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# Effectiveness of zinc given intra-nasally or orally to newly received stocker cattle against bovine respiratory disease and effects on growth performance

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## ABSTRACT

Beef calves (n = 88) were purchased from regional auction barns and delivered as a single group. Upon arrival, cattle were assigned to eight pens. Pens were assigned randomly to one of three treatments; two pens received 3 mL of a nasal spray solution (10.8 mg Zn/mL) into each nostril using a single-use nasal atomizer; three pens received 40 mL of an oral drench (16.25 mg Zn/mL), and three pens received no Zn at processing (negative control). Appropriate treatments were administered at processing on d 0 of the 43-d study. After treatment, cattle were worked and housed so they did not have fence-line contact with any other pens. Cattle were observed daily and rectal temperatures were taken to monitor morbidity. Nasal membranes of four randomly selected calves/pen were swabbed prior to any treatment on d 0 and then on d 1, 2, 4, and 7. Those treated with intra-nasal Zn at processing had lower average daily gain for the first 28 d as compared to controls (P = 0.02) or oral Zn (P = 0.07). Final body weight did not differ. Treatments had no effect on percentage of morbid calves. Treatments had an effect on bacterial cultures from swabs; fewer (P ≤ 0.04) *Escherichia coli*, *Streptococcus* spp., and *Staphylococcus* spp. colonies were cultured from cattle receiving the intra-nasal Zn. Bacterial cultures indicated reduced numbers of microbes in the nasal passages after treatment with intra-nasal Zn, but Zn treatments did not benefit overall morbidity or growth rates of stressed cattle.

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## **MEET THE STUDENT- AUTHOR**



*Amy Guernsey*

I grew up in Joplin, Mo., and graduated from Joplin High School in 2005. Soon after, I ventured south to pursue an animal science degree at the University of Arkansas. I have made the most of my time as a Razorback, enjoying football games, exploring nearby Devil's Den State Park and staying active in the Pre-Veterinary Club and Block and Bridle. Additionally, I have worked in the animal science nutrition laboratory, a local veterinary clinic, and completed an internship in the Edinburgh Zoo (Scotland).

With the tremendous help of Dr. Beth Kegley, Dr. Jeremy Powell, and Doug Galloway, I finished an undergraduate honors research project funded by the Dale Bumpers College of Agricultural, Food, and Life Sciences and the University of Arkansas Honors College. After earning my bachelor's degree I will be attending veterinary school with the hopes of becoming either a mixed or zoo animal medical practitioner. I would like to thank all my family and friends for their ongoing support.

## **INTRODUCTION**

Zinc, an essential dietary trace mineral, has been shown to be required for proper cell function and overall health of an organism. Although Zn may be found in bone or soft tissue, there is no homeostatic mechanism to mobilize this supply. Because of this, a steady intake of this mineral must be available in an individual's diet (Vruwink et al., 1993; Wintergerst et al., 2006). Beef cattle raised on forages are often deficient in Zn, so it is usually supplemented to them (Greene, 2000). Zinc is involved with DNA expression and consequently, protein synthesis and enzyme action. Zinc forms DNA binding proteins known as "fingers" (Klug, 2005), these independently folding domains are found on proteins and help bind the protein to control regions of a gene during the passage of an RNA polymerase molecule (Castro and Sevall, 1993). It is estimated that there are 2,000 transcription factors that need Zn for such structural integrity (Prasad, 2007). Zinc's role as a cofactor in enzymes involved in DNA synthesis and transcription is applicable to the expression of genes in many cell types, including those involved in immune response (Castro and Sevall, 1993). In fact, Zn is important for the expression of gene IL-2 in HUT-78 cells. This in turn contributes to expansion and maintenance of thymocyte and peripheral T cell populations, generation of antiviral and antitumor-specific cytotoxic T cells, delayed type hypersensitivity responses, and upregulation of Natural Killer lymphocyte activity (Prasad, 2007). Also notable is Zn's role in protective en-

zymes such as antioxidants. For example, it is an integral part of superoxide dismutase, which acts as a 'scavenger' for free radicals (Hughes, 2000).

