

## **ABSTRACT**

HANSEN, STEPHANIE LAURA. The Effect of Dietary Manganese on Growth, Reproductive Performance, and Manganese Status of Beef Heifers. (Under the direction of Jerry W. Spears.)

Three trials were conducted to examine the effects of dietary manganese (Mn) on growth, reproductive performance, and Mn status of beef heifers. In Experiment 1, 80 Angus and Simmental heifers, approximately 10 mo of age, were supplemented with 0 (control), 10, 30, or 50 mg Mn/kg DM from  $\text{MnSO}_4$  in addition to a diet containing 15.8 mg Mn/kg DM for 196 d. Performance of heifers was not affected by treatment. Liver Mn concentration increased ( $P=0.04$ ) as dietary Mn level increased. Serum cholesterol was greater ( $P=0.001$ ), in Angus compared to Simmental heifers for the 196-d period, but was not affected by treatment. Reproductive performance was not affected by treatment. Numerically, the number of heifers cycling at 12 mo of age, heifers bred at first service, and pregnancy rate was higher in heifers supplemented with 50 mg Mn/kg DM, compared to control heifers.

In Experiment 2, 20 pregnant heifers from the control ( $n = 10$ ) and 50 mg Mn/kg DM ( $n = 10$ ) treatments were selected from Experiment 1 to continue on their dietary treatments through gestation and early lactation, to observe the effects of dietary Mn concentration on offspring. Serum cholesterol and whole blood Mn concentration of pregnant heifers for the 267 d study was not affected by dietary treatment. Calves born to control heifers were lighter at birth than those born to heifers receiving supplemental Mn ( $P = 0.02$ ). Whole blood Mn concentration of calves at birth were lower ( $P = 0.01$ ) in calves born to control heifers than those born to supplemented heifers. Seventy-one percent of calves born to control heifers exhibited some degree of superior brachygnathism.

Experiment 3 used 70 Angus and Angus-Simmental cross heifers, approximately 9 mo of age, to examine the effect of dietary Mn source and level on growth, reproductive performance, and Mn status of beef heifers. Diets consisted of a corn-silage diet (analyzed 36 mg Mn/kg DM) supplemented with 0, 30 mg Mn/kg DM from MnSO<sub>4</sub> (SO<sub>4</sub>), or 30 mg Mn/kg DM from a chelated Mn source (Mn Bioplex). Performance and liver Mn concentration of heifers was not affected by source or level of dietary Mn. Serum cholesterol was affected by a treatment by breed interaction ( $P = 0.06$ ), with cholesterol in Angus heifers increasing over time in response to treatment. Whole blood Mn concentration was not affected by source or concentration of dietary Mn. Dietary Mn source and concentration did not affect the percent of heifers cycling. Pregnancy rate was affected by dietary treatment ( $P = 0.01$ ). Fewer SO<sub>4</sub> heifers were bred than control ( $P = 0.01$ ) and Bioplex heifers ( $P = 0.04$ ). Results suggest that a diet containing 15.8 mg Mn/kg DM is sufficient for growth of beef heifers. Supplementation of Mn to the control diet tended to improve reproductive performance and was necessary to overcome signs of Mn deficiency in the offspring of the heifers.

**THE EFFECT OF DIETARY MANGANESE ON GROWTH, REPRODUCTIVE  
PERFORMANCE, AND MANGANESE STATUS OF BEEF HEIFERS.**

By

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Chair of Advisory Committee

## **DEDICATION**

This thesis is dedicated to all those who have loved and supported me through my education, but most particularly to...

My parents, Dennis and Susan Hansen, for the sacrifices they have made to give my sister and I such a wonderful life, and for having the strength to let me chase my dreams, even if it meant leaving them behind, for a while anyway.

My grandparents, Jack and Dorothy Powell, for all the knowledge of cattle and life they have imparted on me, and on whose farm I spent endless hours of happiness.

## **BIOGRAPHY**

Stephanie Laura Hansen was born in Sioux City, Iowa on March 13, 1981, to Dennis and Susan Hansen. She was raised in Sergeant Bluff, Iowa with her younger sister, Stacie, and attended high school at Sergeant Bluff-Luton High School. In the fall of 1999 she began her journey in higher education at Iowa State University in Ames, Iowa. Stephanie graduated with Honor and Distinction in December of 2002, receiving a Bachelor of Science degree in Animal Science, as well as the Academic Achievement Award for the College of Agriculture. In August of 2003, Stephanie moved halfway across the country to Raleigh, North Carolina, to pursue her Master of Science degree in Animal Science at North Carolina State University. Upon completion of her Masters degree, Stephanie will begin pursuit of her Doctor of Philosophy degree in Nutrition at North Carolina State University, continuing under the guidance of Dr. Jerry W. Spears.

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## **Introduction**

Manganese (Mn) is a brittle, lustrous metal that is steel gray in color, with an atomic number of 25, and an atomic weight of 54.938 (McDowell, 1992). It was isolated in 1774 by Johann Gahn of Sweden (International Manganese Institute, 2005). The word manganese is of Latin origin, from the word *magnes*, meaning magnet. The name comes from the magnetic properties of manganese oxide, although not all Mn is magnetic (Winter, 2003). Approximately 0.10% of the earth's crust is composed of Mn, with concentrations in the soil averaging 500 to 600 mg Mn/kg DM. Of the heavy metals, only iron (Fe) is more abundant (McDowell, 1992). Manganese has 11 valence states, though +2 and +3 are the most important ones in biological systems (Leach and Harris, 1997).

Industrial uses for Mn increased when the organomanganese compound methylcyclopentadienyl manganese tricarbonyl (MMT) replaced lead as the antiknock agent in gasoline. Canada began using MMT in 1976, and by 1990 MMT had completely replaced lead in gasoline (ATSDR, 2000). Also used as an octane booster, MMT can improve oil combustion, reducing soot levels and boiler clogging (International Manganese Institute, 2005). Typical industrial uses for Mn include the production of batteries, matches, fireworks, and glass-making. It's most important contribution to industry is in the steel making process, where the addition of Mn adds to the resistance of steel to impact (ATSDR, 2000).

Manganese was first determined to be essential for mice by Kremmerer et al. in 1931. Work shortly thereafter determined that Mn was directly involved in a common poultry disease known as perosis, or slipped tendon. Wilgus et al. (1936) found that a deficiency of Mn resulted in twisting or shortening of the leg bones, slipping of the tendon of the

gastrocnemium muscle from the intercondyloid groove, and enlargement of the intertarsal joint. Perotic birds often gave birth to young that suffered from neonatal ataxia (Cotzias, 1958). The first Mn deficiency in ruminants was induced by Bentley and Phillips in 1951, and subsequent research has shown that Mn serves an important role in the reproductive performance and proper skeletal development of adult and young ruminants, respectively.

### **Function of Manganese**

Manganese has been found to have several functions in the body, though it probably affects an animal most greatly through its involvement with enzymatic complexes. Manganese acts as a cofactor in a number of enzymes, including pyruvate carboxylase, mitochondrial superoxide dismutase, and arginase, the final enzyme in the urea cycle (Leach and Harris, 1997). Several kinases, hydrolases, decarboxylases, and transferases are activated by Mn in the body, although when Mn is lacking the element magnesium can often take its place. Manganese is also thought to be involved in cholesterol formation in the body, as well as insulin synthesis in the pancreas (Leach and Harris, 1997).

### **Manganese and glycosyltransferases**

One of the primary functions of Mn is activation of glycosyltransferases, which are necessary for proper skeletal development. Glycosyltransferases are a group of enzymes that are associated with the metabolism of cartilage proteoglycans, affecting the biosynthesis of glycosaminoglycan and oligosaccharide side chains (Leach and Harris, 1997). The role of Mn in cartilage formation makes it essential to the formation of the epiphyseal growth plate, which directly affects longitudinal bone growth. Therefore, it is not surprising that the most easily identified sign of Mn deficiency in young animals is impaired skeletal development (Leach and Muenster, 1962).

### **Manganese and cholesterol synthesis**

Several studies have observed that a Mn deficiency decreases cholesterol levels in the liver (Curran and Azarnoff, 1961; Kawano et al., 1987; Davis et al., 1990; Jenkins and Kramer, 1991). Early work (Curran, 1954) looked at the effects of transition metals on synthesis of cholesterol in the liver of rats. Using chemically pure salts of vanadium (V), Fe, chromium (Cr), and Mn, the author injected 0.5 ml of dissolved salts in isotonic saline or 0.5 ml saline intraperitoneally in rats and sacrificed them one hour later. The researcher found that V and Fe decreased the incorporation of acetate into cholesterol by over 50 percent, while Cr and Mn increased synthesis of liver cholesterol by greater than 100 percent (Curran, 1954).

Manganese deficiency has been shown to reduce plasma cholesterol by decreasing the amount of cholesterol in the high density lipoprotein (HDL) fraction (Davis et al., 1990). Rats fed 0.4 mg Mn/kg DM had lower plasma cholesterol concentrations, specifically lower HDL cholesterol, than rats fed an adequate Mn diet (56 mg Mn/kg DM; Davis et al., 1990). Taylor et al. (1997) examined the effect of Mn deficiency in rats on two subclasses of HDL, HDL<sub>1</sub> and HDL<sub>2</sub>. Using Sprague-Dawley rats and feeding diets containing 0.48 mg Mn/kg DM or 90 mg Mn/kg DM, they found that Mn deficiency caused significantly lower cholesterol and protein characteristics in HDL<sub>2</sub> only, while no differences were seen in the HDL<sub>1</sub> subclass. The results of this study led the authors to suggest that Mn has specific effects in HDL metabolism, and this may be how Mn exerts its effects on cholesterol synthesis (Taylor et al., 1997).

Davis et al. (1990) found no difference in activity of B-hydroxy-B-methylglutaryl CoA reductase, the rate-limiting enzyme in cholesterol synthesis, in rats fed 0.4 mg Mn/kg

DM or 56 mg Mn/kg DM. This led the authors to speculate that a lack of Mn was affecting an enzyme further along the cholesterol biosynthesis pathway. They theorized that Mn may act as a cofactor for two enzymes in cholesterol synthesis, mevalonate kinase, and farnesyl pyrophosphate synthase (Davis et al., 1990). Both of these enzymes are involved in the production of squalene, which is a precursor of cholesterol (Curran and Azarnoff, 1961).

### **Manganese in Superoxide Dismutase**

Manganese is a component of Mn-dependent superoxide dismutase (SOD), which catalyzes the reaction of  $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$  (Weisiger and Fridovich, 1973). Mitochondria consume more than 90% of the oxygen used by cells, and are thus more susceptible to oxygen toxicity than other cellular components (Weisiger and Fridovich, 1973). Manganese SOD is primarily found in mitochondria where it acts as an antioxidant, scavenging  $O_2^-$ , and in general protecting the cell from oxygen toxicity (Leach and Harris, 1997).

Ose and Fridovich (1976) found that Mn was required for catalytic activity of SOD, and that while cobalt, nickel, and zinc (Zn) could bind in place of Mn in SOD, none resulted in enzyme activity. Since Mn is required for activity of Mn SOD it makes sense that a Mn deficiency would cause a decrease in Mn SOD activity. This was shown by Paynter (1980), when he reported that tissues of weanling male rats fed diets containing 0.2, 0.7, 4.7, 9.7, or 29.7 mg Mn/kg DM increased in Mn SOD activity as their dietary Mn increased. Paynter (1980) observed that Mn SOD activity in the heart decreased more rapidly than in other tissues when rats were fed the low Mn diets, with a noticeable difference occurring within 3 weeks on the diets.

### **Manganese transport mechanism**

Manganese is absorbed by the mucosa of the small intestine, transferred to the blood bound to albumin, taken up by the liver, and transported to other tissues via a transport protein, or excreted into the bile (Leach and Harris, 1997). For many years it was unclear as to what particular carrier was responsible for movement of Mn in the body. Among the proposed transporters were serum albumin, transferrin, and transmanganin (Davidsson et al., 1989). Davidsson et al. (1989) reported that transferrin was the major transport protein in plasma for Mn in rats when dosed either orally or intravenously with radiomanganese. Previous research had encountered difficulties distinguishing between transferrin and serum albumin, since they have very similar molecular weights. Davidsson et al. (1989) used a combination of several procedures to determine which of these two proteins the actual transporter of Mn in plasma was. Using fast protein liquid chromatography, SDS-polyacrylamide gradient gel electrophoresis, and Western blot they observed that only transferrin appeared when analyzing the  $^{54}\text{Mn}$ -containing fractions. The researchers also observed that more  $^{54}\text{Mn}$  was bound to transferrin when  $^{54}\text{Mn}^{3+}$  was added to plasma in vitro than when  $^{54}\text{Mn}^{2+}$  was added. This supports work (Aisen et al., 1969) showing that Mn bound to transferrin is exclusively in the trivalent state.

### **Interactions between manganese and other minerals**

Manganese and Fe reside next to each other on the periodic table of elements, and both are transition metals. The metals can exist in similar electron configurations, allowing them to interact with each other in the body (Hill and Matrone, 1970). Manganese and Fe are antagonistic in the body and seem to cause this antagonism via interference with absorption. The intestinal mucosa may not be able to distinguish between the two minerals because of

the similar configuration of their ionic complexes in water (Rossander-Hulten, 1991).

Research in humans by Rossander-Hulten et al. (1991) examined the competitive inhibition of Fe absorption by Mn. Using healthy human subjects with variable Fe status and aged 19-50 years, the authors observed that the addition of 2 mg Mn to radiolabeled ferrous sulfate in either solution or as a hamburger meal caused a reduction of Fe absorption. Iron absorption was decreased by 21% and 34% with the addition of 7.5 or 15 mg Mn to 3 mg Fe in solution, respectively. Similarly, the addition of 15 mg Mn to 3 mg Fe in a hamburger meal caused a reduction of absorbed Fe of 40%. Rossander-Hulten et al. (1991) also found that the absorption rates for 18 mg Fe in solution and 3 mg Fe plus 15 mg Mn in solution were 18% and 15.3%, respectively. The authors speculated that this similarity was due to a failure of the intestinal mucosa to tell the difference between Fe and Mn at the absorption site.

