THESIS

THE EFFECTS OF ZEOLITES (CLINOPTILOLITE) ON NUTRIENT USE IN FISTULATED BEEF STEERS

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ABSTRACT

THE EFFECTS OF ZEOLITES (CLINOPTILOLITE) ON NUTRIENT USE IN FISTULATED BEEF STEERS

The need for a low-cost waste removal method has increased in demand, as the decrease of emissions created by cattle becomes a main focus of agriculture. Zeolites and their adsorptive properties may prove to be beneficial to remove animal excreted elements from volatilizing into the environment. Zeolites can inhibit losses from the conversion of ammonium to nitrate and ammonia that enter the soil or atmosphere when wasted by the animal. The focus of this study was to see if the inclusion of zeolites into beef steers would impact the use and excretion of ADF, NDF, N, and P within the animal. In this study, the effects of the zeolite, clinoptilolite (HEU), on whole-body N and P retention and excretion were evaluated in 12 fistulated Angus steers (initial BW 666 ± 41 kg). In a randomized block crossover design, steers were administered a 0.2 kg dose of limestone or HEU directly into the rumen. Nitrogen and phosphorus balance were measured from day 22 to day 27 of each group period. Urine and fecal samples were collected every 24 hours on each balance day. Urine samples were collected to measure N, P, and Urea-N excretion. Fecal samples were collected to measure ADF, NDF, N, and P excretion. Zeolite treatment did not differ from control ($P < 0.05$) in daily DMI ($P = 0.94$), DM digestibility % ($P =$

0.27), N digestibility % (P= 0.44), Fecal N (P= 0.44), Fecal P (P= 0.15), Urine Urea-N (P= 0.90), N retention (P= 0.94), and P retention (P= 0.21). NDF digestibility trended to be lesser (P= 0.07) in steers dosed with HEU compared to limestone. ADF digestibility trended to be lesser (P= 0.10) among steers dosed with HEU compared to limestone. In summary, N and P excretion levels did not differ between steers dosed with HEU or limestone, but there was a trend of decreased NDF and ADF digestibility between steers dosed with HEU compared to limestone. These data points suggest that directly administering zeolite to the ruminant animal had little impact on nutrient use within the ruminant animal.

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CHAPTER 1: REVIEW OF LITERATURE

Introduction

Nutrition and nutritional management are the basis of livestock production throughout all stages of a food animal's life. Proper nutrition is vital to optimize yield and profits, especially in ruminant livestock production. A current topic of discussion within nutrition and nutritional management is the increased excretion of ammonia and greenhouse gases from livestock animals. Ammonia is a naturally produced compound from the digestion of proteins, amino acids, and non-protein nitrogen. Ammonia is naturally produced in mammals and is excreted through urination and feces. According to the United States Environmental Protection Agency (EPA) in 2022, agriculture comprised 9.4 percent of the United States' total gross greenhouse gas emissions. In that same published inventory, the EPA has stated that the application of agricultural soil management activities, like the use of synthetic and organic fertilizers, application of livestock manure, and growing N-fixing plants, were the largest contributors to N_2O emissions in 2022. This total accounts for 74.6 percent of total N_2O emissions within the United States. Although N_2O emissions comprise 6.1% of total U.S. greenhouse gas emissions, the need for a nitrogen sink within agriculture is necessary. A proposed method of obtaining this goal of decreased emissions is by the application of zeolites to the animal's diet. This review will detail aspects of the ruminant animal, and how zeolites may be effective in the reduction of nitrogenous waste from application to the ruminant diet and when directly given to the animal through a rumen cannula. Nutrition is a vital part in improving animal performance within livestock production. Without proper and adequate nutrition, animals are at risk for nutritional deficiencies and decreased immune function. Nutrition has an impact on every physiological process in the body and nutritional deficiencies have been found to cause poor carcass

characteristics, illness, and even death (Forsberg et al., 2010). Accurate nutritional diet formulation that is specific to an animal or animals within a herd is imperative to the success of a business. To provide a nutritious diet for an animal, the animal's purpose must be clarified. For example, a feedlot animal will need to be in a positive energy balance for efficient anabolism to occur. A dairy animal must consume its basal metabolic rate or more to allow for an increase in milk production while maintaining the same body mass. Proper nutrition will also prevent the prevalence of hypocalcemia within a dairy herd, and alleviating blood calcium depression postpartum via diet has been shown to be a useful strategy to minimize post-calving disorders and enhance performance of cows throughout the lactation period (Nikkhah and Alimirzaei, 2023). Knowledge of nutrition and nutritional management is vital to the success of agriculture, and its application is important to animal performance and animal immune function.

Anatomy of Ruminant Digestion

Foregut Anatomy

The anatomy of the ruminant differs from the monogastric in various ways. The foregut is least similar due to the presence of four distinct chambers of the ruminant stomach compared to the single chambered stomach of the monogastric. The foregut of the ruminant animal is comprised of the reticulum and rumen (often referred to as the reticulorumen), omasum, and abomasum. These chambers allow for the ruminant animal to be the most efficient foregut fermenters. The complexity of the ruminant forestomach is attributed to the presence of bacterial colonies, protozoa, and fungi that assist in the degradation of feedstuffs that enter. Without these components within the foregut, the efficiency of plant material degradation will decrease and as a result, so will animal performance.

The Reticulum

The reticulum is a small pouch that is most cranial compared to the other 3 chambers of the ruminant animal. It is often referred to as the pacemaker of digestion as it plays an important role in ruminal contractions. It is necessary to be aware of its location to recognize the importance of magnet administration to aid in the prevention and decreased incidence of hardware disease in production animals. Hardware disease is the result of the ruminant's non-discriminatory browsing behavior when eating. Due to the location of the reticulum and the ruminant's feeding behavior, the ingestion of foreign metal objects may occur and will result in the puncture of the diaphragm to cause pericarditis, pleuritis, or localized abscesses in the thorax (Miesner and Reppert, 2017). With its honeycomb-like mucosa, the function of the reticulum is to play a decisive role in particle separation and differences among species has been linked to feeding style (Clauss et al., 2010). As stated previously, the reticulum and rumen are often debated in which compartment arises first and is referred to as the reticulorumen, but for the sake of thoroughness and individual differences, they will be separated within this review.

The Rumen

The rumen is the most advanced of the stomach compartments and allows for fermentation, biohydrogenation and degradation of feed components ingested by the animal. The rumen typically accounts for about 15% of the animal's weight, and its large volume serves to increase the retention time of feeds, thereby allowing more extensive feed conversion (Weimer, 2022). The rumen can be further broken up into the layers present within it. The fluid layer is where bacterial populations thrive and is often taken from fistulated ruminants to be transplanted into acidotic ruminants. The inoculation of healthy ruminal fluid, or ruminal transfaunation, has been studied to determine the effects on the ruminal microbiome. Rumen transplantation has been found to accelerate the recovery of rumen fermentation and bacterial homeostasis and

modulates ruminal epithelial morphology and function for sheep suffering from ruminal acidosis (Lui et. al, 2019). The other layers within the rumen are often called the “fiber mat” layer and gaseous layer. The fiber mat is filled with dense ingested plant material. It is the location of feed degradation as bacteria will transport themselves from the rumen wall to the feed within the fiber mat to be digested. The gaseous layer is comprised of gaseous compounds like methane, carbon dioxide, and other gaseous by-products of fermentation. This layer is important in understanding the premise of ruminal bloat and the emissions excreted from ruminants. Together, these three layers allow for the fermentative microenvironment within the rumen.

Volatile Fatty Acids

VFA’s are extremely important for the life of the ruminant animal and are readily present from bacterial fermentation in the rumen or large colon. VFA’s are volatile fatty acids, and are the primary energy source to the ruminant. The VFA’s studied and referred to by literature the most are acetate, propionate, and butyrate. These short chained fatty acids (SCFA) can make up almost 75% of total metabolizable energy for the animal, and these specific SCFAs account for about 95% of total VFA’s produced in the rumen (Bergman, 1990; France and Dijkstra, 2005). The absorptive properties and metabolic uses for these VFA’s within the ruminant are heavily studied. A study performed by Bedford et al. in 2020, proposed the effects of ambient temperature on VFA absorption. It was found that heat-stressed animals fed in an environment 30° C had decreased butyrate absorption. Butyrate is produced in the rumen at less proportions than acetate and propionate, but still has important properties that are useful to the animal. In newborn calves and adult ruminants, the promotion of ruminal butyrate production has been shown to accelerate the growth of ruminal epithelium and speed of maturation (Sakata and Tamate, 1978; Mentschel et al., 2001). A study performed by Sander et al. in 1959 also concluded that the administration of a sodium butyrate solution directly into the rumen of

cannulated calves at two to five weeks of age resulted in an increased rate of papillae development within the rumen. Propionate is another major short chain fatty acid readily used within ruminant digestion. Propionate, a precursor to glucose, is the main energy source for ruminants (Wang et al., 2023). This implies that the primary source of glucose for the ruminant relies on gluconeogenesis within the liver. Bergman et al. in 1966 was able to quantify the amount of glucose created from propionate by infusing the rumen vein of sheep with labeled propionate and found it capable of being utilized for gluconeogenesis. It was also stated in that same study, that out of the three major short chain fatty acids, propionate is the only gluconeogenic compound. Acetate is another major short chain fatty acid used by ruminants. Acetate is primarily used for the synthesis of lipids and ATP from acetyl-Coenzyme A (acetyl-CoA). The formation of ATP is from the catabolism of acetyl-CoA within the tricarboxylic acid (TCA) cycle. Furthermore, during anabolic metabolism of acetyl-CoA, lipids are generated (Bose et. al 2019). This anabolic process is often referred to as de novo lipogenesis. These SCFAs are the backbone to large animal nutrition and diet formulation. Sudden changes in diet will affect VFA production and proportions. It has been found that changes in the composition or physical characteristics of the diet and the level and frequency of feeding produces changes in the rumen ciliate population (Williams 1986). These dietary changes will alter the ruminal microbiome and have recently been the focus of reducing emissions produced by ruminants.

Ruminal Contractions

To properly inoculate and degrade feed with the lytic abilities of the ruminal microbiome, the rumen must have a mixing function. This is achieved by the skeletal muscle contractions of the rumen. As stated, the purpose of ruminal contractions is to allow for the mixing of ruminal contents by minimizing the stratification of feed and to increase particle sorting and passage. Contractions will also allow for the animal to ruminate, eructate, and inoculate incoming feed.

Rumen contraction cycles are separated into primary and secondary contractions. The primary contraction is an orderly process where feed is continuously mixed and churned within the rumen. The source of neuronal signaling for ruminal contractions originates from the gastric centers located in the medulla oblongata of the hind-brain and allow for the primary and secondary contraction cycles to occur (Leek, 1987). Each contraction of the reticulum, compartment, or ruminal sac is followed by a period of relaxation. Ruminal contractions start with the biphasic contraction of the reticulum. The contraction of the reticulum leads to the contraction of the cranial sac of the rumen. As the cranial sac contracts, the reticulum relaxes. The dorsal sac of the rumen then contracts once relaxation of the cranial sac has occurred. The caudal dorsal blind sac of the rumen will then contract after the dorsal sac relaxes. Once the caudal dorsal blind sac contracts, the ventral sac contracts and the caudal dorsal blind sac relaxes. The caudal ventral blind sac then contracts and the ventral blind sac relaxes. These processes are necessary to allow for ruminal bacteria to cover enough surface area to break down feedstuffs within the rumen.