Another aspect of Zn's role in immunology is that this trace mineral actually has some antiviral properties as well. There is evidence to suggest that "adequate intakes of vitamin C and Zn ameliorate symptoms and shorten duration of respiratory tract infections including the common cold" (Wintergerst et al., 2006). A cold is caused by one of 200 types of rhinoviruses. An infection begins when one of these enters the nasal mucosa of a human or animal, from which it is "transported by mucociliary action to the nasopharynx" and proceeds as a more widespread infection (Cohen, 2006). For a rhinovirus to enter the nasal epithelium, it must bind to a cellular receptor, intracellular adhesion molecule-1 (ICAM-1). Zinc acts as "a competitive inhibitor of ICAM -1 in both rhinovirus particles and nasal epithelium" which essentially disrupts the virus's ability to penetrate the cell and replicate (Cohen, 2006). Additionally, because Zn inhibits the binding of leukocyte function associated antigen to ICAM-1 receptor sites, there is a reduction in inflammatory responses associated with colds (Cohen, 2006).

Recognizing Zn's potential, drug companies have developed throat lozenges and intranasal sprays, which aim to reduce the severity and duration of a cold by applying the Zn ion directly to the site of rhinovirus infection (Cohen, 2006). Numerous studies have been conducted on the effectiveness of these products. For lozenges, the best re-

sults were obtained when taken “immediately upon experiencing symptoms” and “taken around the clock (Cohen, 2006).” Similarly, nasal sprays seemed most effective when administered within 24 h of onset of symptoms (Cohen, 2006). These studies determined that the overall effectiveness of throat lozenges and nasal sprays is dependent upon the concentration, rather than the total amount of zinc ions as it is applied directly to mucosa (Wintergerst et al., 2006). This gives it the most contact with ICAM-1 receptors (Cohen, 2006). However taken as a whole, most of these studies were inconclusive at best (Wintergerst et al., 2006).

Bovine respiratory diseases cost farmers in the form of medication, time, quality and quantity of end product (losses due to death or decreased performance) (Bagley, 1997). In its upper-respiratory form, bovine respiratory disease is similar to the common cold in humans with symptoms such as coughing, fever, eye discharge, decreased appetite, and difficulty breathing (Bagley, 1997). It can be caused by a combination of stress and viral or bacterial infection (Bagley, 1997). In the case of a viral infection, no effective treatment can be offered; antibiotics are used only to combat secondary infections. A producer’s best option in controlling this disease is to vaccinate against some of the viruses that initiate the syndrome (Richey, 1994). Alternative routes of vaccination such as intra-rectal and intra-nasal products aim to “generate protective antibody responses at mucosal surfaces” (Sedgmen et al., 2004). Very little research has been conducted on the use of products delivered to the mucosal surface in large animals (Sedgmen et al., 2004). The objectives of our research were to determine whether mucosal applications of Zn solutions could positively affect health and average daily gain of cattle susceptible to bovine respiratory disease, and to explore the effectiveness of intra-nasal and drench Zn applications in combating viral and bacterial loads.

## **MATERIALS AND METHODS**

For this 43 d study, 88 male beef calves averaging 228 kg initial BW were obtained from regional sale barns. Upon receiving (d 0 of the study), cattle were processed as normal. They were assigned a unique ear identification tag and branded with the supplier’s initial. Cattle were vaccinated for respiratory viruses including infectious bovine rhinotracheitis (IBR), bovine respiratory syncytial virus (BRSV), bovine viral diarrhea (BVD), and parainfluenza<sub>3</sub> (PI<sub>3</sub>) (Cattle Master Gold FP5, Pfizer Animal Health, New York, N.Y.) and clostridial diseases (Covexin 8, Schering Plough Animal, Omaha, Neb.). An antihelminthic was administered for internal parasites (Cydectin, Fort Dodge Animal Health, Fort Dodge, Iowa), and external parasites were also addressed (Double Barrel VP ear tags, Schering-

