

## Research Article

# Comparison of Manganese Sulfate and Manganese Threonine Based on Bioavailability and Performance of Broiler Chicks

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## Abstract

The aim of this study was to compare manganese sulfate and manganese threonine chelate based on bioavailability and production performance of broilers and to determine the best consumption level of manganese threonine chelate. This research is in a completely randomized design with 240 day-old Ross 308 chicks and eight treatments including control treatment (no added manganese), three levels of manganese (60, 90 and 120 mg per kg feed) added from manganese sulfate and manganese threonine, and 60 mg of manganese added by a common commercial manganese chelate (Bioplex manganese<sup>®</sup>). In all growing periods, the amount of feed consumed and body weight was measured and the FCR was calculated. On day 44, fecal samples were collected from each experimental unit. On day 45, one bird was slaughtered from each pen and its carcass was cut. Ash and manganese in the tibia were measured and the relative bioavailability of some parameters was calculated. The results showed that the treatments of manganese threonine chelate significantly increased the mean weight and also decreased the FCR compared to the treatments of manganese sulfate in the whole period ( $p < 0.05$ ), the treatment of 90 and 120 ppm level of manganese threonine chelate had the highest ratio of Breast meat and drumstick ( $p < 0.05$ ). Also, according to the results, the amount of manganese excretion in chelate-origin treatments was lower than sulfate treatments ( $p < 0.05$ ), the RBV of manganese threonine chelate relative to the sulfate source based on ash and bone manganese content, body weight and FCR were 202%, 150%, 185% and 433%, respectively.

**Keywords:** Broiler; Manganese threonine chelate; Manganese sulfate; Tibia

## Introduction

Manganese (Mn) is an essential trace element for animals, which plays an important role in the metabolism of carbohydrate, amino acid, and as a necessary cofactor of numerous enzymes or proteins like superoxide dismutase, transferases, and hydrolases [1,2]. Dietary Mn deficiency in animals results in a wide variety of structural and physiological defects, including growth retardation, skeletal, and cartilage malformations [3]. Because of the low availability of inorganic sources and high requirement of Mn in broilers, Mn additive is routinely supplemented into diets for their optimum growth of broilers. Traditionally, trace minerals are supplemented in the form of inorganic salts, such as sulfates, oxides and carbonates, to provide levels of the mineral that prevent clinical deficiencies and allow the bird to reach its genetic growth potential [4]. In the 1990's greater bioavailability for some organic trace mineral sources than inorganic forms was reported, leading to an increased interest in the feed industry for these products. In recent years, organic

Mn supplements have been increasingly used in the feed industry because of their higher bioavailabilities and lower excretions than their inorganic salts [5]. Trace minerals from organic sources would appear to be protected from forming insoluble complexes with feed or endogenous components present in the digestive tract. Moreover, trace mineral complexing or chelating to small size organic molecules would enhance their absorption and even their metabolic utilization [6]. The use of organic complexes of trace elements such as Mn-Met has received more attention because of their potential for greater bioavailability. Farhadi et al. [7] demonstrated that zinc threonine chelate has better results compared to zinc sulfate in broilers and Behjatini et al. [8] showed that zinc threonine is a better source of zinc compared to sulfate and oxide of zinc in layers. But there is no literature cited that examines the effects of gradual substitution of conventional inorganic Mn sources by manganese threonine chelate in poultry nutrition. Therefore, the purpose of this study was to obtain the best level of this chelate to evaluate its bioavailability and performance in comparison with inorganic manganese (manganese sulfate).

