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Evaluation of Chemical Characteristics and Effects of Different Manganese Sources on Kinetics of Manganese Absorption and Performance of Broiler Chickens

Research Article

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ABSTRACT

Three experiments were conducted to evaluate chemical characteristics, intestinal absorption and bioavailability of manganese (Mn) from organic, inorganic and nano sources of Mn. In experiment 1, inorganic sources of Mn including Mn-sulphate and Mn-oxide, organic sources of Mn as Mn-glycinate and Mn-bioplex and FRA® easy dry Mn as a nano source of Mn were subjected to elemental analysis and solubility in deionized water, 0.4% hydrochloric acid, 2% citric acid and neutral ammonium citrate. In the experiment 2, intestinal absorption of Mn from these sources was investigated by *in vitro* everted gut sacs technique in broiler chicks. In the experiment 3, the bioavailability of Mn-sulphate, Mn-Glycinate and FRA® easy dry Mn was determined in chicks fed a corn-soybean meal-basal diet that was supplemented with 0, 40, 100, and 160 mg of Mn from these sources per kg of diet based on body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) for 21 days from d 7 to 28. The results showed Mn-sulfate dissolved completely in all solvents. The solubility of all Mn sources was the lowest and the highest in deionized water and neutral ammonium citrate, respectively. The uptake percentages of Mn as nano Mn and Mn-oxide were significantly the highest and the lowest by duodenal and jejunal sacs, respectively. Mn as either Mn-Glycinate or nano Mn was absorbed more efficiently than Mn from other sources by ileal sacs. Amoung organic and inorganic sources, Mn-Glycinate and Mn-sulfate had the higher Mn absorption, respectively. BWG, FI and FCR did not affect by either Mn level or source. We concluded that ileum was the main site of Mn absorption for broilers and among different Mn sources, Mn-Glycinate and nano Mn had the highest Mn absorption. Furthermore, growth was not appropriate criteria to assess bioavailability of different Mn sources.

KEY WORDS absorption, bioavailability, broiler, chemical characteristics, everted gut sac.

INTRODUCTION

Manganese is an essential trace mineral, plays an important role in the body and bone development, physiological function, biosynthetic processes, amino acid metabolism and various enzymatic systems (Rubio Zapata, 2016). Because of the negative effect of rapid growth on skeletal development, poultry species have relatively high requirement for Mn (Angel, 2007), so Mn is routinely added to poultry diets in the organic and inorganic forms. Several reports indicated that organic forms of Mn had higher absorptivity (Ji *et al.* 2006a; Bai *et al.* 2012) and bioavailability (Li *et al.* 2005; Brooks *et al.* 2012) than the inorganic forms. One of the possible reasons is that there are less chelating or other

unwanted reactions with dietary constituents in the gastrointestinal tract for organic mineral complexes compared with those for inorganic minerals (Ammerman *et al*. 1995). Nano forms of minerals are new forms that have been recently used in poultry nutrition. Nano particles showed new characteristics of transport and uptake pattern and exhibited higher absorption efficiencies (Davda and Labhasetwar, 2002; Zha *et al*. 2008). Bioavailability studies of Mn include *in vitro* (everted gut sacs, solubility of Mn in various solutions) and *in vivo* (ligated gut loops, growth and perosis index, tissue concentration and Mn radioisotopes) estimates of bioavailability. Bioavailability is defined as a part of the mineral that is absorbed, transported to the site of action and converted to its physiological active form (Ammerman *et al*. 1995). Therefore, bioavailability alone does not imply absorption, but refers to mineral consumption for a particular function (Li *et al*. 2005). Although absorption and bioavailability of different organic and inorganic Mn sources have been investigated by *in vitro* and *in vivo* techniques previously, but solubility, absorption and bioavailability of different sources of organic, inorganic and nano Mn have not been compared, simultaneously. Therefore, the main objectives of our studies was firstly evaluation chemical characteristics and absorption of Mn from different organic, inorganic and nano sources of Mn by *in vitro* methods like solubility and everted gut sacs techniques and then evaluation the effects of three Mn sources (that had the highest absorption values in experiment 2) on BWG, FI and FCR of broilers to find relationship between *in vitro* (solubility and everted gut sacs techniques) and *in vivo* (performance) estimates of bioavailability in broilers.

MATERIALS AND METHODS

Experiment 1

Mineral analysis of Mn sources and their solubility in neutral ammonia citrate, citric acid, HCl and deionized H_2O

Different Mn sources included feed grade Mn sulphate ($MnSO_4.H_2O$) and Mn-oxide (Mn_3O_4) as inorganic sources, Mn-chelate of protein hydrolysate (Bioplex®Mn; Alltech Inc., Nicholasville, USA) and Mn-chelate of glycine hydrate (Mn-glycinate) (E.C.O.Trace® Mn; Biochem, Germany) as organic sources and FRA® Easy Dry Manganese (Framelco, The Netherlands) as a nano source of Mn.