The interaction between Mn and Fe does not only affect Fe absorption, high Fe has been shown to have a depressive effect on Mn absorption (Davis et al., 1992). When male weanling Sprague-Dawley rats were fed 0.9, 48, or 188 mg Mn/kg diet, respectively, and a marginal or high Fe level (19 or 276 mg Fe/kg diet) Mn absorption was affected. Rats fed the diet low in Mn and Fe had much higher absorption than rats fed the other treatments, and increasing dietary Mn resulted in increasing endogenous Mn losses when Fe was marginal. However, when Fe was fed at the high level, endogenous Mn losses decreased across treatments and true absorption of Mn was significantly reduced (57.3% and 27.3%, true Mn absorption for low Mn/marginal Fe and low Mn/high Fe, respectively). Manganese concentrations were significantly lower in tibias, livers and mucosal cells of rats fed high Fe levels when compared to rats fed marginal Fe levels, indicating that Fe was affecting the absorption of the dietary Mn (Davis et al., 1992).

Calcium (Ca) and phosphorus (P) have also been shown to affect Mn absorption. Early work in poultry indicates that cases of perosis were due to high dietary Ca and P causing an increased demand for Mn by reducing the Mn bioavailability to the bird (Wilgus et al., 1936). Which mineral, Ca or P, is actually causing the reduction in Mn bioavailability is still the source of some debate. Some research has shown that high levels of both Ca and P are required to interfere with Mn absorption (Wilgus et al., 1936). More recent work by Wedekind and Baker (1990) examined the effects of varying levels of Ca and P on Mn bioavailability in male chicks. Feeding a corn-soybean meal based diet that contained 37 mg Mn/kg DM, and measuring Mn bioavailability as total tibia Mn regressed on supplemental Mn intake, Wedekind and Baker (1990) found that a greater accumulation of Mn in the tibia occurred in those treatments not receiving excess P. They also determined that feeding 0%, 0.4%, and 0.8% P to chicks resulted in a relative Mn bioavailability of 100%, 77.6%, and 62.4%, respectively, when pooled across levels of 0%, 0.5%, and 1% added Ca. A second study conducted by Wedekind and Baker (1990) found similar effects of high P on Mn when pooled across Ca levels of 0%, 0.25%, 0.5%, and 1%. With 0%, 0.22%, 0.44%, and 0.88% added P, the calculated relative Mn bioavailability was 100%, 83.6%, 78.2%, and 69.3%, respectively.

McDermott and Kies (1987) conducted a human study comparing different commercial Ca supplements with milk and their effect on Mn bioavailability in the body. Supplements and milk were fed to provide healthy humans with 500 mg Ca per day. Calcium supplements came from one of the following sources: oyster shells, dolomite, calcium carbonate, calcium gluconate, calcium lactate, and a multi-source calcium. The authors measured Mn excretion in feces, Mn retentions, and whole blood Mn content to

measure bioavailability of Mn in the presence of each source of Ca. They observed that milk had the least impact on Mn bioavailability, with calcium carbonate having the most negative impact on availability of Mn. Since calcium carbonate also functions as an antacid its presence would affect the pH of the gastrointestinal tract, thus interfering with the absorption of Mn, which is more soluble in the +2 state than the +4 state and would prefer a more acidic environment in the intestine for maximum absorptive capacity (Kies, 1994).

### **Absorption of Manganese**

Absorption of Mn is uniformly low across species (Miller, 1973). In 1940, Greenberg and Campbell showed that orally administering Mn to rats resulted in approximately 3-4% absorption of the dose. Work in swine by Gamble et al. (1971) showed that sows in late gestation absorbed 28% of an oral dose of  $^{54}\text{Mn}$  when consuming a diet containing 87.3 mg Mn/kg DM. More recently, growing pigs fed a typical diet of 42 mg Mn/kg DM and given an oral dose of  $^{54}\text{Mn}$  were found to have an apparent absorption of 1.7% and a true absorption of 0.5% (Finley et al., 1997).

In ruminants, the cow has an intestinal absorption rate of about 1% of dietary Mn, regardless of the level of Mn in the diet (Gibbons et al., 1976). Shortly after this report it was observed by Sansom et al. (1978) that cattle fed 1000 mg Mn/kg DM absorbed approximately 0.5% of the Mn in the diet and that the excess was removed from the portal vein by the liver.

The absorption rate of Mn in cattle appears to be different in young animals than in their adult counterparts (Howes and Dyer, 1971; Carter et al., 1974). Howes and Dyer (1971) gave an oral dose of 100  $\mu\text{Ci}$  of carrier free  $^{54}\text{Mn}$  to two young calves, born to dams fed either 13 or 21 mg Mn/kg DM during gestation. The calves were housed in metabolism

crates for 7 days, and received 0.4 mg Mn/kg DM or 14.51 mg Mn/kg DM, respectively, in their daily diet. The authors recorded nearly twice the level of Mn excreted in the feces and urine (76.7 vs. 43.2% in feces and 13.6 vs. 7.8% in urine) from the Mn-supplemented calf verses the non-supplemented calf, indicating that the Mn-supplemented calf absorbed considerably less <sup>54</sup>Mn than the non-supplemented calf. With only one calf per treatment group, the results of the study by Howes and Dyer (1971) do not have any statistical weight, but their findings are useful. After sacrificing the two calves on their radiomanganese study, Howes and Dyer (1971) found the liver, kidney, and spleen, in that order, had the highest concentrations of radiomanganese in both the supplemented and unsupplemented calves. The authors also reported that the percent of <sup>54</sup>Mn dose found in the liver of the unsupplemented calf was 5.5 times higher than that of the supplemented calf; similarly, the activity of radiomanganese in bone was 23.5 times higher in the unsupplemented calf (Howes and Dyer, 1971). Their findings, collectively, suggest that Mn absorption increases as dietary Mn decreases, and that Mn retention in tissue is higher under conditions of low Mn intake. Supporting work by Carter et al. (1974) found that young calves that received a Mn-unsupplemented milk diet had higher absorption and retention of Mn than calves supplemented with 15 mg Mn/kg DM.

### **Excretion of Manganese**

Bile has been shown to be the primary route of excretion for Mn (Bertinchamps et al., 1966; Papavasiliou et al., 1966). In 1951 Bentley and Phillips observed that cows fed 7-10 mg Mn/kg DM had increased incidences of bile duct proliferation, abscesses and hyalinized blood vessels in their livers compared to cows fed 30 mg Mn/kg DM. At harvest, the authors also noted that cows on the low Mn diet had an average of 195 ml of bile in their

gallbladders, while Mn-supplemented cows had an average of 511 ml of bile. Several years later it was shown by Carter et al. (1974) that increasing dietary Mn by 30-fold from 0.5 to 15 mg Mn/kg DM caused a 30-fold increase in bile Mn content in calves. This same level caused an increase of two-fold in liver and gall bladder Mn concentrations, but no measurable difference in other tissues (Carter et al., 1974).

Abrams et al. (1977) observed that young calves fed 1032 mg Mn/kg DM excreted 29.2% of a intravenous dose of  $^{54}\text{Mn}$  in bile compared to 2.3% excretion in bile from calves fed 32 mg Mn/kg DM. They also reported that this noticeably different excretion rate declined rapidly for the first 2 days and by the 6<sup>th</sup> day of measurement the excretion rate of  $^{54}\text{Mn}$  in the bile was similar for both groups of calves (Abrams et al., 1977).

Britton and Cotzias (1966) suggested that variable excretion rather than variable absorption is the most important factor in Mn homeostasis. These findings have been supported in subsequent research from numerous sources (Miller, 1973; Watson et al., 1973). Conversely, research conducted by Carter et al. (1974) suggests that both excretion and absorption are important factors in Mn homeostasis. They observed that the  $^{54}\text{Mn}$  retention rate of low Mn calves (0.5 mg Mn/kg DM) was 60.1% versus a 16.3% retention rate for calves supplemented with 15 mg Mn/kg DM when intravenously dosed with  $^{54}\text{Mn}$ . Similarly, absorption of orally dosed  $^{54}\text{Mn}$  was 9 times higher (18.2 vs. 2.2%) in non-supplemented calves than in Mn-supplemented calves (Carter et al., 1974).

### **Manganese distribution in the body**

Manganese is found in relatively low concentrations throughout the body, with the highest concentrations found in pigmented tissues such as the liver, pancreas, and kidney (McDowell, 1992). These tissues are also higher in mitochondria than other tissues in the

body. Bentley and Phillips (1951) observed no notable increase in liver Mn concentration in cows fed 30 mg Mn/kg DM versus 7-10 mg Mn/kg DM, with liver Mn concentrations of 10.2 and 9.8 mg Mn/kg DM, respectively. However, Rojas et al. (1965) observed a striking difference in liver storage of Mn in newborn calves. A calf born to a dam fed 15.8 mg Mn/kg DM had 6.60 mg Mn/kg DM in its liver while a calf born to a dam fed 25.1 mg Mn/kg DM had 11.84 mg Mn/kg DM in its liver (Rojas et al., 1965). Again, these measurements were taken on just one animal per treatment, and a variety of factors could have caused the difference in Mn concentration in the calves' livers. Other studies have found similar results, with liver Mn concentration increasing or decreasing depending on dietary Mn level (Ivan and Hidirolou, 1980; Howes and Dyer, 1970).

Hidirolou et al. (1978) found that six hours after an intravenous dose of  $^{54}\text{Mn}$  was given to nonpregnant ewes the highest concentration of the radiomanganese was in the pancreas, with the liver having the next highest concentration. Conversely, when 15 nonpregnant ewes fed the same diet were given an oral dose of  $^{54}\text{Mn}$  and sacrificed five days later, the highest concentrations of radiomanganese were found in the liver with slightly lower levels in the pancreas.

The ovary of the bovine has been found to be much more responsive to a change in dietary Mn than other tissues in the body (Bentley and Phillips, 1951). They fed a diet of corn silage, a hay mixture of clover, timothy, and alfalfa, and a ground corn and corn gluten meal supplement. The diet contained less than 10 mg Mn/kg DM and was fed to 9 Holstein heifers, while a second group of 9 heifers received supplemental manganese sulfate sufficient to raise the dietary Mn level to 30 mg Mn/kg DM. These heifers started on study in the fall of 1942, had two calf crops, and were slaughtered in the spring of 1946. Analysis of

tissues for Mn revealed that the low-Mn diet did not reduce the Mn content of the liver, kidney, heart, pancreas or adrenal glands; however, they did find that the ovaries of the low-Mn cows were reduced by more than 50 percent compared to the Mn-supplemented cows (0.85 vs. 2.0 ng Mn/g DM; Bentley and Phillips, 1951).

Hidiroglou (1975) injected ewes intravenously with  $^{54}\text{MnCl}_2$  at a rate of 3.64 mCi per kilogram of bodyweight, at varying points in the estrous cycle. Ewes were slaughtered 6 hours post-injection and several tissues in the reproductive tract were analyzed for the radiomanganese. It was observed that the corpus luteum and Graafian follicle appeared to be the main tissues for Mn uptake in the ovary. Hidiroglou (1975) postulated that these tissues may contain more Mn due to the high mitochondrial population of these tissues, as compared to the low-Mn containing corpus albicans, which is high in collagen content and low in mitochondrial content. Manganese uptake was higher in ewes dosed and killed on day 11 of the estrous cycle verses those dosed and killed on day 4 of the cycle (Hidiroglou, 1975).

Collectively, these studies (Bentley and Phillips, 1951; Hidiroglou, 1975) suggest that the ovary is the major tissue for Mn uptake in the ruminant reproductive tract. Although Bentley and Phillips (1951) found a significant increase in Mn content of the ovaries in their Mn-supplemented cows, they did not report what stage of the estrous cycle the cows were in. Hidiroglou (1975) indicated that a difference of only 7 days in the stage of estrous caused a two-fold increase in Mn content of ovarian tissues, so it is difficult to ascertain whether Bentley and Phillips results are truly a result of increased dietary Mn or that the Mn-supplemented cows were merely in a different stage of the cycle than the non-supplemented group.

### **Manganese deficiency**

There appears to be a relationship between the stage of development during which a Mn deficiency occurs and the severity of signs observed. A Mn deficient diet fed to a gestating female may not negatively affect the animal herself, but could have devastating effects on the growing fetus through impaired skeletal development (Leach and Harris, 1997). A lack of Mn in the diet of an animal can cause a variety of deficiency signs, including skeletal irregularities, reduced growth, atypical reproductive function, ataxia in newborns, and disruptions in lipid and carbohydrate metabolism (McDowell, 1992.)

Doisy (1972) documented a case of Mn deficiency induced in a man on a vitamin K study, when Mn sulfate was inadvertently left out of the diet. The man received 0.34 mg/Mn kg DM a day for 6.5 months, causing a Mn deficiency characterized by hypocholesterolemia, hair depigmentation, scaly dermatitis, and reduced vitamin K-dependent clotting proteins. Supplementation of vitamin K did not cause the symptoms to subside, but they gradually went away after the study was ended and the man returned to a normal diet (Doisy, 1972).

In swine, Mn deficiency can cause a disruption in proper lipid metabolism (Plumlee et al., 1956). Gilts fed a purified diet containing 0.5 or 40 mg Mn/kg had similar weight gain, but different back fat thicknesses. Gilts fed 40 mg Mn/kg DM had 3.61 cm of back fat compared to 4.11 cm of back fat on gilts fed 0.5 mg Mn/kg DM in a purified ration. Skeletal problems in the low-Mn group were also observed by Plumlee et al. (1956). Comparisons of radiograms taken of legs from gilts revealed differences among treatments in several bones. Gilts fed the low-Mn diet displayed shortening of the radius, ulna, tibia, fibula, and metacarpals when compared to gilts fed the high-Mn diet (Plumlee et al., 1956).

