

Selenium Status of Spring-Born Feeder Calves

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ABSTRACT

This study determined the frequency of inadequate selenium status in 532 Missouri feeder calves and identified factors that were associated with selenium status. In conjunction with sample collection, cooperating practitioners completed a questionnaire summarizing exposure to postulated risk factors for selenium deficiency. Whole blood selenium was determined by high-performance liquid chromatography (HPLC). The proportion of calves with low blood selenium concentrations (< 70 ppb wet weight) was compared among agricultural districts. Regression models were developed to predict blood selenium concentration. Logistic regression models were developed to predict selenium status. The calculated rate of blood selenium concentrations less than 70 ppb in the study population was 0.165. Significant regional differences were seen in selenium status. Region, cow body condition, creep feeding of calves, calf age, pasture application of manure, commercial fertilizer and lime, and 4 specific pasture types were significantly associated with blood selenium concentrations. Factors significantly associated with blood selenium concentrations greater than or equal to 70 ppb wet weight included region, pasture application of either lime or manure, and ladino clover pastures. The provision of trace mineralized salt was associated with a decreased probability of adequate (70 ppb or more) blood selenium concentration.

Introduction

Selenium is an essential micronutrient in cattle diets. The primary role of selenium relates to its role as an essential component of glutathione peroxidase, the enzyme that protects cell and organelle membranes from oxidative damage. Selenium deficiency has been linked to a variety of clinical disease manifestations in cattle. These include nutritional myodegeneration (NMD), decreased reproductive performance, retained fetal membranes, increased disease susceptibility, and ill thrift. These clinical signs are not pathognomonic and are readily overlooked by cattle producers and veterinary practitioners.

Selenium deficiency can be diagnosed by measuring blood, serum, liver, or kidney selenium concentrations. Puls reported whole blood selenium concentrations of 4 to 80, 60 to 160, and 200 to 1200 ppb wet weight for selenium-deficient, marginal, and replete categories. Reported ranges for selenium deficient, selenium marginal, and selenium replete cattle often overlap and vary widely. Maas recommended classifying cattle with blood selenium less than or equal to 40 ppb as deficient and cattle with blood selenium less than 70 ppb wet weight as marginal with respect to their selenium status.

Recent studies suggest that these recommended threshold values may be inappropriately low. Swecker et al. showed that calves with blood selenium concentrations more than 160 ppb had greater humoral immune responses than did calves with blood selenium concentrations of 100 ppb. Another study reported that increasing blood selenium concentrations were associated with a decreased prevalence of mastitis and that this association persisted until blood selenium concentrations exceeded 180 ppb.

The purpose of this study was to document the prevalence of low blood selenium concentrations (< 70 ppb wet weight) in Missouri beef calves and to identify risk factors for selenium deficiency.

Materials and Methods

Sample and survey data collection: The data collection process was a systematic attempt to determine the selenium status of feeder calves throughout the state of Missouri. The sampling strategy was based on geographic localities (counties) rather than proportionate sampling of cattle populations. Private veterinary practitioners with a large beef cattle component in their practices were identified throughout the state. These collaborating veterinarians acquired blood samples from 3 randomly selected calves in each enrolled herd and attempted to obtain samples from 3 representative herds in each county. Sampling was performed at the time of routine fall processing and was restricted to calves between the ages of 4 and 10 months. Roughly equal numbers of calves were enrolled in the fall seasons of 1997 and 1998. Whole blood samples were collected into tubes containing an EDTA anticoagulant. Collaborating practitioners were provided with postage prepaid mailers and sampling supplies. In conjunction with sample collection, practitioners completed a short questionnaire summarizing exposure to potential risk factors for selenium deficiency. The survey included the questions regarding region, calf age in months, pasture type and mineral supplementation practices.

Blood selenium determinations: Briefly, the blood samples were oxidized by a nitric acid: magnesium nitrate digestion followed by dry ashing. The oxidized selenium (VI) was reduced to selenium (IV) by concentrated hydrochloric acid, neutralized with ammonium hydroxide and cation interference removed by the addition of disodium EDTA. Selenium IV was mixed with 2,3-diaminonaphthalene to form naphthylpiaszelenole at a pH range of 1.6 to 1.8. The naphthylpiaszelenole was quantitatively extracted into cyclohexane and introduced (50 mL) into an isocratic pump (Perkin-Elmer 250 Isocratic SL Pump, Perkin-Elmer Inc.) and detected with a fluorescence detector (Perkin-Elmer 650S Fluorescence Detector, Perkin-Elmer Inc.) 378 nm excitation, 557 nm emission). This method was based on the modification of a previously described method.

