Effect of Manganese Source on Manganese Absorption by the Intestine of Broilers[1]

F. Ji,*†[2] X. G. Luo,*†[3] L. Lu,*† B. Liu,*† and S. X. Yu*†

*Mineral Nutrition Research Division, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100094; and †State Key Laboratory of Animal Nutrition, Beijing 100094, China

ABSTRACT Two experiments were conducted to investigate the effect of Mn source on Mn absorption by the intestine of broilers. In Experiment 1, the effect of Mn source, including $MnSO_4$, 2 Mn-amino acid chelates (Mn-Gly and Mn-Met) synthesized in our laboratory, 3 Mn-amino acid complexes with different complex strengths (Mn-Met E, Mn-AA A, and Mn-AA B), and 2 mixtures of $MnSO_4$ with Gly or Met, on Mn absorption was assessed with ligated loops of different small intestinal segments of broilers. In Experiment 2, the absorption of Mn from $MnSO_4$, Mn-AA A, and Mn-AA B was compared with intact broilers fed ad libitum. The criterion used for comparison was the Mn content of hepatic portal vein plasma. The absorption of Mn was higher ($P < 0.0002$) by ligated ileal loops than by duodenal and jejunal ones. Met supplementation increased ($P < 0.03$) the absorption of Mn as $MnSO_4$. The absorption of Mn as Mn-AA A and Mn-AA B with moderate and strong complex strengths, respectively, were higher ($P < 0.05$) than those of Mn as $MnSO_4$ and Mn-Met E with weak complex strength. On d 7 and 9 of Experiment 2, the Mn content of portal vein plasma was higher ($P < 0.03$) for Mn-AA B with strong complex strength than for $MnSO_4$. On d 9, Mn content in plasma was higher ($P < 0.01$) for Mn-AA B with strong complex strength than for Mn-AA A with a moderate one. The results from this study confirm that the ileum was the main site of Mn absorption for broilers, and Met was more effective in facilitating Mn absorption than Gly as a ligand. Organic Mn was more efficiently absorbed than inorganic Mn ($MnSO_4$); the absorption of organic Mn with moderate and strong complex strengths was greater than that of the organic Mn, which was weak, and the absorption of organic Mn with strong complex strength was greater than that of the organic Mn with a moderate strength.

Key words: broiler, ligated intestinal loop, organic manganese sources, manganese absorption, hepatic portal vein

2006 Poultry Science 85:1947–1952

INTRODUCTION

Manganese is essential for normal bone formation, enzyme function, and amino acid metabolism in poultry (Scott et al., 1976). The utilization of Mn has become an increasing concern because of the extremely rapid growth rate of commercial broiler strains, which puts additional stress on bone structure. In several studies (Fly et al., 1989; Henry et al., 1989; Lu et al., 2006), organic Mn sources were more bioavailable than inorganic sources. The bioavailabilities of Mn in 3 commercial organic Mn products (Mn-Met E, Mn-AA A, and Mn-AA B) have been compared in our laboratory (Li et al., 2004; Li et al., 2005). Glycine has the smallest molecular weight of all amino acids, thus it might be supposed that Mn as Mn-Gly chelate would be the most easily absorbed of all Mn-AA chelates if the chelates could be absorbed into the mucosal cell and transferred across the gut wall in intact form. Methionine is the first limiting AA for broilers, and Mn-Met might become a commonly used metal-amino acid complex in broiler production. Therefore, both Mn-Gly and Mn-Met chelates synthesized in our laboratory were studied in addition to feed grade Mn-Met E, Mn-AA A, and Mn-AA B.

In an earlier study conducted in our laboratory (Ji et al., 2006), the effect of Mn source on Mn uptake was evaluated using the technique of in vitro everted gut sacs. The results from this study indicated that the ileum was the main site of Mn absorption for broilers, and the uptake of Mn from "complexed" or "chelated" organic sources was higher than that of Mn from inorganic sources. However, the findings of this in vitro study need to be verified by in vivo studies of broilers. To our knowledge, no in vivo study has been reported on the influence of organic and inorganic Mn sources on Mn absorption. The purpose of the present study was to determine the effect of organic

Received March 14, 2006.

Accepted June 5, 2006.

[1]Supported by the National Basic Research Program of China (project no. 2004CB117501), National Natural Science Foundation of China (project no. 30270968), and Chinese Academy of Agricultural Sciences Foundation for First-Place Outstanding Scientists.

[2]Current address: Department of Animal Science, Anhui Agricultural University, Hefei, China.