Plough Animal Health, Summit, N.J.). Cattle were tested for persistent infection-with BVD (PI-BVD) by taking ear notch samples and shipping the samples to CattleStats in Oklahoma City, Okla., for analysis. Bulls were castrated using Callicrate bands (No-Bull Enterprises, St. Francis, Kan.). All cattle were sorted by sex and assigned randomly to 8 pens. Pens were assigned randomly to 1 of 3 treatments. These treatments were administered on d 0: Twenty two cattle (2 pens) received 3 mL of a nasal spray solution (10.8 mg Zn as Zn acetate/mL of 0.9% saline solution) into each nostril using a single-use nasal atomizer; 33 cattle (3 pens) received 40 mL of an oral drench (16.25 Zn as Zn acetate/mL of 0.9% saline solution), and 33 cattle (3 pens) received no Zn at processing to serve as a negative control.

Cattle were housed on eight 0.42-ha grass paddocks and were given ad libitum access to bermudagrass hay. They were offered a daily grain supplement of 1.8 kg as fed/d. This supplement consisted of 68% corn, 28% dried distillers grain, and vitamin and mineral premixes. The diet met and/or exceeded all nutritional requirements for protein and minerals (including Zn) as set by the NRC 1996.

To monitor morbidity, cattle were observed daily. Those that were coughing, appeared lethargic, or had ocular or nasal discharge were pulled from the group to take their rectal temperatures. If the temperatures exceeded 40°C, calves were considered morbid and a pre-planned regimen of antibiotics was administered. An initial treatment of florfenicol (Nuflor, Schering-Plough Animal Health, Summit, N.J.) was given first. Morbid calves were checked again 48 h later. If the re-check temperature was 40°C or higher, a second treatment of enrofloxacin (Baytril, Bayer Health-Care LLC, Animal Health Division, Shawnee Mission, Kan.) was given. After another 48 h, the rectal temperature was checked again. If it was still at or above 40°C, the last antibiotic of ceftiofur crystalline-free acid (Excenel, Pfizer Animal Health, New York, N.Y.) was administered daily for 3 d. No further antibiotics were offered after this final treatment. The rectal temperatures of all cattle were also taken on d 0, 1, 2, 3, 4, and 7 to monitor average trends.

Performance was monitored by observing body weight gain and supplement intake. Cattle were weighed on d 0, 1, 2, 3, 4, 7, 14, 28, 42, and 43 before supplement was offered. Any refusals of the grain supplement were weighed back daily.

To monitor viral and bacterial loads, the nasal membranes of 4 calves in each pen were swabbed prior to any treatment on d 0 and then on d 1, 2, 4, and 7. Viral swabs were packed on ice and immediately shipped via overnight courier to the Oklahoma State University Center for Veterinary Health Sciences (Stillwater, Okla.). Bacterial swabs were taken directly to the University of Arkansas Division of Agriculture Veterinary Diagnostic Laboratory (Fayette-

ville, Ark.) and cultured 24 h on five different media. Each swab was plated on a blood agar of 5% sheep blood, a Columbia CNA agar of 5% sheep blood, a chocolate agar, MacConkey agar, and a hektoen enteric agar. Laboratory personnel monitored and gave qualitative scores to these plates the following day.

Performance and morbidity data were analyzed using the mixed procedure of SAS (SAS Institute, Inc., Cary, N.C.). The model included treatment, gender (arrived as steer or bull), whether or not the calf's nasal membranes were swabbed, and all interactions. Degrees of freedom were calculated using the Kenward-Roger procedure. The random statement included pen, and for repeated measures, the model also included day and its interactions. Bacterial scores were analyzed using the GENMOD procedure of SAS. The model included treatment, gender, day, and all interactions. Binomial distribution of data and Type 3 analysis were specified. The means were generated with the frequency procedure.