Data analysis: For the purpose of this study, low blood selenium concentration was defined as less than 70 ppb whole blood selenium wet weight. Only calves for which blood selenium concentration and complete survey data were obtained were included in the data set. Mean \pm standard deviation (SD) blood selenium concentrations were calculated. Survey responses were summarized, and the proportion of positive responses to each question were reported. Variables for which the proportion of positive responses was either less than 0.05 or greater than 0.95 were deleted from subsequent multivariate analyses.

The proportion of calves with low blood selenium concentrations (< 70 ppb), was reported for each of the 9 agricultural districts (Northwest, North Central, Northeast, West Central, Central, East Central, Southwest, South Central, and Southeast) defined by the Missouri Agriculture Statistics Service (2001 Missouri Farm Facts, Missouri Department of Agriculture, Jefferson City, MO.). These proportions were compared among districts using a chi-squared test (Sigma Stat Statistical Software, Version 2.03, SPSS Inc.) Patterns of deficiency were deemed to differ significantly when the calculated

P value was less than 0.05. The statewide proportions of calves with deficient, marginal, and replete selenium status were calculated.

Stepwise, multivariate, backward-stepping design-variable regression models were developed to predict blood selenium concentration as a function of region, calf age in months, pasture type, 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. All calculations were performed with the aid of a statistical software package (SAS System for Windows, Version 8, SAS Institute, Cary, NC).

Forward stepwise logistic regression models were developed predicting the incidence of replete (≥ 70 ppb blood selenium) selenium status as a function of region, calf breed, calf age, pasture type, 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 greater than 0.10. All calculations were performed with the aid of a statistical software package (SAS System for Windows, Version 8).

Results

Complete data were available for 532 calves originating from 178 herds located in 74 of the 114 counties in Missouri. The mean blood selenium concentration was $133 \text{ ppb} \pm 62$. Survey data describing pasture, management, and nutritional practices are summarized in Table 1. Significant regional differences were seen in selenium status (Table 2). The proportion of calves with low blood selenium concentrations (< 70 ppb) varied from a low of 0.047 in the Northwest region to a high of 0.400 in the Southeast region. The calculated statewide rate of low blood selenium concentration was 0.165.

The stepwise regression model identified 16 variables that were significantly associated with blood selenium concentration (Table 3). These included 6 variables describing agricultural region. Thin cowherds, creep feeding of calves and older calves (> 7 months of age) were associated with higher blood selenium concentrations in calves. Pasture application of either livestock manure or commercial fertilizer were associated with higher blood selenium concentrations and application of lime was associated with lower selenium concentrations. Of the 13 pasture plants considered, 4 were significantly associated with blood selenium concentrations. White clover, ladino clover, and brome grass were associated with lower blood selenium concentrations, and bird's foot trefoil was associated with higher blood selenium concentrations.

Logistic regression models revealed similar patterns. The Northwest and West Central districts were associated with an increased frequency of blood selenium of 70 ppb or more and the Northeast and East Central districts were associated with an increased frequency of low blood selenium concentrations (< 70 ppb). Pasture application of lime, ladino clover, and trace mineralized salt were significantly associated with a decreased probability ($\text{OR} < 1$) of adequate blood selenium concentrations (≥ 70 ppb) and pasture

application of manure was associated with an increased probability of adequate blood selenium concentrations.

Discussion

In the United States, selenium deficiency is most common in the Pacific Northwest, the Northeast, and Southeast. In addition to these regional differences, several factors have been identified that will affect selenium uptake by plants. Acidic soils result in forages that are selenium-deficient, and alkaline soils promote selenium uptake. Sulfur competes with selenium for uptake by plants. Rapid, lush growth typically produces a forage with lower selenium concentration. Plants vary in their ability to absorb selenium. Legumes have been reported to have inferior selenium uptake compared with grasses. Soil and forage selenium concentrations may fail to accurately reflect calf selenium status because a significant portion of the calf's diet is provided by milk intake. Furthermore, health or disease status may not be directly correlated with host selenium status, and deficient states may be ameliorated by dietary vitamin E intake.

We chose to measure blood selenium concentrations in spring-born beef calves at or near the time of weaning. This sampling strategy provided us the optimal opportunity to recognize selenium deficient status. Young, rapidly growing cattle have stringent dietary selenium requirements. Milk is a poor source of selenium. These calves grazed native pastures and suckled their dam. This sampling strategy provided the greatest opportunity to recognize deficiencies.