[3]Corresponding author: wlysz@263.net

Table 1. Composition of the basal diets for broilers in 2 experiments

Ingredient[1]	Amount, %	
	Experiment 1	Experiment 2
Ground yellow corn	56.65	50.21
Soybean meal	34.00	37.00
Fish meal	3.10	5.20
Soybean oil	3.50	3.50
$CaHPO_4$	1.10	1.25
Ground limestone	1.05	1.00
Iodinized salt	0.30	0.30
DL-Met	0.10	0.15
Micronutrients[2]	0.20	0.34
Nutrient composition		
ME, MJ/kg	12.92	12.47
CP,[3] %	21.43	24.08
Lys, %	1.18	1.22
Met, %	0.45	0.58
Met + Cys, %	0.78	0.92
Ca,[3] %	0.93	0.98
Nonphytate P, %	0.37	0.47
Mn,[3] mg/kg	20.95	16.02

[1]Ingredient and nutrient composition are reported on an as-fed basis.

[2]For diets of Experiment 1, the following were provided per kilogram of diet: vitamin A (all-*trans* retinol acetate), 10,000 IU; cholecalciferol, 2,600 IU; vitamin E (all-*rac*-α-tocopherol acetate), 20 IU; vitamin K (menadione Na bisulfate), 2.0 mg; thiamin (thiamin mononitrate), 1.6 mg; riboflavin, 6.0 mg; vitamin B_6, 3.0 mg; vitamin B_{12}, 0.014 mg; pantothenate, 20 mg; niacin, 30 mg; folic acid, 0.8 mg; biotin, 0.12 mg; choline (choline chloride), 500 mg; Cu ($CuSO_4 \times 5H_2O$), 8 mg; Zn ($ZnSO_4 \times 7H_2O$), 40 mg; Fe ($FeSO_4 \times 7H_2O$), 80 mg; I (KI), 0.35 mg; Se (Na_2SeO_3), 0.15 mg. For diets of Experiment 2, the following were provided per kilogram of diet: vitamin A (all-*trans* retinol acetate), 15,000 IU; cholecalciferol, 3,900 IU; vitamin E (all-*rac*-α-tocopherol acetate), 30 IU; vitamin K (menadione Na bisulfate), 3.0 mg; thiamin (thiamin mononitrate), 2.4 mg; riboflavin, 9.0 mg; vitamin B_6, 4.5 mg; vitamin B_{12}, 0.021 mg; pantothenate, 30 mg; niacin, 45 mg; folic acid, 1.2 mg; biotin, 0.18 mg; choline (choline chloride), 700 mg; Cu ($CuSO_4 \times 5H_2O$), 8 mg; Zn ($ZnSO_4 \times 7H_2O$), 40 mg; Fe ($FeSO_4 \times 7H_2O$), 80 mg; I (KI), 0.35 mg; Se (Na_2SeO_3), 0.15 mg.

[3]Determined by analysis.

and inorganic Mn sources on Mn absorption, using ligated loops of broiler intestine or intact living broilers.

MATERIALS AND METHODS

Birds, Diets, and Treatments

Arbor Acres commercial male broilers (Arbor Acres Poultry Breeding Co., Beijing, China) were used in 2 experiments. Birds were housed in electrically heated, thermostatically controlled cages (74 × 70 × 40 cm) with fiberglass feeders and a 24-h constant-light schedule. The birds were allowed ad libitum access to feed and water that contained 106 μg of Ca/mL, 42 μg of Mg/mL, 0 μg of Cu/mL, 0 μg of Fe/mL, 0.01 μg of Mn/mL, and 3.3 μg of Zn/mL. Chicks were managed according to guidelines set by Arbor Acres. Corn-soybean meal basal diets (Table 1) used in the experiments were formulated to meet or exceed all broiler NRC (1994) nutrient recommendations, except for Mn.

