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Effects of Serum Levels of Copper and Zinc on Antibody Titers of Two Breeds of Stocker Calves Injected With *Leptospirosis sp.* Vaccine and Drenched With an Organic Mineral Supplement

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ABSTRACT

This study was conducted to determine the effects of serum levels of copper (Cu) and zinc (Zn) on antibody titers of stocker calves administered *Leptospirosis sp.* vaccine that were given a balanced oral mineral drench. A total of 111 head of stocker calves were injected with *Leptospirosis sp.* vaccine and blood samples were taken from each calf to determine initial serum levels of Cu and Zn. Calves were blocked according to breed (Limousin or Angus based crossbred) and sex (steers or heifers) and allotted to one of two treatments. Treatments were allotted and worked on Day 0 (D 0), treatment one (Trt.-1) received a balanced mineral drench containing chelated forms of Cu and Zn, while treatment two (Trt.-2) did not receive mineral drench and served as control. Blood was obtained via jugular venipuncture on D 14 and D 28 for determination of serum Cu, Zn and titer strength to *Leptospirosis.* Data analysis revealed no differences ($P>0.05$) in mineral levels between control and drench treated calves on D 14 or D28. Pooling calves on breed revealed a higher level ($P<0.05$) of serum Cu levels in Limousin calves, while crossbred calves had higher ($P<0.05$) circulating Zn levels. These results are indicative of previous trials showing substantial breed differences in mineral metabolism and also suggest that a short-term treatment of Zn and Cu will not positively affect a calf's ability to respond to Leptospirosis vaccines.

KEY WORDS: copper, zinc, Leptospirosis, antibody titer, cattle

INTRODUCTION

Factors including cost of vaccines and antibiotics, limited qualified labor, efficiency of gain and overall mortality rates impact management decisions made by cow/calf, stocker and feedyard producers. In an attempt to increase profitability, some producers utilize improved preventative health practices for their cattle marketing. The USDA (2000) defined "preconditioning" as procedures designed to improve an animals' likelihood of successfully adapting to the feedyard environment. Improving preconditioning practices to minimize inputs, health practices and death losses is a high priority for the feedyard industry. Feedyards have found an advantage in purchasing cattle that have undergone a recommended preconditioning regimen. The marketing of cattle through special preconditioned sales continues to increase. Preconditioning procedures may include better matching of rations to cattle requirements, improving formulation and availability of trace mineral mixes and reducing physical stress. Herd (1997) stated that maintenance, growth, lactation and animal health could not be maximized in a situation where minerals were not properly fed. It has also been shown that deficiencies in some minerals compromise immune function (Berger, 1996). An impact on the immune system and growth may be present, if trace minerals are available at less than optimum levels, long before overt deficiency signs such as hair coat and skeletal abnormalities are seen (Mortimer et al., 1999). These works by Mortimer et al. (1999) also indicated that the first biological activity affected by mineral deficiency is the immune system. Suttle and Jones (1989) referred to trace element deficiencies in farm livestock as endemic in many areas of the world. Two minerals that have documented links to immune status in cattle are Zn and Cu (Blezinger, 2000; Zinpro Corp., 2000). Therefore it was the objective of this study to determine if an oral mineral drench containing chelated Zn and Cu would increase circulating plasma levels of these minerals and have an affect on titer levels to *Leptospirosis sp.* vaccine and subsequent serum concentrations of Cu and Zn.

MATERIALS AND METHODS

This trial was conducted in Northeast Texas near the town of Windom during the fall of 2003. The pastures were at approximately 33.6º N latitude and 96º W longitudes with an elevation of approximately 770 ft above sea level. Soil type on both locations was a Houston Black Clay on a limestone base from surface to 30 + ft deep.