## Materials and Methods

All birds were housed in floor pens located in a semi-closed house with the climate control condition. Artificial lighting was used to provide chicks with 24h lighting daily during the whole experimental period. The initial brooding temperature was 32°C in the first week of age and reduced gradually 2°C per week up to 24°C then remains constant. Diet and water were provided ad libitum throughout the experimental period, which lasted for 45 days of age. The experiment was conducted in a completely randomized design with 8 treatments and 5 replicates with 6 birds in each replicate (240 Ross 308 1-day-old chicks) that included control treatment (no added manganese),

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three levels of manganese (60 mg, 90 mg, and 120 mg per kg feed) added from manganese sulfate and manganese threonine and 60 mg of manganese added by a common commercial manganese chelate (Bioplex manganese®). The requirements of birds were met according to the Ross 308 management guideline. The ingredients and chemical composition of the control diet are shown in Table 1. We had no mortality in this experiment. Feed intake and weight gain were measured and the feed conversion ratio was determined in starter, grower, finisher periods, and whole period. The mineral supplement for each treatment was made according to the recommended Ross 308 catalog; the mineral premix made for the first treatment (control diet) had not manganese supplement from any source. It should be noted that the amount of manganese per kg of control feed was 14 mg kg<sup>-1</sup>, and the manganese content in manganese sulfate used in the supplement was 31.43% and the manganese content in manganese threonine chelate used was 14.5% (Table 1).

### Sample collection

At the end of whole period, a bird with approximately the same average weight of that pen was selected. The weight of the hot carcass (without head, blood and hocks removed) was recorded. Carcass yield (weight of the de-feathered eviscerated carcass relative to live weight) and yields of the breast, thigh and drumstick relative to live weight were calculated. The left leg of the birds killed on the 45<sup>th</sup> day was collected for measuring of ash and manganese content of the tibia.

### Collection of excreta for estimation of trace elements

On day 44, a sample of excreta was collected from each experimental unit. According to the method described in AOAC in 1990, the amounts of manganese in the excreta were measured with atomic absorption spectrophotometer by using 1 g of excreta [9].

### Tibia bone manganese

Ash and manganese content of the tibia were measured according to the method of AOAC in 1990 [9]. The amounts of manganese in the tibia bone were measured with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) by using 1g of the tibia bone.

### Statistical analysis

Data were analysed by one-way ANOVA and using SAS software [10]. Comparison of means was performed using Duncan test at 0.05% level [11].

### Relative bioavailability of manganese threonine

It was estimated based on slope ratios from multiple linear regressions of body weight, FCR, ash and manganese of tibia [12,13].

## Results and Discussion

### Production performance

The effects of different levels and sources of inorganic and organic manganese on performance traits in different periods are shown in Table 2. There was no significant difference in feed intake in the starter period (up to 10 days) among all experimental groups. Manganese sulfate treatment at 60 ppm level, manganese chelate treatments at 60 and 90 ppm levels had the highest live weights with significant differences compared to other treatments ( $p < 0.05$ ). However, a significant difference was not observed between these three treatments and the manganese sulfate treatment at 90 ppm and the control group. There is no significant difference in the feed conversion ratio of all experimental groups in the starter period. Manganese chelate treatments at 90 ppm and 120 ppm levels have the highest feed intake in the grower period. There was no significant difference between the treatment of the manganese threonine treatments

**Table 1:** Composition of the experimental basal diet.

Ingredients (%)	Starter (Days1-10)	Grower (Days11-24)	Finisher (Days25-45)
Yellow corn	50.67	58.73	63.62
Soybean meal (44.5%)	43.89	37.4	32.17
Fat powder (Persia Fat®)	1.61	0.17	0.95
Di-calcium phosphate	1.75	1.45	1.25
Calcium carbonate	0.69	1.01	0.72
Sodium Chloride	0.36	0.34	0.06
Sodium bicarbonate	0	0	0.4
DL-methionine	0.33	0.26	0.23
L-Lysine+HCL	0.16	0.1	0.11
Threonine	0.05	0.02	0
Vitamin-mineral premix <sup>a</sup>	0.5	0.5	0.5
Calculated composition	2900	2900	3000
ME (kcal/kg)	2900	2900	3000
Crude protein (%)	22.23	20.11	18.28
Crude Fat (%)	4.89	3.53	4.28
Linoleic acid (%)	1.77	1.49	1.81
Crude Fiber (%)	3.82	3.55	3.3
Calcium (%)	0.93	0.81	0.73
Available phosphorus (%)	0.46	0.41	0.37
Na (%)	0.16	0.16	0.16
DCAB	234.04	212.27	240.09
Lysine (%)	1.39	1.21	1.08
Methionine (%)	0.68	0.59	0.53
Methionine+cysteine (%)	1.04	0.93	0.84
Threonine (%)	0.94	0.82	0.73
Manganese (mg/kg)	14	14	14