Male chicks fed a basal diet containing 2.4 mg Mn/kg DM had reduced hexosamine content in their epiphyseal cartilage, indicating a reduction in mucopolysaccharides that contain this hexosamine (Leach and Muenster, 1962). Chicks supplemented with 10, 20 and 100 mg Mn/kg DM had a reduced incidence of enlarged hocks (93, 39, and 14%, respectively) compared to 100% occurrence of enlarged hocks in non-supplemented chicks (Leach and Muenster, 1962).

Clinical signs of Mn deficiency in young cattle often manifest themselves in the malformation of bones. A broad range of signs may be observed when encountering a Mn deficiency, from no observable changes to severe crippling and bending of the long bones (Hidioglou, 1980). Rojas et al. (1965) found that calves born to cows fed diets containing 15.8 mg Mn/kg DM suffered from enlarged joints, weakness, and twisted legs. They also observed that calves born to Mn deficient dams had 13.9% shorter humeri than calves born to cows fed the control diet containing 25.1 mg Mn/kg DM. Humeri from the deficient calves were also much weaker than humeri taken from control calves, breaking under only 605 kg of vertical pressure as compared to the 1,186 kg of pressure required to break the control humeri.

Howes and Dyer (1971) also found that feeding a low-Mn diet (13 mg Mn/kg DM) to heifers resulted in calves that were weak and had trouble standing while calves from heifers fed 21 mg Mn/kg DM were normal. Calves on this study did not exhibit the extreme malformation of bones observed by Rojas et al. (1965), but heifers were only fed the low Mn diet for the last three months of gestation. However, Howes and Dyer (1971) did observe that calves supplemented with 14.5 mg Mn/kg DM via milk for 7 days had increased Mn concentration in the marrow-free portion of the radius. This difference in Mn

concentration in marrow-free bone when animals were fed different levels of dietary Mn has been seen in other calf as well as sheep studies (Rao, 1963; Watson, 1973).

In addition to controlled studies, Mn deficiency has been suspected in situations where calves born under field conditions displayed skeletal deformities (Ribble et al., 1989; Valero et al., 1990; Staley et al., 1994). The majority of these cases have been in Canada, where newborn calves exhibited a skeletal anomaly known as congenital joint laxity and dwarfism (CJLD). This disease is characterized by a generalized joint laxity, dwarfism, and on occasion, superior brachygnathism (Ribble et al., 1989). Observers noted that in many cases of CJLD births, pregnant cows had been wintered on grass or clover silage exclusively, and when cows were supplemented with rolled barley or hay they did not give birth to calves afflicted with CJLD (Ribble et al., 1989; Hidirolou et al., 1990).

Hidirolou et al. (1990) conducted an experiment to see if CJLD was associated with diet. Over the course of one winter they fed pregnant cows one of three feedstuffs (hay, red clover silage, or grass silage) and measured the frequency of CJLD in calves at birth for each group. While all three feedstuffs had similar levels of Mn (51, 64, and 63 mg Mn/kg DM for hay, red clover, and grass silage, respectively), 38% of calves born to red clover silage fed cows and 28% of the calves born to grass silage fed cows were born with CJLD. No occurrence of CJLD was observed in the group fed hay, and this finding led the authors to speculate that Mn was less available to cattle in silage than in hay (Hidirolou et al., 1990).

### **Manganese and reproduction in ruminants**

A detrimental effect on reproductive performance is the most commonly reported sign of Mn deficiency in adult animals. Several research groups (Bentley and Phillips, 1951; DiCostanzo et al., 1986) have described a reduction in days to first estrus in Mn-

supplemented cows when compared to non-supplemented cows. Bentley and Phillips (1951) found that heifers fed 30 mg Mn/kg DM came into heat approximately 2 months earlier than heifers fed 7-10 mg Mn/kg DM.

Conversely, some authors have observed normal estrous cycles, but have reported some other aspect of reduced reproductive performance due to a deficiency of Mn, such as an increased number of services to conception (Rojas et al., 1965). DiCostanzo et al. (1986) fed beef cows and heifers a corn silage diet that supplied either 40 mg or 52 mg Mn/kg DM, and they found a reduction in the number of services to conception required for heifers and cows fed 52 compared to those fed 40 mg Mn/kg DM. An earlier study (Rojas et al., 1965) indicated that cows fed 15.8 mg Mn/kg DM required four services to conceive compared to only two for cows fed 25.1 mg Mn/kg DM. Bentley and Phillips (1951) also reported that heifers fed 7-10 mg Mn/kg DM on average required slightly more breeding services per conception than heifers fed 30 mg Mn/kg DM, but these data were not statistically significant (Bentley and Phillips, 1951).

In addition to conception and possibly abortion problems with females fed low Mn diets, those that do maintain their pregnancy often give birth to lighter weight offspring. Bentley and Phillips (1951) observed a slight trend for heavier calves born to cows fed 30 mg Mn/kg DM compared to cows fed 7-10 mg Mn/kg DM (42.23 kg versus 39.6 kg for a two year average). Kids born to goats fed 1.9 mg Mn/kg DM were reported to weigh 17% less than the kids born to goats fed 90 mg Mn/kg DM (Anke et al., 1973).

Some researchers have speculated that the reproductive problems caused by a Mn deficiency can be attributed to the affect of Mn on ovarian tissues. Wilson (1966) accredited infertility and lack of estrus in cows that were deficient in Mn to be due to one or both of

the ovaries being inferior in size. Manganese deficiency has also been suspected as a cause of cystic ovaries in cattle. Hidirolou et al. (1977) found that dairy cows with cystic ovaries had lower Mn levels in the cortical stroma of the ovary than cows without cystic ovaries.

Placental transfer of Mn has not been extensively studied, but limited work has been done in rats, pigs, and sheep (Hidirolou and Knipfel, 1981). Rao (1963) found that radioactivity in the rat fetus increased over 16 hours after an intravenous injection of  $^{52}\text{Mn}$  to the dam. Newland and Davis (1961) observed that the percentage of  $^{52}\text{Mn}$  transferred to fetuses of sows fed 6 mg Mn/kg DM was higher than the percent of  $^{52}\text{Mn}$  transferred to fetuses of sows fed 100 mg Mn/kg DM. However, the amount of Mn transferred via the placenta was higher in 60, 80 and 110 day old fetuses of sows fed the high Mn diet, indicating that a higher percentage of absorbable Mn was transferred to the low Mn sows fetuses, but the actual amount of Mn was less (Newland and Davis, 1961). Manganese is rapidly transferred from the ewe to fetus (Hansard, 1972). Placental concentration of  $^{54}\text{Mn}$  at 12 hours post injection was more than 50% of the total fetal Mn concentration when pregnant ewes were administered with  $^{54}\text{Mn}$ . Placental concentration dropped to 25% of the total fetal  $^{54}\text{Mn}$  content by 168 hours after the injection (Hansard, 1972). Few studies have measured Mn in fetal tissues, although Abderahman and Kincaid (1993) found that gestational age did not affect Mn levels in fetal liver and kidney, when fetuses were collected from heifers slaughtered at different stages of pregnancy.

### **Manganese Toxicity**

Manganese toxicosis is relatively rare in animals and humans, and is mostly reported in cases of workers mining Mn or in other situations where there is unusually high exposure to Mn. Manganism, as explained in a recent review of the role of Mn in neurotoxicity

(Dobson et al., 2004), is a disorder caused by excessive Mn in the brain, with the basal ganglia of the brain containing the highest concentration of Mn. Among the signs of Mn toxicosis in humans are neurodegenerative disorders, which cause lesions and symptoms similar to Parkinson's disease. Early signs of manganism are psychiatric in nature, and include violent behavior, hallucinations, erratic emotional outbursts, and often fatigue, headache, and insomnia (Dobson et al., 2004).

The dietary level at which Mn is toxic to cattle is suggested to be 1000 mg Mn/kg DM (NRC, 1980). Manganese toxicosis in animals is often attributed to its antagonistic affect on Fe metabolism (McDowell, 1992). Excessive dietary Mn in cattle resulted in reduced feed intake and decreased growth, while longterm grazing on pastures of 200 mg Mn/kg DM or more caused cows to have impaired reproductive efficiency. The authors suggested that this impairment may be due to antagonism with yet another mineral, suggesting Mn was interfering with iodine metabolism (McDowell, 1992).

Jenkins and Hidirolou (1991) reported that calves fed 40, 200 or 500 mg Mn/kg DM from Mn sulfate via milk replacer from 3 to 38 days of age had no reduction in weight gains or feed efficiencies. Calves on the same trial fed 1000 mg Mn/kg DM had reduced gains and feed efficiency, but appeared healthy throughout the study. Calves fed 5000 mg Mn/kg DM had depressed appetites and appeared listless, and of the seven calves fed this level of Mn three died the first week of the study and the remaining calves died during weeks 2 and 3 (Jenkins and Hidirolou, 1991). These results indicate that the toxic level for Mn in the preruminant calf is somewhere between the 1000 and 5000 mg Mn/kg DM level in the diet, but performance is affected above 500 mg Mn/kg DM.

### **Sources and bioavailability of Manganese**

Manganese concentration in feedstuffs differs greatly due to a variety of factors, including soil pH, plant species and stage of maturity, and soil type. Most forages contain adequate amounts of Mn, although it may not always be available to the animal for absorption (NRC, 2001). Manganese is found in varying levels in cereal grains, with corn often containing as little as 5 mg Mn/kg DM, and wheat and oats having as much as 40 mg Mn/kg DM. Some plant species, such as lupin seeds, have an extraordinarily high Mn content, and may contain as much as 3397 mg Mn/kg DM (Underwood, 1981). Manganese levels from plant protein sources are generally between 30 and 50 mg Mn/kg DM, while animal protein sources contain between 5 and 15 mg Mn/kg DM (NRC, 1996).

The term bioavailability was defined by Forbes and Erdman (1983) as the degree to which an ingested element is absorbed and can be utilized in metabolism by the animal. Supplemental Mn has traditionally been offered as either Mn oxide or Mn sulfate, with research showing that Mn oxide is between 53 and 70% as available as Mn sulfate (Henry et al., 1992). In more recent years, organic Mn sources like Mn-methionine have increasingly been used, with a reported bioavailability that is 120% that of Mn sulfate (Henry et al., 1992).

### **Manganese requirements in ruminants**

The requirements of ruminants for manganese are poorly defined at best. Current Mn requirements, in mg Mn/kg DM of the diet are: 20 for growing beef cattle, 40 for reproducing beef cattle, 20 for sheep, 24.2 for a growing 300 kg dairy heifer (consuming 6 kg DM/day) and 16.7 for a lactating 650 kg dairy cow (consuming 20 kg DM/day and producing 40 kg milk/day; NRC, 1985, 1996, 2001). Manganese requirements for dairy cattle are

substantially lower than those given for beef cattle. Dairy cattle recommendations are calculated using the factorial approach. Recommendations are estimated in this manner by dividing the absorbed mineral requirements for Mn by the estimated absorption coefficient. The factorial approach is based on a series of calculations and assumptions, including the absorption coefficient for Mn in cattle and the specific Mn requirement by an animal.

Early work with limited experimental units indicated that feeding cows a diet containing 15.8 mg Mn/kg DM resulted in skeletal problems in calves and improper reproductive performance in cows (Rojas et al., 1965). Dietary Mn recommendations from the dairy NRC (2001) are at or just slightly above this level at which Mn deficiencies have been observed. Beef cattle recommendations from the NRC (1996) are based on the limited research that has been conducted with Mn. More work is needed to determine the Mn requirement by beef cattle at varying stages of production. The current studies were conducted to further explore the Mn requirements of beef heifers.

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## CHAPTER 1

Growth, reproductive performance, and manganese status of heifers fed varying concentrations of manganese<sup>1,2</sup>

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## **Abstract**

Eighty Angus (n = 40) and Simmental (n = 40) heifers, averaging 248.6 kg, were used to examine the effects of dietary Mn on performance, reproduction, and Mn status of heifers. Heifers were stratified by weight within a breed and randomly assigned to treatments consisting of a control diet (analyzed 15.8 mg Mn/kg DM) supplemented with 0, 10, 30, or 50 mg Mn/kg DM. Average daily gain, DMI, and G:F for the 196-d period were not affected by Mn supplementation. Liver Mn concentration increased ( $P=0.04$ ) as dietary Mn level increased. Control heifers had reduced ( $P=0.04$ ) liver Mn when contrasted with the three levels of supplemental Mn. Heifers supplemented with 30 or 50 mg Mn/kg DM had greater liver Mn compared to control heifers ( $P=0.01$ ). Serum cholesterol was greater ( $P=0.001$ ), in Angus compared to Simmental heifers over the course of the 196-d period but was not affected by treatment. Based on serum progesterone concentrations at or greater than 1 ng/mL, 40% of control heifers were cycling at approximately 12 mo of age compared to 50% of Mn-supplemented heifers. At approximately 13 mo of age, 40% of control heifers exhibited signs of estrus, in response to a two-dose Lutalyse synchronization protocol, compared to 50% of those supplemented with 50 mg Mn/kg DM. The percentage of heifers cycling at 12 or 13 mo of age was not significantly affected by treatment. Based on rectal palpation on d 196, 75% of the heifers supplemented with 50 mg Mn/kg DM were pregnant compared to 50% of the heifers supplemented with 10 mg Mn/kg DM. Sixty percent of heifers supplemented with 50 mg Mn/kg DM settled on one AI service, while 45% of control heifers settled on the first service. Results of this study indicate that 15.8 mg Mn/kg diet DM

appears to be adequate for growth of heifers, but supplemental Mn may improve reproductive performance.

