Growth, reproductive performance, and manganese status of heifers fed varying concentrations of manganese

S. L. Hansen, J. W. Spears, K. E. Lloyd and C. S. Whisnant

J Anim Sci 2006. 84:3375-3380.
doi: 10.2527/jas.2005-667

Growth, reproductive performance, and manganese status of heifers fed varying concentrations of manganese[1,2]

S. L. Hansen, J. W. Spears,[3] K. E. Lloyd, and C. S. Whisnant

Department of Animal Science, North Carolina State University, Raleigh, NC 27695

ABSTRACT: An experiment was conducted to examine the effects of dietary Mn on growth, reproductive performance, and Mn status of beef heifers. Eighty Angus (n = 40) and Simmental (n = 40) heifers, averaging 249 kg, were stratified by BW within a breed and randomly assigned to 1 of 4 treatments providing 0 (control), 10, 30, or 50 mg of supplemental Mn/kg of DM from $MnSO_4$. Heifers were individually fed a diet containing cottonseed hulls, corn gluten feed, citrus pulp, and ground corn, and the control diet contained 15.8 mg of Mn/kg of DM by analysis. Average daily gain, DMI, and G:F for the 196-d period were not affected by Mn supplementation. Control heifers had reduced (P = 0.04) liver Mn when contrasted with the 3 levels of supplemental Mn. Serum cholesterol was greater (P = 0.001) in Angus compared with Simmental heifers over the course of the 196-d experiment but was not affected by treatment. Dietary Mn did not significantly affect measures of reproductive performance. Results of this study indicate that 15.8 mg of Mn/kg of diet DM should be adequate for growth, onset of estrus, and conception of beef heifers.

Key words: cattle, growth, manganese, reproduction

J. Anim. Sci. 2006. 84:3375–3380
doi:10.2527/jas.2005-667

INTRODUCTION

Manganese is an important trace mineral in biological systems, acting as an enzyme component and activator (Leach and Harris, 1997). The importance of dietary Mn in beef cattle has not been researched extensively, and the majority of the work dates back to 1970 or earlier. Early studies were conducted with limited animal numbers, and generally only 2 concentrations of dietary Mn were evaluated. Early work suggests that a deficiency of Mn can impair reproduction, resulting in an increased number of services required for conception, increased days to first estrus, and reduced calf birth weights (Bentley and Phillips, 1951; Rojas et al., 1965).

It has been suggested that Mn may act as a cofactor for mevalonate kinase and farnesyl pyrophosphate synthase, enzymes involved in the production of squalene, a precursor of cholesterol (Curran and Azarnoff, 1961; Davis et al., 1990). Cholesterol is necessary for the production of progesterone, and it is possible that decreased cholesterol in Mn-deficient animals may play a vital role in the delay of the onset of estrus. The role of Mn as an enzyme activator has been well established through work with chicks, demonstrating that Mn is required to activate glycosyltransferase, which is necessary for proper synthesis of cartilage (Leach and Muenster, 1962). Similarly, research with calves also indicates that Mn plays an essential role in proper long bone and epiphyseal growth plate development (Rojas et al., 1965; Howes and Dyer, 1971). Manganese requirements of cattle are poorly defined, and current NRC (1996) suggestions for beef cattle are 20 and 40 mg of Mn/kg of DM for growth and reproduction, respectively.

The objective of the current study was to determine Mn requirements for growth, development, and reproductive performance of beef heifers.

MATERIALS AND METHODS

Animal and Experimental Design

The experimental procedures were reviewed and approved by the North Carolina State University Animal Care and Use Committee. Eighty Angus (n = 40) and Simmental (n = 40) heifers (249 ± 23 kg of initial BW),

[1]Use of trade names in this publication does not imply endorsement by the North Carolina Agric. Research Serv. or criticism of similar products not mentioned.

[2]Appreciation is extended to G. Shaeffer, H. Stahlhut, L. Legleiter, E. Baird, J. Dickerson, J. Woodlief, and B. Matthews for their assistance in sampling and animal care.

