no difference in DMI or eating behavior when biochar was included in the diet, and this is consistent with previous studies performed on cattle fed either high-forage or high-concentrate diets (Terry et al., 2019; Winders et al., 2019).

Conlin et al. (2021) looked at the optimum dose of pine-sourced biochar (0, 1, 2, or 3% of total DMI) required to reduce CH₄ emissions from eight multiparous (late-gestating) commercial Angus cows consuming a high-forage diet (50% alfalfa haylage, 30% wheat straw, 17% corn silage) in a 4 x 4 Latin square design, with animal as the experimental unit. Biochar was sourced from Oregon Biochar Solution (White City, OR) and had a surface area of 456 m²/g, a bulk density of 78.5 kg/m³, and was 83.6% C as a % of total DM. Cows were housed together in a pen equipped with Insentec (Insentec B. C., Marknesse, The Netherlands) bunks to monitor individual intake. A C-Lock GreenFeed trailer (C-Lock Inc., Rapid City, SD, USA) was used to capture CH₄ and CO₂

emissions (g/d) throughout the feeding period. Conlin et al. (2021) found no difference in DMI, ADG, or BW between treatments, and biochar supplementation was ineffective in reducing CH₄ and CO₂ emissions. In a second study by Conlin et al. (2021) looking at the impact of supplementing cows (n = 64; with calf at side) on pasture with biochar pellets at either 0 (control) or 3% of estimated DMI (16 kg/d) on CH₄ emissions (using C-Lock GreenFeed trailer), biochar addition to the diet had no impact on CH₄ production (g/d, g/kg DMI, or g/kg BW) and cow performance was not affected.

Over the last decade, the use of biochar as a feed additive to reduce enteric methane production in livestock has had varying results. The variance in experimental results is impacted by the characterization of biochar utilized in the study, including source of biochar, surface area, moisture content, carbon percentage, processing method, porosity, and bulk density. The basal diet that biochar is added to may also influence whether biochar reduces CH₄ emission from ruminants.

Seaweed

Macroalgae, commonly referred to as seaweed, is widely used in cosmetics, human nutrition, and pharmaceuticals (Paul and Tseng, 2012). Seaweeds are considered a sufficient feed ingredient for livestock due to their high concentrations of organic minerals, protein content, complex carbohydrate content, and broad bioactive substances (Kumar et al., 2008). Seaweed species have a unique makeup, consisting of diverse lipids and tannins, and many have secondary metabolites that possess antimethanogenic properties (Wang et al., 2008).

In an overview of various seaweed species used in livestock diets, Maia et al. (2016) reported that the efficacy of seaweed as a rumen modifier/methane reducer was based on the composition of the biomass including species, growth stage, habitat, and the

basal feed of the livestock ration. In addition, pigmentation of the seaweed, either red, green, or brown algae, further impacts the efficacy of the macroalgae as a rumen modifier because of differences in chemical composition and mineral concentrations between the species. An *in vitro* study comparing five different seaweed species (of the red, brown, and green algae families) incubated at high inclusion level (25% DM basis) against a negative control found that methane production (mL per g of DM) was reduced up to 17% for three of the seaweed species (*Ulva*, *Gigartina*, *Gracilaria*) compared to the control (Maia et al., 2016). Results from this study concluded that seaweed of the brown and red algae families may have greater potential in reducing methane production than green algae (Maia et al., 2016). Furthermore, the red algae *Asparagopsis* has potent antimethanogenic properties *in vitro* when included at 1 to 2% (OM basis), with some reductions in methane production greater than 99% (Maia et al., 2016). The antimethanogenic properties of red seaweeds are due to secondary metabolites (i.e. halogenated compounds with bromine or chlorine) that the plant has derived as a defense mechanism for survival in times of high competition (Liu et al., 2011). When red seaweed is consumed by the ruminant animal, these secondary metabolites inhibit the methyl transfer reactions essential for methanogenesis (Liu et al., 2011) and exert an antimicrobial action on cellulolytic bacteria in the rumen that are key for fiber digestion (Wang et al., 2009), thereby reducing ruminal fiber digestibility. In addition, Li et al. (2018) reported that propionate production increased as the concentrations of *Asparagopsis* in the diet increased, and since propionate is a hydrogen sink, the concentration of hydrogen available to link with carbon for CH₄ production is reduced.

A considerable reduction in CH₄ emissions utilizing *Asparagopsis* species has been reported through varying *in vitro* studies; however, there are limited studies that show a reduction when included as a feed additive in cattle diets. Roque et al. (2019) studied the effect of feeding *Asparagopsis armata* at three levels: 0 (control), 0.5, and 1% inclusion (OM basis) in a 3 x 3 Latin square design on methane production. Twelve multiparous Holstein cows were housed in a freestall barn equipped with Calan gates to measure individual feed intake. Individual cow emissions (CO₂ and CH₄) were measured using the GreenFeed Large Animal System (C-Lock, Inc., Rapid City, SD) over a 7-d period. There was a significant linear reduction in DMI as inclusion of *Asparagopsis* increased. When *Asparagopsis* was fed at 0.5 and 1% of diet OM, methane production (g/d) per cow significantly decreased by 26.4 and 67.2%, respectively. When methane production was adjusted on a DMI basis (g/kg of DMI), methane yield was reduced by 20.3% and 42.7% in cows fed *Asparagopsis* at 0.5 and 1% of diet OM, respectively. Roque et al. (2021) also studied the effect of feeding *Asparagopsis taxiformis* at three levels, 0% (control), 0.25% (low) and 0.5% (high) dietary OM basis, utilizing 21 beef steers, to determine the effect of *Asparagopsis* on CH₄ production, DMI, live performance and carcass characteristics. Steers were fed 3 different diets during the progression of the study to represent the common ration composition offered in most commercial feedlot diets in the USA. These diets were high-forage (starter diet), medium-forage (transition diet), and low-forage (finishing diet), and were fed for a combined total of 147 d (the longest that any study has analyzed the feeding of a seaweed product to beef cattle). Results from this study found that low and high seaweed *A. taxiformis* inclusion yielded CH₄ reductions of 45 and 68%, respectively, without any

loss in efficacy over the 21 weeks. There was a treatment by total mixed ration (TMR) type interaction, where supplementing high *A. taxiformis* on a low-forage TMR had greater CH₄ reduction than supplementing high *A. taxiformis* on a high-forage TMR. There was no difference in ADG between treatments, but DMI tended to decrease in the low-inclusion treatment and was significantly reduced in the high-inclusion treatment. There was no difference between treatments for carcass weight, ribeye area, or marbling, and meat consumer taste preferences were similar between treatments, indicating that supplementation of *A. taxiformis* at these inclusion levels does not alter the sensory properties of the meat.

As CH₄ production decreases, hydrogen and CO₂ emissions increased as a way for excess H to release from the rumen. The CH₄ reductions reported by Roque and associates (2019) are among the largest reductions reported in recent literature when utilizing seaweed as a feed additive, which can be explained by the antimethanogenic effect of the seaweed utilized in their study (Kinley et al., 2016). Although the inclusion of varying species of seaweed in the diet of ruminants have shown significant reductions in CH₄, the use of seaweed as a feed additive for cattle is not currently approved by the Food and Drug Administration (FDA) in the USA. This is because bromoform, a bioactive secondary metabolite found in red seaweed (*Asparagopsis taxiformis*) and the compound suggested to inhibit methanogenesis, is a known carcinogen and is, therefore, of human health concern (Abbott et al., 2020).

3-Nitrooxypropanol

Since development in 2012, research utilizing 3-nitrooxypropanol (3-NOP) as a feed additive to reduce enteric methane production has been well established. Methyl-coenzyme M reductase (MCR) catalyzes methane formation from methanogenic archaea,

and the compound 3-NOP is a structural analogue of methyl-coenzyme M, suggesting that when added to the rumen, it inhibits certain levels of methanogenesis by targeting the active site of MCR during the last steps of methanogenesis (Duin et al., 2016). When included in the diet of beef cattle, 3-NOP has been shown to decrease methane emission without negatively impacting performance (Romero-Perez et al., 2015), and is associated with a shift in ruminal fermentation production of acetate toward propionate (Haisan et al., 2014; Romero-Perez et al., 2015). The inclusion of 3-NOP in the diet reduced methane emissions in sheep, dairy cattle, and beef cattle (Martinez-Fernandez et al., 2014; Haisan et al., 2014; Romero-Perez et al., 2014) by approximately 7 to 60% when corrected for changes in DMI. Based on these studies, methane reduction is dependent on mode of action in which the feedstuff was delivered, either combined as a TMR, top-dressed, or dosed into the rumen (Romero-Perez et al., 2015).

Romero-Perez et al. (2015) included 3-NOP (2 g/d) in a 60% forage diet of beef heifers for an extended period (112 d) to determine the impact of 3-NOP on sustaining a reduction in enteric CH₄ emission long-term compared to non-NOP treatment. Results from their study found that methane emissions were reduced by 59.2% when NOP was included in diet and total VFA concentration was not impacted in a backgrounding diet, suggesting that 3-NOP addition causes a sustained reduction in methanogenesis with no sign of adaptation by the rumen microflora. Following the 112-d treatment period, Romero-Perez (2015) transitioned the heifers into a recovery period with no addition of 3-NOP in diet, and there showed no residual effects of 3-NOP on the variables studied.

The success of 3-NOP in reducing enteric CH₄ production without negatively impacting performance, animal health, or carcass characteristics, has grabbed the

attention of the dairy and commercial feedlot industry. Royal DSM, a global nutrition and sustainability company, has commenced with registration of 3-NOP, renamed Bovaer, on the market in Europe, with sights of approving the feed additive in Canada and the USA in 2022 or 2023 (Guenther, 2021).

Manure emission

Manure from open beef feedlots is a combination of fecal matter, urine, pen topsoil, and other organic compounds. Nitrogen excretion from the animal can be classified into urea, typically from the urine, and fecal N, which combines organic N from the feedstuff and microbial N produced from the hindgut. Methane and nitrous oxide (N_2O) are the predominant GHG emissions produced from manure decomposition, and ammonia (NH_3) is the predominant non-GHG gaseous emission. Of the 10% of total GHG emissions that livestock and crop production account for in the USA, emissions from excreted manure account for approximately 12% of that fraction (USEPA, 2021). Organic matter in manure is converted to CH_4 during anaerobic decomposition (deep lagoons or holding tanks) and denitrification during anaerobic storage or treatment produces N_2O emissions (Gerber et al., 2013). In addition, NH_3 in the manure volatilizes, with some transforming to N_2O via combined nitrification-denitrification (Gerber et al., 2013). These N losses via volatilization are a direct economic loss in value of the manure. Furthermore, N_2O is a particular concern for its global warming potential, estimated to be up to 310 times that of CO_2 (Forster et al., 2007).

Ammonia (NH_3)

Ammonia (NH_3) is a gaseous form of nitrogen, and although it is not a greenhouse gas, it is a common emission produced from agricultural production. The predominant ways in which NH_3 is lost to the environment in agriculture are through the

storage and application of organic (manure) and inorganic N fertilizers, by grazing animals, and during manure storage and treatment. On a global scale, it is estimated that agriculture accounts for 80 to 90% of total NH_3 emissions (Bouwman et al., 1997; Xu et al., 2019) via volatilization from livestock manure and the storage and application of synthetic N fertilizer (Bouwman et al., 1997).

Ammonia emissions are associated with two looming global environmental concerns: acidification and eutrophication. Precipitation that is acidified by 'reactive N', including NH_3 , and direct deposition of certain forms of N fertilizer to agricultural land can lead to acidification of soils and water bodies. Excess soil acidity is harmful for various aquatic and terrestrial species and can negatively impact soil microbial communities. Increased deposition of NH_3 can lead to elevated N concentrations in soil and water bodies, resulting in eutrophication. Eutrophication is defined as the overgrowth of plants and algae due to excess nutrients (N and P) present in water bodies. Following excessive growth, many of the plants will die and decompose. The decomposition process of the dead plant material uses the majority of the O_2 present in the water body, which becomes detrimental for fish and other species that require O_2 to survive, resulting in fish kills.

Once ammonia enters the atmosphere via volatilization from manure (on feedlot pen surfaces and in other storage systems), it can react with atmospheric acids, such as nitric and sulfuric acid. The reaction of ammonia with atmospheric acids can form fine particulate matter, specifically $\text{PM}_{2.5}$, which is a regulated air pollutant associated with respiratory disease in humans. Fine particulate matter can be carried by air currents and

return to soil through dry or wet deposition (Montes et al., 2013) and can be harmful for sensitive ecosystems and contribute to groundwater pollution (Hristov et al., 2013).

The N content of livestock feeds is often in excess of the animal's nutrient requirements, resulting in a large concentration of N that is excreted in urine and feces (Spiehs, Woodbury, & Parker, 2019). Of the total amount of N fed in the animal's diet, only 10 to 30% is retained by the animal for growth and milk production (Stowell, 2018). In a beef feedlot setting, approximately 5% (or less) of the excreted N (representing 70-90% of the N fed) will run off the pen, 30 to 50% will be retained in the manure as organic N, and the remaining 40 to 70% will volatilize into the air as ammonia.

Depending on the diet consumed by the animal, feedlot cattle on a concentrate diet generally excrete 60 to 80% of N intake in the urine and the other 20 to 40% in the feces (Bierman et al., 1999; Bao, Zhou and Zhao, 2018). The concentration of N in excreted feces remains relatively constant, independent from diet, whereas the concentration of N in urine is highly variable and largely dependent on diet (Powell and Rotz, 2015). One of the largest determinants of excreted N in cattle urine is the percentage of dietary crude protein intake (Powell and Rotz, 2015). Menezes et al. (2016) compared feeding beef cattle with a ration of 100, 120, or 140 CP/kg DM, finding that N retention and ADG were not affected by dietary CP inclusion; however, urinary N excretion was lower for the 100 g CP/kg DM treatment compared to the higher inclusion treatments.

