THESIS

INCLUSION OF NATURAL ZEOLITE (CLINOPTILOLITE) IN FINISHING RATION OF FEEDLOT BEEF CATTLE

Submitted by

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ABSTRACT

INCLUSION OF NATURAL ZEOLITE (CLINOPTILOLITE) IN FINISHING RATION OF FEEDLOT BEEF CATTLE

To assess the effects of Zeolite (ZE, clinoptilolite) inclusion on in vitro rumen fermentation. A modified procedure from Tilley and Terry (1963) was used to determine alfalfa in vitro dry matter digestibility (IVDMD) in the presence or absence of ZE. Test tubes (n=96) were placed in a 39 °C bath and were blocked based on hours 0, 1, 2, 4, 6, 8, 12, 24, and 48. Substrate consisted of dried alfalfa grinded through a 1mm screen using a Wily Mill. Treatments consisted of, Control (no ZEl inclusion), and 0.05g ZE, for each 1 gram of alfalfa. After incubation, IVDMD was calculated 24 hours after drying period. Data was analyzed using a randomized block design. The IVDMD was found to be similar across all treatments ($P > 0.8464$). The IVDMD was significantly different when incubated for the varying lengths of time ($P < 0.001$). There was an IVDMD of 20.18 ± 2.89 % for the control and 18.07 ± 5.49 % for the ZE at the zero hour. The IVDMD for Control and ZE for 48 hours were 54.30 ± 1.58 % and 53. 48 ± 1.04 % respectively. To predict the IVDMD of the effects of ZE treatment on digestibility over time, the corresponding regression $f(x) = 20.60 + 0.421X1 + 0.730X2$ with $R2 = 0.8464$. In conclusion these data demonstrate that inclusion of ZE, does not influence the in vitro digestibility of alfalfa. These data indicate that there is likely very little to no impact on feed digestibility when ZE is included in the ration. To assess the effect of ammonia volatilization from manure, ten Holstein calves were selected and placed in calf hutches to evaluate the effects of zeolite efficacy in

reducing ammonia volatilization. Calf hutch was the experimental unit with two treatments being used: 1) Test (n=5) with bedding consisting of zeolite and wood shavings and 2) Control (n=5) with bedding consisting of wood shavings only. Significance was determined using a welch two sample T test with significance being determined at $P \leq 0.05$. Prior to calves being placed in calf hutches, bedding was weighed (~ 55.5 lbs./calf hutch) and zeolite was added to test treatment at 5% of bedding weight. Calves were kept in calf hutches for 105 days. Bedding was added (48.2 lbs./calf hutch) 4 times during the experiment after each weather event or if bedding needed to be added as recommended by dairy workers. At the end of the experiment, calves were removed, and all bedding was stripped, weighed (Test: 334.3 lbs/calf hutch, Control: 289.4 lbs/calf hutch) and sub samples were collected. Sub samples were freeze dried at -65 °C util two identical consecutive weights were obtained. Samples were then homogenized and finely ground using a Thomas-Wiley laboratory mill with a 1mm screen. Sample analysis was done by SDK laboratories (Hutchinson, KS 67501) and consisted of total protein, acid detergent fiber, neutral detergent fiber, and ash. Statistical analysis showed no significant difference in concentrations of nitrogen ($p = 0.0560$), ADF ($p = 0.4366$), NDF ($p = 0.1826$), Ash ($p = 0.7758$), or DM ($p = 0.6508$). To evaluate the effects of zeolite (clinoptilolite) inclusion on feedlot performance, 320 steers were fed a high concentrate, steam flake corn-based finishing ration for 146d. It is hypothesized that the addition of zeolites to a high concentrate ration in a dose dependent fashion may serve as a buffering agent aiding in the improvement of feedlot performance. Cross bred steers (n = 320, initial BW 401 ± 41kg) were evenly distributed in a randomized block design with 4 treatments of zeolite (0, 0.5, 1, and 2% diet DM). Steers were blocked by weight and assigned to one of the 4 treatments which consisted of 8 pens per treatment with 10 hd per pen. Pen was the experimental unit. Steers were individually weighed on days 0, 21, 49, 77, 105, 138

and 167. Initial pen BW was used as a covariant in the statistical analysis with significance being determined at $P \leq 0.05$ and tendency level determined at $P \leq 0.10$. The final BW ($P \geq 0.81$), total average daily gain ($P = 0.76$) and feed efficiency ($P > 0.68$) were found to be similar across treatments. Total dry matter intake was decreased for animals that were fed zeolite at 1% of diet DM ($P < 0.01$). There was no difference ($P = 0.40$) in mortality and morbidity between treatments. Liver abscess rate was found to be independent ($P = 0.54$) of treatment. These data indicate that under the conditions of this experiment the addition of zeolite to steam flaked corn-based finishing diets does not impact final body weight, ADG or feed efficiency but decreases DMI of feedlot cattle when zeolites are added to the diet at 1% of diet DM.

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INTRODUCTION

With the global population expected to increase to 9.7 billion in 2050 (United Nations, Department of Economic and Social Affairs, 2016), there is a need to increase the current output of food production to meet this increase in demand. A 60% increase in agricultural products, estimated by the Food and Agriculture Organization of the United Nations, is expected to meet the demand in 2050 (Alexandratos and Bruinsma, 2012). However, this needs to occur on smaller regions of land due to the expansion of urban areas. As the trend for maximizing nutrients consumed by ruminants for maximum gains in the form of fiber, meat, and milk, extra nutrients are being voided in the feces and urine of production animals. This in turn leads to increased emissions related to food production due to the growing population and raises ethical concerns related to animal health.

Current beef industry practices are to provide nutrients in surplus to increase the yields of beef products. This is done by using high carbohydrate diets which account for 60 – 70% of production costs in feedlots (Becker, 2008). The objective of high carbohydrate diets is to gain monetary profit by converting feed protein into muscle protein which is then sold for consumption by growing populations. Much attention is brought to animal health in these settings as the health and wellbeing of the animal translates into improved gains of lean muscle mass. Any negative effects in the health of the animal translates into increased expenses for the treatment of disease decreasing profit from meat sales. Negative effects related to ruminant health are seen at harvest resulting in the condemnation of livers and reductions in both meat quality and overall carcass weights.

Animal health is affected when intakes of highly fermentable sugars impact the normal rumen flora leading to metabolic diseases. Animals experiencing metabolic diseases in turn have reductions in feed efficiency and dry matter intake. These reductions in feedlot performance manifest from the metabolic disease ruminal acidosis (discussed in the acidosis section) due to fluctuations in ruminal microflora and the inability of the animal to maintain the homeostatic capacity of the rumen. Ruminants with chronic bouts of ruminal acidosis develop liver abscesses resulting in economic reductions in animal carcasses. Efforts to decrease liver abscesses is by feed additives like tylosin. Tylosin is an antibiotic that is used for the prevention of liver abscesses associated with ruminal acidosis. Recently however the FDA began to regulate drugs like tylosin in efforts to prevent antibiotic resistant strains of bacteria, making tylosin an amended drug requiring professional supervision by a licensed veterinarian ((US Food and Drug Administration, 2012). Therefore, there is a need for other options of feed additives with mechanisms that improve feed efficiency and help curb the effects of emissions from production animal diets.

Cattle are known to contribute to greenhouse gas emissions through respiration, feces, and urine. Carbon dioxide (**CO_2**) and methane (**CH_3**) are two compounds produced from microbial digestion within the digestive tracts of cattle and contribute to green-house gas emissions. Other types of emissions like ammonia (**NH_3**) and nitrate (**NO_3**) are produced outside of the animal and can contaminate air, soil, and sources of ground and surface waters (Tamminga, 1992). These sources of nitrogen are generated from microbial environmental enzymes (which will be covered later). Nitrogen losses from cattle, in the form of feces and urine range from 60 – 80% of consumed nitrogen once requirements are met (Varel et al., 1999). These losses of nitrogen, when manure from food producing animals is handled, volatilize to

NH_3 and occurs up to 50% (Bierman et al., 1996). This volatilization is a major environmental concern for animals that are managed on a small, confined spaces which leads to the accumulation of manure. For this reason, nitrogen is considered one of the most critical manure elements concerning environmental stability from food producing animals like beef cattle (Cabrera et al., 2006). As nitrogen leaves production animal sites as emissions, it has the potential to pollute aquatic and terrestrial ecosystems. These environmental impacts from cattle feeding has received much attention, and strategies to curb these effects are currently being researched. (Tamminga, 1992).

One possible strategy is using naturally occurring zeolites. Zeolites (**ZE, clinoptilolite**), can be used to control the excretion of waste products from animal production (Mumpton, 1999). The ZE structure contains entry channels into a crystalline structure allowing the absorption of polar molecules like CO_2 and NH_3 (Mumpton, 1999). When ZE is included in the diet, the molecule has the potential to filter emissions and make manure less odorous by the absorption of charge particles (Mumpton, 1999). Within the rumen, the mineral may improve ruminal pH ameliorating the effects of high starch diets decreasing the occurrence of liver abscesses from cattle fed high grain diets. However little research involving ZE inclusion in rations for ruminant animals has been investigated. More specifically, what effects the molecule has on nitrogen dependent bacteria, which use nitrogen for the synthesis of microbial protein.

Recent research studies have supplemented ZE in cattle diets at concentrations that range from 1-9% of diet dry matter (DM) (Urías-Estrada et al., 2018). These diets consisted of corn-based finishing diets fed to cannulated Holstein steers (Urías-Estrada et al., 2018). Positive effects were seen in ruminal starch fermentation and increases in total tract organic matter (OM) digestion, with increased retention of nitrogen (McCollum and Galyean, 1983, Urias-Estrada et

al., 2018). This indicates improved microbial fermentation, with decreased NH_3 flow to the duodenum with no change in microbial efficiency (Urías-Estrada et al., 2018). At 5% inclusion in the diet it has been found to have the greatest decrease in rumen NH_4 concentrations, although the effects of digestion within the rumen at this concentration are limited (McCollum and Galyean, 1983). Therefore, the aim of experiment 1 is to assess the effects of ZE inclusion on in vitro rumen fermentation of alfalfa at a 5% inclusion rate. Experiment 2 will examine nitrogen retention when ZE is added to dairy calf bedding at 5%. Experiment 3 will focus on the effects on feedlot performance when ZE is added at 0, 0.5, 1, and 2% of diet dry matter.

CHAPTER I: REVIEW OF LITERATURE

PROTEIN METABOLISM

Proteins are the most abundant macromolecule in mammalian systems and comprise hormones and enzymes in systems of organs such as muscle, skin, and blood vessels which are also composed of proteins. Proteins will contain elements of carbon, hydrogen, and oxygen in addition to nitrogen and other elements like sulfur, phosphorus, iron, copper, manganese, and iodine, with proportions of these elements being dependent on the composition of amino acids (**AA**) within the protein (Perry R. C. and Ensminger, 1997). These elements may be contained in enzymes that will catalyze metabolic reactions in the body. Protein turnover, which is a largely enzyme dependent process, is based on the synthesis and breakdown of body proteins which will be influenced by the catabolic and anabolic reactions within the body. The counteractive nature of these catabolic and anabolic reactions will be dictated by the productive state of the animal's body and the difference between these two reactions will ultimately determine the net gain or loss of AA from a given mammalian system. The net gain of AA is transferable to saleable animal products such as meat, milk, fiber, or the fetus (Lobley, 2003). This will be the basis for high protein feedlot diets in addition to implants and steroids to increase efficiency within the United States (Galyean, 1996). This demand for high protein products will be due to a systematic push by markets to increase leaner heavier cattle within the US meat packing industry.

Nitrogen contained in protein can be used to predict if the animal is in a catabolic or anabolic state. Sources of nitrogen within the diet include nucleic acids, amino acids, proteins, peptides, amines, amides, nitrates, nitrites, urea, and NH_3 (Huntington and Archibeque, 2000). Nitrogen from catabolism of protein will be funneled into ureagenesis producing urea. The

primary site of nitrogen excretion will be in the urine, with approximately 97% of this being in the form of urea (Mackie et al., 1998). Increased nitrogen excretion in the form of urea will be seen when animals are consuming protein more than demand from the body. The generation of urea is energy taxing on the body and accounts for 2.5-5% of total oxygen consumption and is energy lost as heat (Lobley et al., 1996). As the animal ages, the need for protein as a percentage of dry matter in cattle diets will decrease (NRC, 2015). Excess protein therefore can be an environmental constraint and negatively correlated to the metabolic efficiency of the animal.

Unlike the non-ruminant, amino acids that arrive in the small intestine of the ruminant will be first subject to alteration by rumen microflora. The rumen microflora will be dependent on the intake of nitrogen by the animal to fulfill the demand for microbial protein synthesis. Microbes that comprise the rumen microflora are bacteria, protozoa, fungi, and archaea and will account for 60-85% of the total protein and nitrogen that is presented to the small intestine of the ruminant (Storm et al., 1983; Hackmann and Firkins, 2015). Microbial protein synthesis is dependent on the fermentation of carbohydrates and the availability of nitrogen and sulfur. Within the rumen there will be two bacterial populations that utilize different forms of nitrogen for the fermentation of carbohydrates. These carbohydrates will be both structural carbohydrates (SC) and non-structural carbohydrates (NSC). Protozoa will influence proteolysis and delay starch digestion of bacteria through predatory behaviors (Leng and Nolan, 1984) while fungi will have a role in fiber digestion (Bauchop, 1981). The symbiotic relationship between the ruminant and the ruminal microflora supplying the ruminant with essential amino acids allows the animal to survive on poor quality forages without having any essential amino acids being supplied by the diet, however some deficiencies may be seen in some high-producing animals that require more than is provided by microbial crude protein (**MCP**).

