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The Effects of Zinc Sulfate on the *in Vitro* Digestibility of Feeds in Cervids

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Abstract

The captive white-tailed deer industry has an estimated impact of 1.6 billion USD in the state of Texas alone. 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 ethically harvested, 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 (+Zn), and the additional two jars received 0.00 mg/kg/d (CON). 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%, respectively. 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 observations of increased digestibility. Formal nutritional guidelines can be created to allow for more efficient feeding of cervids thereby reducing feed costs and continuing the growth of the captive deer industry.

Keywords

Zinc, IVDMD, *In Vitro*, Cervids

1. Introduction

In recent years, species such as axis (*Axis axis*), white-tailed deer (*Odocoileus virginianus*), fallow (*Dama dama*), and red deer (*Cervuselaphus*) have become a specialty livestock in the United States [1]. The captive deer breeding industry produces 1.6 billion USD annually in Texas [1]. 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 [2] 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.

It is well known that the essential trace mineral zinc influences metabolism, growth, and reproduction in domestic animals [2] [3]. Slight over-rationing of zinc may improve the animal's overall well-being, especially during times of stress or disease [4]. 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 [5]. Zinc also increases digestibility in ruminants through a variety of methods [2] [3]. The main mode of action of zinc driving efficiency is through improving microbial efficiency in the rumen [2] [3]. While these differences in ADG, feed efficiency, DMI, and reproduction are well documented in large and small domestic ruminants [2] [3], it is currently unknown if zinc affects white-tailed deer in similar ways.

Reproductive success is tied closely with Zn and other mineral supplementation. [6] sought to determine linkages of Prostaglandin F2α (PGF2α) to zinc plasma levels. An inverse relationship between plasma Zn levels and PGF2α was observed, and plasma Zn levels were determined to be an indicator for potential abortion routes. [7] stated that dairy cows consuming grass containing low amounts of Cu, I, and Zn were more prone to reproductive failures. Non-supplemented cows experienced retained placenta and dystocia while cows that were supplemented experienced no reproductive issues.

[8] 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 [9] used to compare LS means of minerals, vitamins, and cholesterol.

Using [9] reference data, [8] 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**). 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 prior to breeding has been shown to cause decrease oocyte development [10]. Additionally, zinc deficiency has been linked to the synthesis and secretion of vital reproductive hormones such as follicle

Table 1. Reference data averages [9] compared to [8] 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 [8].

Analyte	Open LS Mean	Bred LS Mean	SEM[a]
Zn (µg/mL)	0.42^{x}	0.48^{y}	0.025

[a]Pooled Standard Error of the Mean; [xy]Means with varying superscripts vary ($\alpha < 0.05$).

stimulating hormone and luteinizing hormone [11]. 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.

2. Methodology

2.1. Ethical Statement

Sam Houston State University 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.

2.2. Data Collection

2.2.1. Axis and Red Deer

A protocol for an experimental study was developed to extract rumen fluid from deer to conduct *in vitro* true dry matter digestibility in a DAISY II incubator (Ankom Technology, Macedon, NY, USA), followed by neutral detergent fiber (NDF) and acid detergent fiber (ADF) analysis with an ANKOM 200 fiber analyzer (Ankom Technology, Macedon, NY, USA) based on varying levels of zinc added (0.36, 3.6, and 36 g/d equivalents). A local producer notified the researchers in this study that a series of culling events were taking place. As such, researchers only utilized animals available through this production system. A red

Table 3. *In vitro* True Digestibility, Neutral Detergent Fiber, and Acid Detergent Fiber percentages with different levels of zinc sulfate added in Red Hind and Axis Doe.

Species		Red Hind			Axis Doe		
	Trt[a]	0.36	3.6	36	0.36	3.6	36
NDF (%)		20.63	22.26	27.87	20.03	19.08	18.8
ADF (%)		13.79	12.69	15.74	12.5	12.01	11
IVTD (%)		92.6	90.74	92.16	92.16	90.79	99.91

[a]Treatment added in the form of $ZnSO_4$ at g/d equivalents.

deer hind (*Cervuselaphus*) and an axis doe (*Axis axis*) were utilized in this preliminary study. The deer were collected from a local wildlife producer (Bedias, Texas) in conjunction with privately contracted hunts with the producer. Animals were ethically harvested by trained professionals and immediately transported to an onsite abattoir for processing. The entire gastrointestinal track from the beginning of the rumen to the end of the abomasum was collected and sealed via gut string commonly used in meat processing. The entire organ was placed in 39˚C water housed in a large, insulated cooler and transported back to the laboratory at Sam Houston State University. Once at the laboratory, the rumen was opened, fluid was filtered through 4 layers of cheesecloth to separate from large feed particles. 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 [2]. Cattle supplementation guidelines have been seen to be used for exotic cervids. [12] used cattle supplementation guidelines for copper in red deer. Only one animal from each species was collected and were treated independently, therefore, statistical analysis was not run on this data. 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 (**Table 3**).