Except for P, mineral concentrations of Mn sources were determined by flame atomic absorption spectrophotometry (Model AA-670, Shimadzu). For this aim, 1 g of each Mn source was weighed in triplicate and dried at 105 ˚C for 12 h, ashed at 550 ˚C for 16 h, solubilized in hydrochloric acid and filtered through ashless Whatman 42 filter paper. Phosphorus was determined by a colorimetric method (AOAC, 2000). Mn solubility of all Mn sources was determined in triplicate by mixing 0.1 g sample with 100 mL of neutral ammonia citrate (NAC), 2% citric acid (CA), 0.4% HCl and deionized H_2O. Mixtures were stirred constantly while incubating at 37 ˚C for 1 h and then filtered through Whatman 42 filter paper. The Mn in the filtrates were assumed to be soluble and the values obtained were expressed as a percentage of the total Mn in the source. Soluble and insoluble fractions of Mn in the Mn sources were also determined in deionized H_2O as described by Leach and Patton (1997). A 2 g sample of each source was mixed with 150 mL of deionized H_2O, shaken at 25 ˚C in a water bath for 30 min and then passed through Whatman 42 ashless filter paper into 200 mL volumetric flasks and rinsed with deionized H_2O to bring the flask to volume. Then the filter paper was burned at 500 ˚C to determine insoluble Mn content. After filtration and dissolving the ash of filter paper containing insoluble Mn at 5 mL 20% HCl, the amount of Mn was determined and the solubility of Mn was calculated based on Mn content of the initial sample, filtered sample and the Mn of the residue on the filter paper.

Experiment 2

Evaluation kinetics of Mn absorption

Birds and diets

This study was conducted in the poultry research farm of Tabriz University (Tabriz, Iran). A total of 150 one-day-old male Ross 308 broiler chicks were used in a completely randomized design to evaluate the effect of Mn sources on the intestinal absorption of Mn by everted gut sacs technique. All birds received a corn-soybean meal diet (118.65 mg of Mn/kg of diet; Table 1) formulated according to nutrients recommended by the Brazilian tables for poultry and swine (Rostango *et al*. 2011) from d 1 to 21 and a basal corn-soybean meal diet (19.35 mg of Mn/kg of diet; Table 1) for 1 wk from d 21 to deplete the body Mn stores. Mn-free mineral supplement for preparation basal diet was made by OrumFardaneh company (Livestock and poultry food supplements, Orumieh, West Azarbyjan, Iran). This mixture was formulated so that all minerals (except Mn) met or exceeded the Ross (308) broiler requirements.

During the experimental period, all chicks had *ad libitum* access to feed and water containing no detectable Mn. Birds were exposed to 24 h light during the experiment. The vaccination program was carried out in accordance with the advice of the veterinary administration of the region. On d 28, after an overnight fasting, chicks were weighed (1110±90 g, Means±SD) and thirty chicks of them allotted randomly to 6 replicate groups (1 chicks per replicate cage) for each of 5 treatments so that the average body weight of birds for each treatment was nearly equal to avoid the effect of body weight in the results.

Table 1 Composition of the broilers diets (experiment 2)

Ingredient (%)	Days 1-21	Days 22-28[2]
Ground yellow corn	54.24	56.16
Soybean meal	38.39	35.78
Soybean oil	3.78	4.87
Dicalcium phosphate	1.03	0.86
Limestone	1.24	1.13
Salt	0.44	0.41
DL-Met	0.25	0.21
L-lysine HCL	0.13	0.08
Micronutrients[1]	0.50	0.50
Calculated nutrient analysis		
Metabolizable energy (kcal/kg)	3000	3100
Crude protein (%)	21	20
Dig Lys (%)	1.17	1.07
Dig Met (%)	0.54	0.49
Dig Met + Cys (%)	0.85	0.78
Ca (%)	0.82	0.73
Available P (%)	0.35	0.31
Mn (mg/kg)[3]	118.65	19.35

[1] Provided (per kilogram of diet) for days 1-21: vitamin A (all-trans retinol acetate): 9000 IU; Cholecalciferol: 2000 IU; vitamin E (all-rac-α-tocopherol acetate): 18 IU; vitamin K (menadione Na bisulfate): 2.0 mg; Thiamine (thiamine mononitrate): 1.8 mg; Riboflavin: 6.6 mg; vitamin B_6: 3.0 mg; vitamin B_{12}: 0.0015 mg; Pantothenate: 20 mg; Niacin: 30 mg; Folic acid: 1 mg; Biotin: 0.1 mg; Choline (choline chloride): 500 mg; Cu ($CuSO_4 \times 5H_2O$): 10 mg; Mn (Mn_3O_4): 100 mg; Zn ($ZnSO_4 \times 7H_2O$): 85 mg; Fe ($FeSO_4 \times 7H_2O$): 50 mg and I (KI): 1 mg; Se (Na_2SeO_3): 0.2 mg.
[2] For days 22-28 Mn-free mineral supplement was fed to broilers for depletion body Mn stores.
[3] Determined by analysis.