Amino acids are absorbed by ruminant animals as essential amino acids (**EAA**) and non-essential amino acids (**NEAA**) which are used in signaling, metabolic functions, and are important in energy production. Formulation of diets with adequate protein requires precise estimates in the amount of microbial protein that is absorbed by the animal. Degradable intake protein will be consumed by bacteria and converted to MCP. When microbial protein is the only source of protein, methionine is found to be first limiting, followed by both lysine and threonine. Arginine and histidine were also found to have similar limiting effects when MCP was the main source of protein (Richardson and Hatfield, 1978; Strom and Øskov, 1984). Similarly, when the diet is mostly corn based, lysine has been found to be first limiting. This is thought to be due to the composition of corn containing escape proteins and those proteins containing adequate sulfur containing amino acids but deficient in lysine (Merchen and Titgemeyer, 1992). However, when post ruminal infusion of whole protein and mixtures of EAA's were used, improvements in nitrogen retention were seen in growing steers suggesting single amino acids do not stand out as being completely limiting. Post ruminal infusion of casein along with methionine showed improvements in nitrogen retention compared to when methionine was fed alone (Titgemeyer and Merchen, 1990). This suggests when requirements for methionine are met in growing animals, casein supplies other limiting amino acids for improvements in nitrogen retention and protein synthesis. Therefore, formulations of ruminant diets require supplementation of combinations of EAA's to meet the demands of growing ruminants, provided that all other nutrients like energy are met as well.

Nitrogen that is used by microorganisms of the rumen will be in the form of NH_3 and will be termed non-protein nitrogen (**NPN**). The rumen bacteria will take substrates like urea and amides and convert these compounds to NH_3 that will be used by bacteria to synthesize MCP.

Satter and Slyter (1974) noted no improvement in microbial protein synthesis in concentrations of NH_3 past 50 mg NH_3-N/100 ml of rumen fluid. Alternatively, rumen concentrations at or less than 3 mg NH_3-N/100 ml are too low to support proper rumen fermentation (Kang-Meznarich and Broderick, 1980). Some bacteria will have other requirements for peptides, and amino acids for the synthesis of MCP (Cotta and Russell, 1982). Supply of the peptides and amino acids will be in the form of rumen degradable protein (**RDP**). Rumen undegradable protein (**RUP**) will pass through the rumen avoiding degradation by rumen microbes and will be digested by the animal like a non-ruminant. Animals with increased demand for protein, where MCP does not meet their increased requirements, can have RUP added to the diet to meet the increased demands for lactation and growth (Van Soest, 1994). Increases in the CP offered to the animal will also have improvements of increased intakes and total tract digestion of low-quality forages (Heldt et al., 1999).

Structural carbohydrate (**SC**) fermenting bacteria will use NH_3 as their sole source of nitrogen within the rumen, while Non-structural carbohydrate (**NSC**) fermenters will use both NH_3 and amino acids for their source of nitrogen (Russell et al., 1992). The growth of the two microbial populations will not be static with growth being dependent on the availability of their respective substrates. Increases in the RDP results in improved total tract digestion of structural carbohydrates but the same is not seen when starch is increased in the diet. Decreases in intakes of low-quality forage are seen when there is an increase in the starch consumed by the animal. This may be due to the NSC bacteria outcompeting the SC bacteria for available nitrogen due to the increase of readily fermentable carbohydrates in the diet (Heldt et al., 1999). In a study conducted by Olson et al. (1999) showed improvements in total tract digestion in steers supplemented with RDP. They noted there were linear increases in the digestion rates of

structural carbohydrates with increased intakes of forage when RDP was supplement. Linear decreases of forage intake and rates of digestion were noted when only starch was supplemented (Olson et al., 1999). However, increasing NPN and RDP will result in increases of NH_3 that is absorbed by the animal, and if in excess and depending on the pH of the rumen, acid base changes within the animal may be seen.

Microbial protein digestion consists of two steps, where the microbe will hydrolyze the peptide bonds that hold the amino acids together followed by the degradation of amino acids. These processes are termed proteolysis and deamination respectively. The optimum pH for both of these functions of microbial activity to occur is between 6 and 7 (Blackburn and Hobson, 1960). With deamination of amino acids completely stopping at 4.5 or lower (Lewis and Emery, 1962) and at 7.2 or higher (Tamminga, 1979). The proteolysis activity of microbial enzymes is said to have trypsin like activity and occurs on the cell surface (Craig and Broderick, 1984). As trypsin acts on the peptide linkages of the carboxyl groups located on the arginine and lysine moieties allowing these amino acids to be transported into the bacterial cell. In the bacterial cell the amino acids can be incorporated into MCP or further degraded into volatile fatty acids (**VFA**), CO_2, or CH_3. Deamination can also occur where bacteria cleave the amino group from the carbon skeleton of the amino acid yielding NH_3 to be used for MCP synthesis or absorbed by the animal.

Ruminant protein digestion in the small intestine, like non- ruminants, will be comprised of proteases that will function in the hydrolysis of complete proteins to peptides and ultimately individual amino acids. These enzymes will be in a proenzyme form and will become activated by trypsin activated by enterokinase a brush border enzyme located on intestinal cells (Pond et al., 2005). Active proteolytic enzymes, by trypsin, will be chymotrypsin, and carboxypeptidase.

Pepsin in the stomach is activated at a pH of 3.5 or below which is maintained by hydrochloric acid secretions from the stomach and functions to attack peptide bonds of proteins. Chymotrypsin and trypsin will be endopeptidases which will hydrolyze interior bonds of proteins, liberating smaller peptides than pepsin. Carboxypeptidase will liberate terminal amino acids by cleaving the carboxyl bonds of AA (Pond et al., 2005). The function of both endo and exopeptidases will have a maximum activity at a pH of 7.5 or greater with the pH being maintained at this level from gall bladder secretions. Decreased activities of these enzymes are seen in pylorus and terminal ileum of the small intestine due to the change in pH. (Hutton, 1975).

VFA's produced by rumen microbes will be part of the organic acids that are produced from fermentation. When fermentation increases and these organic acids are allowed to accumulate, health related complications result that will be covered in later sections of this paper. VFA's will be absorbed from the rumen wall and will be part of the portal drained viscera, destined for the metabolism in the liver (Nagaraja and Titgemeyer, 2007). A large portion of MCP that is absorbed in the small intestine will have the same fate (Reynolds, 1992). Once in the liver, AA from both dietary protein and MCP, along with VFA's, will be metabolized for energy via gluconeogenesis depending on the energy state of the animal. Due to little glucose from carbohydrate being absorbed, AA's and VFA's will contribute the carbon needed for the synthesis of glucose for the ruminant animal by the addition of intermediates to the citric acid cycle.

Nitrogen recycling

Ruminants will absorb a substantial amount of NH_3, that will be metabolized to urea in the liver. NH_3 in the rumen will come from the catabolism of amino acids, nucleic acids, and other nitrogen containing compounds. Up regulation or down regulation of the ureagenesis will

be dependent on the expression of enzymes in the urea cycle. Absorption of NH_3 will be based on its concentration in the rumen and the concentration in the blood. The rate of ruminal fermentation will influence the concentration of NH_3 in the rumen while the rate of ureagenesis in the liver will drive the concentration of NH_3 in the blood. Within the blood, NH_3 will almost always be in the protonated form (NH_4^+) acting as an intermediate acid-base regulator. Having a pH less than 7.3 within the rumen will ionize NH_3 to NH_4^+ making it less readily absorbed by the gut epithelium (Abdoun et al., 2006). However, there will also be transporters available for the absorption ammonium, which will couple bicarbonate (HCO_3^-) or VFA anions to this uptake. Ammonium production during periods of acidosis can also excrete excess H^+ ions into the urine to conserve HCO_3^- an important buffer within the body (Weiner and Verlander, 2011)

Enzymes which facilitate the ornithine cycle will largely be in the liver with some enzymes being found in extrahepatic tissues. Extrahepatic tissues include kidney, and intestines and produce substrates (citrulline, ornithine, and arginine) for the ornithine cycle . The cycle will begin with carbamoyl phosphate combining with ornithine in the mitochondria producing citrulline. Citrulline will then be transported to the cytosol of the hepatocyte, where it will combine with aspartate, producing arginosuccinate. The removal of urea from arginosuccinate will produce ornithine to be reused in the in the ornithine cycle. The nitrogen in urea thus will come from NH_3 in carbamoyl phosphate and the amnio group in aspartate. The carbon chain of aspartate will be given off as fumarate to produce energy in the TCA cycle via reducing equivalents (Meijer et al., 1990).

Urea produced in the liver will then be released into the vena cava of the animal to be recycled or excreted in the urine. About 40-80% of the urea that is produced will be recycled into the gastrointestinal tract (Lapierre and Lobley, 2001). Routes of recycling will be in the saliva

and direct transfer from the blood to the rumen. Urea in the rumen will be metabolized back to NH_3 by the enzyme urease, to be used for the synthesis of MCP. Diet will influence this recycling with more recycling occurring with the increased demand for nitrogen by the rumen microflora. Demand for nitrogen recycling is driven by the consumption of carbohydrates during periods of protein deficiency.

Nitrogen in the Environment

Manure excreted from food animals will go through a series of reactions that include decomposition, fermentation, and NH_3 volatilization decreasing the concentration of nitrogen in the manure. Volatilization of NH_3 can contribute to greenhouse gases (**GHG**) and contribute to local water pollution. With 30-50% of the nitrous oxide (**N_2O**) emissions coming from agriculture. The other major GHGs next to N_2O are CH_3 and CO_2 (Li et al., 2012). While there are more GHG out there these are the important gases related to microbial fermentation in an ecosystem. However, livestock production in the United States is said to account for 4.2% of all GHG in world. Livestock sectors contributing to GHG in the US consist of beef cattle (2.2%), dairy cattle (1.37%), swine (0.47%), poultry (0.08%), sheep (0.03%), goats (0.01%) and other (i.e., Horses) 0.04% (Mitloehner, 2018). Manure outside of the animal will be subject to microbial decomposition, hydrolysis, nitrification, denitrification, and fermentation. The action of the environmental microbes will be dependent on the environmental factors that include temperature, moisture, pH, oxygen, amount of substrate, etc. (Li et al., 2012).

Cattle who graze on pastureland will not concentrate manure as is seen on feedlots. Manure management is key in preventing to the loss of nutrients like nitrogen when cattle are intensively managed. Nitrogen and nutrient loss occur during storage, handling, and during application on fields when manure is used as a fertilizer (Eghball and Power, 1994). The cascade

of the loss of nitrogen from manure occurs in aerobic conditions such as those seen in the topsoil, where bacterial urease will metabolize urea from the urine and feces of livestock to NH_3. The NH_3 will have the ability to become nitrate by nitrification. In anaerobic environments such as those in the deeper soils, nitrate will become N_2 gas through denitrification. This gas will have the ability to escape the soil and will form intermediates such as nitric oxide (**NO**), nitrogen dioxide (**NO_2**), and N_2O when N_2 gas reacts with oxygen (Tamminga, 1992). These intermediates then can have harmful effects with the ozone layer and contribute to total greenhouse gases. Other areas of concern for pollution from high levels of environmental nitrogen are bodies of standing water. Nitrates can pollute water systems contributing to eutrophication.

For dairy cattle 75 to 85% of ingested nitrogen will be excreted from the animal. Dietary nitrogen will be a major factor in determining the excretion of nitrogen but some variations in diet and breeds exist (Reynolds and Kristensen, 2008). With much of the nitrogen being excreted from the animal, there is a potential for nitrogen to contribute to environmental pollution (Tamminga, 1992). With close to 50% of the nitrogen being lost before the manure is utilized for fertilizer, there is a need to keep the nitrogen in the manure. Using natural zeolites may help to improve concentrations of manure nitrogen and decrease the amount of nitrogen that is lost to the environment. Zeolites will be covered in a later section.

Urea cycle and Gluconeogenesis

Gluconeogenesis and ureagenesis will be coupled metabolic reactions, requiring similar intermediates. Therefore, in theory, ureagenesis can be regulated by the synthesis of glucose from gluconeogenesis by addition of glucogenic compounds by the addition of highly fermentable carbohydrates in ruminant diets (Huntington, 1989; Agarwal et al., 2015). The

addition of propionic acid to ruminant diets has shown an increase in nitrogen retention and a decrease in ureagenesis.