The Eructation Cycle

Contractions will also allow for eructation when gas accumulates within the rumen. The process of eructation can be described by the secondary contraction process. Secondary contractions are often referred to as the eructation cycle and does not require initiation via the reticulum. The eructation cycle starts with the B-wave or a forward moving contraction, and occurs after the primary contractions have ended. Gas is first pushed toward the esophagus. The reticuloruminal and anterior folds rise to move ingesta away from the cardiac sphincter of the esophagus. The cardiac sphincter relaxes to allow for gas to accumulate within the esophagus, and rapid reverse peristalsis begins. This is achieved by the opening of the diaphragmatic and pharyngeal sphincters and the closure of the nasopharyngeal sphincter. The epiglottis opens and

the thoracic muscles contract causing the gas to enter the trachea. The animal finally eructates out its nasal cavity, muffling the sound in the process. These processes occur to enhance breakdown of feed within the reticulorumen. As feed particles become smaller, degraded feed can enter the omasum to be further digested.

The Omasum

The omasum is the third compartment of the ruminant stomach and acts as a filter for the passing feed particles. The omasum achieves this by the use of over 100 internal laminae or sheets that allow small particles to pass. Per Becker et. al, the pedunculated shape of, and partial blockage by the first-order laminae at the reticulo-omasal orifice, and their extension generally across to the suleus omasi, tend to allow mainly small feed particles to enter, and divert them between the laminae (Becker et al., 1963a). The interior laminae of the omasum project from the greater curvature of the omasum to the lesser curvature, creating the omasal canal. The laminae are of several lengths and those of different sizes alternate and divide the lumen into a series of uniform recesses (Dyce et al., 1987). Feed particles exit the omasum via biphasic contractions. Within the first phase, ingesta is pushed between the recesses of the lamina. The second phase is characterized by the complete contraction of the omasum. The feed particles are then pushed out of the omasum and into the abomasum, where enzymatic digestion occurs resulting in further absorption of nutrients.

The Abomasum

The final compartment of the ruminant stomach is the abomasum. This compartment is similar to the monogastric stomach. It contains regions consistent with other species stomachs: fundic, cardiac, and pyloric. The ruminant stomach also contains sphincters found in other species like the cardiac sphincter and pyloric sphincter. Since the ruminant stomach is elongated, compartmentalized, and complex, the cardiac sphincter is found at the opening of the

reticulorumen orifice and the pyloric sphincter is found at the pylorus of the abomasum. The ruminant stomach also contains similar glands found within the monogastric stomach, as this portion is recognized by the area of enzymatic digestion. Mucous glands and peptic glands are commonly found here. Anatomically, ruminants contain an additional portion of the pylorus called the *torus pyloricus*. This process is a thickened portion of the distal antrum and pyloric region. The literature suggests that the *torus pyloricus* may be capable of erection due to present vasculature, but the functional significance of it is unknown (Dyce et al., 1987). The interior of the abomasum is lined with glandular mucosa and contain ridges referred to as rugae. The abomasum contains specialized secretory cells that contribute to increase digestion of feed particles entering. Of these cells, chief cells are secretory cells located in the fundus of the abomasum and secrete pepsinogen. Pepsinogen is a zymogen, or inactive form of the enzyme that will activate when exposed to hydrochloric acid. The active form of this enzyme is pepsin and will assist in the breakdown of proteins that enter the abomasum. In addition to chief cells, the abomasum also contains parietal cells that secrete hydrochloric acid. The gastric glands stated previously are found in the lamina proper of the mucosal layer of the pyloric region (Ali et al., 2023). Gastric glands are present in the pyloric region and the mucosa of the fundus and body contain the peptic glands (Ali et al., 2023). Out of all regions stated, the esophageal region is the only region that is non-glandular.

Hindgut Anatomy

The hindgut of the ruminant animal is comprised of all digestive structures post-abomasal digestion. The ruminant hindgut includes the small intestine, large intestine, cecum, and rectum. Like monogastric animals, the ruminant hindgut is specific to water absorption and some nutrient absorption. There is not much fermentation and digestion within the large intestine, but it is

possible with a very high passage rate out of the rumen in tandem with a low passage rate out of the lower gastrointestinal system to allow for some fermentation end-products to be synthesized within the large colon. The tissue that lines the walls of the small and large intestine are called villi and are epithelial in nature these villi allow for diffusion and transport of nutrients across the intestinal walls. The villi are tallest within the jejunum and contain microvilli lined with columnar absorptive cells called enterocytes (Volk and Lacy, 2017). This small layer of columnar absorptive cells are called enterocytes and are central to the absorption of triglycerides, sugars, and amino acids.

The small intestine consists of the duodenum, jejunum, and ileum. Small intestine metabolism accounts for 30% of the animal's metabolic processes and 20% of oxygen consumption within the animal (McBride and Kelly, 1990). Unlike the upper gastrointestinal tract, motility within the lower gastrointestinal tract is reliant on smooth muscle contractions in the form of peristaltic waves. The duodenum is a primary site of gastric acid neutralization and the terminal point for pancreatic secretions in response to gastric acid and distention from feed. Secretions from the pancreas are triggered by secretin in response to the acidification of the duodenum. Bicarbonate and proteases are transported from the pancreas through the pancreatic duct and out through the duodenal papillae that opens into the duodenum. A major enzyme released is pancreatic α-amylase. This enzyme is crucial in the degradation of escaped starches from the foregut and allows for increased digestive efficiency of starches (Harmon and Swanson, 2020). With digestive secretions released in the duodenum, the next step in digestion is absorption within the jejunum. The jejunum is the primary site for nutrient absorption as well as some water absorption. The mucosa of the jejunum is comprised of simple columnar epithelium. As stated previously, the functional unit of the small intestinal mucosa is called the enterocyte,

and is the metabolically active portion of the small intestinal mucosa. The processes of lipid absorption and glucose metabolism occur within these cells. These processes can be facilitated by the mucosa themselves or within the liver. The ileum is the final portion of the small intestine and is anatomically identified by the ileocecal junction. The ileum is unique in that it contains lymphoid tissue and contains antiperistalsis properties. The ileum is important in the reabsorption of bile salts and vitamin B12. The ileum in ruminants opens into the colon via the ileocecal junction.

The Colon

The colon of the ruminant is similar to the monogastric, but with a developed and complex ascending colon. Mechanisms are also similar in function but differ in gross anatomy. The ascending colon in ruminants is split into multiple grossly identifiable sections. The portions of the ascending colon of the ruminant animal are the proximal loop of the ascending colon, the spiral colon, and descending loop of the ascending colon. The ruminant also contains a transverse colon and descending colon. Like the monogastric, the function of the large intestine and cecum is to absorb water and nutrients that are not absorbed within the small intestine. The large intestine also contains numerous microvilli that aid in increasing surface area for nutrient absorption. Recent studies have focused on the importance of the large intestine and have stated that gastrointestinal barrier function is considered essential to the preservation of animal health (Sanz-Fernandez et al., 2020). It has also been proposed that, evolutionarily, the immune system was developed from this portion of the gut (Matsunaga and Rahman, 1998). Increased bypass of starch from increased grain consumption has also shown incidence of acidosis within the hindgut (Sanz-Fernandez et al., 2020). The colon is crucial to the absorption of water and must be considered when attempting to increase dietary energy via diet formulation. When damaged or

diseased, the processes of water absorption can be reversed and cause animals to become acidotic from increased water excretion and improper electrolyte balance.

Methods of Data Collection

There are various methods to observe and record nutrient use data. The three most common methods in research are in-vitro, in-vivo, and in-situ. These methods of data collection can work best with fistulated animals when attempting data collection for research. Fistulated animals or cannulated animals are animals who have undergone a surgical cannulation procedure to fit a rubber or plastic cylinder between the anaerobic environment of the rumen and the aerobic environment outside. Cannulation for ruminants was first described in 1928, but has origins from 1822 from Dr. William Beaumont's study on the secretion, motility, and emptying of the stomach in a patient with a gastric fistula (Dougherty, 1955; Castillo and Hernández, 2021). These findings paved the way for large animal nutrition research, and the methods used are still relevant today. Without this procedure, in-vivo rumen data collection would become increasingly difficult and complex. Cannulation allows researchers to quickly gather data points and proves beneficial to animals who are or are losing their bacterial microflora from acidosis.

In-Vivo

The in-vivo research method requires the use of living organisms to allow for the most accurate data collection pertaining to experiments within the animal. For food animal research, in-vivo applications are the most widely used. In the aspect of zeolite application to the ruminant diet, there have been few studies performed. The studies that have been performed have varying results and are often contradicting. A study performed by Sadeghi and Shawrang in 2006 found that acid detergent fiber and neutral detergent fiber digestibility were increased in growing steers that were supplemented with zeolite. Another study performed by Sadeghi and Shawrang in 2008 showed the effects of zeolite on Holstein calves. The zeolite used in the study was clinoptilolite

and was administered through milk. It was proposed that an inclusion of 1.0 g/ kg body weight per day to each steer would prove beneficial to decrease the incidence of diarrhea but not improve passive immunity. It was also concluded that at an inclusion rate over 1.0 g/ kg of body weight per day would cause adverse effects on passive immunity and diarrhea. The reasoning and exact mechanism for this was unclear. In another study done by Klaeui et al in 2020, it was found that NDF digestibility did not differ between treatments when supplemented with zeolite compared to a control diet. The study was performed with cannulated crossbred beef heifers and zeolite treatment was "top-dressed" to treatment animals after feed was administered. Although methods of inclusion differ between studies, many other factors like OM ingestion, feed preference, or zeolite inclusion levels may play a role in the differing results. There have been studies focusing on inclusion rates of zeolites to the ruminant diet. The literature that is published has zeolites included at 3% of the diet or 5% of the diet with varying results on improvements in emissions output and performance characteristics (Dschaak et al., 2010; Urías-Estrada et al., 2018; McCollum and Galyean, 1983). More studies need to be performed and published in order to directly identify the correct concentration of zeolite per animal as the results outlined above have differed in findings.

In-Vitro

An in-vitro method of analysis is used to describe experiments that occur outside an organism. These studies are often easily reproducible and require an artificial environment similar to an in-vivo environment. In 1963, Tilley and Terry proposed a two-step in-vitro technique that would become one of the most influential pieces of literature within digestibility research. This method, often referred as the two-step Tilley and Terry technique, is used to estimate the dry matter digestibility of forages in-vitro. In-vitro experiments are common practice for projects that require a high number of replicates. A zeolite in-vitro study in 2021

performed by El-Nile et al., found that clinoptilolite supplementation form resulted in a linear CH_4 reduction consistent with a linear decrease ($P = 0.004$) in NH_3-N, and linear increases in propionate molar proportions ($P = 0.004$). Although this study found prominent results, the rumen fluid used was taken from three Egyptian buffalo steers prior to slaughter and the lack of diversity could lead to skewed results and failure of application to modern production systems. A recent study performed by Majewska et al. in 2024 found that with an inclusion of 2% zeolite as a treatment within their in-vitro study caused a statistically significant increase in cellulolytic activity and a trend toward an increase in amylolytic activity. The study was performed with the use of rumen fluid of five rumen-fistulated Jersey heifers. Although promising results, Jersey cattle are not regarded as classical feedlot production animals, and it would be difficult to apply these findings to beef producing steers or other meat production systems.