Everted gut sac procedure

The solutions of everted gut sac technique and experimental protocols

The chicks were anesthetized by injection of anesthetic (ketamine 0.1 mg/kg) into the wing vein; a middle abdominal incision was made. The intestine was excised immediately and rinsed free digesta with 39 to 40 ˚C physiologic saline. Sacs of approximately 10 cm length were prepared from duodenum, jejunum and ileum. Everted gut sac procedure, preparation of different solutions injected into different intestinal segments and preparation of incubation solutions were done according to the method described previously by Ji *et al*. (2006a). Phenol red (nonabsorbable reference indicator) was used to correct for changes in Mn concentration resulting from water absorption or intestinal secretion (Ji *et al*. 2006a).

Measurement methods and calculation formulas

Mn concentration in the fluid was determined by flame atomic absorption spectrophotometry (Model AA-670, Shimadzu). The concentrations of phenol red in initial and final serosal fluid were determined by measuring absorbency at 520, 560 and 600 nm (Ji *et al*. 2006a) with an UV-Vis spectrophotometer (Model Milton Roy Spectronic 2OD). The final volume of serosal fluid and uptake percentage of Mn transferred across the gut wall was computed according to equations below (Ji *et al*. 2006a):

$V_f = CP_1 \times V_I / CP_2$

$Up = ((C_{Mn} \times V_f \times 100)/(120 \times 20 \times W)) \times 100$

CP_1 and CP_2: initial and final concentration (mmol/L) of phenol red, respectively.

V_I and V_f: initial and Final volume (mL) of serosal fluid.

C_{Mn}: Mn concentration (mg/L) of final serosal fluid.

120: Mn concentration (mg/L) of initial incubation media.

20: volume (mL) of initial incubation media.

W: wet weight (g) of intestinal segment used to prepare everted sacs.

All calculations of Mn uptake (%) were based on 100 g of wet weight of intestinal segment.

Experiment 3

Evaluation bioavailability of Mn sources

This experiment was conducted with 250 one-day-old male Ross 308 broiler chicks in the poultry research farm of Tabriz University (Tabriz, Iran). The chicks were fed a corn-soybean meal based diet (22.40 mg of Mn/kg of diet; Table 6) without supplemental Mn for 1 week post-hatching for depletion body Mn store. At the end of the 7-d depletion period, chicks were randomly allocated into 5 replicates (5 chicks per pen) for each of 10 treatment groups in a completely randomized design involving a 3 × 3 factorial arrangement of treatments (three sources of Mn×three levels of added Mn plus a control with no added Mn). The grower basal diet (20.50 mg of Mn/kg of diet; Table 6) was supplemented with 0, 40, 100 and 160 mg of Mn/kg from three Mn sources that had higher absorption in each group of organic, inorganic and nano sources of Mn in experiment 2.

Mn-glycinate (in comparison with Mn-bioplex), Mn-sulfate (in comparison with Mn-oxide) and nano-Mn had higher absorption. Each experimental diets were fed for 21 d (d 7 to 28). The basal diet formulated according to nutrients recommended by the Brazilian tables for poultry and swine (Rostango *et al*. 2011) to meet or exceed the requirements except for Mn. Chicks were maintained on a 24 h constant-light schedule and allowed ad libitum access to experimental diets and tap water containing no detectable Mn. Analyzed concentrations of Mn in the experimental diets are shown in Table 7. BW and FI were measured (by pen) weekly for the determination of BWG, FI, and FCR.

Statistical analysis

The GLM procedure of SAS (2003) was applied to analyze data of experiments. Data in experiment 2 were analyzed by one-way ANOVA with a model that included Mn source and different segments of broiler intestine. Data from experiment 3 were subjected to two-way ANOVA with a model that included Mn source and added Mn level as main effects and the interaction of Mn source × Mn level. Differences between means were analyzed with Duncan's multiple tests. The significant difference statements were based on the possibility ($P<0.05$).

RESULTS AND DISCUSSION

Experiment 1

Evaluation chemical characteristics are important in predicting the biological value of the different minerals sources. Constituents and chemical characteristics of organic, inorganic and nano forms of Mn are presented in Table 2.

Dry matter percentage of different sources varied from 85.9% in nano Mn to 99.5% in Mn-sulfate. Mn-bioplex and Mn-oxide showed the lowest (36%) and the highest (95%) amount of ash, respectively. The concentration of Mn in organic, inorganic and nano sources varied from 15.5% in Mn-bioplex to 32% in Mn-sulfate. The amount of phosphorus was low in all sources except the Mn-bioplex (0.84%).