Non-essential amino acids (**NEAA**) will serve as transporters of carbon and nitrogen substrates for both reactions, respectively. The release of NEAA such as alanine and glutamine from tissues following a protein rich meal or injury indicate protein catabolism within those tissues. Alanine can function as an amino group doner in aminotransferase reactions seen predominantly in the liver. The production of pyruvate, a keto acid of alanine, will involve the enzyme alanine aminotransferase. This reaction will require alpha – ketoglutarate (the keto acid for glutamate) plus alanine, and glutamate plus pyruvate. Where pyruvate can be used to produce glucose. Alanine aminotransferase will be found in the liver, kidneys, heart and skeletal muscle and elevated levels will be indicative of tissue injury. Alanine will come from the catabolism of branch chain amino acids (BCAA), aspartate, asparagine, and glutamate. Alanine will serve as transporter of carbon for glucose (in the form of pyruvate) and nitrogen which can be transferred to alpha – ketoglutarate yielding glutamate for ureagenesis (Jungas et al., 1992).

Glutamate will have a gamma carboxylic acid group that can be amidated to glutamine. Glutamine will be the donor of the amide group for the synthesis of asparagine, carbamoyl phosphate, nucleotides, and trans-amination reactions and will be a glucogenic compound within the kidney. However, the kidney can also take glutamate and convert it to alanine that be used in the generation of glucose within the liver. Glutamine synthetase, in a reversible reaction, will catalyze the addition of the NH_3 to the gamma – carboxylic group of glutamate yielding glutamine. Once glutamine is synthesized, it can be hydrolyzed by glutaminase to glutamate and NH_3. Glutamate can be used within the brain as a neurotransmitter, an acid base regulator in the kidney, or an amino group doner. Similarly, aspartate can be converted to asparagine from

addition of an amino group to its beta carboxylic acid group, donated from glutamine by the enzyme asparagine synthetase. This reaction will require the cleavage of ATP to AMP and the addition of inorganic phosphate. Like glutamine, asparagine can be hydrolyzed to aspartate releasing NH_3 by the enzyme asparaginase (Jungas et al., 1992).

Both aspartate and glutamate will bridge this gap between the urea cycle and gluconeogenesis. This will be made possible by the enzyme aspartate aminotransferase, which will take aspartate plus alpha ketoglutarate and oxaloacetate plus glutamate. Similar to the alpha – ketoglutarate being the keto acids for glutamate, oxaloacetate will be the keto acid for aspartate. Aspartate will be responsible for carrying carbon for gluconeogenesis and nitrogen for ureagenesis. The production of glucose from glutamate involves the production of alpha-ketoglutarate from glutamate dehydrogenase or from an aminotransferase reaction. Alpha-ketoglutarate can then be reduced to oxaloacetate. Reducing equivalents from the enzyme's glutamate dehydrogenase, alpha-ketoglutarate dehydrogenase, malate dehydrogenase, and succinate dehydrogenase will generate reducing equivalents ($NADH/FADH_2$) which can be used for the generation of ATP via the electron transport chain (Jungas et al., 1992).

ACIDOSIS IN THE RUMINANT ANIMAL

Ruminant animals consuming diets with elevated concentrations of NSC's will have increased acid production within the rumen that can lead to metabolic diseases. Ruminal acidosis is due to an increase in microbial fermentation outside of the homeostatic capacity of the rumen (Hernández et al., 2014). Microbial fermentation occurs in anaerobic environments with byproducts of fermentation consisting of organic acids as well as other products such as heat, gas, vitamins, etc. Organic acids, if produced in excess, may result in both local and systemic effects with the production of the acids being dependent on the populations of bacteria associated

with NSC fermentation and the concentrations of NSC in the associated diet. Fluctuations in ruminal pH for adapted animals consuming high grain diets range from 5.6 to 6.5. In cases of inadequate adaptation to high grain diets, ruminal pH will decrease below 5.6. Prolonged durations of time with ruminal pH below 5.6, may result in the decrease neutralization of organic acids and shifts in bacterial populations further potentiating the effect of ruminal acidosis (Nagaraja and Titgemeyer, 2007). Saliva promoted from the chewing of forage and is indicative of neutral detergent fiber (**NDF**), will help to buffer ruminal pH and maintain rumen environments. Salivary bicarbonate is responsible for 30-50% of the neutralization of acids (Hernández et al., 2014). Other buffers of ruminal acids in ruminants fed forage, include phosphates, proteins, forage cell walls and VFAs (Russell and Hino, 1985). Low intakes of roughage or decreased mastication leads to fluctuations in growth curves of ruminal bacterial populations that favor more acidic environments. This cascade of events results in metabolic and ruminal acidosis that effect the physiological functions of the rumen consisting of absorption and motility (Russell et al., 1983).

Ruminal acidosis is defined as acute, or subacute depending on the variation of pH within the rumen. Organic acids such as lactic acid (**LA**) and VFA's will cause ruminal pH to decrease past the optimal pH of 6.3, which results in a decrease in organic matter and fiber digestion by SC fermenting bacteria (De Veth and Kolver, 2001). Having a pH less than 5.6 or within a range of 5.0 to 5.6 is considered chronic or sub-acute ruminal acidosis (**SARA**) (Owens et al., 1998; Nagaraja and Titgemeyer, 2007). With incidences of SARA being more transient and seen in periods after eating, or during stress events such as calving (Liebich et al., 1987). In acute cases of acidosis, lactic acid will accumulate due to the increase in lactate producing bacteria and has been termed lactic acidosis. Although lactic acid producing bacteria will be seen in both SARA

and acute acidosis. Acute acidosis is defined as a period where rumen pH is 4.5 or below. At a pH less than 5, there will be more acids that will be ionized increasing their absorption from the ruminal wall. Lactic acid having a pKa of 3.8, will contribute more to the total hydrogen concentration than any other acid (Owens et al., 1998). In prolonged periods of low pH, lactate utilizing bacteria will decrease and lactate producing bacteria will increase. Proportions of isomers of lactic acid will begin to be more skewed toward the production of D-lactic acid. In addition to D-lactic acid other acids such as, VFA's, ethanol, methanol, histamine, tyramine, and endotoxins will also be elevated potentiating the effects of ruminal acidosis (Koers et al., 1976; Slyter, 1976).

Ruminal osmolality and systemic electrolyte disturbances will be seen due to the accumulation of acids and glucose within the rumen. Absorption of ionized VFA's will be based on a counter exchange with bicarb (HCO_3^-) and a normal functioning rumen epithelial layer. In the early stages of acute ruminal acidosis, absorption of VFA's will be maintained by plasma bicarb concentrations but depletion of bicarb will result in a metabolic acidosis. VFA's and lactic acid then will begin to accumulate and damage to the rumen wall will result. Ruminal epithelium will then be subject to keratinization due to high acid concentrations, and ruminal papillae will begin to lose their absorptive capability. Normal ruminal osmolality ranges from 285 to 310 mOsm but during ruminal acidosis there will be an increase to 515 mOsm (Owens et al., 1998). This increase in the ruminal osmolality will then pull water from the blood into the rumen resulting in dehydration of the animal. Osmotically active substances in the rumen contributing to the increase in osmolality of the rumen are due to lactic acid, VFA's, and glucose. The rapid influx of water into the rumen will damage the ruminal wall contributing hyperkeratosis or parakeratosis leading to ruminal abscesses (Slyter, 1976). Damage to the ruminal wall will lead

the movement of ruminal contents freely moving into the portal circulation leading to abscesses in the liver and laminitis.

Ruminal Bacteria Associated with Ruminal Acidosis

Changes in anaerobic microbial populations during ruminal acidosis include changes in populations of amylolytic bacteria, lactic acid producing and utilizing bacteria. Starch fermentation leads to an increase of free glucose within the rumen that promote the growth of bacteria that are normally not competitive. The ruminal acidic environment tends to favor the growth of gram-positive bacteria leading to the destruction of gram-negative bacteria. The lysis of gram-negative bacteria leads to the increase in concentrations of endotoxins within the rumen and the effects of endotoxin absorption will be covered in a later section. A decrease in pH is further potentiated by the increase in VFAs initially in starch fermentation. As previously stated, critical pH levels for SARA are <5.6 and for acute acidosis <5.0. Later VFAs will be seen to decrease in concentration as lactic acid production begins to increase. Lactic acid utilizing bacteria will decrease in numbers in acidic environments further increasing the amount of lactic acid in ruminal fluid. This will lead to an increase in the amount of lactic acid producing bacteria as these strains of bacteria flourish at pH levels <5.6.

Principle bacterial strains that increase greatly in proliferation during starch fermentation are *Selenomonas lactilytica*, *Streptococcus bovis*, and anaerobic *lactobacilli* (Nagaraja and Titgemeyer, 2007). These three strains of bacteria will contribute to the increased concentration of VFAs and DL-lactic acid during low and high grain diets. *S. bovis* is a predominate species that will be seen at higher concentrations during acute acidosis. During high fiber intakes or low glucose availability, rates of growth for *S. bovis* are low, while at high concentrations of glucose, *S. bovis* increases in proliferation and begins to produce lactic acid (Russell and Hino, 1985;

Russell, 1998). Principle compounds produced from *S. Bovis* are dependent on the expression of microbial lactate dehydrogenase (**LDH**) and fructose 1,6 bisphosphatase (**FBP**) enzymes in response to substrate (Russell, 1998). Slow rates of glucose metabolism in *S. Bovis* favor the generation of acetate, formate, and ethanol with the LDH being inactive. The switch to lactic acid production is mainly due to the lower downstream enzymes (pyruvate formate lyase) being sensitive to low pH (Abbe et al., 1982). Pyruvate formate lyase in response to decreases in intracellular pH becomes inactive while higher upstream enzymes are still functional. FBP increases in concentration with the increase in glucose leading to the activation of LDH and production of lactic acid. Lactic acid will then accumulate if the pH falls below threshold suitable for the proliferation of lactic acid utilizing bacteria.

Types of lactic acid utilizing bacteria include *Megasphaera elsdenii* and *Selenomonas ruminatium ssp lactilytica* (Nagaraja and Titgemeyer, 2007). *M. elsdenii* being termed as the most important bacteria in the fermentation of DL-lactic acid (Counotte et al., 1981). However, both strains of bacteria will initially begin with the fermentation of simple sugars such as maltose and glucose. With *M. elsdenii* and *S. ruminatium ssp lactilytica* are dependent on amylolytic bacteria like *S. bovis* to provide simple sugars needed for growth. *M. elsdenii* and *S. ruminatium ssp lactilytica* in the presence of excess sugars will produce lactic acid. Alternatively, when the concentration of simple sugars decreases, they are able to use lactic acid as a carbon source generating butyric acid and propionic acid as by-products respectively (Russell et al., 1981). However, when pH and concentrations of free soluble sugars decrease, the rates of growth by these bacteria slow. Russel et al. (1981) found numbers of *M. elsdenii* grown at a pH of 5.4 decreased in concentration while numbers of *S. bovis* increased in concentration. The production

of lactic acid exceeds the utilization rate of lactic acid utilizing bacteria leading to lactic acid accumulation.

Ruminants well adapted to grain will have similar populations of *S. Bovis* seen in forage fed animals (Wells et al., 1997). These populations will be low and lactic acid production will be manageable by the lactic acid utilizing bacteria in the rumen. However, during prolonged periods of high lactic acid seen in animals not adapted to soluble carbohydrates, the accumulation of lactic acid is not manageable by the lactic acid utilizing bacteria. *S. Bovis* is responsible for the creation of a niche environments suitable for the growth of lactobacilli species through a reduction in pH (Russell and Hino, 1985; Wells et al., 1997). However, *S. bovis* is not a tolerant species of acidotic conditions for prolonged periods of time. If lactic acid concentrations are too high and the ruminal pH falls <5.0, decreases in the growth of *S. bovis* are seen and lactobacilli populations increase (Therion et al., 1982). Lactobacilli species such as L. fermentum can limit the growth of gram-positive bacteria through the production of bacteriocin. Bacteriocin increase the translocation of ions like proton across the cell membrane of gram-positive bacteria leading to a decrease in the efficiency of growth (Wells et al., 1997).

Diet Related Strategies for Prevention of Ruminal Acidosis

Strategies for avoiding exaggerated fluctuations in the ruminal microflora during the transitioning period from an all-forage diet to an all-concentrate diet can be mitigated by limit feeding or by step-up diets. Limit feeding involves a limited amount of concentrate that the animal is allowed to consume over time, while step-up diets utilize a stepwise manner of concentrate introduction over a period time (Slyter, 1976). Adaptation within the rumen involves preventing excessive intakes of fermentable carbohydrates thereby decreasing large fluctuations in ruminal microbial populations, maintaining production of endogenous buffers, and ensuring

proper absorption of VFAs is maintain by the ruminal wall. Inadequate acclimation to high grain diets leads to a reduction in feed intake as well as a reduction in performance. Of the types of acidosis that are more common to cause a reduction in performance leading to economic impacts is SARA. Ruminants that are experiencing SARA have a reduction in performance and feed intake, although these animals may or may not display overt acidotic signs. For this reason, SARA is considered more of an economic issue than acute ruminal acidosis (Tremere et al., 1968).