Experiment 1 was conducted to assess the effect of organic and inorganic Mn sources on Mn absorption by ligated loops of the small intestine of broilers in situ. The chicks were housed in cages of 6 birds each before entering the study. All birds received a corn-soybean meal diet formulated to meet or exceed nutrient requirements of broilers before the age of 22 d, recommended by NRC (1994). The level of Mn in the diet was 110.72 mg/kg by analysis. All chicks were fed the basal diet containing 20.95 mg of Mn/kg (Table 1) from d 22. On d 28, after an overnight fast (12 h), birds were weighed (1,234 ± 70.5 g, means ± SD) and allotted randomly to 1 of 6 replicate cages (1 chick per replicate cage) for each of 8 treatments so that the average BW of birds for each treatment was nearly equal. The 8 treatments of different Mn sources were $MnSO_4 \cdot 7H_2O$, Mn-Gly chelate, Mn-Met chelate, Mn-Met complex with a weak complex strength [Mn-Met E, formation quotient ($\mathbf{Q_f}$) value = 3.2, 8.27% Mn], Mn-AA complex with a moderate complex strength (Mn-AA A, Q_f value = 45.3, 6.48% Mn), Mn-AA complex with a strong complex strength (Mn-AA B, Q_f value = 115.4, 7.86% Mn), the mixture of $MnSO_4$ with Gly at a molar concentration equal to that in the Mn-Gly chelate, and the mixture of $MnSO_4$ with Met at a molar concentration equal to that in the Mn-Met chelate. Manganese concentration in fluids in ligated loops injected with Mn-free solution after dosing were too low to be tested in preliminary studies; therefore, there was no need to set up a blank control to deduct the endogenous Mn from intestinal secretions in this experiment. The complex strengths (Q_f values) of Mn-Met E, Mn-AA A, and Mn-AA B have been determined in our laboratory (Li et al., 2004). The duodenum, jejunum, and ileum of the small intestine of each bird were used as 1 replicate of the ligated loops of corresponding intestinal segments.

In Experiment 2, absorption of Mn as either $MnSO_4 \cdot 7H_2O$, Mn-AA A with the moderate complex strength, or Mn-AA B with the strong complex strength was compared in intact broilers fed ad libitum. All chicks were fed a basal diet containing 16.02 mg of Mn/kg (Table 1) during the first 13 d posthatching. On d 14 posthatching, after overnight fast, the chicks were weighed and allotted randomly to each of 8 replicate cages (6 chicks per cage) for each of 4 experimental treatments (3 Mn sources plus a control with no added Mn). In an earlier study conducted in our laboratory (Ji et al., 2006), the absorption of Mn as Mn source with the weak complex strength was similar to that of Mn as Mn sulfate. Therefore, 2 organic Mn sources with moderate and strong complex strengths were selected in this trial.

Preparation of Chelates

According to the definition by the Association of American Feed Control Officials (2001), metal amino acid chelates are formed from the reaction of a metal ion from a soluble metal salt with amino acids having a mole ratio of 1 mol of metal to 1, 2, or 3 (preferably 2) mol of amino acids to form coordinate covalent bonds. The solution of $MnSO_4$ (0.1% Mn) was mixed with the Gly solution at a 1:2 *M* ratio of Mn and Gly. The solution of NaOH was used to adjust pH of the mixed solution to 6.8 to 7.0. The mixture was then heated to 70 to 80°C in a water bath for 60 min (Dong, 2001). After cooling to room temperature, the solution was filtered to yield the solid chelate. The chelate

was washed with 95% ethanol (Teng and Han, 2001) and evaporated to dryness. An aqueous solution of Mn-Met chelate was synthesized according to the above steps, without any precipitated solid product produced. All chemicals used were reagent grade except for Gly, which was biochemical grade (Beijing Chemical Reagent Co., China). The structural characteristics of the solid Mn-Gly chelate were confirmed using infrared spectroscopy (model IR--435. Shimadu Corp., Kyoto, Japan), as described in the previous study (Ji et al., 2006).

Ligated Loops Procedure

Chicks were fasted overnight, and anesthetized by injection of a complex anesthetic (0.1 mL/kg, Beijing Huadu Animal Health Protection Co., China) into the wing vein. The abdomen was opened by midline incision. The duodenum was ligated 1 cm distal to the pylorus, the jejunum was ligated just anterior to the remnant of the yolk stem, and the ileum was ligated just anterior to the ileocecal junction (Melvin, 1984). Loose ligatures were then placed next to and 10 cm distal to the above ligatures to isolate different regions of the intestine. The isolated segments were rinsed with 30 mL of warm saline to eliminate food residues and debris (Hempe and Cousins, 1989). The distal loose ligatures of duodenal segment, jejunal segment, and ileal segment were secured, respectively. The remaining loose ligature of each intestinal segment was tightened around a needle inserted into the proximal end of the loop, and the Mn dose was injected. Following administration of the dose, the needle was removed and the proximal ligature secured. The intestine was returned to the body cavity. The anesthetized birds were warmed with infrared lamps to maintain their body temperature.