The levels of toxic elements (cadmium, lead and arsenic) in different sources of Mn were zero. The concentration of other micro and macro-elements are shown in Table 2.

Solubility of different Mn sources are presented in Table 3. Mn-sulfate dissolved completely in deionized water, 0.4% hydrochloric acid, 2% citric acid and neutral ammonium citrate and its solubility were also equal in the all solvents. Mn-oxide had low solubility in deionized water (0.19%) but it was soluble 95, 93 and 96% in 0.4% hydrochloric acid, 2% citric acid and neutral ammonium, respectively.

The solubility of Mn-bioplex and Mn-glycinate in deionized water, 0.4% hydrochloric acid, 2% citric acid and neutral ammonium citrate were 86, 93, 89, 95 and 75, 94, 92, 97 %, respectively. The solubility of Mn as nano Mn was 45, 90, 74 and 90% in deionized water, 0.4% hydrochloric acid, 2% citric acid and neutral ammonium citrate, respectively.

The solubility of all Mn sources were the lowest and the highest in deionized water and neutral ammonium citrate, respectively. The average solubility of Mn-sulfate and Mn-oxide in 4 solvents were the highest and the lowest, respectively.

Experiment 2

The absorption percentage of Mn as different sources in everted intestinal sacs of broiler are presented in Tables 4 and 5.

The results showed that the uptake percentages of Mn by ileal sacs had a significant difference with those by duodenal and jejunal sacs for all the Mn sources ($P<0.05$). The uptake percentages of Mn by averted ileal sacs were about 3 to 6 times higher than those by duodenal and jujenal sacs for all Mn sources. Although the differences were not significant ($P>0.10$), the uptake percentages of Mn by jejunal sacs were higher than those by duodenal sacs for all the Mn sources.

The absorption percentage of Mn as Mn-oxide was lower than those of Mn as other Mn sources in the 3 intestinal segments. In duodenal sacs, Mn uptake was affected by Mn sources ($P<0.05$) and the uptake percentages of Mn as nano Mn and Mn-oxide were the highest and the lowest among them, respectively. Amoung organic and inorganic sources, Mn-glycinate and Mn-sulfate had the higher Mn absorption, respectively.

Absorption of Mn among nano Mn, Mn-glycinate and Mn-sulfate were not significant by duodenal sacs ($P>0.10$). In jejunal sacs, the absorption percentages of Mn as nano Mn and Mn-glycinate had the highest and Mn-oxide had the lowest absorption. In ileal sacs, Mn as either Mn-glycinate or nano Mn was absorbed more efficiently ($P<0.05$) than Mn from other sources.

The uptake of Mn as Mn-glycinate in duodenal ($P>0.10$), jejunal ($P>0.10$) and ileal ($P<0.05$) sacs was 41.62, 43.88 and 39.89% higher than Mn-bioplex, respectively. The uptake of Mn as Nano-Mn in duodenal and jejunal sacs was 28.62 ($P>0.10$) and 23.16% ($P>0.10$) higher and by ileal sac was 25.46% ($P>0.10$) lower than Mn-glycinate, respectively.

As well the uptake of Mn as nano-Mn by duodenal, jejunal and ileal sacs was 82.16, 77.21 ($P<0.05$) and 11.50% ($P>0.10$) higher than Mn-bioplex, respectively.

Table 2 Chemical composition of Mn sources on an as-fed basis

Sources	DM (%)	Ash (%)	Mn (%)	Ca (%)	P (%)	Zn (mg/kg)	Fe (mg/kg)	Cu (mg/kg)
$MnSO_4.H_2O$	99.50	88.60	32	0.52	0.005	1.84	0.006	0.45
Mn_3O_4	98.10	95	22	3.60	0.06	2.03	1.85	0.18
Mn-bioplex	88.50	36	15.50	0.50	0.84	7.34	109	1.56
Mn-glycine	98.60	57	20	0.70	0.04	7.14	11.32	0.68
Nano-Mn	85.90	53	16.50	0.60	0.006	0.127	0.08	0.57

Table 3 Solubility of Mn sources in deionized water (dH2O), neutral ammonium citrate (NAC), 2% citric acid (CA) and 0.4% HCl (percent)

Sources	dH2O	NAC	HCl	CA
$MnSO_4.H_2O$	100	100	100	100
Mn_3O_4	0.19	96	95	93
Mn-bioplex	86	95	93	89
Mn-glycinate	75	97	94	92
Nano-Mn	45	90	90	74

Table 4 Uptake percentage (%) of Mn as each source in the everted sacs of duodenum, jejunum and ileum of 28 days old broilers

Intestinal segment	Sources of manganese				
	$MnSO_4.H_2O$	Mn_3O_4	Mn-bioplex	Mn-glycinate	Nano-Mn
Duodenal sac	0.444^{b}	0.146^{b}	0.370^{b}	0.524^{b}	0.674^{b}
Jejunal sac	0.580^{b}	0.186^{b}	0.474^{b}	0.682^{b}	0.840^{b}
Ileal sac	1.933^{a}	0.523^{a}	2.339^{a}	3.272^{a}	2.608^{a}
SE	0.26	0.11	0.29	0.4	0.58
P-value	0.0001	0.0001	0.0001	0.0001	0.0001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SE: standard error.

