THE EFFECTS OF ZINC ON THE *IN VITRO* DIGESTIBILITY OF FEEDS IN CERVIDS

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ABSTRACT

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The captive white-tailed deer industry has an estimated impact of $1.6 billion, annually. However, nutritional requirements for cervids are determined through research based on sheep and goats. The objective of this study was to determine the effects of zinc on differences in dry matter digestibility *in vitro* for white-tailed does (*Odocoileus virginianus*). Deer (*n*=2) were harvested ethically, rumens were collected, and placed into a cooler containing warm water. Rumen contents were agitated, and fluid was filtered using cheese cloth while applying CO_2. Fluid was placed into four separate incubator jars with filter bags containing a 1:1 alfalfa to coastal hay blend. Zinc doses of 0.073 mg/kg/d equivalents were added to two of the jars (treatments), and the additional two jars received 0.00 mg/kg/d (control). Following 48 h of incubation, *in vitro* true digestibility (IVTD) showed no significant differences between the control and the treatment groups. Average dry matter digested *in vitro* was 91.87% and 95.13%, respectfully. There were no differences detected in ADF, NDF, IVTD, or OM between the treatment groups. While no detectable differences were observed in this study, this methodology did prove to be viable and functional for microbial digestion *in vitro*. This study can be replicated with multiple experimental units to confirm the patterns of increased digestibility. Formal nutritional guidelines can be created to allow for more efficient feeding of cervids. In turn, reducing feed costs for the operation and continue the growth of the captive deer industry.

KEY WORDS: Zinc, IVTD, Dry matter, *In vitro*, Cervids.

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I have learned so much in my short amount of time, and I have made many positive connections while being at Sam Houston State University. This program has benefited me greatly overall as both a person and researcher.

“Great researchers always end up with more questions than answers at the end.”

PREFACE

"If you set your goals ridiculously high and it's a failure, you will fail above everyone else's success." -James Cameron

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CHAPTER I

Introduction

Deer are a part of the family Cervidae. Members of this family are more commonly known as cervids or even deer, and there are many species within this family. In recent years, some species have become a specialty livestock in the United States, specifically axis (*Axis axis*), white-tailed deer (*Odocoileus virginianus*), fallow (*Dama dama*), and red deer (*Cervus elaphus*). The captive deer breeding industry produces $1.6 billion annually in Texas (Outlaw et al., 2017). The industry is growing; but there is a need for data to create nutritional baselines to aid in management practices that range from formulating feed rations to breeding protocols and standards of healthcare. The National Academies of Sciences, Engineering, and Medicine (NASEM) has collected research performed on the requirements for microminerals and vitamins in cervids. There is data collected from a myriad of small ruminants, but there is limited information from captive managed cervids. Therefore, research findings using other small ruminants, such as sheep and goats are often applied to cervids.

Zinc and Breeding

Zinc (Zn) is an essential trace mineral, meaning that small amounts of these elements are needed in a balanced diet. Zinc influences metabolism, growth, and reproduction. Additionally, slight over-rationing of trace minerals like zinc may improve the animal's overall well-being, especially during times of stress or disease (Bartoskewitz, et al., 2007). This could be due to the antioxidant characteristics trace minerals like zinc possess; for zinc is a crucial micromineral for the immune system and growth in deer (Gressley, 2009).

Graham et al. (1995), tested the hypothesis of Prostaglandin F2α, (PGF2α) being linked to zinc plasma levels. An inverse relationship between plasma Zn levels and PGF2α was observed, and that plasma Zn levels could be used as an indicator for potential abortions. Additionally, Molefe and Mwanza (2020) stated that dairy cows consuming grass containing low amounts of Cu, I, and Zn were more prone to reproductive failures. This was seen when non-supplemented cows experienced retained placenta and dystocia while cows that were supplemented experienced no reproductive issues. Thus, reproductive success is tied closely with Zn and other mineral supplementation.

Greenwood et al. (2021), observed the microminerals and vitamins in the blood serum of white-tailed deer to establish a baseline for dietary requirements. Does (n=233) were collected from three separate ranches. Table 1 illustrates the reference data from Puls (1994) used to compare LS means of minerals, vitamins, and cholesterol. Using Puls' reference data, Greenwood et al., (2021) observed females that failed to conceive on the day of sampling had lower circulating levels of plasma Zn compared to bred does seen below (Table 2). Thus, there is evidence towards the lack of zinc hindering the ability of these white-tailed does to become pregnant. Feeding a zinc deficient diet 3 to 5 days or longer has been shown to cause decrease oocyte development (Tian and Diaz, 2013). Additionally, zinc deficiency has been linked to the synthesis and secretion of vital reproductive hormones such as follicle stimulating hormone and luteinizing hormone (Nasiadek et al., 2020). Thus, the importance of zinc in the diet can be viewed in terms of reproductive performance. However, if zinc is to be supplemented to aid in reproductive functions for the herd, the chemical pathways, usages, and effects of zinc should be considered.

Table 1

Reference Data Averages (Puls, 1994) Compared to Greenwood et al., (2021) Study LS Means of Serum Micromineral and Fat-Soluble Metabolites in Sampled Does

Analyte	Reference Data Average Ranges	Current LS Mean	SE
Co (ng/mL)	Unknown	6.31	0.194
Cu (µg/mL)	0.60-1.30	1.04	0.012
Fe (µg/mL)	152.00- 277.00	220.41	12.134
Mn (ng/mL)	Unknown	4.43	0.449
Mo (ng/mL)	Unknown	4.23	0.141
Se (ng/mL)	60.00- 150.00	172.48	1.383
Zn (µg/mL)	0.50-1.00	0.54	0.010
Vitamin A (ng/mL)	Unknown	275.25	15.421
Vitamin E (µg/mL)	Unknown	1.80	0.055
Cholesterol (mg/dL)	Unknown	79.61	1.920

Table 2

Pregnancy Status of Does determined by Blood Test 30-37 D following Breeding Procedure that occurred in Conjunction with Sampling for Micromineral and Fat-Soluble Analyte Analysis (Greenwood et al., 2021)

Analyte	Open LS Mean	Bred LS Mean	SEM[a]	*P*-Value
Zn (µg/mL)	0.42[x]	0.48[y]	0.025	<.01

Note. [a]Pooled Standard Error of the Mean
[xy]Significant difference determined as $P < 0.05$

Objective

Knowing that zinc is an essential nutrient and key for reproductive success, improving digestibility, and microbial efficiency in the rumen, the objective of this study is single fold.