In-Situ

An in-situ method of analysis is often used to determine the digestibility of forages within the rumen of fistulated ruminants. For this method, a dry nylon bag is weighed without feed and then weighed again to determine both the weight of the feed, weight of the bag, and weight of both items together. The nylon bag of feed is then tied to the fistulas of the cannulated animals and left within the rumen to be degraded over time. The samples are then weighed again every few hours and recorded to see the rate of digestion for the feedstuff in question. The time recorded, in conjunction with the weight of the sample, is used to determine the rate of digestion. This method was first used by Quin et al. in 1938 while studying rumen kinetics. Both the in-situ method and in-vitro method allow researchers to compare the forage qualities of feeds with their nutritive values. Forage quality is a measure of animal gain or milk production when forage is not limiting, whereas nutritive value is measured by laboratory methods that are disconnected from the biological component of the animal consuming the forage (Diao et al., 2020). In-situ

studies do not necessarily provide the information needed for live animal applications of zeolites but may prove beneficial on understanding the effects of zeolites on both the rumen microbiome as well as the effects on feed degradation within the rumen.

Zeolites

The History of Zeolites

The term “zeolite” was first used by the Swedish minerologist Axel Fredrick Cronstedt in 1756 to describe a mineral with properties that allowed it to be hydrated and once heated, show unique frothing features. The term zeolite comes from the Greek roots zéō and líthos, meaning "boiling stone.” Since 1756 there have been over 40 naturally formed zeolite frameworks discovered and over 253 unique frameworks discovered and reported to the International Zeolite Association (IZA). The IZA classifies all known zeolites by framework using a three-letter code. The zeolite framework is attributed to the molecularly dense network of AlO_4 and SiO_4 that form microporous channels within the mineral. These channels allow for the exchange of water and other polar molecules within the framework and allow for a zeolite’s high adsorbancy and ion exchange capacity (Mastinu et al., 2019). These properties of zeolites are the main driver of proposed agriculture applications. Zeolites can be both natural and synthetic. The purity of natural zeolites are lower compared to synthetically derived zeolites due to the presence of impurities. Although a lower purity, natural zeolites can increase in purity when acids are applied. The application of sulfuric acid or hydrochloric acid to treat these minerals will yield a higher purity due to the removal of aluminum from the aluminosilicate framework (Mansouri et al. 2013; Silva et al. 2019). This increases the porosity of the mineral and subsequently increases its adsorbancy and surface area for exchange. The properties of zeolites make them a potential use for attempts to decrease total ammonia emissions from cattle by encasing them within the pores of the mineral. Both natural and synthetic zeolites have been used within research studies,

though the use of natural zeolites are common when applied to ruminant animals for the sole purpose of increasing production outputs. Other systems besides agriculture may find the application of zeolites useful, but it is not the sole purpose of this review. For this paper, the focus is on the natural zeolite, Clinoptilolite (HEU).

Clinoptilolite

Clinoptilolite is one of the most widely used natural zeolites found and its most common application is to filter out or sieve out harmful substances like heavy metals and ammonia (Mastinu et al., 2019). Like other aluminosilicates, clinoptilolite is recognized by its molecular structure and unique properties. All zeolites are aluminum or silica-based compounds that form a rigid molecular tetrahedra that attributes to its crystalline structure. Typically, this mineral is associated with low-temperature environments (60–110 °C), resulting from the alteration of volcanic rocks. This alteration occurs due to a reaction between acidic volcanic glass and interstitial solutions during burial diagenesis (Iijima, 1980). The creation of natural zeolites takes thousands of years, yet are less expensive than synthetic zeolites. The price of synthetic zeolites are increased compared to the natural type due to the use of expensive substrates during the creation process. The process of creating a clean synthetic zeolite requires expensive equipment, the use of clean substrates, and requires a lot of energy (Król, 2020). Each aluminosilicate contains a different ratio of aluminum to silicon and have different affinities toward metals. The highly organized framework of zeolites allows for the exchange of metal cations within the molecular pores of the tuff. For clinoptilolite, the affinities for different metal elements have been studied and published with differing findings. In 1984, a study done by Blanchard et. al states clinoptilolite cation exchange potential as $Ba^{2+} > Cu^{2+}$, $Zn^{2+} > Cd^{2+}$, $Sr^{2+} > Co^{2+}$. A study done by Zamzow et. al in 1990 found similar evidence with different metal elements. The cation exchange potential found in that study was $Pb^{2+} > Cd^{2+} > Cs^{+} > Cu^{2+} > Co^{2+} > Cr^{3+} > Zn^{2+} > Ni^{2+} >$

Hg^{2+}. In Blanchard's 1984 study, the metal Zinc (Zn^{2+}) was found to have a lower exchange potential than copper (Cu^{2+}) and was supported by Zamzow et. al in 1990. A study that found differing evidence was done by Erdem et. al in 2004. This study found the metal cobalt (Co^{2+}) had a higher exchange potential than both copper and zinc metals. These differences in findings can be due to the different methods of analysis and metal types. For example, the 1990 study performed by Zamzow et al. used 22 different types of zeolites and analyzed them via X-ray diffraction and inductively coupled plasma analysis (ICP). X-ray diffraction (XRD) is used to determine what elements comprise a material and is regarded as non-destructive. ICP is regarded as destructive (loss of the material used) and measures elements at trace levels in biological fluids (Laur et al. 2020). The study performed in 1984 by Blanchard et al. uses X-ray diffraction and standard wet chemical analysis to determine the make-up of zeolites used in the study. The procedure to identify clinoptilolite is to determine the ratio of Al to Si. In various studies, the ratio found typical of clinoptilolite is 4.5- 5.5 (Loizidou et al. 1992; Langella et al. 2000). Erdem et al. in 2004 also found that that low-silica zeolites are enriched with calcium, whereas high-silica clinoptilolites are enriched with potassium, sodium, and magnesium. Clinoptilolite has been applied within lab settings numerous times but has also been applied to situations outside of a typical lab setting. In a study performed by Jaynes et al. in 2007 it was found that clinoptilolite was found to capture ammonium ions, reducing the rate of their release and absorption from the ammonium wall, and act as adsorbents for mycotoxin. A method of controlling the amount of nitrogenous waste excreted from ruminants is paramount and the use of clinoptilolite seems promising. In the livestock industry, most emissions are in the form of carbon dioxide (CO_2), nitrous oxide (N_2O), methane (CH_4), and ammonia (NH_3) (Leytem, 2011). The ammonia production is a result of the wasted nitrogen from the ruminant animal. This wasted nitrogen is

created from the synthesis of microbial protein in the rumen and from the urea cycle within the animal's liver. Some wasted nitrogen is excreted from the animal via urine in the form of urea. It is then broken down into ammonia and carbon dioxide by urease, an enzyme excreted by bacteria naturally found in soil, feces, and rumen of the ruminant animal. Clinoptilolite has a wide variety of uses and often exploited for its cation exchange capacity. Most notably, clinoptilolite is used for wastewater treatment as its properties make it excellent for adsorbing harmful metals in wastewater and causing the metals to be transferring from an aqueous solution and into the zeolite's solid structure. The use of these compounds may have beneficial effects with little to no side effects of use.

Zeolites and Livestock Production

Zeolites are important within livestock production because of their adsorptive properties toward positively charged ions like ammonium. Adsorption is a reversable process that involves the transfer of a molecule or molecules from a fluid to a surface either from physical force or chemical bonds. The reversable process of adsorption is known as desorption. Adsorption can be commonly confused with absorption, but unlike adsorption, absorption is the process of molecules entering a bulk phase (gas, liquid, solid) that are taken up by the volume of the substance and not the surface. Zeolites have excellent adsorptive properties and as such, zeolites like clinoptilolite, can be used as feed additives within livestock diets to potentially decrease the presence of harmful toxins and increase performance within livestock animals.

Zeolite Properties

Zeolites are naturally forming aluminosilicates with a high affinity for positively charged ions, otherwise known as cations. These cations can include molecules such as Na^+, K^+, NH_4^+, and H^+. Zeolite structure consists of a three-dimensional tetrahedron where the vertices are composed of a framework of oxygen atoms. This crystalline structure creates small channels or

pores nanometers wide (<2 nm) that act as molecular sieves. Molecular sieves are commonly used for drying or purifying substances within disciplines like organic chemistry and are often made from zeolites. Zeolites can be both naturally made or synthetically created. The versatility of zeolites allows them to have a variety of uses and are prominent within laundry detergents, used as catalysts for oil refinery, and used as sieves for wastewater treatment.

Intended Uses

Zeolites are not limited to the application of production animal diets. Zeolites have been used in wastewater treatment to sequester heavy metals from aqueous solutions rendering heavy metals to a solid form for easy transport and removal. Zeolites have also been used to remove radioactive material from the production of nuclear waste. Zeolites were most notably used to sequester radioactive material from the Chernobyl disaster in 1986. The Dnieper River system and reservoir were the main source of water to the major city of Kiev, Ukraine. In the Dnieper Waterworks Station, activated charcoal and zeolite were added to water filtration systems to remove radioactive compounds like I^{131}, Ru^{106}, $Cs^{137,134}$ and Sr^{90} (Tsarik, 1993). Zeolites also have use within the United States military. The on-board oxygen generation system (OBOGS) in military aircraft like Lockheed Martin's F-22 "Raptor" utilize zeolites. OBOGS generates in-flight O_2 to pilots. Air from outside the aircraft is filtered by zeolites to remove N_2 and provides up to 94% pure oxygen to pilots (USAF-Scientific Advisory Board, 2012). It has also been found that the inclusion of zeolite as an additive in the diets of ruminants stabilizes the rumen environment during the first stages of fermentation in terms of pH and ammonia concentration, especially in high-concentrate diets (Amanzougarene and Fondevila, 2022). Zeolites have many intended uses within the livestock industry and even more outside of livestock. Zeolites have been claimed to increase average daily gain, increase ruminal pH, and to prevent the volatilization of ammonia excreted from the ruminant animal. Many companies have claimed the

substantial benefits of zeolites, but these claims have not been backed by science or do not have enough data to state that these claims are true as the data published differ in methods, analysis, and results.

Ruminant Nitrogen Metabolism and Pathways

Microbiology of the Rumen and Intestines

The microbiome within the rumen is vast and not all species that inhabit the rumen have been discovered. The rumen alone contains a variety of bacterial microflora, protozoa, archea, and fungi that assist in the degradation of plant material. Each microorganism within the rumen contain three properties: they are anaerobic or facultative anaerobic, they produce end-products found in the rumen or are utilized by other microorganisms, and are needed and present in high numbers. Discovering species within the rumen has been difficult due to the it being impossible to reproduce the complex system within laboratory settings (Castillo and Hernández, 2021). There is difficulty in separating particulate bound bacteria and additional difficulty in counting the viable but non-dividing cells within the colonies. The bacterial flora within the rumen are said to be strictly anaerobic, but have been found to also be facultative anaerobic. These bacterial populations are important to ruminant life. If the animal were to be defaunated, the animal would have decreased performance. The bacterial populations within the rumen allow for degradation of cellulose, hemicellulose, and other nutrients unavailable to the monogastric. In the rumen, bacteria are estimated to constitute 50–90%, protozoa 10–50%, fungi 5–10% and archaea <4% of the total microbial biomass (Williams and Coleman, 1997). These microbes play a role in the degradation of feeds, protein synthesis, and B vitamin synthesis. Degradation via microbes will yield fermentation by-products useful to the animal. Within the rumen, a food bolus can be degraded into methane, carbon dioxide, VFA's, ammonia, amino acids, triglycerides, and heat. Among the various populations, the main bacterial populations that will be stated in this review

are the cellulolytic and hemicellulolytic populations that are important in degrading plant fibers that enter the forestomaches.