Table 5 Uptake percentage (%) of Mn as different source in each part of the intestinal everted sacs of 28 days old broilers

Added Mn source	Intestinal segment		
	Duodenal sac	Jejunal sac	Ileal sac
$MnSO_4.H_2O$	0.444^{ab}	0.580^{b}	1.933^{b}
Mn_3O_4	0.146^{c}	0.186^{c}	0.523^{c}
Mn-bioplex	0.370^{b}	0.474^{b}	2.339^{b}
Mn-glycinate	0.524^{ab}	0.682^{ab}	3.272^{a}
Nano-Mn	0.674^{a}	0.840^{a}	2.608^{ab}
SE	0.18	0.17	0.58
P-value	0.0009	0.0001	0.0001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SE: standard error.

The uptake of Mn as Mn-glycinate by duodenal, jejunal and ileal sacs was 18.01, 17.58 (P>0.10) and 69.27% (P<0.05) higher than Mn-sulphate and 258.90, 266.67 and 525.62% (P<0.05) higher than Mn-oxide, respectively. The absorption percentage of Mn as Mn-bioplex was 20 and 22.36% lower and 21.01% higher than that of Mn as Mn-sulphate by duodeal, jejunal and ileal sacs, respectively, although this differences were not significant (P>0.10). The absorption of Mn as Mn-bioplex in duodenal, jejunal and ileal sacs was 153.42, 154.83 and 347.23% (P<0.05) higher than Mn-oxide, respectively.

Experiment 3

The effects of different Mn sources and different levels of Mn on broiler performance at 28 d of age are shown in Table 8. The results showed that different sources and different levels of Mn did not have significant effects on BWG, FI and FCR of broilers during the whole experimental period.

The high level of phosphorus in Mn-bioplex probably related to the hydrolyzed proteins used as ligands in the chelated products (Cao *et al*. 2000).

Solubility of Mn-bioplex in deionized water was higher than Mn-glycinate. It was reported that proteinates as ligands are weaker chelate and they have less stability than glycine complexes when dissolved in H_2O (Cao *et al*. 2000).

The low solubility of nano-Mn in deionized water is probably due to its structure (lauric acid and glycerin salts). Solubility of minerals in different solvents is an *in vitro* method to predict bioavailability of these sources in animals body, based on relative solubility values averaged over the four solvents, the relative bioavailability of different Mn sources could be predicted (Ledoux *et al*. 1991).

Table 6 Composition of the broilers diets (experiment 3)

Ingredient (%)	Days 1-7	Days 8-28
Ground yellow corn	48.90	55.17
Soybean meal	43.23	37.65
Soybean oil	3.97	3.45
Dicalcium phosphate	1.23	0.83
Limestone	1.30	1.13
salt	0.46	0.41
DL-Met	0.24	0.21
L-lysine HCL	0.17	0.15
Micronutrients[1]	0.50	0.50
Corn starch + Mn[2]	-	0.50
Calculated nutrient analysis		
Metabolizable energy (kcal/kg)	2950	3100
Crude protein (%)	22.60	20.80
Dig Lys (%)	1.31	1.17
Dig Met (%)	0.55	0.49
Dig Met + Cys (%)	0.86	0.78
Ca (%)	0.90	0.73
Available P (%)	0.39	0.31
Mn (mg/kg)[3]	22.40	20.50

[1] Provided (per kilogram of diet) for days 1-21: vitamin A (all-trans retinol acetate): 9000 IU; Cholecalciferol: 2000 IU; vitamin E (all-rac-α-tocopherol acetate): 18 IU; vitamin K (menadione Na bisulfate): 2.0 mg; Thiamine (thiamine mononitrate): 1.8 mg; Riboflavin: 6.6 mg; vitamin B_6: 3.0 mg; vitamin B_{12}: 0.0015 mg; Pantothenate: 20 mg; Niacin: 30 mg; Folic acid: 1 mg; Biotin: 0.1 mg; Choline (choline chloride): 500 mg; Cu ($CuSO_4 \times 5H_2O$): 10 mg; Mn (Mn_3O_4): 100 mg; Zn ($ZnSO_4 \times 7H_2O$): 85 mg; Fe ($FeSO_4 \times 7H_2O$): 50 mg and I (KI): 1 mg; Se (Na_2SeO_3): 0.2 mg.
[2] Mn supplements added in place of equivalent weights of cornstarch.
[3] Determined by analysis.

