

Serum Zinc Concentrations in Spring-Born Missouri Feeder Calves

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Abstract

This study determined the prevalence of zinc deficiency in Missouri feeder calves. Additionally, this study identified factors related to zinc status and determined whether serum zinc concentrations were related to owner perceptions of disease. Serum samples and survey data were collected from calves throughout Missouri. Regression models were developed predicting serum zinc concentrations and zinc status. Associations between zinc status and owner perceptions of disease were examined using Chi-squared tests. The statewide zinc deficiency rate was 2.1%. An additional (24.4%) of the calves had marginal serum zinc concentrations. Low serum zinc concentrations were associated with owner perceptions that adult cow diarrhea, poor hair coats, and reproductive failure were problems in the herd of origin. The Northwest, Northeast, Central, Southwest, and South Central regions were associated with increased serum zinc concentrations. Commercial fertilizer was associated with increased serum zinc concentrations and pasture application of lime was associated with decreased serum zinc concentrations. Red clover and orchard grass were associated with increased serum zinc concentrations and fescue, white clover, ladino clover, and birdsfoot trefoil were associated with decreased serum zinc concentrations. Provision of trace mineralized salt was associated with increased serum zinc concentrations. Logistic regression models revealed that application of a commercial fertilizer, orchard grass, and trace mineralized salt were associated with a decreased probability of serum zinc concentrations

<0.8 ppm, and lespedeza and calf age over 7 months were associated with an increased probability of serum zinc concentrations ≥ 0.8 ppm.

INTRODUCTION

Zinc is an essential micronutrient in cattle diets. Clinical signs and abnormalities present in cattle with zinc deficiency include decreased growth rate, diarrhea, poor appetite, salivation, abnormal hooves, swollen joints and coronary bands, stiff gait, hair loss, parakeratosis, thymic atrophy, lymphoid depletion, decreased disease resistance with specific depression of cell-mediated responses, and decreased reproductive performance.^{1–10} These clinical signs are not pathognomonic and zinc deficiency could readily be overlooked. Although experimental zinc deficiency readily induces clinical disease, the diagnosis of zinc deficiency is rarely made by veterinarians.

Cattle have a small, labile zinc storage pool. Clinical signs and laboratory abnormalities associated with zinc deficiency occur rapidly after removal of zinc from diets and rapidly return to normal after supplementation.^{10,11} Consequently, optimal zinc nutrition requires ongoing, adequate, and continuous intakes. Zinc deficiency can be induced either by low dietary zinc concentrations or excess dietary calcium, sulfates, iron, or molybdenum.

Zinc deficiency is usually diagnosed on the basis of serum or plasma zinc concentrations; however, liver and hair concentrations have been used.^{12,13} None of these measures is optimal or consistently accurate. Serum and plasma concentrations appear accurate, but inflammatory conditions cause decreased serum and plasma zinc concentrations, creating the potential for the spurious diagnosis of zinc deficiency.¹⁴ Cattle with serum concentrations of zinc ≥ 0.8 ppm wet weight are zinc replete, cattle with serum zinc concentrations ≥ 0.4 ppm and < 0.8 ppm have marginal zinc status, and cattle with serum zinc concentrations < 0.4 ppm are deficient.^{12,13}

The purpose of this study was to determine the prevalence of zinc deficiency in Missouri feeder calves. A secondary goal of this study was to identify management and husbandry factors related to zinc status and serum zinc concentrations. Finally, we determined whether serum zinc concentrations were related to owner perceptions of disease.