Cellulolytic Bacteria

Cellulolytic bacteria found to inhabit the rumen digest cellulose that enters their environment. Cellulose is a molecule that can only be broken down by microorganisms that contain cellulase. This makes it increasingly difficult for monogastric animals and other enzymatic digesters to harness the full caloric capability of cellulose containing feedstuffs. The three bacterial species considered to be the predominant cellulolytic species within the rumen are *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens* (Weimer 2022; Russel 2002; Hungate 1966). For cellulolysis to occur, these bacterial species must adhere to the surface of the feedstuffs that enter the rumen. Adherence of the bacteria to its substrate allow for the most efficient cellulolytic activity from the direct contact of cellulases to the substrate without loss from within the liquid layer (Lynd et al. 2002). The pH which supports optimal cellulolytic bacterial growth is a pH of 6-7. When ruminal pH drops below 6, growth of these species will decrease, and the growth of lactic acid producing bacteria will increase. In order for these colonies to grow efficiently, a source of free ammonia must be available. This is a frequent by-product of bacterial digestion of proteins in the rumen. Phenolic acids, carbon dioxide in the form of bicarbonate, and sulfur are also needed. Cellulolytic fermentation by these bacterial species will yield end-products like cellobiose, acetic acid, carbon dioxide, and ethanol and hydrogen.

Hemicellulolytic Bacteria

Hemicellulolytic bacteria also inhabit the rumen. Hemicellulose is unique in that it is a non-cellulose polysaccharide that is not recognized by mammalian enzymatic digestion. This means it cannot be broken down directly into glucose. Hemicellulose can only be broken down

into pentose sugars like xylose or six carbon sugars like galactose. Unlike cellulose, hemicellulose composition has been found to vary with different plant species (Van Soest, 1994). These compounds differ from the arrangement of their β-(1→4)-linkages. Cellulose forms in a straight chain, while hemicellulose will branch out in a less uniform manner. These glycosidic linkages are crucial in understanding how ruminant bacterial enzymes degrade these compounds. A polysaccharide that is also closely associated with hemicellulose is lignin (Sullivan 1966). Both lignin and hemicellulose form parts of plant cell walls and provide rigidity. These polysaccharides form covalent cross links within plant cell walls. The process of cross-linking between hemicelluloses and lignin occurs from the occurrence of two mechanisms: the incorporation of lignin by the coupling of ferulate to xylan in commelinid monocots, and incorporation of hemicellulosic glycosyl residues by re-aromatisation of lignification intermediates (Terrett and Dupree, 2019). As such, lignin will either form a cross-link with hemicellulose by the pairing of ferulate to xylan in ferulic acid containing plant cell walls or by incorporating hemicellulose residues with lignin intermediates. The hemicellulases produced in the rumen arise from some bacterial populations as well as some ciliate protozoa. Rumen hemicellulases are also more soluble than cellulases (Dekker and Richards, 1976). Hemicellulose is quite important in understanding the ruminant microbiome as it is a polysaccharide in the largest proportion beneath cellulose.

Protozoa

Besides bacteria, protozoa are also one of the more distinct inhabitants of the rumen. These microorganisms move via chemotaxis, are larger than bacteria in size, but are smaller in numbers. Among the various microbes that inhabit the rumen, protozoa make up 10-50% of total microbial biomass (Williams and Coleman, 1997). These protozoa also have an important function in ruminant health and microbial protein turnover. The first discovery of rumen

protozoa within literature was from a study done by Gruby and Delafond in 1843. The protozoa within the rumen can be classified into one of two ciliated species, holotrichs and entodiniomorphs. Holotrichs comprise 5-10% of the total ruminal protozoa populations, have cilia on the entire cell surface, and increase in numbers after feeding. Once feed enters the rumen, holotrichs detach from the rumen wall and migrate toward feed particles in solution. Many factors can change the overall make-up of holotrich protozoa. Composition and overall size of the rumen holotric population is determined by various interacting factors, the more important of which are the type of host, its geographical location, the diet consumed, and protozoal interspecies antagonisms (Williams 1986). Entodiniomorphs have cilia that are restricted to zones of the cell and make up 95% of protozoa within cattle. This species of protozoa is responsible for most of bacterial turnover within the rumen (Newbold et al., 2015). Entodiniomorphs are known for their predatory behavior toward the rumen's bacterial inhabitants. It has been estimated that in every minute, 0.1% of the rumen prokaryote population is digested by the rumen protozoal population (Coleman and Sandford, 1979; Anderson et al., 2023). An in vitro study performed by Ranilla et al. in 2007 detailed the effects of the predatory behavior of protozoa on the rumen microbiome. It was found that the presence of protozoa increased the concentration of ammonia and altered the VFA balance with more acetate and butyrate produced at the expense of propionate (Ranilla et al., 2007). Understanding the predatory behavior of these ciliates and their effects on the amount of energy available to the animal is important in understanding potential methods of decreasing total emissions from ruminants.

Fungi

Other inhabitants of the rumen are fungi. The fungi found within the rumen are cellulolytic and are the primary fiber digestors within the rumen (Wang et al. 2022). Although

fungi only make up 20% of the biomass within the rumen, they are regarded as the most efficient contributor to fiber digestion (Rezaeian et al., 2004). A study done by Bouchop in 1979 used an in-situ method to learn about the microorganisms that inhabit the rumen. In this study, partially digested plant fragments were obtained from fistulated bovids and ovids. It was found that large numbers of phycomycetous fungal zoospores were found attached to the fibrous plant fragments, particularly the vascular tissues of those plant fragments. Bouchop credits an earlier study in proposing the presence of fungi in the rumen. This study, performed by Orpin in 1975, proposed that some of the sheep rumen microorganisms believed to be protozoan flagellates were in fact zoospores of anaerobic phycomycetous fungi. It is later stated that the fungi proposed in the 1975 study remained classified as protozoa.

The Ornithine Cycle

The ornithine cycle, or urea cycle, was first discovered by Hans Krebs and Kurt Henseleit in 1932. It was the first metabolic cycle to be discovered and explains the synthesis of urea within mammals. The urea cycle transforms the toxic ammonium (NH_4^+) ion into urea and ornithine. Urea is an organic compound produced within the liver from ammonia, is useful in the excretion of nitrogenous waste from the body, and is water soluble (Adeyomoye et al., 2022). Ornithine is a non-essential amino acid that is an intermediate within the urea cycle. It is a key substrate for organic acids, most notably, citrulline (Sivashanmugam et al., 2017). Ammonium is produced by the deamination of amino acids and is fatal in high concentrations. Within ruminants, the urea cycle begins with the free ammonium ions available inside the rumen. These ammonium ions travel to the liver via the blood stream to ultimately be converted to urea and ornithine. Free ammonium undergoes a chemical reaction to form carbamoyl phosphate. This reaction requires ATP and carbamoyl phosphate synthase (the catalyst) to begin. Carbamoyl

phosphate then combines with ornithine, a non-protein amino acid to form another non-protein amino acid called citrulline. Citrulline can create another amino acid called arginine by undergoing a series of chemical reactions with specific enzymes. If the body does not need arginine, the molecule is hydrolyzed to once again create urea or ornithine, thus completing the urea cycle. Urea is important in regulating the amount of free ammonia within the blood of the animal, useful in concentrating urine, and is ultimately excreted from the animal.

Nitrogen Recycling

The urea that is metabolized within the liver is heavily involved in the process of removing metabolic waste or retaining it for the animal. Retention of nitrogenous waste and its recycling allow ruminants to be able to survive in areas of low nutritional protein. This process is known as nitrogen recycling, and it refers to the reabsorption of nitrogenous compounds within the ruminant animal when feed nitrogen levels are poor. The process of nitrogen recycling revolves around the reuse of the urea synthesized from protein digestion in the rumen and its ammonia reduction in the liver. Once synthesized in the liver, urea can be transported to either the urine to be excreted when protein levels are high or to the saliva and rumen to be re-digested and absorbed to yield ammonia for nitrogen metabolism when feed protein levels are low.

Urine

Once urea is produced from the liver, it will leave via the vena cava to be either excreted through urine or recycled back into the body via the rumen, gut walls, or salivary glands. One of the methods urea is excreted from the ruminant animal is from production of urine by the animal. Once urea is formed and leaves the liver via the vena cava, it will enter the kidneys and be bound to urea transporters. Urea transporters are proteins that are located in the renal medulla and play a central role in urea excretion and water balance within the kidney (Hediger et al., 1996). Urea transporters allow for the accumulation of urea within the renal medulla and allows the kidney to

concentrate urine to allow for a high concentration of urea within a small volume of water. Urea excretion through the kidneys can fluctuate with changes in blood urea levels. Once blood urea is too high, the body will increase urea concentrations into the kidneys and allow for subsequent excretion via urination.

Saliva

All mammalian species utilize saliva to lubricate food and assist the process of mastication for ingestion. Within the ruminant, saliva is used as a buffer as the animal does not have the capability to secrete saliva within its forestomach tissues. Of the salivary glands present within the ruminant, the lingual and submaxillary salivary glands secrete saliva of a low alkalinity and high mucous composition while the parotid salivary gland secretes a saliva of high alkalinity and high buffering capability (McDougall, 1947). Urea present within the saliva allow for its buffering capability. It has been estimated that under normal conditions, 60-70% of sheep saliva can contain urea and no ammonia (Somers, 1961). This allows for the excellent buffering ability of saliva. Since nitrogen within the saliva is mostly urea, it can be assumed that an increase in nitrogen present in the rumen can indicate an increase in salivary urea, it is not useful in determining the quantitative amount of nitrogen consumed by the animal. This is due to microbial cell protein utilized and degraded by ruminants.

Rumen Degradation

It is known that urea that enters the rumen is quickly degraded to yield ammonia. Urea that is reintroduced to the rumen via rumen blood flow also utilizes that same degradation mechanisms. The transport of urea back to the animal is important for the overall retention of nitrogen within the body. Once diffusing back through the rumen papillae and into the rumen, urea will become hydrolyzed by bacterial urease to be converted back into free ammonia (Berends et al., 2018). Ammonia can be used by the bacterial population within the rumen to synthesize proteins that

the animal can use for anabolic purposes. In the rumen, free ammonia can be used by microbial cells or transferred back through the rumen wall to restart the cycle of nitrogen recycling. Increased microbial cells will increase the available MCP available to the animal and will either influence nitrogenous retention or excretion.

Protein Metabolism

Protein that is used by the animal can be classified into non-protein nitrogen (NPN), plant and animal protein, and microbial cell protein (MCP). Once ingested, the availability of each type will change depending on the location of digestion. Protein digestion within the ruminant starts at the mouth, where mastication grinds the animal's chosen feedstuffs into smaller particles. These ground particles, with an increased surface area from the process of mastication, enter the reticulorumen and are further digested. Dietary protein entering the rumen is broken down rapidly to yield peptides and amino acids. This results in ammonia formation and the subsequent loss of N from the animal (Walker et al., 2005). Microbes within the rumen will attach to feed particles and create various fermentation end-products that the animal can use. Rumen degradable protein (RDP) within the reticulorumen is reduced into amino acids that can be synthesized into volatile fatty acids. Volatile fatty acids are one of the most energy dense molecules the ruminant animal can use. VFA's can pass through the rumen gut wall or be turned into ammonia for microbial cells to use. The ammonia and amino acids synthesized from fermentation can readily pass through the rumen wall and be absorbed via the bloodstream. Ammonia, with the addition of the free carbon skeleton from protein digestion in the rumen, can be synthesized back into VFA's thus allowing for efficient energy production. Once rumen undegradable protein (RUP) exits the reticulorumen, it enters the omasum. From the omasum, the feed particles will then enter the abomasum, or the "true stomach". Within the abomasum, the

distention of the abomasum or vagal nerve stimulation causes an increased release of gastrin. The vagal nerve is important in the regulation of various digestive processes. It has been found that with stimulation it can stimulate acid production (Lloyd et al. 1993), increase pancreatic secretions (Li and Owyang, 1993), inhibit the emptying of the stomach (Schwartz and Moran 1998), and send signals to the brain to inhibit the intake of food (Moran et al. 1997). Gastrin is produced by the pyloric antrum and causes the abomasum to increase gastric secretions from the parietal cells and chief cells of the abomasum. The parietal cells increase HCl production, and the chief cells increase pepsinogen secretion within the abomasum. Pepsinogen is a gastric enzyme in its zymogen form. Once contacted with HCl, pepsinogen undergoes allosteric modification and becomes pepsin. Pepsin acts on the protein and breaks peptide bonds and interrupts R groups by breaking the bond between the protein's amino end and carboxyl end. As the protein is denatured by the low pH of the abomasum, the surface area of the protein increases and allows pepsin to act more efficiently. The pylorus then opens, allowing digesta to enter the duodenum. From this point forward, any formation of ammonia from protein digestion is regarded as midgut and hindgut ammonia formation.