- Determine the effects of zinc on differences in dry matter digestibility *in vitro* in white-tailed does (*Odocoileus virginianus*)

CHAPTER II

Literature Review

Research and Nutrition

The classification of an animal being wild, semi-domesticated, or domesticated can be determined by the level of human involvement. Semi-domesticated can be stated as, "partial control over breeding, mortality, space use, food supply, and that have not been greatly modified by artificial selection," (Mysterud, 2010). Deer raised in more controlled environments such as high-fenced properties could be considered semi-domesticated animals based on this definition. Additionally, knowledge pertaining to the nutritional requirements for wild deer is limited, but data from other domesticated ruminants and farmed deer can be applied given that the differences of the species are considered (Shin, et al., 2000).

In general, higher quality forages have higher palatability. The palatability of the feed is determined through characteristics such as taste, odor, appearance, texture, and temperature (Anguah et al., 2017). Certain species of forage crops will have superior nutritional values, and the quality or density of the forages can dictate the nutritional values the animal receives (Rye et al., 2016).

McMahan (1964) stated that deer primarily consume browse and forbs. Browse are woody plants such as trees, shrubs, mast, and lichens. Forbs are broad leafed herbaceous plants (Tomeček and Redmon, 2016). Lyons and Machen (2018) stated that a white-tailed deer's natural diet is on average made up of 52% browse, 36% forbs, and 12% grass. Animals may preform poorer than expected if not receiving proper nutrition, and this can be especially true for animals that have limited access to resources. If only natural forages are available, and they are poor quality or low density, then various nutrients may be lacking amongst the herd. Understanding these nutrients through various analyses is helpful to producers. This study as many other before it, hope to expand how zinc interacts within the rumen.

Neutral Detergent Fiber and Acid Detergent Fiber

Neutral detergent fiber (NDF) is a measurement for the main components in the cell wall of the plant which are cellulose, hemicellulose, and lignin (Van Soest, 1991). Acid detergent fiber (ADF) refers to the cellulose and lignin portions mainly found in the primary cell wall. Structurally, lignin is bonded to both cellulose and hemicellulose independently with either hydrogen or covalent bonding (Zhang et al., 2015). NDF is useful for measuring the cell wall components, but the analysis can underestimate the concentration of the cell wall. This is due to minor compounds like fructans, gums, mucilages, β-glucans, and most pectin are solubilized and not captured (Sharpe, 2018). Compounds that are soluble in neutral detergent solution include organic acids, simple sugars, oligosaccharides, starch, and other carbohydrates, (Hall et al., 1999). A low NDF is desirable when feeding ruminants, for it is a good indicator for voluntary intake (Penn State Extension, 2013).

Fiber is an important constituent in the diet of ruminants, and largely contains cellulose. Cellulose is chemically made up of glucose molecules that are bound together with a β-1,4 glycosidic linkage. Microbial enzymes that are found in the rumen can digest β-1,4 linked glucose found in cellulose (Kung, 2014). Hemicellulose is the other major sugar found in the cell wall that is also digestible by microbial enzymes. Hemicellulose is structurally mirroring to cellulose, but hemicellulose ranges 50 to 3,000 glucose molecules per polymer while cellulose ranges 7,000 to 15,000 glucose molecules per polymer (Brunner, 2014). ADF is another measure of fiber, and it can be considered a derivative of NDF. ADF is a good predictor for the energy content in feeds (Penn State Extension, 2013). A lower ADF concentration is directly correlated to a more digestible forage as there are less poorly digestible substances in the forage, and vice versa. As mentioned, lignin is linked to both cellulose and hemicellulose, but lignin is very indigestible. As a plant matures the lignin content increases, and it can even act as a barrier to microbial enzymes (Moore & Jung, 2001). As forage crops mature, more NDF and ADF components are present and, this can lower voluntary intake of available forage (Penn State Extension, 2013).

Minerals and Breeding

Cope (2021) illustrated that minerals are essential to the diet of ruminants for overall production, tissue development, and immunity support, and that deficiencies in minerals lead to poor performance and health issues race minerals impact reproductive health in all animals greatly. Hidiroglou (1979) stated that testicular size and zinc concentration in semen may be used as indicators for zinc deficiency, since Zn is involved in the final stages of spermatozoa maturation. Therefore, understanding and fulfilling dietary mineral requirements is necessary for male reproductive functions as well. Most captive deer are bred through artificial insemination (AI) or embryo transfer (ET). This is due to high costs related to animal transportation if bred naturally, or the possible stress added to the animals. Additionally, controlled breeding allows more oversight of genetics, population, and environmental conservation (Jacobson, et al., 1989). There are multiple types of breeding programs such as natural cover, AI, and ET.

For any program, there are costs associated with animal health, estrus synchronization, the genetic material being used, transportation of the semen, embryo, or the animal as well. Natural cover can be problematic because of transportation of the buck(s). The wellbeing, stress levels, weight loss, the costs associated with transporting a live animal, and any expenses related to daily care of additional livestock present should be considered (Penn State Extension, 2015). These concerns may influence how a producer’s breeding program operates.

Supplementing trace minerals has been seen to improve reproductive performance. In humans, minerals have been seen to affect fertility, embryogenesis, and placentation (Cetin et al., 2010). For livestock operations, the producers have influence on the herd’s diet, and how they are bred. Therefore, the producer must consider how the herd’s reproductive performance will be affected by their diet. This could lead to additional costs associated with adjustments in feeding and breeding protocols. Thus, ensuring proper mineral supplementation included in a balanced diet could assist in preventing further issues or costs in the operation.

Bioavailability

The bioavailability of zinc in ruminants has been shown to vary based on how the mineral is administered. The percentage of Zn may alter greatly based on the compound. Spears (1996) stated that absorption of the mineral may not be related to the quantity supplemented, but the type of compound may prove more influential. Determining the type of supplementation may be more heavily influenced by the economic efficiency of the product rather than the animals or environment (McDowell, 1996). Table 3 shows different forms of inorganic zinc supplementation that are commonly used. This highlights how when comparing inorganic forms to each other, the amount of zinc varies.