MATERIALS AND METHODS

Sample and Survey Data Collection

The sampling strategy was premised on geographic localities (counties) rather than random sampling. Private veterinary practitioner-collaborators having a large beef cattle component in their practices were identified throughout the state. Collaborator veterinarians collected blood samples from 3 calves in each enrolled herd and obtained samples from no more than 3 herds in each county. Sampling was performed at the time of routine fall processing of calves and was restricted to calves between the ages of 4 and 10 months. Practitioners were cautioned that sampled calves should be representative of herd. Calves that were overtly ill were not sampled. Approximately half of the samples were collected in the fall of 1998 and the remainder in the fall of 1999. Whole blood samples were collected into tubes specifically designed to limit zinc contamination

(Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Collaborating practitioners shipped samples to a central laboratory using postage-prepaid mailers. Blood centrifugation and serum collection was performed at the central laboratory. Practitioners completed a questionnaire summarizing exposure to potential risk factors for zinc deficiency. The survey included the questions regarding region, calf age in months, pasture type, soil amendment practices, including commercial fertilizer use, lime application, and cattle, swine, and poultry manure application, and mineral supplementation practices. Information regarding whether calves were weaned was not available. Potential risk factors that were present in less than 5% of study population were removed from further consideration. Owners also were asked whether diarrhea of mature cows, calf diarrhea, pneumonia, fractures, abnormal hair coats, lameness, and cow fertility were perceived as ongoing health problems in their herd.

Serum Zinc Determinations

Briefly, serum zinc was analyzed by atomic absorption spectrophotometry at 213.9 nm. The method used was identical to the published method for the assessment of serum copper with the exception of the substitution of 0.5% Triton X-100 for the glycerol in preparation of standard solutions (2, 1, 0.5, and 0.2 ppm) and the selection of an appropriate detection wavelength.¹⁵ Plastic tubes were used throughout the procedure to limit the binding of zinc to glassware. The external control contained 2.86 ppm zinc. Five percent of samples were processed in duplicate to verify analytic quality. Sample sets were reanalyzed if more than 10% variation occurred in duplicate samples.

Data Analysis

For the purpose of this study, low serum zinc concentration was defined as a serum zinc concentration <0.4 ppm wet weight. Marginal serum concentration was defined as greater than or equal to 0.4 ppm wet weight and <0.8 ppm wet weight. Adequate serum zinc concentrations were defined ≥ 0.8 ppm wet weight. Only calves for which blood zinc concentration and complete survey data were obtained were included. The proportion of calves with low, marginal, and replete serum zinc concentrations was reported for each of the 9 agricultural districts: Northeast, North Central, Northwest, West Central, Central, East Central, Southeast, South Central, and Southwest, defined by the Missouri Agriculture Statistics Service (2001 Missouri Farm Facts, Missouri Department of Agriculture, Jefferson City, MO). Initially, low and marginal serum zinc concentration groups were combined to eliminate cells with less than 5 observations. These proportions were compared among districts using a 2- ∞ -9 Chi-squared test (Triton X-100, Fisher Scientific Co., Fair Lawn, NJ).¹⁹ Patterns of deficiency were deemed to differ significantly when the calculated P value <0.05 . The statewide proportions of calves with deficient, marginal, and replete zinc status were calculated as follows. The proportion of calves with low blood zinc concentrations in each region was multiplied times the number of calves in the respective region to calculate the number of calves in each region with less-than-optimal zinc status. These numbers then were summed and divided by the total state beef calf population, yielding statewide weighted results.

Associations between owner perceptions of herd health and calf serum zinc concentrations were explored using a series of Chi-squared tests. Observations were cross-classified using 2- \times -2 tables defined by disease status (0, 1) and serum zinc concentrations (<0.4 ppm). Disease status variables included the presence of diarrhea in mature cows, calf diarrhea, pneumonia, fractures, abnormal hair coats, lameness, and cow fertility. These analyses were repeated using serum zinc <0.8 ppm as a threshold for marginal zinc status as a cut point. For all significant associations ($P < 0.05$), odds ratios were calculated.

Stepwise, multivariate, backward-stepping design-variable regression models were developed to predict blood zinc concentration as a function of district, calf age in months, husbandry and animal health practices, pasture type, soil amendment practices, and mineral supplementation practices. Initially, all variables were forced to enter the model. Thereafter, the variable with the largest P-to-enter was removed at each step. The process was repeated until no variable had a P-to-enter greater than 0.10.