## **RESULTS AND DISCUSSION**

There were no differences in supplement intake ( $P = 0.97$ ) or final body weights ( $P = 0.15$ ). However, rates of gain varied between treatment groups (Table 1). Cattle that received the Zn nasal spray had lower average daily gain for the first 28 d of the study when compared to the control and oral Zn treatment groups ( $P = 0.04$ ). Average daily gain up to 42 d reflected similar results. The Zn nasal spray treatment group again had lower gain when compared to the control group ( $P = 0.06$ ), but the oral Zn treatment group was intermediate.

Although we randomly assigned cattle to treatment groups, those receiving the Zn nasal spray happened to have higher initial rectal temperatures (Fig. 1) (treatment by day interaction,  $P = 0.01$ ). There were no other differences in rectal temperature observed. Likewise, there were no differences in percentage morbidity, number of calves pulled, or medication costs (Table 1). There was a 73% morbidity rate, but this was not different due to Zn treatments ( $P = 0.43$ ). One calf on the control treatment died during the study.

We found numerous species of bacteria (Table 2), four of which are notable. *Pasteurella multocida* was by far the most prevalent in the cultures, and its occurrence seemed to be affected by a treatment by day interaction ( $P = 0.07$ ; Fig. 2). There were treatment differences for three other species of bacteria (Fig. 4). Cattle that received Zn nasal spray had fewer ( $P \leq 0.04$ ) colonies of *Escherichia coli*, *-Streptococcus* spp., and *Staphylococcus* spp.

There are no virus results to report. Although we packed and shipped our swabs exactly as instructed by Oklahoma State University, there were no viruses detected on any of them by the time they arrived.

In exploring why we obtained these results, it has been suggested that the cattle receiving the Zn nasal spray solution were under more stress than those in the other treatment groups. The nasal spray apparatus was awkward for the handler to use and for the animal to receive. The extra time spent handling the heads of the cattle may have increased stress which in turn could have suppressed the immune system, negatively impacting performance. As mentioned earlier, however, there were no recorded differences in morbidity between treatment groups. Additionally, cattle from each group had members that experienced the similar stressor of having their nasal membranes swabbed. When comparing cattle that were swabbed to those that were not, there were no differences detected in morbidity or growth performance.

In humans, anosmia, or a loss of sense of smell, has been noted as a potential side effect of using Zn nasal sprays (Cohen, 2006). If this were to occur in the cattle, decreased appetites may have also resulted. We observed no differences among treatment groups for grain supplement intake. However, we had no way of measuring hay consumption. There may have been differences in total feed intake that went undetected.

Finally, it appears that the Zn nasal spray had some antimicrobial effects. The question remains as to whether or not this was a positive outcome. Two of the more notable species found, *Pasteurella multocida* and *Escherichia coli*, are gram-negative bacteria. As such, they release endotoxins upon their death, potentially causing inflammation in the host animal. Additionally, by altering the natural flora of the mucosal membranes, the cattle may have become more susceptible to infection by more detrimental microbes. Killing the normally non-pathogenic bacteria of the nasal passages may have done more harm than good. While these were not the results we expected, they are interesting nonetheless. It does seem that these particular Zn applications had no positive impact on growth performance or against bovine respiratory disease in stressed cattle.

In conclusion, bacterial cultures indicated a reduced number of microbes in the nasal passages of cattle that received Zn nasal spray. However, neither Zn application appeared to have a positive impact on average daily gain or bovine respiratory disease in stressed cattle.

## **ACKNOWLEDGMENTS**

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**Table 1.** Growth performance and morbidity data for cattle receiving zinc solution as an oral drench, zinc solution as a nasal spray, or no zinc treatment.