2.2.2. White-Tailed Does

In this study, rumen fluid from white-tailed does (*Odocoileus virginianus*) was 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.

[13] 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 45˚C. The temperature goal was 39˚C 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. Two does were used in this study due to the nature of cull deer tags received through permitting through the Texas Parks and Wildlife Department. Utilizing fluid from multiple deer 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 [14]. 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 one of four incubator jars in a DAISY Incubator (Ankom Technology, Macedon, NY, USA). 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 [3]. 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 [15]. 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 [16]. 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.

2.3. 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.

3. Results

3.1. Axis and Red Deer

In vitro true digestibility (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 population. The standard deviation 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. [17] 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 support rumen microbial digestion.

3.2. White-Tailed Does

The IVTD was not significantly different ($P > 0.05$) between the non-zinc supplemented group (control) and the zinc supplemented group. The average dry matter digested is 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**).

Neutral Detergent Fiber (NDF) did not show significant difference ($P > 0.05$) between the two groups (**Figure 2**). The control group had a mean of 56.03% NDF and the zinc supplemented group had a mean of 57.11% NDF.

Acid Detergent Fiber (ADF) did not show any significant difference ($P > 0.05$) between the two groups (**Figure 3**). The control group had an average of 74.56% ADF and the zinc supplemented group had a mean of 76.90% ADF.

There was no statistical difference ($P > 0.05$) in organic matter (OM) between the control group and the zinc supplemented group (**Figure 4**). The control group had a mean of 78.51% OM and zinc supplemented group had a mean of 79.38% OM.

4. Discussion

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.

[18] showed no difference in digestibility in bulls when zinc was supplemented. [19] did not see a change in digestibility of DM, NDF and ADF with zinc sulfate supplementation. [20] 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

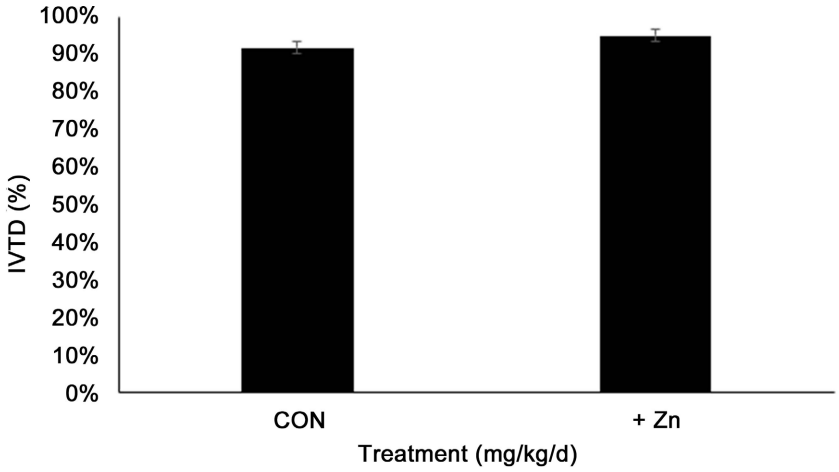

Figure 1. *In vitro* true digestibility (IVTD) of a forage-based diet in rumen fluid collected from white-tailed does given differing doses of zinc sulfate (0 mg/kg/d: CON; 0.073 mg/kg/d: +Zn).