[3]Corresponding author: Jerry_Spears@ncsu.edu

Received November 16, 2005.
Accepted July 5, 2006.

Table 1. Ingredient and calculated chemical composition of basal diet[1]

Ingredient, % of DM	
Ground corn	11.7
Cottonseed hulls	40.0
Corn gluten feed	25.0
Citrus pulp	20.0
Urea	0.75
Calcium carbonate	0.5
Vitamin premix[2]	0.03
Mineral premix[3]	0.01
Corn supplement[4]	2.0
Chemical composition	
CP,[5] % of DM	12.4
NDF,[5] % of DM	50.9
ME,[5] Mcal/kg of DM	2.4
Mn,[6] mg/kg of DM	15.8
Ca,[6] % of DM	0.47
P,[6] % of DM	0.24

[1]Contained Rumensin (Elanco Animal Health, Indianapolis, IN) at 33 mg/kg.
[2]Provided per kilogram of diet: 1,980 IU of vitamin A; 456 IU of vitamin D_3; and 1.98 IU of vitamin E.
[3]Provided per kilogram of diet: 30 mg of Zn as $ZnSO_4$; 10 mg of Cu as $CuSO_4$; 0.5 mg of I as $Ca(IO_3)_2(H_2O)$; 0.2 mg of Se as Na_2SeO_3; and 0.1 mg of Co as $CoCO_3$.
[4]Provided supplemental Mn as $MnSO_4$.
[5]Calculated value (NRC, 1996).
[6]Analyzed value.

approximately 10 mo of age, were used in this study. Heifers were from the cow herd at the Butner Beef Cattle Field Lab and grazed tall fescue pasture, with ad libitum access to water and plain white salt before starting on study. Before initiation of the study, heifers were vaccinated for protection against infectious bovine rhinotracheitis, bovine viral diarrhea (I and II), parainfluenza-3, bovine respiratory syncytial virus (Titanium 5, AgriLabs, St. Joseph, MO) and clostridial organisms (Vision 7, Intervet, Millsboro, DE). Heifers were also treated for internal and external parasites (Bovimec, Virbac, Fort Worth, TX). Heifers were housed in a covered facility with slatted floors and individually fed via electronic feeders (American Calan, Northwood, NH). Heifers were stratified by BW within a breed and randomly assigned within a pen to treatments. There were 6 pens of 12 heifers each and 1 pen housing 8 heifers. Age of heifers in each treatment averaged 310 ± 1 d, with a range of 49 d between the youngest and oldest heifers on the study.

Treatments consisted of 0 (control), 10, 30, and 50 mg of supplemental Mn/kg of DM. Supplemental Mn was supplied from $MnSO_4$. Ingredient composition and calculated chemical composition of the basal diet are shown in Table 1. Diets were formulated to allow heifers to gain 1 kg/d, and to meet or exceed all NRC (1996) requirements, except that for Mn. The control diet contained 15.8 mg of Mn/kg of DM. Heifers were fed once daily, with feed amounts based on ad libitum consumption in the previous 24-h period, and feed refusals collected daily. Initial and final BW for the 196-d study were the average of BW measured on 2 consecutive days. Interim heifer BW were recorded at 28-d intervals. Jugular blood samples were collected 2 h postfeeding on d 0, 63, 98, 168, and 196 for determination of serum cholesterol and on d 0, 98, and 168 for plasma Mn concentrations. Liver biopsy samples were obtained as described by Engle and Spears (2000) on d 98 and 196 for Mn determination.

Two 25-mg doses of Lutalyse (Pfizer Animal Health, New York, NY) were administered i.m. on d 90 and 104 to synchronize estrus. On d 104, the heifers were moved to a covered building, with dry lots grouped by treatment into 8 pens of 10 heifers with the breeds mixed, and bunk fed for 8 d to avoid injuries during estrual activity. Heifers were observed for signs of estrus at 4 times each day (0700, 1000, 1300, and 1800). Heifers detected during the 2 morning periods were artificially inseminated in the afternoon, and heifers detected during the 2 afternoon periods were bred the following morning. After the 8-d bunk-feeding period, heifers were returned to their original pens with the Calan feeders, with estrus detection and breeding continuing the same protocol.

