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Evaluation of Mintrex® Manganese as a Source of Manganese for Young Broilers[1]

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Abstract: The study was conducted to evaluate the relative bioavailability of MINTREX® Mn, an organic source of trace minerals, compared to reagent grade Mn sulfate and reagent grade Mn monoxide in diets for young broiler chicks. Nutritionally complete diets were formulated based on nutrient specifications of top broiler producers. Each of the Mn sources was added at 0, 100, 200, 400, 600, and 800 mg/kg. Diets were adjusted for the amount of 2-hydroxy-4 (methylthio) butanoic acid added from the MINTREX® Mn. All diets were fortified with 50 mg/kg Fe from ferrous sulfate, 100 mg/kg Zn from zinc sulfate and 10 mg/kg Cu from copper sulfate. Diets were fed in mash form. Five male chicks (Cobb 500) were placed in each of 96 pens in battery brooders; six pens were assigned to each dietary treatment. Diets were fed from 1 to 20 d at which time body weight and feed consumption were determined and birds killed by CO_2 inhalation. Tibias of all surviving birds, grouped by pen, were analyzed for bone ash and Mn content. There were no significant differences among treatments for body weight, feed conversion, feed intake, mortality, or tibia ash. Significant differences in tibia Mn content were observed among source and level of Mn. Slope-ratio analysis of the response to the various products indicated that birds fed MINTREX® Mn had 15.81% higher levels of tibia Mn than those fed the sulfate form and 53.89% higher levels of tibia Mn than those fed the oxide form, indicating greater biological availability of the Mn from the MINTREX® Mn than provided by commonly used inorganic forms of Mn.

Key words: Manganese, organic mineral complex, bioavailability, broiler

Introduction

Manganese is an essential trace mineral and is routinely added to practical poultry diets. The common Mn sources used in animal feed are inorganic Mn salts, primarily the sulfate form. The absorption of minerals in inorganic form by broilers is limited, primarily due to their tendency to form complexes with other dietary constituents that are less available or unavailable, and their tendency to interfere with each other in the digestive tract. The absolute bioavailability of inorganic minerals is generally low and a significant portion of these mineral nutrients pass through the gastrointestinal tract and end up in the excreta. The peer-reviewed publications from 1985-2003 were summarized to find out that the average P retention was 49.3% for broilers younger than 32 d (from 22 reports), and 41% for broilers older than 32 d of age (from 5 reports) (Applegate *et al*., 2003). The utilization of trace minerals is even less efficient. The true Mn absorption in chicks from d 8 to 16 determined by isotope-dilution method was 8.4% when a corn-soybean diet with 100 mg/kg supplemental Mn was fed, and 2.8% when the diet was not supplemented with Mn (Wedekind *et al*., 1991). With increasing concern of the potential pollution to the environment of the excess nutrients in the excreta of chickens, more emphasis is placed on improving the biological availability of nutrients and therefore reducing their excretion.

Organic trace minerals have been reported to be greater in bioavailability compared to the inorganic sources by some researchers (Henry *et al*., 1989; Fly *et al*., 1989; Smith *et al*., 1995). 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*., 1998). An organic source of trace minerals, MINTREX®, has recently been introduced[3]. It is a chelate of two 2-hydroxy-4 (methylthio) butanoic acid (HMTBa) ligands per atom of trace mineral, i.e. zinc, copper or manganese. HMTBa is an organic acid with a structure identical to methionine except for a hydroxyl group on the α-carbon instead of an amino group. MINTREX® Zn has been shown to be able to travel intact to the small intestine and to be equivalent to ALIMET® feed supplement[4] as a methionine source for chicks (Richards *et al*., 2005).

