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Bioavailability of manganese from manganese proteinate, manganese sulfate and manganese oxide in broilers reared under heat distress

Isaac Larry Sherman

To the Graduate Council:

I am submitting herewith a thesis written by Isaac Larry Sherman entitled "Bioavailability of manganese from manganese proteinate, manganese sulfate and manganese oxide in broilers reared under heat distress." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Michael O. Smith, Major Professor

We have read this thesis and recommend its acceptance:

Kelly Robbins, James Miller

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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BIOAVAILABILITY OF MANGANESE FROM MANGANESE PROTEINATE, MANGANESE SULFATE AND MANGANESE OXIDE IN BROILERS REARED UNDER HEAT DISTRESS

A Thesis

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Isaac Larry Sherman

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QUE DIOS LES BENDIGA.

DEDICATION

To my father NATALIO SHERMAN KATTOFF who this month, seven years ago, passed unto the Lord's hands; my sisters NATALIA and CATALINA SHERMAN; my mother MIRIAM MEJIA and my nephew SHAJAR COHEN whom I adore.

ABSTRACT

The relative bioavailabilities of manganese from manganese monoxide, manganese sulfate and Mn-proteinate were compared in two different environmental conditions. Birds were raised at a cycling temperature of 18.3 to 23.9°C or at a cycling temperature of 23 to 35°C. Experimental animals used were day-old male commercial broiler chicks (Arbor Acre X Arbor Acre). Treatments were prepared from a starter and a grower diet (26 ppm Mn dry matter basis) which were supplemented with 0, 1000, 2000, and 3000 ppm Mn as Mn-proteinate, manganese sulfate or manganese monoxide. Birds had *ad libitum* access to feed and water.

Bone was the most sensitive tissue followed by kidney to manganese supplementation from all sources. Tibia manganese concentration increased linearly ($P < 0.05$). Based on ratios of slopes from multiple linear regression analysis of bone manganese on manganese intake from various sources, the relative bioavailabilities were 120 and 91% from manganese proteinate and manganese oxide, respectively, compared with 100% from manganese sulfate in three-week old chicks and 125 and 83% from manganese proteinate and manganese oxide, respectively, compared with 100% from manganese sulfate in chicks older than three weeks. All values were significantly different from 100% ($P < 0.05$).

Heat distress was observed to increase the manganese bioavailabilities of the various manganese sources ($P < 0.05$). Manganese from Mn-proteinate was more available than manganese from manganese sulfate followed by manganese oxide under each environmental temperature regimen.

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CHAPTER I

INTRODUCTION

Manganese is an essential element for many biological processes in living organisms. The essentiality of manganese for normal growth in mice and reproduction in rats was first established by Orent and McCollum (1931) and Kemmerer and co-workers (1931) when feeding diets deficient in this ion.

Manganese plays an essential role in a variety of enzyme systems which regulate carbohydrate metabolism, bone growth, and cartilage mucopolysaccharide synthesis. It is also essential in maintenance of acid-base balance and in the manganese superoxide dismutase activity. Enzymes for which manganese is an essential cofactor in carbohydrate metabolism include phosphatases, kinases and decarboxylases. Manganese deficiency can adversely affect bone and muscle growth, reproduction and brain development (Cotzias and Greenbough, 1958; Schroeder et al., 1966).

The nutritional importance of manganese for chicks has been an area of intensive investigation. Numerous studies have been conducted on the mechanisms of absorption (Southern et al., 1987; and Lonnerdal et al., 1987), tissue distribution (Underwood, 1977), homeostasis and excretion (Bertinchamps et al., 1966; Suzuki and Wada, 1981). Research has also been conducted on manganese availability from different feedstuffs and manganese supplements (Baker and Halpin, 1987; Fly et al.,

1989) used in poultry diets. Supplementation of poultry diets with inorganic manganese is necessary because of the high manganese requirement of chicks as a result of their extremely rapid growth rate (Henry et al., 1989). Furthermore, feedstuffs most commonly used in poultry diets such as corn and sorghum grains are low in manganese (Henry et al., 1989), and, as Halpin and Baker (1987) reported, may actually decrease intestinal uptake of manganese by chicks.

The selection of diet manganese sources is usually based on two factors: bioavailability and cost. The biological availability of several manganese sources including manganese oxide, manganese sulfate, manganese chloride, manganese carbonate and manganese-methionine chelate have been compared. Manganese from manganese sulfate and Mn-methionine chelate were reported to be more bioavailable for chicks than manganese from oxide and carbonate (Baker and Halpin, 1987). Tissue accumulation of manganese proved to be a very useful response variable in the determination of manganese bioavailibility from different sources. Bone followed by kidney, plasma, liver and muscle are the tissues of choice commonly used (Black et al., 1984a).

Bioavailability of manganese from these various supplements under elevated temperatures has not been well studied. All previous studies of manganese bioavailability were conducted in normal environmental conditions. Belay and

Teeter (1991) demonstrated that manganese excretion is elevated during heat exposure. Large amounts of potassium in the excreta of chicks were also reported by Smith and Teeter (1987). El Husseiny and Creger (1981) reported that broilers subjected to 32°C environment for 42 days had lower retention of Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn. Reduced growth, decreased performance, and high mortality are also observed in heat distressed chicks. It thus seems probable that bioavailability of manganese may be reduced and manganese requirement increased due to high temperature conditions.

This study was designed to evaluate the relative biological availability of different sources of manganese at two environmental temperatures. The main objective was to examine the bioavailability of manganese when fed as an organic, Mn-proteinate, and inorganic, manganese sulfate and manganese oxide, sources under high temperature conditions.

CHAPTER II

REVIEW OF LITERATURE

MANGANESE ABSORPTION

Manganese is poorly absorbed from the intestinal tract into mucosa cells throughout the small intestines. Absorption has been reported to be passive and mediated by a low affinity carrier and can be inhibited by excess Fe or Co (Thomson, et al., 1971). Two uptake processes have been suggested (Lonnerdal et al., 1987), a low capacity, saturable process, which may involve a membrane receptor; and a non-saturable process, which may occur via diffusion and may allow very high uptake of manganese.

Manganese absorption is proportional to the amount of ion in the brush border which is readily available for intestinal uptake (Keen et al., 1987). Studies with mice have shown that manganese absorption and retention are higher at younger ages (Lonnerdal et al., 1987). Bile is a principal route of manganese elimination; therefore, immature biliary function in young animals may enhance manganese retention. Kinetics of manganese uptake by purified brush border membranes of rats was reported more rapid in 14 day old rats than 18-21 day old rats. Maximum uptake capacity was also higher in 14 day old

rats. Higher permeability of the brush border was suggested as the mechanism for the higher rate and capacity for manganese uptake at younger age.

Dietary factors affecting manganese absorption

Poultry diets require manganese supplementation since manganese in most commonly used poultry feedstuffs is frequently poorly available to chicks. Furthermore, these ingredients may contain chemical fractions including neutral detergent fiber and phytate which depress the manganese bioavailability of inorganic supplements (Southern et al., 1987). Knowledge of interactions among trace minerals is also imperative since such interactions can change the intestinal absorption pattern of some minerals.