Volatilization of NH_3 from cattle manure occurs rapidly and is altered by the ambient air temperature, wind, and pH of the pen surface. Nitrogen is primarily excreted through urine, around 60 to 80% on concentrate diet (Bierman et al., 1999), where

approximately 97% of the N in urine is in the form of urea (Mackie et al., 1998). When urine and feces mix, urea is rapidly converted to NH_3 (Mobley and Hausinger, 1989) via the enzyme urease, which is derived from microbes in the feces. It is well defined in literature that manure N loss as NH_3 is greater in summer months (May to October) than winter months (November to May), because warmer temperatures ($>21^\circ\text{C}$) increase the speed of hydrolysis of urea (Dari, Rogers, & Walsh, 2019).

A meta-analysis completed by Homolka et al. (2021) from data collected in Nebraska determined that manure N loss as a percentage of fed N averaged around 70% in the summer and 50% in the winter. The winter months range from 40 to 70% loss and the summer months from 65 to 71%, making it clear that the warmer ambient air temperature in the summer months favors volatilization. Kissinger et al. (2007) summarized the manure characteristics of 15 open-lot pens of cattle with 40 separate lots of cattle fed in those pens ($n = 6,366$) sourced from Nebraska feedlots over the course of a 1-yr feeding period. They observed N losses from volatilization and runoff to be 53 and 67% of fed N for winter and summer, respectively, and also concluded that the total mass of manure harvested after winter-feeding is about 20% more than the mass of manure harvested after summer-feeding. Todd et al. (2008) conducted a feedlot study in Texas to determine the NH_3 emission rates of a 77-ha, 45,000-head commercial yard. Emissions were quantified using profiles of NH_3 concentration, air temperature, and wind speed. They found the N loss from manure via NH_3 volatilization to be 68% in the summer months and 36% in the winter months.

The pH of the pen surface influences the speed of NH_3 volatilization as well, where the ideal pH conditions for rapid volatilization are neutral (pH 7) to basic (pH 10);

Hartung and Phillips et al., 1994). Water and urease are present on the pen surface and available for interaction with urea, and as the pH of the pen surface becomes more alkaline ($\text{pH} > 8$), a single molecule of urea rapidly hydrolyzes into two molecules of NH_3 (Rhoades et al., 2010). When surface pH drops below 6.5, research has shown that little NH_3 volatilization will occur, due to the pKa of ammonium (NH_4) being less volatile than NH_3 (Rhoades et al., 2010). The pKa for ammonia and ammonium equilibrium is 9.24 under normal conditions and temperatures. As pH of the pen surface becomes more alkaline, the relative proportions of NH_3 to NH_4 increase. Court et al. (1964) reported relative proportions of NH_3 to NH_4 of 0.1, 1, 10 and 50% at pH of 6, 7, 8, and 9, respectively.

The mechanisms of action by which urea and organic N undergo microbial transformation upon contact with the feedlot pen surface are different. As mentioned above, N excreted in the feces originates from the feed source, bacterial cells, and other endogenous sources. The undigested feedstuff is mostly excreted as true protein in the form of amino acids, bacterial N is excreted in nucleic acids (partly) and amino acids, and the remaining fecal N is excreted as ammonium (around 50% of the total fecal N content). The N content in urine is predominantly excreted as urea and comes from excess N in the diet above the requirements of the animal.

Nutrients on the feedlot pen surface

Concentrate finishing diets incorporate high concentrations of N, P and soluble salts from the various feedstuffs, and not all of the nutrients consumed are retained by the animal. Of total N and P offered in the diet, beef cattle retain approximately 12% of fed N and 15% of fed P (Kissinger et al., 2007). According to Kissinger et al. (2007), overfeeding N (protein) does result in greater N excreted but does not result in greater N

content of the manure; instead, a greater amount of N is lost via volatilization and runoff. This is not the case with P, however, where an increase in P in the diet is directly related to a greater concentration of P recovered in the harvested manure (Kissinger et al., 2007). Approximately 0.16, 0.022, and 0.11 kg per animal per day of N, P and K, respectively, are excreted in the manure (ASAE, 2005) of feedlot beef cattle. Once excreted onto the pen surface, the vast majority of manure nutrients: 1) volatilize and leave as ammonia emissions (only N); 2) wash off the pen surface via rainfall runoff; and 3) are removed during pen cleaning.

The nutrient availability in soils, specifically carbon bioavailability, largely impacts the metabolic and physiological processes of the microbial populations that inhabit it. Carbon cycling is a large part of the metabolic process within the soil microbial population. Microbes drive C cycling, as they are constantly in search of C, energy (in the form of ATP), and reducing power (NADH). When soil organic matter concentration increases, the concentration of carbon in the soil also increases. Soil organic carbon helps to improve soil structure, specifically by stabilizing aggregates within the soil, which in turn, protect microbes. The biological activity within the soil (microorganism growth and reproduction) is impacted by the availability of N, P, and K, substrate bioavailability, and redox reactions. The addition of fresh manure to the soil provides a source of organic matter (C and N) that is then consumed by soil organisms, to be utilized in growth, reproduction, and metabolism. Manure is also then subject to decomposition, which impacts the C pools in the soil. The readily available C in the soil decreases as time progresses, and the rate of decomposition of the microbes declines as the pool of C becomes more stabilized and harder to degrade.

The soil profile of open-air, dirt feedlot pens is altered by animal activity and feedyard management practices. The feedlot soil profile can be divided into an organic layer (fresh accumulation of manure), interface layer (mixture of OM and mineral soil), and the natural mineral soil (Vaillant et al., 2009). The surface layer (roughly top 0.15 m) is compacted by cattle hoof action, resulting in a high bulk density (1600 to 1870 kg cm³), and has poor aeration (Mielke et al., 1974; Olson et al., 2005). Moisture content of the feedlot pen surface is dependent on animal waste and normal precipitation. In addition to being a moisture source, manure provides a habitat for microorganisms, such as bacteria and protozoa, that utilize the manure nutrients (specifically N, P, and C). Select groups of these microorganisms can produce organic gels and polysaccharides, which increase the compaction of the soil by plugging soil pores (Vaillant et al., 2009). The compaction of the top organic layer and the activity of the microorganisms all help to stabilize and seal the interface layer, preventing seepage into the natural mineral layer and potential contamination of groundwater. Vaillant et al. (2009) looked at soil physical and chemical profile under the feedlot pen in four Kansas feedlots and found that a limited amount of excreted nutrients had accumulated in the soil beneath the pens, suggesting that the compaction of the manure and surface soil mixture acts as a soil liner to reduce infiltration. The average organic N concentrations on the pen surface ranged from 500 to 22,000 mg/kg, rapidly dropping to a stable 150 to 600 mg/kg at a depth of 0.25 m. The extractable P concentration on the pen surface ranged from 20 to 9000 mg/kg and rapidly dropped to a range between <1.0 and 80 mg/kg at a depth of 0.50 m. Therefore, leaching of N and P from the feedlot pen surface represent a very small

component of the overall nutrient balance of the feedlot pen, and are not significant contributors to N and P found in groundwater (Vaillant et al., 2009; Elliot et al., 1972).

It is estimated that less than 5% of nutrient loss from the pen surface is via runoff (Bierman et al., 1999; Erickson et al., 2000). In a beef cattle feeding operation, runoff from the pen surface generally contains nutrients (N, P, K, C), organic matter, microorganisms, and soil sediment (Eghball and Power, 1994). This runoff is then either collected in a runoff retention pond or may infiltrate into the soil, potentially posing a risk to ground water quality (Vaillant et al., 2009). In a recent meta-analysis by Homolka et al. (2021) including 15 mass-balance feedlot studies in Nebraska, it was suggested that N and P loss due to runoff represent 1.5 to 2.5% of fed N and 4 to 6% of fed P. These values are considerably smaller than values estimated by Liu and Nienaber (1996) based on a review of 13 studies that estimated N runoff to be 0.1 to 7% of manure N and P runoff to be 0.02 to 12.5% of manure P. Previous literature has noted seasonal variation in nutrients captured in runoff (Gilbertson et al., 1979; Homolka et al., 2021), with summer-feeding periods having greater nutrient recovery in runoff compared to winter, likely due to greater precipitation and rainfall intensity in summer months.

Manure characteristics on the open-lot pen surface vary according to distance from the feed bunk, climatic conditions, and season (Sweeten et al., 1985; Kissinger, 2005). Kissinger (2005) summarized 11 studies representing 244 pens of cattle over 10 years and concluded that there is almost twice the manure mass harvested following a winter-feeding period compared to a summer period and that manure harvested after winter feeding has significantly greater N retention compared to summer. The significant improvement in excreted N recovery in manure is likely due to lower losses of N

volatilization during winter feeding months compared to summer feeding months (Kissinger, 2005). Kissinger et al. (2007) summarized the manure characteristics of open-lot beef systems for 15 pens of cattle ($n = 6,366$) sourced from 6 central and eastern Nebraska feedlots over the course of a 1-yr feeding period to determine factors that impact manure quantities and mass balance of N and P. Results from this study found that harvested manure contains 33% of N excreted from the animal and 91% of excreted P (Kissinger et al., 2007). Homolka et al. (2021) reported N losses of 50 and 73% (of excreted N) for winter and summer-feeding periods, respectively, from a meta-analysis of 15 feedyard studies in Nebraska. These seasonal differences in manure N content and overall manure N retention are impacted by feed N intake (Homolka et al., 2021) and organic matter (OM) content on the feedlot pen surface (Bierman et al., 1999; Erickson and Klopfenstein, 2001b; Lory et al., 2002). Increasing OM content may retain more N in the manure and reduce N loss. Organic matter removal from the pen surface during pen cleaning ranges from 0.3 to 4.0 kg of OM/steer per day (Homolka et al., 2021). Manure that is removed from the feedlot pen surface consists of OM and ash. Organic matter includes all carbon-based compounds (cellulose, lignin, proteins, lipids, sugars) within the manure and topsoil, while ash represents various other elements (Ca, Mg, K, P). When manure is cleaned from the feedlot pen surface, a large quantity of soil can be removed with the manure, with this amount varying with moisture content of the pen surface. When a larger volume of soil is removed with the manure, ash concentration of the manure increases (Kissinger et al., 2007).

Kissinger et al. (2007) suggests that over a 150-d feeding period, approximately 1100 kg of manure is removed per finishing animal. Furthermore, spring cleaning

harvests about 20% more manure than fall cleaning, with much of this increase attributed to a greater ash content (soil) from the spring cleaning (Kissinger et al., 2007). In general, the winter and spring months bring increased precipitation and less evaporation potential, resulting in a wetter feedlot pen surface. The wetter the feedlot pen surface, the more mixing of manure and soil results from cattle hoof action. When pen cleaning is completed in the spring (following a winter/spring feeding period), the elevated moisture content of the pen surface can create challenges in pen cleaning and manure harvest, resulting in greater soil inclusion with the manure solids, and nutrient concentrations (specifically phosphorus) that are greater than what was excreted by the animal (Kissinger et al., 2007).

Pen surface amendments

To reduce nutrient loss from feedlot cattle manure, a variety of pen amendments have been utilized, including, but not limited to, woodchips, straw, aluminum sulfate, and biochars sourced from various organic materials. The addition of organic matter sources to the feedlot pen surface can increase the C:N ratio of the pen surface, decreasing overall N losses from the manure by immobilizing active N. In beef feedlots, it is estimated that 50 to 75% of excreted N is lost before manure is mechanically removed from the feedlot pens (Eghball and Power, 1994), resulting in a decrease in nutrient value of the manure (N specifically). Therefore, improving retention of N in livestock excreta can increase the value of the manure while also producing environmental and air quality benefits.

Lory et al. (2002) looked at the relationship between application of sawdust to the feedlot pen surface and N losses in winter and summer feeding phases, concluding that application of a product high in C to the pen surface reduced N losses during summer

months. Adams et al. (2004) utilized sawdust as an OM addition to the pen surface, comparing it to a treatment where cattle had a dietary inclusion of 30% greater corn bran in the diet, and a control treatment with no sawdust application or dietary bran addition. The higher inclusion of bran in the diet was designed to decrease diet digestibility, thereby increasing OM excretion onto the pen surface. Results from their study found that adding OM to the pen surface, either as a pen amendment (sawdust) or by increasing OM excretion from the steers (bran), increased manure N content by 20% compared to the control in winter-feeding. Adding OM to the pen surface numerically reduced overall N losses from the manure, with approximately 70% of the N excreted from the cattle in bran and sawdust treatments captured in the manure during manure removal, and only 49% of N excreted in control treatment captured in the manure during pen cleaning.

Similarly, Embertson and Davis (2009) looked at the impact of harrowing woodchips into the open drylot pen surface at various dairy operations and found that pens with woodchips harrowed in had up to 40% lower ammonia emissions compared to control pens, as measured using a real-time NH₃ analyzer (Nitrolux-S, Pranalytica). Embertson and Davis (2009) also noted that utilizing woodchips on the pen surface reduced the moisture content of the pen surface, thereby improving cow health during excessive wet periods.

Feeding less digestible diets has been shown to increase OM excretion onto the pen surface and improve manure nutrient capture (Bierman et al., 1999; Erickson and Klopfenstein, 2001a; Farran et al., 2006). Bierman et al. (1999) conducted a mass-balance experiment to evaluate the impact of decreasing digestibility in feedlot diets on N mass balance. Three treatments were evaluated from June until September: a corn control

diet with no roughage, a diet with 7.5% alfalfa, and a diet with 41.5% wet corn gluten feed (WCGF). Results from their study showed that excreted N was the greatest for steers fed WCGF and lowest for the control diet with no roughage, and that the concentration of N in the collected manure was greatest for the WCGF treatment. The percent N loss was greatest for the WCGF and alfalfa diets compared to control, where the difference in N excretion between diets was via fecal N excretion.