The traditional method to evaluate the bioavailability of Mn for chickens is to use a basal diet deficient in Mn and supplement the Mn from the test sources in graded levels, feed the test diets to birds and then collect responding measurements, i.e. incidence of perosis (Gallup and Norris, 1939), growth rate, leg abnormality and bone Mn concentration (Watson *et al*., 1970; 1971). However, due to the low requirement for Mn and the amount of Mn contained in the commonly used feed ingredients, semi-purified diets are needed, which usually makes the diets less palatable for chickens and adds to the expense of the assay. In addition, potential

Mn contamination from water, feeding and drinking equipment, and housing facilities is difficult to avoid which can make the method difficult to perform and less accurate. A new method to examine the bioavailability of some minerals was established (Southern and Baker, 1983, Black *et al.*, 1984a, b, 1985), where high levels of Mn, many times above the requirement but nontoxic and without significant effects on feed intake and growth rate, were added in the diets and fed to chickens. Tissue accumulation of Mn in bone and kidney was found to respond linearly to these graded high levels of Mn in the diet and can be used as a measure of bioavailability. Compared to the traditional method, this method is less expensive by eliminating use of semi-purified diets and easier to perform because contamination of the mineral from the environment is of less significance. The bioavailability values obtained from this method have been proven to be similar to those from the traditional method (Henry *et al.,* 1986). This technique has also been proven to work for Cu (Ledoux *et al.*, 1991, Zanetti *et al.*, 1991) and Zn (Sandoval *et al.*, 1997), only with different sensitive tissues.

The objective of this study was to evaluate the bioavailability of MINTREX® Mn, compared with reagent grade Mn sulfate or reagent grade Mn monoxide, as a source of Mn in diets of young broiler chicks as measured by tissue uptake of Mn from elevated dietary levels.

Materials and Methods

Nutritionally complete diets were formulated based on nutrient specifications of the top five broiler producers in a leading agricultural survey (Agri-Stats, Fort Wayne IN). Three different sources of Mn were compared. These included MnO, $MnSO_4 \cdot 5H_2O$, and MINTREX® Mn. The MnO and $MnSO_4$ sources were ACS reagent grade (Mallinckrodt Baker, Inc., Philipsburg NJ 08865). A low Mn basal diet without supplemental Mn and three high Mn basals containing 800 mg/kg Mn from MINTREX®, $MnSO_4$ or MnO were prepared. These test diets were also fortified with 50 mg/kg Fe from ferrous sulfate, 100 mg/kg Zn from zinc sulfate, and 10 mg/kg Cu from copper sulfate. Composition and calculated nutrient analysis of the basal diets is shown in Table 1. Appropriate portions of the low Mn basal diet were blended with portions of the high Mn basal diets to provide test diets with added Mn levels of 0, 100, 200, 400, 600, and 800 mg/kg for each Mn source. Diets were fed in mash form.

Male chicks of a commercial broiler strain[5] were obtained from a local hatchery where they had been vaccinated in ovo for Marek's disease and had received vaccinations for Newcastle Disease and Infectious Bronchitis post hatch via a coarse spray. Five males were placed in each of 96 pens in electrically heated battery brooders with raised wire floors. Six pens were assigned to each dietary treatment, replicated across tiers in the battery brooder. Care and management of the birds followed recommended guidelines (FASS, 1999). Birds were placed on test diets on day of hatch and fed test diets to 20 d of age. Measurements included starting and ending bird weights by pen, feed consumption during the test period, and the weight of dead birds to adjust for feed conversion ratio. At the conclusion of the trial surviving birds were killed by CO_2 inhalation; the right tibias were removed, boiled for 10 min, and cleaned of adhering tissue. Tibias including epiphyses were dried at 105°C for 12 h, and extracted with ethyl ethanol for 24 h and with petroleum ether for another 24 h in a Soxhlet apparatus. The dry fat-free tibias were pooled by pen and analyzed for bone ash (AOAC, 1990) and bone Mn content by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES). All test diets were analyzed for Mn content, crude protein, and activity of HMTBa.

Growth performance data and tibia ash content were subject to one-way ANOVA. Bone Mn content was analyzed by two-way ANOVA with the model including the main effect of Mn source, dietary supplemental Mn level and their interaction. Slope ratio analysis was performed by regressing tibia Mn concentration on added Mn intake to compare the bioavailability of the Mn sources tested in the trial. The slope ratio test followed the procedures given by Littell *et al.* (1997) where three validity checks (linearity, common intercept, equality of the basal diet mean to the common intercept) were first performed before parameter estimates for calculation of relative bioavailability was obtained by the multiple linear regression method. All analyses used General Linear Models procedure of SAS (SAS institute, 1991). Pen served as the experimental unit. All statements of significance were based on $P<0.05$.