All doses contained 2.18 mmol of Mn/L (120 mg of Mn/L) as different Mn sources in 3 mL of saline. This Mn amount was chosen because of the dietary intake of Mn for broilers (Luo et al., 1991). Because the pH values of chymes in the duodenum, jejunum, and ileum of 28-d old broilers have been measured to be 6.0, 6.0, and 7.0, respectively (Zhang, 2002), the doses injected into the duodenal and jejunal loops were buffered with 15.5 mmol/L of morpholineoethanesulfonic acid, and the doses injected into the ileal loops were buffered with 15.5 mmol/L of Tris at the pH indicated above. At 5 min after dosing, the intestinal loops were sectioned and the contents drained for analysis. Each chick was killed by anesthesia overdose. All chemicals used were reagent-grade, except for morpholineoethanesufonic acid and Tris, which were biochemical grade (Beijing Jingke Chemical Reagent Co., China).

Experimental Procedures for Oral Mn Source Study

The dietary treatments included a corn-soybean meal basal diet (Table 1) and the basal diet supplemented with 100 mg of Mn/kg as reagent-grade $MnSO_4$ or 1 of the 2 organic Mn sources with moderate or strong complex strengths. The diets, in mash form, were fed from d 14 to 23.

On d 21 and 23, 3 birds per cage for each of those 4 treatments were selected according to the average BW of the cage and killed by cervical dislocation. The blood of the hepatic portal vein was collected, and plasma was separated for Mn determination. The samples were pooled for each cage, resulting in 8 composite samples per treatment.

Methods of Assays and Calculations

Collected samples were frozen (–20°C) for analysis. Manganese concentrations in samples of diet and fluid content in loop tissue were determined by inductively coupled Ar plasma spectroscopy (model 9000, Thermal Jarrell Ash, Waltham, MA). Manganese content in plasma samples were determined by furnace atomic absorption spectrophotometry (model Unicam Solaar M-6, Thermo Electron Corp., Camb, UK; Zhao et al., 1988; Liu and Bi, 1995).

A nonabsorbable reference indicator (phenol red) was used to correct for changes in Mn concentration resulting from water absorption or intestinal secretion (Schedl et al., 1966) in Experiment 1. The concentrations of phenol red in initial and final fluid content were determined by measuring absorbency at 520, 560, and 600 nm with a UV-Vis spectrophotometer (model 756MC, Shanghai Optical Apparatus Corp., China). The 3-wave length correction was applied, because phenol red concentration tends to be overestimated in samples if absorbency is measured at 560 nm only (Schedl et al., 1966). Final volumes of content and absorption percentages of Mn were computed according to equations outlined in Table 2.

Statistical Analysis

Data from Experiments 1 and 2 were analyzed by 1-way ANOVA using the GLM procedure of SAS Institute (1989). Cage was used as an experimental unit, and the statistical models included the effect of Mn source in the 2 experiments.

RESULTS

Experiment 1

Our preliminary studies showed that Mn absorption declined during a period of 5 to 40 min after the initiation of the experiment in the 3 intestinal segments, with maximal absorption peaks occurring at 5 min for all treatments (Ji, 2003). Therefore, standard conditions of sample collections at 5 min after dosing were adopted for a comparison of the absorption of Mn in different forms to obtain the most sensitive response. The observed mean values for the absorption percentage of Mn as different Mn sources from ligated duodenal, jejunal, and ileal loops are reported in Table 3. The absorption percentages of Mn by ligated ileal segments were about 6 to 15 times ($P < 0.0002$) those by duodenal segments and significantly higher ($P < 0.0002$) than those by jejunal segments. The absorption percentages of Mn by jejunal sacs were similar ($P > 0.10$) to those by

Table 2. Equations used for deriving volume of fluid content and absorption percentage of Mn[1]

Term	Symbol	Formula
Experiment 1: final volume of content (mL)	V_F	$V_F = \frac{C_{P\,(1)} \times V_I}{C_{P\,(2)}}$
Experiment 1: absorption percentage of Mn (%)	AP	$AP = \frac{C_{Mn\,(1)} \times V_I - C_{Mn\,(2)} \times V_F}{C_{Mn\,(1)} \times V_I \times T} \times 100$

[1]$C_{P\,(1)}$, $C_{P\,(2)}$ = initial and final concentration (mmol/L) of phenol red, respectively; V_I = initial volume (mL) of injected dose; $C_{Mn\,(1)}$, $C_{Mn\,(2)}$ = Mn concentration (mg/L) of initial and final fluid content, respectively; and T = the sampling time (min) after initiation of dosing.

duodenal segments. Therefore, it appeared that ileum was the main site of Mn absorption for broilers.