Successful strategies are those that modulate ruminal pH by preventing large fluctuations in rumen microflora. Reductions in feed intake are seen when the pH reaches 5.6, the physiological threshold for SARA (Fulton et al., 1979). Absorption and neutralization of acids within the rumen are needed to maintain the pH of the rumen. Absorption is maintained by the lining of the rumen which is lined with papilla. Papillae are absorptive structures in the rumen that will increase in development with increased concentrations of VFAs from carbohydrate fermentation (Dirksen et al., 1985). Increases in the amount of VFAs and lactic acid can also lead to these papillae becoming keratinized leading to parakeratosis. With parakeratosis being defined as the thickening of the stratum cornea of the rumen mucosa. Ruminitis can also be seen in response to increased acid production. The absorptive capacity of the rumen wall will decrease with increasing keratinization of the ruminal wall (Dirksen et al., 1985). The loss of the absorptive capacity of the rumen wall leads to accumulation of acid and the decrease in the absorption of VFAs. VFAs will account for 65-75% of the metabolizable energy supply for ruminants and a decrease in the absorption of VFAs leads to a reduction in both feed efficiency and production of meat and milk products (Bergman, 1990). This accumulation of acids is buffered by endogenous buffers.

Endogenous buffers coming from saliva, will account for almost half of the bicarb entering the rumen, which is then able to buffer the acids produced from fermentation. The secretion of saliva will be based on the time the animal spends chewing and ruminating which is based on the amount of fiber in the diet. Fiber in the diet will be the fraction of feed that is indigestible known as acid detergent fiber (ADF) and accounts for slowing the rate of fermentation (Mertens, 1997). To high of fiber can also decrease the performance of the animal due to decreasing the intake of energy, however not enough fiber will increase the risk of SARA. NDF measures the total portion of fiber in the feed and is related to intake, density and digestibility of the feed, and the time spent chewing. Mertens (1997) further defined NDF by the physical size of the fiber which contributes to the formation of a fiber mat in the rumen and the amount of time the animal spends chewing as physically effective NDF (**peNDF**). Processing effects the composition peNDF that is composed within the diet and is based on the feed variety and harvesting conditions of the forage. Corn silage is said to vary greatly in the peNDF due to processing increasing the starch availibity within the corn kernel. This increase in processing of silage increases the risk of SARA. Alternatively, to little processing leaves longer particle sizes which increases the risk of sorting which can also lead to increased incidences of SARA. Proper consumption of total mixed ration without sorting of ingredients was studied by Maekawa et al. (2002). In this study, lactating diary cows fed a 50:50 diet of forage to concentrate had higher intakes of concentrate (67%) to forage (43%) when ingredients were fed separately. These cattle also had lower ruminal pH when compared to cattle that were fed a total mixed ration (**TMR**) (Maekawa et al., 2002). The study highlights the importance of feeding a properly mixed TMR and avoiding excessive intake of concentrate.

The amount of feed required to induce acidosis depends on factors related to the intake of the animal and the composition of the diet. These factors include the response of the rumen microflora to the type of feed, and the animal's ability use or excrete the absorbed products of fermentation. The time required to properly adapt cattle to a high concentrate diet ranges 3-4 weeks and should be done in a gradual fashion (Bevans et al., 2005). Pacing in grain adaptation ensures less variation in ruminal pH during the grain introduction. Bevans et al. (2005) found heifers abruptly introduced to a high grain diet compared to heifers gradually introduced to a high grain diet, were more likely to experience acidosis. This is likely due to longer durations of time with ruminal pH <5.6 and larger variations in pH likely leading acidosis. However, the gradual adaptation to high grain diets does not completely avoid acidosis. Acidosis can still be seen grain adapted animals who consume an excessive amount of soluble carbohydrates when normal feed intake is interrupted. In a study conducted by Goad et al. (1998), steers either adapted to a grain-based diet or a hay-based diet were challenged with a high grain diet to determine their resistance to acidosis. Both groups of steers, fasted for 24 hours before they were fed an all-grain diet, had similar changes in ruminal fermentation patterns. This study suggests abrupt changes in the intake of soluble carbohydrates in animals either adapted to grain or hay are likely to experience acidosis. Abrupt changes in the intake of soluble carbohydrates needs to be avoided during the adaptation phase and the exposure to soluble carbohydrates needs to be gradual and consistent.

Secondary diseases of Ruminal Acidosis

As previously stated, SARA is more of concern for cattle that are fed high concentrate diets. The prevalence in the United States was reported to range from 19-26% for dairy cattle that are fed a high concentrate TMR (Enemark, 2008). While every animal being fed on feedlots

is said to experience some form of ruminal acidosis during the step-up periods to a high concentrate diet. Factors that contribute to the occurrence of acidosis include those that prevent normal consistent intake of feed. Interruptions include storms, where cattle are seen to consume more feed prior to, while other environmental factors such as mud and heat also disrupt the normal feeding pattens of cattle. High mud accumulation will decrease the intake of the animal and high heat will shift the eating to overnight when temperatures are mild (Nagaraja and Lechtenberg, 2007a). Due to these factors, SARA is more prevalent than acute ruminal acidosis (Dohme et al., 2008) and repeated exposure leads to secondary diseases that include laminitis, liver abscesses, rumenitis, and polioencephalomalacia (Brent, 1976). There are some variations in the clinical signs for SARA, while acute acidosis will have more overt symptoms.

Clinical symptoms for acute acidosis will be seen 12-36 hours after grain engorgement. Animals suffering from acute acidosis will appear lethargic, anorexic, with death proceeding 8-16 hours later in serious cases. While the clinical signs of SARA, appearing as obvious as acute acidosis, can affect feed intake, milk production, rumen microflora, rumen digestion, leading diarrhea, rumen mucosal damage laminitis, and liver abscesses (Zhao et al., 2018). The more obvious symptom of SARA is a reduction in feed intake. This reduction in feed intake is described as cyclical in nature as cattle refuse feed due to the decrease in ruminal pH, but appetite returns with the re-establishment of normal ruminal pH. Unfermented and undigested feed particles in the hind gut will be fermented by hindgut bacteria leading to issues with diarrhea. Diarrhea can be seen in both acute acidosis and SARA and appears as a yellow soupy foamy mixture with a fetid odor (Enemark, 2008). Feces may have undigested feed particles in them indicating more a homogenous mixture in the rumen devoid of an adequate fiber mat. Rumen stasis will be evident and is indicative of a high organic acid concentrations, particularly

butyrate (Crichlow and Chaplin, 1985). The increase in the VFA and lactic acid as previously stated will be due to ruminal microflora which favor acidotic environments. Other actions related ruminal microflora in acidic environments involve the expression of the enzyme such as thiaminase.

Increased concentration of thiaminase as been identified in the rumen of animals with polioenceophalomalacia (**PEM**). Cattle on high grain diets are more at risk of developing PEM due to the activity of microbial thiaminases resulting in a thiamine deficiency. Other routes of acquiring PEM are through the ingestion of sulfur, salt, and lead toxicity, as well as water deprivation followed by immediate rehydration (Gould, 1998). PEM develops in animals who are fed high grain diets in as little as four days (Brent, 1976). Clinical signs of PEM involve dullness, head pressing, and blindness persisting to convulsions and nystagmus with permanent opisthotonos. Upon necropsy, the gross appearance of the brain will be swollen with edema and gyri will be compressed. The increased pressure will displace the brain caudal, leading to herniation of the cerebellum and medulla through the foramen magnum. The cerebrocortical grey matter will appear soft with liquefactive necrosis, indicating there is loss of blood flow to regions of the cerebrum. Treatment for PEM involve the administration of thiamine, with rapid recovery depending on the duration of PEM (Brent, 1976). Other toxic insults that lead to vascular destruction are seen with the aseptic inflammation leading to laminitis.

Causes of laminitis are related to conformation, body condition, and environmental factors. However, nutrition is a common component in the development of laminitis in animals fed high grain diets (Nocek, 1997). During the latter portions of a feeding period close to slaughter, it is common to see some lameness in about 60% of cattle (Nagaraja and Lechtenberg, 2007b). The nutritional link to laminitis is multifactorial and may be related to the release of vasoactive

substances that lead to the destruction of the normal hemodynamic flow in the extremities. These vasoactive substances are said to be histamine and endotoxins which have been shown to cause vasodilation and vasoconstriction respectively. With histamine being seen in high concentrations in animals that are fed a high grain diet (Brent, 1976). This increase may be related to the action of lactobacilli producing histamine from the decarboxylation of the amino acid histidine. However, the amount of the histamine that is absorbed from the rumen may be metabolized by the liver once absorbed into the portal circulation and has been shown to be elevated in animals with laminitis (Nocek, 1997). The action of histamine in the extremities is to vasodilate increasing capillary permeability. The increase in capillary permeability leads to edema and the destruction of tissues within the foot leading to the formation of fibrous tissue in chronic cases (Nocek, 1997). A similar cause is linked to parakeratosis leading to the weakening the rumen wall leading to the absorption of endotoxins may also be a cause. Endotoxins absorbed by the animal may trigger an inflammatory response leading to the release of histamine or vasoconstrict of vessels in the extremities leading to hypoxic conditions.

LIVER ABSCESSES

Ruminitis is commonly seen in cattle that are fed high grain diets and liver abscesses are a common sequalae. The action of microbes as previously stated leads to a production of organic acids that increase in concentration leading to a chemical impairment of rumen function. Rumen papillae have been shown to be directly linked to the presence of VFA's within the tissue of the rumen. Butyrate and propionic acid have been linked to the growth of rumen papillae which their growth promotes the absorption of VFA's from the rumen. However, during period of acidosis, the concentrations of the VFA's increase leading to parakeratosis within the rumen. Parakeratosis leads to rumenitis, and absorption of organic acids decreases, further decreasing

the pH in the rumen fluid. As a result, the ruminal mucosal barrier becomes impaired resulting in absorption of rumen microflora by the portal circulation resulting in liver abscesses (Kleen et al., 2003). Liver abscesses result in economic losses related to a reduction in daily gains translating to a reduction in dressing percentages or condemnation of carcasses at time of harvest. Strategies for the mitigation of liver abscesses is through proper feeding management and targeting rumen microbes responsible for causing liver abscesses. However, the use of anti-microbials for increasing the feed efficiency of food animals is under much scrutiny leading to an increased need for other sources of feed additives that promote feed efficiency.

The prevalence of liver abscesses in animals that consume high grain diets is variable and depends on the diet of the animal, days on feed, cattle breed, and gender (Amachawadi and Nagaraja, 2016). There are higher incidences of liver abscesses seen in Holstein cattle when compared to other beef breeds. The increase in liver abscesses seen in Holstein cattle is likely due to the high dry matter intake, and the amount of time they are on feed when fed for beef in feedlots. Holstein cattle in comparison to other beef breeds, will need to be on high concentrate feeds for much longer (>300 d) (Hicks et al., 1990). As a result, there will be higher incidences of liver abscess in Holstein cattle. With reference to gender, heifers are seen to have less liver abscesses when compared to steers. This observation may have something to do with the difference in feed intakes between the two genders of cattle (Amachawadi and Nagaraja, 2016). There is also a direct link between liver abscesses and ruminal acidosis. With liver abscesses be a common sequalae to rumen acidosis. Therefore, proper feed management ensures a gradual adaptation period to a high grain diet, and that there is consistent bunk management preventing irregular feedings. However, of the 20.9% of livers condemned at harvest, two thirds of those livers have abscesses (Mckeith et al., 2012).

Upon harvesting beef cattle for meat, liver abscesses are identified and scored using the Elanco Scoring System. Cattle with liver abscesses will not display outward clinical signs but can have subtleties in performance with reference to feed efficiency. The Elanco Scoring System ranks liver abscesses on a scale from 0, A, and A+. This scoring system will be based on size and number of abscesses within the liver. The appearance of the liver with a score of 0 is considered normal but with increasing severity and appearance of abscesses, livers are designated with a letter. Score of A will have up to four abscesses, but the liver is still considered healthy. A score of A+ indicates an inflammatory response with the presence of large diffuse abscesses (Elanco, 2014). In a study conducted by Brown and Lawrence, livers with scores of A– (livers with 1 to 2 small abscesses) and A+ (livers with multiple large abscesses) had carcasses that were less valuable than carcasses with normal livers. Liver abscesses scored A+ are also noted to have diaphragm adhesions and other visceral organs with adhesions leading to a reduction in animal feed performance and reductions in carcass yield, grade, and value. These animals also will require more trimming at time of harvest and condemnation of all of the visceral organs may be needed (Brown and Lawrence, 2010). Rupturing of abscesses on the slaughter floor leads to interruptions and delays of the assembly line due to possible contamination of infectious bacteria. (Mckeith et al., 2012).