Purebred Limousin calves (n = 60; 30 male, 30 female) were in one location and Angus-based crossbred calves (n = 51, 30male, 21 female) were in another location that were approximately one mile apart. Calf initial weights ranged from 450 to 600 lbs. On October 18, Angus crossbred calves were weaned and placed on grass hay and fresh water until processing. Limousin cattle were weaned on October 19 and also placed on grass hay and fresh water until processing. On October 24 all calves were processed using similar handling facilities in a squeeze chute. The same crew of four men performed all processing. They used methods to attempt to minimize stress on calves and create a safe environment for calves and workers.. Penning and processing of the Angus crossbred calves began at 0700 h and concluded at 1000 h. Penning and processing of the Limousin calves began at 1100 h and concluded at approximately 1400 h. All calves were processed in accordance with Texas Cooperative Extension and the Lamar County Cattleman's Association in preparation for the Cattleman's Vac-45 preconditioned calf sale held in Paris, TX on November 25, 2003. All calves were dewormed with Ivomec Plus® (Merial Ltd., Iselin, NJ) as indicated on label recommendations at a rate of 1 ml per 100 lbs of body weight. Injections were given subcutaneously in the elbow pocket, just behind the shoulder. Ultrabac 7 / Somnubac®, Clostridial vaccine with ***Hemophilus somnus***, (Pfizer Inc., New York, NY) was administered in a 5 ml dose, subcutaneously in the neck. Vira Shield 4® (Grand Labs., Larchwood, IA) and a 4 way respiratory vaccine for IBR, BVD, BRSV and PI-3 was administered in a 2 ml dose intramuscularly into the neck. Presponse HM®, a *Pasteurella hemolyticum* vaccine (Fort Dodge Labs., Fort Dodge, IA) was administered at 2 ml per head intramuscularly in the neck. All calves received Origin Lepto 5® vaccine, (Agropfarm, Grapevine, TX) at a rate of 2 ml per head intramuscularly in the neck. Origin Lepto 5® vaccine contains inactivated cultures of *Leprospirosis Pomona*, *L. canicola*, *L. grippotyphosa*, *L. hardjo* and *L. icterohaemorragia*. These five *Leptospira* organisms are commonly known collectively as “Lepto.”

Calves were blocked by sex and breed and assigned to the two treatments. One treatment (Trt. 1) received a Pull-Thru® cattle drench (Albion Laboratories Inc., Clearfield, UT) while the calves assigned to the control treatment (Trt. 2) did not receive the mineral drench. Guaranteed analysis of the Pull-Thru® cattle drench, which is used primarily as a mineral and vitamin supplement drench, was Mg 1.97%; K 1.97%; Cu 2.27%; Zn 2.27%; Se 0.02%; Mn 0.93%; Co 0.13% and Vitamin E 15,350 I U/ 45 kg (Albion Laboratories, Clearfield, UT). The Pull-Thru® drench was mixed by adding water to one packet containing 283.5 g of drench powder to create a 950 ml suspension. This resulting suspension was administered at the recommended level of 30 ml for calves 400 to 600 lb. The drench suspension was given orally using an oral hook connected to an automatic draw, pistol grip dosing gun. The dosing gun was connected to a bottle containing 950 ml of the drench suspension.

On D 0, prior to administration of the oral drench, two blood samples were taken via venipuncture from each calf. Blood was collected with a 10 ml Vacutainer glass test tube utilizing red topped tubes for later analysis of Cu and blue topped tubes containing samples for Zn analysis. Immediately following blood collection, tubes were placed on ice in a cooler and transported to the Texas A&M University-Commerce Nutrition Laboratory. Blood was centrifuged at 1600 RPM for 15 min. Serum was aspirated from each tube and placed into a sterile 10 ml test tube for shipping. Serum was packed with cool packs and shipped to the Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX for analysis of serum Cu and Zn. Following processing all calves were placed on grass hay and pasture with ad libitum access to water.

On D 14 calves were revaccinated with Vira Shield 4® and Presponse HM®. Blood samples were taken and treated as previously described, but samples collected on D 14 were also analyzed for titers against all five serovars of Lepto given on D 0 as well as analysis for Cu and Zn. On D 28, blood samples were collected again as previously described and analyzed by Texas A&M Veterinary Medical Diagnostic Laboratory for Cu, Zn and the five serovars of Lepto given on D 0. Data were analyzed using analysis of variance generated by a statistics software package called Statview produced by SAS.

RESULTS AND DISCUSSION

Serum samples collected on D 0 were not analyzed for Zn levels due to extreme hemolysis, however, Cu analysis was performed at this collection date. Treatment with Pull-Thru® cattle drench had no effect ($P>0.05$) on Lepto titers (Table 1). There were no differences in the basal level of Cu on antibody titers for Lepto on any date, showing no response due to the mineral drench administered on D 0 (Table 2). These findings are consistent with Underwood and Suttle (1999), Spears and Kegley (2002) and Richardson (2002) who reported no response in serum Cu level to supplemental Cu being fed.