Forward stepwise logistic regression models were developed predicting the incidence of low or marginal serum zinc status (<0.80 ppm) as a function of district, calf age, animal health and husbandry practices, pasture type, soil amendment practices, and mineral supplementation practices. In each regression model, the independent variable with the smallest P-to-enter was added to the model at each step until no remaining variable had a P-to-enter >0.10. Logistic regression models were not developed to predict serum zinc concentrations <0.4 ppm because only 11 of the 529 calves had serum zinc concentrations <0.4 ppm.

RESULTS

Five hundred twenty-nine calves drawn from 177 farms were enrolled. At least 3 calves were sampled in 74 of Missouri's 114 counties. Of the 529 calves studied, 34% had access to creep feed and 71% of the calves had access to trace mineralized salt. Twelve percent of the calves originated from herds that were provided with supplemental hay and 17% of the calves originated from herds that were provided with supplemental concentrates. The predominant pasture plant was fescue (94% of pastures); however, orchard grass (31%) and red clover (44%) also were common. The presence of reed canary grass, coastal Bermuda grass, and timothy in pasture and the use of poultry or swine manure as soil amendments were all very infrequent (<5%). Consequently, these variables were removed from further consideration.

The proportion of zinc deficient calves varied from 0% to 5.9% by agricultural district (Table 1). No deficient calves were observed in 5 districts, and the highest proportion of deficient calves was observed in the Northwest district. The regional proportion of calves with marginal serum zinc status varied from 0.138 to 0.458. The regional proportion of calves with adequate serum zinc status varied from 0.542 to 0.862. Of the 529 calves enrolled in the study, only 11 had serum zinc concentrations less than 0.4 ppm. The calculated statewide zinc deficiency rate was 2.1% and an additional 24.4% of calves had marginal serum zinc concentrations, ≥ 0.4 ppm and <0.8 ppm. The patterns of zinc status varied among the 9 agricultural districts ($P < 0.05$).

Low serum zinc concentrations (<0.4 ppm) were associated with owner perception that adult cow diarrhea was a problem in the herd of origin (P = 0.013, odds ratio [OR] = 6.82). Low serum zinc concentrations were associated with owner perception that poor hair coats were common in the herd (P = 0.019, OR = 6.33). Low serum zinc concentrations were associated with owner perception that infertility was common in the herd of origin (P <0.0001, OR = 12.35). Low serum zinc concentrations were not significantly associated with owner perceptions that calf diarrhea, pneumonia, fractures, or lameness were problems in the herd. Marginal or low serum zinc concentrations (<0.8 ppm) were not significantly associated with any of the surveyed owner perceptions of disease.

The developed regression model revealed a number of significant associations between independent variables and serum zinc concentrations. The Northwest, Northeast, Central, Southwest, and South Central districts were associated with increased serum zinc concentrations (Table 2). Pasture fertilization, red clover or orchard grass pastures, and access to trace mineralized salt were associated with increased serum zinc concentrations. Pasture application of lime, fescue, white clover, ladino or trefoil pastures, thin cows, feeding of supplemental hay, and increased age were associated with decreased serum zinc concentrations (Table 2).

Logistic models predicting the presence of marginal to deficient serum zinc concentrations (<0.8 ppm) reveal similar patterns (Table 3). Fertilization, orchard grass pastures, and access to trace mineralized salt were associated with a decreased probability of serum zinc <0.8 ppm. Lespedeza pastures and increased calf age were associated an increased probability of serum zinc <0.8 ppm.