Bacterial Pathology

Liver abscesses are part of the acidosis-rumenitis-liver abscess complex, with up to 32% of cattle who have rumenitis also having liver abscesses (Rezac et al., 2014). A primary etiologic agent related to liver abscesses is *Fusobacterium necrophorum* subsp. *necrophorum*. Subsp. *necrophorum,* is a gram-negative aerotolerant anaerobe commonly found in ruminants and ferments lactic acid. As grain consumption increases the proliferation of subsp. *necrophorum*

increases 10 fold in response to increasing lactic acid concentrations (Tan et al., 1994). For the optimal growth of subsp. *necrophorum* the pH needs to be near 7.4. During acute ruminal acidosis subsp. *necrophorum* is not detectable in rumen fluid. However, adherence to the ruminal wall where pH is closer to the optimal pH of 7.4, subsp. *necrophorum* can survive (Coe et al., 1999). Rumenitis leads to the damage to the ruminal wall allowing for the bacteria to enter the portal circulation of the animal. Within the liver tissue, a high vascularization is seen which increases the amount of oxygen. Subsp. *necrophorum* can survive in these environments but establishes itself in an anerobic environment through the secretion of leukotoxins and endotoxins. Leukotoxins are cytotoxic to white blood cells such as neutrophils and macrophages allowing for the bacteria to evade the immune system, while the endotoxins help to create an anaerobic environment suitable for the growth and proliferation of the pathogen within the liver tissue (Pillai et al., 2021). An additional bacterium responsible for the facilitation of an anaerobic environment is by the bacteria *Trueperella pyogenes*.

T. pyogenes is a lactate producing facultative gram-positive anaerobe that is seen secondary to subsp. *necrophorum* within liver abscesses. This bacterium is found to be a commensal organism on the surfaces of mucus membranes and is found in the contents and wall of the rumen. Location of *T. pyogenes* is located on the ruminal wall next to capillaries which are oxygen rich. Increased ruminitis with abrasions in the ruminal wall leads to the absorption of *T. pyogenes* along with subsp. *necrophorum.* Principle toxins produced by *T. pyogenes* is hemolysin which acts to lyse red blood cells causing coagulation within the liver leading to establishment of an anaerobic environment (Pillai et al., 2021). Both *T. pyogenes* and subsp. *necrophorum* have been shown to have a synergistic effect in benefiting one another in growth. Subsp. *necrophorum* creates leukotoxins which aids *T. pyogenes* in avoiding the immune system

while *T. pyogenes* produces lactic acid needed for the growth of subsp. *necrophorum* (Takeuchi et al., 1983)

Prevention

The use of antimicrobials as feed additives has received much concern due to the increased risk of antimicrobial resistance. There are 6 approved antibiotics for the use of preventing liver abscesses and include, bacitracin, methylene disalicylate, chlortetracycline, neomycin sulfate, oxytetracycline, tylosin, and virginiamycin (Lundeen, 2013). Tylosin phosphate is a macrolide antibiotic commonly used as a feed additive for the prevention of liver abscesses in the United States. The effects of the inhibition of subsp. *necrophorum* are seen in the liver and in the rumen. Recently however due to the increase concern for antimicrobial resistance, veterinary oversight is needed for the use of antibiotics like tylosin as feed additives. Veterinary oversight is needed to limit the use of antimicrobial drugs used for assuring animal health, and to limit the use of medically important drugs that require veterinary oversight or consultation. This was enacted in April 2012 by the Center of Veterinary Medicine of the US food and Drug Administration (US Food and Drug Administration, 2012).

Both subsp. *necrophorum*, a gram-negative bacterium and *T. Pyogenes* a gram-positive bacterium are said to be susceptible to the tylosin. However even with the use of tylosin, there is still a prevalence for liver abscesses which may be due to other infectious forms of bacteria or that subsp. *necrophorum* is resistant to tylosin (Müller et al., 2018). In a study conducted by Nagaraja et al., (1999), two groups of cattle were fed a high concentrate diet either containing tylosin and not containing tylosin were compared measuring bacterial susceptibilities and predominant bacterial isolates. Cattle fed tylosin had fewer liver abscesses containing subsp. *necrophorum* but had more liver abscesses containing *T. Pyogenes*. Microbial susceptibilities

were seen not to differ between the groups. In a similar study, comparing both sustained vs intermittent use of tylosin bacterial resistance was found not to differ (Müller et al., 2018). Weinroth et al, mentioned that resistance and microbiome composition identified from the feces of cattle fed tylosin and not fed tylosin, may be due to location of cattle production. There is much interest regarding the use of antimicrobials as feed additives although there is a need for more research regarding the etiology and pathogenesis regarding bacterial resistance (Weinroth et al., 2019).

ZEOLITES

Zeolites are minerals that come in both synthetic and natural forms and can be used as a molecular sieve for trapping and releasing cations (Step et al. 2008). Origins of the natural forms of zeolites are volcanic in origin and are soft, friable, and light weight (Mumpton. 1999). On the molecular scale the mineral has a three-dimensional porous structure containing cations of aluminum ($\mathbf{AlO_4}$) and silicon ($\mathbf{SiO_4}$). The oxygen contained in these structures are the linkages that make the three-dimensional structure creating pores which are effective ion exchangers and can gain or lose water irreversibly. With the use of heat or the application of other cations, filtered cations can be washed out of the crystalline lattice. Zeolites can be used for filtering toxins and elements that may be deleterious to humans and animals. This wide range of applications gives the mineral the ability to be used in industrial applications for water treatment, waste treatment, catalysis, nuclear waste, agriculture, and animal feed additives.

The filtering ability of the mineral is dependent on the size of the pores which contain the negatively charged channels bound to positively charged monovalent and divalent alkali ions which include Na^+, K^+, Ca^{2+} , OH-groups, or H_2O molecules. These ions can be exchanged for cations from the surrounding environments which include NH_3, NH_4, nitrates, and other metals

like lead, copper, and zinc. Of the many forms of zeolites, clinoptilolite has been used in the agricultural setting. Clinoptilolite has been placed in 450 °C heat without being destroyed when compared to other forms of zeolites. Clinoptilolite has a higher silicon to aluminum ratio and will have a higher affinity for binding Na and K over Ca, Sr, and Ba. Clinoptilolite also has a higher silica content which will make it more stable in acidic environments (Ghiara et al., 1999). The use of zeolite may be used in animal waste treatment to reduce malodor, control moisture, and prevent the loss of nutrients from anaerobic fermentation (Mumpton, 1999).

Clinoptilolite has a selectivity for NH_4 and potassium ($\mathbf{K}^+$) ions and can be used as a soil conditioner which will act as a slow-release fertilizer. If clinoptilolite is added to compost it can bind the available nitrogen and prevent the leaching of nitrates from soils (He et al., 2002). In a study conducted by He et al., (2012) clinoptilolite added to compost at a rate of 15 g kg^{-1} reduced the amount of NH_4 volatilization from agricultural soils. Results from this study were seen to amplify when cellulose was added to the soil increasing the soil microbial biomass. This effect is likely due to the increased nutrient retention when clinoptilolite and cellulose are combined. In a similar study, the addition of 21% clinoptilolite added to manure had lower levels of nitrogen leaching compared to when soils were treated with urea alone. Soils when treated with a concentration of 21% clinoptilolite had 36% less nitrogen leaching when compared to urea treated soils. This may be due to the absorption of NH_4 by clinoptilolite preventing the access to ammonium by nitrifying bacteria due to the small pore size of clinoptilolite (Gholamhoseini et al., 2013).

Animal Studies

Recently the use of zeolites like clinoptilolite have been used to improve the quality and performance of animals in decreasing the amount of mycotoxins in the milk upon exposure

(Katsoulos et al., 2016) and improving the absorption of immunoglobulins in calves consuming colostrum (Marc et al., 2018). However, the integrity of the clinoptilolite when added to food animal diets needs to be considered to determine if it is generally recognized as safe (**GRAS**). The movement of clinoptilolite through the animal digestive tract may become hydrolyzed releasing silicon and aluminum ions that will impair the absorption of other minerals by the animal. In a study conducted by Karatazia et al., (2011), clinoptilolite was fed at 200 g per day to dairy cattle and noted no change in ruminal and blood concentrations of aluminum or phosphorus. The effects in the rumen showed that when clinoptilolite was fed to cattle there was also an increase in ruminal pH. This shows that there is an effect on ruminal fermentation when clinoptilolite is added to the ruminant diets however there are some variations in these effects.

The addition of clinoptilolite to ruminant diets has shown to influence concentrations of NH_4 thereby decreasing the amount of nitrogen that is excreted from animals on high protein diets. Within in vivo and in vitro studies it was shown that zeolites are able to sequester up to 15% of ammonium ions within in ruminal contents (White and Ohlrogge, 1974). In a study conducted by McCollum and Gaylean (1983), crossbred steers fed 2.5 and 5% clinoptilolite had a notable decrease in ruminal NH_3 concentration at 6 and 9 hours post feeding when compared to animals not fed with clinoptilolite. Similar results were seen in a study conducted by Sadeghi and Shawrang (2007), with ruminal ammonium concentrations being decreased when Holstein steers were fed 30 g/kg of clinoptilolite with no improvement in feedlot performance. The use of zeolites within food animal diets and manure management may have some benefits as a means for controlling the excretion of products from microbial fermentation like ammonium. Which have negative impacts on sensitive ecosystems. However due to the limited information

concerning the effects of including zeolite in food animal diets, the need for more research is warranted.

CHAPTER III: EFFECTS OF CLINOPTILOLITE INCLUSION ON RUMINANT INVITRO DIGESTIBILITY

INTRODUCTION

With the use of feed additives in ruminant diets, feed performance can be improved by manipulation of rumen fermentation patterns and ultimately lead to improvements in nutrient utilization. Being that feed costs account for 60 to 70% of expenses in cattle production (Becker, 2008), increasing feed efficiency could increase profitability for beef producers. Improvements in feed conversions by rumen microbiota can be seen in the production of volatile fatty acids and the animal's ability to absorb and utilize nutrients. Therefore, improvements in rumen fermentation translate to improvements in activities of microorganisms within the rumen (Lima et al., 2019). These improvements in rumen fermentation can also help mitigate the production enteric greenhouse gases which have been linked to environmental issues (Hernandez-Sanabria et al., 2012). However, common feed additives such as tylosin used by the feedlot industry for improving rumen fermentation by reducing ruminal acidosis may be linked to antimicrobial resistance (Weinroth et al., 2019). Therefore, there is a need alternative feed additives that function in improving rumen fermentation.

Zeolites have been shown to alter the patterns of fermentation within the rumen which may lead to improvements in feed efficiency and organic matter digestion (Urías-Estrada et al., 2018). This is thought to be through the minerals properties as an aluminosilicate which can trap and release cations (Mumpton and Fishman, 1977). Within the rumen, zeolites can be used to sequester ruminal ammonium concentrations and increase pH when ruminates are consuming a high grain diet. Thus, mitigating the negative effects seen with ruminal acidosis leading to a reduction in liver abscesses. This is due to the minerals ability to exchange constituent ions for

hydrogen ions thus increasing the ruminal pH (Mumpton and Fishman, 1977). However, the sequestration of ruminal ammonium and the effects on microbial protein synthesis are not known. The use of zeolites within beef cattle being feed high grain diets is limited and the impacts on rumen fermentation need to be researched further. The aim of this study was to assess the effects of zeolite inclusion on in vitro fermentation at a 5% inclusion rate.

MATERIAL AND METHODS

Experimental Feed Additives

Natural zeolite was purchased from BioGreen Technologies Inc., Denver Colorado. Zeolite is a mineral composed of silicon and aluminum with tetrahedra microporous arrangement. The general formula for the clinoptilolite used in the following studies was N_2(Al, Si, O_{12}) x 6 H_2O. The chemical composition for the zeolite used in experiment 1, 2, and 3 was provided by Dr. Jan Krason, GeoExplorers International, Inc., Denver Colorado (table 1).

Experimental Procedures and Treatments

A modified procedure from Tilley and Terry (1963) was used to determine alfalfa in vitro dry matter digestibility (**IVDMD**) in the presence or absence of ZE at a 5% inclusion rate. The experiment was conducted at the Agriculture, Research, Development, and Education center in Fort Collins, Colorado.

Whatman filter papers (#54) were weighed, labeled, and placed into a desiccator. These papers were used to weigh residue after the DM digestibility assay was complete. 1.0 gram (g) of dried alfalfa was weighed into each test tube. Test tubes were then placed in a 39 °C oven for 24 hours prior to in vitro run. Artificial saliva or McDougall's solution (1 L Deionized H_2O, 9.80 g $NaHCO_3$, 3.70 g Na_2HPO_4, 0.57 g KCl, 0.47 g NaCl, 0,12 g $MgSO_4$ * 7H2O, and 0.50 g Urea) was then prepared prior to the collection of ruminal fluid. At this time blank tubes (6) containing

ruminal fluid and artificial saliva without substrate were made to account for endogenous losses. Ruminal fluid was collected 2 hrs after feeding fistulated steers grass hay maintenance diet. Ruminal fluid after collection was filtered through 4 layers of cheese cloth twice while bubbling CO2. Tubes containing ctrl (1 g of alfalfa without ZE) and treatment (trt) (1 g with ZE) samples were wet with 4mls of deionized water 1 hr prior to mixing with ruminal fluid and artificial saliva. 10mls of ruminal fluid was combined with 40mls of 39 °C artificial saliva in a large prewarmed beaker. A 50ml mixture of ruminal fluid/artificial saliva was then added to each digestion tube. CO2 was then bubbled into the top of the tube for 15 seconds before placing a rubber one way stopper on the top of the tube beginning the IVDMD. Tubes were then allowed to incubate for 0, 1, 2, 4, 6, 8, 12, 24, and 48hrs in a 39 °C water bath. Tubes during this time were swirled every 4hrs (making sure not to splash sides of tube with fermenting contents). Fermentation was stopped by placing the tubes in 18 °C ice bath. Samples were then immediately filtered through a pre-weighed # 54 Whatman filter paper after rising papers with deionized water. IVDMD was calculated 24hrs after drying period of Whatman filter paper and sample residue (Tilley and Terry, 1963).