Table 3

Percentage of Mineral Element and Relative Bioavailability (McDowell, 1996)

Source Compound	% Zinc in Compound	Bioavailability
Zinc carbonate	52.0	High
Zinc chloride	48.0	Intermediate
Zinc sulfate	22.0 - 36.0	High
Zinc oxide	46.0 - 73.0	High

Feeds and forages are the main sources of zinc for animals. A forages zinc content is influenced by the condition of the soil, species, and maturity (Mir et al., 2018). Thus, the condition of the feedstuff influences the amount of zinc available to the animal. Different species of forages have different chemical compositions and metal constituents (Kumar and Kewalramani, 2011). Therefore, the type and quality of the forage influences the availability of the mineral for the animal. The additional zinc requirements for livestock varies with the chemical form administered and the diet of the animal (McDowell, 1996). Factors influencing mineral availability for feedstuffs include the soil quality, the species of crop, the chemical compound of the mineral, and the quality of the feedstuff as mentioned. The mineral contents within the feedstuffs (or supplemented) should be evaluated to ensure adequate amounts are being taken in and utilized by the animal. However, the pathways for minerals are influenced by the digestion of the contents as well.

Ruminant Digestion and Absorption

The rumen hosts a unique anaerobic environment with an optimal temperature around 39ºC, and an optimal pH range of 6 to 7. Ruminant saliva is rich in sodium and potassium that acts as a buffer against acidic environments. Carbon dioxide is produced by the neutralization of acids with bicarbonate, and in turn, helps maintain pH levels of the rumen (Millen, 2016). Microbes present in the rumen include bacteria, protozoa, fungi, and archaea. The rumen can be sectioned into three segments where these microbes are mainly found. Those areas are the liquid phase making up 25% of the microbial mass, the solid phase making up 70% of the microbial mass, and the rumen epithelial cells and protozoa, containing 5% of the microbial mass (Mathews, et al., 2019). There are numerous levels of microbes flourishing inside of the rumen, and the proportions of these microbes depend largely on the animal's diet.

The breakdown of feed begins with mechanical digestion prior to reaching the rumen. Rumination aids in this process by increasing surface area of the feed making it more accessible to microbial enzymes. Most microbes are predominantly anaerobic, and some are associated with binding with feed particles (Millen, et al., 2016). The rumen, reticulum, and omasum surfaces are lined with stratified squamous epithelia that contain tight junctions that regulate nutrient absorption (Goff, 2018). The rumen does not allow large particles to pass, thus it can be considered a major feed intake regulator (Moran, 2005). Feed is eventually exposed to the liquid portion of the rumen, and when the rumen contracts, it causes the entire contents of the rumen to shift. This allows for microbes to be exposed to new feedstuffs and moves materials out of the rumen to continue the digestive process (Goff, 2018). The symbiotic relationship between ruminants and the microbes within them is crucial for their survival. The microbes will produce volatile fatty acids (VFA) as a product of fermentation, and the VFAs are the main energy source for the ruminant animal. Loor, et al., (2016) stated that the main volatile fatty acids are acetate, propionate, and butyrate which are necessary for fatty acid synthesis and the production of energy (ATP). Dietary carbohydrates are used as the main substrates for fermentation that lead to the intermediate product pyruvate, and the final VFA product is influenced by the chemical pathway taken (France and Dijkstra, 2005). The volatile fatty acids can be absorbed across the rumen wall and enter the systemic circulation where tissues like the liver, adipose, mammary gland, and muscles can utilize them.

As mentioned, there is a myriad of microbes living congruently within the host. *Fibrobacter succinogenes* and *Ruminococcus albus* have a far better digestion rate of cellulose compared to other cellulolytic bacteria (Mathews, et al., 2019) The main products of fermentation from cellulolytic bacteria are acetate, butyrate, propionate and carbon dioxide. Hydrogen, ethanol, succinic acid, formic acid, and lactic acid are formed as well, but are usually utilized by other microbes. If the lactic acid is not metabolized by the animal, then there will be an accumulation of lactic acid in the rumen that will be absorbed to the blood causing a decrease in blood pH (Mathews, et al., 2019). High grain diets will supply the rumen with starch. As a result, the glucose levels rise from the starch introduced, and the acidity increases in the rumen. *Streptococcus bovis* which is an amylolytic bacterium has lower optimum pH. This allows it to function normally in a lower pH and utilize the starch. This exemplifies the variety of bacteria within the rumen with unique functionalities that allow the animal to breakdown a variety of feedstuffs.

Protozoa in the rumen are anaerobic that are divided into two groups, ciliated and flagellated. Certain species of ciliated protozoa possess an organelle called hydrogenosomes. These structures make protozoa relatively aerotolerant. They are deemed responsible for scavenging for the oxygen in the rumen to maintain anaerobiosis. Flagellates interact with only soluble nutrients (Moran, 2005). Ciliated protozoan can be categorized into holotrichids and entodiniomorphs. Ciliated protozoa possess hydrolytic enzymes to ferment major components of feedstuffs. Holotrichid ciliates primarily use the soluble sugars, while entodiniomorphs use a variety of substrates such as small plant particles and cell wall carbohydrates. Protozoa engulf bacteria and other smaller microbes including larger molecules like proteins and carbohydrates as well. While the exact functions of the protozoa are not fully understood, they are still appreciated as efficient carbohydrate and protein metabolizers (Williams, et al., 2020).

Fungi are categorized into yeasts and molds. The functionally important fungi are molds known as Chytridomycetes. These fungi produce hydrogen by degrading plant material that can be used by other microbes. Being in the phylum Chytridiomycota, they are reproduced through motile zoospores (Moran, 2005). In the life cycle of the fungi there are two stages. A flagellated zoospore stage in the ruminal fluid, and a non-motile stage known as thalli that is associated with binding to feed particles. Zoospores that are freely floating in the ruminal fluid attach to plant particles, and eventually create a mycelial structure that are responsible for the production of hydrolytic enzymes (Nagaraja, 2012). The rhizoidal development of the thalli is believed to penetrate plant tissue better than bacteria and protozoa, which could lead to greater degradation of forage (Ho et al., 1988).

The rumen contains a group of archaea known as methanogens. They function normally utilizing hydrogen and formate as their energy sources and reduce carbon dioxide to form methane. Since methanogens utilize hydrogen as a substrate, the pH is regulated allowing other microbes to grow (Mathews, et al. 2019). Most of the methanogens live freely in rumen liquid or are adhering to feed particles (Patra, et al., 2017). Methane contributes to the system by prohibiting increases in the partial pressure of H_2 to levels that might inhibit the normal functions of microbial enzymes (Morgavi, et al., 2010).