The absorption percentages of Mn as $MnSO_4$ were lower ($P < 0.01$) than those of Mn as other Mn sources in the 3 intestinal segments. In the duodenum, Gly and Met supplementations significantly increased ($P < 0.001$) the absorption of Mn as $MnSO_4$ by 218.3 and 204.2%, respectively. In the jejunum and ileum, Met supplementation significantly increased ($P < 0.03$) the absorption of Mn as $MnSO_4$ by 82.0 and 50.2%, respectively. The absorption of Mn as Mn-Gly chelate was 233.2 and 90.2% ($P < 0.02$) higher than that of Mn as $MnSO_4$ in the duodenum and jejunum, respectively. The absorption of Mn as Mn-Met chelate was 271.5, 132.6, and 70.1% ($P < 0.002$) higher than those of Mn as $MnSO_4$ in the duodenum, jejunum, and ileum, respectively. The absorption of Mn as Mn-Met chelate was higher ($P < 0.01$) than that of Mn from the mixture of $MnSO_4$ and Gly in ligated jejunum and ileum. In ileal segments, the absorption of Mn from the mixture of $MnSO_4$ and Met was higher ($P = 0.0653$) than that of Mn from the mixture of $MnSO_4$ and Gly. This experiment indicated that both Gly and Met could facilitate Mn absorption, but Met was more effective in facilitating Mn absorption than Gly as a ligand.

The absorptions of Mn as Mn-AA A in the duodenum, jejunum, and ileum were higher ($P < 0.05$) than those of Mn as $MnSO_4$. Manganese from Mn-AA B was absorbed more efficiently ($P < 0.01$) than Mn as $MnSO_4$ in the 3 intestinal segments. The absorptions of Mn as Mn-Met E were 58.9, 9.8, and 16.7% higher than those of Mn as $MnSO_4$ in the 3 intestinal segments, respectively, although the differences were not significant ($P > 0.10$). Manganese as either Mn-AA A or Mn-AA B was absorbed more efficiently ($P < 0.02$) than Mn from the mixture of $MnSO_4$ and Gly in the ileum. The absorption of Mn as Mn-AA B was higher ($P = 0.04$) than that of Mn from the mixture of $MnSO_4$ and Gly in the jejunum. The absorption percentages of Mn as Mn-AA A in the duodenum, jejunum, and ileum were higher ($P < 0.05$) than those of Mn as Mn-Met E. Manganese as Mn-AA B was absorbed more efficiently ($P < 0.01$) than Mn from Mn-Met E in the 3 intestinal segments. The absorptions of Mn as Mn-AA B were 32.3, 11.2, and 5.4% higher than those of Mn as Mn-AA A in the 3 intestinal segments, respectively, although the differences were not significant ($P > 0.10$). The results showed that the absorptions of Mn as Mn-AA B with strong complex strength and Mn-AA A with moderate complex strength were higher than those of Mn from inorganic Mn sources and Mn-Met E with weak complex strength.

Experiment 2

Significant differences ($P < 0.001$) were observed in Mn contents of plasma from hepatic portal vein among treatments on d 7 and 9 of this experiment (Table 4). On d 7, Mn content in plasma was increased by 60.7 ($P = 0.008$), 71.4 ($P = 0.004$), and 107.1% ($P = 0.0001$) for $MnSO_4$, Mn-AA A, and Mn-AA B treatments, respectively, compared with the control. Manganese content in plasma was 6.7% higher for Mn-AA A treatment than for $MnSO_4$ treatment

Table 3. Absorption percentage (%) of Mn from different Mn sources in ligated duodenal, jejunal, and ileal loops of 4-wk-old broilers (Experiment 1)

	Mn absorption percentage				
Added Mn source	Duodenal loops	Jejunal loops	Ileal loops	Pooled SE	*P*-value
$MnSO_4 \times H_2O$	$0.40^{B,b}$	$0.59^{B,d}$	$6.35^{A,c}$	0.47	0.0001
$(MnSO_4 \times H_2O)$ + Gly	$1.27^{B,a}$	$0.75^{B,bcd}$	$7.03^{A,bc}$	0.53	0.0001
$(MnSO_4 \times H_2O)$ + Met	$1.22^{B,a}$	$1.08^{B,abc}$	$9.54^{A,ab}$	0.46	0.0001
Mn-Gly	$1.33^{B,a}$	$1.13^{B,a}$	$8.81^{A,abc}$	1.08	0.0002
Mn-Met	$1.49^{B,a}$	$1.38^{B,a}$	$10.8^{A,a}$	0.57	0.0001
Mn-Met E (weak complex strength)	$0.64^{B,b}$	$0.65^{B,d}$	$7.42^{A,bc}$	0.39	0.0001
Mn-AA A (moderate complex strength)	$1.09^{B,a}$	$1.08^{B,ab}$	$10.5^{A,a}$	0.52	0.0001
Mn-AA B (strong complex strength)	$1.44^{B,a}$	$1.20^{B,a}$	$11.1^{A,a}$	0.35	0.0001
Pooled SE	0.16	0.14	0.94		
P-value	0.0001	0.0061	0.0028		

[A,B]Means within rows with different superscripts differ significantly ($P < 0.05$).