Erickson and Klopfenstein (2001b) looked at the impact of feeding bran at 0, 15, and 30% of dietary DM on N mass balance in summer and winter feeding periods. Nitrogen intake and excretion increased linearly with bran inclusion, and manure N loss decreased linearly with bran inclusion level in winter. The same patterns in intake and excretion were observed in the summer feeding period. Manure OM content increased as inclusion of corn bran in the diet increased, resulting in improved manure N capture at time of pen cleaning.

Farran et al. (2006) evaluated alfalfa hay level (0, 3.75, and 7.5% dietary DM) and WCGF (0 and 35% dietary DM) on nitrogen mass balance from crossbred steers in a summer feeding period (June to October). Nitrogen intake, retention, and excretion increased linearly as hay inclusion increased; however, the N removed in manure and % N loss were similar among hay levels. The OM removed increased linearly with hay inclusion. The addition of WCGF to the diet increased N intake and retention, and N and OM removal from the pen surface, but the amount of N lost was numerically greater for WCGF compared to control. These data suggest that, although a greater amount of N was excreted and lost, there was greater N recovered in the manure.

Reducing the pH of the pen surface reduces NH_3 volatilization and, therefore, amendments that reduce the pH of the bedded pack, feedlot pen surface, or litter pile may reduce N volatilization and improve N retention in the manure. The addition of an acidifying agent, aluminum sulfate (alum), to livestock waste has been well developed in the poultry industry as a litter amendment to reduce NH_3 emissions from poultry barns. Research results have shown up to 70% lower NH_3 fluxes with alum-treated compared to untreated litter (Moore et al., 2000). Spiels, Woodbury and Parker (2019) explored the addition of alum to lab-simulated cattle manure bedpacks at varying concentrations (0, 2.5, 5 and 10%) to determine its impact on NH_3 and hydrogen sulfide (H_2S) emissions. Results from their study found that NH_3 emissions were reduced with 10% alum treatment, but H_2S and CH_4 emission increased as the percentage of alum added to the bedded packs increased. Although alum has been shown to reduce NH_3 emissions, it can be costly when added to the feedlot pen surface, estimated to be approximately \$43 per head per year, and may only be a short-term solution, with limited long-term effectiveness (Embertson and Davis, 2009). Another acidifying agent, calcium chloride, is a proposed method to reduce NH_3 emission from livestock excreta, and when added to fresh poultry slurry, Witter (1991) reported a decrease in ammonia loss via volatilization.

As discussed above, upon excretion from the animal, urea is rapidly converted to NH_3 via the enzyme urease. The addition of urease inhibitors to livestock excreta has been shown to reduce N loss via volatilization. Phenyl phosphorodiamidate (PPDA) and cyclohexylphosphoric triamide (CHPT) are urease inhibitors that can prevent hydrolysis of urea for a period. Varel (1997) evaluated the effect of PPDA and CHPT on urea hydrolysis in beef cattle manure slurries. Results from this study found that both PPDA

and CHPT were adequate in preventing hydrolysis of urea for 4 to 11 d. When PPDA was added to the manure slurry weekly, it prevented 38 to 70% of urea from being hydrolyzed after 28 d. As a follow up, Varel et al. (1999) applied CHPT and N-(n-butyl) thiophosphoric triamide (NBPT) to open beef cattle feedlot pens. The NBPT treatment was applied every 7 d for 6 weeks and results revealed that the peak concentration of urea (17 g per kg dry manure) was stabilized at d 31 and remained at that level until week 6. Once NBPT application was halted, urea concentration in the manure decreased over the next two weeks, suggesting that the urease inhibitor lost potency over time. Parker et al. (2005) utilized NBPT in a simulated beef open-lot experiment to determine the effect of increasing application rates of NBPT (either 5 kg per ha every 4 d or 5 kg per ha doubled every 4 d up to 40 kg ha) on ammonia emissions. Results from their study showed that NBPT application at varying rates was effective in reducing ammonia emissions from manure by 26 to 33% of the non-NBPT treatments. Parker et al. (2012) looked at the interaction of NBPT application and rainfall in a lab-simulated feedlot pen surface study and reported that all NBPT treatments, regardless of rainfall or not, were successful in reducing NH₃ emissions compared to non-NBPT treatments.

The application of lignite (brown coal), an acidic product of the first stage of coalification, to the feedlot pen surface has also been shown to reduce NH₃ volatilization. Lignite has a strong adsorption for ammonium and can biologically immobilize N due to its high C content (Maharjan and Wilke, 2021). When lignite was applied to the pen surface at 3 to 6 kg per m² prior to cattle entering the pen, results showed a reduction in N volatilization from manure of 30 to 66% compared to manure volatilization in non-lignite treated pens (Chen et al., 2015). Impraim et al. (2020) evaluated the combination of

lignite amendment and forced-aeration composting in cattle feedlot pens and found that lignite-treated pens retained N in the manure by reducing NH_3 loss by 35 to 54%, and ultimately increased the N content of the harvested manure.

The addition of varying sources of biochar to the feedlot pen surface have been proposed in recent literature to improve manure nutrient capture. Biochar is often referred to as a carbonized charcoal as most biochar products have a high C percentage; however, this differs depending on the source of the biochar. A Canadian feedlot study reported the C:N ratio for fresh manure, interim manure, and composted manure to be 18.3, 13.6, and 10.6, respectively, noting that straw bedding had been used on the pen surface during the winter feeding period (Larney et al., 2007). The recommended C:N ratio for feedlot manure to be of value as a fertilizer is between 25:1 and 40:1, so the application of a carbon source to the pen surface should improve the C:N ratio. When C:N ratio is low (<15:1), ammonia losses increase because the C available for microbial growth is limiting and mineralization and release of N occurs rapidly (Brust, 2019). When biochar is utilized as a soil amendment, improvements in crop yields and soil fertility (Atkinson, Fitzgerald & Hipps, 2010) and decreases in emissions of N_2O and CH_4 from fields have been reported (Cayuela et al., 2014; Karhu et al., 2011). During pen cleaning and manure management at cattle feeding operations, a significant amount of N can be lost from the manure, decreasing the value of that manure as a fertilizer, as N is a valuable addition to cropping systems.

Maharjan and Wilke (2021) utilized coal char, a coal combustion residue sourced from a sugar factory in Nebraska. The coal char was 30% C by weight and was utilized as a manure amendment in three different experiments, two of which will be discussed

below. In experiment 1, manure was scraped from pens and composited into 8 piles (4 piles per treatment) in a storage plot, and char was added at 454 kg char for 7258 kg manure. Manure grab samples were collected from each pile to measure ammonium-N of the manure piles as-is and after drying for 24 h in 100°C to determine the potential for manure N volatilization. Results from experiment 1 found that N loss potential was reduced from 68% (control) to 44% with char addition. In experiment 3, char was added to the feedlot pen surface of 5 treatment pens at a rate of 0.625 ton per animal prior to cattle entering the pens and was compared to 5 control pens. Soil moisture sensors were fixed in each pen, and results showed that following a series of snowfall events in November, the char-amended pens were significantly drier than the controls. Manure samples were collected from each of the 10 pens at the completion of the experiment, showing a decrease in ammonium and total N and P concentration for the char treatment compared to the control, however, total amounts of nutrients were not calculated.

In a simulated lab-based cattle manure study utilizing wood-sourced biochar at 0, 5, and 10% of manure DM and its impact on manure N retention, researchers found that biochar addition limited C losses, but did not impact N, P, or K retention (APPENDIX A). Aguilar et al. (2013) completed a laboratory evaluation of organic residues (sorghum straw, prairie grass, and woodchips) and biochar created from those organic residues and their impact on CO₂, N₂O and CH₄ production over time when surface-amended to moist beef cattle manure. They found that topical application of the organic residues on manure did not significantly reduce GHG emissions; however, the organic residue biochars applied to a thickness of 3 and 5 mm significantly reduced GHG emissions after 5 d of measurement.

The addition of biochar to poultry litter has also shown significant reductions in GHG production. Agyarko-Mintah et al. (2016) utilized biochar produced from either green waste or poultry litter and incorporated them into a poultry litter and straw compost mix at 10% of mixture (dry weight) to determine their impact on GHG emissions when compared to a negative control. Emissions were measured *in situ* using a Fourier Transform Infrared Spectroscopy (FTIR). Results from their study found that despite the differences in chemical and physical properties of the two biochars, the cumulative N₂O and CH₄ emissions were similar between the two and were significantly lower than the control treatment (no amendment). In addition, total retained N content in the poultry litter was significantly higher for the two biochar treatments relative to the control.

Livestock manure lagoons and runoff retention ponds are associated with ammonia emissions to the atmosphere due to volatilization from the stored material. Dougherty et al. (2017) studied the efficacy of utilizing wood-sourced biochar as a manure lagoon cover to trap gases and reduce ammonia emission in a lagoon-simulated laboratory study. Four treatments were utilized in their study: 1) biochar made from the combination of Douglas fir chips and center wood pyrolyzed at 600°C, 2) biochar made via gasification of Douglas fir chips at 650°C, 3) wheat straw, and a 4) control with no biocover. The treatment covers were applied to the manure surface at a thickness of 5 cm. Polyethylene pails (26.5 L) with 17 L of manure slurry were fit with gas sampling apparatuses to capture NH₃ and NH₂. Slurry was collected from 3 different dairy operations in Oregon. Biochar made from the combination of Douglas fir chips and center wood reduced the ammonia concentration of emissions by 72 to 80% (dependent on manure slurry used) compared to control. There were no significant reductions with

biochar made via gasification of Douglas fir chips, and researchers attributed this to a spike in ammonia emission at the onset of the study. The wheat straw treatment reduced the average NH_3 concentration in the air (headspace of bucket) by 53 to 70%. Odor analysis from trained sensory panelists was also conducted with this research, noting that biochar covers may be an effective tool to reduce odor from livestock manure lagoons.

Although the application of many of the aforementioned pen amendments have proven successful in reducing NH_3 emissions and subsequently improving manure N content, the rigorous application of the products, including time and labor, make many of these amendments an economic burden for commercial feedlot application.

Implications and future research

The exact mechanisms of action of feeding biochar on the rumen microbiome are still widely unknown. Since biochar can be created from a multitude of different organic materials, there needs to be characterization of these chars in order to gain true insight into the product. Future large-scale research on the use of biochar included as a feed additive to reduce enteric methane emissions will likely offer greater insight into the mechanism of action and system influence that this forestry byproduct holds.

Conclusion

Ruminant enteric methane production is a concern both for the environment, as a GHG, and as a waste of fed energy to the animal. A plethora of research has been completed on the topic of methane abatement strategies in livestock production, including alterations to the diet and level of feed intake, feed processing, addition of lipids or ionophores to the diet, and alterations in the microflora of the rumen. Three newly developed methane reduction strategies with promising preliminary results include the addition of biochar, seaweed, or 3-NOP to the diet of ruminants. The development and

implementation of these methane reduction strategies can enhance utilization of dietary energy while decreasing the GHG impact of ruminant animals on the environment. The loss of N from manure in the form of NH_3 via volatilization is a growing environmental and economic concern for livestock producers. The addition of a C source to the feedlot pen surface, like straw, woodchips, biochar and varying other organic matter's, has shown potential in reducing N loss from manure. As an industry, there is an opportunity to reduce our overall imprint on gaseous emissions. Reducing the volatilization of ammonia from livestock manure is an area that should be of current industry focus.

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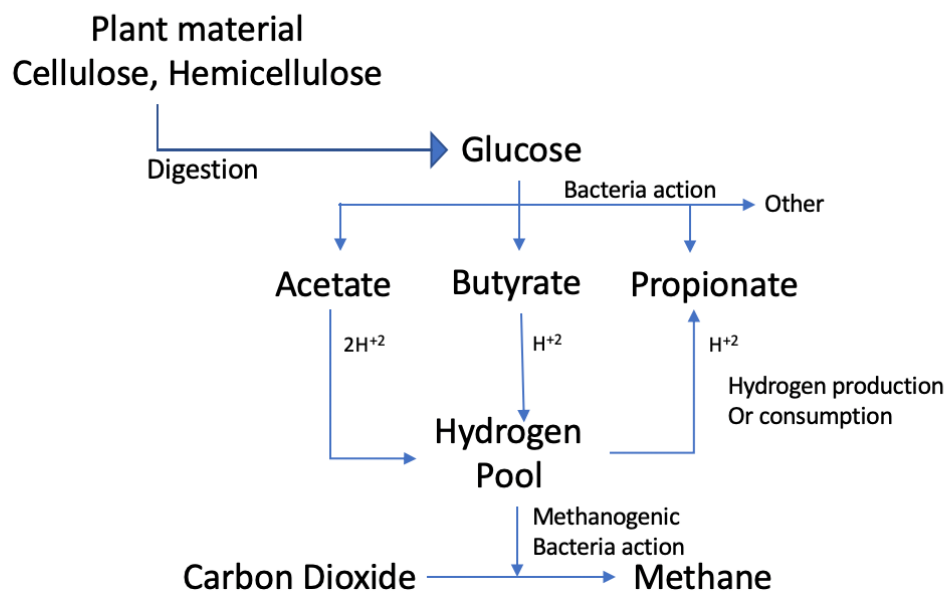


Fig. 1.1. Mechanism of enteric CH_4 production during carbohydrate digestion in the rumen (Adapted from Sejian et al., 2013).