Results and Discussion

The analyzed crude protein and Mn content of the test diets were found to be in agreement with the calculated values (data not shown). The effects of diets containing different sources and levels of Mn on performance and tibia ash content are shown in Table 2. There were no significant differences among treatments for body weight, feed conversion, feed intake, mortality, or tibia ash content. The results were not unexpected as the Mn basal diet in this trial was analyzed to contain 34 mg/kg Mn. Watson *et al.* (1970) reported that 10 mg/kg Mn added to a semi-purified diet containing 4 mg/kg Mn was adequate to maintain normal growth and bone ash and to prevent the occurrence of perosis for chicks up to 28 days of age when supplied from reagent grade sulfate form or one of the two commercial feed grade oxide sources tested. In another trial, Watson *et al.* (1971) showed that when 20 mg/kg Mn from reagent grade sulfate form was supplemented to a basal diet with 5

Table 1: Composition (%) and calculated nutrient content of basal diets

Ingredient	Low Mn Basal	800 mg/kg MINTREX® Mn	800 mg/kgMn sulfate	800 mg/kgMn oxide
Soybean meal 47%	35.130	35.252	35.225	35.171
Yellow corn	53.863	55.556	53.331	53.638
Poultry oil	4.132	4.369	4.317	4.210
Limestone	1.242	1.240	1.241	1.241
Dicalcium phosphate	1.710	1.712	1.711	1.711
Feed grade salt	0.569	0.570	0.570	0.570
Vitamin premix[1]	0.500	0.500	0.500	0.500
L-Lysine Hcl	0.092	0.090	0.090	0.091
L-Threonine	0.023	0.023	0.023	0.023
ALIMET®-10 premix[2]	2.666	0.000	2.672	2.668
MINTREX® Mn[3]	0.000	0.615	0.000	0.000
Mn oxide[4]	0.000	0.000	0.000	0.104
Mn sulfate[5]	0.000	0.000	0.247	0.000
Copper sulfate	0.004	0.004	0.004	0.004
Ferrous sulfate	0.025	0.025	0.025	0.025
Zinc sulfate	0.044	0.044	0.044	0.044
Total weight	100.000	100.000	100.000	100.000
AME, kcal/lb	1400.00	1400.00	1400.00	1400.00
AME, kcal/kg	3085.00	3085.00	3085.00	3085.00
Crude protein %	22.03	22.03	22.03	22.03
Calcium %	0.92	0.92	0.92	0.92
Phosphorus %	0.68	0.68	0.68	0.68
Nonphytate P %	0.44	0.44	0.44	0.44
Met %[1]	0.59	0.76	0.59	0.59
TSAA %[1]	0.98	1.15	0.98	0.98
Lys %	1.30	1.30	1.30	1.30
Thr %	0.88	0.88	0.88	0.88
Trp %	0.27	0.27	0.27	0.27
Added HMBTA	0.30	0.47	0.30	0.30

[1]Provides per kg of diet: vitamin A 7714 IU; cholecalciferol 2204 IU; vitamin E 16.53 IU; vitamin B_{12} 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione 1.5 mg; folic acid 0.9 mg; choline 1040 mg; thiamin 1.54 mg; pyridoxine 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg; Se 0.1 mg. [2]Mixture of ground corn with ALIMET® to provide 10% methionine activity. [3]MINTREX® Mn providing 13% Mn activity. [4]Manganous oxide calculated to contain 76.67% Mn. [5]Manganese sulfate calculated to contain 32.41% Mn.