Key Words: Manganese, heifers, growth, reproduction

### **Introduction**

Manganese is an important trace mineral in biological systems, acting as both 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. These studies were conducted with limited animal numbers and generally only two 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). The role of Mn in skeletal development 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, 1970). Manganese requirements of cattle are poorly defined and current NRC (1996) suggestions for beef cattle are 20 and 40 mg Mn/kg DM for growth and reproduction, respectively. The objective of the current study was to estimate Mn requirements for growth, development, and reproductive performance of beef heifers.

## Materials and Methods

### *General*

Eighty Angus (n = 40) and Simmental (n = 40) heifers (248.6 kg initial BW), approximately 10 months of age, were used in this study. Experimental procedures were reviewed and approved by the North Carolina State University Animal Care and Use Committee. Prior to initiation of the study heifers were vaccinated with Titanium 5 (AgriLabs, St. Joseph, MO) and Vision 7 (Intervet, Millsboro, DE), and wormed with Bovimec (Virbac, Fort Worth, Texas). Heifers were housed in a covered facility with slotted floor pens, and individually fed via electronic feeders (American Calan, Northwood, NH). Heifers were blocked by weight and randomly assigned within a pen to treatments. There were six pens of 12 heifers each and one pen housing 8 heifers.

Treatments consisted of 0 (control), 10, 30, and 50 mg of supplemental Mn/kg DM. Supplemental Mn was supplied from manganese sulfate. Ingredient composition and calculated chemical composition are shown in Table 1. The diets were formulated to meet or exceed all NRC (1996) requirements, except Mn. The control diet analyzed 15.8 mg Mn/kg DM. Heifers were fed once daily with feed amounts based on ad libitum consumption in a 24-h period. Initial and final weights for the 196-d study were the average of weights taken on two consecutive days. Interim heifer weights were recorded at 28 d intervals. Jugular blood samples were collected 2 h post-feeding on d 0, 63, 98, 168 and 196 for determination of serum cholesterol and on d 0, 98, and 168 for plasma Mn concentrations. Two blood samples, 3 d apart, were taken when heifers were approximately 12 mo of age (d 63 and d 66), for serum progesterone analysis. Heifers with greater than 1 ng progesterone/mL serum

were considered to be cycling. Liver biopsy samples were obtained as described by Engle and Spears (2000) on d 98 and 196 for Mn determination.

At approximately 13 mo of age (d 90 and 104) two doses of Lutalyse were administered to synchronize estrus in the heifers. Heifers were moved to a covered building with dry lots and were group fed by treatment for one week to avoid injuries among animals on the concrete slatted floors. Heifers that exhibited signs of estrus were bred artificially and heat detected for 30 d. Heifers that had not been bred by d 139 were given a dose of Lutalyse and bred on d 142 regardless of exhibition of estrus. Heifers bred early in the breeding season were ultra sounded and rectally palpated at 30 and 60 d post breeding for pregnancy determination. Rectal palpation was performed on all heifers on d 196 for final determination of pregnancy.

#### *Analytical Procedures*

Blood was collected in heparinized vacuum tubes designed for trace mineral analysis (Becton Dickenson, Rutherford, NJ), centrifuged at 1,200 x g for 20 min at 20°C and wet ashed prior to Mn analysis. The ashing procedure was a modification of procedures described by Johnson et al. (1991). One mL of plasma was put in a 30 mL beaker and 6 mL of trace mineral grade nitric acid was added to the sample. The nitric was boiled off on a hotplate, and then 2 mL of 30% hydrogen peroxide was added to the beaker and again boiled off. After cooling, 1 mL of 5% nitric acid was used to reconstitute the dried sample. Flameless atomic absorption spectroscopy (GFA-6500, Shimadzu Scientific Instruments, Kyoto, Japan) was used to determine Mn content in the plasma.

Blood for serum cholesterol samples was collected in vacuum tubes, and allowed to clot at room temperature for 1 h. They were then centrifuged at 1,200 x g for 20 min at

20°C and analyzed using a colorimetric end-point assay kit from ThermoDMA (Arlington, TX). Serum samples for progesterone were collected in vacuum tubes and centrifuged at 1,200 x g for 20 min at 20°C. Progesterone concentrations were determined using a radioimmunoassay kit (Coat-A-Count; Diagnostic Products Corp, Los Angeles, CA).

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).

### *Statistics*

Performance data was statistically analyzed using the Mixed procedure of SAS (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, and day of sampling and all related interactions. The individual animal served was a random effect, with treatment by breed serving as the error term. Pre-planned contrasts were used and included the following comparisons: 0 mg Mn vs. 10, 30, and 50 mg Mn/kg DM, 0 mg Mn vs. 30 and 50 mg Mn/kg DM, and 30 mg Mn vs. 50 mg Mn/kg DM. Reproductive data were analyzed using the Genmod procedure of SAS. The model included the fixed effects of breed, treatment, and the treatment x breed interaction. Interactions that were not significant ( $P < 0.05$ ) for the measurement of interest were removed from the models.

## Results

One heifer was humanely put down on d 68 after becoming severely ill from coccidiosis. Data from this heifer was not included in statistical analysis. Average daily gain, DMI, and gain:feed for the 196-d study did not differ among treatments (Table 2).

Plasma Mn concentration was affected by sampling time ( $P = 0.001$ ), but not by treatment, treatment x sampling time or breed (Table 3). Liver Mn concentrations on d 98 and 196 were adjusted for initial liver Mn content. Liver Mn concentrations were greater ( $P = 0.04$ ) in heifers supplemented with Mn than in controls (Table 3). Manganese concentrations in liver were lower ( $P = 0.001$ ) on d 196 than on d 98. However, liver Mn was not affected by breed or a sampling time x treatment interaction. Serum cholesterol concentrations were not affected by dietary Mn (Table 3). Angus heifers had greater ( $P = 0.006$ ) serum cholesterol concentrations than Simmental heifers on all sampling dates (Figure 1). Cholesterol concentrations were also affected by sampling time ( $P = 0.001$ ), 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.

Numerically, the percent of heifers cycling at 12 mo of age, and pregnancy rate were greater for heifers supplemented with 30 or 50 mg Mn/kg DM compared with controls (Table 4). However, pregnancy rate, conception rate, services to conception, and percent of heifers cycling were not significantly affected by dietary Mn. Pregnancy rate and first service conception rate were greater ( $P = 0.01$ ) in Simmental than in Angus heifers.

## Discussion

Manganese supplementation to the control diet containing 15.8 mg Mn/kg DM did not affect growth characteristics of beef heifers in the present study. Similar results have

been reported by Bentley and Phillips (1951), who observed similar gains in dairy heifers fed diets containing 7-10 mg Mn/kg DM and those fed 30 mg Mn/kg DM during a 12 mo growing period. Howes and Dyer (1970) also found that beef heifers receiving diets containing 13 or 21 mg Mn/kg DM from 3 mo prior to gestation through early lactation had no differences in performance characteristics. Gain, feed intake and feed efficiency of gestating beef cows fed diets containing 15.8 mg Mn/kg DM did not differ from cows receiving 25.1 mg Mn/kg DM (Rojas et al., 1965). Similar results have also been found in sheep, where no significant difference among treatments was observed for average daily gain, feed intake, and feed to gain ratio for wethers fed diets containing 22, 300, or 3000 mg Mn/kg DM for 56 days (Ivan and Hidirolou, 1980). The current NRC (1996) recommendation for growing beef cattle is 20 mg Mn/kg DM; however, the previously mentioned research in conjunction with the present study would suggest that the Mn requirement for maximum growth in heifers does not exceed the level (15.8 mg Mn/kg DM) found in our control diet.

Plasma Mn was not affected by supplemental Mn in the current study. This result is consistent with previous work in sheep (Masters et al., 1988) which demonstrated that plasma Mn concentrations are uniformly very low, and not affected by dietary Mn concentration. Watson et al. (1973) observed that following an oral dose of  $^{54}\text{Mn}$ , plasma  $^{54}\text{Mn}$  levels were very low and not affected by the concentration of Mn in the diets of sheep. Conversely, the same laboratory found that plasma Mn was higher in lambs fed 4030 mg Mn/kg DM than in lambs fed 30 mg Mn/kg DM, with supplemental Mn provided in the form of  $\text{MnCl}_2$ . However, Watson (1968) previously observed that plasma Mn concentration was not increased by supplementation of up to 1200 mg Mn/kg DM in sheep. 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 shown 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). Following 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 in the present study were increased slightly by supplementation of Mn to the control diet. Previous work in ruminants (Howes and Dyer, 1970; Ivan and Hidirolou, 1980) has shown that liver Mn concentration increases as dietary Mn increases. Similar results were found by Hidirolou et al. (1978), who observed that sheep fed 5 mg Mn/kg DM had reduced liver Mn concentrations when compared to those fed 60 mg Mn/kg DM. Conversely, Bentley and Phillips (1951) found that feeding dairy cows a diet containing 7-10 or 30 mg Mn/kg DM for more than 3 years did not affect liver Mn concentration.

Unlike some minerals, Mn does not have an established level in liver or plasma below which an animal is classified as being in a deficient state. It is possible that initial body Mn stores were sufficient for heifers to draw on throughout the study, and thus prevent a greater response to Mn supplementation of the control diet. 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 (Hidirolou, 1979), however, whole blood Mn values as reported are inconsistent, reflecting laboratory analysis difficulties and

individual animal variability (Underwood and Suttle, 1999). Using young dairy calves fed a basal diet containing no more than 1.7 mg Mn/kg DM, Hawkins et al. (1955) observed an increase in whole blood Mn when the basal diet was supplemented with 50 mg Mn/kg DM. In sheep, similar work performed by Hidioglou et al. (1978) found a significant increase in whole blood Mn concentration when a basal diet of 8 mg Mn/kg DM was supplemented with 60 mg Mn/kg DM. These studies would suggest that whole blood Mn concentration may be a suitable indicator of Mn status.

Manganese deficiency has been associated with a decrease in activity of certain Mn-dependent enzymes (Masters et al., 1988). Manganese-dependent superoxide dismutase (MnSOD) is an important antioxidant in the body, responsible for catalyzing the dismutation of superoxide anion to hydrogen peroxide and oxygen (Leach and Harris, 1997). Ose and Fridovich (1976) found that Mn was required for catalytic activity of MnSOD, and that while cobalt, nickel, and zinc (Zn) could bind in place of Mn in MnSOD, none resulted in enzyme activity. Since Mn is required for activity of MnSOD it makes sense that a Mn deficiency would cause a decrease in MnSOD activity. Paynter (1980) reported that tissue Mn concentration and MnSOD activity in the heart and kidney were significantly increased in rats fed 9.7 or 29.7 mg Mn/kg DM versus rats fed a diet containing 0.2, 0.7 or 4.7 mg Mn/kg DM. The same laboratory also observed that MnSOD activity in the heart declined more rapidly than in other tissues when rats were fed low Mn diets, with noticeable differences occurring within 3 weeks on the diets. Similar results have been demonstrated in sheep, where both tissue Mn concentration and MnSOD activity in the heart increased linearly as the concentration of Mn in the diet increased (Masters et al., 1988). In the present study liver

Mn concentrations were slightly lower in control heifers; however, it is unclear whether or not this reduction in liver Mn affected activity of Mn-dependent enzymes.

Serum cholesterol levels in the present study were not affected by dietary Mn concentration. It has been suggested that Mn may act as a cofactor for two enzymes in cholesterol synthesis, mevalonate kinase, and farnesyl pyrophosphate synthase (Davis et al., 1990). Both of these enzymes are involved in the production of squalene, which is a precursor of cholesterol (Curran and Azarnoff, 1961). Several studies with rats have observed that a diet severely deficient in Mn results in significantly lower cholesterol levels in the plasma or liver (Curran and Azarnoff, 1961; Kawano et al., 1987; Davis et al., 1990). The lack of cholesterol response in the present 196-d study may be due to the fact that our control diet was not severely deficient in Mn. However, no differences in serum cholesterol were seen among lambs fed diets containing 0.8 or 29.9 mg Mn/kg DM for 16 wk (Lassiter and Morton, 1968).

Although not statistically significant, the number of heifers cycling at 12 mo of age and pregnancy rate was numerically higher for heifers supplemented with 30 or 50 mg Mn/kg DM compared with those fed the control diet containing 15.8 mg Mn/kg DM. This suggests that the control diet may have been marginally deficient in Mn for proper onset of estrus and conception. However, a greater number of animals will likely be needed to show a significant reduction in cycling or conception rate in heifers fed a basal diet with a Mn concentration similar to that used in the present study.

Several other research groups have reported reduced reproductive performance in cattle fed a low Mn diet (Bentley and Phillips, 1951; Rojas et al., 1965; DiCostanzo et al., 1986). Bentley and Phillips (1951) reported that dairy heifers fed a diet containing 7-10

mg Mn/kg DM expressed first estrus approximately 2 mo later than those fed a diet containing 30 mg Mn/kg DM, and that heifers fed the low Mn diet required slightly more breeding services per conception than their supplemented counterparts, but these data were not significant. Rojas et al. (1965), in a study with limited animal numbers ( $n = 4$ ), observed that beef cows fed a low Mn diet (15.8 mg Mn/kg DM) required four services to conceive compared to two services for cows fed 25.1 mg Mn/kg DM. Another study found that heifers and cows fed a corn-silage based diet containing 40 mg Mn/kg DM required an average of 1.6 services to conception compared to 1.1 services required by those fed a diet containing 52 mg Mn/kg DM (DiCostanzo et al., 1986).

At the termination of the present study, 10 pregnant heifers from the control group and 10 pregnant heifers from the 50 mg supplemental Mn/kg 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 lower whole blood Mn concentrations than those born to supplemented heifers (Hansen et al., 2005). Several calves born to control heifers exhibited signs linked to a deficiency of Mn, including dwarfism and superior brachygnathism. These results indicate that 15.8 mg Mn/kg DM is not sufficient for normal calf development in the gestating beef heifer.