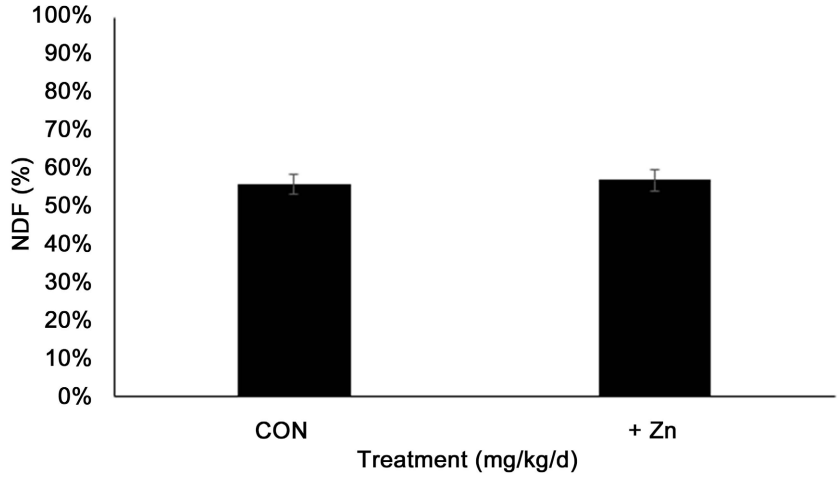

Figure 2. NDF of forage-based diet in white-tailed doe rumen fluid given differing doses of zinc sulfate (0 mg/kg/d: CON; 0.073 mg/kg/d: +Zn).

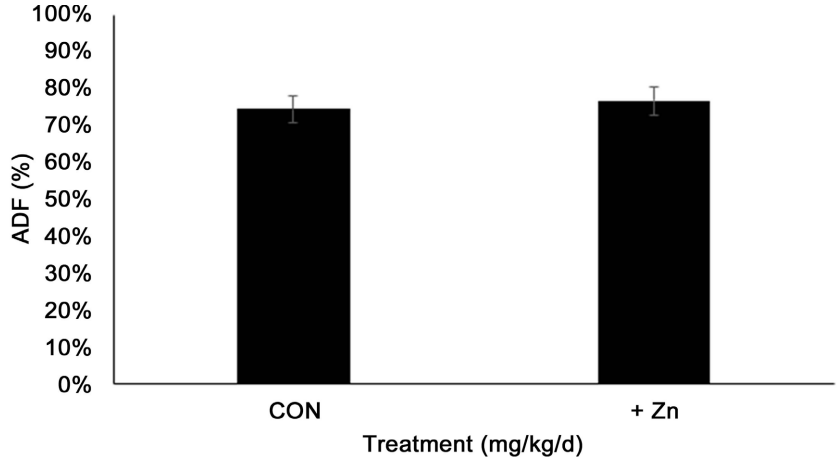

Figure 3. ADF of forage-based diet in white-tailed doe rumen fluid given differing doses of zinc sulfate (0 mg/kg/d: CON; 0.073 mg/kg/d: +Zn).

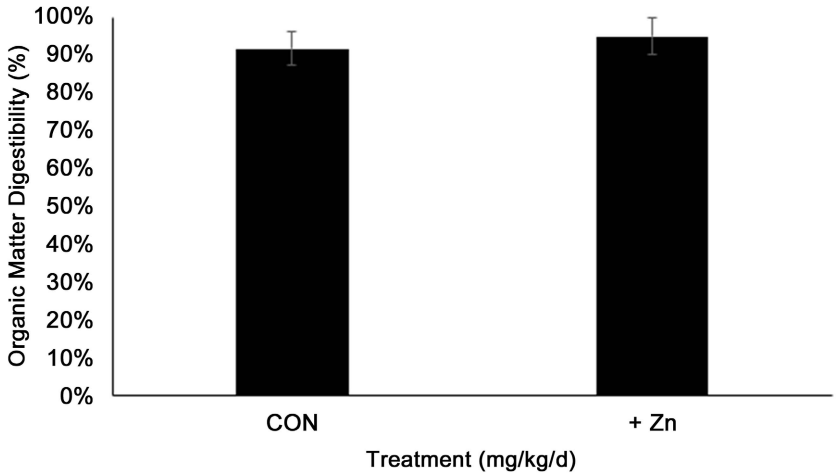

Figure 4. OM Digestibility of forage-based diet in white-tailed doe rumen fluid given differing doses of zinc sulfate (0 mg/kg/d: CON; 0.073 mg/kg/d: +Zn).

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 [19]. In contrast, [21] observed an increased average daily gain (ADG) in goats when supplementing with zinc sulfate. [22] 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 matters for both groups were approximately 79% and highlighting that the white-tailed deer exhibited a higher digestibility of organic matter comparably.

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 [23]. 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 [24]. Thus, exploring the effects of different zinc compounds may assist in improving i*n vitro* digestibility.

5. Conclusions

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 shows 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 allows fewer 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 [1]. 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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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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