Halpin and Baker (1986a) have shown that fish meal, soybean meal, corn, wheat bran, and rice bran reduce manganese accumulation in bone, bile, and pancreas when added to a casein-dextrose diet containing adequate levels of manganese. However, they observed that rice bran does not reduce manganese deposition when the diet is at or below the chick's manganese requirement. In a related study Halpin and Baker (1986b) reported a reduction in chick performance when fish meal, wheat bran and a corn-soybean meal mixture were added to a casein-dextrose diet containing 14 ppm manganese. When

added to a manganese deficient diet (7 ppm manganese) only fish meal reduced weight gain, feed efficiency, tissue manganese concentration, and increased the incidence of perosis. Wheat bran and the corn-soybean mixture showed no effect at that manganese level. Halpin and Baker (1987) indicated that the neutral detergent fiber (NDF) fraction in the corn-soybean mix and wheat bran and the mineral content of fish meal were found to negatively affect manganese absorption. Both the NDF and the ash content in rice bran were observed to decrease tissue Mn deposition. Halpin and Baker (1987) also demonstrated that the main site of action of the negative effect of some poultry feedstuffs is the intestine. Tissue manganese deposition was slightly reduced when manganese was administered via intraperitoneal injection in chicks fed 10% dietary wheat bran.

Iron has been observed to interact with manganese by reducing manganese intestinal uptake. Experiments using albino rats have shown that manganese absorption from ingested milk was reduced when the milk was enriched with iron (Gruden, 1976). Iron and manganese share some common transport mechanisms which have higher affinity for iron over manganese when both ions are present. Indeed, only under iron deficiency conditions can manganese replace iron in the binding sites (Gruden, 1987). Cadmium and phosphorus have also been suggested to reduce manganese absorption. Gruden

(1985) observed that the cadmium effect was more apparent when iron was present in the diet (Gruden, 1985). Wedekind et al. (1991) reported that chicks fed diets containing supplemental phosphorus either 100 mg Mn/kg or 1000 mg Mn/kg had reduced absorption of dietary manganese and reduced excretion of endogenous manganese. It was also reported that absorption of manganese was more affected at the level of 1000 mg Mn/kg.

MANGANESE DISTRIBUTION AND EXCRETION

After intestinal absorption, manganese is transported via alfa-2 macroglobulin, transferrin as Mn (II), or serum albumin also as Mn(II) (Bertinchamps et al., 1966). Manganese is found in all tissues; however, higher concentrations are present in bone, liver, kidneys, and in pancreas (Underwood, 1977). This is consistent with the assumption that mitochondria-rich tissues contain higher manganese levels.

Black and co-workers (1984a) observed a positive linear relationship between dietary manganese concentrations and manganese accumulation in bone, liver, and kidney. Bone, liver, and kidney store sufficient manganese to sustain normal cellular function during manganese deficient episodes (Southern et al., 1987).

High manganese concentration in liver and kidney is related to manganese role as a cofactor for pyruvate carboxylase, superoxide dismutase and arginase, all of which are abundant in these tissues. Manganese is an activator of other enzymes such as phosphoenopyruvate carboxykinase, galactosyl transferase and insulin receptor protein kinase which are also mainly found in liver and kidney (Brandt and Schramn, 1986). Pyruvate carboxylase and arginase have been identified as the major manganese binding proteins (Brandt and Schramn, 1986).

Within cells, manganese content is very high in the mitochondria and the lysosomes, and very low in the cytosol of rat hepatocytes (Thiers and Vallee, 1957; Smeyers-Verbeke et al., 1977; Suzuki and Wada, 1981). Most cellular manganese is in the bound form. The subcellular location of free manganese has not been identified (Brandt and Schramn, 1986).

Factors affecting cellular uptake

Studies using Sprague-Dawley rats with streptozotocin-induced diabetes revealed higher manganese accumulations in their livers (Failla, 1986) and pancreas (Korc, 1983) than in livers and pancreas of normal rats (Failla, 1986). A depression in the plasma insulin-glucagon ratio rather than hypoinsulinemia per se was determined to be the cause of the

enhanced liver manganese accumulation. Adrenalectomized diabetic rats did not increase their manganese levels as much as intact diabetic rats did (Failla, 1986). This is consistent with epinephrine-stimulated release of glucagon and inhibition of insulin secretion. Hypoinsulinemia thus enhances cellular uptake of manganese to provide the cell with this essential cofactor for several enzymes involved in amino acid degradation, gluconeogenesis, glycogenolysis, and urea synthesis which are biochemical events stimulated by insulin deficiency (Failla, 1986). Enhanced manganese accumulation may also be a function of reduced turnover since Papavasilion et al. (1968) reported that high glucagon doses reduce billiary excretion of Mn.

The main route of manganese excretion is through biliary excretion; nevertheless, some manganese is eliminated through pancreatic juice. Lysosomes have been described to be involved in manganese excretion by concentrating and transporting manganese to the bile canaliculus when the liver is loaded with this metal ion (Suzuki and Wada, 1981). Homeostatic control of tissue manganese is maintained at the level of excretion rather than at the level of absorption since its intestinal uptake operates independently of the tissue manganese status (Bertinchamps et al., 1966; Britton and Cotzias, 1966; Papavasiliou et al., 1966).

Keen et al. (1987) demonstrated that the rate of intestinal absorption in rats does not respond to manganese deficiency. This is consistent with the observation that absorption of dietary manganese is correlated to manganese concentration. In addition, it has been reported that high levels of dietary or injected manganese can increase manganese concentration in bile (Brandt and Schramn, 1986).

FUNCTIONS OF MANGANESE

Manganese is an essential activator of kinases, glycosyltransferases, phosphatases, decarboxylases and an important component of pyruvate carboxylase, arginase, and superoxide dismutase. Manganese deficiency has been reported to lower the activity of glycosyltransferases which are enzymes involved in the synthesis of polysaccharide sidechains present in the core protein of proteoglycans (Leach, 1986). Proteoglycans, collagen, and ash are the major extracellular constituents of epiphyseal growth cartilage which is responsible for longitudinal bone growth (Smith et al., 1944). Reduction of proteoglycan synthesis increases bone deformation due to the inability of the growth plate to withstand compressive loads and depresses longitudinal bone growth (Leach, 1986).

Shortened and thickened limbs, swollen and enlarged joints, and reduced bone ash content and breaking strength are skeletal abnormalities which have been observed in most species affected with manganese deficiency (Leach, 1986; Amdur et al., 1945; and Wachtel et al., 1943). Hurley et al. (1980) indicated that perosis, a very common disease in poultry, is induced by both Mn and Zn deficiencies. This disease is characterized by gross enlargement and malformation of the tibiometatarsal joint, twisting and bending of the distal end of the tibia and the proximal end of the tarsometatarsus, thickening and shortening of the leg bones and slippage of the gastrocnemius or Achilles tendon from its condyles (Scott, 1969).

As a component of one of the two superoxide dismutases present in mitochondria of mammalian cells, manganese participates in the preventive effect of this enzyme against the release of substantial amounts of O_2 , H_2O_2, and OH free radicals which can cause excessive damage to cellular and subcellular membranes (Zidenberg-Cherr and Keen, 1987). Increased peroxidation of polyunsaturated fatty acids contained in the cellular membranes by free radicals results in the loss of membrane integrity and function.