As digesta enters the duodenum along with stomach acid, the acidification of the duodenum causes the duodenal mucosal cells to secrete secretin. Secretin was first identified by in 1902 by Bayliss and Starling. It acts on the pancreas to make the pancreas secrete more pancreatic enzymes as well as bicarbonate to de-acidify the duodenum (Christodoulopoulos et al., 1961). The pancreatic enzymes specific to protein digestion that are released from the pancreas from the binding of secretin are the zymogens trypsinogen, chymotrypsinogen, and procarboxypeptidase. In addition to pancreatic enzymes stated, there are digestive enzymes released from the small intestine brush border that assist in protein digestion. Enterokinase and

aminopeptidase are enzymes released by the brush border that act on polypeptides present in the digesta to further digest the dietary protein. Aminopeptidase converts polypeptides into amino acids and dipeptides, while enterokinase acts on trypsinogen to convert it into its active enzymatic form of trypsin. Trypsin acts on chymotrypsinogen to yield chymotrypsin, and chymotrypsin acts on procarboxypeptidase to yield carboxypeptidase. The active forms of these enzymes break down polypeptides into tripeptides, dipeptides, and most importantly, amino acids. These molecules can be absorbed through the walls of the small intestine and large intestine through the intestinal epithelial cells and released into the blood. Nitrogen metabolism begins during or once all the previous events have occurred. Within the rumen, the carbon skeleton yielded from the microbial breakdown of RDP can be synthesized into the amino acids needed for the bacterial populations to survive within the rumen. In order for the bacterial populations to synthesize amino acids from this carbon skeleton, bacteria need sulfur, a carbon source, a source of energy (commonly a CHO source), and ammonia. This allows the bacterial populations to synthesize any amino acid that is needed for those populations to grow. Once those bacterial populations turn over or are lysed during death, the amino acids within those bacterial cells can be used as a source of amino acids for the ruminant animal.

Effects of Zeolites on Nutrient Pathways

The efficacy and outcome of zeolites used within the body is dependent on the type of zeolite used, the degree of dispersion within the animal, and the size of the granules used. A study was conducted by White and Ohlrogge in 1983 to patent the use of zeolites in ruminants to increase the use of NPN within the diet without causing toxic effects from ammonium. It is stated that the degree of dispersion of zeolite is of major importance, and that powder or granular form zeolites take up and release ammonium ions substantially more rapidly than pellet form particles (White and Ohlrogge, 1983). It is further stated that the ion exchange ability of zeolites

are inhibited by rumination activity. The process of rumination will create sodium rich saliva that will disturb the sodium-ammonium equilibrium between the zeolite and ruminal fluids. This interaction will displace some of the ammonium ion from the zeolite (White et al. 1974). A study performed by Kotoulas et al. in 2019 found that zeolite particle size is associated with ammonium adsorption rate. It was reported that as particle size decreased, the ammonium adsorption rate increased due to an increased surface area (Kotoulas et al., 2019). Zeolites have also been proposed to be a low-cost buffer replacement for sodium bicarbonate in ruminant diets. A study performed by Dschaak et al. in 2010 found evidence that supplementation with zeolite to lactating dairy cows had no negative effects on productive performance and ruminal fermentation. Zeolite supplementation trended toward a reduced VFA production and increased milk protein (Dschaak et al., 2010). A study performed by Sherwood and Klopfenstein in 2005 evaluated the effects of clinoptilolite in finishing crossbred steers. Statistically, there were no differences in control and zeolite treatments, but it is stated that numerically, the zeolite steers had a 3.4% increase in ADG over the control group (Sherwood and Klopfenstein, 2005). A study performed by Koknaroglu et al. in 2005 evaluated the effects of zeolites on the feedlot performance of Brown Swiss Cattle. It was observed that there were no effects on gain efficiency between treatments (0.114 vs 0.116 kg gain/kg DMI) but found that there was a trend of increased average daily gain and feed intake on lighter cattle compared to their heavier counterparts. In that same study it was found that steers supplemented with zeolite had a trend of increased ADG and feed intake compared to the control group. Previously published papers based on feeding zeolites to cattle have differing results within the literature. This may be a result of differing diet types or differing methods of delivery. Most published papers do not disclose what purity or type of zeolite used within the study. The effects of zeolites on the animal needs to

be further studied in order to identify the efficacy of the mineral on ruminant performance characteristics and the resulting effects on the emissions released.

Post-Abomasal Digestion and Pathways

Nutrient use within the intestines

Nutrients broken down from digestive processes are absorbed through either rumen epithelia or intestinal epithelia. The epithelia are commonly referred to as papillae and are finger-like projections of epithelial tissue that possess absorptive properties. The functional units of these papillae are called enterocytes and are the site of aerobic synthesis of ATP for use within the animal. Rumen papillae are vital in the process of nitrogen recycling in ruminant animals. They also act as a protective barrier to protect the host from microorganisms, toxins, and chemicals in the lumen and to prevent unregulated movement of these compounds into the lymphatic or portal circulation (Gäbel et al., 2002). As ammonia is created within the rumen, the rumen papillae absorb NH_3 via diffusion. Yet, the transport of nutrients across rumen epithelium is not considered to be purely passive (Graham and Simmons, 2005). Absorption within the rumen is possible due to the distinct structure of rumen epithelia. There are four different layers of epithelial tissues that make up the rumen papillae. From outermost layer to innermost layer, there is the stratum corneum, stratum granulosum, stratum spinosum, and stratum basale. The stratum corneum contains no nuclei or mitochondria and can be sloughed off to associate with the rumen digesta. The stratum granulosum is comprised of flat epithelial cells that are connected by very tight junctions that allow for selective absorption of molecules and nutrients in and out of the rumen. Cells within the stratum spinosum layer are tortuous in shape, have mitochondria, and become oval shaped toward the stratum granulosum and stratum basale. The stratum basale contains more mitochondria than the stratum granulosum, is comprised of columnar epithelium, and is regarded as a site of energy metabolism (Na and Guan, 2022). These layers of epithelial

tissue that form the structure for rumen papillae are not well studied due to the difficulty in separating each epithelial layer.

Intestinal Digestion and Metabolism

Protein digestion

Complete digestion of protein within the ruminant animal depends on the type of protein that is contained within the ruminant diet. The type of protein found in ruminant diets can be simplified into 3 distinct categories. Rumen degradable protein (RDP) is protein that is readily digested in the rumen. Rumen undegradable protein (RUP) is not readily digested within the rumen and needs to enter the abomasum to be broken down into simpler parts to be further digested. Non-protein nitrogen, or NPN is a form of protein that can be added to increase nitrogen levels in the diet. The most common form of NPN is urea and it is readily digested within the rumen. Another form of protein available to the ruminant animal is microbial cell protein (MCP). This form of protein is the amino acids from direct lysis of bacterial populations that escape the rumen.

Digestion of RDP begins within the rumen after mastication and deglutition of the food bolus. Within the reticulorumen, this form of protein is hydrolyzed into amino acids, NH_3 and a carbon skeleton. Compounds like amino acids and ammonia can be readily absorbed within the rumen via the rumen papillae to enter the blood stream. The carbon skeleton resulting from protein digestion can be used by the rumen bacteria to yield more microbial cell protein for the animal to use. In order for the synthesis of amino acids by bacteria to occur, rumen bacteria need a source of energy in the form of a carbohydrate, a source of ammonia and sulfur, as well as a carbon skeleton. With these components, rumen bacteria can synthesize amino acids for the ruminant animal to use.

Digestion of RUP begins at the abomasum. The highly acidic environment of this ruminant stomach compartment allows for the denaturation or unfolding of the amino acids within the food bolus that was ingested. Within the abomasum, HCl and pepsinogen are secreted. When there is distention of the abomasum or vagal nerve stimulation, gastrin is produced and secreted by the pyloric antrum to allow for more gastric secretions within the abomasum. Pepsinogen is a zymogen that undergoes allosteric modification when in contact with HCl. Pepsinogen is converted into pepsin by contact with HCl and will act on proteins by interrupting R groups via the breaking of the bonds of the amino end and carboxyl end of the protein. HCl allows for the denaturation of the protein to increase the total surface area. This process allows for pepsin to be more efficient in disrupting the amino acid bonds. The breaking of peptide binds by pepsin will cause the protein to break down into simpler components, notably polypeptides and amino acids.

From the abomasum, the undigested protein enters the duodenum. Once the duodenum is acidified from the abomasal acid, the dudodenal mucosal cells secrete secretin. Secretin is a hormone that is released by the duodenal mucosal cells and enters the bloodstream to bind to the pancreas. The binding of secretin to the pancreas causes the pancreas to create and secrete more enzymes for digestion and secrete bicarbonate to neutralize the stomach acid. Like the digestion of glucose, protein digestion begins at the mouth and the bulk of RUP digestion begins in the abomasum. In the abomasum, protein in the partially digested food bolus will unravel in the acidic environment of the abomasum. This process is known as denaturation of protein and is important in the complete breakdown of the protein components in the food bolus within the abomasum.

Protein that is utilized by the animal can be classified into non-protein nitrogen (NPN), plant and animal protein, and microbial cell protein (MCP). Once ingested, the availability of each type will change depending on the location of digestion. Protein digestion within the ruminant starts at the mouth, where mastication grinds the animal's chosen feedstuffs into smaller particles. These ground particles, with an increased surface area from the process of mastication, enter the reticulorumen and are further digested.

Microbes within the rumen will attach to feed particles and create various fermentation end-products that the animal can use. Rumen degradable protein (RDP) within the reticulorumen is reduced into amino acids that can be synthesized into volatile fatty acids (VFA's). Volatile fatty acids are one of the most energy dense molecules the ruminant animal can use. VFA's can pass through the rumen gut wall or be turned into ammonia for microbial cells to use. The ammonia and amino acids synthesized from fermentation can readily pass through the rumen wall and be absorbed via the bloodstream. Ammonia, with the addition of the free carbon skeleton from protein digestion in the rumen, can be synthesized back into VFA's thus allowing for efficient energy production. Once rumen undegradable protein (RUP) exits the reticulorumen, it enters the omasum.

From the omasum, the feed particles then enter the abomasum, or the "true stomach". Within the abomasum, the distention of the abomasum or vagal nerve stimulation causes an increased release of gastrin. Gastrin is produced by the pyloric antrum and causes the abomasum to increase gastric secretions from the parietal cells and chief cells of the abomasum. The parietal cells increase HCl production, and the chief cells increase pepsinogen secretion within the abomasum. Pepsinogen is a gastric enzyme in its zymogen form. Once contacted with HCl, pepsinogen undergoes allosteric modification and becomes pepsin. Pepsin acts on the protein and

breaks peptide bonds and interrupts R groups by breaking the bond between the protein's amino end and carboxyl end. As the protein is denatured by the low pH of the abomasum, the surface area of the protein increases and allows pepsin to act more efficiently. The pylorus then opens, allowing digesta to enter the duodenum. From this point forward, any formation of ammonia from protein digestion is regarded as hindgut ammonia formation.