Table 2: Effect of source and level of manganese on performance of broilers

Supplemental Zn source	Added Mn mg/kg	20 dBW kg	Feed:gain 1 to 20 d kg:kg	Feed intake kg	Mortality %	Tibia ash %
None	0	0.782	1.429	1.054	6.67	44.68
Oxide	100	0.780	1.413	1.030	3.33	45.38
Oxide	200	0.771	1.405	1.015	6.67	44.95
Oxide	400	0.772	1.405	1.018	6.67	45.41
Oxide	600	0.785	1.391	1.026	3.33	44.43
Oxide	800	0.802	1.367	1.032	6.67	44.78
SO_4	100	0.760	1.426	1.015	0.00	44.59
SO_4	200	0.777	1.391	1.014	3.33	44.77
SO_4	400	0.783	1.416	1.043	3.33	45.49
SO_4	600	0.796	1.388	1.039	13.33	44.54
SO_4	800	0.758	1.415	1.010	0.00	44.62
MINTREX®	100	0.799	1.386	1.044	6.67	44.68
MINTREX®	200	0.789	1.402	1.040	6.67	45.38
MINTREX®	400	0.799	1.407	1.059	10.00	44.41
MINTREX®	600	0.787	1.422	1.052	10.00	44.44
MINTREX®	800	0.777	1.427	1.041	0.00	45.43
P-value		0.9837	0.8631	0.9576	0.8644	0.4576
SEM		0.020	0.024	0.028	4.92	0.42
CV		6.18	3.87	6.06	203.33	2.09

mg/kg Mn, normal growth rate and bone ash was achieved and leg abnormity of 28-d-old chicks was prevented. Manganese supplementation up to 1000 mg/kg from various sources has been reported to be non-toxic (Gallup and Norris, 1939, Black *et al.*, 1985, Miles *et al.*, 2003). The highest Mn level included in this

Table 3: Effect of source and level of Mn on tibia Mn concentration (mg/kg of 20 d old broilers

Level (mg/kg)	MINTREX®	Oxide	Sulfate	Mean
0	4.32^{j}	4.32^{j}	4.32^{j}	4.32^{f}
100	8.48^{ghi}	7.46^{hi}	6.87^{i}	7.60^{e}
200	11.19^{ef}	9.16^{gh}	9.72^{fg}	10.03^{d}
400	15.07^{d}	11.87^{e}	14.06^{d}	13.67^{c}
600	18.03^{c}	14.81^{d}	17.69^{c}	16.84^{b}
800	25.58^{a}	17.37^{c}	21.14^{b}	21.36^{a}
Mean	13.78^{a}	10.83^{c}	12.30^{b}	
	P-value	SEM		
Source	<0.0001	0.28		
Level	<0.0001	0.40		
Source*Level	<0.0001	0.74		

$^{a-j}$ means within comparison with common superscripts do not differ significantly (P<0.05).

Table 4: Multiple linear regression of tibia Mn concentration on added dietary Mn intake

Parameter	Estimate	Standard error	P-value
Intercept	5.578	0.358	<.0001
Mn oxide	0.01466	0.00091	<.0001
Mn Sulfate	0.01948	0.00096	<.0001
MINTREX® Mn	0.02256	0.00090	<.0001

Table 5: Slope ratio comparison of Mn sources

Source	Oxide	Sulfate	MINTREX
Mn oxide	----	123.87	153.89
Mn sulfate	75.26	----	115.81
MINTREX® Mn	64.98	86.34	-----

trial was 800mg/kg, which was expected to have no negative effect on growth performance.

Tibia levels of Mn as affected by Mn source and level are shown in Table 3. Both Mn source and Mn level had significant effects on tibia Mn content and there was a significant interaction between source and level of Mn. Birds fed MINTREX® Mn as a source of Mn had significantly higher tibia levels of Mn than did birds fed the sulfate or oxide forms of Mn. Birds fed the sulfate form had significantly higher levels of Mn than did those fed the oxide form. Tibia concentration of Mn increased in a linear manner with the increase in dietary supplemental Mn levels for all forms of Mn; however, the rate of increase with respect to the increase in dietary Mn level differed among the three sources which accounted for the significant interaction between Mn source and level.