Statistical Analysis

The statistical analysis was done using RStudio (Team, 2019) packages car (John Fox, 2019) , emmeans (Lenth, 2019), ggplot2 (Hadley Wickham, 2016) and dplyr (Hadley Wickham, Romain François, Lionel Henry, 2019) with the significance level $\alpha = 0.05$.

The study was designed as a random complete block design and was analyzed using a multiple regression, where hours served as a blocking variable. Outliers were determined by using both the outliers test function in Rstudio and creating a data frame using the arrange function from the dplyr package to return the largest Rstudent residuals. Diagnostic plots used

for the second model had issues with normality and equal variance due to outliers included within the data. Outliers (n = 5) were then removed, and the third model was used for the rest of the analysis. Diagnostics plots were run on the final model, residuals vs. fitted plots showed a small pattern but this was thought be ok. QQplot testing for normality also showed no issues.

Digestibility was the response variable; predictor variables were hours and treatment with hours functioning as a blocking variable. Table 1 lists the sample sizes for each treatment with means and standard deviations. The data was unbalanced, with some samples having to be removed due to incomplete incubation of substrate. Which may have resulted in damage of rumen micro flora due to light, temperature, or some other unknown factors. A graph was chosen to represent the means for both treatments visually. Outliers were removed from 0-, 4-, and 6-hours' time blocks from the control group and from 12- and 24- hours' time blocks from the treatment group.

The first model included an interaction between treatment and hours. This interaction was found not to be significant and was removed from the final model (p value = 0.42). Summary Statistics were then created and included in a table 1 and figure 1. Data was unbalanced, therefore a type 3 Anova, and emmeans for pairwise comparisons were used. The final model focused on the main effects for both treatment and hours. Summary of the final model gave the adjusted R2 value of 0.95. Emmeans for pairwise comparisons were analyzed to compare the treatment effects at each time period with hours held constant. These pairwise comparisons showed no difference in treatment means during the 48-hour period. Additional pairwise comparisons for hours averaged over treatment were analyzed and showed significant differences between hours. With the biggest difference being seen in hour 0 and 48.

RESULTS

These data demonstrate that inclusion of ZE, does not influence in vitro digestibility of alfalfa. From fig. 1, digestibility for both treatment and control showed similar digestibility percentages during the 48-hour incubation period. The pairwise comparison for treatment when averaged over hours had no significant differences (mean ± SE, 30.5 ± 0.38 and 31.1 ± 0.37 % Digested/hour, p value = 0.266). From table 2, the only significant difference was seen in hours. During the 48-hour incubation period, fermentation in digestion tubes was significantly different (p value = <2e-16). With the largest differences seen in hours 0 and 48 when average over treatments (p value = <.0001).

Table 1. Chemical composition of natural zeolite (clinoptilolite). Major exchangeable cations are: Rb, Li, K, Na, Ag, Cd, Pb, Zn, Ba, Sr, Cu, Hg, Fe, Co, Al, and Mg. With major exchanging gases consisting of: CO, CO_2, SO_2, H_2S, NH_3, C_2H_2, CH_3OH, CH_3NH_2, CH_3Cl, and H_3Br.

Item	Zeolite Characteristics
Chemical Composition	
SiO_2	81.13
Al_2O_3	9.66
K_2O	6.18
CaO	1.96
Na_2O	0.33
Fe_2O_3	0.90
MgO	0.38
Si/Al ratio	7.12
Mean Pore Diameter (nm)	9.49

Table 2. Effects of ZE supplementation on in vitro fermentation for trt (alfalfa + ZE (5%)) and ctrl (alfalfa). Mean was a percentage of total feed fermented during incubation.

Item	Hours	N	Mean[1]	Std. dev	SE
Treatment	0	6	18.1	5.47	2.23
	1	6	19.0	1.64	0.67
	2	6	22.8	2.51	1.02
	4	6	24.9	2.15	0.88
	6	6	28.9	1.59	0.65
	12	5	32	1.32	0.59
	24	2	44.9	0.71	0.5
	48	6	53.5	1.05	0.43
Control	0	5	20.2	2.89	1.29
	1	6	22.1	3.26	1.33
	2	6	21.1	1.14	0.46
	4	5	27.2	1.67	0.75
	6	4	27.4	0.79	0.39
	12	6	30.9	1.98	0.81
	24	6	45.3	1.55	0.63
	48	6	54.3	1.58	0.65

[1]Mean: The response variable digestibility was calculated by using the equation : 1.00 – ((R-F) – blank/oven-dried sample weight)) * 100 = percent digestibility (R = weight of residue and filter paper, F = weight of filter paper).

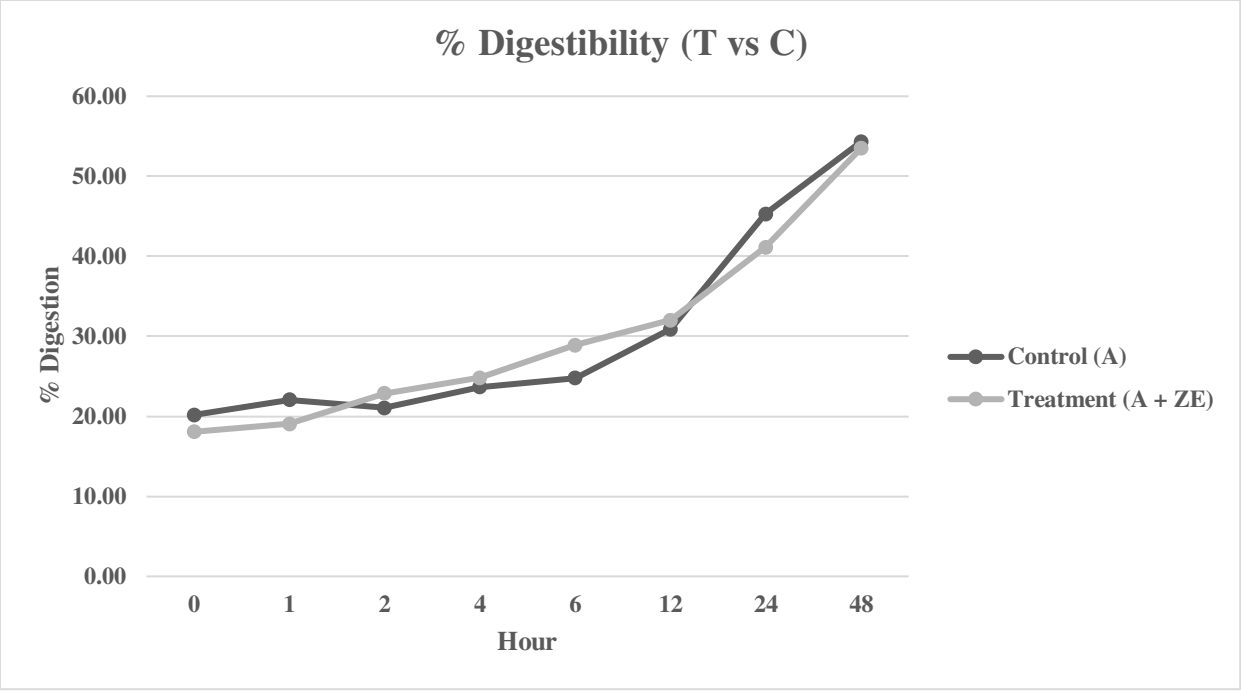

Figure 1. Differences in mean digestibility's for treatment and control. Mean digestibility's for treatment (ZE + alfalfa) and control (alfalfa) taken over a 48-hour incubation period.

DISCUSSION

ZE inclusion had no negative or positive effects on in vitro digestibility's in our experiment. While El-Nile et al., (2021) noted linear increases in total degraded organic matter when using clinoptilolite. The ZE used in that experiment had a different formula and chemical composition than the ZE used in our experiment. The formula consisted of $(Ca, K_2, Na_2, Mg)_4$ Al_8 Si_{40} O_{96} x $24H_2O$ with a silicon to aluminum ratio ranging from 4.80-5.40 (El-Nile et al., 2021). While the silicon to aluminum ratio for the ZE used in our experiment was 7.20. This may be the reason for the noted effects and differences seen in digestibility's for our experiment. Concentrations of NH_3 and proportions of VFA's were not considered in this experiment. However, nitrogen concentrations of NH_3 within the in vitro experiment conducted by El-Nile et al., (2021) had decreasing amounts with changes in proportions of VFA's. Proportions of acetate decreased, with proportions of propionate increasing due to ZE inclusion (El-Nile et al., 2021).

This may suggest there are some improvements in carcass characteristics in beef cattle fed diet with ZE inclusion.

CONCLUSION

Supplementing ZE showed no positive or negative effects on in vitro dry matter digestibility, however the data from this study was limited. Future studies would like to consider final nitrogen concentrations, proportions of VFA's, and pH to determine if there is an effect with ZE supplementation on rumen fermentation.

CHAPTER IV: IN VIVO DIFFERENCE IN NITROGEN CONCENTRATION WITH OR WITHOUT CLINOPTILOLITE INCLUSION IN DAIRY CALF HUTCHES

INTRODUCTION

The volatilization of ammonia seen from cattle manure is mainly due to urinary nitrogen with urea constituting 65-90% of the total urine nitrogen (Whitehead and Raistrick, 1993). Urea will become hydrolyzed to ammonium bicarbonate by microbial derived urease enzymes within the environment. Increased rates of hydrolysis will lead to ammonium volatilization in the form of ammonia. Volatilization will be seen when manure is applied to agricultural land and will decrease the agronomic value of manure (Pfluke et al., 2011). Volatilization of ammonia will be dependent on environmental factors such as temperature, pH, cation exchange capacity, and the soil nitrification activity. Ammonia volatilization increases during high environmental temperatures, dry soil conditions, and when soil pH is high (Whitehead and Raistrick, 1993). Negative effects of ammonia volatilization are linked to damages seen in the surrounding soils and water systems. Ammonia volatilization has been linked to acidification and eutrophication of forest water sheds and soils and can contribute to changes flora ecosystems (Pfluke et al., 2011). Ammonia once in the atmosphere can also react with nitric acid leading to increase nitrate particles within the air and is linked to negative impacts on human health.

The addition of zeolite to manure may help to reduce the amount of ammonia volatilization due to the mineral's high affinity for ammonium and increase in water retention in soils. The mineral has a high cation exchange capacity with great affinity for ammonium (He et al., 2002). In the past zeolite has been used to minimize ammonia emissions from manure (Amon et al., 1997) and reduce ammonia toxicity in plants (Gupta et al., 1997). When zeolite binds the ammonium in soils it slowly releases ammonium (Kithome et al., 1998) decreasing the rate of

eutrophication and concentration of nitrate production from soil bacteria. Therefore, we predict the use of zeolite top dressed in calf hutches will reduce the amount of nitrogen that is lost during handling and may have increased concentrations of nitrogen. We hypothesize that the addition of zeolite to calf hutch bedding at 5% will reduce nitrogen volatilization.

MATERIAL AND METHODS

This study was in collaboration with the Department of Animal Science in 2020 and was performed to determine the effects of nitrogen retention with the inclusion of clinoptilolite at a 5% inclusion rate in calf hutch bedding. All procedures involving live animals were approved within the guidelines of the Colorado State University Animal Care and Use Committee (Approval # IACUC 20-151).

Experimental Design and Experimental Treatments

Ten calf hutches were used to determine if there was an effect of clinoptilolite (**ZE**) inclusion to the bedding of diary calf hutches as a method for the reduction of NH_3 emissions and for the ameliorative effects related to animal health. Factors for the study included the use of top dressed ZE (5%) added to wood shavings for a duration of 105 days. Factors for the experiment consisted of two treatments with 5 replicants per treatment: Treatment 1 (ZE) and Treatment 2 (No-ZE).

Calves, Hutches Parameter and Sample Collections

Calves recently born were place in a calf hutch and assigned to ZE treatment or No-ZE treatment groups. Bedding was weighed (~55.5 lbs./Calf hutch) and placed in each calf hutch prior to calf introduction. After a weather event or if calf bedding needed to be added, new bedding was weighed (48.2 lbs./calf hutch) and place in each calf hutch with additional ZE added to ZE replicates. When calves were removed from calf hutches and all bedding was

collected and weighed (ZE; 334.3 lbs./Calf hutch, No-ZE; 289.4 lbs./Calf hutch). Sub samples from each calf hutch were collected and homogenized for analysis of chemical composition. No calves died or displayed sickness during the study.