As digesta enters the duodenum along with stomach acid, the acidification of the duodenum causes the duodenal mucosal cells to secrete secretin. Secretin acts on the pancreas to make the pancreas secrete more pancreatic enzymes as well as sodium bicarbonate to de-acidify the duodenum. The pancreatic enzymes specific to protein digestion that are released from the pancreas from the binding of secretin are trypsinogen, chymotrypsinogen, and procarboxypeptidase. In addition to pancreatic enzymes stated, there are digestive enzymes released from the small intestine brush border that assist in protein digestion. Enterokinase and aminopeptidase are enzymes released by the brush border that act on polypeptides present in the digesta to further digest the dietary protein. Aminopeptidase converts polypeptides into amino acids and dipeptides, while enterokinase acts on trypsinogen to convert it into its active enzymatic form of trypsin. Trypsin acts on chymotrypsinogen to yield chymotrypsin, and chymotrypsin acts on procarboxypeptidase to yield carboxypeptidase. The active forms of these enzymes break down polypeptides into tripeptides, dipeptides, and most importantly, amino acids. These molecules can be absorbed through the walls of the small intestine and large intestine through diffusion. The free peptides created from RUP digestion can interact with urea that has entered through the intestinal lumen. This process will yield ammonia and hydrogen that will be converted into ammonium and excreted through the feces of the animal or converted into

urea, where it will either be reintroduced into the rumen as a non-protein nitrogen source or excreted through the urine.

Carbohydrates

The digestion of carbohydrates within the ruminant animal relies on its intake form. Simple carbohydrates like starches are easily broken down within the rumen. Complex carbohydrates like cellulose and hemicellulose must be broken down further to be absorbed. These complex carbohydrates have the potential to escape the rumen to become rumen bypass starch, but it's rate of absorption will decrease. Approximately 40% of rumen bypass starch cannot be digested and utilized by ruminants (Moharrery et al., 2014). Like all other nutrient forms, the digestion of carbohydrates begin within the mouth with mastication and deglutition. Once ingested, the food bolus enters the reticulorumen where bacteria begin the process of fermentation of carbohydrate compounds, mainly starch and cellulose contained within the ruminant diet. Cellulose is broken down by microbes to yield cellobiose that can be further degraded into glucose. Glucose is rapidly absorbed within the rumen. VFA's are a byproduct of bacterial fermentation of these carbohydrates along with heat, carbon dioxide, and methane. Rumen bypass starch will enter the small intestine to be broken down into simpler components. The small intestine is the primary site of digestion of and absorption of escape starch, lipids, RUP, and MCP besides the VFAs absorbed within the forestomaches (Harmon and Swanson, 2020). Pancreatic amylase is the main driver of the digestion of complex carbohydrates and escape starch within the small intestine. Pancreatic amylase acts on these carbohydrates by hydrolyzing the bonds between the glucose molecules within complex carbohydrates. This process will break these complex carbohydrates into smaller units. Pancreatic amylase specifically targets the alpha-1, 4 glycosidic linkages present in starches (Fend, 2023). The resulting subunit varies depending on the complex carbohydrate broken down. Common starch

forms that may enter the small intestine depending on diet fed are amylose, amylopectin, lactose, or sucrose. Amylose and amylopectin are common plant starches that can escape the rumen to be digested in the small intestine. The digestive enzyme that acts on amylose and amylopectin is pancreatic amylase. When hydrolyzing the alpha-1, 4 glycosidic linkages present, amylose will yield two maltose molecules and amylopectin will yield a maltose and an isomaltose molecule. Maltose is a natural disaccharide consisting of two molecules of glucose joined by an alpha-1, 4 glycosidic linkage (Li et al., 2022). Maltose can be acted on further with the enzyme maltase to yield two glucose molecules. Isomaltose, when acted on by isomaltase, will yield two molecules of glucose. Ingestion of lactose by the ruminant animal is typical of newborns but not of adults. Lactose is another type of disaccharide that must be broken down by the enzyme lactase to be efficiently digested. This reaction of enzyme and substrate will yield glucose and galactose (Mirzaei-Aghsaghali, 2011). This process must occur before it can be absorbed within the small intestine to be utilized by the animal. Sucrose is a disaccharide utilized within the diet as a source of readily fermentable energy (Ravello et al., 2022). Sucrose is a molecule of fructose and glucose bonded by an α-1,4 glycosidic bond (Campbell et al., 2005). Sucrose can be enzymatically cleaved into the respective glucose and fructose molecules via sucrase or isomaltase glycoside hydrolases (White Jr. 2018). The glucose and fructose molecules are rapidly absorbed by the body. The fructose molecule does not follow the same physiologic pathway of glucose as it is a lipogenic sugar. Fructose does not stimulate the release of insulin and will be transported to the liver to produce glycogen, lactate, glucose, and triglycerides (White, 2018). Digestion of these complex carbohydrates and the breakdown of their subunits will occur within the small intestine if not already fermented within the rumen. Once these sugars are fully reduced, the body can absorb these nutrients via diffusion through the intestinal wall. Any

undigested carbohydrates may be fermented within the large intestine if they escape in large amounts. Absorption will not be as efficient, and the remaining carbohydrates will be excreted from the animal as waste.

Lipids

Fat is mainly digested within the small intestine, but some important processes must occur before fat can be fully digested and absorbed by the ruminant animal. As with all digestion processes, digestion of fats begins in the mouth where mastication occurs to increase surface area and decrease particle size of the feed. In the mouth, lingual lipase is secreted to cleave fatty acids from the glycerol backbone to yield a monoglyceride and two fatty acids. Within the rumen, biohydrogenation occurs to convert unsaturated fats into saturated fats. Unsaturated fats are those that contain one double bond or more within the fatty acid carbon framework. Saturated fats are fats that contain no double bonds within its framework and are saturated with hydrogen. The trans-configuration within an unsaturated fatty acid is described as a double bond between a carbon-to-carbon bond, where the hydrogens connected to those carbon-carbon double bonds are on opposite sides. The cis-configuration is similar to the trans configuration, but instead of containing the hydrogen ion to opposite sides, both hydrogens are on the same side of the molecular plane. The process of biohydrogenation is described as the exchange of electrons to remove the carbon-carbon double bond to allow for the addition of hydrogen to the fatty acid molecule. As an unsaturated fatty acid is biohydrogenated, it becomes saturated. The term saturated can be differentiated from the unsaturated state due to the removal of double bonds to "saturate" the fatty acid with hydrogens. Once a triglyceride or fatty acid is saturated, it can move into the omasum and abomasum where triglycerides can be further digested. Within the abomasum, there is some gastric lipase that can act on a triglyceride to yield more fatty acids. Once fat enters the duodenum, pancreatic lipase acts on triglycerides to yield two fatty acids and

a monoglyceride. Bile salts produced by the liver and stored in the gallbladder are secreted into the duodenum to act on the monoglycerides and fatty acids produced from pancreatic lipase digestion of fats. These bile salts bind to the fatty acids and monoglyceride to make them soluble in water. Bile salts are amphipathic meaning hydrophobic and hydrophilic. The end-product of this binding process creates a soluble product called a micelle and allows this fat molecule to be absorbed.

Absorption

Once a micelle is created, it can be absorbed by the small intestine through the enterocytes found within the small intestine epithelia. The enterocyte is the functional unit of the small intestine and is the site of many metabolic processes. As the process of lipid metabolism continues, the micelle will deposit its contents into the enterocyte to allow for the eventual creation of a triglyceride. The two fatty acids within the micelle can undergo either the phosphatidic acid phosphatase pathway (PAP) or the monoacylglycerol pathway (MAG). The PAP pathway is upregulated within normal metabolic conditions, while the MAG pathway is downregulated. The PAP pathway is the process of converting the two fatty acids from the micelle into a triglyceride by the addition of a glycerol backbone from glucose metabolism. During glycolysis, a glycerol backbone can be yielded from the triose. This glycerol backbone can be used to form a triglyceride within the enterocyte. The MAG pathway is similar to the PAP pathway, but the glycerol backbone can only be formed from components in the diet. It cannot use amino acids, glucose, or other fats to create a glycerol backbone for the formation of a triglyceride in the enterocyte. Once a triglyceride is formed within the enterocyte it will be deposited into a very-low-density lipoprotein or VLDL so that it can move freely in the lymph. The VLDL contains both cholesterol and triglycerides and is transported from the bloodstream to adipose cells. High-density lipoproteins or HDLs are used to add lipoproteins to the VLDL. The

VLDL deposits fatty acids into the adipose cell by a membrane bound protein called lipoprotein lipase or LPL. Within the adipose cell, the fatty acids are converted back to triglycerides by the glycerol backbone created by glycolysis. The resulting low-density lipoprotein (LDL) created from the deposition of fat from the VLDL will transport the cholesterol contained in its structure to either tissues or the liver where it is recycled.

Conclusion

The metabolism and nutrient pathways of the ruminant is complex and has been heavily studied within the literature. The mechanisms of digestion and absorption are a vast network of individual pieces that work together to support the animal's fitness. The end-products of these mechanisms, and their contributions to the environment can cause detrimental effects to the surrounding areas. The release of nitrogenous wastes and other greenhouse gases are a topic of study within current literature. A low-cost and environmentally safe method must be implemented to slow or reverse the effects of, or reconstitute the released form of, these animal waste products without inhibiting the animal's normal metabolic processes. Zeolites like clinoptilolite have been proven useful in lab settings and have been proven to be effective in the adsorption of heavy metals and nitrogenous wastes, but its application to food animal research has had varied results. The limited data supplied within the literature as well as the dissimilar results presented within a live animal setting may be detrimental to the success of implementing this mineral as a method to improve production traits and decrease livestock emissions. In order for clinoptilolite to be regarded as a useful product within the animal, the literature must reflect replicable studies of similar methods of delivery, breed, age, and sex of animal used, as well as the mineral's commensal effects. Until that time, zeolites may only provide useful to productions that prefer it as an alternative low-cost mineral supplement.

CHAPTER 2: THE EFFECTS OF ZEOLITES (CLINOPTILOLITE) ON NUTRIENT USE IN FISTULATED BEEF STEERS

INTRODUCTION

Ammonia is a major by-product of protein digestion that volatilizes into the atmosphere when excreted. Concern over the amount of nitrogenous waste excreted from ruminant livestock animals grow over the effects on the environment. Facilitating the method of ammonia removal within the rumen has been proposed as a method of decreasing total ammonia emissions to combat this concern of increased nitrogenous emissions by cattle. Most of the ammonia excreted from livestock operations were produced by the hydrolysis of urinary urea to form ammonium and carbon dioxide (Mobley et al., 1995). In addition to the urinary excretion of nitrogen, the nitrogen within excreted manure has also been linked to negative effects on the environment. Manure N from cattle contributes to nitrate leaching, nitrous oxide, and ammonia emissions emitted into the environment (Bougouin et al., 2022). Thus, an adequate method of removal and/or sequestration of nitrogenous waste within the environment is needed.

A possible method of decreasing nitrogenous emissions would be the application of zeolite to the ruminant diet. The proven use of zeolites in wastewater treatments (Pérez-Botella et al., 2022) and use in agriculture to improve soil quality after excess fertilization (Cataldo et al., 2021) gives rise to the potential uses in-vivo to retain waste products and decrease nitrogenous waste into the environment. Numerous theories have been proposed and tested within laboratory settings. Few papers exist highlighting the effects of zeolite on the live animal. There are numerous contradictions within papers published on the use of zeolites in-vivo. Thus, finding

methods to decrease the total amount of nitrogenous waste excreted from the live animal is necessary.