Table 7 Calculated and analyzed manganese concentrations of diets fed to broilers (7-28 d)[1]

Mn sources	Added Mn (mg/kg)	Dietary Mn[2] (mg/kg)
Control	0	20.50
	40	58
$MnSO_4.H_2O$	100	119.50
	160	180.22
	40	59.10
Mn-glycinate	100	120.34
	160	181.10
	40	60.45
Nano-Mn	100	123.90
	160	180.62

[1] See Table 6 for composition of the basal diet.
[2] Values based on chemical analysis of triplicate samples of diets. Values for Mn concentrations are reported on an as-fed basis.

Black *et al*. (1984) reported that there was relationship between the solubility of inorganic Mn sources in neutral ammonium citrate and their bioavailability in chicks and sheep. In the present experiments, no relationship between solubility and bioavailability values (absorption and growth parameters) were observed. Li *et al*. (2004) also found no relationship between solubility and bioavailability values of different Mn sources. For organic mineral sources, solubility is partly a function of the ligand, thus the solubility results may be misleading (Li *et al*. 2004). The results obtained from everted gut sacs technique showed that the main absorption site of Mn is everted ileal sac of broilers and this is consistent with previously *in vitro* (Ji *et al*. 2006a) and *in vivo* studies (Ji *et al*. 2006b; Bai *et al*. 2008) showed that Mn as different sources was absorbed more efficiently by everted and ligated ileal sacs, respectively.

Some researchers reported inconsistent results, Moshtaghie *et al*. (2006) found that absorption percentage of Mn by averted duodenal sacs of rats' intestine was significantly highest. They reported the absorption of Mn by duodenum is approximately 12 and 14% higher than jejunum and ileum, respectively.

The discrepancies among the research reports can be explained by the techniques used, the species of animals and the length of experimental period . As well as, decreasing the thickness of ileum and presence of a gradient of absorption capacity, increased absorption of Mn in ileum (Ji *et al*. 2006a). It seems that the main absorption site of most minerals is ileum. Yu *et al*. (2008) reported that zinc absorption by ileum was 1 to 5 times greater than those by duodenum and jejunum at different Zn concentrations (as $ZnSO_4.7H_2O$).

Table 8 Effects of different levels and sources of manganese on growth performance of broiler chicks fed with corn-soybean meals from 7 to 28 days of age

Main effects	BWG (g)	FI (g)	Feed / gain (g/g)
Control	934.12	1686.10	1.81
Mn sources			
$MnSO_4.H_2O$	854.77	1546.83	1.83
Mn-glycinate	816.97	1560.70	1.95
Nano-Mn	896.80	1574.17	1.76
Mn level (mg/kg)			
40	838.95	1525.37	1.85
100	861.65	1550.67	1.82
160	867.94	1605.67	1.87
P-value			
Mn source	0.16	0.84	0.15
Added Mn level	0.76	0.23	0.87
Source × level	0.68	0.08	0.71
SE	15.91	19.63	0.03

BWG: body weight gain and FI: feed Intake.
SE: standard error.

Uptake percentage of Mn as organic Mn (Mn-glycinate and Mn-bioplex) was greater than inorganic sources (Mn-sulphate and Mn-oxide). Ji *et al*. (2006b) reported that organic Mn sources (Mn-Met or Mn-AA) had higher absorption than inorganic source of Mn (Mn-sulphate) in the 3 intestinal segments of broilers.

Several studies indicate that organic sources of trace minerals such as amino acid complexes, chelates and proteinates have higher absorbility than inorganic forms (Li *et al*. 2005). Organic sources of minerals increase the intestinal absorption of trace elements, they prevent the formation of insoluble complexes with micro elements (Nollet *et al*. 2007; Rubio Zapata, 2016).

Significant differences were not found (P>0.10) in Mn uptake as Mn-sulphate with nano-Mn, Mn-glycinate and Mn-bioplex by duodenal sacs. In addition, these differences between Mn-sulphate, Mn-glycinate and Mn-bioplex were not significant (P>0.10) by incubating jejunal sacs. At ileal sacs, the differences between Mn-sulphate, Mn-bioplex and nano-Mn were not significant (P>0.10).

Ji *et al*. (2006a) consistently showed the differences between Mn-sulphate and Mn-glycinate were not significant in 3 intestinal segments.

It has been reported that there was not significant difference between the uptake of organic (Zn-Met and Zn-Lys) and inorganic ($ZnCl_2$) zinc at everted duodenal sacs of rats. The reasons may be due to different species and different physiological state of the animals used in experiments (Ji *et al*. 2006a).