DISCUSSION

Overt zinc deficiency (serum zinc <0.4 ppm) was rare in the study population. Only 11 of 529 calves (2%) had serum zinc concentrations <0.4 ppm. An additional 24% of the population had marginal serum zinc concentrations, and the majority of calves had replete serum zinc concentrations. However, the frequency of marginal zinc status, serum zinc concentrations less than 0.8 ppm, is cause for concern. Deficient and marginal zinc status (serum zinc <0.8 ppm) has been associated with suboptimal growth in cattle and sheep.^{16,17} Impaired reproductive performance has been associated with zinc deficiency in cattle, sheep, and laboratory animals.^{17,18}

In the present study, zinc deficiency was significantly associated with owner perceptions regarding 3 clinical observations: adult cow diarrhea, poor hair coats, and infertility. The association between low serum zinc and perceived infertility (OR = 12.35) raises serious concerns. The limited number of deficient calves supports the possibility that this association was spurious. However, the strength of this association (OR = 12.35) and the degree of statistical significance (P <0.0001) suggest this association is representative of a biologically meaningful relationship. Marginal serum zinc concentrations (<0.8 ppm) were not significantly associated with any owner-perceived disease problem. Consequently, if low serum zinc concentrations are associated with overtly impaired

health, this relationship appears to be restricted to cattle with profound deficiency and is less closely associated with marginal zinc status. Previous experimental studies support the possibility that zinc deficiency could be a cause of reproductive failure.^{18–20} Increased prostaglandin F₂ synthesis has been observed in zinc-deficient females.^{20–22} This increased production of PGF₂ could either result in an increased potential for luteolysis or increased myometrial activity. Either could potentially have an impact on fertility. Additional manifestations of reproductive failure that have been associated with zinc deficiency include decreased spermatogenesis, abortion, fetal mummification, decreased birth weight, uterine inertia, and delayed testicular development.^{10,18–23} However, it should be noted that owner perception of disease, in this case reproductive failure, is an imprecise end point.

Models predicting serum zinc concentration and models predicting serum zinc status were consistent. The majority of factors that were associated with decreased serum zinc concentrations also were associated with an increased risk of serum zinc concentrations less than 0.8 ppm.

Provision of a trace mineralized salt was associated with a small but significant increase in serum zinc concentration (Table 2). Similarly, the probability of low zinc concentrations (<0.8 ppm) was decreased in calves that were provided access to trace mineralized salt (OR <1). A cautionary note regarding the potential impact of trace mineralized salt on calf zinc status is appropriate. A mineral source with a high concentration of highly bioavailable zinc would likely increase serum zinc concentration and decrease the probability of zinc deficiency. However, a mineral source with low zinc concentrations or unavailable zinc sources might actually increase the probability of zinc deficiency if the mineral contains high concentrations of calcium, iron, sulfur, and molybdenum.^{3,10,11} The composition of mineral sources and intake were not documented in this study.

Pasture amendment practices were associated with serum zinc concentrations and calf zinc status. Use of commercial fertilizer was associated with increased serum zinc concentrations and a decreased probability of serum zinc concentrations less than 0.80 ppm. This contradicts previous reports in which commercial fertilizers containing either nitrogen or phosphorus predisposed zinc deficiency.^{10,11} The negative relationship between pasture application of lime and serum zinc concentrations was anticipated. Soil pH greater than 6.5 has been associated with decreased serum zinc concentrations.^{10,11} Consequently, the increased pasture pH caused by lime application could cause decreased serum zinc concentrations. Furthermore, commercial lime application could increase the pasture concentrations of calcium impairing absorption of zinc.¹³

The decreased serum concentrations of zinc in calves on white clover, ladino clover, and birdsfoot trefoil pastures and the increased probability of serum zinc concentrations <0.8 ppm in calves on lespedeza pastures were in agreement with previous studies. Previous studies have substantiated the presence of lower serum zinc concentrations in cattle grazed on legume pastures than cattle grazing on grasses.^{10,11} This generalization was

not consistent. Red clover was associated with increased serum zinc concentrations and fescue pastures were associated with decreased serum zinc concentrations.

Increasing calf age was associated with both lower serum zinc concentrations and an increased probability of lower serum zinc concentrations. This could reflect an actual age effect or, alternatively, an effect caused by the shift from a dam's milk-based diet to pasture or supplemental forage and concentrates.