Low manganese superoxide dismutase (MnSOD) activity has been observed in liver, lung, heart and brain of rats and chickens with manganese deficiency (Zidenberg-Cherr and Keen,

1987). In chickens, reduced MnSOD activity was observed in the liver after only seven days of feeding a manganese-deficient diet. Extensive damage in mitochondrial membranes is characteristic in manganese-deficient animals. This is consistent with the observation of large vacuoles in the matrix of the mitochondria in addition to open spaces between the inner and outer membranes in manganese-deficient animals (Zidenberg-Cherr and Keen, 1987). These authors suggested that the damage was due to decreased MnSOD activity resulting in elevated lipid peroxidation from free radicals (Zidenberg-Cherr and Keen, 1987).

Manganese is also an important component of arginase which is an enzyme essential in the urea cycle. Arginase activity has been described to increase in liver of diabetic rats (Duncan and Bond, 1981). The increase in arginase activity is associated with a high rate of protein degradation that occurs in diabetes as a compensatory biochemical adaptation to low glucose levels in the tissues.

Pyruvate carboxylase is another enzyme which contains manganese. Avian liver pyruvate carboxylase has manganese firmly incorporated into its structure (Scrutton and Mildvan, 1968); however, magnesium can replace manganese in this enzyme when manganese is deficient without affecting enzyme activity (Scrutton et al., 1973). A large number of diseases are associated with the alteration of pyruvate carboxylase

activity. Biotin deficiency along with stress can cause fatty livers and kidney syndrome (FLKS) in chickens as well as foot lesions, perosis, poor growth, and poor feather development as a result of reduced pyruvate carboxylase activity. Metabolic acidosis (Iles et al., 1977), phenylketonuria (Patel et al., 1973), several drugs, and heavy metals (Amatruda et al., 1977; Meraldi et al., 1974), are some other factors which decrease pyruvate carboxylase activity. It is thus probable that manganese deficiency could cause similar pathologies characteristic of reduced pyruvate carboxylase activity since manganese is important for the function of this enzyme.

Manganese also plays an important role in the pancreas. Everson and Shrader (1968), and Baly et al. (1985) using rats and guinea pigs described a decrease in pancreatic manganese content and insulin synthesis in both animals when they were manganese deficient.

It has been suggested that epilepsy could be caused by manganese deficiency. Papavasiliou and co-workers (1979) reported that some epileptics exhibited lower than normal blood manganese concentrations and suggested that low manganese concentration could be the cause for epilepsy. It was suggested (Carl et al., 1987) however that the observed low blood manganese concentrations were of genetic origin and not due to trace mineral deficiency.

MANGANESE REQUIREMENT AND SUPPLEMENTATION

The manganese requirement for poultry is very high. Sixty mg Mn/kg feed for young chicks and 30 mg Mn/kg feed for hens have been established as requirements for poultry (NRC, 1984). This relatively high requirement for manganese is due to low and poorly available dietary manganese levels in poultry feed ingredients, low intestinal manganese absorption in chicks, and the rapid rate of growth and development characteric of this species (Henry et al., 1989). Rapid growth and development require increased rates of protein, carbohydrate, and fat metabolism by enzymes for which manganese is either an essential cofactor or an activator. Glycosyltransferase is necessary for normal bone growth; pyruvate carboxylase is important in gluconeogenesis and protein synthesis; arginase is involved in protein metabolism; and manganese superoxide dismutase is essential in maintaining mitochondrial membrane integrity and normal cell function. The net effect of manganese-deficiency is thus reduced growth and performance. Scott (1969) reported reduced egg production, decreased hatchability, and increased incidence of thin shelled and shell-less eggs in manganese-deficient laying hens. In chicks, manganese deficiency results in perosis, ataxia, poor feather development, increased lipid peroxidation, poor growth, and other lesions associated with

abnormal protein metabolism. Scott (1969) described chondrodystrophy in young chicks as a condition caused by manganese deficiency. Chondrodystrophy is characterized by shortened and thickened legs and shortened wings, parrot beak, edema, portruding abdomen, and poor growth.

Several inorganic manganese supplements including manganese chloride ($MnCl_2$), manganese oxide (MnO), manganese sulfate ($MnSO_4.H_2O$), and manganese carbonate ($MnCO_3$) are routinely added to conventional poultry diets to meet the manganese requirement. However, there are differences among these compounds with regard to manganese bioavailability. Using tissue uptake of manganese (bone followed by kidney and liver) as a criterion of bioavailability, it was reported that manganese from some inorganic supplements is more bioavailable than from others. Tissue manganese accumulation is used to evaluate bioavailability since it has been reported as the most sensitive variable to changes in manganese intake (Black et al., 1984a; Black et al., 1984b; and Henry et al., 1986).

Baker and Halpin (1987) reported the bioavailability of manganese from manganese sulfate to be superior to the bioavailability of manganese oxide, and similar to that of manganese chloride. No effect of diet manganese concentration on chick weight gain or feed efficiency was observed. This was supported by Black et al. (1984b) who reported manganese fed as manganese sulfate more available than manganese fed as

manganese oxide followed by manganese carbonate, and observed no difference in feed intake or feed conversion. Southern and Baker (1983) reported similar tissue manganese values in chicks fed either manganese sulfate or manganese chloride. They also observed that high levels of manganese (>3000 ppm) in either form tended to depress weight gain and to cause a mild anemia in chicks.

Chelates of manganese with protein or single amino acids have been recently evaluated and have shown potential value in permitting enhanced gut absorption of manganese in the presence of mineral binding factors in the gut (Fly et al, 1989). It appears that the chelate prevents manganese from binding to ligands which render it unavailable (Henry et al., 1989) or perhaps it enhances intestinal absorption due to its high solubility and small particle size (Fly et al., 1989).

Fly et al. (1989) reported manganese fed as Mn-methionine to be more available than manganese fed as MnO. It was reported that the difference was more apparent in the presence of phytate. Weight gain and food intake were observed not to be affected by either source or level of supplementation. In addition, Henry et al. (1989) reported that manganese fed as Mn-met was more bioavailable than manganese fed as $MnSO_4$ followed by MnO. Likewise, birds fed Mn-met exhibited more efficient feed conversion. Baker and Halpin (1987), however, reported no difference between Mn-met and $MnSO_4$.

Most studies on manganese bioavailability, however, were conducted in ideal growth environments. Less is known about the effect of high temperatures on manganese metabolism and bioavailability from different sources. High temperatures have been shown to increase mineral excretion (Smith and Teeter, 1987; Belay and Teeter, 1992). El Husseiny and Creger (1981) reported that broilers subjected to 32°C environment for 42 days had lower rates of Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn retention. Reduced bone weight and strength and increased incidence of leg problems have also been described as a result of high environmental temperatures (Siegel et al., 1973). The high manganese levels in feces of heat stressed birds could be either of endogenous or exogenous origin. Endogenous manganese loss results from the altered-mineral balance caused by several physiological changes such as increased urinary output (Belay, 1992) and evaporative cooling (Jukes, 1971). Exogenous manganese could be due to decreased manganese biovailability from dietary sources; however more research needs to be done in this area.