	Control	Oral	Nasal	SE	P-value
Initial body weight, kg	229	228	228	3.9	0.96
Final body weight, kg	268	263	256	3.8	0.15
Supplement intake, kg/d	1.08	1.10	1.07	0.024	0.76
Average daily gain, kg (d 1 to 28)	0.92 <sup>a</sup>	0.75 <sup>a</sup>	0.65 <sup>b</sup>	0.065	0.04
Average daily gain, kg (d 1 to 42)	0.93 <sup>a</sup>	0.81 <sup>a,b</sup>	0.67 <sup>b</sup>	0.061	0.06
Morbidity, %	60	72	86	12.3	0.43
Number of pulls	0.7	1.3	1.3	0.25	0.21
Medication cost, \$/calf	10.14	18.44	18.50	3.63	0.22

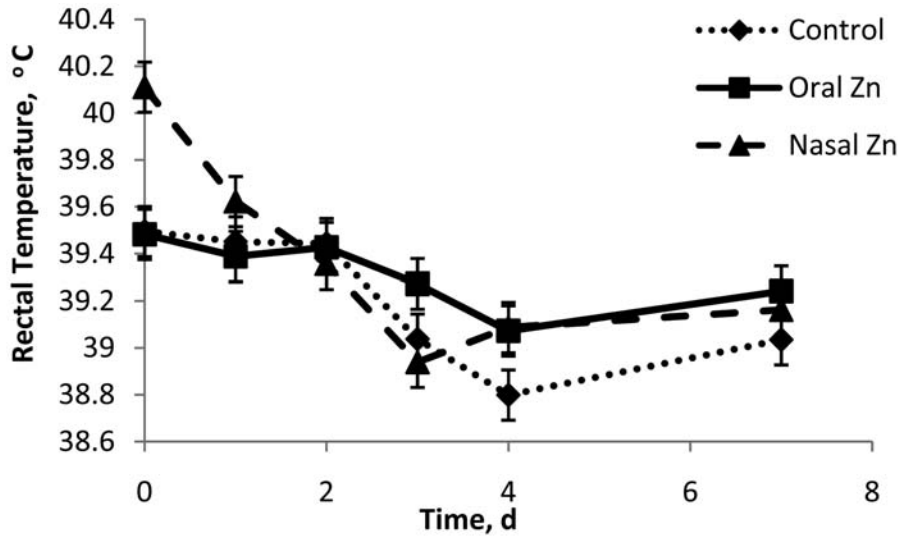
<sup>a,b</sup>  $P < 0.10$

**Table 2.** A list of bacteria cultured from the nasal membrane swabs of cattle treated with zinc solution as an oral drench, zinc solution as a nasal spray, or no zinc solution.

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*Pasteurella multocida*  
 $\beta$ - *Escherichia coli*  
*Escherichia coli*  
 $\alpha$ - *Streptococcus* sp.  
*Staphylococcus* sp.  
*Bacillus* sp.  
*Moracella lacunata*  
*Serratia marcescens*  
*Lactose-E. coli*  
*Pseudomonas aeruginosa*  
*Enterobacter* sp.

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**Fig. 1.** Average rectal temperatures of cattle receiving no zinc treatment (Control), zinc solution as a drench (Oral), or zinc solution as a nasal spray (Nasal). Treatment by day interaction ( $P = 0.01$ ).