In conclusion, marginal serum zinc concentrations were common in the study population, but overt, frank deficiency (<0.4 ppm) was rare. In the present study, sampling was restricted to feeder calves. Consequently, this study only provides an indirect measure of whole-herd zinc status and not a direct measure of either cow or bull zinc status. It should be noted that the impact of zinc deficiency on reproductive performance has been documented best in laboratory animals, and evidence supporting a direct association between zinc status and reproductive performance in cattle is less compelling.

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REFERENCES

1. Andresen E, Flagstad T, Basse A, et al: Evidence of lethal trait A 46 in black pied Danish cattle of Fresian descent. *Nord Vet Med* 22:473–485, 1970.
2. Flagstad T: Lethal trait A 46 in cattle intestinal zinc absorption. *Nord Vet Med* 28:160–169, 1976.
3. Underwood EJ: *The Mineral Nutrition of Livestock*, 2nd ed; 1981.
4. Apgar J, Fitzgerald JA: Effect on the ewe and lamb of low zinc intake throughout pregnancy. *J Anim Sci* 60:1530–1538, 1985.
5. Vogt DW, Carlton CG, Miller RB: Hereditary parakeratosis in Shorthorn beef calves. *Am J Vet Res* 49:120–121, 1988.
6. Keen CL, Hurley LS: Zinc and reproduction: Effects of deficiency on foetal and postnatal development. In: Mills CF (ed). *Zinc in Human Biology*. London, Springer-Verlag; 1989:183.
7. Perryman LE, Leach DR, Davis WC, et al: Lymphocyte alterations in zinc-deficient calves with lethal trait A 46. *Vet Immunol Immunopathol* 21:239–248, 1989.
8. Keen CL, Gerschwin ME: Zinc deficiency and immune function. *Annu Rev Nutr* 10:415–430, 1990.
9. Machen M, Montgomery T, Holland R, et al: Bovine hereditary zinc deficiency: lethal trait A 46. *J Vet Diagn Invest* 8:219–227, 1996.
10. Radostits OM, Gay CC, Blood DC, et al: *Veterinary Medicine*, 9th ed. Philadelphia: WB Saunders Co; 2000:1510–1513.

11. Lamand M: Zinc deficiency in ruminants. *Irish Vet J* 38:40–47, 1984.
12. Beeson WM, Perry TW, Zurcher TD: Effect of supplemental zinc on growth and on hair and blood serum levels of beef cattle. *J Nutr* 45:160–165, 1977.
13. Puls R: *Mineral Levels in Animal Health*, 2nd ed. Clearbrook, British Columbia: Sherpa International; 1994.
14. Erskine RJ, Bartlett PC: Serum concentrations of copper, iron, zinc during *Escherichia coli*-induced mastitis. *J Dairy Sci* 76:408–413, 1993.
15. Osheim DL: Atomic absorption determination of serum copper: collaborative study. *J Ass Off Anal Chem* 66:1140–1142, 1983.
16. Mayland HF, Rosenau RC, Florence AR: Grazing cow and calf responses to zinc supplementation. *J Anim Sci* 51:966–985, 1980.
17. Eng K: Trace minerals, BST, starch-amino acid bypass examined. *Feedstuffs* 61:16, 1989.
18. Campbell MH, Miller JK: Effect of supplement dietary vitamin E and zinc on reproductive performance of dairy cows and heifers fed excess iron. *J Dairy Sci* 81:2693–2699, 1998.
19. Miller WJ, Blackmon DM, Gentry RP, et al: Zinc absorption, metabolism, and endogenous excretion in zinc-deficient and normal calves over an extended time. *J Dairy Sci* 74:3535–3543, 1991.
20. Graham TW, Thurmond MC, Gerschwin ME, et al: Serum zinc and copper concentrations in relation to spontaneous abortion in cows: implications for human fetal loss. *J Reprod Fertil* 102:253–262, 1994.
21. Meydani SN, Dupont J: Effect of zinc deficiency on prostaglandin synthesis in different organs of the rat. *J Nutr* 112:1098–1104, 1982.
22. O'Dell BL, Browning JD, Reeves PG: Plasma levels of prostaglandin metabolites in zinc-deficient female rats near term. *J Nutr* 113:760–765, 1983.
23. Browning JD, Reeves PG, O'Dell BL: Effect of zinc deficiency and food restriction on the plasma levels of prostaglandin metabolites in male rats. *J Nutr* 113:755–759, 1983.