Either endogenous or exogenous losses of manganese increase manganese requirements by chicks. Therefore, additional manganese supplementation of poultry diets may be necessary. Smith and Teeter (1989) reported a positive response of heat distressed broilers excreting large amounts of potassium to additional sodium and potassium salt

supplementation. Providing broilers with a manganese source with highly bioavailable manganese may alleviate the problem of increased manganese requirement and perhaps decrease the cost of manganese supplementation.

CHAPTER III

MATERIALS AND METHODS

Three hundred day-old male commercial broiler chickens (Arbor Acre X Arbor Acre) were individually weighed, wing banded and randomly assigned to wire-floored starter batteries containing 50 compartments and maintained on a 23h:1h light:dark schedule using incandescent lighting. Five replicate groups of six chicks each were randomly assigned to each of ten treatments. The experimental treatments were prepared from a common starter diet (Table 1) and a grower diet (Table 2). The basal diet contained 26 ppm Mn on dry matter basis which is below the manganese requirements for starting and growing chicks (National Research Council, 1984). The treatments consisted of four manganese levels (0, 1000, 2000, or 3000 mg Mn/kg diet) and three manganese sources (MnO, MnSO4 and Mn-proteinate). All manganese additions to the basal diet were made at the expense of corn starch. Feed and water during the first three weeks were provided in trough feeders and drinkers attached to the battery compartments. Chicks were allowed *ad libitum* access to feed and water. Body weight and feed intake were recorded weekly.

Table 1. Composition of basal starter diet

Ingredient (as fed basis)	Amount (%)
Ground corn	51
Soybean meal	36.35
Fats	6.0
Fish Meal	1.5
Dicalcium phosphate	1.5
Limestone	1.1
Vitamin mix[1]	.6
Salt	.4
Coban	.1
DL-Methionine	.15
Corn Starch[2]	1.15
Trace mineral mix[3]	.15
Composition:	
Dry matter, %	89.42
CP, %	23.48
Manganese, ppm	26

[1]Supplied per kilogram of diet: vitamin A, 4,175 IU; cholecalciferol, 750 ICU; choline, 468 mg; niacin, 42 mg; pantothenic acid, 7.3 mg; riboflavin, 478 mg; vitamin B_{12}, .011 mg

[2]Manganese supplementation was added at the expense of equivalent weights of corn starch

[3]Provided per kilogram of diet: Zn, 80 mg; Fe, 60 mg; Cu, 10 mg; I, 1 mg

Table 2. Composition of basal grower diet

Ingredient (as fed basis)	Amount
	(%)
Ground corn	57
Soybean meal	32
Fats	5.4
Fish Meal	0.0
Dicalcium phosphate	2.20
Limestone	0.9
Vitamin mix[1]	.6
Salt	.4
Coban	.1
DL-Methionine	.1
Corn Starch[2]	1.15
Trace mineral mix[3]	.15
Composition:	
Dry matter, %	89.25
CP, %	20.48
Manganese, ppm	26

[1]Supplied per kilogram of diet: vitamin A, 4,175 IU; cholecalciferol, 750 ICU; choline, 468 mg; niacin, 42 mg; pantothenic acid, 7.3 mg; riboflavin, 478 mg; vitamin B_{12}, .011 mg;

[2]Manganese supplementation was added at the expense of equivalent weights of corn starch

[3]Provided per kilogram of diet: Zn, 80 mg; Fe, 60 mg; Cu, 10 mg; I, 1 mg

On day 22, chicks were weighed individually and feed consumption for each replicate group was determined. One chick from each pen was randomly selected and euthanized by cervical dislocation. Right tibias were removed and frozen for later analysis. Twelve birds were randomly selected from each treatment and transferred to individual cages fitted with feed- and water-dispensing equipment in each of two environmental chambers. Chicks were fed a practical grower diet (Table 2) maintaining the same dietary manganese sources and supplemental levels as in the starter diet. Chicks were allowed *ad libitum* access to feed and water. Body weight, feed consumption and water consumption were recorded weekly. One chamber was allowed to cycle between 18.3 and 23.9 oC (thermoneutral) over each 24-hour period. The temperature was held at 18.3°C for 10 hours and then gradually increased to 23.9°C, maintained such as for two hours, and then gradually decreased to 18.3°C. In the other chamber, temperature cycled between 23.9 and 35 °C (heat distress). Birds were exposed to 8 h of 23 °C, 4 h of 23.9 to 35 °C, 4 h of 35 °C and 8 h of 35 to 23.9 °C

On day 45, blood samples were collected from brachial veins of birds (six birds per treatment) and placed in heparinized tubes. Plasma was separated by centrifugation using a table top centrifuge; samples were stored frozen for later analysis.

On day 47, temperature was raised to 37 and 39°C (acute heat distress) in the thermoneutral and heat distress environments, respectively, and maintained for 4 consecutive hours. Finally, on day 49, birds were weighed and after a 12 hour fast, six birds per treatment were euthanized by cervical dislocation. Liver, kidneys, and right tibias were removed and stored for further manganese analysis.

Tibias were labeled, wrapped in cheesecloth and boiled in petroleum ether in a soxhlet apparatus for 8 h to remove fat. The fat extracted bones were weighed, dried at 100 °C and ashed at 600 °C 24 hr. Dry matter and ash content were recorded. After ashing, tibias were powdered and 1 g samples were solubilized in 3 ml of 6N HCl. Samples were diluted to 25 ml using deionized water and analyzed for Mn content using flame atomic absorption spectrophotometry (IL model 551 spectrophotometer). Liver and kidney samples were homogenized in 1:3 ratio with deionized water. Dry matter was determined on homogenates. Ash content was calculated by wet digestion of homogenates using nitric followed by perchloric acid and digested samples were diluted to 10 ml using deionized water. Manganese level in samples was determined by flame atomic absorption spectrophotometry. Plasma samples were diluted in 1:3 ratio with deionized water prior to taking atomic absorption readings. Laboratory analysis was carried out on duplicate samples of all tissues.

Analysis of variance was done on all the data using the General Linear Models procedure of SAS software (SAS Institute, 1987). Least square treatment means were compared to check for statistical differences (5% level probability). Linear regression analysis of tissue manganese concentration on dietary manganese were conducted.

CHAPTER IV

RESULTS

Performance data for 7-week old chicks are shown in Table 3. Several interactions between manganese sources and levels were observed in the thermoneutral environment. Weight gain of birds fed Mn-proteinate at 3000 ppm was significantly lower than at 1000 and 2000 ppm ($P < 0.05$). This condition was also observed in 3-week old chicks whose weight was low when fed Mn-proteinate at 3000 ppm Mn. The addition of manganese oxide and manganese sulfate at 3000 ppm resulted in birds gaining more weight than their counterparts fed the same level as Mn-proteinate ($P < 0.05$). However, birds fed manganese oxide or sulfate ate more than those fed Mn-proteinate ($P < 0.05$). The low gain and feed intake of birds fed Mn-proteinate at 3000 ppm manganese was not clearly understood. But it is hypothesized that this high level of manganese from this source resulted in toxicosis.