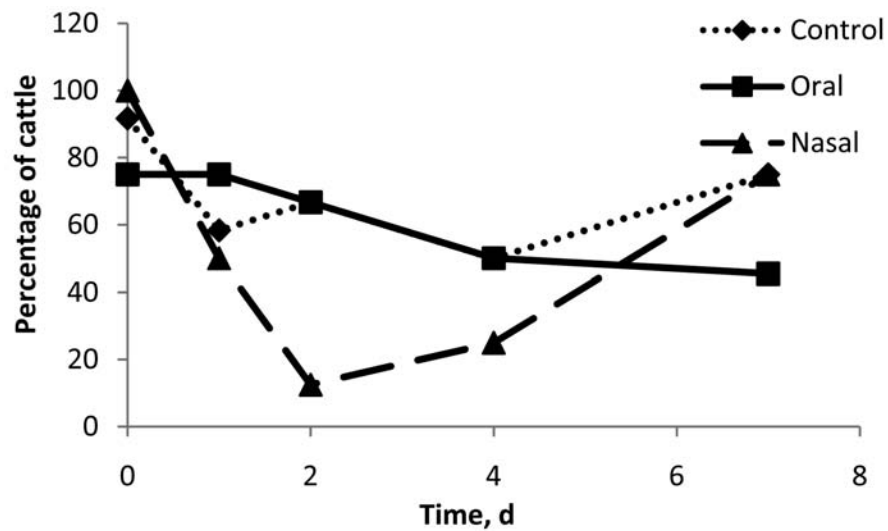


Fig. 2. Percentage of cattle receiving no zinc treatment (Control), zinc solution as a drench (Oral), or zinc solution as a nasal spray (Nasal) with positive nasal membrane swabs for *Pastuerella multocida*. Treatment by day interaction ( $P = 0.07$ ).

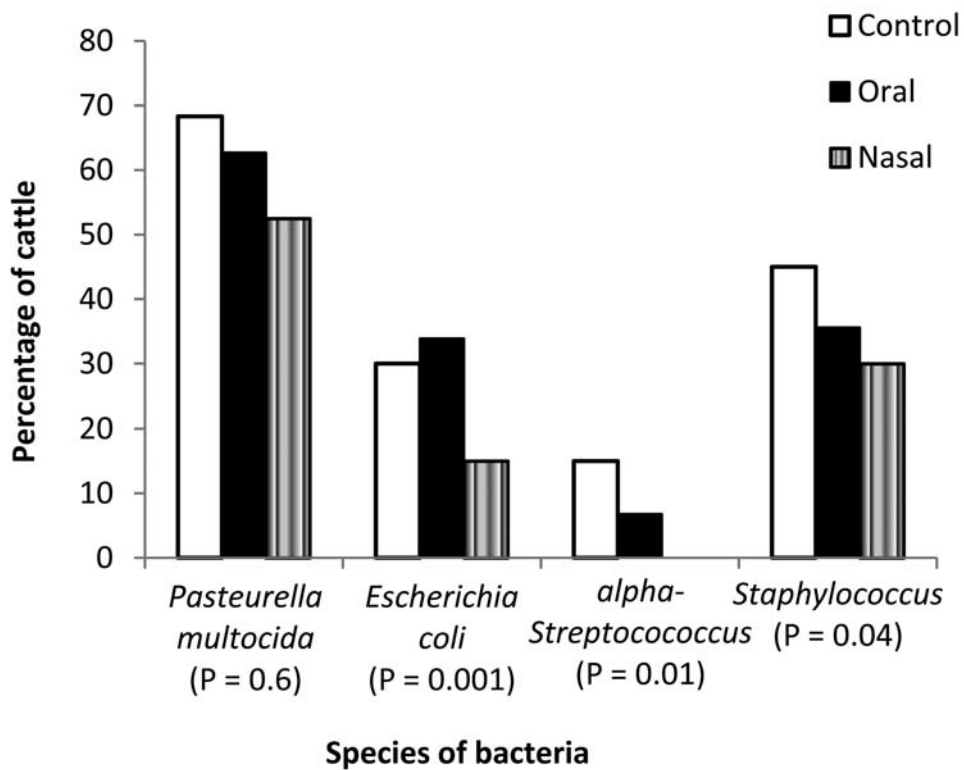


Fig. 3. Percentages of different bacterial species found on nasal membranes swabs of cattle receiving no zinc treatment (Control), zinc solution as a drench (Oral), or zinc solution as a nasal spray (Nasal).