Prior to reaching the small intestine the digested feed travels through the omasum. The purpose during this stage is water absorption and continue the flow of feed. This is performed by papillae on the laminae of the omasum which assist in grinding up the feed further and transporting it to the abomasum (Goff, 2018). The abomasum allows for acid digestion because it contains hydrochloric acid and enzymes. The pH of the abomasum is approximately 2. The pH level causes the microbes to perish, and the pepsins conduct initial microbial and dietary protein digestion. The remaining digested components are transferred to the small intestine where bile is introduced from the liver. The pH of the bile is high causing a change of pH to a more neutral environment. This allows for further digestion and absorption of different nutrients (Moran, 2005).

Zinc Digestion and Absorption

Zinc can be consumed the animal through feeds or by supplementation. Zinc is released from foodstuffs as free ions during digestion. These ions may then bind to ligands before transported into the enterocytes in the small intestine (Roohani et al., 2013). Goff (2018) mentions the potential electrostatic effect that causes minerals like zinc to become bound to the plant fibers. This may allow the zinc to pass through the rumen or be broken down later in the digestive tract if it remains with the plant fiber.

Zinc chelates are zinc bound to chelating agents that allow it to be a stable and water-soluble compound. The feed is broken down through the digestive process and zinc can become bound to a chelating agent. Typically, chelating agents contain amino acids or small peptides. An amide or hydroxyl group on the ligands allow the formation of a ring structure with the mineral (F.D.A., 2002). The chelate allows protection for the trace mineral as the digestion process continues. The main site of mineral absorption is the small intestine. However, indigestible ligands known as phytates can prohibit zinc absorption if bound (Stanstead and Freeland, 2014). At this point, the zinc can either be utilized or excreted.

Zinc transporter proteins fall into two soluble ligand carrier groups. There is the SLC30A group that are also known as the ZnT group which are a part of the cation diffusion facilitator family of proteins. The SLC39A group are known as the ZIP group which are Zrt and Irt-like proteins (Kimura and Kambe, 2016). The ZnT group transports zinc out of the cell, and the ZIP group transports zinc into the cell as their primary functions (Kimura and Kambe, 2016). Along the lining of the intestines, there are enterocytes. Enterocytes contain organelles such as mitochondria, lysosomes, and endoplasmic reticulum which support normal cellular functions. Furthermore, enterocytes exhibit specific digestive and absorptive functions key in mineral uptake. The enterocytes are connected through tight junctions that moderate mineral and water absorption. Paracellular absorption is the main mechanism for zinc to pass through tight junctions and eventually pass through the basolateral cell membrane by the way of an electrochemical gradient. An increased mineral concentration in the interstitial space can also allow minerals to move into the lumen, resulting in secretion of the mineral (Goff, 2018). Zinc has two methods of passing through the tight junctions. Ligands can be co-transported through a ligand mediated transport protein, or a free radical zinc can enter the cell through the ZIP4 transport protein. ZIP4 transporter causes an increase in zinc concentration in the cytoplasm of the cell. Zinc is used in the cell as needed and maintained with the use of cell organelles, zincosomes and metallothionein. Mir et al., (2018) stated that the cysteine residues of the metallothionein molecule bind with zinc ions in a complex pattern which is not seen in other metalloproteins. Zinc is used up by reactions or is stored uniquely in metallothionein or a zincosome. This stage allows for homeostatic maintenance, preventing oxidative stress, and metal detoxification as needed (Williams, et al., 2020). Zinc is escorted out the cell by either a ligand protein or in a free radical state to be transported by a ZnT1 transporter. Carrier proteins such as albumin or α-2 macroglobulin pick up the free radical zinc to enter the bloodstream because free radical zinc may prove harmful if present in the blood (Goff, 2018). Zinc is stored throughout the entirety of the body, but it is primarily found in striated muscles and bones. Additionally, it is stored in the liver, brain kidneys, and many other organs (Mir et al., 2018).

As seen, zinc utilizes multiple complex pathways. Zinc is a key factor in the process of cell transcription, and therefore, having influence on cellular replication and growth (Mir, et al., 2018). Previously mentioned, zinc has been seen to be beneficial in growth rates, embryo development, and immunity in offspring for ruminants. However, there is still limited knowledge on how this information applies to cervids. Deer producers rely on their herd as a major source of income, and feed is a major cost component to any livestock operation. On average, feed for breeding and hunting purposes are over $175,000 per year in Texas (Outlaw et al., 2017). Therefore, using feed more efficiently can prove to benefit the operation from a financial standpoint. If zinc is to be used for the long-term goal of reducing feed costs, how zinc affects digestibility in the rumen needs to be examined. By increasing the digestibility of feedstuffs in the rumen, the animal can utilize the nutrients more efficiently. In turn, the animals can benefit from the additional utilized nutrients which would lead to the operation benefitting financially as well.

CHAPTER III

Materials and Methods

Ethical Statement

SHSU Institutional Animal Care and Use Committee (IACUC) granted exemption (IACUC Approved: 21-01-05-1044-10-01) for Field Studies by SHSU IACUC Form X.

Data Collection

Phase 1: Experimental Study

A protocol for an experimental study was developed to extract rumen fluid from deer to conduct *in vitro* digestibility in a DAISY II incubator (Ankom Technology, Fairport, NY), followed by neutral detergent fiber (NDF) and acid detergent fiber (ADF) analysis with an ANKOM 200 fiber analyzer (Ankom Technology, Fairport, NY) based on varying levels of zinc added (0.36, 3.6, and 36 g/d equivalents). A red deer hind (*Cervus elaphus*) and an axis doe (*Axis axis*) were utilized in this preliminary study. The deer were collected from a local wildlife producer (Bedias, Texas). The rumen fluid was utilized in the incubator along with filter bags containing the commercial feed the herd was currently consuming. Three treatment groups per deer were made using the rumen fluid collected with 0.36, 3.6, and 36 g/d equivalents of zinc sulfate ($ZnSO_4$) added to each respective jar according to beef cattle supplementation guidelines (NRC, 2007). Cattle supplementation guidelines have been seen to be used for exotic cervids. Serrano et al., (2019) used cattle supplementation guidelines for copper in red deer. Only one specimen from each species was collected and were treated independently. The sample size (*n*=1) limits the statistical capabilities of this experiment, but as a preliminary trial, it may serve as a proof of concept for subsequent research. Therefore, statistical analysis was not run on this data.