Semen from 3 sires was used, with sires assigned to equal numbers of heifers in each treatment. Artificial insemination was conducted by 1 of 4 certified technicians. Heifers that had not been bred by d 139 were given a dose of 25 mg of Lutalyse administered i.m. and bred on d 142 regardless of exhibition of estrus. Heifers bred early in the breeding season were examined by ultrasonography at 30 d and rectally palpated at 60 d postbreeding for pregnancy determination. Rectal palpation was performed on all heifers on d 196 for final determination of pregnancy.

Analytical Procedures

Blood was collected into heparinized vacuum tubes designed for trace mineral analysis (Becton, Dickenson, Rutherford, NJ), centrifuged at 1,200 × *g* for 20 min at 20°C, and the plasma was wet-ashed before Mn analysis, as described by Legleiter et al. (2005). Flameless atomic absorption spectroscopy (GFA-6500; Shimadzu Scientific Instruments, Kyoto, Japan) was used to determine Mn content in plasma.

Blood for serum cholesterol was collected in vacuum tubes and allowed to clot at room temperature for 1 h. Samples were then centrifuged at 1,200 × *g* for 20 min at 20°C and analyzed for cholesterol using a colorimetric end-point assay kit from ThermoDMA (Arlington, TX).

Feed and liver samples were prepared for Mn analysis by wet ashing using microwave digestion (Mars 5, CEM Corp., Matthews, NC), as described by Gengelbach et al. (1994). Manganese content of feed and liver samples was determined by flame atomic absorption spectroscopy (Shimadzu Scientific Instruments).

Table 2. Effect of supplemental Mn on performance of growing beef heifers

	Supplemental Mn,[1] mg/kg of DM				
Item	0	10	30	50	SEM
ADG, kg	0.98	0.97	0.98	1.01	0.02
DMI, kg/d	10.18	9.84	10.23	10.42	0.2
G:F	0.11	0.11	0.11	0.11	0.002

[1]Basal (0 supplemental Mn) diet contained 15.8 mg of Mn/kg of diet DM, and supplemental Mn was provided as $MnSO_4$.

Statistical Analysis

Statistical analysis of performance data was performed by ANOVA for a completely randomized design using the MIXED procedure (SAS Inst. Inc., Cary, NC). Liver Mn, plasma Mn, and serum cholesterol were analyzed as repeated measures, with individual animals serving as the experimental unit. The model included the fixed effects of breed, treatment, day of sampling, and all related interactions. Individual animal served as a random effect, with treatment × breed serving as the error term, and day of sampling as the repeated statement. For liver Mn, d 0 concentrations were used in a covariate analysis. Reproductive data were analyzed using the GENMOD procedure of SAS. The model included the fixed effects of breed, treatment, and the treatment × breed interaction. Interactions that were not significant ($P < 0.05$) for the measurement of interest were removed from the models. When treatment was significant ($P < 0.10$), differences among means were separated using single degree of freedom orthogonal contrasts. The comparisons made were 0 mg of Mn vs. 10, 30, and 50 mg of Mn/kg of DM; 0 mg of Mn vs. 30 and 50 mg of Mn/kg of DM; and 30 mg of Mn vs. 50 mg of Mn/kg of DM. One heifer was removed from the study on d 68 after becoming severely ill from coccidiosis. Data from this heifer were not included in statistical analysis. All values in the tables are reported as least squares means.