MATERIALS AND METHODS

This study was conducted between July and September of 2023 at Colorado State University's Agricultural Research Development and Education Center (ARDEC) in Fort Collins, Colorado. All methods were approved through the Institutional Animal Care and Use Committee (IACUC).

Nine fistulated angus steers and three fistulated ½ angus ½ Hereford steers were transferred to an enclosed barn after three weeks on either zeolite (clinoptilolite) or limestone supplementation. Supplementation was administered through a rumen cannula once a day for four weeks. Supplement consisted of either 0.2 kg of limestone once per day or 0.2 kg of clinoptilolite once per day, directly into the rumen via rumen cannula. After 3 weeks of treatment supplementation in an open dirt pen, each steer within the group was moved to an enclosed barn for 5 days. Each steer within the enclosed barn was housed in an individual stall (87cm x 214cm) with free access to water from water feeding cups. The steers were fed once per day for three weeks when in an open dirt pen, and then twice per day when housed in individual stalls. When in individual stalls, the steers were fed an *ad libitum* diet with access to 110% of the previous day's fed amount if leftover feed was not present.

On the first day of the study, the steers were assigned to two groups by weight, and then randomly assigned to either a treatment group or control group. The mean weight among all steers on day 1 of supplementation was 666.25 kg (624.84, 707.65). Group 1's treatment offset Group 2 by one week to allow for ample working room within the enclosed barn. Treatment

application of each group was randomly selected. Treatments were alternated after each animal finished their balance trial within the enclosed barn, yielding an experimental unit of steer within period and group. During the first three weeks of their respective supplementation, Group 1 and 2 were allowed to exercise freely in an open dirt pen for as long as they wanted. Each steer had access to socialize when in the open dirt pen. After 3 weeks of supplementation, each steer within the group selected was moved to the enclosed barn for the 5 day collection period.

Balance Trial

Within the enclosed barn, each stall was outfitted with a feed bunk and waterer cups that could be activated when the steers pushed their noses to the nozzle. The waterers and steers were checked three times each day to make sure water was clean and free of debris, and that no steer succumbed to dehydration, sickness, or injury. Water was also checked to make sure it was provided *ad libitum*. Feed that had fallen out of the bunk was returned to the steer's feed bunk to allow accurate ORTs measurements. Each stall was outfitted with a slatted floor with a rubber mat on top to increase welfare and decrease the potential for injury due to loose footing.

Total collections of urine, feces, and ORTs were conducted during a 5-day period. ORTs, feces, urine, and feed were collected each day for 5 days after the three weeks of direct supplementation into the rumen. ORTs, feces, urine, and feed were weighed following exact feeding times within 10-minute increments to keep data collection as accurate as possible within a 24-hour time frame. ORTs are the amount of feed left over after 24 hours. This term is synonymous with the amount of feed that the steer refuses to eat after each 24-hour time period. ORTs were collected to determine dry matter intake and dry matter digestibility of each individual steer. Feces and urine were collected to determine the digestibility of various nutrients in the diet such as N, P, TDN, ADF, and NDF

Urine collection from each steer was completed using a urine collection harness and was aspirated into a polypropylene jug under a vacuum. Each jug contained 100 mL of 6N HCl to prevent volatilization and loss of ammonia. Feces were collected using a canvas bag fastened to a harness. Urine, feces, and orts were collected daily and weighed. A 5% sample of daily feces and urine were taken from each steer's total and then frozen. Each 5% subsample of each feces, ORTs, and urine were composited and frozen for future analysis. Composited ORTs, feces, and a weekly feed sample were then dried in a forced air oven at 60 degrees Celsius to determine DM content. The dried samples were ground in a Wiley Mill (Arthur Thomas Co., Philadelphia, PA) With a 2mm sieve. Samples were then sent out for analysis after drying and grinding. Total N content, P content, NDF, ADF, and DM content of feces, ORTs, and a feed sample were subsequently tested.

Statistical Analysis

Feces, feed, ORTs, and urine were analyzed using the Dairy One Forage Analysis service. Feces, feed, and ORTs were analyzed for % DM, N, P, ADF, NDF, and TDN for each individual animal. Urine was analyzed for % urea and % total nitrogen for each individual animal. The experimental design of this study was a randomized block crossover design, where our experimental unit was steer within period and group. We fit a one-way ANOVA model with nutrient use and excretion variables as our outcome, and period, group, and treatment as predictors. Period, group, and treatment were treated as fixed effects. Significance of heterogeneity was assessed using an F-test. A significance value of 0.05 was used to determine the significance of data, and values below 0.1 reflected a trend toward significance. The model fit was completed using R, using the "performance" package and group means were calculated using the "emmeans" package.

Dry Matter Calculations

Dry Matter Intake (DMI) was calculated by subtracting the amount of dry matter fed to each animal, each week, by the ORTs recovered by each animal, each week. The value for dry matter fed was calculated by multiplying the total amount of dry matter fed each week by percent dry matter of the feed offered. ORTs recovered was calculated by multiplying the total amount of dry matter fed that was untouched by each animal (ORTs), each week by the percent dry matter of the ORTs recovered. DMI was converted from lbs/d to g/d by multiplying the DMI value by a conversion factor of 453.592. Dry Matter Digestibility (DM%) was calculated by subtracting DMI g/d by the Fecal DM g/d, then dividing this computed value by DMI g/d. This value is multiplied by 100 to get a percentage. Fecal DM g/d was calculated by drying weekly fecal amounts and subtracting weight of feces before drying by the weight of feces after drying. Daily fecal amounts were calculated by dividing total g of feces for that period by the 5-day feeding period. Fecal DM, % was calculated by dividing Fecal DM g/d after drying by Fecal g/d before drying for that period.

ADF and NDF Calculations

ADF intake was calculated by multiplying DM offered g/d by % ADF analyzed from the feed. This value is subtracted by the product of ORTs g/d and % ADF of ORTs. Fecal ADF g/d was calculated by multiplying Fecal DM g/d by the Fecal ADF % found by fecal analysis. ADF Digestibility % was calculated by multiplying ADF intake g/d by Fecal ADF g/d and dividing that value by ADF intake g/d. This calculated value is multiplied by 100 to yield a percentage.

NDF intake was calculated by multiplying DM offered g/d by % NDF analyzed from the feed. This value is subtracted by the product of ORTs g/d and % NDF of ORTs. Fecal NDF g/d was calculated by multiplying Fecal DM g/d by the Fecal NDF % discovered by fecal analysis.

NDF digestibility % was calculated by multiplying NDF intake g/d by Fecal NDF g/d and dividing that value by NDF intake g/d. This calculated value was multiplied by 100 to yield a percentage.

Nitrogen Calculations

Nitrogen intake was calculated by multiplying DM g/d by the Feed N % found by laboratory analysis. This value is subtracted by the product of ORTs DM g/d and ORTs N % found by laboratory analysis to yield N intake g/d. Nitrogen digestibility % was calculated by subtracting N intake g/d by Fecal N g/d. This value is divided by N intake g/d and multiplied by 100 to yield N digestibility %. N Balance was calculated by subtracting N intake g/d by Fecal N g/d and Urine N g/d. Urine N g/d was calculated by multiplying urine g/d by the urine N % found by laboratory analysis. N balance is a measure of

Phosphorus Calculations

P intake was calculated by multiplying DMI g/d by the Feed P % found by feed analysis. This value is subtracted by the product of ORTs DM g/d and ORTs P% found by laboratory analysis to yield P intake g/d. P digestibility % was calculated by subtracting P intake g/d by Fecal P g/d. This value is divided by P intake g/d then multiplied by 100 to yield P digestibility %. P balance was calculated by subtracting P intake g/d by Fecal P g/d and Urine P g/d. Urine P g/d was calculated by multiplying urine g/d by the urine P % found by laboratory analysis. P balance is a measure of

RESULTS

Results reflect a 5-day total collection of urine, ORTs, and feces with the exception of 2 occurrences. A steer within the first group and first period was thrown out of the study due to refusing to eat within the enclosed barn. In the other instance, a steer within the first group and second period was thrown out due to complications within the enclosed barn's stall. Data from these steers were used up until date of termination and was accounted for within the analysis. The steers involved in these occurrences were both supplemented with the control at the time of termination.

The diet used within the study was designed to be isocaloric and isonitrogenous and reflected a ration similar to a maintenance cow ration. Four weekly feed samples compiled by period were planned for analysis, but only one weekly sample was able to be compiled and analyzed due to communication error. Thus, weekly samples could not be compared due to having only one sample compiled and analyzed. The feed sample analyzed reflected the diet provided to the first group and first period, and may not be indicative of the entire population.

Dry matter intake did not differ across control and treatment groups. The lowest DMI observed was 2951.82 g/d while the highest intake observed was 7738.1 g/d. Within the control group DMI g/d averaged at 6389 g/d and the zeolite treatment group averaged 6434 g/d. There was no significance between groups in daily DMI g/d due to the nature of dosing the treatment directly into the rumen via cannula. DM digestibility % was also not significant between the two treatment groups (P= 0.27).

ADF and NDF digestibility % trended toward significance (ADF: P= 0.07, NDF: P= 0.10) between the two treatment groups. In contrast, ADF and NDF intake per day did not differ

significantly between treatment groups (ADF: P= 0.94, NDF: P= 0.96). Analysis of nutrients within the feces showed that fecal NDF did trend toward significance (P=0.07) while fecal ADF did not trend towards significance (P=0.11).

Nitrogen intake and utilization did not differ among treatments. N intake did not differ among treatments (P=087). N digestibility % also did not differ among the limestone and zeolite treatments (P=0.44). Excretion N values were determined to also have no significance (Fecal N, g/d: P=0.44, Urine N, g/d: P=0.71). Urinary Urea N excretion, g/d, also had no significance (P=0.90). N retention, g/d, was also shown to be similarly insignificant (P=0.94).

Phosphorous intake and utilization had similar P-values compared to nitrogen utilization and intake. P intake and digestibility % did not differ between treatments (P intake, g/d: P= 0.87, P digestibility, %: P= 0.30). P excretion values were also found to be insignificant (Fecal P, g/d: P=0.15, Urine P, g/d: P= 0.73). P retention, g/d, was also shown to have no difference between treatment and control groups (P= 0.21).

Weight gain and Performance characteristics

Initial weights of each steer did not differ significantly (P=0.90). The mean initial body weight of the control group was 677 kg (95% CI= 645, 709). The mean initial body weight of the zeolite treatment group was 675 kg (95% CI= 643, 706). The final mean body weights post-balance trial had no difference between treatments (P=0.97). The final mean body weight of the control group was 651 kg (95% CI= 614, 686). The final mean body weight of the treatment group was 650 kg (95% CI= 614, 685). There was no difference between average daily gain (ADG) among groups (P= 0.79) and each group lost weight during the balance trial. The control group lost an average of 4.3 kg/d and the zeolite group lost an average of 5 kg/d. These values

may indicate that the steers were in a negative energy balance during the periods of daily urine, fecal, and ORTs collections.

DISCUSSION

The purpose of this study was to evaluate the effects of supplemental zeolite on intake, performance characteristics, and the metabolism of nutrients within the ruminant when dosed directly into the rumen through a rumen cannula. Each variable was included in this study to hypothesize zeolite treatment effects on typical cost-based factors within ruminant production systems.