<sup>a</sup>Vitamin-mineral premix provided the following values per kg of control feed: vitamin A, 9000 IU; vitamin D3, 2000 IU; vitamin E, 36 mg; vitamin K3, 2 mg; vitamin B1, 75.1 mg; vitamin B2, 6.6 mg; calcium pantothenate, 8.9 mg; nicotinic acid, 7.29 mg; vitamin B6, 94.2 mg; folic acid, 1 mg; vitamin B12, 0.015 mg; vitamin H2, 1.0 mg;

choline chloride, 250 mg; BHT, 1 mg; Zn, 110 mg; Fe, 20 mg; Cu, 16 mg; I, 1.25 mg; Se, 0.3 mg

at 90 ppm and 120 ppm levels, but both of them had a significant difference compared to the other treatments and the control group ( $p < 0.05$ ). Manganese threonine treatments at 90 and 120 ppm levels had significantly the highest weight gain compared to other groups ( $p < 0.05$ ). Levels of 90 and 120 ppm manganese threonine had lower FCR compared to other groups and it was significant with the control group and Bioplex® manganese treatment ( $p < 0.05$ ). In the finisher period, the highest feed intake was for manganese sulfate treatment at 60 ppm level that had a significant difference with all groups ( $p < 0.05$ ) except manganese threonine treatment at 120 ppm level. Weight gain of manganese threonine treatment at 120 ppm level in the finisher period compared to other treatments and the control group was the highest and had a significant difference ( $p < 0.05$ ). The treatment with the highest level of manganese threonine chelate (120 ppm) had the lowest FCR, which was significantly different compared to other treatments and the control group ( $p < 0.05$ ). In total period, the treatment with 60 ppm level of manganese with sulfate origin had the highest feed intake, which was not significantly different from the treatments of manganese threonine at 90 ppm and 120 ppm levels, but compared to other treatments and the control group had significant difference ( $p < 0.05$ ). Manganese threonine at 120 ppm level had the highest body weight with significant difference compared to others ( $p < 0.05$ ) and after that manganese threonine at 90 ppm level had the second-highest body weight with significant difference compared to other treatments. The treatment of manganese threonine at 120 ppm level had the best FCR ( $p < 0.05$ ), which was not significantly different from manganese threonine at 90 ppm level (Table 2).

### Carcass yield

The effects of different levels and sources of inorganic and organic manganese on carcass yield are shown in Table 3. Manganese threonine at 90 ppm and 120 ppm levels significantly had the highest ratio of breast meat to body weight ( $p < 0.05$ ). The treatment of a high level of threonine-manganese chelate (120 ppm) significantly had the highest ratio of drumstick ( $p < 0.05$ ). The ratio of carcass and thigh had no significant difference between treatments.

### Ash and manganese in tibia

The effects of different levels and sources of inorganic and organic manganese on ash and manganese in the tibia are shown in Table 4. The amount of tibia ash in the treatments receiving 90 ppm and 120 ppm levels of manganese threonine chelate is the highest and is significantly different from others ( $p < 0.05$ ). Also, as the level of dietary

manganese supplementation (organic and inorganic) increases, the amount of ash present in the tibia increases. The amount of tibia manganese in the treatments receiving 90 ppm and 120 ppm levels of manganese threonine chelate was the highest and had significantly different from others ( $p < 0.05$ ) plus as the level of dietary manganese supplementation (organic and inorganic) increased, the amount of manganese present in the tibia increased (Table 4).

### Bioavailability

Relative bioavailability of manganese threonine to manganese sulfate based on body weight, feed conversion ratio, ash, and manganese content in tibia were 185%, 433%, 202% and 150%, respectively (Table 5).