In general, heat distress decreased weight gain of all birds ($P < 0.01$) except only for those fed Mn-proteinate at 3000 ppm Mn whose weights were already very low. There was a trend for heat distress to reduce feed intake of all birds ($P < 0.01$). The effect of heat distress on feed intake of birds fed Mn-proteinate at 3000 ppm could not be elucidated because

Table 3. *Effects of manganese sources, manganese levels, and heat distress on chick performance(7 weeks)*

Mn Source	Mn Supplement Level (ppm)	Gain[1](g) TN[2]	Gain[1](g) HD[3]	Feed[1](g) TN	Feed[1](g) HD	Gain:Feed TN	Gain:Feed HD
	0	1478[a]	1278[ab]	3515[a]	3318[ab]	0.42	0.39
	1000	1384[a]	1208[ab]	3803[a]	3054[b]	0.36	0.41
MnO	2000	1393[a]	1211[ab]	3373[ab]	3307[ab]	0.42	0.38
	3000	1412[a]	1234[ab]	4135[a]	3260[b]	0.35	0.35
	1000	1554[a]	1297[ab]	3894[a]	3259[b]	0.41	0.41
$MnSO_4$	2000	1452[a]	1304[ab]	3623[a]	3534[ab]	0.41	0.38
	3000	1516[a]	1320[ab]	3901[a]	3286[b]	0.40	0.41
	1000	1514[a]	1361[ab]	3929[a]	3030[b]	0.33	0.45
Mn-Pro	2000	1490[a]	1344[ab]	3535[ab]	3034[ab]	0.43	0.38
	3000	1190[b]	1272[ab]	2653[b]	3021[b]	0.39	0.43

[a,b]Means within columns having unlike superscript differ ($P < 0.05$)
[1]The main effect of environment (TN vs. HD) was significant ($P < .01$)
[2]Thermoneutral environment
[3]Heat distress environment

of the poor feed intake of those birds in the thermoneutral temperatures. Feed intake reduction was significant in birds fed oxide and sulfate at 1000 and 3000 ppm manganese and proteinate at 1000 ppm manganese ($P < 0.01$). The feed consumption of heat distressed birds did not diverge significantly among the various manganese sources. Feed efficiency was not affected by either manganese source or manganese level. This is inconsistent with the research of Henry et al. (1989) who reported birds fed diets with Mn-proteinate had more efficient feed conversions. Feed efficiency was not affected by elevated temperatures.

Bone was the tissue that showed the greatest response to dietary manganese supplementation in both environments. This is consistent with findings by Henry et al. (1986) and Black et al. (1984ab) who indicated that bone was the most sensitive tissue to dietary manganese levels. In 3-week old chicks, tibia manganese concentration increased linearly ($P < 0.05$) as supplemental manganese from all sources increased (Table 4). Large tibia manganese concentrations were generated as dietary manganese from the various manganese sources increased. Manganese from Mn-proteinate was more bioavailable than manganese from manganese sulfate and oxide ($P < 0.05$), Figure 1 shows the relationships between bone manganese concentration and dietary manganese intake for the various sources in 3-week old chicks. Regression analysis indicates that manganese from

Table 4. *Mean bone manganese concentration in 3-week old birds with respect to manganese source*

Source	Mn Level	Manganese intake (g)	Mean Mn concentration (ppm)
			Bone[1]
	0	0.1±0.01	16.1±2.43
	1000	1.3±0.43	37.4±5.37
Sulfate	2000	1.9±1.21	52.8±4.83
	3000	2.6±1.35	70.2±5.98
	1000	0.9±0.64	32.4±1.74
Oxide	2000	2.2±1.71	50.7±3.91
	3000	3.0±1.93	68.8±6.35
	1000	1.3±1.07	33.4±3.29
Mn-Proteinate	2000	1.9±0.84	60.7±5.40
	3000	2.7±1.39	86.5±7.13

[1]Dry fat-free bones

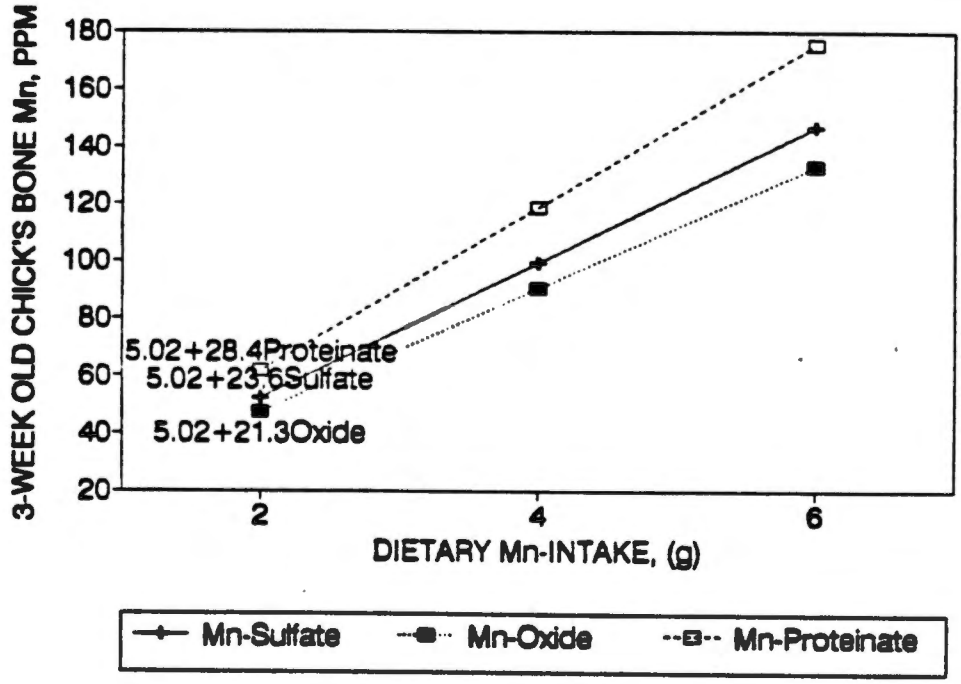


Figure 1. Relative availability of Mn sources based on regression analysis of tibia manganese on dietary manganese

and manganese oxide were 120 and 91% bioavailable relative to manganese in manganese sulfate (Table 5).

The same trend was observed in older birds whose tibia manganese concentration increased linearly ($P < 0.01$) in either the thermoneutral or the heat distress environment when dietary manganese increased (Tables 6 and 7). This relationship is illustrated in Figures 2 and 3, where tibia manganese was regressed on supplemental manganese intake. Mn-proteinate produced greater bone manganese concentration than the sulfate followed by oxide at the three levels in both environments (Tables 6 and 7). The slope representing birds fed Mn-proteinate was greater ($P < 0.05$) than slope representing birds fed sulfate or oxide (Table 8); ratios of slopes indicated manganese in the proteinate and the oxide were 125 and 83% bioavailable, respectively, relative to manganese in the sulfate, as measured in chicks housed in the thermoneutral environment. Manganese in manganese sulfate was considered 100% bioavailable and used as standard.