Table 1. Guaranteed Analysis of Pull-Thru® Cattle Drench (Albion Laboratories, Clearfield, UT)

Magnesium (Mg)…………………………………………..1.97%
Potassium (K)………………………………………………1.97%
Copper (CU)………………………………………………..2.27%
Zinc (ZN)…………………………………………………...2.27%
Selenium (Se)………………………………………………0.02%
Manganese (MN)…………………………………………...0.93%
Cobalt (Co)…………………………………………………0.13%
Vitamin E……………………………..minimum 15,350 IU/.45 kg

Ingredients: MAAC® Magnesium Amino Acid Chelate, Potassium Amino Acid Complex, MAAC® Copper Amino Acid Chelate, MAAC® Zinc Amino Acid Chelate, MAAC® Manganese Amino Acid Chelate, Cobalt Sulfate, Vitamin E Supplement (di-alpha tocopherol acetate), Sodium Selenite, Saccharine Sodium, Modified Starch, Silicon Dioxide, Molasses Flavoring and Dextrose.

Table 1. Effect of Pull-Thru® Cattle Drench on *Leptospirosis sp.* Titer Response in Growing Calves.

	L. pomona	*L. ictero*	*L canicola*	*L. grippo*	*L. hardjo*
Trt. 1*	4.31 ± .8	.86 ± .5	9.48 ± 1.0	.86 ± .5	32.3 ± 3.3
Trt. 2+	5.66 ± .9	3.77 ± 1.2	8.49 ± 1.1	1.89 ± 1.1	25.38 ± 3.1

*Received one 30 ml oral Pull-Thru® cattle drench on D 0
+Received no supplemental mineral treatment

However, other research has indicated that Cu supplementation resulted in variable immune responses depending on the class of immune cell being studied, as well as the source and concentration of the supplemental Cu (Dorton et al., 2003). Treatment with Pull-Thru® cattle drench had no effect on serum concentrations of Cu and Zn on either day 14 or day 28 (Table 2). This could possibly be attributed to the homeostatic functions metallothionein has on hepatic Cu and Zn storage (Bremner, 1987; Richards, 1989).

Table 2. Effect of Pull-Thru® Cattle Drench on Serum Cu and Zn Concentrations 14 and 28 Days Post-administration in Growing Calves.

	-----------------Cu ppm------------		----------------Zn ppm----------------	
	D 14	D 28	D 14	D 28
Trt 1*	0.86 ± .04	0.73 ± .03	0.87 ± .03	0.59 ± .02
Trt 2+	0.79 ± .04	0.69 ± .03	0.78 ± .03	0.59 ±.02

*Received one30 ml oral Pull-Thru® cattle drench on D 0
+Received no supplemental mineral treatment

Breed differences existed in this study with Limousin calves having a higher (P<0.05) serum Cu level across all collection dates than did Angus crossbred calves. There were no differences (P>0.05) between steers and heifers for any treatments and data was pooled. However, Angus crossbred calves had higher serum concentrations of Zn (P<0.05) across all collection dates (Table 3). In a similar study done by Mullis et al. (2003a), Simmental steers had lower serum and liver Cu concentrations than did their Angus counterparts. These data as well as an accompanying study suggest that Simmental cattle may have a higher Cu requirement than Angus (Mullis et al., 2003b). Other supporting data found that Angus calves whose dams were fed supplemental Cu exhibited higher serum concentrations than either Charolais or Simmental calves treated similarly (Ward et al., 1995). Cumulatively, these studies suggest a higher Cu requirement for Simmentals and/or inherently lower serum Cu levels when compared to Angus.

Table 3. Effect of Breed on Circulating Concentrations of Cu and Zn in Growing Calves During a 28 Day Period.