Appendix A is the guaranteed analysis and ingredients list of the feed used from the local producer. The ingredients list will assist in finding *in vitro* digestibility studies with similar feed ingredients used. Appendix B is the IVTD, NDF, and ADF percentages obtained from the red hind and axis doe based on varying levels of zinc to be used to compare to other IVTD studies.

Phase 2: White-tailed Does

In this study, rumen fluid from white-tailed does (*Odocoileus virginianus*) were utilized. Fluid was used in conjunction with the protocol developed in the previous preliminary study. This study utilized deer ethically harvested at Gibbs Ranch in Huntsville, Texas through use of antlerless deer tags supplemented by the Texas Parks and Wildlife Managed Lands Deer Program. At harvest, researchers collected the rumen and transported it to the agricultural science laboratory on the SHSU campus.

McDougall's (1948) artificial saliva was created and used as a buffer. The buffer solution was mixed on the day of collection. A 1:1 alfalfa to coastal hay blend was used to create a replicable feedstuff mix. All feedstuffs were dried in a drying oven for 24 hours. Feed was ground using a 1mm MF 10 basic Microfine grinder (IKAWerke, Staufen, Germany). There were 10 bags with feed and one correction factor bag per jar with four incubator jars used in this experiment.

An insulated cooler was used with water at approximately 45ºC. The temperature goal was 39ºC when placing the rumen into the cooler. Therefore, overheating allowed for heat loss while accommodating for transportation time. Once the gastrointestinal tract was removed from the animal, gut string was used to tie the ends of the esophagus and small intestine to prevent leakage. The rumen was then transported back to the research lab.

In this procedure, the rumen fluid from two does (*n*=2) were combined. This allowed for reduced variation between the deer that we were able to collect because combining of the rumen fluid allows for reduced variation between rumen fluid activity (Cone et al., 1996). Rumen fluid was filtered and agitated from the feed using multiple layers of cheese cloth. Carbon dioxide was used to maintain an anaerobic environment. The fluid was poured into the four incubator jars. Two jars remained as a control with no zinc sulfate added. The other two jars were administered 0.073 mg/kg/d based on sheep and goat zinc supplementation guides (NRC, 2007). Since white-tailed deer were collected in this phase, sheep and goat supplementation guidelines were used instead. The size of the ruminoreticulum relative to their body weight determines the digestive capabilities for the animal (Henke et al., 1988). Thus, white-tailed deer are more similar to sheep and goats than cattle in terms of digestion. White-tailed deer have diets resembling sheep and goats where deer compete against sheep primarily for forbs and compete for browse against goats (McMahan, 1964). Thus, white-tailed deer share nutrient sources with sheep and goats closely. The filter bags containing 0.5 g of the hay blend mixture were placed in the incubator jars with the fluid and allowed to incubate for 48 hours. Following fermentation, NDF and ADF procedures were conducted to measure the digestibility of these respective feedstuff constituents. Samples were then ashed to measure remaining organic matter.

Data Analysis

A paired t-test was utilized in SAS Enterprise v9.4 (SAS Institute Inc., Cary, NC, USA) was used to determine differences in the white-tailed doe data. Data obtained from the exotic cervids was not subjected to statistical analysis due to the lack of power in the experimental design. Each jar was an experimental unit.

CHAPTER IV

Results

Phase 1

IVTD was noticeably higher compared to other studies. The IVTD for the red hind ranged from 90.74% to 92.60%. The axis doe had a IVTD range of 90.71% to 99.91%. The percentage increase in digestibility may be explained by the sample size, standard error, and that a commercial feed was used. Only one specimen from each species was utilized in this phase. Thus, it is not a strong representation of the populations. The standard deviation (SD) for IVTD for most groups were greater than 1, illustrating that the feeds in the filter bags were not digested uniformly, and that the ranges for IVTD may be higher numerically due to standard error. However, the commercial feed may have increased IVTD since it contained a prefabricated balanced diet that the deer were consuming prior to the time of harvest. Kamal et al., (2020) analyzed crude fiber (CF), ADF, and NDF with commercial beef cattle feeds *in vitro*. NDF ranged from 30.42% to 33.08%, and ADF ranged from 17.66% to 22.91%. The NDF and ADF ranges found in the beef cattle feeds were lower than the ranges in the deer feed. However, the maximum CF of the deer feed was higher than all the beef cattle feeds. This may have influence on the NDF and ADF values obtained with the deer comparably. This phase of the study exemplified that the methodology will produce viable and functional IVTD results that supports rumen microbial digestion.

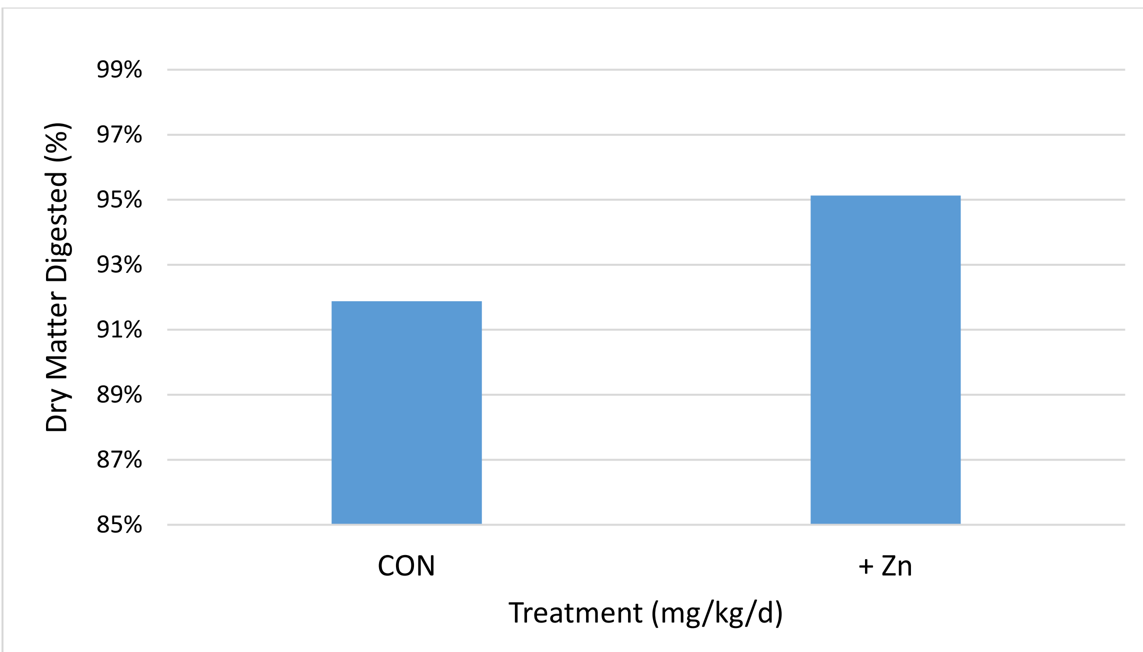
Phase 2

IVTD

The amount of dry matter digested *in vitro* was not significantly different ($P >$ 0.05) between the non-zinc supplemented group (control) and the zinc supplemented group. The average dry matter digested are displayed as a percentage of the original sample weight. The control group had an average digested dry matter of 91.87%. The zinc supplemented group displayed an average digested dry matter of 95.13%.

