

# Factors Related to Copper Status in Spring-Born Missouri Feeder Calves

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## Abstract

This study determined the prevalence of copper deficiency in Missouri feeder calves and described the relationship between management factors and copper deficiency. Five hundred twenty-eight beef calves aged 4 to 10 months were included in the study. Serum samples and survey data were collected from calves throughout Missouri. Serum copper concentrations were determined by atomic absorption spectrophotometry. Apparent and real prevalence of copper deficiency were calculated for each agricultural district as well as the overall prevalence for the entire state. Regression models were developed predicting both serum copper concentration and copper status. Associations between copper status and owner perceptions of disease were examined with the use of Chi-squared tests. Calculated real prevalence of the 9 agricultural districts ranged from 0% to 53.1%. None of the questions regarding animal health were significantly associated with copper deficiency. A number of management factors were significantly associated with either serum copper concentration or copper status ( $P < 0.05$ ). These included a number of agricultural regions as well as legume-type forages, cow body condition, and calf age. Access to creep feed and trace mineralized salt were significantly associated with serum copper concentration and copper status. Copper deficiency was commonly identified in many of the agricultural districts. The most consistent risk factor identified in this study was agricultural region. The relationship between geographic areas of copper deficiency occurrence and the state's 2 major rivers, the Missouri and Mississippi, was the most

intriguing factor identified. The true influence of these rivers could not be determined with this study, but it warrants further investigation.

## INTRODUCTION

Copper is an essential micronutrient.<sup>1,2</sup> Copper deficiency has been associated with disease states that decrease commercial beef production. Clinical manifestations of copper deficiency include anemia, diarrhea, long bone fractures, generalized ill thrift, and decreased fertility.<sup>2–4</sup>

Recent studies have demonstrated that copper deficiency is common in North American beef cattle.<sup>2,5</sup> Dargatz et al. found that 40.6% of the beef cows and heifers were either deficient or marginally deficient. This study demonstrated that copper deficiency was common even though half of the producers reported using a copper supplement. It should be noted that Dargatz et al. used a test end point of less than 0.65 µg/g serum copper concentrations to define marginal deficiency. Results of a recent study suggested that this test end point might have resulted in wholesale misclassification of copper replete calves as copper deficient.<sup>6</sup> A test end point of less than or equal to 0.45 µg/g best optimizes test performance.

The primary purpose of this study was to identify management factors that would affect serum copper concentration and copper deficiency. In addition, we wanted to determine whether copper status was related to owner perceptions regarding the occurrence of disease. Although in this study only herds from Missouri were surveyed, the conclusions reached should be applicable in other areas. General management practices for beef herds are similar across the Midwest and the vast majority of Missouri calves are fed in other states.

## MATERIALS AND METHODS

### Sample and Survey Data Collection

The data collection process was a systematic attempt to determine the copper status of feeder calves throughout Missouri. The sampling strategy was premised on geographic localities (counties) rather than proportionate sampling of cattle populations. Private veterinary practitioner-collaborators whose practice included a large beef cattle component were identified throughout the state. Collaborator veterinarians collected blood samples from 3 representative 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 completed a questionnaire summarizing exposure to potential risk factors for copper deficiency. The survey included questions regarding region, calf age in months, pasture type, and mineral supplementation practices. 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 Copper Analysis

Serum samples were analyzed by use of atomic absorption spectrophotometry (Perkin Elmer 2380 Atomic Absorption Spectrophotometer, Perkin Elmer, Norwalk, CT; wavelength, 324.7 nm). Serum copper determination was performed with external controls containing 1.99 µg/g copper, and copper standards of 1, 0.5, 0.2, and 0.1 µg/g were prepared by use of calibration reference solution (Fisher Scientific Co., Fair Lawn, NJ) and 0.5% Triton X-100 (Fisher Scientific Co.). A standard curve was generated through regression analysis of the copper standards. One milliliter of serum was added to 1 mL of 0.5 % Triton X-100 in a plastic tube and processed through a vortex before analysis. Copper concentrations of individual samples were determined by comparison to the standard curve.

### Data Analysis

Low serum copper concentration was defined as a serum copper concentration  $\leq 0.45$  µg/g wet weight.<sup>6</sup> Adequate serum copper concentrations were defined as  $>0.45$  µg/g wet weight. Only calves for which serum copper concentration and complete survey data were obtained were included. Apparent prevalence (the proportion of calves with serum copper concentrations  $\leq 0.45$  µg/g) was reported for each of the 9 agricultural districts (Northeast, North Central, Northwest, West Central, Central, East Central, Southeast, South Central, and Southwest) as defined by the Missouri Agriculture Statistics Service (Missouri Department of Agriculture, Jefferson City, MO). Additionally, real prevalence was defined using the following equation:  $RP = (AP + S_p - 1)/(S_s + S_p - 1)$ ,<sup>7</sup> where RP is real prevalence, AP is apparent prevalence,  $S_s$  is sensitivity, and  $S_p$  is specificity. Values used for sensitivity and specificity, 53% and 89%, respectively, in the calculation were drawn from a previous study.<sup>6</sup> Proportions were compared among districts using a 2-∞-9 Chi-squared test (SAS System for Windows, version 8, SAS Institute Inc., Cary, NC). Patterns of deficiency were deemed to differ when the calculated P value was less than 0.05. To calculate the statewide proportions of calves with deficient copper status, we multiplied the proportion of calves with low serum copper concentrations in each region times the number of calves in the respective region to get the number of calves in each region with less-than-optimal copper status. These numbers then were summed and divided by the total state beef calf population, yielding statewide weighted proportions.