CHAPTER II. Evaluation of the effects of pine-sourced biochar on cattle performance and methane and carbon dioxide production from growing and finishing steers

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Abstract

A feedlot growing (77-d) and finishing (111-d) experiment was conducted to evaluate the effects of feeding biochar on steer performance, methane and carbon dioxide emissions, and carcass characteristics. Two treatments were evaluated, a control diet without biochar and the same diet with biochar included at 0.8% of dietary DM (growing) or 1.0% of dietary DM (finishing). The growing diet consisted of 40% corn silage, 40% wheat straw, 15% modified distillers grains plus solubles, and 5% supplement, with 0.8% biochar replacing fine ground corn in supplement. The finishing diet consisted of 55% high-moisture corn, 35% Sweet Bran, 5% wheat straw, and 5% supplement, with biochar replacing 1.0% HMC and added as an ingredient. Biochar was sourced from ponderosa pine wood waste (High Plains Biochar, Laramie, WY) and was 83% C with 426 m²/g surface area for both studies. Crossbred steers were utilized in the growing (n = 160; initial BW = 363 kg; SD = 16 kg) and finishing (n = 128; initial BW = 480 kg; SD = 17 kg) experiments, blocked by BW, and assigned randomly to pens. Pens were assigned randomly to one of two treatments (biochar vs. control) with eight replications per treatment. Four pen replications per treatment were paired within BW block and rotated randomly through an emissions barn with two chambers (each treatment was evaluated simultaneously and for two rotations) to capture average weekly emissions of CH₄ and

CO₂. Pen was the experimental unit and chamber was included as a fixed effect for emissions data. There were no statistical differences in performance outcomes between treatments for the growing experiment ($P \geq 0.23$). Dry matter intake (DMI; $P < 0.01$) and average daily gain (ADG; $P = 0.02$) were 2.2 and 5.9% lower for biochar-fed steers in the finishing experiment, respectively, resulting in a lighter hot carcass weight ($P = 0.10$) and lower calculated USDA yield grade ($P = 0.02$). Emissions of CH₄ and CO₂ were not affected by biochar inclusion in the growing ($P \geq 0.22$) or finishing experiment ($P \geq 0.60$). Results from these studies show no indication that feeding biochar, supplemented at 0.8% (growing) and 1.0% (finishing) of diet, reduces methane emissions in growing or finishing cattle.

Keywords: beef cattle, biochar, methane

Introduction

Methane (CH₄) emissions have been of growing environmental concern over the last few decades based on their contribution to climate change. Methane is emitted to the atmosphere via natural sources, such as wetlands and enteric fermentation from wildlife, and by human activities, including the petroleum and natural gas sectors and enteric fermentation from domesticated ruminant animals (NASEM, 2016). Although modern beef production resulted in a 16% decrease in carbon footprint per unit of beef compared to the 1970s (Capper, 2011), the beef industry has been further challenged to lower its contribution to greenhouse gas (GHG) emissions. The rumen serves as a fermentation vat equipped with various microbial populations allowing cattle to digest and convert plant product such as cellulose into high-quality proteins like meat and milk (Layman, 2018). Enteric CH₄ production is critical in anaerobic fermentation and ruminal H₂ recycling

(Sharp et al., 1998), but does represent an energetic loss for the animal ranging from 2 to 12% of gross energy intake (GEI) depending on diet (Johnson and Johnson, 1995).

One proposed method to reduce CH₄ production in cattle is by feeding a product called biochar. Biochar is produced by burning organic matter at high temperatures in the absence of oxygen (Hansen et al., 2012), resulting in a carbonized charcoal product.

When included in the ruminant diet, there are several theories on the mode of action of biochar for affecting methane production, which stem from the porous nature and large surface area of the product. Biochar may adsorb CH₄ gas in the rumen, may increase the inert surface area in the rumen impacting the microbial community, or may alter the rumen microbial population (Leng et al., 2013, 2014; Saleem et al., 2018). When biochar was included *in vitro* in high-forage diets, such as cassava root (Leng et al., 2012b) and barley silage (Saleem et al., 2018), a reduction in CH₄ production was observed.

However, when biochar was included *in vitro* in a combined oaten pasture, maize silage, and concentrate diet, no difference in CH₄ production was observed (Teoh et al., 2019).

Previous literature evaluating the effect of feeding biochar on enteric CH₄ production *in vivo* has mixed results. Leng et al. (2012a) reported a 24% reduction in CH₄ (ppm) production from cattle fed a basal diet of cassava root chips and foliage supplemented with 0.62% biochar produced from rice husks. Winders et al. (2019) included pine-sourced biochar at 0, 0.8 and 3% of dietary DM in a growing and finishing experiment, reporting numerical reductions in CH₄ production of 9.5 and 18.4% (g/kg DMI) at the 0.8% inclusion rate in the growing and finishing trials, respectively. Biochar utilized in Winders et al. (2019) had a C content of 85%, bulk density of 88.10 kg/m³, and surface area of 323 m²/g. Contradictory to the findings of Leng et al. (2012a) and Winders et al.

(2019), Terry et al. (2019) reported no effect of pine-sourced biochar supplemented at 0, 0.5, 1.0, and 2.0% dietary DM with a basal diet of 60% barley silage and 35% barley grain on CH₄ production (g/kg DMI). There are a broad variety of biochars available, and the characterization of the product can differ significantly (McFarlane et al., 2017) based on organic matter source, burning or processing method, management, and transport. The variability between biochars utilized throughout the literature may be a factor in the mixed results as well as differences in basal diet fed and cattle type. The objectives of the following experiments were to determine the effects of wood-sourced biochar on cattle performance, carcass characteristics, CH₄ and CO₂ emissions from growing and finishing beef steers.

Materials and Methods

All procedures and animal management practices were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee (IACUC # 1785). Biochar is not currently approved by the U. S. Food and Drug Administration (FDA) to be fed to cattle intended for human consumption. Prior to experiment initiation, a food use authorization from the FDA was obtained for cattle utilized in this experiment to enter the human food chain.

Growing Experiment

A 77-d feedlot growing experiment was conducted at the University of Nebraska-Lincoln (UNL) Eastern Nebraska Research, Education, and Extension Center (ENREEC) near Mead, NE, to evaluate the impact of biochar inclusion in a forage-based diet on performance and CH₄ emissions from cattle. Biochar utilized for both the growing and finishing experiments was provided by High Plains Biochar LLC (Laramie, WY) and was sourced from forest wood waste, primarily ponderosa pine trees. Biochar was processed

to a small particle size to reduce opportunity of cattle sorting in the bunk. Six randomized grab samples of biochar collected through the growing and finishing periods were sent to Control Laboratories (Watsonville, CA) for chemical analysis. Dry matter of the biochar fluctuated with ambient moisture from 57 to 76% DM with an average of 70%. On a DM basis, biochar carbon content was 82.8%, with a surface area of 426 m²/g, bulk density of 107.8 kg/m³, total N content of 0.7% of total dry mass, and pH of 9.49. Biochar particle size distribution ranged from less than 0.5 mm to 8 mm, with approximately 66% of sampled biochar measuring less than 2 mm and 1% of sampled biochar measuring greater than 4 mm.

Steers (n=160; initial BW = 363 kg; SD = 16 kg) were assigned to two treatments (Table 2.1); a negative control growing diet (no biochar inclusion) and a growing diet with 0.8% biochar inclusion. Biochar was included at 0.8% in the growing experiment based on results from Winders et al. (2019), who demonstrated that biochar included at 0.8% of the dietary DM had the greatest reduction in CH₄ emissions when compared to a 3.0% inclusion. Diets fed were identical between treatments other than the biochar inclusion, which replaced fine ground corn in the supplement. Biochar was weighed and mixed into the feed truck as an ingredient each day. Steers were stratified into 3 BW blocks, 3 reps in the light block, 4 reps in the middle block, 1 rep in the heavy block, and assigned randomly to pen (10 steers/pen). Pens were assigned randomly to treatment (n = 16). Prior to initiation of the growing experiment, steers were individually identified and processed upon arrival to the ENREEC research feedlot. Steers were administered a modified live vaccine for prevention of infectious bovine rhinotracheitis, bovine viral diarrhea, parainfluenza 3, bovine respiratory syncytial virus, mannheimia haemolytica,

and *Pasteurella multocida* (Vista, Merck Animal Health, Summit, NJ), a killed vaccine for clostridial toxoids and *Histophilus somni* (Ultrabac 7/Somubac, Zoetis Inc, Kalamazoo, MI), and an injectable solution for the treatment and control of gastrointestinal roundworms, lungworms, eyeworms, lice, and mites (Dectomax, Zoetis Inc., Kalamazoo, MI). Before experiment initiation, steers were limit-fed a common diet of 50% alfalfa hay and 50% Sweet Bran (Cargill, Blair, NE) offered at 2% of BW for five days to equalize gut fill (Watson et al., 2013). Steers were weighed in the morning before feeding on days 0 and 1 of the experiment and weights were averaged to establish initial BW. Steers were implanted with 80 mg trenbolone acetate and 16 mg estradiol (Revalor-IS; Merck Animal Health, Summit, NJ) on day 1 of the experiment.

Finishing Experiment

A 111-d feedlot finishing experiment was conducted immediately following the growing experiment, utilizing the same group of steers. Steers (n=128; initial BW=480 kg; SD=17 kg) remained in the same treatment groups as the growing experiment, and 2 steers (lightest BW) were removed from each pen to better accommodate chamber space in the emission barn, reducing pen count from 10 to 8 steers per pen. Two treatments were evaluated in the finishing experiment (Table 2.2); a negative control finishing diet (no biochar inclusion) and finishing diet with 1.0% biochar inclusion, which replaced 1.0% high-moisture corn (HMC) in the ration. Diets were identical other than biochar inclusion, and contained wheat straw, HMC, and Sweet Bran (Cargill, Blair, NE). Biochar was weighed and mixed into the feed truck as an ingredient each day. Steers were limit-fed a common diet of 50% alfalfa hay and 50% Sweet Bran offered at 2% of BW for 5 d to equalize gut fill. Steers were weighed in the morning before feeding on

days 0 and 1 of the finishing experiment and weights were averaged to establish initial BW. Steers were implanted with 200 mg trenbolone acetate and 20 mg estradiol (Revalor-200; Merck Animal Health, Summit, NJ) on d 1 of experiment.

Feed was delivered to pens once daily at approximately 0800 h, aiming for trace amounts of feed in the bunk during time of feeding. Weekly grab samples of dietary ingredients were completed for determination of DM and as-fed proportions of ration ingredients were adjusted weekly if required. Weekly feed samples were composited by month and composites were analyzed for DM, OM, crude protein (CP), and neutral detergent fiber (NDF) content. Cattle were adapted to the finishing diet in 4 steps over 21 d. Step 1 diets were fed from d 1 to 5 and contained (DM-basis) 35% Sweet Bran, 31% wheat straw, 29% HMC, and 5% supplement. Step 2 diets were fed from d 6 to 11 and contained 35% Sweet Bran, 24% wheat straw, 36% HMC, and 5% supplement. Step 3 diets were fed from d 12 to 16 and contained 35% Sweet Bran, 17% wheat straw, 43% HMC, and 5% supplement. Step 4 diets were fed from d 17 to 21 and contained 35% Sweet Bran, 10% wheat straw, 50% HMC, and 5% supplement. Biochar at 1.0% of the dietary DM replaced HMC in each of the above steps for the biochar treatment.

Steers were harvested at a commercial abattoir (Greater Omaha, Omaha, NE) at experiment completion. On the day of shipping, pens were offered 50% of the previous day's feed offering at regular time of feeding. Cattle were loaded and shipped to the abattoir in the afternoon for slaughter the next morning. Hot carcass weights (HCW) were recorded on day of slaughter and USDA marbling scores, 12th rib fat thickness, and LM area were recorded after a 48-hr chill. Calculated yield grade was determined using the following equation (USDA, 2016): $2.50 + (0.98425 \times 12\text{th rib fat, cm}) + (0.2 \times 2.5 \text{ KPH}$,

$\%$) + $0.00837 \times \text{HCW, kg}$) – ($0.0496 \times \text{LM area, cm}^2$), where KPH fat was assumed to average 2.5%. Carcass adjusted final BW was calculated from HCW divided by a common dressing percentage of 63%.

Gas Emissions

The UNL ENREC emission barn, equipped with a negative pressure system to monitor and record CH₄ and CO₂ production, was utilized for 8 consecutive weeks to monitor emissions from growing steers, followed by an additional 8 consecutive weeks to monitor emissions from finishing steers. The emission barn, as described by Winders et al. (2020), has 2 isolated pens (no emission cross-over) and operates using two air sensors, the LI-COR 7500 and LI-COR 7700 (LI-COR, Lincoln, NE) to monitor CO₂ and CH₄, respectively. Eight pens of cattle, 4 control and 4 biochar, were randomly selected to rotate through the methane barn by pairing replications within BW block, representing 1, 2, and 1 rep from light, middle, and heavy block, respectively. Pairings were rotated through the barn for two 5-d periods, with each treatment represented in the barn concurrently. Each week, steers entered the barn Wednesday morning and remained in the barn until Monday morning when they were returned to their respective feedlot pen. Manure CO₂ and CH₄ emissions were measured from the accumulation of 5 d of manure buildup and was calculated for the remainder of Monday when cattle were absent from barn. The barns were scraped clean using a skid steer each Tuesday to develop a baseline emission level post manure removal. Baseline emission levels of CO₂ and CH₄ were subtracted from manure emission levels of CO₂ and CH₄ and final values were divided over 5 days and 10 steers (growing experiment) or 8 steers (finishing experiment), to account for individual animal emissions. Following these steps, average CO₂ and CH₄

values of 16.89 g per steer and 0.08 g per steer, respectively, were subtracted from the daily emissions for CO₂ and CH₄ in the growing trial due to manure emissions, and an average CO₂ and CH₄ values of 17.45 g per steer and 0.07 g per steer, respectively, were subtracted from the daily emissions for CO₂ and CH₄ in the finishing trial.

Statistical Analysis

Performance and emissions data were analyzed using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. Performance data included BW block as a fixed effect. For emissions data in the growing experiment, day was a repeated measure. During the growing trial, 6 days (out of 40 total) were not usable for emissions measurement due to complications with the barn sensor recording.

Concentrations of CO₂ and CH₄ reached greater than 60 ppm at certain points throughout the day, which may be beyond the capacity of the sensor for accurate measurement (Winders et al., 2020). Unexpectedly high concentrations of CO₂ and CH₄ in the growing experiment were due to housing 10 steers per chamber and the high inclusion of low-quality forage in the diet. Emissions data in the finishing experiment utilized chamber as a fixed effect. Due to complications with the CO₂ analyzer, CO₂ emissions were averaged from one replication per treatment for each period. In addition, one replication (week two of finishing experiment barn rotations) had unusable data for CH₄ emissions. Significance was considered at $\alpha \leq 0.05$ and a tendency was considered at $0.05 < \alpha \leq 0.10$.