[a–d]Means within columns with different superscripts differ significantly ($P < 0.05$).

Table 4. Effect of Mn source on plasma Mn content (μg/L) of blood from the hepatic portal vein of 21 to 23 d-old broilers (Experiment 2)

	Plasma Mn content	
Added Mn source	d 7 (21 d old)	d 9 (23 d old)
Control group	12.63[c]	11.84[c]
$MnSO_4 \times H_2O$	20.30[b]	17.77[b]
Mn-AA A (moderate complex strength)	21.66[ab]	20.19[b]
Mn-AA B (strong complex strength)	26.16[a]	25.39[a]
Pooled SE	1.99	1.36
P-value	0.0006	0.0001

[a–c]Means with different superscripts within the same column differ significantly ($P < 0.05$).

and 20.8% higher for Mn-AA B than for Mn-AA A, although the differences were not significant ($P > 0.10$). Manganese content in plasma was 28.9% higher ($P = 0.0297$) for Mn-AA B treatment than for $MnSO_4$. On d 9, Mn content in plasma was increased by 50.1 ($P = 0.004$), 70.6 ($P < 0.0001$), and 114.5% ($P < 0.0001$) for $MnSO_4$, Mn-AA A, and Mn-AA B treatment, respectively, compared with the control. Manganese content in plasma was 13.7% higher ($P > 0.10$) for Mn-AA A than for $MnSO_4$, 42.9% higher ($P = 0.0006$) for Mn-AA B than for $MnSO_4$, and 25.8% higher ($P = 0.0083$) for Mn-AA B than for Mn-AA A. The results showed that the absorption of Mn as Mn-AA B with strong complex strength was higher than that of Mn from inorganic Mn sources and Mn-AA A with moderate complex strength.

DISCUSSION

The results obtained in Experiment 1 for the main absorption site of Mn are consistent with the previous work in our laboratory with everted intestinal sacs in vitro (Ji et al., 2006). Once again, the experiment has clearly demonstrated the importance of the ileum as the main site at which Mn is absorbed by ligated loops of the small intestine of broilers in situ. The main intestinal absorption site for Mn has been investigated mainly with rats by several researchers; however, the problem has remained controversial (Sahagian et al., 1966; Thomson et al., 1971; Kaloudin and Ganovski, 1981). The techniques used, the length of the experimental periods, the species of animals, and different physiological conditions of animals in studies may explain the discrepancies among the research reports. Sahagian et al. (1966) found that Mn was absorbed maximally in the duodenum, and Mn uptake was least in the ileum by intact strips of rat intestine in vitro. However, in their study, the transport of Mn across intestinal wall was not considered. Garcia-Aranda et al. (1983) reported that Mn was better absorbed in the jejunum than in the ileum of rat using an in vivo perfusion system. But our data of in vitro and in vivo studies consistently showed that the absorption of Mn was greater in the ileum than in the duodenum or jejunum of broilers.

The data from Experiment 1 also indicated the differences of Mn absorption among several organic and inorganic sources. It could be concluded that Met was more effective in facilitating Mn absorption than Gly as a ligand. Organic Mn was more efficiently absorbed than inorganic Mn ($MnSO_4$) under the conditions of the experiment. Similar enhancements of Mn absorption have been reported in our previous study (Ji et al., 2006). Garcia-Aranda et al. (1983) found, in segments of either the jejunum or ileum of rats, that Mn absorption at a 2:1 ratio of ligand (either histidine or citrate) to Mn was 3 times greater than in the absence of any ligand with an in vivo perfusion system . Wapnir et al. (1983) quantitatively investigated the role of some amino acids, dipeptides, and organic acids as ligands facilitating the intestinal absorption of Zn and determined optimum ligand:Zn ratios for Zn absorption by in vivo perfused ileal segments of rats. A variety of small-molecular-weight compounds that chelated Zn and prevented its precipitation at physiological pH could facilitate the intestinal absorption of Zn in vivo. These complexes were most effective when the ligand:Zn ratios were 3:1 or less. It was postulated that the active transport of the 2:1 L-His:Zn complex occurred by the same intestinal translocation mechanisms as L-His alone, by finding that an excess of L-His acted as a competitive inhibitor of the L-His:Zn complex. However, Bonewitz et al. (1982) found that the role of low-molecular-weight compounds was to render dietary Zn more absorbable rather than providing an indispensable component for the translocation.