Table 1. Numbers and Proportions of Calves With Deficient, Marginal and Replete Serum Zinc Concentrations by Agricultural District in a Survey of 529 Missouri Feeder Calves*

Calves No. at Risk†	Deficient Proportion		Marginal Proportion		Replete Proportion			
	Calves	Deficient Calves	Marginal	Calves	Replete			
	(≥0.80 ppm)		(0.40–0.79 ppm)		(<0.40ppm)			
1 Northwest	223,000	85	5	0.059	16	0.188	64	0.753
2 North Central	222,000	48	0	0.000	22	0.458	26	0.542
3 Northeast	121,000	102	1	0.010	30	0.294	71	0.696

4 West Central	240,000	36	0	0.000	12	0.333	24	0.667		
5 Central	460,000	80	3	0.038	16	0.200	61	0.763		
6 East Central	136,000	55	0	0.000	14	0.255	41	0.745		
7 Southwest	312,000	50	2	0.040	10	0.200	38	0.760		
8 South Central	320,000	58	0	0.000	8	0.138	50	0.862		
9 Southeast	39,000	15	0	0.000	5	0.333	10	0.667		
Summary estimates	2,073,000		529		11	0.021	133	0.244	385	0.735

*Summary estimates are weighted on the basis of calf numbers in each of the districts.

†Triton X-100, Fisher Scientific Co., Fair Lawn, NJ.

Table 2. Results of a Forward-Stepping Stepwise Regression Model Predicting Serum Zinc Concentration (ppm) in 529 Spring-Born Missouri Beef Calves as a Function of Agricultural District, Cow Condition, Calf Age, Pasture Fertilization Practices, and Predominant Pasture Forage

Analysis of variance

Source of Variation	F Value	P
Model	3.15	<0.0001

Regression summary

Variable	Coefficient	SE
Intercept	0.894	0.082
Northwest	0.085	0.050
Northeast	0.135	0.048

Central	0.040	0.049
Southwest	0.115	0.057
South Central	0.242	0.055
Thin cows	-0.098	0.086
Fertilizer	0.050	0.039
Lime	-0.088	0.045
Fescue	-0.051	0.066
Orchard grass	0.032	0.034
Red clover	0.061	0.032
White clover	-0.082	0.061
Ladino clover	0.038	0.037
Birdsfoot trefoil	-0.119	0.067
Supplemental hay	-0.035	0.050
Trace mineralized salt	0.034	0.035
Older (>7 months) calves	-0.070	0.038

R² = 0.0951.

Table 3. Results of a Forward Stepwise Logistic Regression Model Predicting the Occurrence of Serum Zinc Concentrations <0.80 ppm in 529 Feeder Calves as a Function of Geographic, Management, and Nutritional Risk Factors

Variable	Coefficient	SE	P	Odds Ratio (95% CI)
Intercept	-0.247	0.265	NA	NA
Fertilizer	-0.690	0.237	0.004	0.502 (0.315, 0.798)
Orchard grass	-0.475	0.230	0.039	0.622 (0.396, 0.975)
Lespedeza	0.839	0.294	0.004	2.315 (1.300, 4.120)

Trace mineralized salt	-0.435	0.213	0.041	0.647 (0.426, 0.983)
Older calves (>7 months)	0.874	0.225	<0.001	2.396 (1.543, 3.722)