Sample Evaluation

Sub samples collected from calf hutches were freeze dried at -65 °C until there were two identical consecutive weights. The samples were then finely ground using a Thomas-Wiley laboratory mill with a 1mm screen. Composite samples were analyzed for protein, acid detergent fiber, neutral detergent fiber, and ash utilizing SDK laboratories (Hutchinson, KS 67501).

Statistical Analysis

The statistical analysis was done using RStudio (Team, 2019) packages car (John Fox, 2019) , and emmeans (Lenth, 2019). Calf hutch was the experimental unit with significance being determined with least square means and a Welch Two Sample T-Test with significance level $\alpha = 0.05$.

Diagnostic plots were used to determine outliers. Calf hutch four was found to have issues with normality and equal variance and was removed from the final analysis. Reasons for this are unknown but may have been related to the environment such as a wind or rain. Sampling methods may have also been as issue.

RESULTS

Table 3 provides mean data and statistical results for calf hutch bedding with and without ZE inclusion at 5%. Statistical analysis for the comparison for bedding between treatment and control showed no significant difference ($p = 0.107$) in nitrogen concentrations (Figure 2). Kilograms (Kgs) differences for ADF were found not to be different between the treatments ($p = 0.935$) (Figure 3). Kgs differences for NDF between treatments were not significantly different

(p = 0.537) (Figure 4). Similarly, Ash was also found not to be significantly different between treatments (p = 0.513) (Figure 5).

Table 3. Manure composition for pounds of Nitrogen, ADF, NDF, and Ash are in pounds of DM. Data represents mean with standard deviation in parentheses.

	Treatment	Control	P-Value
Nitrogen	0.974 (0.047)	1.040 (0.051)	0.107
ADF	44.852 (4.034)	45.097 (4.120)	0.935
NDF	49.908 (4.107)	51.869 (4.362)	0.537
Ash	19.352 (5.313)	16.920 (4.541)	0.513
DM	0.603 (0.107)	0.535 (0.055)	0.317

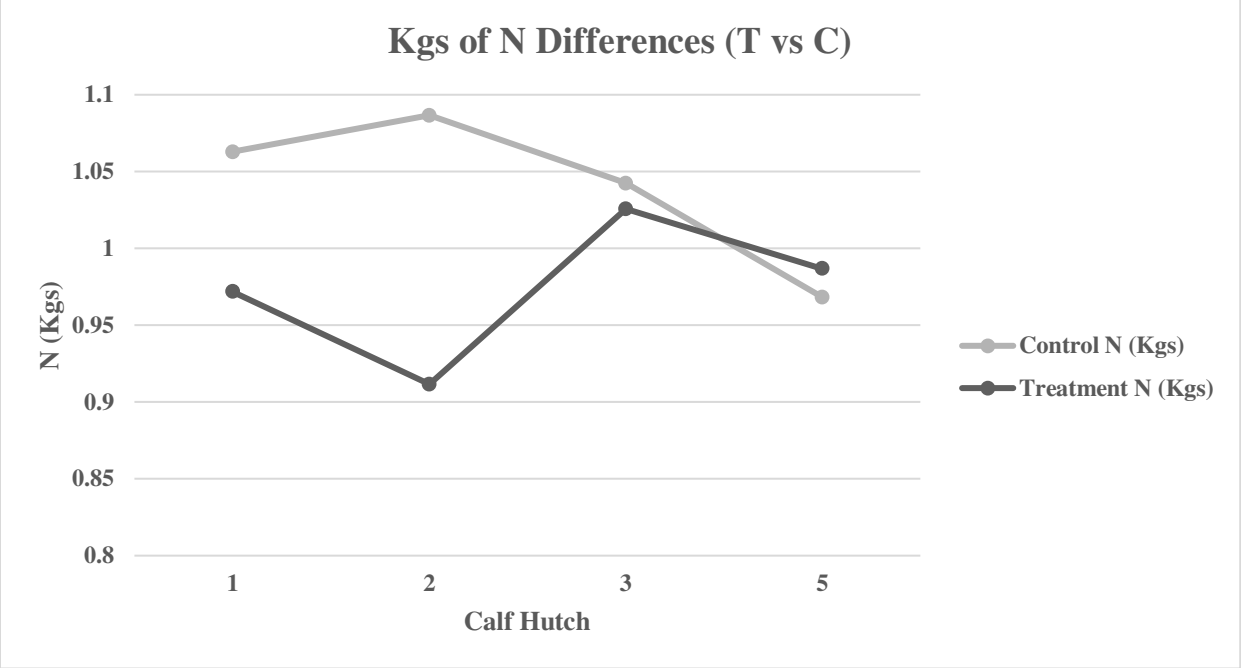

Figure 2. Kilograms of nitrogen differences for calf hutches with or without clinoptilolite inclusion at 5%.

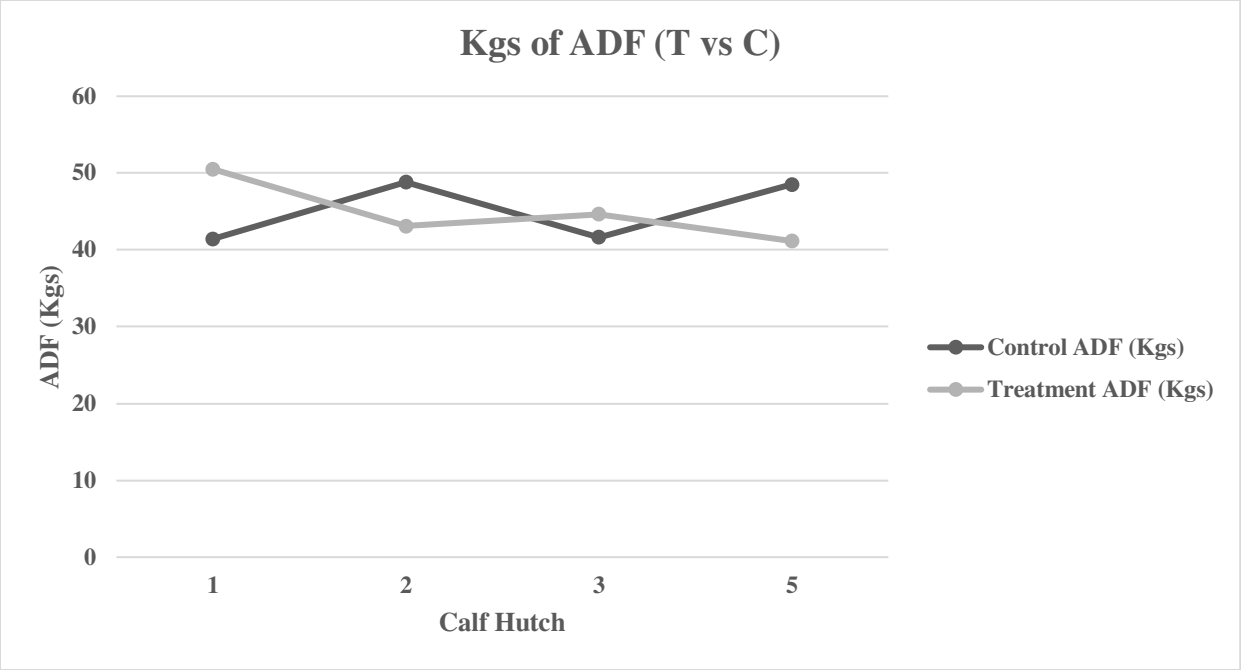

Figure 3. Kilograms of acid detergent fiber (ADF) differences for calf hutches treated with or without clinoptilolite inclusion at 5%.

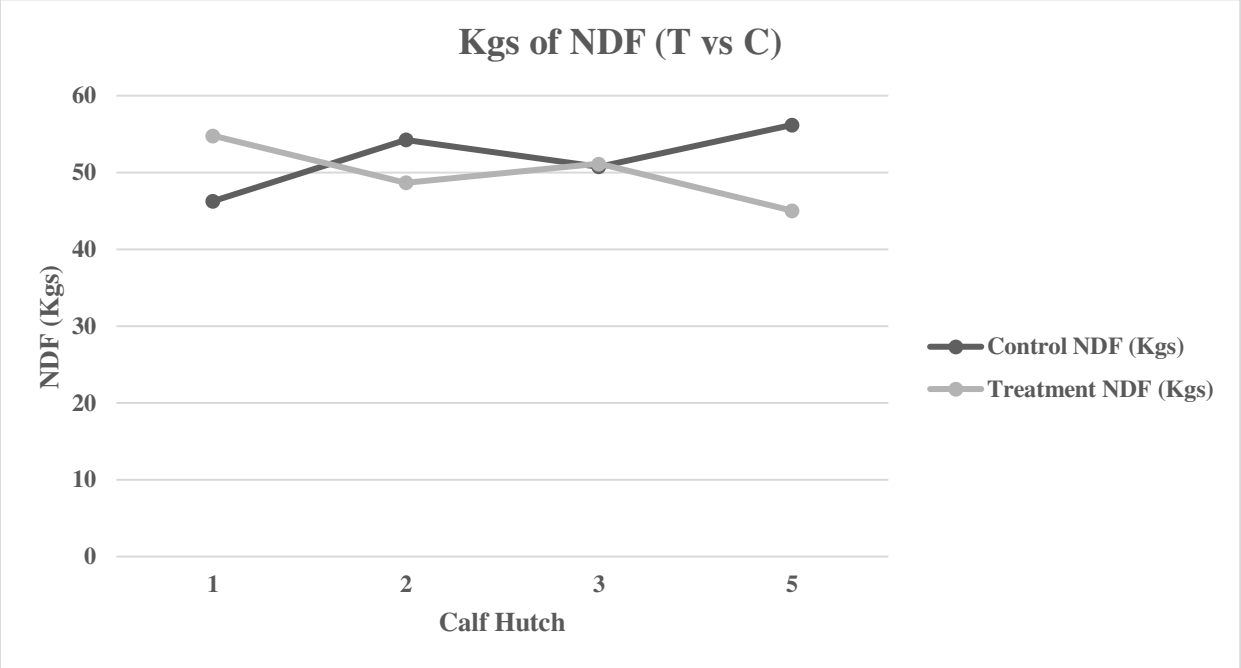

Figure 4. Kilograms of neutral detergent fiber differences for calf hutches with or with clinoptilolite inclusion at 5%.

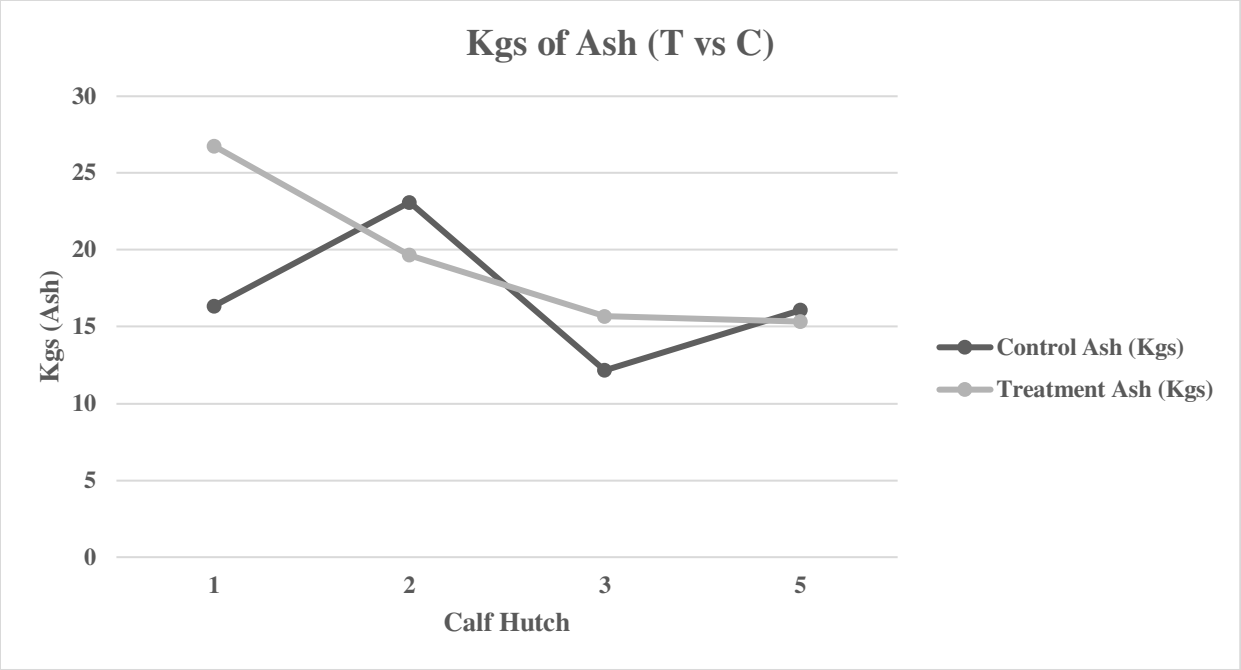

Figure 5. Kilograms of ash differences for calf hutches with or without clinoptilolite inclusion at 5%.