Associations between owner perceptions of herd health and calf serum copper concentrations were explored using a series of Chi-squared tests. Observations were cross-classified using 2-∞-2 tables defined by disease status (0, 1) and serum copper concentrations ( $\leq 0.45$  µg/g). Disease status variables included the presence of diarrhea in mature cows, calf diarrhea, pneumonia, fractures, abnormal hair coats, lameness, and cow fertility. Forward stepwise logistic regression models were developed to predict the incidence of low serum copper status ( $\leq 0.45$  µg/g) as a function of region, calf breed, calf age, animal health and husbandry practices, pasture type, and mineral supplementation practices. In each regression model, the independent variable with the smallest P-to-enter was added at each step until no remaining variable had a P-to-enter  $<0.05$ . Calculations were performed with the aid of a statistical software package (SAS Institute Inc., Cary, NC).

## RESULTS

Of the 528 calves studied, 34% had access to creep feed and 71% of the calves had access to trace mineralized salt. Eleven percent of the calves originated from herds provided

with supplemental hay and 16% of the calves originated from herds with supplemental concentrates. Many of the operations fertilized pastures (84%). Fifty-eight percent of the operations used only commercial fertilizer, 5% used only manure from various species, and 21% used both. The predominant pasture plant was fescue (94% of pastures); however, orchard grass (31%) and red clover (44%) were common. More detailed description of the study population is available on request.

Serum copper concentrations varied from 0.06 to 2.25  $\mu\text{g/g}$ . Eighteen percent, or 96 of the total number of calves sampled, had serum copper concentrations less than or equal to 0.45  $\mu\text{g/g}$  (Fig. 1). Apparent prevalence of low serum copper concentration ( $\leq 0.45 \mu\text{g/g}$  wet weight) varied from 4% to 33% among the 9 agricultural districts. The calculated real prevalence of copper-deficient calves varied from 0% to 53% by agricultural district. The highest proportion of deficient calves was observed in the Southeast district. Two districts, Southwest and South Central, were defined as zero calculated real prevalence because the actual value calculated was less than zero. The calculated statewide real prevalence of copper deficiency was 17.1% (Table 1). The proportion of copper-deficient calves differed among the 9 agricultural districts ( $P < 0.05$ ). Low serum copper concentrations ( $\leq 0.45 \mu\text{g/g}$ ) were not significantly associated with owner perceptions that cow diarrhea, poor hair coats, infertility, calf diarrhea, pneumonia, fractures, or lameness were problems in the herd.

The regression model predicting serum copper concentration revealed a large number of associations between serum copper concentration and independent variables (Table 2). The Southwest and South Central regions, calves more than 6 months of age, lespedeza pastures, creep feed, and trace mineralized salt were associated with an increase in serum copper concentration. The West and East Central regions, poor cow condition, and white clover pastures were associated with a decrease in serum copper concentration.

The logistic regression model predicting copper deficient status identified several significant associations with postulated independent variables (Table 3). Ladino clover pastures were associated with copper-deficient status. The Southwest and South Central regions, alfalfa pastures, and providing creep feed and trace mineralized salt were associated with a decreased likelihood of copper-deficient status.

## DISCUSSION

Copper deficiency ( $\leq 0.45 \mu\text{g/g}$ ) was found in 7 of the 9 agricultural districts. Two districts, Southwest and South Central, with no copper deficiency are located in the Ozark Plateau, a region of relatively higher elevation. We estimated that more than 300,000 calves were copper-deficient. Inspection of a map of Missouri indicates that the patterns of most severe copper deficiency were observed in the Missouri and Mississippi River basins (Fig. 2). This observation agrees with the observation that river silt pastures could be associated with trace element deficiencies.<sup>8</sup> Explanation of this phenomenon could lie in a recent report describing the complex interaction of trace elements, most notably zinc and copper, with dissolved organic matter in fresh water.<sup>9</sup> Highly stable complexes that are resistant to disassociation are formed.

Although fescue pastures were the most common forage provided across the state (94%), this was not significantly associated with copper status or serum copper concentration. This is contrary to recent reports of endophyte-infected fescue being associated with decreased available copper.<sup>10</sup> The survey made no attempt to differentiate endophyte versus non-endophyte-infected fescue. In addition, the small number of operations (31 herds) that did not use fescue limits the ability to detect differences.