There was a highly linear relationship between tibia Mn concentration and added Mn consumption in the diet for Mn oxide (R^2=0.88), Mn sulfate (R^2=0.91), and MINTREX® Mn (R^2=0.92) (Fig. 1, 2, 3). The common intercept and equality of the basal diet mean to the common intercept were also satisfied for the slope-ratio assay. Slope-ratio analysis of the response to the various products is shown in Tables 4 and 5. With ACS reagent grade Mn sulfate as reference and based on tibia Mn accumulation, the relative bioavailability of Mn was 75.26% for Mn oxide and 115.81% for MINTREX® Mn, both were significantly different from the sulfate form. The birds fed MINTREX® Mn had 15.81% higher levels of tibia Mn than those fed the sulfate form and 53.89% higher levels of tibia Mn than those fed the oxide form.

Black *et al.* (1984a) reported a bioavailability of 65.6% for reagent grade Mn oxide relative to Mn sulfate based on multiple linear regression on bone Mn concentration and 79.4% based on multiple linear regression on liver Mn concentration for broiler chicks. Henry *et al.* (1986) reported an average bioavailability of reagent grade Mn oxide of 66% relative to Mn sulfate as determined by a combination of linear regression, multiple linear regression and tissue (bone, kidney and liver) Mn increase for chicks up to 21 d old. Based on multiple linear regression from bone Mn concentration, Wong-Valle *et al.* (1989) observed the bioavailability of 81.9, 91.3, 75.0, and 70.3% for reagent grade Mn oxide and three feed grade Mn oxides A, B, and C respectively. Ammerman *et al.* (1995) summarized eight studies and found an average bioavailability of 75% for Mn oxide in comparison with Mn sulfate for chicks. The relative bioavailability value of 75.26% of Mn oxide relative to Mn sulfate obtained from this study generally agrees with the above reports. Part of the variation in the results of the reported trials can be explained in that different tissues were analyzed for Mn concentration, and different regression methods used to generate the bioavailability values.

Fly *et al.* (1989) reported that a Mn-methionine chelate had a relative bioavailability of 130% in a semi-purified diet compared to feed grade Mn oxide based on bone Mn accumulation. Henry *et al.* (1989) found that compared to Mn sulfate, the relative bioavailability of Mn-methionine was 108% based on bone Mn concentration and 132% based on kidney Mn concentration for chicks. Based on bone Mn concentration of 7-week-old birds, Smith *et al.* (1995) found a bioavailability value of 125% for manganese-proteinate relative to Mn sulfate under normal temperature, and 145% under heat stress. The bioavailability value of 115.81% obtained in this study for MINTREX® Mn relative to Mn sulfate and 153.89% with respect to zinc oxide is in agreement with these reports, which suggested greater biological activity for organic Mn complex. However, some studies showed that bioavailability of organic complexes were no greater than the inorganic sources. Baker and Halpin (1987) reported that the Mn bioavailability of a manganese-proteinate was not significantly different from that of Mn sulate based on bone Mn accumulation during a 14-d feeding period for chicks. Based on bone Mn concentration regression on added dietary Mn concentration and using Mn sulfate as reference, Miles *et al.* (2003) reported a relative bioavailability value of 84% for a Mn amino acid chelate. Several possible reasons may account for some of the differences in the results regarding organic

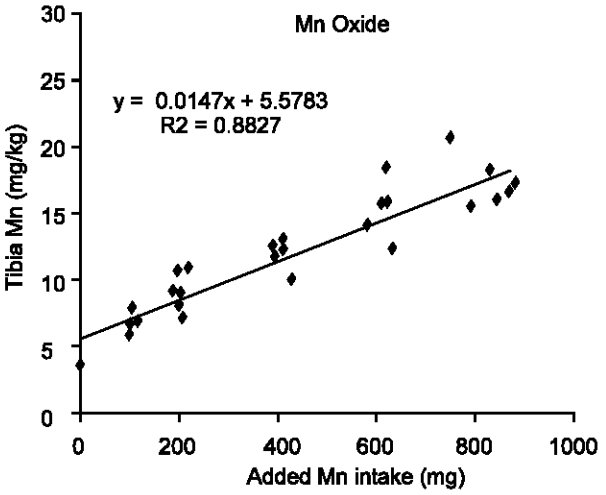

Fig. 1: Tibia Mn concentration of 20-d-old broilers as affected by dietary supplementation of Mn from reagent grade oxide form