Figure 1

In Vitro True Digestibility of a Forage-based Diet in Rumen Fluid Collected from White-Tailed Does given Differing Doses of Zinc Sulfate (0 mg/kg/d: CON; 0.073 mg/kg/d: +Zn)

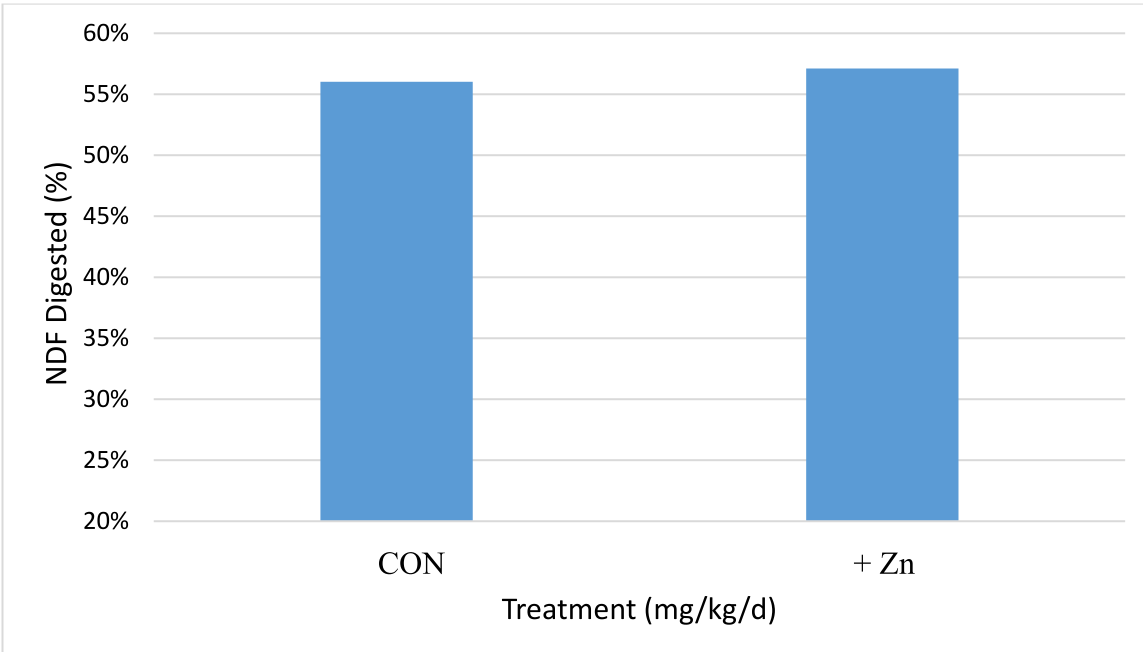


NDF

The digested NDF components after *in vitro* digestibility are displayed in Figure 2. There was not a significant difference ($P > 0.05$) in digested NDF components between the two groups. The control group had an average of 56.03% for digested NDF components. The zinc supplemented group had a mean of 57.11% for digested NDF components.

Figure 2

Digested Ndf of Forage-Based Diet in White-Tailed Doe Rumen Fluid given Differing Doses of Zinc Sulfate (0 Mg/Kg/D: Con; 0.073 Mg/Kg/D: +Zn)

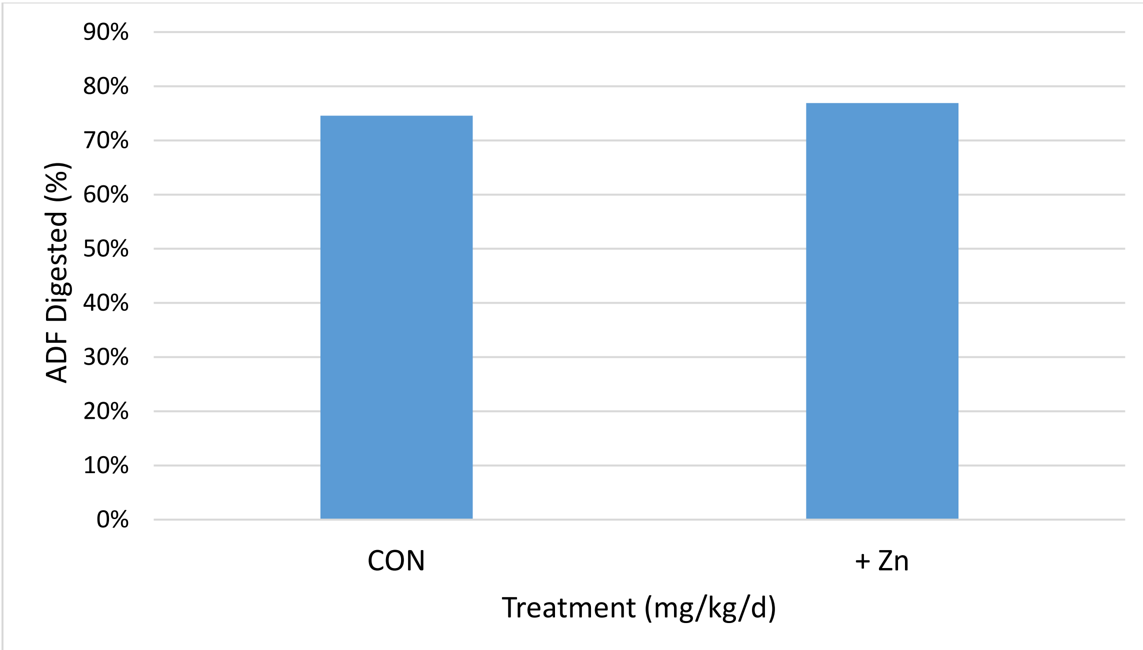


ADF

The digested ADF portions after IVTD and NDF analysis are displayed in Figure 3. There was not a significant difference ($P > 0.05$) in digested ADF components between the two groups. The control group had an average of 74.56% for digested ADF components. The zinc supplemented group had a mean of 76.90% for digested ADF components.