Breed	Cu ppm
Limousin[¥]	$0.78^{a} \pm .01$
Angus Cross[§]	$0.73^{b} \pm .01$

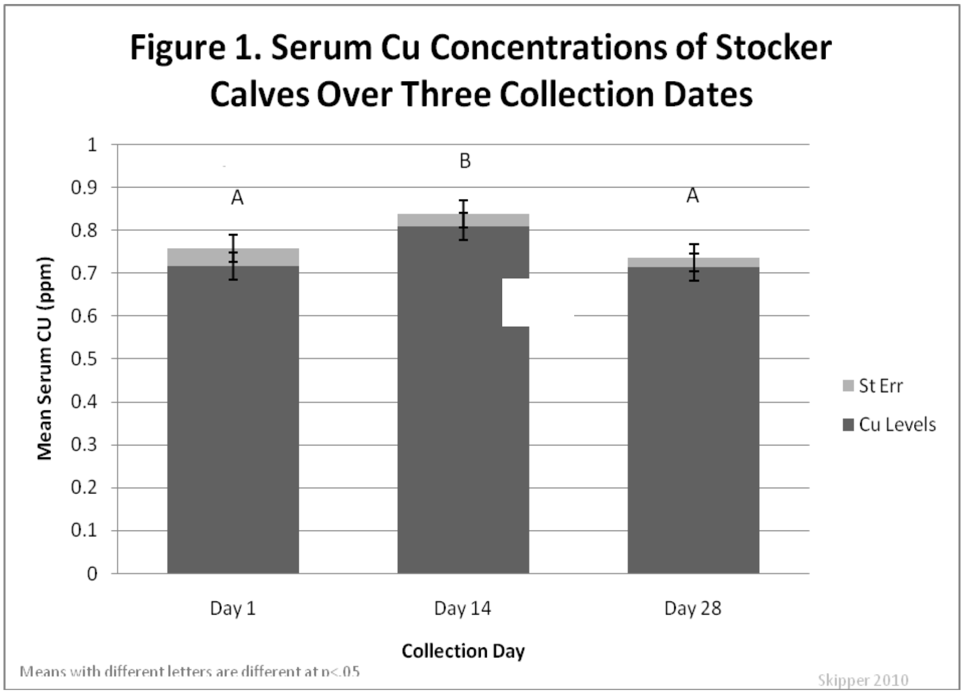
[¥]Registered purebred Limousin cattle

[§]Angus sired cattle with crossbred Dams

Means indicated with different letters are significant at (P<0.05)

Serum Cu concentrations on D 0 ranged from 0.10 ppm to 1.00 ppm with a mean of 0.72 ppm. On day 14 Cu levels ranged from 0.30 to 1.50 ppm with a mean of 0.71 ppm. Barr (2004) stated that cattle with serum Cu concentrations of less than 0.40 ppm are considered deficient. Marginal when serum levels are between 0.40 ppm and 0.60 ppm and optimum levels should be within a range of 0.80 ppm and 1.50 ppm. Based on these levels given by Barr (2004), initial Cu levels of calves on D 0 revealed that 19% were considered deficient, 47% fell in the marginal range and the remaining 34% were optimum. When calves were sampled on D 14, 5% of calves would be considered deficient, 24% had Cu levels which placed them in the marginal range, while the remaining 71% were optimum in serum Cu levels. Numbers changed at the D 28 collection, where it was found that only 1% of calves were deficient. However, the number of calves in the marginal range increased to 44%, with the remaining 55% of calves being within the optimum circulating Cu range. These data may indicate a continual reduction in the number of deficient calves from D 0 through D 28.

As shown in Figure 1 average serum Cu levels on D 14 were higher (P<0.05) than day 0, but returned to baseline levels on D 28. An increase in circulating levels of Cu on day 14 could have been the administration of the drench or this increase may be the result of cattle being subjected to processing stress on day 0, including vaccinations, blood sampling and handling. This explanation would concur with findings by Orr et al. (1990) who found that cattle stressed with infectious bovine rhinotracheitis virus or with market-transit showed increases in serum levels of Cu. Additional research has shown that stress was accompanied by a decrease in fecal and urinary excretion of Cu as compared to baseline levels in studies where stress was induced (Nockels et al., 1993). The return to near baseline levels may be attributed to the homeostatic functions the metallothionein has on storage of Cu and Zn indicated by Bremner (1987) and Richards (1989).



Although insufficient data exists in this trial as to the change in Zn from D 0 through the remaining two collections dates, Nockels et al. (1993) found urinary Zn excretion was lower during stress periods than in baseline. They suggested the decrease in feed consumption led to reduced urinary volume that increased metallothionein synthesis in the kidney which may have contributed to a negative Zn balance during the period of stress.

Levels of Zn and Cu in relation to immune response tend to be inconclusive. As Dorton et al. (1993) stated, these relationships tend to be related to immune cell type being studied as well as the source and concentration of supplements in the diet. These levels may be influenced by the relationship between Cu, Zn and the synthesis of metallothionein in the homeostatic binding and storage of both Cu and Zn. Breed differences and stress also seem to be major influencing factors in absorption, excretion and utilization of Cu and Zn.

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