### Manganese excretion

The effects of different levels and sources of inorganic and organic manganese on manganese excretion are shown in Table 6. The treatments receiving 90 ppm and 120 ppm levels of manganese sulfate had the highest manganese excretion in the feces, while the control treatments, Bioplex® manganese, and low levels of threonine-manganese chelate had the lowest manganese excretion in the feces, respectively ( $p < 0.05$ ). Also, in the treatments with manganese supplement with organic source, the excretion of manganese in the feces was less than manganese of sulfate origin.

### Discussion

In agreement with the results of this experiment, Kumar Singh et al by studying protein chelate, methionine chelate, and sulfate salt of trace elements of copper, iron, manganese, and zinc in broilers found that organic sources of trace elements had lower feed intake and more weight than the control group and sulfate treatments [14]. Rodolf Vieira et al. [15] have shown that increasing the replacement of trace elements with the organic source will improve body weight and feed conversion ratio. Jasek et al. [16] by comparing different levels of organic manganese found that with increasing manganese level, the performance of birds improved and also the treatment with the highest manganese level had the highest weight and the lowest conversion ratio. Also, the organic form of the elements is hardly soluble at neutral pH, which increases the stability of the mineral in the intestine [17]. Organic trace minerals are environment-friendly because of their lower excretion rate and it remains a long time in the gut consequently improves the growth performance [18]. Manganese threonine at (90 ppm and 120 ppm levels) had a good effect on some

**Table 2:** The effects of different levels and sources of inorganic and organic manganese on performance traits in different periods.

Treatment	Added manganese (ppm)	Amount of manganese in whole feed (ppm)	Feed intake (g)			Body weight gain average (g)			FCR			Total		
			starter	Grower	Finisher	starter	Grower	Finisher	Starter	Grower	Finisher	FI(g)	BW (g)	FCR
Control	0	14	149	58402 <sup>c</sup>	2578 <sup>c</sup>	143.66 <sup>ab</sup>	434.73 <sup>d</sup>	1131.6 <sup>d</sup>	1.03	1.34 <sup>a</sup>	2.27 <sup>bc</sup>	3380 <sup>d</sup>	1710 <sup>f</sup>	1.97 <sup>a</sup>
	60	74	157	749.2 <sup>b</sup>	3319 <sup>a</sup>	151.13 <sup>a</sup>	597.20 <sup>b</sup>	1332.2 <sup>cb</sup>	1.03	1.25 <sup>ab</sup>	2.49 <sup>a</sup>	4225 <sup>a</sup>	2080 <sup>c</sup>	2.03 <sup>a</sup>
Manganese sulfate	90	104	144	686 <sup>c</sup>	3079 <sup>b</sup>	144.32 <sup>ab</sup>	596.87 <sup>b</sup>	1257.8 <sup>c</sup>	0.99	1.15 <sup>c</sup>	2.45 <sup>ab</sup>	3909 <sup>bc</sup>	1999 <sup>de</sup>	1.95 <sup>a</sup>
	120	134	146	643.4 <sup>d</sup>	3068 <sup>b</sup>	139.33 <sup>b</sup>	548.93 <sup>c</sup>	1280.4 <sup>c</sup>	1.04	1.15 <sup>c</sup>	2.40 <sup>ab</sup>	3848 <sup>bc</sup>	1968 <sup>de</sup>	1.95 <sup>a</sup>
Manganese threonine	60	74	152	633 <sup>d</sup>	2943 <sup>b</sup>	149.93 <sup>a</sup>	524.87 <sup>c</sup>	1359.6 <sup>b</sup>	1.01	1.20 <sup>bc</sup>	2.16 <sup>cd</sup>	3727 <sup>c</sup>	2034 <sup>dc</sup>	1.83 <sup>b</sup>
	90	104	147	810 <sup>a</sup>	3063 <sup>b</sup>	150.33 <sup>a</sup>	687.53 <sup>a</sup>	1403.6 <sup>b</sup>	0.98	1.17 <sup>bc</sup>	2.18 <sup>cd</sup>	4020 <sup>ab</sup>	2241 <sup>b</sup>	1.79 <sup>cb</sup>
120	134	136	776 <sup>ab</sup>	3161 <sup>ab</sup>	139.73 <sup>b</sup>	687.53 <sup>a</sup>	1549.2 <sup>a</sup>	0.97	1.12 <sup>c</sup>	2.04 <sup>d</sup>	4073 <sup>ab</sup>	2376 <sup>a</sup>	1.71 <sup>c</sup>	
Bioplex® manganese	60	74	152	696 <sup>c</sup>	3079 <sup>b</sup>	139.93	528.33 <sup>c</sup>	1266.8 <sup>c</sup>	1.09	1.32 <sup>a</sup>	2.43 <sup>ab</sup>	3928 <sup>bc</sup>	1935 <sup>e</sup>	2.03 <sup>a</sup>
SEM			6	15.7	74.7	2.46	12.18	25.3	0.04	0.03	0.06	73	25	0.03
P value			0.5	<0.0001	<0.0001	0.0023	<0.0001	<0.0001	0.48	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
CV (%)			9.3	4.7	5.5	3.8	4.7	4.2	8.9	5.6	6.2	4.2	4.2	4.2