Results and Discussion

Growing Experiment

There were no statistical differences in performance outcomes between biochar supplemented steers and control ($P \geq 0.23$; Table 2.3). Numerically, average daily gain (ADG) was greater ($P = 0.46$) and dry matter intake (DMI) was lower ($P = 0.23$) for

biochar supplemented steers, resulting in a 2.9% improvement in feed efficiency for biochar treatment ($P = 0.25$). Although 8 replicates were analyzed per treatment, the limitation of studying only two treatments leads to insufficient statistical power, and G:F response should be further evaluated to determine repeatability. Although not significant, the numerical response in DMI was similar to that of previous research analyzing the impact of biochar supplementation in high-forage diets (Winders et al., 2019; Terry et al., 2020). Winders et al. (2019) demonstrated that as biochar inclusion in a high forage diet (21% brome hay, 20% wheat straw, 30% corn silage, 22% WDGS) increased from 0 to 3.0%, DMI numerically decreased. In a backgrounding experiment by Terry et al. (2020) evaluating the inclusion of 0, 0.5, 1.0, or 2.0% enhanced pine biochar in a 60% barley silage and 30% barley grain diet on steer performance, DMI and ADG were numerically the lowest at the highest biochar inclusion level (2.0%). Additionally, Conlin et al. (2021) fed varying pine-sourced biochar inclusions at 0, 1, 2 and 3% of diet DM to multiparous cows fed a high-forage diet (50% alfalfa haylage, 30% wheat straw, 17% corn silage) and found no impact on DMI or ADG between treatments. These results were dissimilar to Leng et al. (2012a), who reported a tendency for *Bos indicus* type cattle fed a high-forage diet supplemented with rice husk biochar to have improved live weight gain. Diet composition (specifically forage quality) and type of cattle may be the reason for the differing results.

Finishing Experiment

Biochar-supplemented steers had a significant decrease in dry matter intake (DMI; $P < 0.01$) and average daily gain (ADG; $P = 0.02$) and tended to have a lighter carcass adjusted final BW ($P = 0.10$) compared to the control (Table 2.4). Feed efficiency

did not statistically differ between the two treatments, however, steers fed biochar had a reduction of 3.2% in feed efficiency compared to control steers ($P = 0.22$). The significant reduction in DMI observed for biochar-fed steers in the finishing experiment is dissimilar to the results in previous literature (Winders et al., 2019; Terry et al., 2020). Winders et al. (2019) looked at the effects of pine-sourced biochar inclusion at 0, 0.8, and 3.0% of dietary DM in a finishing diet (53% dry-rolled corn, 15% corn silage, 15% WDGS) on steer performance, observing the greatest numerical DMI (kg/d) for steers supplemented biochar at the 0.8% level. Terry et al. (2020) demonstrated that supplementing enhanced pine biochar at 0, 0.5, 1.0 or 2.0% in a 85% barley grain and 10% barley silage finishing diet did not significantly impact DMI, and although intake was lowest (kg/d) for the 2.0% biochar inclusion, intake was the greatest for 0.5% biochar inclusion.

The reduction in ADG for biochar-supplemented steers in the finishing experiment are congruent with Terry et al. (2020) who reported a numerical decrease in overall ADG and total weight gain when enhanced pine biochar was included at 2.0% of a high-grain diet. This reduction in gain may be attributed to the replacement of 1.0% HMC in the current finishing experiment and 2.0% of the TMR in Terry et al. (2020), as biochar has been described as largely indigestible within the rumen (Terry et al., 2019).

Biochar-fed steers tended to be lighter in hot carcass weight (HCW; $P = 0.10$) with numerically reduced 12th rib fat ($P = 0.12$), resulting in a significantly lower USDA calculated yield grade ($P = 0.02$) compared to the control. Reduced HCW and improved yield were a function of the significant reduction in DMI and ADG for biochar-fed steers compared to the controls. A Canadian experiment by Terry et al. (2020) reported no

significant difference in HCW between steers with or without biochar supplemented in a high-grain diet; however, the numerical trend of their data showed a reduction in HCW as biochar inclusion in the diet increased, where steers fed biochar at 2.0% of dietary DM were approximately 10 kg lighter than control steers. The Canadian experiment also reported a significant reduction in USDA yield grade for cattle fed biochar at 2.0% of diet, which may be influenced by the lighter HCW of steers supplemented with biochar. Results from the finishing experiment showed no difference in LM area or marbling ($P \geq 0.93$) which was congruent with Terry et al. (2020).

Production of CH₄ and CO₂

Gas emissions of CH₄ and CO₂ were reported as g/steer and g/kg of DMI, where reported DMI (kg/d) used for the gas emission calculations were averaged from the weekly intakes of each treatment during rotation through the respective emission chambers. Emissions of CH₄ and CO₂ did not statistically differ between steers fed biochar and control treatments for the growing experiment ($P \geq 0.24$; Table 2.3) or the finishing experiment ($P \geq 0.60$; Table 2.4). In the growing experiment, CO₂ and CH₄ emissions were numerically lower for control steers compared to biochar-supplemented steers when reported as g per day (4.0% lower) or g per kg of DMI (2.8% lower). Based on results from this experiment, there was no indication that feeding biochar reduces CH₄ emissions in growing steers, especially when considering biochar-fed steers had numerically lower DMI in the growing experiment and significantly lower DMI in the finishing experiment.

Consistent with previous literature (Beauchemin and McGinn, 2006; Winders et al., 2019), CH₄ production (reported as g/steer and g/kg of DMI) was greater in the

growing experiment compared to the finishing experiment due to differences in diet composition and quality. Beauchemin and McGinn (2006) estimated losses due to enteric CH₄ production as around 6% of gross energy intake (GEI) for forage-fed cattle and 3.5% for concentrate-fed cattle. Leng et al. (2012a) reported a 24% reduction in CH₄ (ppm) production from cattle native to Southeast Asia when supplemented rice husk biochar at 0.62% of a high-forage (cassava root chip) diet. Winders et al. (2019) reported numerical reductions of 9.5 and 18.4% CH₄ production (g/kg DMI) with pine-sourced biochar inclusion of 0.8% in high-forage and high-concentrate diets, respectively. Although the reductions in CH₄ production from feeding biochar reported by Winders et al. (2019) were numerical differences, the limitation of statistical power with headbox trials encouraged us to further expand on the implications of feeding biochar in a pen setting.

The mechanism by which dietary biochar inclusion reduces enteric CH₄ production is based on one or more of the following theories: biochar may adsorb ruminal CH₄ gas, biochar may increase the inert surface area of the rumen resulting in greater opportunity for microbial colonization, and biochar may alter the rumen microbial community (Leng, 2014; Leng et al., 2013; Leng et al., 2012a). The mechanism by which biochar may adsorb CH₄ in the rumen is no longer the leading theory accepted in literature, considering that previous studies reporting reductions in enteric CH₄ production included biochar at < 1.0% dietary DM (Winders et al., 2019; Leng et al., 2012a), which is seemingly not an adequate quantity of biochar to adsorb the volume of gas. The porous nature and large surface area of processed biochar supports the theory by which biochar increases the opportunity for microbial colonization, potentially resulting in an alteration of the rumen microbial community. The increase in surface area provided

by dietary biochar may provide a functional site for microbial biofilm formation within the rumen (Leng, 2014). Improved biofilm formation may support improved efficiency of microbial growth and proliferation and, therefore, increased feed degradation (Leng, 2014; Leng et al., 2012a).

Dissimilar to Leng et al. (2012a), Conlin et al. (2021) fed multiparous cows a high-forage diet with pine-sourced biochar inclusions at 0, 1, 2, and 3% of diet, and reported no impact on CH₄ emissions. Biochar utilized in Conlin et al. (2021) was of similar characterization to the biochar utilized in the present experiment, with a carbon content (as % of dry mass) of 83.6%, surface area of 456 m²/g, bulk density of 78.5 kg/m³, and pH of 10.5. Type of diet, physical properties of the biochar, and inclusion percentage of biochar in the diet are all potential reasons for differing emission and performance results between studies.

In conclusion, pine-sourced biochar included at 0.8% of diet DM during the growing experiment did not impact cattle performance or CH₄ emissions; however, during the finishing experiment, biochar inclusion at 1.0% diet DM reduced DMI and ADG, resulting in a tendency for reduced HCW and a significant improvement in lean carcass yield grade, with no impact on CH₄ emissions.

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Table 2.1. Composition of diet (DM) fed to steers in growing experiment (77 days on feed)

Ingredient, % of diet DM	Treatments	
	Control	Biochar
Wheat straw	40	40
Corn silage	40	40
MDGS ¹	15	15
Supplement ²		
Finely ground corn	2.188	1.408
Biochar	-	0.800
Limestone	1.310	1.310
Tallow	0.125	0.105
Urea	1.000	1.000
Salt	0.300	0.300
Beef trace mineral	0.050	0.050
Vitamin A-D-E	0.015	0.015
Rumensin-90 ³	0.012	0.012
<i>Nutrient analysis, %</i>		
DM	65.3	65.1
OM	90.3	90.3
Crude Protein	9.7	9.7
Neutral Detergent Fiber	57.5	57.5

¹MDGS = Modified distillers grains plus solubles.

²Supplement fed at 5% of dietary DM.

³Formulated to supply monensin (Rumensin-90; Elanco Animal Health; Greenfield, IN) at 200 mg/steer daily

Table 2.2. Composition of diet (DM) fed to steers in finishing experiment (111 days on feed)

Ingredient, % of diet DM	Treatments	
	Control	Biochar
High-moisture corn	55	54
Sweet Bran ¹	35	35
Wheat straw	5	5
Biochar ²	-	1
Supplement ³		
Finely ground corn	2.879	2.879
Limestone	1.630	1.630
Tallow	0.100	0.100
Salt	0.300	0.300
Beef trace mineral	0.050	0.050
Vitamin A-D-E	0.015	0.015
Rumensin-90 ⁴	0.016	0.016
Tylan-40 ⁵	0.010	0.010
Nutrient analysis, %		
DM	69.3	69.3
OM	88.8	88.8
Crude Protein	13.4	13.4
Neutral Detergent Fiber	24.2	24.2

¹Sweet Bran = branded wet corn gluten feed produced by Cargill (Cargill corn milling, Blair NE).

²Biochar added as an ingredient to the feed truck and replaced high-moisture corn inclusion in the diet

³Supplement fed at 5% of dietary DM.

⁴Monensin (Rumensin; Elanco Animal Health, Indianapolis, IN) targeted to provide 33 mg/kg dietary DM

⁵Tylosin (Tylan; Elanco Animal Health) targeted to provide 90 mg/steer daily

Table 2.3. Effect of pine-sourced biochar addition at 0.8% of dietary DM on performance and gas emissions of growing steers

	Treatments		SEM	<i>P</i> -value
	Control	Biochar		
<i>Performance</i>				
Initial BW, kg	363	363	0.91	0.96
Ending BW, kg	477	479	2.04	0.50
DMI, kg/d	8.57	8.45	0.08	0.23
ADG, kg	1.45	1.47	0.023	0.46
Gain:Feed	0.170	0.175	0.003	0.25
<i>Gas emissions</i>				
DMI, kg/steer ¹	9.6	9.7	0.11	0.52
CH ₄ , g/steer	196.2	203.8	6.62	0.45
CH ₄ , g/kg of DMI	20.5	20.9	0.66	0.60
CO ₂ , g/steer	5725	5982	143.1	0.25
CO ₂ , g/kg of DMI	561.3	581.4	10.8	0.24

¹Dry matter intake (DMI) was used to unitize reported emissions and was averaged from the weekly intakes of each treatment during rotation through the respective emission chambers

Table 2.4. Effects of pine-sourced biochar addition at 1.0% dietary DM on performance, carcass characteristics, and gas emissions of finishing steers

	Treatments		SEM	P-value
	Control	Biochar		
<i>Performance</i>				
Initial BW, kg	479	481	2.08	0.55
Carcass Adjusted Final BW ¹ , kg	667	658	4.00	0.10
DMI, kg/d	13.4	13.1	0.06	<0.01
ADG, kg	1.69	1.59	0.03	0.02
Gain:Feed	0.126	0.122	0.002	0.22
<i>Carcass characteristics</i>				
HCW, kg	420	415	2.5	0.10
LM area, cm ²	95.5	94.8	0.90	0.93
Marbling ²	455	455	10.2	0.97
12 th rib fat ³ , cm	1.55	1.45	0.046	0.12
Calculated yield grade	3.23	3.18	0.041	0.02
<i>Gas emissions</i>				
DMI, kg/steer ⁴	11.8	12.0	0.55	0.59
CH ₄ , g/steer	168.7	165.7	5.60	0.71
CH ₄ , g/kg of DMI	15.0	14.3	0.95	0.60
CO ₂ , g/steer	6282	6173	375	0.87
CO ₂ , g/kg of DMI	589.7	523.6	143	0.80

¹Carcass adjusted final BW was determined from hot carcass weight (HCW) divided by common dressing percentage of 63%.