In conclusion, supplementation of Mn to the control diet of 15.8 mg Mn/kg DM did not improve growth characteristics of beef heifers; however, it may have had a beneficial effect on sexual maturity and development of heifers. While it was one of the earliest trace minerals to be recognized as nutritionally essential, limited scientific research has made Mn 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 the Mn status of cattle is necessary before current NRC (1996) recommendations for Mn in beef cattle can be further refined.

### **Implications**

Manganese supplementation to a diet containing 15.8 mg Mn/kg DM did not affect performance characteristics. Numerically, the addition of 50 mg Mn/kg diet to the control diet increased the percent of heifers cycling and pregnancy rate of heifers, though results were not statistically significant. The results of this study indicate that a dietary Mn concentration of 15.8 mg Mn/kg DM is adequate for growth of beef heifers, but may be marginal for maximum fecundity. Further research is needed to determine the beef heifer requirement for Mn during her reproductive life cycle. Beneficial information could also come from investigation of the bioavailability of Mn in various beef cattle feedstuffs and forages.

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**Table 1. Ingredient and calculated chemical composition of growing basal diet fed to beef heifers<sup>a</sup>**

|                              | Growing |
|------------------------------|---------|
| 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 <sup>b</sup>  | 0.03    |
| Mineral premix <sup>c</sup>  | 0.01    |
| Corn Supplement <sup>d</sup> | 2.0     |
| Chemical Composition         |         |
| CP, % of DM                  | 12.38   |
| NDF, % of DM                 | 50.88   |
| ME, Mcal/kg DM               | 2.40    |
| Mn, mg/kg DM <sup>e</sup>    | 15.8    |

<sup>a</sup>Diet contained Rumensin (Elanco Animal Health, Indianapolis, IN) at 33 mg/kg.

<sup>b</sup>Contained per kg of premix: 6,600,000 IU of vitamin A, 1,520,000 IU of vitamin D, and 6,600 IU of vitamin E.

<sup>c</sup>Provided per kg of diet: 30 mg of Zn as ZnSO<sub>4</sub>; 10 mg of Cu as CuSO<sub>4</sub>; 0.5 mg of I as Ca(IO<sub>3</sub>)<sub>2</sub>(H<sub>2</sub>O); 0.2 mg of Se as Na<sub>2</sub>SeO<sub>3</sub>; and 0.1 mg of Co as CoCO<sub>3</sub>.

<sup>d</sup>Provided supplemental Mn as MnSO<sub>4</sub>.

<sup>e</sup>Analyzed value.

**Table 2. Effect of supplemental manganese on heifer performance**

| Item      | Supplemental Mn, mg/kg |      |       |       | SEM   |
|-----------|------------------------|------|-------|-------|-------|
|           | 0                      | 10   | 30    | 50    |       |
| ADG, kg/d | 0.98                   | 0.97 | 0.98  | 1.01  | 0.02  |
| DMI, kg/d | 10.18                  | 9.84 | 10.23 | 10.42 | 0.2   |
| Gain:Feed | 0.11                   | 0.11 | 0.11  | 0.11  | 0.002 |

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

|                                | Supplemental Mn, mg/kg |       |       |       | SEM  | Significance <sup>a</sup>                     |
|--------------------------------|------------------------|-------|-------|-------|------|-----------------------------------------------|
|                                | 0                      | 10    | 30    | 50    |      |                                               |
| Plasma Mn <sup>b</sup>         | 16.39                  | 17.12 | 15.54 | 17.10 | 0.94 | A <sup>**</sup>                               |
| 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 <sup>c</sup>          | 9.08                   | 9.43  | 9.69  | 10.25 | 0.29 | A <sup>**</sup> B <sup>*</sup> C <sup>*</sup> |
| d 98                           | 9.95                   | 10.26 | 10.83 | 11.01 | 0.43 | B <sup>†</sup> C <sup>*</sup>                 |
| d 196                          | 8.19                   | 8.57  | 8.69  | 9.40  | 0.32 | B <sup>*</sup> C <sup>*</sup>                 |
| Serum Cholesterol <sup>d</sup> | 196.3                  | 212.0 | 203.0 | 200.6 | 9.20 | A <sup>**</sup>                               |

†P < 0.10

\*P < 0.05

\*\*P < 0.01

<sup>a</sup>A =sampling time effect; B =control vs. supplemental Mn; C =control vs. 30 and 50 mg supplemental Mn/kg DM.

<sup>b</sup>Means across all sampling times expressed as ng/mL.

<sup>c</sup>Means across all sampling times expressed as mg/kg DM.

<sup>d</sup>Means across all sampling times expressed as mg/dL.

**Table 4. Effects of supplemental manganese on reproductive performance of heifers**

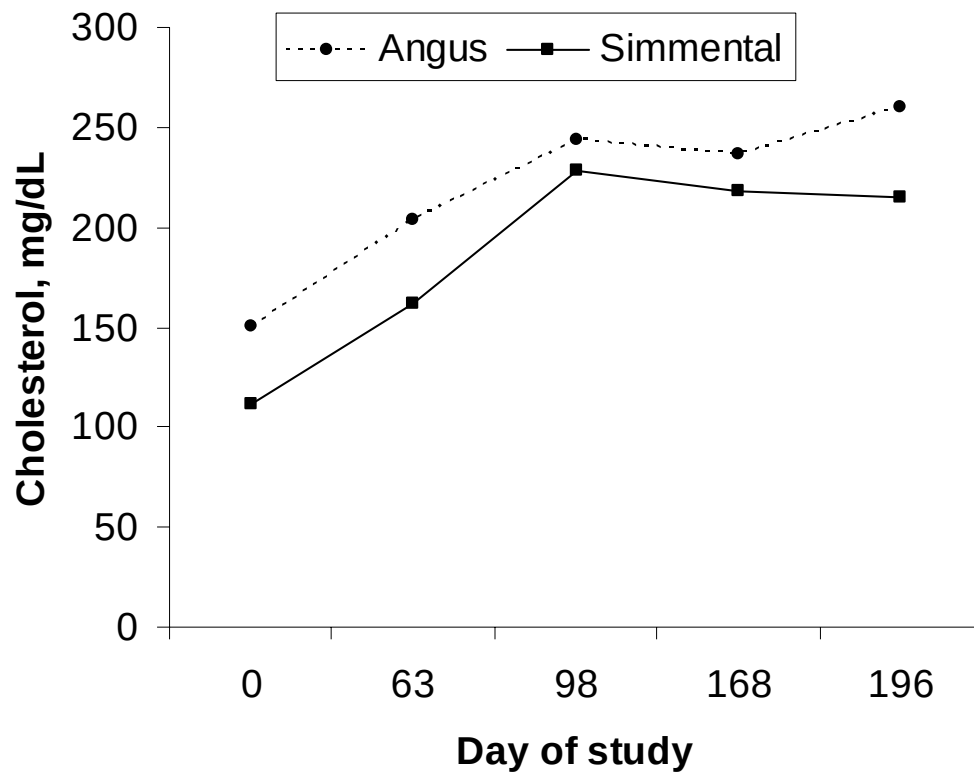
| Item                                       | Supplemental Mn, mg/kg |      |      |      |
|--------------------------------------------|------------------------|------|------|------|
|                                            | 0                      | 10   | 30   | 50   |
| Cycling <sup>a</sup> , %                   | 40                     | 50   | 47   | 55   |
| Response to Lutalyse dose <sup>b</sup> , % | 40                     | 40   | 47   | 50   |
| Services to Conception                     | 1.20                   | 1.15 | 1.26 | 1.20 |
| Conception Rate <sup>c,d</sup> , %         | 45                     | 40   | 47   | 60   |
| Pregnancy Rate <sup>d</sup> , %            | 60                     | 50   | 67   | 75   |

<sup>a</sup>Based on serum progesterone of > 1.0 ng/mL.

<sup>b</sup>Heifers that showed estrus in response to Lutalyse protocol.

<sup>c</sup>At first service.

<sup>d</sup>Breed effect ( $P < 0.01$ ).



**Figure 1. Effect of breed on serum cholesterol level in heifers (Breed effect,  $P < 0.01$ ; sampling time effect,  $P < 0.01$ )**

## CHAPTER 2

Effects of dietary manganese concentration on performance and manganese status of pregnant beef heifers and their offspring<sup>1,2</sup>

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## **Abstract**

A study was conducted to examine the effects of dietary manganese (Mn) concentration on the performance and Mn status of pregnant beef heifers and their offspring. Twenty pregnant Angus (n = 9) and Simmental (n = 11) heifers averaging 17 mo of age and 447.6 kg initial BW were used in the 267 d study. Heifers were selected from a previous study examining the effects of supplemental Mn on growth and reproductive performance of beef heifers. Heifers started on the 196-d study at approximately 10 mo of age (248.6 kg initial BW). Treatments consisted of a control diet (analyzed 15.8 mg Mn/kg DM) supplemented with 0, 10, 30 and 50 mg Mn/kg DM from MnSO<sub>4</sub>. Ten pregnant heifers per treatment from the control and 50 mg Mn/kg DM treatments were randomly selected at the conclusion of the 196-d study to continue on their respective dietary treatments through gestation and early lactation. Serum cholesterol for the 267-d period was not affected by treatment. Whole blood Mn concentration of heifers on d 267 was not affected by treatment. Whole blood Mn concentration at birth was lower (P = 0.01) in calves born to control heifers than those from supplemented heifers. Calves born to control heifers weighed significantly less at birth than those born to the heifers receiving supplemental Mn (P = 0.02). Seventy-one percent of calves born to control heifers exhibited some degree of superior brachygnathism. Calves born to control heifers suffered from other varying signs of Mn deficiency, including unsteadiness and swollen joints. Results suggest that feeding gestating beef heifers a diet containing 15.8 mg Mn/kg DM is not adequate for proper fetal development. Supplementation of 50 mg Mn/kg DM to the control diet was sufficient to overcome any signs of Mn deficiency in calves.

## **Introduction**

Previous work in cattle has shown that Mn is an important part of proper skeletal development of young animals, and normal reproductive performance in adults (Bentley and Phillips, 1951; Rojas et al., 1965). Manganese deficiency in the gestating female is often difficult to diagnose, with no apparent visual signs in the female. However, insufficient amounts of Mn in the diet of a pregnant heifer may lead to a variety of Mn deficiency signs in her calf, including ataxia and skeletal malformation (McDowell, 1992).

As an essential trace element, Mn plays a crucial role in several enzymatic processes in the body, including glycosyltransferase (Leach and Harris, 1997). Glycosyltransferases are a group of enzymes that are involved in the metabolism of cartilage proteoglycans, affecting the biosynthesis of glycosaminoglycan and oligosaccharide side chains (Leach and Harris, 1997). The role of Mn in cartilage formation makes it essential to the formation of the epiphyseal growth plate, which directly affects longitudinal bone growth. Thus, it is not surprising that the most frequently observed sign of Mn deficiency in young animals is skeletal malformation (Leach and Muenster, 1962).

Recently, the NRC (2001) for dairy cattle reduced recommendations for Mn requirements for the gestating dairy cow. For example, while the Mn recommendation for a gestating dairy cow was previously 40 mg/Mn kg DM, the new NRC (2001) recommends 17.8 mg Mn/kg DM for a dry dairy cow (BW=650 kg) on day 270 of gestation. The current dairy cattle (NRC, 2001) recommendation for Mn in gestating cows is similar to concentrations (16 to 17 mg Mn/kg DM) in beef cow diets that resulted in Mn deficiency in their offspring (Rojas et al., 1965). The most recent beef cattle NRC (1996) recommends 40

mg Mn/kg DM for reproducing beef cattle. The present study was designed to observe the effects of long term low Mn diets on gestating beef heifers and their offspring.

Key words: Manganese, gestation, deficiency, calves, superior brachygnathism

## **Materials and Methods**

### *General*

Experimental procedures were reviewed and approved by the North Carolina State University Animal Care and Use Committee. Twenty pregnant Angus (n = 9) and Simmental (n = 11) heifers (447.6 kg initial BW) approximately 17 mo of age were used in a 267-d study examining the effects of long term feeding of a low Mn diet on the offspring of beef heifers. Heifers were selected from a previous 196-d study examining the effects of varying levels of dietary Mn on growth and reproductive performance of beef heifers (Hansen et al., 2005). Heifers began on study at approximately 10 mo of age (248.6 kg initial BW) and were fed 0 (control), 10, 30, or 50 mg supplemental Mn/kg DM in addition to the basal diet (analyzed 15.8 mg Mn/kg DM). Supplemental Mn was supplied from manganese sulfate. Heifers were synchronized for estrus and artificial inseminated at approximately 13 mo of age. Heifers were rectally palpated and ultrasounded for pregnancy determination at the termination of the study.

Ten pregnant heifers per treatment were randomly selected from the control and 50 mg supplemental Mn/kg DM treatments. Heifers were blocked by dietary treatment and weight with 2 heifers in each pen. Pregnant heifers were housed in a covered facility with slotted floors that connected to dirt runs, and were bunk fed by pen. Ingredient and chemical composition of the diets fed to the growing and gestating heifers are shown in Table 1. Diets were formulated to meet or exceed all NRC (1996) requirements for beef heifers with the

exception of Mn. The control diet analyzed 15.8 mg Mn/kg DM for the growing phase and first two trimesters of pregnancy, and 16.3 mg Mn/kg DM for the third trimester and early lactation. Heifers were fed once daily, at a rate of 1.4% of their BW until calving, after which feed amounts were increased due to lactation requirements. Weights were taken on d 0, 28, 54, 84, 112, 140, 170 and 267 of the study. Jugular blood samples were taken on d 0, 54, 112, and 160 of the study, for analysis of serum cholesterol concentration. Weights and blood samples were not taken during the period between calving and the end of the study. Heifers calved from d 179 to d 226 of the study and calf birth weights and any signs of Mn deficiency were noted. A jugular blood sample for analysis of whole blood Mn concentration was taken from calves within 24 h of birth and from both heifers and calves at the termination of the study (d 267), when calves averaged 67 d of age.