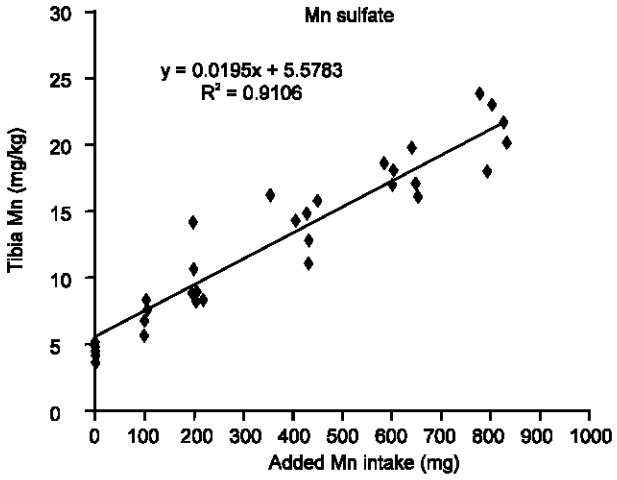

Fig. 2: Tibia Mn concentration of 20-d-old broilers as affected by dietary supplementation of Mn from reagent grade sulfate form

Mn complexes. The organic ligands for different organic complexes vary. The most common ligands include single amino acid, multiple amino acids, hydrolyzed protein, and organic acids. The organic complexes formed by various ligands have different characteristics that might affect their absorption in the gut, i.e. stability constants, effective binding constants over a range of pH levels, charge of functional groups, molecular weight and size of the molecule. Physicochemical factors that reduce uptake of mineral nutrients from the intestinal lumen are the predominant influence on mineral bioavailability (Dreosti, 1993). Inorganic minerals are present in chyme as ions which interact with other dietary constituents to form less available or unavailable complexes for absorption. Organic trace mineral

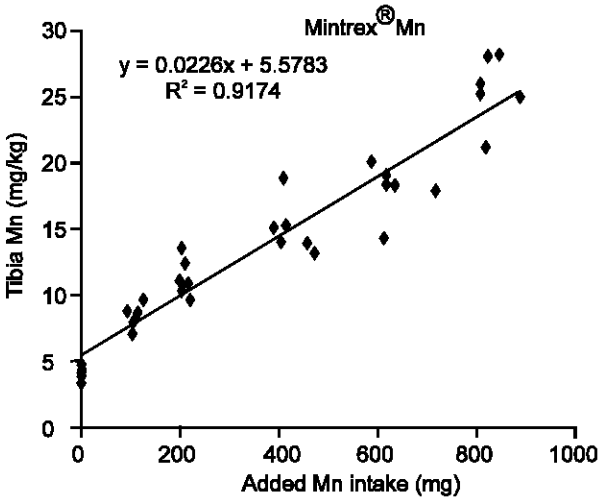

Fig. 3: Tibia Mn concentration of 20-d-old broilers as affected by dietary supplementation of Mn from MINTREX® Mn

chelates are believed to have less undesired reactions in the gastrointestinal tract due to the protection of chelate structure (Ammerman *er al.*, 1998). For this to happen, organic complexes need to be stable in the acid gastric condition and travel intact to the intestinal absorption sites. The binding between organic ligands and trace minerals, however, cannot be too strong that the minerals cannot be released from the complex to allow for mineral uptake by the appropriate metal transporters, or for metabolism and utilization in the body after absorption. MINTREX® Zn, sharing similar structure with MINTREX® Mn, has been reported to be able to travel intact to the small intestine and to be equivalent to free HMBTa as a methionine source (Richards, 2005). These data indicate that the binding between Zn and its organic ligand HMBTa in MINTREX® Zn is strong enough to deliver the Zn in protected form to the small intestine for absorption, but not too strong to prevent dissociation either immediately prior to or after absorption.

In summary, these results indicate the Mn provided by MINTREX® Mn has greater biological availability than the Mn provided by the reagent grade sulfate form or oxide form for young broilers. Use of this product at adjusted dietary Mn levels should reduce fecal excretion of Mn in broilers.

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