Figure 3

Digested Adf of Forage-Based Diet in White-Tailed Doe Rumen Fluid given Differing Doses of Zinc Sulfate (0 Mg/Kg/D: Con; 0.073 Mg/Kg/D: +Zn)

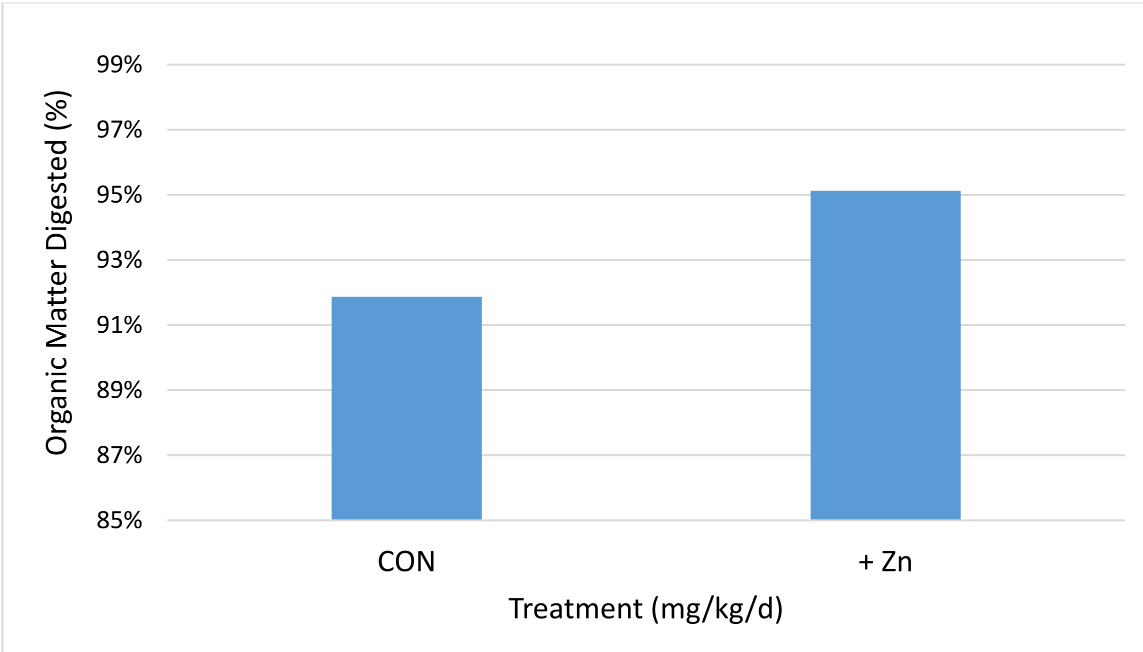


Organic Matter

The results showed no statistical difference ($P > 0.05$) in digested organic matter between the control group and the zinc supplemented group. The organic matter digested in the control group averaged 78.51%. The organic matter remaining in the zinc supplemented group averaged 79.38%.

Figure 4

Digested Organic matter of Forage-Based Diet Post White-Tailed Doe Rumen Fluid given Differing Doses of Zinc Sulfate (0 mg/kg/d: CON; 0.073 mg/kg/d: +Zn)



CHAPTER V

Discussion

Data

The sample size (*n*=2) should be noted, future studies with larger sample sizes will illustrate more accurate results with less standard error. A more controlled study with the capabilities of conducting trials with multiple experimental units may further confirm the observations from this study.

Mandal (2007) showed no difference in digestibility in bulls when zinc was supplemented. Jia et al., (2008) did not see a change in digestibility of DM, NDF and ADF with zinc sulfate supplementation. Mabjeesh et al., (2000) observed a digested NDF range for roughage products from 47% to 61% in a daisy incubator which this range aligns with the amounts of digested NDF components observed in this study. The data for ADF was non-significant ($P > 0.05$) as well, showing marginal differences if any in digested ADF components. No differences in digested components may be due to the zinc requirements of the microbes being met by the basal diet supplied (Jia et al., 2008). In contrast, Jia et al., (2009) observed an increased average daily gain (ADG) in goats when supplementing with zinc sulfate. Anassori et al., (2012) produced an average DMD of organic matter (OM) of about 55% using sheep rumen fluid *in vitro*. The feedstuffs used were mainly alfalfa hay and corn silage. However, the digested organic matter for both groups were approximately 79% and highlighting that the white-tailed deer exhibited a higher digestibility of organic matter comparably.

Experimental Factors

The 1:1 alfalfa and coastal hay blend proved to be beneficial for experimental purposes. However, this is not a natural diet for wild deer, and the variety of browse, forbs, and grass with varying consumption amounts could prove difficult to recreate. Changes in diet cause alterations in the substrates available to the microbes for fermentation, and this will ultimately cause changes in structure and function of the microbial community (Petri et al., 2013). While this feed blend provides a standard that could be potentially replicated, it may be more applicable to captive operations. This experiment may have better applications to captive deer being fed strictly commercial diets. This would allow researchers to know the complete diet of the deer prior to experimental use, and this could be used to mimic the substrates that are entering the rumen more precisely. Thus, replicating the functionality of the rumen for captive deer more closely than wild deer.

Additionally, monitoring the pH levels could prove beneficial for data analysis as well. The pH was not monitored for the duration of this experiment. While there were no indications of human error in preparing the buffer solution, it is not guaranteed that the pH was maintained. Observing pH during the incubation may prove beneficial in data analysis, for it could be used as an additional reference to gauge microbial activities.

Future studies may include the usage of different zinc compounds. The bioavailability of zinc compounds differs based on the type of source. Organic forms such as zinc methionine and zinc proteinate have illustrated to improve the digestibility of OM and ADF more effectively than Zn-sulfate (Alimohamady, 2018). Thus, exploring the effects of different zinc compounds may assist in improving i*n vitro* digestibility.

CHAPTER VI

Conclusion

Phase 1 of the experiment using the exotic deer illustrated to be both viable and functional for the usage of rumen fluid in deer for IVTD experimentation. Additionally, adequate NDF and ADF ranges were obtained when compared to similar studies. Although the exotic deer data holds no statical leverage, it served as a proof of concept for trials with white-tailed does. Phase 2 with white-tailed deer saw no differences in any parameters tested.