#### *Analytical Procedures*

Whole blood was collected in heparinized vacuum tubes designed for trace mineral analysis (Becton Dickinson, Rutherford, NJ), and wet ashed prior to Mn analysis. The ashing procedure was a modification of procedures as described by Hansen et al. (2005). Five mL of trace metal grade nitric acid was added to 1 mL whole blood in 30 mL beakers and boiled off on a hot plate. One mL of 30% reagent grade hydrogen peroxide was added to the beaker and boiled off. Dried samples were reconstituted using 1 mL of 5% nitric acid. Flameless atomic absorption spectroscopy (GFA-6500, Shimadzu Scientific Instruments, Kyoto, Japan) was used to determine Mn content of whole blood.

Blood for serum cholesterol samples was collected in vacuum tubes and allowed to clot at room temperature for 1 h. They were then centrifuged at 1,200 x g for 20 min at 20°C and analyzed 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).

### *Statistics*

Calf performance data was statistically analyzed using the Mixed procedure of SAS (SAS Inst. Inc, Cary, NC). Serum cholesterol and whole blood Mn data 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 was the random effect, with treatment by breed serving as the error term. Age was used as a covariant in analysis of calf whole blood Mn on d 267 of the study. Interactions that were not significant ( $P < 0.05$ ) for the measurement of interest were removed from the models.

## **Results and Discussion**

Heifers started on their respective dietary treatments at approximately 10 mo of age. The pregnant heifers began calving at approximately 23 mo of age, at which point they had been on their respective dietary treatments for an average of 375 d. Two Simmental heifers from the control treatment, and 1 Angus and 1 Simmental heifer from the supplemented treatment were found to be open on d 168 and were removed from the study. One control heifer gave birth to a stillborn calf, and one calf born to a supplemented heifer died within 3 days of birth. One heifer receiving supplemental Mn delivered twins, one born alive and another dead. Data from these heifers and the surviving twin calf were not included in

statistical analysis. In all, 7 calves born to control heifers and 6 born to supplemented heifers were included in statistical analysis.

Serum cholesterol of beef heifers was affected by time ( $P < 0.001$ ), but not treatment (Table 2). Similar findings have been reported in sheep, where no differences in serum cholesterol were seen among lambs fed diets containing 0.8 or 29.9 mg Mn/kg DM for 16 wk (Lassiter and Morton, 1968). Several studies in rats have demonstrated that a low Mn diet caused a reduction in serum cholesterol; however, all of these studies fed diets that were severely deficient in Mn (Curran and Azarnoff, 1961; Kawano et al., 1987; Davis et al., 1990). In the present study, the control diet was probably not low enough in Mn to see the dramatic serum cholesterol response demonstrated in rats.

Whole blood Mn concentration of beef heifers was measured on d 267 of the present study, by which time the heifers had been on their respective diets for 463 d. Manganese concentration in whole blood did not differ among treatments (Table 2). Similarly, Bentley and Phillips (1951) reported no differences in whole blood Mn concentrations of heifers fed diets containing 7 to 10 or 30 mg Mn/kg DM for as long as 3 years. Conversely, pregnant ewes fed diets containing 8 mg Mn/kg DM were found to have lower whole blood Mn concentrations than those fed 68 mg Mn/kg DM, with measurements taken up until lambing (Hidioglou et al., 1978). Previously, we reported that plasma Mn concentrations in beef heifers were not affected by dietary Mn concentrations (Hansen et al., 2005). It is apparent that a rigorous homeostatic mechanism for Mn exists in the body, as whole blood Mn concentrations were not affected in the present study even after heifers were fed two very different concentrations of Mn for more than a year.

Calves born to control heifers weighed less at birth ( $P = 0.02$ ) than calves born to heifers supplemented with 50 mg Mn/kg DM (Table 3). Similar findings have been observed by Bentley and Phillips (1951), who reported a slight trend for heifers fed a diet containing 7 to 10 mg Mn/kg DM to give birth to lighter calves than heifers who were fed diets containing 30, 40, or 60 mg Mn/kg DM. Comparable results have been observed in other ruminants, where kids born to goats fed a diet containing 1.9 mg Mn/kg DM were reported to weigh 17% less than kids born to goats fed a diet containing 90 mg Mn/kg DM (Anke et al., 1973 as cited by Hidioglou, 1979). In a study with limited animal numbers ( $n = 2$  or  $3$ ), Rojas et al. (1965) noted no differences in calf birth weights among cows fed dietary Mn concentrations of 15.8 mg or 25.1 mg Mn/kg DM, although marked skeletal deformities were observed in calves born to the low Mn heifers.

Whole blood manganese concentrations in calves were affected ( $P < 0.01$ ) by a time x treatment interaction (Table 2). Manganese concentration at birth was lower ( $P = 0.01$ ) in calves born to control heifers than in calves born to supplemented heifers (Table 2). Similarly, calves born to dams fed 15.8 mg Mn/kg DM had lower whole blood Mn concentrations than calves born to cows fed 25.1 mg Mn/kg DM (Rojas et al., 1965). In the present study, the reduced whole blood Mn concentration of control calves at birth suggests that calves born to control heifers had a lower supply of Mn available during fetal development than calves born to supplemented heifers. It has been demonstrated in swine that placental transfer is the method of fetal acquisition of Mn (Newland and Davis, 1961; Gamble et al., 1971). It has also been observed that storage of Mn in fetal tissues appears to be dependent on dietary intake of the dam (Rojas et al., 1965; Howes and Dyer, 1971).

In the blood sample taken on d 267 of the study, when calves averaged 67 d of age, whole blood Mn concentration was higher ( $P = 0.02$ ) in control calves than in calves whose dams received supplemental Mn (Table 2). It is unclear why control calves had increased whole blood Mn levels when compared to their supplemented counterparts. It is possible that the lowered Mn status of the control calves caused increased absorption of dietary Mn, and thus resulted in increased blood Mn levels.

Twenty-nine percent of calves born to control heifers were much smaller in stature when compared to age matched counterparts born to supplemented heifers. At least one control calf seemed to have equilibrium problems, holding his head slightly tilted. A few of the calves born to control heifers were unsteady for several days after birth, moving stiffly and suffering from swollen joints. In agreement with the present study, young lambs exhibited poor movement and balance and increasing joint pain the longer they remained on a diet containing 0.8 mg Mn/kg DM (Lassiter and Morton, 1968). Lambs receiving a diet containing 29.9 mg Mn/kg DM did not exhibit any of these signs.

Several clinical studies have observed a broad range of signs in young animals when a lack of Mn is suspected, from no observable changes to severe crippling and bending of the long bones (Rojas et al., 1965; Lassiter and Morton, 1968; Howes and Dyer, 1971). Although limited in animal numbers, Rojas et al. (1965) observed that calves born to cows fed diets containing 15.8 mg Mn/kg DM suffered from enlarged joints, weakness, and twisted legs. The calves born to Mn deficient dams had 13.9% shorter humeri than calves born to cows fed the control diet containing 25.1 mg Mn/kg DM. Humeri from the deficient calves required only 605 kg of vertical pressure to break, nearly half that required to break the humeri of a control calf (1, 186 kg of pressure; Rojas et al., 1965). Howes and Dyer (1971)

also found that feeding a low-Mn diet (13 mg Mn/kg DM) to heifers resulted in calves that were weak and had trouble standing while calves from heifers fed diets containing 21 mg Mn/kg DM were normal. Calves on Howes and Dyer's (1971) study did not exhibit the extreme malformation of bones observed by Rojas et al. (1965), but heifers were only fed the low Mn diet for the last three months of gestation.

In the present study, approximately 70% of the calves born to control heifers exhibited some degree of superior brachygnathism, or over extension of the lower jaw (Figure 1). Severity of the ailment varied in each calf, in one calf the entire bottom row of teeth was exposed. None of the calves born to supplemented heifers exhibited any signs of superior brachygnathism. It is interesting to note that in rams the bone found to have the highest concentration of Mn is the lower jaw bone, suggesting that decreased Mn availability may have caused the jaw problems observed in control calves (Odynets and Lysenko, 1966 as cited by Hidirolou, 1980). Superior brachygnathism has not been a commonly reported sign of Mn deficiency in controlled studies; however, it has been found in several field observations where a deficiency of Mn was suspected (Ribble et al., 1989; Valero et al., 1990; Staley et al., 1994). Several of these cases have been in Canada, where newborn calves exhibited a skeletal anomaly known as congenital joint laxity and dwarfism (CJLD). This disease is characterized by a generalized joint laxity, dwarfism, and on occasion, superior brachygnathism (Ribble et al., 1989). Observers noted that in many cases of CJLD births, pregnant cows had been wintered on grass or clover silage exclusively, and supplementation of cows with rolled barley or hay seemed to overcome any signs of CJLD (Ribble et al., 1989; Hidirolou et al., 1990). Hidirolou et al. (1990) reported a link between serum Mn concentrations and appearance of CJLD in calves. The authors fed

pregnant cows one of three feedstuffs (hay, red clover silage, or grass silage) and measured the frequency of CJLD in calves at birth for each group. While all three feedstuffs had similar levels of Mn (51, 64, and 63 mg Mn/kg DM for hay, red clover, and grass silage, respectively), 38% of calves born to red clover silage fed cows, and 28% of the calves born to grass silage fed cows were born with CJLD. None of the calves born to cows fed hay displayed any signs of CJLD. Serum Mn concentrations were significantly lower in cows fed either of the silages than in cows fed hay.

Decreased whole blood Mn concentrations suggest that Mn-dependent enzymatic processes may have been limited in control calves compared to calves born to supplemented heifers in the present study. Manganese is required for the activation of glycosyltransferases, a group of enzymes involved in cartilage metabolism. Manganese deficiency in poultry has been shown to cause a reduction in mucopolysaccharide content of epiphyseal cartilage, which leads to skeletal deformities (Leach and Muenster, 1962). Similarly, other ruminant studies have reported a direct correlation between dietary Mn concentration and Mn content of marrow-free bone (Rao, 1963; Howes and Dyer, 1971; Watson et al., 1973). The dwarfism, swollen joints, and superior brachygnathism observed in the control calves in the present study are likely due to decreased glycosyltransferase activity in the absence of Mn.

Previously, we investigated the effects of supplemental Mn on growth, reproductive performance, and Mn status of beef heifers (Hansen et al., 2005). The addition of 50 mg Mn/kg DM to the control diet (15.8 mg Mn/kg DM) did not affect performance characteristics of the heifers, but tended to improved reproductive performance. Few studies have examined the long term effects of feeding low Mn diets to heifers, and these typically had limited animal numbers (Bentley and Phillips, 1951; Rojas et al., 1965). We

hypothesized that while the control diet of 15.8 mg Mn/kg DM did not adversely affect growth in growing heifers, it might not be sufficient for proper fetal development in gestating heifers.

Results of the present study suggest that a diet containing 16 mg Mn/kg DM is insufficient for proper fetal development in gestating beef heifers. Calves born to heifers fed a low Mn diet had lower blood Mn concentrations, and exhibited varying degrees of dwarfism, superior brachygnathism, and weakness following birth. Addition of 50 mg Mn/kg DM to the control diet did not affect whole blood Mn concentration of heifers, but was sufficient to overcome any signs of Mn deficiency in calves born to supplemented heifers. No criteria for the evaluation of the Mn status of animals have been established, but it is evident that the control calves were at least marginally deficient in Mn. While the most apparent signs of Mn deficiency were visually detectable, whole blood Mn concentration may be an appropriate indicator of Mn status in the young bovine. Clearly, the Mn requirement of the gestating beef heifer is higher than that of the growing heifer; however, the exact time frame in which this increased dependency occurs has yet to be elucidated.

### **Implications**

A dietary Mn concentration of 16 mg Mn/kg DM was sufficient for growth of young beef heifers, but reproductive performance tended to improve with supplementation of Mn to the diet. Diets fed to gestating beef heifers containing less than 20 mg Mn/kg DM probably require supplementation of Mn, to avoid deficiency signs as observed in this study. While supplementation of 50 mg Mn/kg DM to the control diet of 16 mg Mn/kg DM was sufficient to overcome signs of Mn deficiency in offspring, it is unclear if such a high level was necessary. The economics of supplementation and differences in bioavailability of Mn

from various feedstuffs suggest that further research is necessary to determine a more exact Mn requirement for pregnant beef heifers.

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**Table 1. Ingredient composition of growing and gestating basal diets fed to heifers<sup>a</sup>**

|                              | Growing/Early<br>Gestation | Mid-Gestation | Late Gestation/ Early<br>Lactation |
|------------------------------|----------------------------|---------------|------------------------------------|
| Ingredient, % of DM          |                            |               |                                    |
| Cottonseed hulls             | 40.0                       | 40.0          | 40.0                               |
| Citrus pulp                  | 20.0                       | 20.0          | 15.0                               |
| Corn gluten feed             | 25.0                       | 5.0           | 10.0                               |
| Ground Corn                  | 11.71                      | 31.75         | 31.96                              |
| Urea                         | 0.75                       | 0.75          | 0.5                                |
| Calcium carbonate            | 0.5                        | 0.50          | -                                  |
| Dicalcium phosphate          | -                          | -             | 0.5                                |
| Vitamin premix <sup>b</sup>  | 0.03                       | 0.03          | 0.03                               |
| Mineral premix <sup>c</sup>  | 0.01                       | 0.01          | 0.01                               |
| Corn Supplement <sup>d</sup> | 2.0                        | 2.0           | 2.0                                |
| Chemical Composition         |                            |               |                                    |
| CP, % of DM                  | 12.38                      | 9.39          | 9.76                               |
| NDF, % of DM                 | 50.88                      | 45.44         | 46.13                              |
| ME, Mcal/kg DM               | 2.40                       | 2.46          | 2.47                               |
| Mn, mg/kg DM <sup>e</sup>    | 15.8                       | 18.64         | 16.3                               |

<sup>a</sup>Diet contained Rumensin (Elanco Animal Health, Indianapolis, IN) at 33 mg/kg.