The Mn-proteinate also generated larger bone manganese concentrations than the sulfate and the oxide at the same manganese levels in the heat distress environment. The slope of Mn-proteinate was higher than the slopes of manganese sulfate and oxide ($P < 0.05$). Comparison of slopes (Table 8) shows manganese in Mn-proteinate and manganese oxide were 145 and 82% bioavailable, respectively, relative to manganese in

TABLE 5. *Relative values of manganese sources based on linear regression analysis of 3-week old chick's bone manganese on Mn-intake*

Mn Source	Regression coefficient (Slope + SE)	Relative Value
		(%)
$MnSO_4$	23.64 ± 3.696[b]	100
MnO	21.33 ± 2.527[b]	91 ± 9
Mn-Proteinate	28.43 ± 2.791[a]	120 ± 11

[a-c]Means within column having unlike superscript differ ($P < 0.05$)

Table 6. *Mean tissue manganese concentration with respect to manganese source*[1]

Source	Mn Level	Manganese intake (g)	Mean[2] manganese concentration (ppm)			
			Bone[3]	Plasma[4]	Liver[4]	Kidney[4]
	0	0.1±0.02	9.4±3.32	2.1±0.23	10.7±0.98	8.6±1.15
	1000	3.6±0.64	24.4±6.44	2.8±0.55	14.8±1.74	16.8±1.90
Sulfate	2000	5.9±1.62	38.7±5.45	2.1±0.36	17.9±2.20	24.0±2.93
	3000	8.9±1.26	46.6±6.83	3.7±3.13	17.8±3.30	27.5±3.46
	1000	3.4±0.95	19.6±1.42	2.4±0.82	14.3±1.35	17.2±1.22
Oxide	2000	5.9±1.34	29.5±4.15	3.8±1.65	17.1±3.18	20.6±2.50
	3000	11.0±2.79	51.4±9.90	4.1±0.92	18.7±4.30	30.3±3.69
	1000	4.6±0.72	24.6±2.95	2.9±1.87	15.7±2.37	19.2±2.13
Prot	2000	6.8±0.83	40.0±4.87	3.6±1.84	16.7±0.72	24.9±1.55
	3000	7.9±0.77	61.1±9.93	5.3±4.52	21.1±3.81	32.0±3.42

[1]Thermoneutral environment
[2]Mn concentration was significantly ($P < .01$) affected by diet for bone, liver and kidney
[3]Dry fat-free bones
[4]Dry matter basis

Table 7. *Mean tissue manganese concentration with respect to manganese source*[1]

Source	Mn Level	Manganese intake (g)	Mean[2] manganese concentration (ppm)			
			Bone[3]	Plasma[4]	Liver[4]	Kidney[4]
	0	0.1±0.03	9.1±3.34	2.7±0.25	9.6±0.98	8.9±1.15
	1000	3.3±0.65	23.6±6.43	3.6±0.56	14.6±1.77	16.7±1.90
Sulfate	2000	5.2±1.67	35.4±5.48	3.8±0.33	17.9±2.24	25.2±2.94
	3000	7.5±1.23	48.9±6.83	4.5±3.18	17.6±3.30	30.2±3.42
	1000	2.4±0.90	24.0±1.42	3.3±0.80	14.9±1.33	19.9±1.25
Oxide	2000	6.1±1.33	35.7±4.14	4.2±1.61	19.8±3.16	27.8±2.53
	3000	8.6±2.72	42.7±9.92	3.5±0.97	22.2±4.34	34.0±3.66
	1000	3.2±0.77	27.7±2.93	3.8±1.83	16.1±2.33	19.9±2.13
Prot	2000	6.2±0.85	48.4±4.80	3.8±1.89	15.7±0.74	23.9±1.56
	3000	7.2±0.75	63.9±9.97	4.3±4.54	17.9±3.80	30.4±3.47

[1]Heat distress environment

[2]Mn concentration was significantly ($P < .01$) affected by diet for bone, liver and kidney

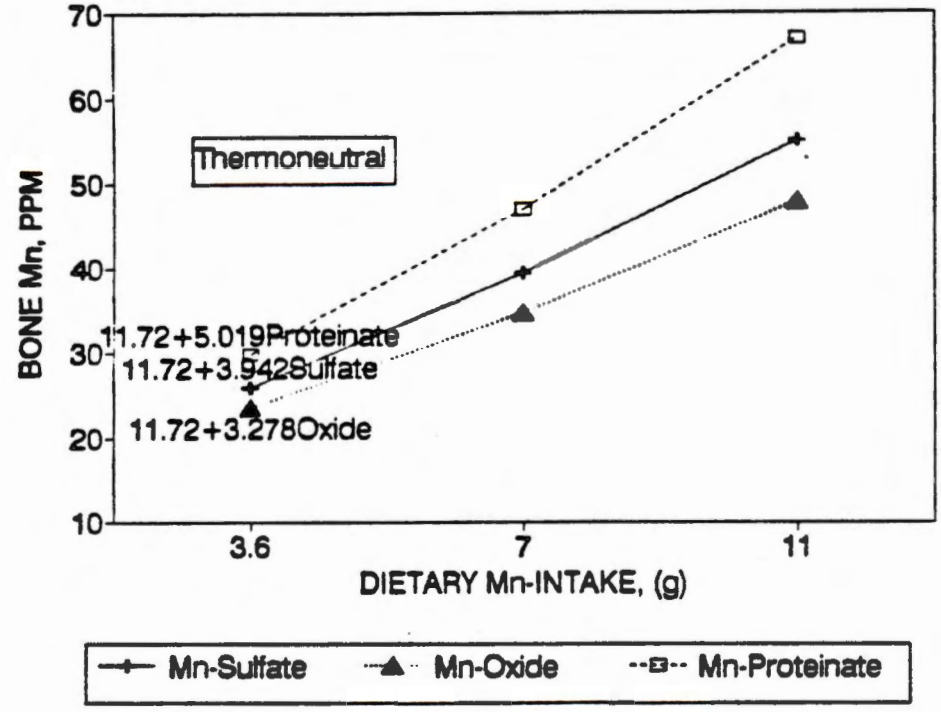
[3]Dry fat-free bones

[4]Dry matter basis

TABLE 8. *Relative values of manganese sources based on linear regression analysis of tissue manganese on Mn-intake*

Tissue	Mn Source	Regression Coefficient (Slope+SE)	Relative Value	Regression Coefficient (Slope+SE)	Relative Value
		Thermoneutral		Heat distress	
Bone	Sulfate	3.9 ± 0.56^{d}	100	4.5 ± 0.67^{c}	100
	Oxide	3.3 ± 0.45^{f}	83 ± 10	3.7 ± 0.55^{e}	82 ± 12
	Proteinate	5.0 ± 0.56^{b}	125 ± 14	6.3 ± 0.68^{a}	145 ± 17
Kidney	Sulfate	1.6 ± 0.28^{d}	100	2.1 ± 0.34^{bc}	100
	Oxide	1.4 ± 0.23^{d}	91 ± 16	2.5 ± 0.28^{a}	120 ± 13
	Proteinate	1.9 ± 0.28^{c}	125 ± 22	2.2 ± 0.34^{b}	105 ± 10

[a-f]Means within tissue having unlike superscript differ ($P < 0.05$)