While not observed in this study, previous literature outlined in this paper show digestibility can be increased with an optimal amount of zinc supplementation. However, these patterns need to be confirmed *in vivo* as well. There is a financial benefit to increasing digestibility of feedstuffs amongst the herd for an operation. The herd can utilize more nutrients from the feed, and potentially see improvements in terms of microbial functions, ADG, and conception rates (mentioned previously). Increasing conception rates allow less expenses to be wasted on females that are not producing offspring. In turn, the producer receives more revenue in the long run by producing more fawns that can be sold in the future. Overall, this can mitigate costs associated with feed and breeding to increase profitability for the producer. This could also potentiate formal nutritional guidelines for cervids to be created and standardized which would allow for better management practices in the deer industry. Standardized guidelines for cervid nutrition would ultimately benefit the producer by feeding deer more efficiently than the industry is now. If properly managed and supported, the captive deer industry can begin seeing improvements in production. The deer industry supplies almost 17,000 jobs to the state of Texas (Outlaw, et al., 2017). This contributes a substantial amount of funds to the Texas economy. Exemplifying that the deer industry is a major economic entity that needs proper support. Nutritional guidelines for cervids can be created to begin assisting the further development of the deer industry.

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APPENDIX A

Guaranteed Analysis and Ingredients of Feed

The guaranteed analysis and ingredients list of the feed used from the local producer. The feed tag will serve as a reference to compare other NDF and ADF values from related studies.

This feed is designed to be fed to breeding deer and elk.

GUARANTEED ANALYSIS:

Crude Protein........	Min.	16.0 %
Lysine..............	Min.	0.7 %
Methionine..........	Min.	0.25 %
Crude Fat...........	Min.	6.0 %
Crude Fiber.........	Max.	22.0 %
Calcium...Min. 1.6 %	Max.	2.1 %
Phosphorus..........	Min.	0.8 %
Salt.......Min. 0.5 %	Max	0.75 %
Copper..............	Min.	50 ppm
Manganese...........	Min.	200 ppm
Selenium............	Min.	0.3 ppm
Zinc................	Min.	200 ppm
Vitamin A...........	Min.	11,000 IU/LB
Vitamin E...........	Min.	24 IU/LB

INGREDIENTS:

Roughage Products (27.4%), Plant Protein Products, Processed Grain By-Products, Grain Products, Calcium Carbonate, Cane Molasses, Soybean Oil, Lignin Sulfonate, Salt, Dicalcium Phosphate, Monocalcium Phosphate, Propionic Acid (a preservative), Aspergillus Oryzae, L-Lysine, Natural and Artificial Flavors Added, Iron Oxide, Saccharin Sodium, DL-Methionine, Sodium Selenite, Vitamin E Supplement, Manganous Oxide, Zinc Sulfate, Copper Sulfate, Calcium Iodate, Cobalt Carbonate, Ferrous Sulfate, Dried Bifidobacterium thermophilum Fermentation Product, Dried Enterococcus faecium Fermentation Product, Dried Lactobacillus acidophilus Fermentation Product, Dried Lactobacillus casei Fermentation Product, Cobalt Glucoheptonate, Copper Lysine Complex, Manganese Methionine Complex, Zinc Methionine Complex, Vitamin A Supplement, Vitamin D3 Supplement, Vitamin B12 Supplement, Riboflavin Supplement, Niacin Supplement, d-Calcium Pantothenate, Folic Acid, Thiamine Mononitrate, Biotin.

APPENDIX B

In Vitro True Digestibility, Neutral Detergent Fiber and Acid Detergent Fiber percentages with different levels of zinc sulfate added

The remaining neutral detergent fiber and acid detergent after *in vitro* digestibility with axis and red deer. Used to compare to other related studies. This data was not given a statistical analysis due to samples size.

Species	Treatment $ZnSO_4$ Added (g/d)	Mean NDF, DM (%)	Mean ADF, DM (%)	Mean IVTD (%)
Red Hind	36.0	27.87	15.74	92.16
Red Hind	3.60	22.26	12.69	90.74
Red Hind	0.36	20.63	13.79	92.60
Axis Doe	36.0	18.80	11.00	99.91
Axis Doe	3.60	19.08	12.01	90.79
Axis Doe	0.36	20.03	12.50	92.19

VITA

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Sam Houston State University: 2022
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Sam Houston State University- Graduate Assistant: 2022-Present
Assist professors with daily tasks such as grading, managing data in excel, and proctoring lectures. Communicate with students concerning grades, deadlines, and attendance. Help gather resources to host FFA career development events for the agribusiness portions.

Texas A&M Forest Service- Conservation Leader: 2021
Attend forest conservation workshops across the state to gain more knowledge of the industry. Interact and observe professionals in the industry. Apply learned concepts to activities prepared for our area for participants to learn more about forest and wildlife conservation.

Tarleton State University- Research Assistant: 2019-2020
Conduct research to analyze if Treponema bacteria are transmitted through flies. This bacterium causes lesions in the hooves of cattle that can be very painful and lead to lameness. Conducting DNA extraction and running qPCR protocols. Meeting with multiple experts to determine the best methods. Drafting short communications paper to submit to be published.

Publications

Short Communication: Screening stable flies and house flies as potential vectors of digital dermatitis in dairy cattle, *J. Dairy Sci.*: 2021
Analyzing the presence of Treponema bacteria in flies as vectors for digital dermatitis. https://doi.org/10.3168/jds.2020-18550

Abstracts

NCUR, Treponema Bacteria Presence on Flies Captured on a Dairy Farm: 2020
Poster presentation presented virtually due to Covid-19. https://apps.cur.org/ncur2021/archive/Display_NCUR.aspx?id=115714

ASAS Southern Section, Determination of Various Dietary Levels of Inorganic Zinc on *In Vitro* Dry Matter Disappearance of Diets Fed to Cervids as Compared to Other Established Protocols: 2022

Oral presentations presented at southern section conference in Ft. Worth, Tx.

Activities

American Society of Animal Science, Southern Section: 2022

Present current research on the *in vitro* digestibility of feeds with zinc in cervids. Allowed for a 3-minute thesis competition against other graduate students in the section.

Academic Quadrathlon Champions: 2019

Represent Tarleton at the Southern Region competition in Tennessee. Applying all knowledge obtained in animal science to undergo, quizzes, practicums, presentations, and oral questions.