²Marbling score: 400= small⁰⁰, minimum required for U.S. Low Choice

³12th rib fat, cm: calculated by back-calculating from the USDA YG equation

⁴Dry matter intake (DMI) was used to unitize reported emissions and was averaged from the weekly intakes of each treatment during rotation through the respective emission chambers

CHAPTER III. Evaluation of the effects of wood-sourced biochar as a feedlot pen surface amendment on manure nutrient capture

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Abstract

Two nutrient mass balance experiments were conducted during winter and summer seasons to evaluate the effects of spreading unprocessed Eastern red cedar biochar onto the feedlot pen surface on manure nutrient capture and cattle performance. A 186-d feedlot finishing experiment was conducted from December to June (WINTER) and a subsequent 153-d finishing experiment was conducted from June to November (SUMMER). The WINTER experiment evaluated three treatments, including biochar spread to pen surface during the feeding period (24 kg/steer; 10 steers per pen), hydrated lime spread to pen surface at end of feeding period (31 kg/steer), and control (no treatment applied). The SUMMER experiment evaluated biochar treatment (31 kg/steer; 8 steers per pen) against control. There was no difference in N and P intake, retention, or excretion ($P \geq 0.38$) between WINTER treatments. Steer performance ($P \geq 0.10$) and carcass characteristics ($P \geq 0.50$) were not impacted by pen treatment in WINTER. Nitrogen and P intake and excretion ($P \geq 0.35$) were similar between treatments in SUMMER and retention of N and P was significantly greater for the biochar treatment ($P \leq 0.04$) due to greater ADG ($P = 0.05$). There was no difference in DMI ($P = 0.48$) in SUMMER, steers on biochar pen treatment had heavier HCW ($P = 0.05$) and increased ADG, resulting in improved feed efficiency ($P = 0.08$). In both experiments, biochar

addition to the pen surface tended ($P = 0.07$) to increase manure N as a percent of manure DM, but this increase in N concentration did not impact kg of N removed from the feedlot pens ($P \geq 0.15$) or N losses ($P \geq 0.68$). The addition of red cedar biochar to the feedlot pen surface did not improve manure nutrient capture of N or P and did not reduce N losses associated with soil-based feedlot pens.

Keywords: biochar, feedlot manure, mass balance

Introduction

Typical beef feedlot finishing diets in the U.S. combine high inclusions of concentrate (grains) with a variety of byproducts, crop residues and forages that incorporate high quantities of N, P and soluble salts of which not all are retained by the animal. Of total N offered in the diet, beef cattle retain approximately 12% of fed N and 15% of fed P (Kissinger et al., 2007), with the remainder excreted onto the feedlot pen surface. Once excreted onto the pen surface, the manure nutrients can be 1) volatilized off as ammonia (NH_3) emission (only N); 2) lost as precipitative runoff and captured in a runoff retention pond; or 3) removed in manure during pen cleaning. The opportunity for manure N loss on open dirt feedlot pens is a risk to the environment, public health, and poses a potential economic loss in the value of manure as a fertilizer. The environmental risks include water quality concerns from the deposition of N from manure fertilizer application contributing to eutrophication, air quality degradation due to NH_3 volatilization, and the potential for nitrous oxide (N_2O) formation and its implications as a GHG on climate change (USDA Agricultural Air Quality Task Force, 2014; USEPA, 2004).

Immediately following excretion of urine and feces from the animal, NH_3 formation and volatilization occur rapidly due to abundant urease activity in the feces and soil (Bussink and Oenema, 1998), with manure N losses via volatilization ranging from 43 to 64% of fed N (Homolka et al., 2021). Seasonal differences in manure N loss via NH_3 volatilization occur due to the impacts of temperature and moisture conditions on feedlot pen surface microbial activity and the speed of chemical reactions with greater losses occurring during summer feeding periods (Gilbertson et al., 1971; Homolka et al., 2021; Hristov et al., 2011; Kissinger et al., 2007). Various feedlot management strategies, such as sawdust application (Lory et al., 2002) and increased dietary bran inclusion (Adams et al., 2004), have shown that as OM content is increased on the pen surface, greater N is retained in the manure and N losses via NH_3 volatilization are reduced.

One proposed method of improving manure nutrient capture of N and P is to apply biochar to the feedlot pen surface. Biochar is produced by burning OM (typically forest industry byproducts) at high temperatures in the absence of oxygen (Hansen et al., 2012) resulting in an end-product that is high in C content. When biochar is utilized as a soil amendment, improvements in crop yields and soil fertility (Atkinson, Fitzgerald and Hips, 2010; Ding et al., 2016) and reductions in emissions of N_2O and CH_4 from crop fields have been observed (Cayuela et al., 2014; Karhu et al., 2011). The addition of biochar to various livestock wastes has resulted in reductions of N_2O emission, potentially due to the high surface area and sorptive capacity of biochar, reducing the availability of N for N_2O formation (Agyarko-Mintah et al., 2017; Kammann et al., 2015). Agyarko-Mintah et al. (2017) reported a 65 to 75% reduction in N_2O emissions (mg/kg of compost-biochar mixture) when biochar made from litter and green waste was

added to a compost mixture of poultry litter and straw at 10% of compost DM. Similarly, Li et al. (2016) observed a 54% reduction in N₂O emissions when wheat straw biochar was added to cattle manure and rice straw compost mix at 3% (w/v). In addition to reducing N loss from livestock waste, adding biochar to the feedlot pen surface may also improve pen surface (moisture) conditions (Maharjan and Wilke, 2021).

The objective of these experiments was to evaluate the effects of applying wood-sourced biochar to the feedlot pen surface during winter and summer feeding periods on manure nutrient capture of N and P.

Materials and Methods

All procedures and animal management practices were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee (IACUC # 1785).

Feedlot Performance

Two experiments were conducted at the University of Nebraska-Lincoln (UNL) Eastern Nebraska Research, Extension and Education Center (ENREEC) near Mead, NE, to evaluate the impact of biochar addition to the feedlot pen surface on manure nutrient capture. A 186-d feedlot finishing experiment was conducted from December to June (WINTER) and a subsequent 153-d feedlot finishing experiment was conducted from June to November (SUMMER) of 2020.

In WINTER, crossbred calves (n=150; initial BW=274 kg; SD=7 kg) were assigned to three treatments; negative control, biochar application to pen surface, and hydrated lime (calcium hydroxide) application to pen surface. Unprocessed biochar made from Eastern red cedar trees was applied to the pen surface in equal weights (approximately 123 kg dry matter (DM) per pen) at trial initiation in December and again

in February (total of 246 kg DM per pen). Lime treatment was applied to the pen surface (approximately 308 kg DM per pen) one day prior to shipping cattle for harvest. The application of lime to the feedlot pen surface was in cooperation with UNL Environmental Engineering to determine the impact of lime on microbial activity on the pen surface. The alkaline stabilization properties of lime are hypothesized to reduce antimicrobial resistant bacteria in cattle manure. However, increasing the pH of the pen surface may increase NH_3 volatilization, as the speed of hydrolysis from NH_4 to NH_3 is increased as pH of the pen surface approaches an equilibrium point at pK_a greater than 9.

Pens were assigned randomly to treatments (5 pens/treatment) and steers were assigned randomly to pens (10 steers/pen). The WINTER finishing diet contained 51% high-moisture corn (HMC), 20% Sweet Bran (Cargill Corn Milling, Blair, NE), 15% corn silage, and 10% modified distillers grains, with a mean dietary crude protein (CP) level of 13.7% and dietary P level of 0.45% (Table 3.1).

In SUMMER, crossbred yearlings ($n=80$; initial BW=339 kg; SD=7 kg) were assigned to two treatments; negative control and biochar application to pen surface. Unprocessed biochar was applied to the pen surface in equal volumes (approximately 123 kg DM per pen) at trial initiation in June and again in August (total of 246 kg DM per pen). Pens were assigned to the same treatments (5 pens/treatment) as in the WINTER phase and steers were assigned randomly to pens (8 steers/pen). The SUMMER finishing diet contained 51% HMC, 40% Sweet Bran, and 5% cornstalks, with a mean dietary CP level of 14.5% and dietary P level of 0.53% (Table 3.1).

Prior to initiating each of the experiments (WINTER and SUMMER), all steers were individually identified and processed upon arrival at the ENREEC feedlot. Steers

were administered a modified live vaccine for prevention of infectious bovine rhinotracheitis, bovine viral diarrhea, parainfluenza 3, bovine respiratory syncytial virus, mannheimia haemolytica, and pasteurella multocida (Vista, Merck Animal Health, Summit, NJ), a killed vaccine for clostridial toxoids and *Histophilus somni* (Ultrabac 7/Somubac, Zoetis Inc, Kalamazoo, MI), and an injectable solution for the treatment and control of gastrointestinal roundworms, lungworms, eyeworms, lice, and mites (Dectomax, Zoetis Inc., Kalamazoo, MI). Steers were limit-fed a common diet of 50% alfalfa hay and 50% Sweet Bran offered at 2% of BW for five days to equalize gut fill (Watson et al., 2013). Steers were weighed in the morning before feeding on days 0 and 1 of the experiments and weights were averaged to establish initial BW. Feed was delivered to pens once daily at approximately 0800 h, aiming for trace amounts of feed in the bunk during time of feeding. Weekly grab samples of dietary ingredients were collected for determination of DM and as-fed proportions of ration ingredients were adjusted weekly as required. Weekly feed samples were composited by month and composites were sent to Ward Laboratories LLC (Kearney, NE) for nutritional and chemical analysis. On all samples, total N was determined using a combustion method N analyzer (AOCAC International, 1999: method 4.2.04). Ashing and digestion (AOAC, 1990: method 648.08) of samples, followed by colorimetric analysis using the molybdovanadate method (AOAC, 1990: method 965.17) on a spectrophotometer (Molecular Devices SpectraMAX 250; 400nm) was used to determine total P content.

Steers were implanted with 80 mg trenbolone acetate and 16 mg estradiol (Revalor-IS; Merck Animal Health, Summit, NJ) on d 1 of the experiments and reimplanted with 200 mg trenbolone acetate and 20 mg estradiol (Revalor-200; Merck

Animal Health, Summit, NJ) on d 76 and d 68 for WINTER and SUMMER, respectively. Steers were harvested at a commercial abattoir (Greater Omaha, Omaha, NE) upon completion of the WINTER and SUMMER feeding periods. On the day of shipping, cattle were offered 50% of the previous day's offering at the regular time of feeding. Cattle were loaded and shipped to the abattoir in the afternoon for slaughter the next morning. Hot carcass weights (HCW) were recorded on the day of slaughter and USDA marbling scores, 12th rib fat thickness, and LM area were recorded after a 48- and 72-h chill for WINTER and SUMMER, respectively. Carcass chill for SUMMER included an additional 24-h compared to WINTER because of plant shutdown during the [USA] Thanksgiving holiday. Calculated yield grade was determined using the following equation (USDA, 2016): $2.50 + (0.98425 \times 12\text{th rib fat, cm}) + (0.2 \times \text{KPH, \%}) + 0.00837 \times \text{HCW, kg} - (0.0496 \times \text{LM area, cm}^2)$, where KPH fat was assumed to average 2.5%. Performance traits including final body weight (BW), average daily gain (ADG), and Gain:Feed (G:F) were calculated based on HCW adjusted to a common dressing percentage of 63.

Nutrient Mass Balance

Biochar utilized for the WINTER and SUMMER experiments was provided by Sawle Mill (Springview, NE), and was sourced from Eastern red cedar trees. Dry matter of the biochar fluctuated with moisture in the air from 85% to 95% DM with an average of 90%. Biochar samples were collected on both application dates for WINTER and SUMMER experiments and were sent to Control Laboratories (Watsonville, CA) for chemical analysis. On a DM basis, carbon (C) content of the biochar was 80.3%, with a surface area of 233 m²/g, bulk density of 155.4 kg/m³, total N content of 0.6% of DM

mass, and pH of 6.3. Biochar particle size distribution ranged from 0.5-mm to 50-mm, with approximately 70% of biochar sampled sizing >8-mm. Unprocessed biochar was utilized in both WINTER and SUMMER, expecting that cattle hoof action would reduce particle size.

The nutrient mass balance experiments were conducted similar to experiments described by Bierman et al. (1999), Erickson and Klopfenstein (2001a), and Luebke et al. (2012) on open dirt feedlot pens. Pens utilized in both WINTER and SUMMER experiments had a soil-based surface area of 176 m² and total pen surface area of 262 m², with a pen slope of 3%, and feed bunk space of 17.6 and 22 cm per steer in WINTER and SUMMER, respectively.

Twelve soil core samples (15-cm depth) were taken from each pen at the start (before cattle entered pens) and end of each experiment to correct for any change in soil nutrient concentration and to determine pen cleaning equivalence. Soil cores were collected by dividing the pens into 12 grids and collecting one core sample per grid to represent pen average. Once cattle were removed from pens on day 186 (WINTER) and 153 (SUMMER) for slaughter, the pen surfaces were cleaned (< 24 h) in replication across treatments with a box scraper to remove waste material with minimal soil removal, and a skid steer to scrape the concrete apron and pile manure. The manure pile was mixed using the skid steer and during loadout from the concrete apron, 2 separate sets of manure samples were collected for nutrient analysis (n = 20 samples per pen) and DM determination (n = 10). Manure trucks were weighed to determine the weight of manure removed from each individual pen before transferring it to a storage lot.

All manure samples were frozen at -4°C to conserve N until either being oven-dried for DM analysis or freeze-dried for nutrient analysis. Manure samples (analyzed in duplicate) were oven-dried at 60°C for 48 h (AOAC International, 1999; method 4.2.03) to determine DM content and, subsequently, DM removal from each pen. Pen soil core samples and manure samples collected for nutrient analysis were freeze-dried, ground to pass through a 1-mm screen (Wiley Mill), composited by pen (2 composites per pen for manure), and sent to a commercial laboratory (Ward Laboratories LLC, Kearney, NE) for analysis of N, P, K, and S contents (outlined by Homolka et al., 2021) utilizing the same procedures described for diet ingredients.

Statistical Analysis

Cattle performance, carcass characteristics, and nutrient mass balance data were analyzed using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC) with pen as the experimental unit for both WINTER and SUMMER experiments.

Nutrient mass balance data were calculated using the methods outlined by Luebke et al. (2012) and Homolka et al. (2021). Nutrient intake was determined based upon monthly feed ingredient composites and feed delivery and refusals on a pen basis. Nutrient retention and excretion were calculated utilizing methods established by NASEM (2016) and ASABE (2014). The N and P retained by the animal were calculated utilizing energy, protein, and P retention equations (NASEM, 2016). Nutrient excretion was then calculated by subtracting nutrient retention from nutrient intake. Runoff was not measured in this experiment, and generally only accounts for 1.5 to 2.5% of fed N loss and 4 to 6% of fed P loss from an open dirt lot (Homolka et al., 2021). Total nutrient loss (kg/steer) was calculated by subtracting recovered manure nutrients (corrected for soil

cores) from excreted nutrients. Significance was considered at $\alpha \leq 0.05$ and a tendency was considered at $0.05 < \alpha \leq 0.10$.