Research from our laboratory (Li et al., 2004) has shown that the Mn-AA A source with a moderate complex strength was the most available, and the Mn-AA B source with the strong complex strength had a tendency to be more available than the Mn-Met E source with the weak complex strength numerically, whereas the Mn-Met E source was similar to Mn sulfate. The criterion used to evaluate bioavailability values of organic Mn sources relative to Mn sulfate was heart MnSOD mRNA. The results indicated an effect of complex strengths of the organic Mn sources on their relative bioavailability values for broilers. The data obtained in Experiment 1 showed that the absorption of Mn as Mn-AA B and Mn-AA A were higher than inorganic Mn source and Mn-Met E. The regression of Mn absorption on complex strengths was not calculated, but the effects of complex strengths of these organic Mn sources on Mn absorption for broilers are consistent with the in vitro study reported previously (Ji et al., 2006).

Absorbed Mn is concentrated in the liver, and it is transported in the plasma bound to a β1-globulin, most likely transferrin (Tichy and Cikrt, 1972). The appearance of Mn in the hepatic portal plasma was evidence that the absorption of Mn from the Mn source was higher. Our findings on the Mn content of plasma from the hepatic portal vein indicated that the absorption of Mn from the organic Mn source with strong complex strength was higher than that of Mn from the organic Mn with moderate strength in broilers. The above results are in agreement with those of Experiment 1 in this study and the previous study (Ji et al., 2006).

There are 2 assumptions about the absorption mechanism of mineral complexes (Ashmead, 1993). One is that "complexed" or "chelated" trace minerals are absorbed in intact form, and the metal atoms remain safely bound or

protected within organic molecular structures or "ligands" during absorption. The other is that organic ligands can prevent the harmful effect of competitive ligands such as phosphate, phytate, and other compounds, which can bind free metal ions and render the minerals unavailable for absorption. Maintaining a dietary mineral in solution allows maximum opportunity for contact with intestinal mucosa. Neither the Association of Official Analytical Chemists (1995) nor the Association of American Feed Control Officials (2001) has approved definite methods to test the complex bonding of a mineral element to an organic ligand. This may be the reason why no direct evidence for any assumption could be found in the literature. The data from our study did not show the absorption mechanism of Mn as Mn complexes or chelates, but they indicated the differences of Mn absorption among several organic and inorganic sources. Therefore, further studies are needed to elucidate the absorption mechanism of Mn as Mn complexes or chelates.

In conclusion, the results from the present study clearly demonstrated that the absorption of "complexed" or "chelated" organic Mn was much higher than inorganic Mn in the small intestinal segments of broilers, which might be due to different absorption modes for organic and inorganic Mn. The absorptions of organic Mn with moderate and strong complex strengthes were higher than that of organic Mn with weak complex strength, and the absorption of organic Mn with strong complex strength was better than that with moderate complex strength. The previous finding in our laboratory that the ileum is the main site of Mn absorption for broilers was verified by the results of ligated intestinal loops in situ in this study. As a ligand, Met was more effective in facilitating Mn absorption than Gly.

REFERENCES

Association of American Feed Control Officials. 2001. Pages 282–283 in Official Publication. Assoc. Am. Feed Control Offic. Inc. Atlanta, GA. .

Association of Official Analytical Chemists. 1995. Official Methods of Analysis. 16th ed. Assoc. Offic. Anal. Chem., Arlington, VA.

Ashmead, H. D. 1993. Comparative intestinal absorption and subsequent metabolism of metal amino acid chelates and inorganic metal salts. Pages 32–57 in The Roles of Amino Acid Chelates in Animal Nutrition. Noyes Publishers, Park Ridge, NJ.

Bonewitz, R. F., C. Voner, and E. C. Foulkes. 1982. Uptake and absorption of zinc in perfused rat jejunum: The role of endogenous factors in the lumen. Nutr. Res. 2:301–307.

Dong, X. H. 2001. Pages 46–54 in Study on absorption, transportation characteristics of zinc amino acid chelate and influencing factors. PhD dissertation. Northeast Agricultural University, Harbor City, Heilongjiang, China.