Different letters (a, b, c, etc.) in each column indicate a significant difference ( $p < 0.05$ )

**Table 3:** The effects of different levels and sources of inorganic and organic manganese on carcass yield.

Treatment*	Added manganese (ppm)	Amount of manganese in whole feed (ppm)	Carcass/BW (%)	Breast/BW (%)	Thight/BW (%)	Drumstick/BW (%)
Control	0	14	69.35	21.1 <sup>d</sup>	10.41 <sup>b</sup>	9.79
	60	74	70.7	23.39 <sup>bcd</sup>	10.38 <sup>b</sup>	9.83
Manganese sulfate	90	104	68.85	24.74 <sup>abc</sup>	10.88 <sup>b</sup>	9.97
	120	134	68.91	24.71 <sup>abc</sup>	10.69 <sup>b</sup>	10.32
Manganese threonine	60	74	71.84	25.41 <sup>ab</sup>	11.04 <sup>b</sup>	9.95
	90	104	73.84	27.3 <sup>a</sup>	10.88 <sup>b</sup>	9.97
	120	134	75.02	26.34 <sup>a</sup>	13.03 <sup>a</sup>	10.26
Bioplex® manganese	60	74	75.5	26 <sup>a</sup>	11.19 <sup>b</sup>	10.75
SEM			2.09	0.83	0.39	0.28
P value			0.1418	0.0001	0.0003	0.2797
CV (%)			6.5	7.5	9	6.2

\*Different letters (a, b, c, etc.) in each column indicate a significant difference (p<0.05).

**Table 4:** The effects of different levels and sources of inorganic and organic manganese on ash and manganese in tibia.

Treatment*	Added manganese(ppm)	Amount of manganese in whole feed (ppm)	Ash of tibia (%)	Manganese in tibia (ppm)
Control	0	14	37.10 <sup>f</sup>	21 <sup>d</sup>
	60	74	37.33 <sup>ef</sup>	26 <sup>c</sup>
Manganese sulfate	90	104	37.99 <sup>d</sup>	27 <sup>c</sup>
	120	134	39.12 <sup>b</sup>	34 <sup>ab</sup>
Manganese threonine	60	74	38.34 <sup>c</sup>	30 <sup>b</sup>
	90	104	39.85 <sup>a</sup>	34 <sup>ab</sup>
	120	134	40.10 <sup>a</sup>	35 <sup>a</sup>
Bioplex® manganese	60	74	37.59 <sup>e</sup>	23 <sup>cd</sup>
SEM			0.09	1.2
P value			<0.0001	<0.0001
CV (%)			0.05	9.4

\*Different letters (a, b, c, etc.) in each column indicate a significant difference (p<0.05).