DISCUSSION

Bedding calf hutches with 5% ZE showed no differences in nitrogen concentrations when compared to control ($p = 0.107$). A similar effect was also seen when ZE was added to solid dairy manure on sandy soil in a study conducted by Tarkalson and Ippolito et al., (2011). Researchers attempted to slow the rates of nitrification of NH_3 with the use of ZE over a period of a year but found no difference in rates of nitrification or mineralization in nitrogen over time. The researchers speculated that potassium may be out competing NH_3 for exchange sites on the ZE molecule (Tarkalson and Ippolito, 2011). In the current experiment, other cations such as Rb, Li, K, Na etc., were not identified but differences in Ash compositions between treatments and control were found not to be significantly different ($p = 0.513$). ZE was not applied to bedding during the termination of the study suggesting losses in nitrogen from volatilization due to handling and sampling.

Other samples such as the soil under the bedding were not sampled nor was the soil outside of the calf hutch. All samples collected from the experiment consisted of bedding that was within the calf hutch. Future experiments should focus on collecting samples from all locations within the calf hutch and collect samples over time to determine if there is an effect related to handling and to see nitrogen accumulation over time.

CONCLUSION

Top dressing ZE in calf hutches showed not differences in end nitrogen concentrations. However, the data from this study is limited and only considers the end nitrogen concentrations. Future studies should focus on the concentrations of nitrogen that are accumulated over time, collect representative samples from all locations in the calf hutch, and determine if other cations within the bedding are out competing nitrogen for ZE cationic exchange sites.

CHAPTER IV: INCLUSION OF NATURAL ZEOLITE (CLINOPTILOLITE) IN FINISHING RATION OF FEEDLOT BEEF CATTLE

INTRODUCTION

Clinoptilolite (**ZE**), a common form of zeolite, can be used as a feed additive to improve organic matter digestion, average daily gain (**ADG**), and feed efficiency in ruminant animals (Pond, 1984; Urías-Estrada et al., 2018). However, there is lacking research with the use of ZE within the feedlot setting regarding the improvement in animal performance. ZE in the feedlot industry can be used to help control the levels of ammonium in the rumen preventing metabolic issues due to the cationic exchange with ionic compounds ameliorating acidity levels seen during increased fermentation rates. This can be done by sequestering excess nitrogen and slowly releasing it during periods of low ammonium levels to help stabilize microbial synthesis. The addition of the ZE may also help stabilize the pH within rumen to help alleviate complications related to ruminal acidosis. These complications are related to the ruminitis – acidosis – liver abscess complex which is related to reductions in both carcass quality and feed efficiency. However, there is little understanding concerning the type and amount of zeolite that provides the greatest enhancements and is the focus of this study.

Of the studies that do exist, natural zeolite added to the diet at 2% was found to be beneficial for weight gain in growing lambs when compared to synthetic zeolite (**zeolite Na-A**) when added to a corn based diet containing soy-bean meal or fish meal for dietary nitrogen sources (Pond, 1984). This study conducted by Pond et al., (1984), demonstrated that the growth response was dependent on the source of nitrogen within the diet of growing male lambs when zeolite Na -A or ZE was used. Growth and feed utilization was found to be greater in lambs fed soybean meal when compared to urea when either ZE or zeolite Na-A was included at 3% of the

diet (Pond et al., 1984). These studies suggest there is an effect on the patterns of rumen fermentation when ruminants are fed either natural or synthetic forms of zeolite and this may be related to the NH_4 binding capacity of zeolite within the rumen. Therefore, the use of natural forms of zeolite in the diet of ruminants may help alleviate fluctuations in microbial synthesis and aid in mitigating ruminal acidosis in cattle fed high concentrate diets and decrease the incidences in liver abscesses seen in cattle at slaughter. This study is interested in the effect on average daily gain, feed efficiency, dry matter intake, and overall weight gain when ZE is included in the diet. We hypothesize that the addition of clinoptilolite will provide a ruminal buffer promoting increased production efficiency reducing the number of hepatic abscesses found during carcass evaluation.

MATERIAL AND METHODS

Experimental design, and Experimental Treatments

Three – hundred and twenty cross bred steers were used to evaluate the effects of natural zeolite inclusion on the performance of feedlot steers fed a high concentrate, steam flake corn-based finishing diet for 146d. The study was a completely randomized block design consisting of steers supplemented with or without ZE inclusion. The design of the study consisted of 4 experimental treatment groups with 8 replicates per treatment (10 head per pen) consisting of ZE added to a finishing ration at concentrations of 0, 0.5, 1, and 2% of diet DM. Steers were blocked by weight and randomly assigned to one of the four treatment groups, with pen (n = 32) serving as the experimental unit for nutrient intake data.

Animals and Experimental Procedures

The study was conducted at the CSU Agriculture, Research, Development, and Education Center (ARDEC) in Fort Collins, Colorado. All steers were weighed (n = 320, initial BW 401 ±

41 kg), given an ear tag used for identification, and vaccinated prior to the start of the study with the following: Pyramid 2 + Type II BVD (Fort Dodge Animal Health, Fort Dodge, IA), ProMectin (ivermectin; IVX Animal Health, Inc., St. Joseph, MO), Presponse SQ (Fort Dodge Animal Health), and Safe-Guard Suspension 10% (fenbendazole; Intervet / Schering-Plough Animal Health Inc.). After processing animals were allowed ad libitum access to grass hay and water.

Steers were fed once a day to allow ad libitum access to feed. Feed bunks were managed with a slick bunk protocol so little orts being collected. Diet formulations were balanced to meet or exceed the recommendations of the NRC (2015) for growing beef steers (Table 4). Steers were adjusted to the finishing ration by being fed an initial grower ration and incrementally increasing the grain within the diet by 25% each week in a step-up fashion. When steers were adjusted to the finishing ration, steers were place in one of the 4 treatments (0, 0.5, 1, and 2% ZE). Steers were individually weighed on days 0, 21, 49, 77, 105, 138, and 167.

All steers were sent to the commercial abattoir Diamond R Livestock Services Inc., Yuma, CO for slaughter. Hot carcass weights were obtained at time of slaughter and allowed to cool prior to the obtaining carcass data. Carcass data included, marbling, yield grade, rib eye area, and calculated yield grade. Liver inspections were done by a trained independent USDA inspector.

Analytical Procedures

Total mixed ration subsamples were dried in a 60° C oven until 2 consecutive weights were obtained. Subsamples were then finely grounded in a Thomas – Wiley laboratory mill with a 1 mm screen. Composite samples were then sent to SDK laboratories, Hutchinson, KS for proximate analysis.

Statistical Analysis

Data was analyzed using a mixed procedures of SAS (Version 9.3, SAS Inst. Inc., Cary, NC). Pen served as the experimental unit. Significance was determined with least square means, which was done with an F test ($P \leq 0.05$) and tendencies were declared when $P \leq 0.10$. Initial weight was used as a covariant with fixed effects being final BW, total average daily gain, and feed efficiency. Effect of diet on liver abscesses was analyzed used a Fisher's Exact Test in RStudio (Team, 2019).

RESULTS

Feed lot performance

The effect of feeding ZE on feedlot performance is summarized in table 5. The only noted difference that was seen was a decrease in DM intake when steers were fed 1% ZE ($p = <0.01$). This was an approximate 0.34 kg per day decrease in DM intake when compared to the other diets .

Carcass Characteristics

The effects of ZE inclusion on carcass characteristics is summarized in table 6. The effects of ZE inclusion did not improve carcass characteristics, nor did it have any deleterious effects when fed at 0.5, 1, and 2% of diet DM. When looking at condemned liver proportions (table 7), there were no effects of treatment on liver abscess rates ($p = 0.544$) with rate of liver abscesses being independent of treatment. However, there was a non-significant decrease of 50% seen in A+ liver abscess scores for cattle consuming 1 and 2% ZE of diet DM. A score of "0" indicated no abscesses and represents the highest per pound value for livers. A score of "A" indicates on or two small abscesses, representing a reduced per pound value for livers. A score of "A+" indicates multiple abscesses and represents no monetary value.

Table 4. Step-up Ration Composition on a Dry Matter Basis.

	Ration Composition, % Step			
Ingredient	Starter	Step 1	Step 2	Finish
		Base Ration		
Steam Flaked Corn	33	49.51	64.17	79.07
Corn Silage	14	13.38	13.38	10.37
Alfalfa Hay	43.93	28.19	12.44	0
Dried Distillers Grains	0	0	0	5
Liquid supplement	2.23	2.17	3.25	3.81
Mineral Supplement	4.69	4.76	4.76	4.76
		Treatment		
	1	2	3	4
Limestone	2.00	1.50	1.00	0.00
Zeolite	0.00	0.50	1.00	2.00

Table 5. Effects of natural zeolite on performance of feedlot steers.

Item	0[a]	0.5[b]	1[c]	2[d]	SEM	P-value <
Initial body weight, kg	413.23	412.13	414.66	409.66	3.18	0.38
Final body weight, kg	687.66	691.46	694.84	692.76	8.01	0.81
Overall gain, kg (per animal)	274.43	279.33	280.18	283.48	6.94	0.59
Average daily gain, kg/hd/d						
ADG1[1]	1.32	1.33	1.56	1.48	0.13	0.22
ADG2[2]	1.44	1.51	1.60	1.58	0.06	0.10
ADG3[3]	1.66	1.68	1.72	1.67	0.48	0.72
10ADG4[4]	1.61	1.61	1.66	1.65	0.51	0.72
ADG5[5]	1.57	1.60	1.61	1.62	0.04	0.69
ADG Final[*]	1.99	2.03	1.98	2.09	0.09	0.76
Dry matter intake, kg/hd/d	11.16	10.93	10.82	11.00	0.29	<0.01
Feed efficiency, g/f	0.18	0.19	0.18	0.19	0.008	0.68

[a]Diet consisting of control with no zeolite.
[b]Diet consisting of zeolite at 0.5% of diet DM.

[c]Diet consisting of zeolite at 1% of diet DM.
[d]Diet consisting of zeolite at 2% of diet DM.
[1]Calculated between day 0 and day 21.
[2] Calculated between day 21 and day 49.
[3] Calculated between day 49 and day 77.
[4] Calculated between day 77 and day 105.
[5] Calculated between day 105 and day 138.
[*] Calculated between day 138 and day 167.

Table 6. Carcass performance characteristics for finishing cattle fed BioGreen Zeolite (clinoptilolite) at differing percent of final diet dry matter.

Item	0[a]	0.5[b]	1[c]	2[d]	SEM	P-value <
Marbling numerical	443.06	464.75	457.64	459.69	21.62	0.57
Yield grade	3.03	3.22	2.95	3.15	0.13	0.16
Hot carcass weight, lbs	897.45	903.12	908.42	905.50	10.75	0.72
Rib eye area	12.74	12.90	12.32	13.05	0.55	0.55
Calculated Yield Grade	2.99	3.30	2.95	3.09	0.16	0.11
Liver abscess Scoring						
0	42	44	55	48		
A	24	25	21	24		
A+	8	8	4	3		

[a]Diet consisting if the control with no zeolite.
[b]Diet consisting of zeolite at 0.5% of diet DM.
[c]Diet consisting of zeolite at 1% of diet DM.
[d]Diet consisting of zeolite at 2% of diet DM.

Table 7. Liver scores across treatment types.

Treatment	0 (No Abscess)	A (1-2 Abscesses)	A+ (Multiple Abscesses)	No scores reported
1	46	24	9	1
2	44	25	8	3
3	55	21	4	0
4	48	24	3	5

DISCUSSION

The inclusion of ZE at concentrations 0.5, 1, and 2% of diet DM showed no difference in feedlot performance. ZE inclusion showed no impact on final body weights ($p = 0.81$), final ADG ($p = 0.76$), liver abscess rates ($p = 0.5445$), or feed efficiency ($p = 0.68$). There was no difference ($p = 0.40$) in mortality and morbidity between treatments. However, there was a decrease in DMI for steers fed ZE at 1% of diet DM and a non-significant 50% decrease in A+ liver abscess scores for cattle being fed 1 and 2% ZE of diet DM. These findings suggest that there may be an effect related to cost of gain and liver abscess rates and may come at a cost of reduced digestibility of feed. However, from the previous in vitro study, alfalfa digestibility was found unaffected suggesting there are other unidentified factors at play for the decrease in A+ liver abscess scores and decrease dry matter intake for steers supplemented with ZE.

The decrease in DMI has the potential to reduce feed intakes to approximately 0.35 kg per day. This could transfer to a reduction in feed costs but cannot be interpreted as higher feed efficiency. However, this group of animals showed similar quantities of final beef products when compared to other treatments. If feed costs are assumed to be $0.33/kg, then this would equate to savings of $0.11/animal each day on feed. With the average finishing period in the U.S. currently being around 150 days, producers would save up to $16.86 per animal over the entire finishing period. On a hypothetical 30,000 head feed yard with 2.5 rotations per year this could translate to $1.2 million less feed each year.

CONCLUSION

The decrease in liver abscess scores and reduction in dry matter intake for steers consuming 1 and 2% ZE of diet DM may have some improvements on feedlot performance.

However, this needs to be research further in a commercial setting with larger sample sizes to determine if there is an effect on liver abscess scores and dry matter intake.

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