Based on our data, 16.5% of these calves had less than optimal selenium status (blood selenium concentration < 70 ppb). Calves with low blood selenium concentrations were seen in all 9 agricultural districts with 4.7% to 40.0% of calves affected. Profound regional differences were seen in calf selenium status (Table 2). The 3 regions with the lowest proportion of selenium-deficient calves were the 3 northernmost districts. These districts have decreased rainfall and higher elevations above sea level than the remainder of the state (2001 Missouri Farm Facts). From west to east, the proportion of deficient calves increased as elevation fell toward the Mississippi River flood plain. The 3 districts with the highest proportion of deficient calves were East Central, Southeast, and South Central. These 3 districts have higher rainfalls than the remainder of the state.

These conclusions were supported in part by regression models predicting blood selenium concentration (Tables 3). Positive coefficients for regions indicate blood selenium concentrations greater than the baseline exposure level (98 ppb). Negative coefficients indicate blood selenium concentrations less than the baseline. The logistic regression model (Table 4) supported these observations. The negative coefficients, and thus odds ratios less than 1, seen in the logistic models indicated a lower frequency of blood selenium concentrations greater than 70 ppb, and hence, a greater frequency of selenium deficiency in the East Central and Northeast regions (Table 4).

Although the Northeast region had a low prevalence of selenium concentrations less than 70 ppb (Table 2), residence in this district actually resulted in a decreased probability of blood

selenium greater than or equal to 70 ppb (Table 4). This apparent disparity was probably caused by regional variation in dietary and management factors impacting calf selenium status. Although a significant regional effect existed that caused a decreased probability of blood selenium concentrations greater than or equal to 70 ppb, this negative influence was counterbalanced by other risk factors that raised blood selenium concentration. The net effect was a regional prevalence of low blood selenium concentrations that was lower than the statewide prevalence (Table 2). The multivariate logistic model presented (Table 4) should be considered more representative of these regional effects than the tabular accounting of deficient calves (Table 2), because the influences of management and dietary factors have removed from regional effects.

Commercial fertilizer and manure application were associated with higher blood selenium concentrations. However, pasture application of lime was associated with lower blood selenium concentrations. Historically, alkaline soils have been associated with increased selenium uptake by plants, and consequently, higher blood selenium concentrations. In the present study, the application of lime, a soil amendment used to raise soil pH, was associated with lower blood selenium concentrations, an increased frequency of selenium deficiency, and an increased frequency of marginal to deficient selenium status. This inverse relationship between lime application and blood selenium concentrations is not surprising. Farmers are unlikely to use lime unless soil acidity has already been documented. Furthermore, lime application is probably associated with legume pastures. Grasses tolerate acid soils better than do legumes. In many instances, soils will receive supplemental lime only if farmers are attempting to replace pasture grasses with legume monocultures. Legumes have been documented as having an inferior uptake of selenium.

This inverse relationship between pasture legumes and blood selenium was substantiated by all 3 regression models. Two legumes, white clover and ladino clover, were associated with decreased blood selenium concentration. However, another legume, bird's foot trefoil, was associated with increased blood selenium concentrations, and one grass, brome grass, was associated with decreased blood selenium concentrations. Ladino clover was associated with an increased frequency of selenium deficiency and white clover was associated with an increased frequency of marginal to deficient selenium status.

Older calves had higher blood selenium concentrations, suggesting that these calves are voluntarily shifting from milk-based diets to diets that include superior selenium sources. Creep feeding was associated with increased blood selenium concentrations (Table 3). Most prepared feeds contain supplemental selenium.

The provision of trace mineralized salt was associated with a decreased probability of blood selenium concentrations greater than or equal to 70 ppb. This observation raises questions regarding the efficacy of free-choice trace mineralized salt as a prophylaxis strategy for selenium deficiency in calves. Selenium concentrations in trace mineralized salt may be appropriate to prevent deficiencies in adult cattle and still be inadequate for prevention of deficiency in nursing calves. Either dietary supplementation of cows does

not result in adequately increased milk selenium or direct calf intake of trace mineralized salt is adequate to provide selenium supplementation. Both hypotheses are probably true. Parenteral injections of selenium might address this problem. However, it should be noted that less than 3% of the calves studied received parenteral selenium (Table 1).

No definitive explanation is apparent for the association between thin cows and higher blood selenium concentrations. However, we hypothesize that thinner cow herds may have inferior milk production. The calves of these thin cows will likely receive a greater proportion of their dietary intake by grazing, potentially a superior source of selenium relative to cows milk.