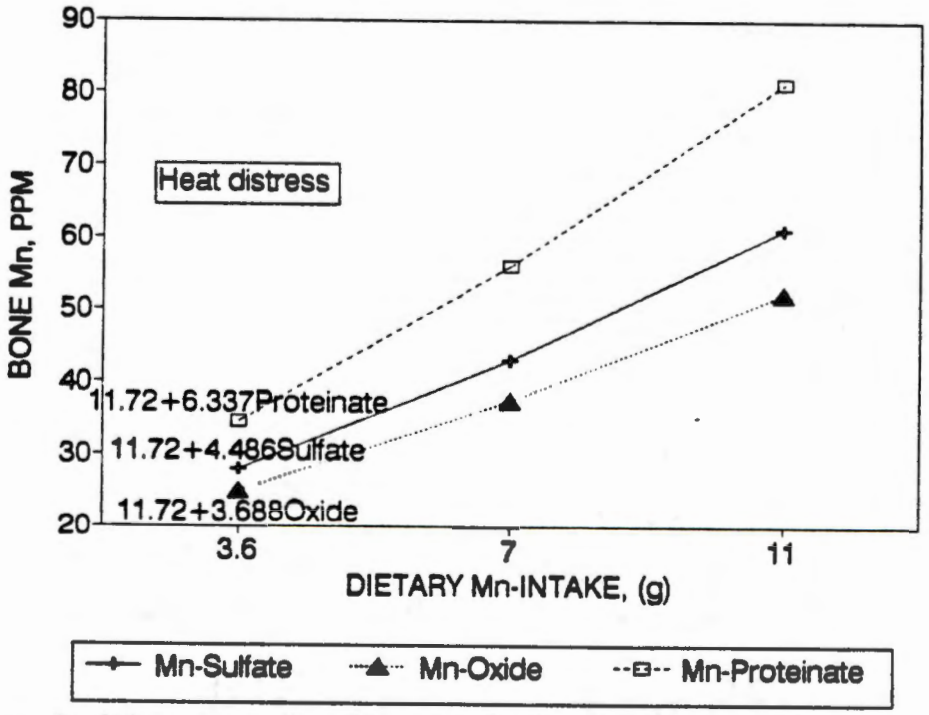


Figure 2 (thermoneutral) and 3 (heat distress) show the relative availability of Mn sources based on regression analysis of bone manganese on dietary manganese

manganese sulfate, as measured in birds housed in the heat distress environment. The manganese bioavailabilities of manganese sulfate, oxide, and proteinate were significantly increased ($P < 0.05$) by high temperatures. However, the difference in bioavailability between Mn-proteinate and manganese sulfate was amplified by heat distress.

Plasma manganese concentrations did not increase linearly ($P > 0.1$) in the thermoneutral and heat distress environments as supplemental dietary manganese from all manganese sources increased (Tables 6 and 7). These findings are inconsistent the work of Black et al. (1984a) who reported plasma manganese concentration increased linearly as dietary Mn increased. Plasma was far less sensitive to dietary manganese than bone, as indicated by regression analysis from all manganese sources (Table 8). Manganese sources and levels did not affect plasma manganese concentrations (Tables 6 and 7). Ratios of slopes indicated similar manganese bioavailabilities among manganese sources within each environment. High temperatures had no effect on plasma manganese concentration for the various manganese sources ($P > 0.1$).

Liver responded linearly ($P < 0.01$) to dietary manganese as indicated by regression analysis from manganese sources in both environments (Table 9). These results are consistent with the reports of Black et al. (1984b) who described a highly linear uptake of Mn by liver as dietary Mn increased.

Table 9. *Relative biological availability of manganese sources based on linear regression analysis of tissue manganese concentration on manganese intake*

Regression equation	
Neutral environment	Heat distress environment
Bone[1]	
$Y = 11.72 + 5.019$ Proteinate $+ 3.278$ MnO $+ 3.942$ $MnSO_4$	$Y = 11.72 + 6.337$ Proteinate $+ 3.688$ MnO $+ 4.486$ MnSO
Liver[1]	
$Y = 13.79 + 0.629$ Proteinate $+ 0.448$ MnO $+ 0.447$ $MnSO_4$	$Y = 13.79 + 0.503$ Proteinate $+ 0.925$ MnO $+ 0.540$ MnSO
Kidney[1]	
$Y = 13.05 + 1.945$ Proteinate $+ 1.401$ MnO $+ 1.555$ $MnSO_4$	$Y = 13.05 + 2.194$ Proteinate $+ 2.466$ MnO $+ 2.078$ $MnSO_4$

[1]Where Y equals tissue Mn (ppm), and proteinate, MnO, and $MnSO_4$ equal to dietary Mn intake (g). Each regression equation represents 17 chicks.

Liver manganese concentrations within manganese levels and sources did not differ substantially (Tables 6 and 7). There were no differences among the slopes of manganese sources in either environment ($P > 0.05$). Elevated temperatures did not affect the slopes of manganese sources as measured by liver response to manganese supplementation.

Kidney was the second most sensitive tissue to dietary manganese. Kidney manganese concentration increased linearly ($P < 0.01$). Its concentration was much greater than plasma and liver manganese in either the thermoneutral or the heat distress environment as dietary manganese increased (Table 6 and 7). Regression analysis from sources showed that Mn-proteinate produced larger bone manganese concentration than manganese sulfate and oxide (Table 8). Ratios of slopes indicated manganese in the proteinate and the oxide forms were 125 and 91% bioavailable, respectively, relative to manganese in the sulfate, as measured in chicks housed in the thermoneutral environment. Elevated temperatures increased kidney manganese accumulation for the various manganese sources ($P < 0.05$). Ratios of slopes indicated manganese in the oxide and proteinate were 120 and 105% bioavailable, respectively, relative to manganese in the sulfate (Table 8).

The effect of Mn-supplementation on bone ash (old birds) is shown in Table 10. Bone ash did not respond to increased Mn-supplementation. This is consistent with the work of

TABLE 10. *Relative values of manganese sources based on linear regression analysis of bone ash on Mn-intake*

	Mn Source	Regression coefficient (Slope + SE)	Relative Value
		Thermoneutral environment	
			(%)
Bone ash	$MnSO_4$	$0.30^{a} \pm 0.19$	100
	MnO	$0.09^{c} \pm 0.15$	26 ± 49
	Mn-Proteinate	$0.08^{c} \pm 0.19$	25 ± 56
		Heat distress environment	
			(%)
Bone ash	$MnSO_4$	$0.31^{ab} \pm 0.25$	100
	MnO	$0.49^{a} \pm 0.20$	157 ± 94
	Mn-Proteinate	$0.24^{b} \pm 0.24$	76 ± 92

[a-c]Means in columns having unlike superscript differ ($P < 0.01$)

Southern and Baker (1983) who reported bone ash unchanged when dietary manganese increased. The ash content in tibias of chicks fed $MnSO_4$ was significantly higher ($P < 0.05$) than the ash content of those fed either manganese oxide or Mn-proteinate in the thermoneutral environment. Ratios of slopes (Table 10) showed ash content in chicks fed Mn-proteinate and manganese oxide was 25 and 26%, respectively, relative to ash content in chicks fed $MnSO_4$.

Elevated temperatures affected bone ash concentration among sources. The slopes of manganese oxide and Mn-proteinate increased, whereas the slope of manganese sulfate decreased ($P < 0.01$). The ash content in tibias of heat distressed chicks fed MnO was significantly higher ($P < 0.05$) than the ash content of chicks fed Mn-proteinate. Ratios of slopes indicated ash content in chicks fed Mn-proteinate and manganese oxide were 76 and 157%, respectively, relative to ash content in chicks fed $MnSO_4$.