RESULTS AND DISCUSSION

Average daily gain, DMI, and G:F for the 196-d study did not differ among treatments (Table 2). Dry matter intakes in this study were rather high, most likely due to the high inclusion level of cottonseed hulls in the diet. As Galyean and Defoor (2003) have previously suggested, it seems that diets high in cottonseed hulls cause animals to consume greater amounts because of a dilution in the energy content of the diet. The lack of differences in growth characteristics among treatments suggests that the control diet, containing 15.8 mg of Mn/kg of DM, was adequate in Mn to support maximum performance of growing heifers. Earlier studies (Bentley and Phillips, 1951; Howes and Dyer, 1971) also indicated that Mn supplementation to diets containing 10 to 13 mg of Mn/kg of DM did not improve performance of heifers. More recently, Legleiter et al. (2005) reported that performance of growing and finishing steers did not differ among treatments of 0, 10, 20, 30, 120, or 240 mg of supplemental Mn/kg of DM. The control diet used by Legleiter et al. (2005) contained 29 mg of Mn/kg of DM during the 84-d growing phase and 8 mg of Mn/kg of DM during the finishing phase. The current NRC (1996) recommendation for growing beef cattle is 20 mg of Mn/kg of DM; however, the previously mentioned research in conjunction with the current study would suggest that the Mn requirement for maximum growth in heifers does not exceed the level (15.8 mg of Mn/kg of DM) found in our control diet.

Plasma Mn concentration was affected by sampling date ($P = 0.001$), but not by treatment, treatment × sampling date, or breed (Table 3). Similarly, Legleiter et al. (2005) found no differences in plasma Mn concentrations in steers fed supplemental concentrations of Mn ranging from 0 to 240 mg of Mn/kg of DM. Previous work in sheep (Watson et al., 1973; Masters et al., 1988) also demonstrated that plasma Mn concentrations were very low and not affected by supplementation of physiological concentrations of Mn. The consistently low concentration of Mn in plasma, regardless of dietary Mn concentration, implies that the body has a very effective mechanism by which Mn homeostasis is maintained. Cattle have been reported to have an intestinal absorption rate of about 1% of dietary Mn, regardless of the level of Mn in the diet (Gibbons et al., 1976). After absorption, Mn is bound to albumin for transport to the liver, the principal organ involved in Mn metabolism (Leach and Harris, 1997). At least 95% of the absorbed Mn is removed on the first pass through the liver and is excreted in bile (Gibbons et al., 1976).

Liver Mn concentrations on d 98 and 196 were adjusted using d 0 values as a covariate. Manganese concentrations in liver were affected by date of sampling, with concentrations being greater ($P = 0.001$) on d 98 than on d 196. Liver Mn concentrations were greater ($P = 0.03$) in heifers provided supplemental Mn than in control heifers (Table 3). Previous work in ruminants (Howes and Dyer, 1971; Ivan and Hidiroglou, 1980) has shown that liver Mn concentration increases as dietary Mn increases. Legleiter et al. (2005) found a linear increase in liver Mn concentrations of steers as supplemental Mn increased from 0 to 240 mg of Mn/kg of DM. Conversely, Bentley and Phillips (1951) found that feeding dairy cows a diet containing 7 to 10 or 30 mg of Mn/kg of DM for more than 3 yr did not affect liver Mn concentration. Whereas liver Mn concentrations in the current study showed a dose response, growth of the control heifers was not negatively affected when compared with the supplemented treatments. However, although it is apparent that the level of Mn in the control diet (15.8 mg of Mn/kg of DM) was adequate for growth of beef heifers, it is unclear what concentration of liver Mn is necessary for optimum activity by Mn-dependent enzymes in the liver.

Table 3. Effects of Mn supplementation on plasma and liver Mn concentrations and serum cholesterol concentrations of growing beef heifers

	Supplemental Mn,[1] mg/kg of DM					
Item	0	10	30	50	SEM	Significance[2]
Plasma Mn,[3] ng/mL	16.39	17.12	15.54	17.10	0.94	A**
d 0	9.85	13.55	9.83	13.23	1.58	
d 98	19.71	20.15	20.47	17.90	1.65	
d 168	19.61	17.67	16.31	20.15	1.63	
Liver Mn,[3] mg/kg of DM	9.1	9.4	9.7	10.3	0.29	A** B* C*
d 98	10.0	10.3	10.8	11.0	0.43	C*
d 196	8.2	8.6	8.7	9.4	0.32	B* C*
Serum cholesterol,[3] mg/dL	196.3	212.0	203.0	200.6	9.20	A**