Fly, A. D., O. A. Izquierdo, K. L. Lowry, and D. H. Baker. 1989. Manganese bioavailability in a manganese-methionine chelate. Nutr. Res. 9:901–910.

Garcia-Aranda, J. A., R. A. Wapnir, and F. Lifshitz. 1983. In vivo intestinal absorption of manganese in the rat. J. Nutr. 113:2601–2607.

Hempe, J. M., and R. J. Cousins. 1989. Effect of EDTA and zinc-methionine complex on zinc absorption by rat intestine. J. Nutr. 119:1179–1187.

Henry, P. R., C. B. Ammerman, and R. D. Miles. 1989. Relative bioavailability of manganese in a manganese-methionine complex for broiler chicks. Poult. Sci. 68:107–112.

Ji, F. 2003. Page 53 in In vitro and in vivo studies on absorptions of manganese as organic manganese sources in the small intestine of broilers. PhD dissertation. Chinese Academy of Agricultural Sciences, Beijing.

Ji, F., X. G. Luo, L. Lu, B. Liu, and S. X. Yu. 2006. Effects of manganese source and calcium on manganese uptake by in vitro everted gut sacs of broiler's intestinal segments. Poult. Sci. 85:1217–1225.

Kaloudin, I., and H. Ganovski. 1981. On the absorption of manganese and zinc in infantile, mature and pregnant animals. Vet. Sci. 18:64–69.

Liu, W., and G. Bi. 1995. Determination of micro amounts of Mn, Ni, Co and Cr in blood serum using graphite furnace atomic absorption spectrometry. Spectrosc. Spec. Anal. 15:83–86.

Li, S., X. Luo, B. Liu, T. D. Crenshaw, X. Kuang, G. Shao, and S. Yu. 2004. Use of chemical characteristics to predict the relative bioavailability of supplemental organic manganese sources for broilers. J. Anim. Sci. 82:2352–2363.

Li, S., X. G. Luo, L. Lu, T. D. Crenshaw, Y. Q. Bu, B. Liu, X. Kuang, G. Z. Shao, and S. Yu. 2005. Bioavailability of organic manganese sources in broilers fed high dietary calcium. Anim. Feed Sci. Technol. 123–124:703–715.

Lu, L., C. Ji, X. G. Luo, B. Liu, and S. X. Yu. 2006. The effect of supplemental manganese in broilers diets on abdominal fat deposition and meat quality. Anim. Feed Sci. Technol. 129:49–59.

Luo, X. G., Q. Su, J. C. Huang, and J. X. Liu. 1991. A study on the optimal manganese Mn level in a practical diet of broiler chicks. Chin. J. Anim. Vet. Sci. 22:313–317.

Melvin, J. S. 1984. Page 359 in Duke's Physiology of Domestic Animals. 10th ed. Cornell Univ. Press, Ithaca, NY.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Sahagian, B. M., I. Harding-Barlow, and H. M. Perry Jr. 1966. Uptake of zinc, manganese, cadmium and mercury by intact strips of rat intestine. J. Nutr. 90:259–267.

SAS Institute. 1989. SAS User's Guide: Statistics. Version 6.03. SAS Inst. Inc., Cary, NC.

Schedl, H. P., D. Miller, and D. White. 1966. Use of polyethylene glycol and phenol red as unabsorbed indicators for intestinal absorption studies in man. Gut 7:159–163.

Scott, M. L., M. C. Nesheim, and R. J. Young. 1976. Nutrition of the Chicken. M. L. Scott of Ithica, NY.

Teng, B., and Y. W. Han. 2001. The synthesis and preparation of zinc methionine chelate. Feed Extension Suppl.: 1–4.

Thomson, A. B. R., D. Olatunbosun, and L. S. Valberg. 1971. Interrelation of intestinal transport system for manganese and iron. J. Lab. Clin. Med. 78:642–655.

Tichy, M., and M. Cikrt. 1972. Manganese transfer into the bile in rats. Arch. Toxicol. 29:51–58.

Wapnir, R. A., D. E. Khani, M. A. Bayne, and F. Lifshitz. 1983. Absorption of zinc by the rat ileum: Effects of histidine and other low-molecular-weight ligands. J. Nutr. 113:1346–1354.

Zhang, T. Y. 2002. Pages 29–34 in Technique of phytase evaluation by digestion in vitro. PhD dissertation. Chinese Academy of Agricultural Sciences. Beijing.

Zhao, J., J. Sun, and S. Jiang. 1988. Zeeman graphite furnace atomic absorption spectrometric determination of trace chromium and manganese in human serum. Environ. Chem. 7:29–33.