Pasture types that were significantly associated with serum copper concentration include lezpedeza (increased concentration) and white clover pastures (decreased concentration). Pasture types significantly associated with copper status are ladino clover pasture (positive) and alfalfa (negative). In general, legumes tend to be higher in copper concentration than grasses.<sup>11</sup> This observation presents a conundrum because all of these pasture types are legume-type forages. It is possible that these disparities are the result of random chance. Given the strength of the associations (Tables 2 and 3), this seems unlikely.

Not surprisingly, access to creep feed and trace mineralized salt were positively associated with serum copper concentration and negatively associated with the probability of copper deficiency. Both of these practices are recommended as therapeutic preventatives for copper deficiency.<sup>1,8</sup>

Older calves had increased serum copper concentrations and calves in herds with poor cow body condition had decreased serum copper concentrations. Both of these relationships are intuitively logical. Older calves will rely more heavily on pasture forage and concentrates. Therefore, the risk of copper deficiency associated with a cow's milk diet will be decreased.<sup>12,13</sup> In the instance of cows with poor body condition, one can intimate that this is a reflection of overall herd management. The fact that the cows themselves are in poor condition suggests an overall lack of good-quality available feedstuffs. This lack of quality feed easily could lead to a copper deficiency as well as deficiencies in other micronutrients.

None of the health-related questions in the survey are significantly associated with copper deficiency in this study. This is of particular interest because most of the questions posed pertain to disease syndromes that have been historically linked to copper deficiency.<sup>1,2,8</sup> It is possible that there were too few of those syndromes observed to make a statistically significant association. Other explanations could include a lack of owner awareness to the various disease entities.

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Figure 1. Proportion of calves within each of 8 classes of serum copper concentration. The numbers appearing at the top of each bar represent the total number of calves in each class. The vertical dashed line (- -) represents the test end point for copper deficiency ( $\leq 0.45 \mu\text{g/g}$ ). Classes are intervals of serum copper concentration of 0.15 mg/g, except for the last class, which is any value greater than 1.05 mg/g.



Table 1. Apparent and Calculated Real Prevalence of Copper Deficiency by Agricultural Region\*

	Calves at Risk§	Deficient		Apparent Prevalence	Real Prevalence†	No. of Calves Copper-Deficient
		Calves No. ( $\leq 0.45 \mu\text{g/g}$ )	Calves No. ( $\leq 0.45 \mu\text{g/g}$ )			
1 Northwest	223,000	86	15	0.174	0.153	34,203
2 North Central	222,000	48	9	0.188	0.185	40,964
3 Northeast	121,000	99	22	0.222	0.267	32,330
4 West Central	240,000	36	7	0.194	0.201	48,253
5 Central	460,000	81	18	0.222	0.267	122,910
6 East Central	136,000	55	16	0.291	0.431	58,580
7 Southwest	312,000	50	2	0.040	0.000‡	0
8 South Central	320,000	58	2	0.034	0.000‡	0
9 Southeast	39,000	15	5	0.333	0.531	20,738
Summary estimates	2,073,000	528	96	0.182	0.171	357,980

\*Proportion of copper-deficient calves differed significantly by region, ( $P < 0.05$ ).

†Real prevalence was calculated using the formula,  $RP = (AP + S_p - 1)/(S_c + S_p - 1)$ , and the sensitivity and specificity, 0.53 and 0.89, respectively.

‡Valculated value is below zero.

§2001 Missouri Farm Facts, Missouri Department of Agriculture, Jefferson City, MO.

Table 2. Results of Forward Stepping Regression Model Predicting Serum Copper Concentration ( $\mu\text{g/g}$ ) as a Function of Various Husbandry Practices

Variable	Coefficient	P Value
Intercept	0.53997	<0.0001
West Central	-0.10937	0.0068
East Central	-0.10168	0.0015
Southwest	0.12965	0.0001
South Central	0.09887	0.0020
Thin cows	-0.21109	<0.0001
Calves aged 7 to 10 months	0.07255	0.0019
White clover	-0.09013	0.0196
Lespedeza	0.14705	<0.0001
Creep feed	0.11264	<0.0001
Trace mineralized salt	0.04573	0.0382

Table 3. Results of Logistic Regression for Predicting Copper Deficiency ( $\leq 0.45 \mu\text{g/g}$ ) in 528 Spring-Born Missouri Feeder Calves as a Function of Various Husbandry Practices

Parameter	Coefficient	P Value	Odds Ratio
Intercept	-0.4945	0.0204	
Southwest	-1.9913	0.0069	0.137
South Central	-2.2241	0.0029	0.108

Ladino clover	0.6524	0.0225	1.920
Alfalfa	-2.4606	0.0215	0.085
Creep feed	-1.0001	0.0004	0.368
Trace mineralized salt	-0.8770	0.0004	0.416

Figure 2. Map of Missouri depicting the 9 agricultural districts and the Missouri and Mississippi Rivers. Gray-shaded regions have estimated real prevalence of copper deficiency greater than 20%. Key for districts; 1 = Northwest, 2 = North Central, 3 = Northeast, 4 = West Central, 5 = Central, 6 = East Central, 7 = Southwest, 8 = South Central, and 9 = Southeast.