<sup>b</sup>Contained per kg of premix: 6,600,000 IU of vitamin A, 1,520,000 IU of vitamin D, and 6,600 IU of vitamin E.

<sup>c</sup>Provided per kg of diet: 30 mg of Zn as ZnSO<sub>4</sub>; 10 mg of Cu as CuSO<sub>4</sub>; 0.5 mg of I as Ca(IO<sub>3</sub>)<sub>2</sub>(H<sub>2</sub>O); 0.2 mg of Se as Na<sub>2</sub>SeO<sub>3</sub>; and 0.1 mg of Co as CoCO<sub>3</sub>.

<sup>d</sup>Provided supplemental Mn as MnSO<sub>4</sub>.

<sup>e</sup>Analyzed value.

**Table 2. Effect of dietary manganese concentration on whole blood manganese and serum cholesterol concentrations of beef heifers and their offspring**

| Item                                   | <u>Supplemental Mn, mg/kg</u> |        | SE    | P <  |
|----------------------------------------|-------------------------------|--------|-------|------|
|                                        | 0                             | 50     |       |      |
| Whole Blood Mn, ng/mL                  |                               |        |       |      |
| Calves <sup>a,b,c</sup>                | 27.87                         | 28.16  | 1.17  | 0.87 |
| Birth <sup>d</sup>                     | 31.53                         | 38.90  | 3.31  | 0.01 |
| End of study <sup>e</sup>              | 24.21                         | 17.42  | 3.28  | 0.02 |
| Cows <sup>f</sup>                      | 20.00                         | 21.22  | 2.05  | 0.68 |
| Serum Cholesterol <sup>b</sup> , mg/dL | 286.22                        | 288.15 | 13.69 | 0.92 |

<sup>a</sup>Time x treatment effect ( P < 0.01).

<sup>b</sup>Time effect ( P < 0.05).

<sup>c</sup>Breed effect, treatment x breed effect ( P < 0.10).

<sup>d</sup>Samples taken within 24 h of birth.

<sup>e</sup>Samples taken on d 267, average calf age of 67 d.

<sup>f</sup>Samples taken on d 267, approximately 60 d post-calving.

**Table 3. Effect of dietary manganese concentration fed to beef heifers on performance of their offspring**

| Item                          | Supplemental Mn, mg/kg |       | SEM   | P <  |
|-------------------------------|------------------------|-------|-------|------|
|                               | 0                      | 50    |       |      |
| Birth weight, kg <sup>a</sup> | 31.54                  | 38.51 | 1.75  | 0.02 |
| ADG, kg <sup>b</sup>          | 0.81                   | 0.84  | 0.097 | 0.80 |

<sup>a</sup>Weights taken within 24 h of birth.

<sup>b</sup>Final weights taken on d 267 of study when calves averaged 67 d of age.



**Figure 1. Calves born to heifers receiving a low manganese diet exhibiting varying degrees of superior brachygnathism.**

## CHAPTER 3

Effect of dietary manganese source and concentration on growth, reproductive performance,  
and manganese status of heifers<sup>1, 2</sup>

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## ABSTRACT

Seventy Angus (n=34) and Angus-Simmental cross (n=36) heifers, averaging 9 mo of age and 261 kg initial BW, were used in a 219-d study to evaluate the effects of supplemental source and concentration of manganese on growth and reproductive performance of beef heifers. Heifers were blocked by weight within breed and randomly assigned to one of three treatments: 0 mg supplemental Mn (control), 30 mg supplemental Mn from Mn proteinate (Bioplex; Alltech, Lexington, KY), or 30 mg supplemental Mn from MnSO<sub>4</sub> (SO<sub>4</sub>). Heifers were fed a corn-silage based diet (analyzed 36 mg Mn/kg DM). Average daily gain, DMI, G:F, and liver Mn concentration were not affected by source or level of dietary Mn. Serum cholesterol was affected by a treatment by breed interaction (P = 0.06), with cholesterol in Angus heifers increasing over time in response to treatment. There was a tendency (P = 0.16) for SO<sub>4</sub> supplemented heifers to have higher serum cholesterol when compared to the control and Bioplex treated heifers. Serum cholesterol tended to be higher (P= 0.11) in heifers receiving the SO<sub>4</sub> supplement than those receiving the Bioplex supplement. Concentration and source of Mn did not affect whole blood Mn levels of heifers. Blood samples were taken when heifers were approximately 12 mo of age to be analyzed for serum progesterone levels to determine the number of heifers cycling per treatment. There was a tendency for a greater number of Simmental x Angus heifers to be cycling than Angus heifers (P = 0.17). Dietary Mn source and concentration did not affect the percent of heifers cycling or the percent of heifers that responded to a synchronization protocol with standing heat. Pregnancy rate was affected by dietary treatment (P = 0.01). Fewer SO<sub>4</sub> heifers were bred than control (P = 0.01) and Bioplex heifers (P = 0.04). There was a tendency for more Simmental x Angus heifers to be bred than Angus heifers (P = 0.17). Results of this study

suggest that a silage-based diet containing 36 mg Mn/kg DM was sufficient for proper growth, development, and reproductive performance of beef heifers.

Key Words: Manganese, heifers, reproduction, cholesterol, manganese sulfate, manganese proteinate

## **Introduction**

Manganese (Mn) is an essential trace mineral that serves as a cofactor or activator in several enzymatic processes in the body (Leach and Harris, 1997). Manganese requirements for beef heifers are not well defined, and are based on limited research, with recommendations set at 20 mg Mn and 40 mg Mn/kg DM for growth and reproduction, respectively (NRC, 1996). DiCostanzo et al. (1986) found that supplementing a corn-silage based diet (40 mg Mn/kg DM) with 14 mg Mn/kg DM reduced the number of days to estrus and services to conception in heifers and cows. Previously, we reported that supplementation of  $\text{MnSO}_4$  to a basal diet containing 16 mg Mn/kg DM did not affect growth characteristics of young heifers, but tended to improve reproductive performance (Hansen et al., 2005).

Manganese levels in the body are tightly controlled, with excess Mn rapidly removed via the portal vein and liver into the bile (Leach and Harris, 1997). It has been demonstrated that about 1% of dietary Mn is absorbed in cattle (Gibbons et al., 1976). With this low absorption rate, a source of supplemental Mn that is readily absorbed is desirable. Organic sources of trace minerals have been shown to have higher absorption levels than those of inorganic trace minerals (Henry et al., 1992). It has been observed that Mn from manganese-methionine has a relative bioavailability of 121% compared to a baseline 100% for  $\text{MnSO}_4$  (Henry et al., 1992). Providing supplemental Mn in the form of a Mn-proteinate may allow for increased absorption of Mn, thereby increasing Mn availability to biological processes

in the body which require it. Therefore, the current study was conducted to evaluate the effects of dietary Mn source and concentration on growth, development, and reproductive performance of beef heifers.

### **Materials and Methods**

Experimental procedures were reviewed and approved by North Carolina State University's Animal Use and Care Committee. Seventy Angus (n=34) and Angus-Simmental cross (n=36) heifers (approximately 9 mo of age, 261 kg initial BW) were used in this experiment. Prior to initiation of the study heifers were vaccinated with Titanium 5 (AgriLabs, St. Joseph, MO) and Vision 7 (Intervet, Millsboro, DE), and wormed with Bovimec (Virbac, Fort Worth, Texas). Animals were housed in a covered facility with slotted floor pens. Twenty-two pens contained three heifers each and two pens contained two heifers. Heifers were blocked by weight within breed and randomly assigned to treatments.

Treatments consisted of a corn-silage based diet with no supplemental Mn (control), 30 mg/kg DM supplemental Mn supplied from Mn proteinate (Bioplex; Alltech, Lexington, KY), and 30 mg/kg DM supplemental Mn from  $\text{MnSO}_4 (\text{SO}_4)$ . The diet was formulated to meet or exceed all NRC (1996) requirements, with the exception of Mn. Ingredient composition and chemical composition of the control diet is shown in Table 1. Heifers were bunk fed in their pens by treatment, and were fed once daily. Heifers were fed based on ad libitum consumption in a 24 h period until d 160, after which they were restricted to 2% of their BW on a DM basis for the remainder of the study. Water was available to heifers at all times.

Initial and final weights for the 219-d study were the average of weights taken on two consecutive days, and heifers were weighed at 28 d intervals in the interim. Liver biopsies for Mn analysis were taken on d 0 and 219 of the study as described by Engle and Spears (2000). Blood samples for serum cholesterol were taken on d 0, 58, 98, 170, and 219. Whole blood samples for analysis of Mn concentration were taken on d 58, 98, 170, and 129. Blood was collected via jugular venipuncture, and all samples were collected at 2 h post-feeding. Blood samples for serum progesterone analysis were collected on d 84, 91, 98, and 104, when heifers were approximately 12 mo of age. Heifers with serum progesterone greater than 1 ng/mL on two or more dates were considered to be cycling.

At approximately 13 mo of age, heifers that were cycling were synchronized for breeding using a two dose Lutalyse protocol (d 121 and 132). Heifers were bred by artificial insemination upon visual detection of standing heat. Synchronized heifers that did not exhibit estrus by d 135 were given a dose of GnRH and bred regardless of exhibition of estrus. On d 136 heifers were split into treatment groups and put into pastures with bulls for 3 weeks. Three bulls were rotated among the three pastures, with sires in each pasture for one week, to avoid a sire effect. Pastures were heavily grazed previous to arrival of heifers, and little herbage mass was left in the paddocks. Heifers continued on their dietary treatments while in the pastures, and were group fed via cement bunks lining the pasture fence. Rectal palpation was performed on all heifers on d 219 for final determination of pregnancy, and ultrasound was utilized on pregnancies less than 60 d.

#### *Analytical Procedures*

Blood was collected in heparinized vacuum tubes designed for trace mineral analysis (Becton Dickinson, Rutherford, NJ) and wet ashed prior to analysis for Mn content. The

ashing procedure was a modification of procedures described by Hansen et al. (2005). Five mL of trace metal grade nitric acid was added to 1 mL whole blood in 30 mL beakers and boiled off on a hot plate. One mL of 30% reagent grade hydrogen peroxide was added to the beaker and boiled off. Dried samples were reconstituted using 1 mL of 5% nitric acid. Flameless atomic absorption spectroscopy (GFA-6500, Shimadzu Scientific Instruments, Kyoto, Japan) was used to determine Mn content in the whole blood.

Blood for serum cholesterol samples were collected in vacuum tubes, and allowed to clot at room temperature for 1 h. They were then centrifuged at 1,200 x g for 20 min at 20°C and analyzed using a colorimetric end-point assay kit from ThermoDMA (Arlington, TX). Serum samples for progesterone were collected in vacuum tubes, allowed to clot at room temperature for 1 h, and centrifuged at 1,200 x g for 20 min at 20°C. Progesterone concentrations were determined using a radio immunoassay kit (Coat-A-Count; Diagnostic Products Corp, Los Angeles, CA).

Feed and liver biopsy samples were prepared for Mn analysis by wet ashing, using a microwave digestion (Mars 5™, CEM Corp., Matthews, NC) procedure as described by Gengelbach et al. (1994). Atomic absorption spectroscopy (Shimadzu Scientific) was used to determine Mn content of feed and liver samples.

### *Statistics*

Performance data were analyzed as a RCBD with 3 treatments and 4 blocks, using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). Liver Mn, whole blood Mn, and serum cholesterol were analyzed as repeated measures with pen means serving as the experimental unit. The model included the fixed effects of treatment, breed, and block, and all related interactions. Pen served as the random effect, with treatment by breed as the error term.

Pre-planned contrasts were used and included a comparison between no supplemental Mn (control) and the two supplemental Mn groups, and a comparison of Mn sources (organic Mn verses inorganic Mn). Reproductive data were analyzed using the Genmod procedure of SAS. The model included the fixed effects of breed, treatment, and the treatment x breed interaction. Interactions that were not significant ( $P < 0.05$ ) for the measurement of interest were removed from the models.

### **Results and Discussion**

One heifer was humanely euthanized on d 44, suffering from a broken back. Using an outlier test, two heifers were found to be consistent outliers in the data set, regardless of the type of measurement being examined, and along with the heifer that died were removed from the data set before statistical analysis. Manganese source and level did not affect average daily gain of heifers (Table 2). Angus x Simmental heifers gained more ( $P = 0.0001$ ) than Angus heifers and had a higher daily dry matter intake than Angus heifers ( $P = 0.0003$ ). Daily dry matter intake and gain:feed ratio were not affected by Mn source or concentration. Previously, we reported that supplementation of a growing diet (15.8 mg Mn/kg DM) with  $\text{MnSO}_4$  did not affect growth performance of beef heifers (Hansen et al., 2005).

Liver Mn concentration did not differ among treatments (Table 3). It is unclear why diets containing nearly twice the Mn concentration of the control diet did not result in increased liver Mn concentration. A silage-based diet was used in this study, and silage has been shown to vary widely in Mn content, with some samples containing as low as 3.2 mg Mn/kg DM (Hidiroglou, 1979). The silage in the present study contained approximately 42 mg Mn/kg DM, which is similar to the NRC (1996) value of 41 mg Mn/kg DM. It is possible that the high Mn content of the silage used in the present study is why little

differences were observed between control heifers and those receiving supplemental Mn, regardless of source.