CHAPTER V

DISCUSSION

Although the bioavailability of manganese from manganese sulfate has been reported equal to that of Mn-proteinate (Baker and Halpin, 1987), the data from this experiment clearly demonstrate that manganese in Mn-proteinate was significantly more bioavailable than manganese in manganese sulfate when measured by bone response to dietary manganese supplementation. Manganese in Mn-proteinate was found more bioavailable than manganese in manganese sulfate followed by manganese oxide. These results are supported by the report of Henry et al. (1989) who described manganese in Mn-methionine more available than from manganese sulfate and oxide. Fly et al. (1989) also indicated that manganese in Mn-methionine was substantially more bioavailable to the chick than manganese from manganese oxide.

In 3-week old birds, manganese from Mn-proteinate was significantly more bioavailable than manganese from manganese sulfate and oxide ($P < 0.05$) as measured by bone manganese concentration. Likewise, in older birds housed in either thermoneutral or heat distress environments, the manganese bioavailability of Mn-proteinate was higher than that of

manganese sulfate and oxide. Interestingly, 3-week old birds tended to accumulate more manganese in their bones than older birds did in response to dietary manganese supplementation. This phenomenon was probably attributed to the higher manganese absorption and lower manganese excretion frequently observed in young birds. Since bile is the main route of manganese elimination, immature biliary function possibly enhances manganese retention (Lonnerdal et al., 1987). At the same time, Lonnerdal et al. (1987) indicated that higher permeability of the brush border may increase the rate and capacity for manganese uptake.

Similar manganese bioavailabilities were observed between Mn-proteinate and manganese sulfate in older birds under the different environmental conditions when measured by kidney manganese response to dietary manganese supplementation. Plasma and liver did not facilitate the evaluation of manganese bioavailabilities among the various because these tissues showed similar responses to dietary manganese level.

Heat distress increased the manganese concentrations of bones and kidneys as dietary manganese supplementation increased from all manganese sources. Although heat distress has been reported to decrease manganese retention in broilers (Belay et al., 1992), results in this experiment (Table 5 and 6) show that bone and kidney manganese concentrations in heat distressed birds for the various sources were significantly

higher ($P < 0.05$) than in birds housed in thermoneutral conditions. At the same time, high temperatures amplified the difference in manganese bioavailibility between Mn-proteinate and manganese sulfate as measured in bone tissue.

It seems possible that manganese intestinal absorption probably increased due to high temperatures thus permitting more manganese retention and increased bone manganese concentration, provided that endogenous manganese excretion did not increase. Heat distress may create physiological or anatomical intestinal changes which could favor manganese absorption. If this assumption is correct, the much higher bioavailability of manganese in Mn-proteinate relative to manganese in manganese sulfate could be explained by the fact that chelates have preference for intestinal absorption over other chemical forms.

Another possibility could be that heat distress affects the chemical structure of manganese sources making manganese more available for absorption. But, this would account for the much larger difference in manganese bioavailability between Mn-proteinate and manganese sulfate only if the effect of heat distress on Mn-proteinate was similar or higher relative to manganese sulfate and oxide.

Decreased endogenous manganese excretion during heat distress may also explain this phenomenon of higher bone manganese concentration in heat distressed birds. Perhaps,

due to high mineral loss during heat distress (Belay et al., 1992), homeostatic mechanisms are activated to reduce excessive mineral loss. It should be recalled that manganese homeostatic control is maintained at the level of excretion (Bertinchamps et al., 1966; Britton and Cotzias, 1966 and Papavasiliou et al., 1966).

Besides Mn-proteinate, many other mineral-protein chelates, especially those containing Cu, Zn and Fe have been shown to have commonly greater bioavailabilities than inorganic sources of the mineral (Kratzer and Vohra, 1986). The high bioavailability of manganese proteinate relative to manganese sulfate and manganese oxide was probably attributed to its high relative solubility and small particle size (Henry et al., 1989), its ability to protect manganese from binding by ligands (Henry et al., 1989) and its chelation value which is fundamental for the absorption of metal ions from the intestinal tract (Reddy et al., 1992).

The particule size and molecular weight of the chelates must be very tiny (less than 1000), so that the particles can penetrate the membranes of intestinal cells. A larger chelate can not be absorbed without further degradation or digestion (Reddy et al., 1992). Because they are prepared using acids, bases and enzymes involved in intestinal absorption, chelates are very soluble in the intestinal lumen and move rapidly through the intestinal wall (Reddy et al., 1992).

Manganese-protein chelates have the potential value in facilitating enhanced manganese absorption in the presence of mineral antagonizing factors in the gut (Fly et al., 1989). The chelating agent binds the metal ion within its structure in such a way that the ion is protected against chemical reactions in the gut including reactions with phosphates, hydroxides, phytates, oxalates or the bulk density of diets (Reddy et al., 1992). According to Wedekind and Baker (1991), inorganic phosphorus or calcium and phosphorus together can cause a decrease in manganese utilization. Phytate, fiber, or both in corn and soybean meal were also reported to decrease manganese availability from supplemental manganese sources (Halpin and Baker, 1986a and Halpin et al., 1986).

Chelation of metal ions by integral proteins at the absorption site in the gut or with amino acids prior to digestion is essential for their absorption (Reddy et al., 1992). This is in agreement with Underwood (1977) who reported that copper, zinc and iron must be bound in the gastro-intestinal lumen prior to absorption and reports of increased bioavailability of these minerals when chelated with amino acids. Increased absorption of manganese from ileum or jejunum in rats was also described by Garcia-Aranda (1983) when he included L-histidine or citrate in the perfusion solution.

Having found manganese from Mn-proteinate more bioavailable than from manganese sulfate and oxide in either environment and much more bioavailable during heat distress, it leads us to believe that factors including high solubility, small particle size, chelation and strong ion binding make proteinates potential candidates in mineral supplementation. Since manganese is more bioavailable from Mn-proteinate, manganese requirement of chicks would be met at lower manganese dietary levels in thermoneutral conditions and at much lower levels in heat distress episodes if Mn-proteinate were to be included in the broiler feed formulation.

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VITA

Isaac Larry Sherman was born in San Pedro Sula, Honduras on March 7, 1966, to Natalio Sherman and Miriam Mejia. He is the brother of two sisters, Catalina and Natalia Sherman. He grew up in San Pedro and was educated at La Salle catholic high school where he obtained his high school diploma in 1983. In 1986, two months after his father death, while enrolled in Electrical Engineering at The University of Honduras, he was awarded a scholarship to pursue a BS degree in Agriculture in the United States.

In May of 1987 he began his studies in Animal Science at The University of Tennessee, Knoxville, where he obtained his Bachelor of Science in Animal Science in May of 1991. Before moving back to his native country, Isaac was awarded an assistantship in the Animal Science Department to continue his graduate education in Animal Nutrition. In July of 1993 he completed the requirements for the Master of Science degree in Animal Nutrition at The University of Tennessee, Knoxville.

Isaac Sherman will return to his country where he will be joining his family and where he is planning to work providing assistance in the area of poultry management.