Results and Discussion

Cattle Performance

There were no significant differences in dry matter intake (DMI; $P = 0.10$), average daily gain (ADG; $P = 0.50$) or Gain:Feed ($P = 0.98$) due to pen treatment in WINTER (Table 3.2). Carcass characteristics were not impacted by pen treatments for cattle in WINTER ($P \geq 0.50$). There was a significant increase in carcass-adjusted final BW ($P = 0.05$) and ADG ($P = 0.05$) for steers in biochar-amended pens in SUMMER (Table 3.3) compared to control, and no difference between treatments for DMI ($P = 0.48$). This improvement in gain tended to improve feed efficiency ($P = 0.08$) for steers in biochar-treated pens compared to control and resulted in significantly heavier HCW ($P = 0.05$) for biochar treatment. Results from SUMMER showed no difference in other USDA carcass parameters, including LM area, marbling, 12th rib fat, or yield grade ($P \geq 0.76$).

The significant increase in ADG and final BW for SUMMER steers on biochar-amended pens may have been influenced by the moisture content of the pen surface (Maharjan and Wilke, 2021); however, pen surface moisture across time was not measured in this experiment. Maharjan and Wilke (2021) spread coal char (30% C) sourced from a sugar factory on feedlot pens in Nebraska at a rate of 625 kg per steer. Results from soil moisture sensors fixed on the pen surface indicated that following a series of snowfall events, the char amended pens were significantly drier than the controls. The biological and chemical properties of wood-sourced biochar may absorb water (Zhang, Chen, and You, 2016), thereby reducing the impact of moisture on the pen

surface and lessening the negative impacts of mud. The volume of biochar added to the pen surface per steer was greater in the SUMMER (31 kg) compared to WINTER (25 kg) experiment (because of fewer steers per pen in SUMMER), and the SUMMER feeding period had greater precipitation compared to WINTER (Table 3.4). In addition, biochar in the SUMMER experiment was spread at experiment initiation in June and again in August, with precipitation for the month of July reaching levels that were over double that of the 25-y average of annual precipitation. These factors suggest that the increased volume of biochar and the timing of application (during higher-than-average precipitation conditions) may have reduced the negative implications that moisture causes on the feedlot pen surface. Muddy feedlot pens contribute to poor animal performance and increased labor for feedyard personnel, ultimately increasing cost of gain (Mader, 2011). The 25-yr average of annual precipitation near Mead, NE, equates to approximately 76 cm per year (WRCC, 2021; Table 3.4). Grandin (2016) suggests that controlling mud in open-lot pens becomes increasingly difficult when precipitation is greater than 51 cm per year. On average, steers on the biochar-amended pens in SUMMER were 17 kg heavier in carcass-adjusted final BW compared to controls, suggesting that the addition of biochar to the pen surface indirectly benefited steer performance.

Nutrient Mass Balance

In the WINTER experiment (Table 3.5), N intake, retention, and excretion were similar between treatments ($P \geq 0.42$). The concentration of N in manure tended to differ between treatments ($P = 0.07$), with biochar-amended pens having the greatest manure N as a percent of manure DM. The manure N concentration as a percent of OM was the greatest for the control pens, and lowest for the lime-amended pens, with biochar-

amended pens as an intermediate ($P < 0.01$) suggesting the change in manure N concentration on a DM basis is related to soil contamination in as-removed manure from pens. Less soil was removed from biochar-amended pens based on ash/OM concentrations resulting in greater manure N concentration as a % of manure DM due to less soil removed from the pen surface. The concentration of N as a % of OM being greatest for control pens suggests the change in N concentration as % of manure DM is not due to capturing more N due to biochar. In WINTER, P intake, retention, and excretion were all similar between treatments ($P \geq 0.38$) and there was no difference between treatments in concentration of manure P ($P = 0.23$) as a percent of manure DM. Manure nutrient amounts (with correction for soil) were numerically greatest in the lime-amended pens and lowest in the biochar-amended pens for N ($P = 0.15$) and P ($P = 0.75$). Manure nutrient losses were similar for all treatments and averaged 54% loss of N ($P = 0.37$) and 0.43% loss of P ($P = 0.87$). The lime treatment had the highest numerical retention of N in the manure ($P = 0.15$), which was not expected based on the relationship between pH and NH_3 volatilization and the high alkalinity of calcium hydroxide. The pH of the pen surface influences the speed of NH_3 volatilization, where the ideal pH conditions for rapid volatilization are neutral (pH 7) to basic (pH 10; Hartung and Phillips et al., 1994). When surface pH drops below 6.5, research has shown that little NH_3 volatilization will occur, due to the pKa of ammonium (NH_4) being less volatile than NH_3 (Rhoades et al., 2010). Conversely, the application of hydrated lime to the pen surface may have reduced the pen surface absorption of radiant energy resulting in a cooler surface, thereby reducing NH_3 volatilization, however, the lime treatment was only applied to the pen surface on the final day of the experiment.

The quantity of DM removed from the pen surface in WINTER was numerically lowest for the biochar treatment ($P = 0.17$), which may have been influenced by the abnormally dry pen conditions at the conclusion of the WINTER experiment. Oven-dried manure samples averaged 92, 91, and 92% DM content for control, biochar, and lime, respectively, which was drier than expected for WINTER feeding periods, averaging around 64% in previous literature (Homolka et al., 2021).

In the SUMMER experiment (Table 3.6), N intake and excretion were similar between treatments ($P \geq 0.35$) and steers in biochar-amended pens had significantly greater N retention compared to the control ($P = 0.04$). The intake and excretion of P was similar between treatments ($P \geq 0.35$), and P retention was significantly greater for the biochar treatment compared to control ($P = 0.03$). Steers fed in biochar-treated pens had significantly greater ADG ($P = 0.05$), and final BW ($P = 0.05$), resulting in greater N and P retention compared to control steers. Manure N concentration as a percent of manure DM tended to be greatest for biochar treatment ($P = 0.07$) with no difference in manure P concentration as a percent of manure DM ($P = 0.36$). The increase in manure N as a % of DM was a reflection of removing more OM (originating feces and urine from cattle) and less ash (soil). When corrected for OM, the manure N concentration as a percent of OM tended to be the greater for the control treatment ($P = 0.09$). Manure nutrient losses were similar for biochar and control pens with 71% of excreted N (26.3 kg/steer; $P \geq 0.79$) and 10% of excreted P (0.85 kg/steer; $P = 0.88$) lost during the SUMMER experiment. Loss of 71% of excreted N is consistent with a 15-study analysis from 1999 to 2015 measured at the same location as these experiments, reporting an average of 73% loss of excreted N in SUMMER feeding periods (Homolka et al., 2021).

Oven-dried manure samples averaged 55 and 56% DM content for control and biochar, respectively, suggesting that the feedlot pen surfaces in SUMMER were considerably wetter than the 15-study average of 70% DM reported by Homolka et al., 2021. The quantity of manure DM removed from the pen surface in SUMMER tended to be less for the biochar amended treatment ($P = 0.08$). The percent of ash removed in the manure tended to be less for the biochar-amended pens compared to the control ($P = 0.06$), indicating that less soil was removed from the pen surface in biochar-amended pens. Due to the wet conditions during feedlot pen cleaning, the manure-soil interface may have been difficult for the operator to identify.

It was hypothesized that biochar addition to the feedlot pen surface would improve manure N capture because of the high C content (80.3% C on DM basis) of the biochar utilized in this experiment. In both WINTER and SUMMER, biochar addition to the pen surface tended to increase manure N as a percent of manure DM ($P = 0.07$) and manure OM content ($P \leq 0.09$), but this increase in N concentration did not translate into increased kg of N or P removed from the biochar-amended pens, because there was less manure DM removed from these pens. Results from these experiments were incongruent with previous literature regarding various feedlot management strategies aiming to increase C content of the pen surface to reduce nutrient loss. Lory et al. (2002) looked at the impact of sawdust application to the feedlot pen surface on N losses in winter and summer feeding phases, concluding that application of a product high in C to the pen surface (such as sawdust) reduced N losses during summer months. Adams et al. (2004) utilized sawdust as an OM addition to the pen surface, comparing it to a treatment where cattle had a dietary inclusion of 30% greater corn bran in the diet, and a negative control

treatment (no sawdust or dietary bran addition). The higher inclusion of bran in the diet was designed to decrease diet digestibility, thereby increasing OM excretion onto the pen surface. Adams et al. (2004) found that adding OM to the pen surface, either as a pen amendment (sawdust) or by increasing OM excretion, increased manure N content by 20% compared to the control during winter/spring months. Bierman et al. (1999) and Erickson and Klopfenstein (2001b) reported similar results to Adams et al. (2004), where feeding less-digestible diets resulted in increased OM excretion on the pen surface and improved manure nutrient capture.

As expected, manure N recovery in the WINTER experiment was greater than the SUMMER experiment, averaging 54 and 71% loss of excreted N across treatments in the WINTER and SUMMER feeding experiments, respectively. These observations are consistent with Homolka et al. (2021) who reported losses of 50 and 73% of excreted N for winter and summer feeding periods, respectively. The greater N loss observed in summer feeding experiments compared to winter is well defined in literature, where warmer temperatures ($>21^{\circ}\text{C}$) increase the speed of hydrolysis of urea, equating to faster rates of NH_3 volatilization (Dari, Rogers, and Walsh, 2019). Kissinger et al. (2007) summarized the manure characteristics of 15 open-lot pens of cattle ($n = 6,366$) sourced from Nebraska feedlots over the course of a 1-yr feeding period, reporting N losses from volatilization and runoff to be 53 and 67% of fed N for winter and summer, respectively. In a Texas feedlot experiment conducted by Todd et al. (2008), N loss from manure via NH_3 volatilization was 68% in the summer months and 36% in the winter months. The quantity of manure P collected in SUMMER, 48 g/steer daily, and WINTER, 36 g/steer daily, were substantially greater than values reported by Homolka et al. (2021), who

observed 17 g/steer daily in summer and 28 g/steer daily in winter. Kissinger et al. (2007) reported an average of 26 and 37 g/steer daily in summer and winter feeding periods, respectively, from 6 commercial feedlots. Of the 15 experiments that Homolka et al., 2021 reported, the average dietary P concentration was 0.36%, with the maximum dietary P concentration at 0.49% of dietary DM. The diets fed in SUMMER and WINTER had a P concentration of 0.53 and 0.45% of dietary DM, respectively, influenced by the greater inclusion of byproduct in the diet compared to Homolka et al., 2021.

The DM removal from the WINTER experiment was 27% greater and manure N capture was improved by 28.5% averaged across treatments compared to the SUMMER experiment. These results are consistent with Kissinger et al. (2007), who summarized data from six Nebraska feedlots representing 15 feeding pens with 40 separate lots of cattle fed in those pens, equating to 6,366 head of cattle; concluding that manure harvested after a winter feeding period is about 20% more than that of manure harvested following a summer feeding period, and that manure harvested after winter feeding has significantly greater retention of excreted N compared to summer. The significant improvement in N recovery in manure during winter is likely due to less N volatilization during winter feeding months compared to summer feeding months (Kissinger et al., 2007).

Results from these experiments suggest that the addition of unprocessed Red cedar biochar to the feedlot pen surface (25 to 31 kg per steer) did not improve manure nutrient retention and did not reduce N losses. In both experiments, biochar addition to the pen surface tended to increase manure N as a percent of manure DM, but this increase in N concentration did not translate into increased kg of N or P removed from the feedlot

pens. Biochar addition to the feedlot pen surface did improve growth performance of steers in the SUMMER feeding experiment, although no differences were found in growth performance for the WINTER feeding experiment.

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Table 3.1. Composition of diet (DM) fed to steers in WINTER and SUMMER mass balance experiments

Item	Experiment	
	WINTER	SUMMER
Ingredient, % dietary DM		
High-moisture corn	51	51
Sweet bran ¹	20	40
Corn silage	15	--
MDGS ²	10	--
Cornstalks	--	5
Supplement ³		
Finely ground corn	1.89	1.89
Limestone	1.63	1.63
Salt	0.300	0.300
Tallow	0.100	0.100
Beef trace mineral	0.050	0.050
Rumensin-90 ⁴	0.015	0.015
Vitamin A-D-E	0.014	0.014
Tylan-40 ⁵	0.010	0.010
Nutrient analysis, %		
Dry matter	57.7	66.9
Organic matter	92.6	92.4
Crude protein	13.7	14.5
Neutral detergent fiber	18.89	20.07
P	0.45	0.53
K	0.69	0.73
S	0.20	0.21

¹Sweet Bran = branded wet corn gluten feed produced by Cargill (Cargill Corn Milling, Blair, NE).

²MDGS = Modified distillers grains plus solubles.

³Supplement fed at 4% of dietary DM. Ractopamine hydrochloride (Optaflexx; Elanco Animal Health; Indianapolis, IN) was fed for last 28 d prior to harvest in WINTER experiment targeted to provide 300 mg/steer daily and replaced finely ground corn in the supplement.

⁴Monensin (Rumensin; Elanco Animal Health) targeted to provide 33 mg/kg dietary DM.

⁵Tylosin (Tylan; Elanco Animal Health) targeted to provide 90 mg/steer daily.