[1]Basal diet (0 supplemental Mn) contained 15.8 mg Mn/kg of diet DM and supplemental Mn was provided as $MnSO_4$.
[2]A = sampling date effect; B = control vs. supplemental Mn; C = control vs. 30 and 50 mg of supplemental Mn/kg DM.
[3]Pooled means across all sampling dates.
*$P < 0.05$; **$P < 0.01$.

Unlike minerals such as Cu or Zn, Mn does not have an established level in liver or plasma below which an animal is classified as being in a deficient or marginally deficient state. It is possible that initial body stores in the current study were sufficient to provide adequate Mn during the 196-d study for Mn-dependent functions. However, because no criteria have been established for the evaluation of Mn status in beef cattle, it is unclear if control heifers reached a state of marginal Mn deficiency. Whole blood Mn concentration has been suggested as an appropriate indicator of Mn status (Hidiroglou, 1979); however, whole blood Mn values as reported are inconsistent, reflecting laboratory analysis difficulties and individual animal variability (Underwood and Suttle, 1999).

Serum cholesterol concentrations were not affected by supplemental Mn (Table 3). Several studies using rats as a model have noted that a diet severely deficient in Mn results in decreased cholesterol levels in the plasma or liver (Curran and Azarnoff, 1961; Kawano et al., 1987; Davis et al., 1990). The lack of cholesterol response to Mn supplementation in the present 196-d study may be due to the fact that our control diet was not severely deficient in Mn. Cholesterol concentrations were affected by sampling date ($P = 0.001$), with serum concentrations increasing ($P = 0.001$) between d 0 and 63 and between d 63 and 98 (Figure 1). Serum cholesterol values were similar on d 98, 168, and 196. Angus heifers had greater ($P = 0.006$) serum cholesterol concentrations than Simmental heifers on all sampling dates (Figure 1). In a previous study, Angus heifers had greater serum cholesterol concentrations than Simmental × Angus heifers (Hansen, 2005).

Pregnancy rate, conception rate, age at conception, and services to conception were not significantly affected by supplemental Mn (Table 4). Several research groups have reported reduced reproductive performance in cattle fed a low Mn diet, including delayed estrus expression (Bentley and Phillips, 1951; Rojas et al., 1965; DiCostanzo et al., 1986). Other studies have also noted a decreased number of services required to conceive when cows were provided supplemental Mn in addition to a low-Mn basal diet (Rojas et al., 1965; DiCostanzo et al., 1986). These studies were conducted with limited numbers and only evaluated 2 levels of Mn. Pregnancy rate (63 and 33%, respectively) and first service conception rate (63 and 31%, respectively) were greater ($P = 0.01$) in Simmental than in Angus heifers (Table 4).

The mode of action by which Mn deficiency may impair reproduction has not been elucidated. Based on the previous literature, we hypothesized that the control diet in the current study would be low enough in Mn to cause greatly impaired reproductive performance when compared with heifers supplemented with Mn. Even with 20 heifers per treatment, we were not able

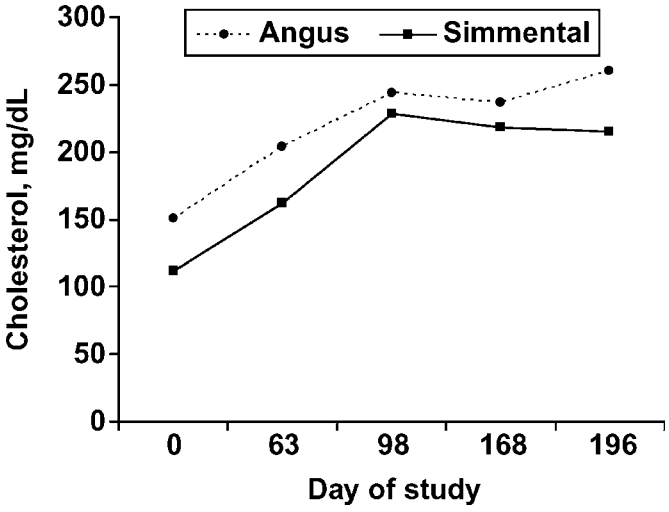

Figure 1. Effect of breed on serum cholesterol level in heifers (breed effect, $P < 0.01$; SEM = 7.87; sampling date effect, $P < 0.001$).