Mn as Mn-glycinate was absorbed more efficiently than Mn from Mn-bioplex in the 3 intestinal segments of broilers, although except ileum, the differences were not significant in duodenum and jejunum (P>0.10). Low molucular weight of Glycine in comparison to hydrolyzed protein could be explained this.

Absorption of Mn as nano Mn was higher than other sources by duodenal and jejunal sacs, but absorption of Mn as Mn-glycinate was higher than nano-Mn by ileal sacs.

Although the differences were not significant but the probably reason of highly absorption of nano-Mn in duodenum and jejunum is special propertise of nano materials. Highly absorption of nano-Mn (FRA-easy dry Mn) is probably because of its nano scale. Every particle of FRA-easy dry Mn is individually coated and embedded in a fat matrix.

Once the material is in the form of small sized particles is rapidly cooled in the liquid medium. These coating benefits are improved target release, improved stability in the low pH environment of the upper gastrointestinal tract. It has been reported that nanoparticle showed new characteristics of transport and uptake and exhibited higher absorption efficiencies, some properties of nano materials are great specific surface area, high surface activity, a lot of surface active centers and high catalytic efficiency (Zhang *et al*. 2001; Mohapatra *et al*. 2014).

Effects of different nano minerals like selenium (Mohapatra *et al*. 2014) chromium (Sirirat *et al*. 2012) and zinc (Zaboli *et al*. 2013) on performance, immune system and minerals retention at broilers tissues have been investigated and positive results have been reported. Probably reason of high absorption of Mn-glycinate in comparison to nano-Mn in ileum is change of the thickness of the intestinal wall, which decreased as the more distal intestine, so absorption of Mn as Mn-glycinate increased more than nano-Mn in ileum.

BWG, FI and FCR were not affected by Mn source or level. Similar results were reported by Brooks *et al*. (2012) when feeding Mn-propionate and Mn-sulphate at levels of 0, 20, 100 and 500 mg Mn/ kg diet. Also Wang *et al*. (2012) found different levels of Mn (0, 60, 120 and 180

mg/kg) from Mn-sulphate and Mn-proteinate did not affect BWG, FI and FCR. Li *et al*. (2004) and Li *et al*. (2011) also reported that different sources, levels of Mn and their interactions did not have significant effects on BWG, FI and FCR. Yan and Waldroup (2006) reported that reagent-grade $MnSO_4$ and a 2-hydroxy-4-(methylthio) butanoic acid chelate of Mn at levels of 0 to 100 mg of Mn/kg did not have significant effects on performance of broilers.

CONCLUSION

As a result of this study, Mn-sulfate dissolved completely in all solvents. The solubility of all Mn sources were the lowest and the highest in deionized water and neutral ammonium citrate, respectively. The avearage solubility of Mn-sulfate and Mn-oxide in 4 solvents were the highest and the lowest, respectively. Also, it has been found that ileum being the main absorption site of Mn. Mn-glycinate and nano-Mn was absorbed more efficiently than other Mn sources. Mn-glycinate and Mn-sulphate had the highest absorption among organic and inorganic sources, respectively. There was no relationship between *In vitro* and *In vivo* estimates of Mn bioavailability like solubility, averted gut sacs technique and performance of broilers. It is recommended to use either Mn-glycinate or nano-Mn in broilers nutrition, because of high absorption values.

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REFERENCES

Ammerman C.B., Baker D.H. and Lewis A.J. (1995). Bioavailability of Nutrients for Animals Amino Acids, Minerals, and Vitamins. Academic Press, Massachusetts, USA.

Angel R. (2007). Metabolic disorders: limitations to growth of and mineral deposition into the broiler skeleton after hatch and potential implications for leg problems. *J. App. Poult. Res*. **16,** 138-149.

AOAC. (2000). Official Methods of Analysis. 17th Ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Bai S.P., Lu L., Luo V. and Liu B. (2008). Kinetics of manganese absorption in ligated small intestinal segments of broilers. *Poult. Sci*. **87,** 2596-2604.

Bai S.P., Lu L., Wang R.L., Xi L., Zhang L.Y. and Luo X.G. (2012). Manganese source affects manganese transport and gene expression of divalent metal transporter 1 in the small intestine of broilers. *Br. J. Nutr*. **108,** 267-276.

Black J.R., Ammerman C.B., Henry R.P. and Miles R.D. (1984). Biological availability of manganese sources and effects of high dietary manganese on tissue mineral composition of broiler-type chicks. *Poult. Sci*. **63,** 1999-2006.

Brooks M.A., Grimes J.L., Lloyd K.E., Valdez F. and Spears J.W. (2012). Relative bioavailability in chicks of manganese from manganese propionate. *J. Appl. Poult. Res*. **21,** 126-130.