**Table 5:** Relative Bioavailability Value (RBV) of manganese threonine compared with manganese sulphate.

Response criterion	Manganese sulfate equation	Manganese threonine equation	Manganese sulfate R2	Manganese threonine R2	Manganese threonine to manganese sulfate relative bioavailability (%)
Body weight	Y=3.0376x+1710	Y=5.6426x+1710	0.3348	0.9667	185
FCR	Y=0.0006x+2.021	Y=-0.0026x+2.021	0.2389	0.8851	433
Ash in tibia	Y=0.0129x+37.104	Y=0.0261x+37.104	0.7289	0.9215	202
Manganese in tibia	Y=0.0002x+0.0574	Y=0.0003x+0.0574	0.6713	0.7683	150

**Table 6:** The effects of different levels and sources of inorganic and organic manganese on manganese excretion.

Treatment*	Added manganese (ppm)	Amount of manganese in whole feed (ppm)	Manganese in excreta (%)
Control	0	14	0.001 <sup>f</sup>
	60	74	0.023 <sup>bc</sup>
Manganese sulfate	90	104	0.028 <sup>b</sup>
	120	134	0.077 <sup>a</sup>
Manganese threonine	60	74	0.016 <sup>d</sup>
	90	104	0.020 <sup>cd</sup>
	120	134	0.020 <sup>cd</sup>
Bioplexmanganese®	60	74	0.011 <sup>e</sup>
SEM			<b>0.001</b>
P value			<0.0001
CV(%)			16.1

\*Different letters (a, b, c, etc.) in each column indicate a significant difference (p<0.05).

carcass characteristics. According to our results, Zhao et al. [19] found that the source of chelate produced more breast meat and reduced footpad dermatitis in chelate treatments. Manganese is a cofactor for the enzyme manganese superoxide dismutase, which is the body's first line of antioxidant defense against superoxide produced by the respiratory chain. Also, the antioxidant system and respiratory chain efficiency are effective in breast muscle synthesis [20]. Therefore, it can be concluded that the amount of lower breast muscle ratio in

the treatment of low-level sulfate (60 ppm) and control groups is reasonable. In our experiment, manganese threonine improved bone characteristics in broilers. Bone stores about 40% of the body's total manganese. So bone can be a good biomarker for manganese [21]. The half-lives of manganese in the femur, tibia, and humerus are 77, 263, and 429 days, respectively [22]. Rudolf Vieira et al. [15] have shown that the amount of tibia ash increases with the increasing use of trace elements with organic sources by replacing the organic source

of trace elements with their sulfated source. Black et al. [23] showed that the amount of manganese in the tibia changes with changes in manganese levels, and therefore the retention of manganese in chicken bones is the best indicator of manganese source bioavailability. Henry et al also observed greater retention of manganese in the tibia by supplementation of the manganese methionine chelate instead of sulfate, which increased with increasing dietary manganese levels [17]. Osama et al. [24] reported that organic Zn, Cu, Mn has improved the Tibia weight and length. In 2009, a study was conducted on turkeys and the researcher observed that organic trace minerals supplementation improved biochemical properties of bone [25]. Manganese threonine showed good bioavailability in our trial. Most organic sources of manganese are amino acids, which are thought to increase bioavailability by reducing the effects of calcium phytate, phosphorus, and fiber during the digestion process. Poultry also needs more manganese than other domestic animals, so using less inorganic sources of manganese can increase bioavailability and absorption of manganese and reduce its excretion in animal feces [26]. Henry et al. [17,27] founded that the bioavailability of manganese from manganese methionine is greater than manganese from manganese sulfate and manganese oxide. We founded that manganese threonine can reduce manganese excretion into the litter. In agreement with the results of this experiment, Scott and Leeson [28] found that the use of trace elements with organic sources reduced the excretion of these elements into the litter [28,29]. Also, Bao et al. [4] by studying the organic source of trace elements showed that using the organic source in broiler diets can be observed less excretion of these elements. Due to less retention and bioavailability of inorganic sources, the excretion of trace elements from the inorganic source is higher [30,31]. Wang et al. [27] indicated that the replacement of dietary inorganic trace minerals by organic trace minerals improved mineral deposition in tissues and reduced fecal mineral excretion in broiler breeders under the conditions of this study.

## Conclusion

According to the results of performance and other traits measured in this study, it seems that using 90 ppm and 120 ppm levels of manganese from manganese threonine chelate in the diet of broilers is recommended in comparison with manganese sulfate levels and another level of manganese threonine.

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## Compliance with Ethical Standards

Conflict of Interest the authors declare that they have no conflict of interest.

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