Low blood selenium concentrations were common in the study population. Ad libitum access to trace mineralized salt was associated with a decreased probability of adequate serum selenium concentrations. The frequency of low blood selenium concentrations in calves and the lack of apparent response to common supplementation practices suggests that beef calves in this region should be monitored for selenium deficiency. Alternative supplementation practices, such as parenteral injection of selenium, can be implemented in herds in which low blood selenium concentrations are common. In conclusion, it should be emphasized that the model predicting blood selenium concentration accounted for less than 25% of observed variation in blood selenium concentration ($R^2 = 0.23$).

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Table 1. Frequency of Positive Responses to Survey Questions Describing Pasture, Animal Husbandry and Animal Health Practices in 532 Missouri Feeder Calves

Question	Proportion positive responses
Have pastures received commercial fertilizer in the last 2 years?	0.791
Have pastures received supplemental lime in the last 2 years?	0.150
Is livestock manure spread on pastures?	0.262
Does the pasture contain fescue?	0.938
Does the pasture contain orchard grass?	0.309
Does the pasture contain Bermuda grass?	0.038
Does the pasture contain red clover?	0.437
Does the pasture contain white clover?	0.073
Does the pasture contain ladino clover?	0.251
Does the pasture contain bluegrass?	0.073

Does the pasture contain birdsfoot trefoil?	0.058
Does the pasture contain alfalfa?	0.049
Does the pasture contain lespedeza?	0.107
Does the pasture contain bromegrass?	0.050
Does the pasture contain timothy?	0.040
Does the pasture contain reed canary grass?	0.006
Are calves provided access to creep feed?	0.335
Are cows provided supplemental hay in addition to pasture forage?	0.115
Are cows provided supplemental concentrates in addition to pasture forage?	0.166
Are cows and calves provided access to trace mineralized salt?	0.712
Do calves receive injections of selenium and vitamin E?	0.026

Table 2. Numbers and Proportions of Calves With Blood Selenium Concentrations Less Than 70 ppb by Agricultural District in a Survey of 532 Missouri Feeder Calves

	Calves at risk	n	Number of calves with blood selenium concentrations	
			< 70 ppb	Proportion of calves with blood selenium concentrations < 70 ppb
1 Northwest	223,000	86	4	0.047
2 North Central	222,000	48	4	0.083
3 Northeast	121,000	102	12	0.118

4 West Central	240,000	36	6	0.166
5 Central	460,000	80	15	0.188
6 East Central	136,000	55	14	0.255
7 Southwest	312,000	50	7	0.140
8 South Central	320,000	58	14	0.241
9 Southeast	39,000	15	6	0.400
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Summary estimates	2,073,000	532	332,750	0.165

Chi-squared = 60.26 with 16 degrees of freedom, P < 0.001

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

Table 3. Results of a Backward Stepping Stepwise Regression Model Predicting Blood Selenium Concentration (Ppb) in Spring-Born Missouri Beef Calves as a Function of Agricultural District, Cow Condition, Calf Age, Pasture Fertilization Practices and Predominant Pasture Forage

Variable	Coefficient	P-value
Intercept	98.09	< 0.0001
Northwest	55.77	< 0.0001
North Central	23.70	0.0149
West Central	32.75	0.0023
Central	25.49	0.0011
East Central	-20.31	0.0272
South West	21.17	0.0211
Cow condition (thin)	23.19	0.0758

Calf age (> 7 months)	20.68	0.0743
Pasture fertilization	15.63	0.0154
Pasture lime application	-25.91	0.0005
Pasture manure application	17.63	0.0030
White clover	-24.74	0.0120
Ladino clover	-9.75	0.0966
Birdsfoot trefoil	18.41	0.0889
Bromegrass	-21.45	0.0543
Creep feeding	19.50	0.0004

R² = 0.2313

Table 4. Results of a Forward Stepwise Logistic Regression Model Predicting the Occurrence of Blood Selenium Concentrations ≥ 70 Ppb as a Function of Geographic, Management and Nutritional Risk Factors

Variable	Coefficient	Odds Ratio P	(95% CI)
Intercept	-1.0290	0.0001	*
Northwest	1.0430	0.0004	2.84 (1.59, 5.08)
Northeast	-1.1820	0.0032	0.31 (0.14, 0.67)
West	0.911	0.0372	2.49 (1.06, 5.85)
Central			
East	-1.877	0.0013	0.15 (0.05, 0.48)
Central			
Lime	-1.179	0.0212	0.31 (0.11, 0.84)
Manure	1.475	< 0.0001	4.37 (2.53, 7.54)

Ladino -0.914 0.00690.40 (0.21, 0.78)

clover

Trace -0.792 0.00270.45 (0.27, 0.76)

mineralized salt