Whole blood Mn concentration was not affected by dietary treatment. The body has a rigorous homeostatic mechanism for Mn, and as a result, Mn is removed rapidly from the blood (Carter et al., 1974). Contrary to our findings, Hawkins et al. (1955) found that supplementation of Mn increased whole blood Mn concentration. Using young dairy calves, they found that supplementation of 50 mg Mn/kg DM to a basal diet of no more than 1.7 mg Mn/kg DM resulted in an increase in whole blood Mn concentration (Hawkins et al., 1955). The fact that our control diet was relatively high in Mn content (36 mg Mn/kg DM) prior to supplementation may explain why we did not see differences in whole blood Mn concentration.

Addition of Mn to the control diet increased serum cholesterol on day 98 when compared to control heifers ( $P = 0.06$ ). Serum cholesterol increased ( $P = 0.0001$ ) over time in heifers, regardless of treatment. There was a tendency ( $P = 0.11$ ) for heifers fed the  $\text{SO}_4$  treatment to have higher serum cholesterol when compared to heifers fed the Bioplex treatment. There was a trend ( $P = 0.16$ ) for serum cholesterol to increase over time in heifers fed the  $\text{SO}_4$  treatment when compared to heifers fed either the control or the Bioplex diet. Cholesterol was affected by a treatment by breed interaction ( $P = 0.06$ ; Figure 1). Manganese supplementation increased serum cholesterol in Angus but not in Simmental x Angus cross heifers. It has been demonstrated that a severe deficiency of Mn can lead to a decrease in cholesterol concentrations (Davis et al., 1990). Rats fed 0.4 mg Mn/kg DM had lower plasma cholesterol concentrations, specifically lower HDL cholesterol, than rats fed an adequate Mn diet (56 mg Mn/kg DM; Davis et al., 1990). The authors found no difference

in activity of B-hydroxy-B-methylglutaryl CoA reductase, the rate-limiting enzyme in cholesterol synthesis among rats fed 0.4 and 56 mg Mn/kg DM. This led them to speculate that a deficiency of Mn was affecting enzyme activity further along the cholesterol synthesis pathway. They theorized that Mn may act as a cofactor for two enzymes in cholesterol synthesis, mevalonate kinase, and farnesyl pyrophosphate synthase (Davis et al., 1990). Both of these enzymes are involved in the production of squalene, which is a precursor of cholesterol (Curran and Azarnoff, 1961). Further research is needed to elucidate the mechanism by which Mn affects cholesterol levels in the body.

Previous work has indicated that the reproductive performance of heifers may benefit from supplementation of Mn to the diet. Supplementing a corn-silage based diet (40 mg Mn/kg DM) with 14 mg Mn/kg DM caused a reduction in days to first estrus in heifers, and days to conception, and services per conception in heifers and cows was observed when a corn-silage diet was supplemented with Mn (DiCostanzo et al., 1986). Based on serum progesterone levels of greater than 1 ng/mL, we saw no difference between treatments in the number of heifers cycling (Table 4). There was a tendency for a greater number of Simmental x Angus heifers to be cycling than Angus heifers ( $P = 0.17$ ). The percent of heifers that responded to a synchronization protocol with standing heat was not affected by dietary Mn source or concentration. Pregnancy rate was affected by dietary treatment ( $P = 0.01$ ). Fewer SO<sub>4</sub> heifers were bred than control ( $P = 0.01$ ) and Bioplex heifers ( $P = 0.04$ ). There was a tendency for more Simmental x Angus heifers to be bred than Angus heifers ( $P = 0.17$ ). It is unclear why so few heifers supplemented with inorganic Mn were bred. It is possible that the reduced pregnancy rate was due to a sire effect. Although sires were rotated

among the treatment groups at 7-d intervals, it is possible that the majority of the SO<sub>4</sub> heifers exhibited estrus while one sire was in the pasture.

Results of the present study indicate that supplementation of Mn to a silage-based diet containing 36 mg Mn/kg DM did not affect growth and development of heifers, regardless of source. Reproductive performance was affected by source of supplemental Mn, although it is unclear why. Serum cholesterol was increased in heifers supplemented with inorganic Mn. Further research focusing on the bioavailability of Mn from forages and feedstuffs like corn-silage is needed.

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**Table 1. Ingredient and chemical composition of basal diet fed to heifers**

|                              | Basal Diet |
|------------------------------|------------|
| Ingredient, % of DM          |            |
| Corn Silage                  | 78.00      |
| Ground Corn                  | 12.98      |
| Corn gluten feed             | 5.00       |
| Urea                         | 1.25       |
| Calcium carbonate            | 0.75       |
| Vitamin premix <sup>b</sup>  | 0.01       |
| Mineral premix <sup>c</sup>  | 0.01       |
| Corn Supplement <sup>d</sup> | 2.00       |
| Chemical Composition         |            |
| DM, % of diet <sup>e</sup>   | 43.05      |
| CP, % of DM <sup>f</sup>     | 14.06      |
| NDF, % of DM <sup>f</sup>    | 38.26      |
| ME, Mcal/kg <sup>f</sup>     | 2.48       |
| Mn, mg/kg DM <sup>e</sup>    | 36.46      |

<sup>a</sup>Diet contained Rumensin (Elanco Animal Health, Indianapolis, IN) at 33 mg/kg.

<sup>b</sup>Contained per kg of premix: 6,600,000 IU of vitamin A, 1,520,000 IU of vitamin D, and 6,600 IU of vitamin E.

<sup>c</sup>Provided per kg of diet: 30 mg of Zn as ZnSO<sub>4</sub>; 10 mg of Cu as CuSO<sub>4</sub>; 0.5 mg of I as Ca(IO<sub>3</sub>)<sub>2</sub>(H<sub>2</sub>O); 0.2 mg of Se as Na<sub>2</sub>SeO<sub>3</sub>; and 0.1 mg of Co as CoCO<sub>3</sub>.

<sup>d</sup>Provided supplemental Mn as Mn Bioplex or MnSO<sub>4</sub>.

<sup>e</sup>Analyzed value.

<sup>f</sup>Calculated values from NRC (1996).

**Table 2. Effect of supplemental manganese concentration and source on heifer performance**

| Item                   | Supplemental Mn, mg/kg |            |                    | SEM   | P <  |
|------------------------|------------------------|------------|--------------------|-------|------|
|                        | 0                      | 30 Bioplex | 30 SO <sub>4</sub> |       |      |
| ADG, kg/d              | 0.91                   | 0.86       | 0.94               | 0.02  | 0.09 |
| DMI, kg/d              | 8.48                   | 8.49       | 8.74               | 0.16  | 0.44 |
| Gain:Feed <sup>a</sup> | 0.10                   | 0.10       | 0.10               | 0.002 | 0.91 |

<sup>a</sup>Breed effect (P < 0.001)

**Table 3. Effects of manganese supplementation level and source on liver manganese concentrations of beef heifers**

| Liver Mn, mg/kg DM | Supplemental Mn, mg/kg |            |                    | SEM  |
|--------------------|------------------------|------------|--------------------|------|
|                    | Control                | 30 Bioplex | 30 SO <sub>4</sub> |      |
| Day 0 <sup>a</sup> | 10.17                  | 9.45       | 10.61              | 0.67 |
| Day 219            | 11.53                  | 12.23      | 11.12              | 0.59 |

<sup>a</sup>Day of sampling effect ( P < 0.001).

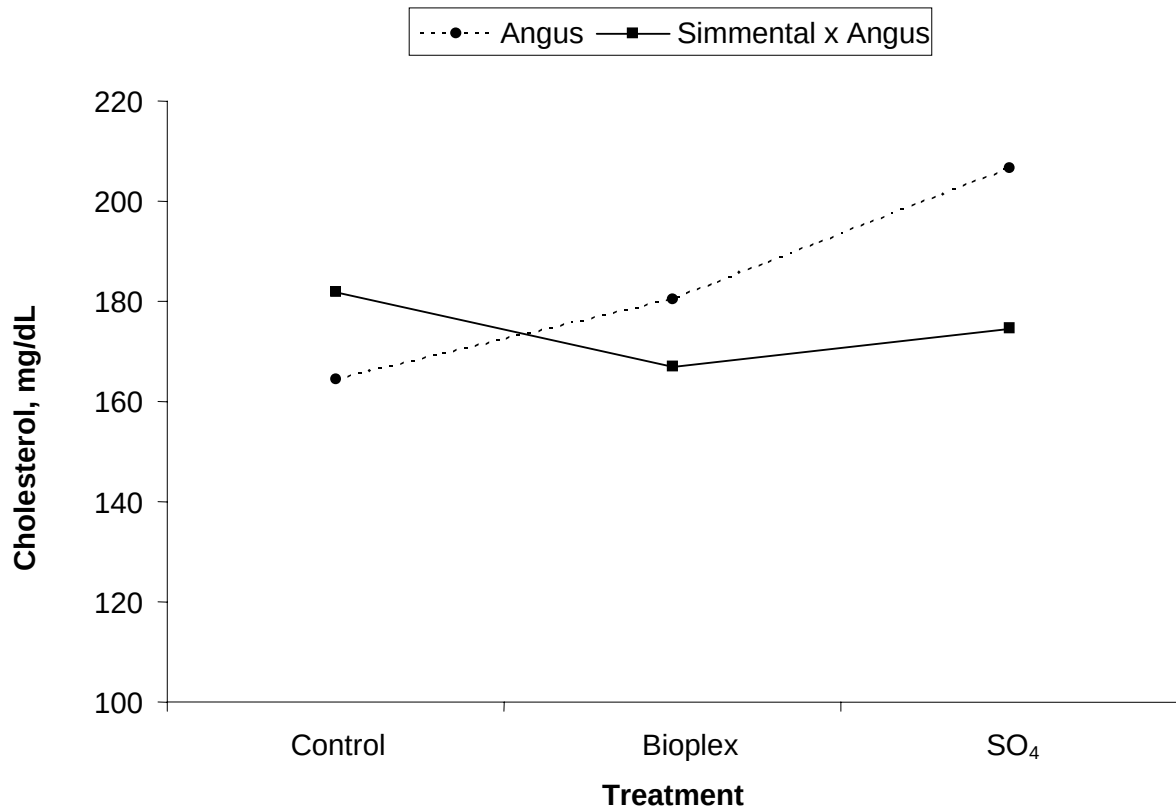
**Table 4. Effects of supplemental manganese source and concentration on reproductive performance of heifers**

| Item                                       | Supplemental Mn, mg/kg |            |                    | P <  |
|--------------------------------------------|------------------------|------------|--------------------|------|
|                                            | Control                | 30 Bioplex | 30 SO <sub>4</sub> |      |
| Cycling <sup>a</sup> , %                   | 87                     | 78         | 74                 | 0.30 |
| Response to Lutalyse dose <sup>b</sup> , % | 43                     | 50         | 44                 | 0.90 |
| Pregnancy Rate <sup>c</sup> %              | 78                     | 67         | 36                 | 0.01 |

<sup>a</sup> Based on serum progesterone of > 1.0 ng/mL.

<sup>b</sup> Heifers that showed estrus in response to Lutalyse protocol.

<sup>c</sup> Breed effect (P = 0.10)



**Figure 1. Effect of treatment and breed on serum cholesterol levels of beef heifers (treatment x breed interaction  $P < 0.10$ ).**

## SUMMARY

Three experiments were conducted to evaluate the effects of dietary manganese on growth, reproductive performance, and manganese status of beef heifers. In experiment 1, Angus and Simmental heifers were individually fed a control diet containing 15.8 mg Mn/kg DM for 196 d. Addition of manganese, from manganese sulfate, at concentrations ranging from 0 to 50 mg Mn/kg DM, to the control diet did not affect growth characteristics of heifers, but tended to increase the number of heifers cycling at 12 mo of age, as well as pregnancy rate of heifers. Plasma manganese and serum cholesterol levels were not affected by the addition of manganese to the control diet. Angus had higher serum cholesterol levels than Simmental heifers. Liver manganese concentrations increased as dietary manganese increased. Results of this study suggest that a diet containing 15.8 mg Mn/kg DM is adequate for growth of young beef heifers, but addition of higher concentrations of dietary manganese may benefit sexual maturity of heifers.

In experiment 2, pregnant Angus and Simmental heifers from the previous study were kept on their diets (0 or 50 mg supplemental Mn/kg DM) through gestation and early lactation to further explore the effects of a low-manganese diet on beef heifers and their offspring. Calves born to control heifers were lighter at birth than those born to supplemented heifers. Whole blood manganese concentrations at birth were lower in calves born from control heifers than in calves born to supplemented heifers. Calves born to control heifers exhibited a variety of manganese deficiency signs, including superior brachygnathism, swollen joints, unsteadiness, and dwarfism. No problems were observed in calves born to supplemented heifers. Results of this study indicate that the manganese requirement for gestating beef heifers is greater than 15.8 mg Mn/kg DM for proper fetal

development. The addition of 50 mg Mn/kg DM from manganese sulfate was sufficient to prevent any signs of manganese deficiency in calves.

Experiment 3 evaluated the effect of manganese level and source on growth and reproductive performance of beef heifers. Angus and Angus x Simmental heifers were fed a corn silage-based diet, containing 36 mg Mn/kg DM. Supplemental manganese was supplied from either manganese sulfate or a chelated form of manganese (Bioplex). Source and concentration of manganese did not affect growth characteristics of heifers. Liver manganese concentration was not affected by dietary manganese source and concentration. Across all sampling days, manganese supplementation increased serum cholesterol in Angus but not in Simmental x Angus cross heifers. On d 98 heifers fed supplemental manganese, regardless of source, had higher serum cholesterol than those fed the control diet. Heifers supplemented with manganese sulfate had lower pregnancy rates than those fed control diets or supplemented with manganese Bioplex, although it is unclear why. Results of this study suggest that a silage-based diet containing 36 mg Mn/kg DM was adequate for growth and development of beef heifers.

In conclusion, a series of studies conducted with beef heifers suggest that the dietary manganese requirement of growing beef heifers is no greater than 15.8 mg Mn/kg DM. Reproductive performance of heifers may improve with the addition of supplemental manganese. Proper fetal development of calves requires a diet containing greater than 16 mg Mn/kg DM.