Dry Matter Intake and Digestibility

Consumption of dry matter did not differ among the treatment groups (P=0.94). Numerically, the data shows little variation between group means. The control group ingested an average of 6.39 kg/d compared to the 6.43 kg/d ingested by the treatment group. It was expected that both the control and treatment groups would ingest similar amounts due to the similar ages and weights of the steers within the groups. Directly dosing zeolite into the rumen cannula allowed for more accurate supplementation compared to other published papers that included zeolite at a set percentage within the diet itself. Dosing directly into the rumen cannula ensured an accurate dosage administered without concerns for feed palatability or individual animal preference affecting the amount of zeolite entering the animal. The effects of zeolite on DMI have been widely debated. Studies have shown zeolites can decrease (Grabherr et al., 2009; Frizzarini et al., 2024), have no effect on (Dschaak et al., 2010) or potentially increase DMI (Hu and Murphy, 2005; Kawas et al., 2007; Polat et al., 2004). Decreased DMI during supplementation has been observed in studies that apply zeolite directly to the diet via total mixed ration (Grabherr et al., 2009; Frizzarini et al., 2024). The palatability of zeolite has been

stated to be poor and could have resulted in decreased DMI within these studies. Other studies contradict the observed decrease in DMI and show no change in DMI when zeolite was supplemented into the diet via TMR (Dschaak et al., 2010). Zeolites have been theorized to increase DMI in dairy cows in early to mid-lactation. This theory is attributed to its effects as an alkaline buffer (Polat et al., 2004). Inclusion of zeolite in this period could be used to influence acid-base balance and regulation, leading to an increase in DMI. Studies have shown that the use of alkaline buffers in dairy cows has led to observed increases in DMI (Hu and Murphy, 2005; Kawas et al., 2007) further supporting this theory of the proposed buffer effects of zeolites. The available data on the effects of zeolite supplementation on DMI differ in conclusions. The effect of increased DMI can only be theorized and published studies often have contradictory results. Currently, the effects of zeolite inclusion on DMI are unclear.

Dry matter digestibility % also did not differ between the treatment groups (P=0.27). The group means were also similar with control group average DM digestibility of 58.8% and treatment DM digestibility of 55.3%. The little difference in group means and the insignificance between groups document that zeolites do not have an effect on dry matter digestibility. This warrants further studies on percent inclusion of zeolite in the diet, as most published papers only include zeolite at upwards of 5% of the diet. With both DMI and DM digestibility unaffected by zeolite administration, we can assert that zeolites do not inhibit the digestion of dry matter within ruminants. Studies also differ on the effects of zeolite supplementation on DM digestibility %. One study showed a decrease in DM digestibility % in cannulated dairy cattle directly supplemented with zeolite (Johnson et al., 1988) while another study showed no change in DM digestibility % when given zeolite via TMR (Dschaak et al., 2010). Differing results could be caused by the different inclusion methods used to supplement zeolite. This study has shown no

effect of zeolite supplementation when dosed directly to the rumen. More data must be collected before a true effect can be stated.

Fiber utilization

Digestible fiber components were evaluated to determine possible effects of zeolite inclusion into the diet. Like DM intake, ADF and NDF intake also had no significance (ADF intake, g/d: P=0.94 NDF intake, g/d: P=0.96). Digestibility of ADF and NDF trended toward significance (ADF digestibility, %: P= 0.07, NDF digestibility, %: P=0.10). ADF and NDF digestibility tended to decrease when supplemented with zeolite. Control group ADF digestibility tended to be 8.6% greater compared to zeolite supplementation. Control group NDF digestibility tended to be 8.2% greater compared to zeolite supplementation. This trend may indicate a shift toward cellulolytic bacteria within the rumen. Cellulolytic bacteria are in greater proportions within the rumen when ADF and NDF digestibility is greater. This shift is proposed to be caused by an increase in cellulose within the diet. Cellulolytic bacteria also create methane as a waste-product to remove excess hydrogen atoms within the rumen. These findings may indicate an opportunity for future studies to determine if inclusion of zeolite into the diet can increase or decrease methane emissions from ruminants. The best hypothesis for the findings in this paper is a decreased rate of passage causing increased rate of digestion and retention time within the rumen. This will result in the increased digestibility of ADF and NDF seen within this study. We also observed a reduced dry matter intake compared to our anticipated DMI. A low DMI causes increased time of feed in the rumen, further supporting this hypothesis. Organic matter digestibility has also been shown to increase when supplemented with zeolite (Klaeui et al., 2020). Organic matter, ADF, and NDF intake has also been shown to increase in Bos Indicus bulls when supplemented with zeolite (Brixner et al., 2025). A study performed by Johnson et al.

in 1988 found no change in ADF digestibility % and a decrease in OM, CP, and DM digestibility. The differences in results make it difficult to state the true effect of zeolite supplementation on fiber digestion and degradation.

Fecal excretion values for fiber digestion did not differ significantly. Fecal ADF did not differ between treatments (P= 0.11). Fecal NDF did trend toward significance with a P- value of 0.09, but this trend may or may not be attributed to the effects of zeolites. Published literature often contradicts itself on the effects of zeolites on fiber digestion. A study performed by Sadeghi and Shawrang in 2007, states a statistically significant increase in ADF and NDF digestibility in steers supplemented with zeolite (P< 0.05). Another study supports this study by documenting that organic matter digestibility had an increased trend in cattle fed high energy diets supplemented with zeolite (McCollum and Galyean, 1983). Contradictory to the other papers, a study performed by Klaeui et al., in 2020 stated that there was no significant difference in NDF digestibility with cattle supplemented with zeolite. The differing lab findings could be attributed to zeolite purity, administration methods, or composition of diet fed. These findings may indicate the need for the replication of previously performed studies.

Nitrogen utilization

Concentrations of fecal nitrogen and phosphorus in manure are important in determining the amount of manure you can spread on a plot of land which must be included in a comprehensive nutrient management plan (CNMP) required by the USDA. There was no significant difference in N intake and digestibility % between treatment groups (N intake, g/d: P=0.87, N digestibility,%: P= 0.44). Excretion of N remained insignificant with fecal excretion (P= 0.44) and urinary excretion (Urine N, g/d: P= 0.71, Urine Urea-N, g/d: P= 0.90). The study performed in 2020 by Klaeui et al. also found no statistical difference in fecal N excretion and

supports the finding in this study. This is supported by Urinary-N excretion increasing in a study comparing feedlot cattle supplemented with zeolite versus a control diet (Myers et al., 2024). Nitrogen retention was also insignificant (P= 0.94) but yielded negative values. A study performed by Sherwood et al. in 2005 also found no difference in nitrogen balance between feedlot steers supplemented a control diet and a zeolite diet. Other studies also showed no difference in nitrogen utilization (Myers et al., 2021; Sherwood et al., 2006). This negative retention rate seen may be attributed to the nature of the steers within this study. The cannulated steers used within this study were not growing steers and were of mature weight exceeding the weight of growing steers. The movement limitations of a stall, in addition to the maintenance ration, could indicate the loss of N through breakdown of muscle tissue causing more N to be excreted and not retained in these animals. Particle size of zeolite has also been shown to affect N metabolism (Papaioannou et al., 2005; Leung et al., 2006). Although most studies do not indicate the particle size of zeolite used, particle size should be considered when evaluating N metabolism.

Phosphorous utilization

Phosphorous utilization within an animal can signify growth within a young animal and is also used, with nitrogen, to determine the amount of manure allowed on a plot of land. There was no statistical difference between P intake and P digestibility % between each treatment in this study (P intake g/d: P= 0.87, P digestibility, %: P= 0.3). Fecal and urinary excretion of P did not statistically differ (Fecal P, g/d: P= 0.15, Urine P, g/d: P= 0.73). Similarly to N retention, P retention was not significant and also yielded negative values (P= 0.21). The negative value could be attributed to the mobilization of tissues resulting in a reduced retained value. The negative value could also be associated with a deficiency and as such, the animal will mobilize

its own bodily tissues to decrease the severity of that deficiency. More data should be collected on P utilization and zeolite application to the diet to definitively state the cause of a negative P retention. The focus of P utilization in other studies has been the effects of zeolite on calcium and phosphorus balance in the animal. The dose and particle size of zeolite used has been shown to influence calcium and phosphorus metabolism and regulation within hypocalcemic dairy cows (Su et al., 2025). This may be attributed to its potential to act as an alkaline buffer stated by Polat et al. in 2004. Serum P concentrations were shown to be reduced in dairy cows fed diets supplemented with zeolite compared to a control diet (Kerwin et al., 2019). This study also found significant increases in serum Ca levels, further supporting the potential for addition within a high DCAD diet.

CONCLUSION

The data provided demonstrates the need for a consistent method of zeolite delivery and the need for consistent results from future studies. This experiment investigated the addition of zeolite directly dosed into the rumen of cannulated angus steers, and due to the circumstances of the feed analysis, should only be used to make further hypotheses. This study found that the addition of zeolite had a trend of decreasing ADF and NDF digestion, yet no other significant findings were observed. The addition of zeolite directly to the animal had no significance on the total amount of ammonia excreted from the animal. Our study demonstrates that, although zeolite administration to a diet has been shown to decrease total rumen ammonia levels within the literature, the amount excreted from the animal is unchanged with direct application.

Table 1. Composition of Diet Fed

Ingredient	% of, AF Basis	% of, DM Basis
Corn Silage- Processed- 41 NDF – Medium	47.7	25.0
Corn Grain- Cracked	21.0	30.0
Wheat Straw 5 – 79 NDF	11.1	16.7
Alfalfa Hay 20- 37 NDF	10.2	15.0
Corn Distillers- Ethanol	6.0	9.1
Hay Treat- Liquid Supplement	3.3	3.0
Limestone- Ground	0.4	0.7
Salt- White	0.3	0.5
Total	100.0	100.0

Table 2. Nutrient Composition of Diet (DM Basis)[1]

Nutrient	%, DM Basis
Dry Matter %	93.2
Crude Protein, %	12.5
Phosphorus, %	0.4
ADF, %	29.5
NDF, %	40.1
TDN, %	74.0

[1]Nutrient composition from weekly compiled feed sample

1

Table 3. Descriptive Data Summary

	Treatment			
Item	Control	Zeolite	SE	*P*-value
Dry Matter				
DMI, g/d	6,389	6,434	419	0.94
DM digestibility, %	58.8	55.3	2.25	0.27
Fiber				
ADF intake, g/d	1860	1874	132	0.94
NDF intake, g/d	2523	2536	184	0.96
Feces ADF, g/d	743	864	52.1	0.11
Feces NDF, g/d	1075	1346	107	0.09
ADF digest, %	60.1	51.9	3.04	0.07
NDF digest, %	53.0	44.4	3.55	0.10
Nitrogen				
N intake, g/d	123	125	10.12	0.87
Feces N, g/d	50.6	53.8	2.94	0.44
Urine N, g/d	85.1	83.1	3.76	0.71
N digestibility, %	57.6	54.5	2.87	0.44
N retained, g/d	-12.5	-11.4	9.66	0.94
Phosphorus				
P intake, g/d	23	25.5	2.15	0.42
Feces P, g/d	17.7	20.6	1.39	0.15
Urine P, g/d	7.05	7.59	1.09	0.73
P digestibility, %	24.5	24.6	1.57	0.97
P retained, g/d	0.47	-2.710	1.49	0.14

n = 23 observations

2

3

Table 4. Performance Characteristics

	Treatment			
Item	Control	Zeolite	SE	*P*-value
DMI, g/d	6,389	6,434	419	0.94
DM digestibility, %	58.8	55.3	2.25	0.27
ADG lb/steer	-9.43	-11.03	4.19	0.79
Initial BW	1490	1484	35.3	0.90
Final BW	1431	1429	40.3	0.97

4

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