Cao J., Henry P.R., Guo R., Holwerda R.A., Toth J.P., Little R.C., Miles R.D. and Ammerman C.B. (2000). Chemical characteristics and relative bioavialability of supplemental organic zinc sources for poultry and ruminants. *J. Anim. Sci*. **78,** 2039-2054.

Davda J. and Labhasetwar V. (2002). Characterization of nanoparticle uptake by endothelial cells. *Int. J. Pharm*. **233,** 51-59.

Ji F., Luo X.G., Lu L., Liu B. and Yu S.Y. (2006a). Effects of manganese source and calcium on manganese uptake by in vitro everted gut sacs of broilers' intestinal segments. *Poult. Sci*. **85,** 1217-1225.

Ji F., Luo X.G., Lu L., Liu B. and Yu S.Y. (2006b). Effects of manganese source on manganese absorption by the intestine of broilers. *Poult. Sci*. **85,** 1947-1952.

Leach G.A. and Patton R.S. (1997). Analysis techniques for chelated minerals evaluated. *Feedstuffs*. **69,** 13-15.

Ledoux D.R., Henry P.R., Ammerman C.B., Rao P.V. and Miles R.D. (1991). Estimation of the relative bioavailability of inorganic copper sources for chicks using tissue uptake of copper. *J. Anim. Sci*. **69,** 215-222.

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

Li S., Lu L., Hao S., Wang Y., Zhang L., Liu S., Liu B., Li K. and Luo X. (2011). Dietary manganese modulates expression of the manganese-containing superoxide dismutase gene in chickens. *J. Nutr*. **141,** 189-194.

Mohapatra P., Swai R.K., Mishra S.K., Behera T., Swain P., Mishra S.S., Behura N.C., Sabat S.C., Sethy K., Dhama K. and Jayasankar P. (2014). Effects of dietary nano selenium on tissue selenium deposition, antioxidant status and immune function in layer chicks. *Int. J. Pharmacol*. **10,** 160-167.

Moshtaghie A.A., Badii A.A. and Hassanzadeh T. (2006). Investigation of manganese and iron absorption by rat everted gut sac. *Pakistan J. Biol. Sci*. **9,** 1346-1349.

Nollet L., Van der klis J., Lensing M. and Spring P. (2007). The effect of replacing inorganic with organic trace minerals in broiler diets on productive performance and mineral excretion. *J. App. Poult. Res*. **16,** 592-597.

Rostango H.S., Teixeira Aalbino L.F., Donzele J.L., Gomez P.C., Oliveria R.F.D., Lopes D.C., Ferreira A.S., Toledo Barreto S.L. and Euclides R.F. (2011). Brazalian Tables for Poultry and Swine. Universidade Federal de Vicosa, Mato Grosso do Sul, Brazal.

Rubio Zapata N.K. (2016). Effect of Increasing Levels of Dietary Zinc (Zn), Manganese (Mn), and Copper (Cu) from Organic and Inorganic Sources on Egg Quality and Egg Zn, Mn, and

Cu Content in Laying Hens. MSc, Louisiana State Univ. Zamorano.

SAS Institute. (2003). SAS®/STAT Software, Release 9.3. SAS Institute, Inc., Cary, NC. USA.

Sirirat N., Lu J., Alex Tsung-Yu H., Shih-Yi C. and Tu-Fa L. (2012). Effects different levels of nanoparticles chromium picolinate supplementation on growth performance, mineral retention and immune responses in broiler chickens. *J. Agric. Sci*. **12,** 48-58.

Wang F., Lin L., Sufen L., Songbai L., Liyang Z., Junhu Y. and Xugang L. (2012). Relative bioavailability of manganese proteinate for broilers Fed a conventional corn soybean meal diet. *Biol. Trace Elem. Res*. **146,** 181-186.

Yan F. and Waldroup P.W. (2006). Evaluation of Mintrex® manganes as a source of manganese for young broilers. *Int. J. Poult. Sci*. **5,** 708-713.

Yu Y., Lu L., Luo X.G. and Liu B. (2008). Kinetics of zinc absorption by *in situ* ligated intestinal loops of broilers involved in zinc transporters. *Poult. Sci*. **87,** 1146-1155.

Zaboli K.H., Aliarabi H., Bahari A.A. and Abbasalipourkabir R. (2013). Role of dietary nano zinc oxide on growth performance and blood levels of mineral. A study on in Iranian Angora (Markhoz) goat kids. *J. Pharm. Health Sci*. **2,** 19-26.

Zha L., Zeng J., Sun S., Deng H., Luo H. and Li W. (2008). Chromium (III) nanoparticles affect hormone and immune responses in heat-stressed rats. *Biol. Trace Elem. Res*. **129,** 157-169.

Zhang J.S., Gao X.Y., Zhang L.D. and Bao Y.P. (2001). Biological effects of a nano red elemental selenium. *Biofactors*. **15,** 27-38.