Table 3.2. Performance and carcass characteristics for steers fed the same diet with different pen amendments in WINTER phase

	Treatments ¹			SEM	P-value
	Control	Biochar	Lime		
<i>Performance</i>					
Initial BW, kg	274	274	274	1.3	0.95
Final BW ² , kg	618	622	628	5.7	0.50
DMI, kg/d	10.0	10.1	10.3	0.04	0.10
ADG, kg	1.86	1.87	1.90	0.029	0.50
Gain:Feed	0.185	0.186	0.185	0.0022	0.98
<i>Carcass characteristics</i>					
HCW, kg	390	392	396	3.5	0.50
LM area, cm ²	86.5	87.7	87.7	1.29	0.76
Marbling ³	472	463	476	13.71	0.79
12 th rib fat ⁴ , cm	1.45	1.40	1.42	0.051	0.79
Calculated yield grade	3.43	3.38	3.40	0.050	0.78

¹Control = no treatment applied; Biochar = red cedar biochar applied in Dec and Feb at 123 kg per pen for each application; Lime = applied 1 d prior to cattle harvest approximately 308 kg per pen.

²Carcass adjusted final BW determined from HCW divided by common dressing percentage of 63%.

³Marbling score: 400= small⁰⁰, minimum required for U.S. Low Choice.

⁴12th rib fat, cm: calculated by back-calculating from the USDA YG equation.

Table 3.3. Performance and carcass characteristics for steers fed the same diet with different pen amendments in SUMMER phase

	Treatments ¹		SEM	P-value
	Control	Biochar		
<i>Performance</i>				
Initial BW, kg	339	339	1.08	0.92
Final BW ² , kg	665	682	5.99	0.05
DMI, kg/d	12.1	12.2	0.045	0.48
ADG, kg	2.13	2.24	0.039	0.05
Gain:Feed	0.176	0.184	0.0032	0.08
<i>Carcass characteristics</i>				
HCW, kg	419	429	5.3	0.05
LM area, cm ²	92.7	93.0	1.67	0.89
Marbling ³	492	499	15.1	0.76
12 th rib fat ⁴ , cm	1.50	1.50	0.090	0.98
Calculated yield grade	3.48	3.48	0.065	0.98

¹Control = no treatment applied; Biochar = red cedar biochar applied in June and August at 123 kg per pen for each application.

²Carcass adjusted final BW determined from HCW divided by common dressing percentage of 63%.

³Marbling score: 400= small⁰⁰, minimum required for U.S. Low Choice.

⁴12th rib fat, cm: calculated by back-calculating from the USDA YG equation.

Table 3.4. Monthly precipitation (cm) for WINTER and SUMMER compared to 25-year average monthly precipitation for the Eastern Nebraska Research, Education and Extension Center located near Mead, NE.

Month	Phase		25-Year Average ¹
	WINTER	SUMMER	
December	6.50	-	2.95
January	3.28	-	1.47
February	0.28	-	2.08
March	4.24	-	3.71
April	1.91	-	7.70
May	11.63	-	12.75
June ²	1.63	6.27	11.63
July	-	14.66	6.81
August	-	3.23	9.83
September	-	4.01	8.05
October	-	0.94	5.79
November	-	3.18	3.18
Total precipitation ³	29.47	32.29	75.95

¹Monthly average precipitation (cm) from 1995 to 2020 for Mead, NE, sourced from the Western Regional Climate Center (WRCC, 2021).

²WINTER phase included precipitation from June 1st through 18th, SUMMER phase included precipitation from June 19th through 30th.

³Total precipitation for WINTER and SUMMER combined = 61.76 cm

Table 3.5. Effect of biochar and lime pen amendments on manure nitrogen (N), phosphorus (P) and organic matter (OM) during WINTER¹

	Treatments ²			SEM	P-value
	Control	Biochar	Lime		
Nitrogen					
N Intake, kg/steer	41.4	41.8	42.4	0.5	0.46
N Retention ³ , kg/steer	7.3	7.4	7.4	0.1	0.60
N Excretion ⁴ , kg/steer	34.1	34.4	34.9	0.5	0.42
N Manure ⁵ , kg/steer	15.4	15.2	16.8	0.6	0.15
N Lost, kg/steer	18.7	19.2	18.1	0.9	0.68
N Lost, % ⁶	54.9	55.7	51.7	2.0	0.37
Phosphorus					
P Intake, kg/steer	8.5	8.6	8.7	0.1	0.43
P Retention ³ , kg/steer	1.8	1.8	1.8	0.01	0.60
P Excretion ⁴ , kg/steer	6.7	6.8	6.9	0.1	0.38
Manure P ⁵ , kg/steer	6.7	6.6	7.0	0.4	0.75
P Lost, kg/steer	0.005	0.181	-0.086	0.363	0.87
P Lost, % ⁶	-0.1	2.7	-1.3	5.3	0.87
Manure characteristics					
DM, %	92.0	91.1	91.7	0.01	0.42
DM Removed, kg/steer	446.7	360.1	450.8	35.6	0.17
OM, %	35.1	40.3	37.8	1.5	0.09
OM Removed, kg/steer	157.8	144.7	168.7	13.7	0.48
Manure N, % of DM	1.57	1.71	1.51	0.06	0.07
Manure N, % of OM	4.50 ^a	4.25 ^b	4.00 ^c	0.07	<0.01
Manure P, % of DM	0.69	0.76	0.69	0.031	0.23
Ash, %	64.9	59.7	62.2	1.5	0.09

¹Values expressed as kg/steer over entire feeding period (186 days on feed).

²Control = no treatment applied; Biochar = red cedar biochar applied in Dec and Feb at 123 kg per pen for each application; Lime = applied 1 d prior to cattle harvest approximately 308 kg per pen.

³Calculated using the NASEM (2016) net energy, protein, and P retention equations.

⁴Calculated as nutrient intake –retention.

⁵Manure N or P with correction for soil N or P. Soil nutrient concentration evaluated before and after WINTER experiment to account for all nutrients remaining or in excess on the pen surface.

⁶Calculated as nutrient (N or P) lost divided by nutrient (N or P) excretion.

Table 3.6. Effect of biochar as a pen amendment on manure nitrogen (N), phosphorus (P) and organic matter (OM) during SUMMER¹

	Treatments ²		SEM	P-value
	Control	Biochar		
Nitrogen				
N Intake, kg/steer	43.4	43.9	0.6	0.35
N Retention ³ , kg/steer	6.5	6.8	0.1	0.04
N Excretion ⁴ , kg/steer	36.9	37.1	0.5	0.67
N Manure ⁵ , kg/steer	10.5	11.2	1.8	0.78
N Lost, kg/steer	26.4	25.9	2.0	0.85
N Lost, % ⁶	71.6	69.6	5.0	0.79
Phosphorus				
P Intake, kg/steer	9.8	9.9	0.1	0.35
P Retention ³ , kg/steer	1.6	1.7	0.01	0.03
P Excretion ⁴ , kg/steer	8.2	8.3	0.1	0.69
Manure P ⁵ , kg/steer	7.5	7.3	1.0	0.90
P Lost, kg/steer	0.7	1.0	1.1	0.88
P Lost, % ⁶	8.5	11.4	13.1	0.88
Manure characteristics				
DM, %	55.2	55.8	0.01	0.72
DM Removed, kg/steer	267.1	233.6	16.6	0.08
OM, %	44.2	50.1	1.94	0.06
OM Removed, kg/steer	117.0	116.1	5.2	0.87
Manure N, % of DM	2.01	2.20	0.06	0.07
Manure N, % of OM	4.57	4.39	0.07	0.09
Manure P, % of DM	1.06	1.13	0.06	0.36
Ash, %	55.8	49.9	1.94	0.06

¹Values expressed as kg/steer over entire feeding period (153 days on feed).

²Control = no treatment applied; Biochar = red cedar biochar applied in June and Aug at 123 kg per pen for each application.

³Calculated using the NASEM (2016) net energy, protein, and P retention equations.

⁴Calculated as nutrient intake –retention.

⁵Manure N or P with correction for soil N or P. Soil nutrient concentration evaluated before and after SUMMER experiment to account for all nutrients remaining or in excess on the pen surface.

⁶Calculated as nutrient (N or P) lost divided by nutrient (N or P) excretion.

APPENDIX A: BIOCHAR ON NUTRIENT LOSS FROM CATTLE MANURE

Sperber, J. L., T. Spore, G. E. Erickson, and A. K. Watson. 2021. Evaluation of biochar on nutrient loss from fresh cattle manure. Neb. Beef Cattle Report. pp. 93-94.

Summary with Implications

An experiment was conducted to evaluate the impact of biochar and time on manure nutrient retention. Pans were used to simulate feedlot pens with 10 replications per treatment. Biochar was included at 0, 5, or 10% of manure dry matter with 30 and 60 d durations to evaluate pan contents over time. There was a 13-percentage unit increase in organic matter losses from day 30 to 60 for pans without biochar, and a 3-percentage unit increase for pans containing biochar. The least nitrogen loss was measured on the pans without biochar harvested at 30 d. Pans harvested at 60 d all had similar nitrogen loss. Phosphorus losses were not impacted by treatment while potassium losses decreased over time but were not impacted by biochar treatment. In this study biochar included at 5 and 10% of manure dry matter limited carbon losses but did not impact manure nutrient retention of nitrogen, phosphorus, or potassium.

Procedure

A simulated feedlot pen study was conducted using 60 aluminum pans (10 × 9 × 2 inches) to represent the hard interface of a feedlot pen. Each pan was weighed and filled with a 60:40 blend of feedlot top soil and manure, respectively. Biochar was included at 0, 5, and 10% of manure dry matter (DM), and all contents of the pan were mixed to mimic the hoof action of cattle in a feedlot pen. A 3 × 2 factorial design was utilized, with biochar inclusion at 0, 5, or 10% of manure DM and samples harvested at 30 and 60 days with 10 replications per treatment. All pans were randomized onto 2 screened, metal shelving units located in a temperature-controlled room in the University of Nebraska-

Lincoln Metabolism Lab (Lincoln, NE). Biochar, manure, and soil samples were analyzed for DM and nutrient content prior to study initiation.

Biochar was provided by High Plains Biochar (Laramie, WY) and was sourced from forest wood waste, primarily ponderosa pine trees. Biochar had a DM content of 97.5%, and on a DM basis carbon (C) content was 75.4%, with a surface area of 306 m²/g, bulk density of 129.8 kg/m³, and pH of 8.45. Biochar particle size measured \leq 2-mm for 72.3% of total sample, 22.7% of sample measured between 2- and 4-mm and the remainder measured $>$ 4-mm. Manure was sourced from a commercial feedlot near Mead, NE, that houses cattle in covered pens with slatted flooring. Slatted flooring allows for elevated manure and urine capture, with no soil contamination, thereby producing a liquified manure slurry. Nutrient content of manure at a DM of 10.4% measured 72.8% OM, 5.87% N, 1.33% P, and 2.66% potassium (K) on a DM basis.

Original intent was to harvest thirty pans at 30 d after trial initiation and thirty pans at 60 d. Due to UNL research restrictions onset from COVID-19, thirty pans selected for harvest at 30 d were placed in plastic bags (to avoid cross-contamination), placed in a 4°C freezer, and were ground at a later date. Thirty pans selected for 60 d harvest, were harvested on d 52 of study and ground immediately, due to Phase 4 restrictions on UNL research.

At time of harvest, pans were weighed, and contents were ground through a 1-mm screen. Ground samples were sent to Ward Laboratories, Inc. (Kearney, NE), and analyzed for DM, OM, and nutrient (N, P, K specifically) content. Data were analyzed using the MIXED procedure of SAS (SAS Institute, Inc., Cary, N.C.) with pan as the experimental unit.

Results

Nutrient losses from the manure:soil mixture are reported as a % of nutrients weighed into each pan on d 1 (Table 1). There was an interaction ($P = 0.05$) between biochar inclusion and day for OM loss. At the 30-day harvest there were no differences between treatments (9.12% OM loss). The biochar treatment was effective at limiting OM losses at 60 days, with the 10% biochar treatment being most effective. The pans with no biochar had an increase in OM losses of 13-percentage units from day 30 to day 60 while the pans with biochar had a 3-percentage unit increase.

A biochar inclusion by day interaction ($P < 0.01$) was observed for nitrogen losses. With no biochar, N losses increased 7 percentage units from day 30 to day 60. With biochar inclusion (both the 5 and 10% biochar treatments) N losses did not increase from day 30 to day 60. The least N loss was measured on the 0% biochar pans harvested at day 30 while the greatest N losses were for 10% biochar pans harvested at day 30.

Phosphorus losses were not impacted by treatment ($P \geq 0.37$) and averaged 5.98%. There was an effect of day for K ($P < 0.01$) with pans harvested at 30 d having greater K losses compared to pans harvested at 60 d. Biochar inclusion did not impact K losses ($P = 0.53$). The quantities and losses of both P and K were small and there is a challenge in accurately measuring these small quantities.

Results from this study suggest that biochar, included at 5 or 10% of manure DM content, is not a sufficient method to improve nutrient capture from cattle manure. These results are dissimilar to previous literature on the use of biochar inclusion to capture manure nutrients although previous studies focused on manure from animals other than cattle. One primary difference in this study is that manure was collected from covered

feedlot pens with slatted floors, thus DM content of the manure was less than 20% and N content was over 5% of DM. Increasing the amount of biochar added may impact the results but could also become expensive, depending on the type and source of biochar.

Table 1. Simple effects of biochar inclusion and time on manure nutrient loss

	Biochar 0%		Biochar 5%		Biochar 10%		SEM	<i>P</i> -Value		
	30d	60d	30d	60d	30d	60d		Inclusion	Day	Inclusion × Day
OM lost, %	7.50 ^b	20.6 ^a	9.94 ^b	14.0 ^{ab}	9.91 ^b	11.8 ^b	2.38	0.40	<0.01	0.05
N lost, %	26.3 ^b	33.3 ^a	34.8 ^a	32.7 ^a	37.9 ^a	33.2 ^a	1.85	0.01	0.96	<0.01
P lost, %	3.16	4.75	8.25	4.00	9.75	5.94	2.93	0.42	0.37	0.54
K lost, %	6.36 ^{ab}	1.26 ^{bc}	10.6 ^a	0.22 ^c	9.34 ^a	3.06 ^{bc}	2.15	0.53	<0.01	0.44

^{abc}Within a row, least squares means without a common superscript differ ($P \leq 0.05$).