Table 4. Effects of supplemental Mn on reproductive performance of growing beef heifers

	Supplemental Mn,[1] mg/kg of DM			
Item	0	10	30	50
Estrus response to Lutalyse,[2] %	40 (8/20)	40 (8/20)	47 (9/19)	50 (10/20)
Age at conception, d	431 ± 17	436 ± 15	432 ± 16	432 ± 18
Services to conception	1.20	1.15	1.26	1.20
First service conception rate,[3] %	45 (9/20)	40 (8/20)	47 (9/19)	60 (12/20)
Pregnancy rate to AI,[4] %	73 (11/15)	71 (10/14)	79 (11/14)	93 (14/15)
Pregnancy rate to mass breed,[5] %	40 (2/5)	0 (0/6)	40 (2/5)	20 (1/5)
Overall pregnancy rate,[6] %	60 (12/20)	50 (10/20)	67 (13/19)	75 (15/20)

[1]Basal diet (0 supplemental Mn) contained 15.8 mg Mn/kg of diet DM and supplemental Mn was provided as $MnSO_4$.
[2]Heifers that exhibited estrus in response to Lutalyse protocol.
[3]Breed effect (Simmental 63%, Angus 31%; $P < 0.01$).
[4]Percentage of heifers conceiving when bred after exhibition of estrus.
[5]Percentage of heifers conceiving when mass bred after d 139 Lutalyse dose.
[6]Breed effect (Simmental 63%, Angus 33%; $P < 0.01$).

to show significant improvements in pregnancy rates of beef heifers. Although the number of heifers conceiving when artificially inseminated in response to estrus appeared to be numerically higher in those supplemented with 50 mg of Mn/kg of DM, there was no statistical improvement. A greater number of animals will likely be needed to show a significant reduction in pregnancy rate in heifers fed a basal diet with a Mn concentration similar to that used in the current study.

At the termination of the current study, 10 pregnant heifers from the control group and 10 pregnant heifers from the 50 mg of supplemental Mn/kg of DM group were selected to remain on treatments through gestation and early lactation. This study was designed to observe the effects of long term feeding of a low Mn diet on pregnant heifers and their offspring. Calves born to control heifers were lighter at birth and had lesser whole blood Mn concentrations than those born to supplemented heifers (Hansen et al., 2006). Several calves born to control heifers exhibited signs previously linked to Mn deficiency, including dwarfism and superior brachygnathism. These results indicate that 15.8 mg of Mn/kg of DM is not sufficient for normal calf development in the gestating beef heifer.

In conclusion, supplementation of Mn to a control diet containing 15.8 mg of Mn/kg of DM did not affect growth performance of beef heifers. Although Mn was one of the earliest trace minerals to be recognized as nutritionally essential, limited scientific research has made it one of the least understood of all the vital metals. Manganese is found in very low concentrations in tissues but has a number of important roles in biochemical systems in the body. The level of dietary Mn below which Mn stores and Mn-dependent processes in the body begin to be affected is unclear. Therefore, the establishment of criteria to evaluate Mn status of cattle is necessary before current NRC (1996) recommendations for Mn in beef cattle can be further refined.

LITERATURE CITED

Bentley, O. G., and P. H. Phillips. 1951. The effect of low manganese rations upon dairy cattle. J. Dairy Sci. 34:396–403.

Curran, G. L., and D. L. Azarnoff. 1961. Effect of certain transition elements on cholesterol biosynthesis. Fed. Proc. 20:109–111.

Davis, C. D., D. M. Ney, and J. L. Greger. 1990. Manganese, iron and lipid interactions in rats. J. Nutr. 120:507–513.

DiCostanzo, A., J. C. Meiske, S. D. Plegge, D. L. Haggard, and K. M. Chaloner. 1986. Influence of manganese, copper and zinc on reproductive performance of beef cows. Nutr. Rep. Int. 34:287–292.

Engle, T. E., and J. W. Spears. 2000. Effects of dietary copper concentration and source on performance and copper status of growing and finishing steers. J. Anim. Sci. 78:2446–2451.

Galyean, M. L., and P. J. Defoor. 2003. Effects of roughage source and level on intake by feedlot cattle. J. Anim. Sci. 81(E. Suppl. 2):E8'E16.

Gengelbach, G. P., J. D. Ward, and J. W. Spears. 1994. Effect of dietary copper, iron, and molybdenum on growth and copper status of beef cows and calves. J. Anim. Sci. 72:2722–2727.

Gibbons, R. A., S. N. Dixon, K. Hallis, A. M. Russell, B. F. Sansom, and H. W. Symonds. 1976. Manganese metabolism in cows and goats. Biochim. Biophys. Acta 444:1–10.

Hansen, S. L. 2005. Effect of dietary manganese source and concentration on growth, reproductive performance, and manganese status of heifers. M.S. Thesis, North Carolina State Univ., Raleigh.

Hansen, S. L., J. W. Spears, K. E. Lloyd, and C. S. Whisnant. 2006. Feeding a low manganese diet to heifers during gestation impairs fetal growth and development. J. Dairy Sci. 89:4305–4311.

Hidiroglou, M. 1979. Manganese in ruminant nutrition. Can. J. Anim. Sci. 59:217–236.

Howes, A. D., and I. A. Dyer. 1971. Diet and supplemental mineral effects on manganese metabolism in newborn calves. J. Anim. Sci. 32:141–145.

Ivan, M., and M. Hidiroglou. 1980. Effect of dietary manganese on growth and manganese metabolism in sheep. J. Dairy Sci. 63:385–390.

Kawano, J., D. M. Ney, C. L. Keen, and B. O. Schneeman. 1987. Altered high density lipoprotein composition in manganese-deficient Sprague-Dawley and Wistar rats. J. Nutr. 117:902–906.

Leach, R. M., Jr., and E. D. Harris. 1997. Manganese. Pages 335–356 in Handbook of Nutritionally Essential Mineral Elements. B. L. O'Dell and R. A. Sunde, ed. Marcel Dekker Inc., New York, NY.

Leach, R. M. J., and A. Muenster. 1962. Studies on the role of manganese in bone formation. J. Nutr. 78:51–56.

Legleiter, L. R., J. W. Spears, and K. E. Lloyd. 2005. Influence of dietary manganese on performance, lipid metabolism, and carcass composition of growing and finishing steers. J. Anim. Sci. 83:2434–2439.

Masters, D. G., D. I. Paynter, J. Briegel, S. K. Baker, and D. B. Purser. 1988. Influence of manganese intake on body, wool and testicular growth of young rams and on the concentration of manganese and the activity of manganese enzymes in tissues. Aust. J. Agric. Res. 39:517–524.

NRC. 1996. Nutrient Requirements of Beef Cattle. 7th ed. Natl. Acad. Press, Washington, DC. Rojas, M. A., I. A. Dyer, and W. A. Cassatt. 1965. Manganese deficiency in the bovine. J. Anim. Sci. 24:664–667.

Underwood, E. J., and N. F. Suttle. 1999. Pages 283–342 in The Mineral Nutrition of Livestock. Commonwealth Agricultural Bureaux, Slough, UK.

Watson, L. T., C. B. Ammerman, J. P. Feaster, and C. E. Roessler. 1973. Influence of manganese intake of metabolism of manganese and other minerals in sheep. J. Anim. Sci. 36:131–136.