IJAS Iranian Journal of Applied Animal Science

Determining Relative Bioavailability of Different Manganese Sources in Broiler Diets

Research Article

A. Yaghobfar[1*], H.R. Ghalamkari[2], N. Sanei[2] and M. Ebrahimnejad[2]

[1] Department of Animal Nutrition, Animal Science Research Institute of Iran (ASRI), Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran
[2] Sepahan Daneh Parsian Co., Isfahan, Iran

Received on: 13 Jul 2020
Revised on: 6 Apr 2021
Accepted on: 1 May 2021
Online Published on: Mar 2022

*Correspondence E-mail: yaghobfar@yahoo.com

Online version is available on: www.ijas.ir

ABSTRACT

This study aimed to evaluate the relative bioavailability value (RBV) of different manganese mono oxide (MnO) sources and manganese sulfate ($MnSO_4$) in broiler diets as well as their effect on tibial characteristics, and serum inorganic phosphorous (P), calcium (Ca) and Mn concentrations. The experiment was carried out based on a completely randomized design (CRD) with 660 broiler chicks (Ross 308) assigned to 12 dietary treatments with 6 replicates of 10 birds each. The experimental diets consisted of one basal diet (as control), and 10 treatment groups which were supplemented with 400 or 800 mg/kg of feed of 1-5 MnO sources with 35%, 25%, 45-55%, 30%, and 40% purities, respectively; MnSO4 was also used (800 mg/kg of feed) in a treatment group as a reference standard with 100% bioavailability. The mean RBV of MnO sources (27.27 to 181.82%) showed a significant difference ($P<0.05$). The results also showed sera Ca and Mn concentrations, and retention of Mn in diet and tibia bone were significantly affected by different Mn sources used ($P<0.05$). Although no differences were observed for sera P, Ca, and Mn concentrations, regardless of the level of supplementation ($P<0.05$). The results of this study demonstrated that the purity of Mn supplement sources has a relationship with RBV of Mn, Mn absorption, and its retention in broiler bone and ileum.

KEY WORDS ileal digestibility, manganese mono oxide, Mn retention, relative bioavailability value (RBV), tibia bone.

INTRODUCTION

Manganese (Mn) is an essential trace element for birds, which plays an important role in the metabolism of carbohydrates, amino acids, and as a necessary cofactor of numerous enzymes, hydrolases, and normal bone formation, in poultry (Keen *et al.* 1999; Moomaw *et al.* 2009). Inorganic Mn supplements, including manganese oxide (MnO) or sulfate ($MnSO_4$), are routinely added to conventional poultry diets to meet the Mn requirements of birds for their optimum growth. However, the bioavailability of these inorganic Mn supplements (mineral salts) is very poor, leading to high concentrations in the excreta. It may be related to the formation of complexes with other substances in the digestive tract, such as phytate, which reduces the solubility of these elements and their absorption, thereby increasing their excretion (Bao *et al.* 2007). Numerous studies have been conducted to show the Mn homeostasis and excretion (Bertinchamps *et al.* 1966; Suzuki and Wada, 1981), tissue distribution (Underwood, 1977), and mechanism of absorption (Lonnerdal *et al.* 1987; Southern *et al.* 1987). It has been shown that ileum was the main site of Mn absorption for broilers (Khakpour *et al.* 2019), therefore, Mn bioavailability from Mn supplements used in poultry diets (Baker and Halpin, 1987; Fly *et al.* 1989). Baker and Halpin (1987) reported that the Mn bioavailability from $MnSO_4$ is roughly

similar to that of $MnCl_2$ and it is higher than that of MnO and $MnCO_3$. The high amount of Mn content observed in broiler feces is due to a high level of supplementation and/or indigestibility of the Mn source used. The supplement indigestibility is affected by endogenous or exogenous factors. The change in mineral balance caused by physiological status alteration and low Mn bioavailability of Mn supplement source led to endogenous and exogenous Mn indigestibility, respectively bioavailability. Therefore, additional Mn supplementation for poultry diets may be necessary to maintain the correct Mn level (Scott *et al.* 1976). The purpose of the present study was to evaluate the relative bioavailability of MnO from different sources in broilers diets. Followed by an investigation of the effect of Mn bioavailability on tibial characteristics, and serum inorganic phosphorous (P), calcium (Ca), and Mn concentration.

MATERIALS AND METHODS

Experimental design

The experiment was carried out based on a completely randomized design (CRD) using 660 male broiler chickens (Ross 308) assigned to 12 dietary treatments by 6 replicates including 10 birds each. The birds were fed the experimental diets for 21 to 42 days and water was offered *ad libitum*. The experimental diets consisted of one basal diet (as control), and 10 treatment groups which were supplemented with 400 or 800 mg/kg of feed of 1-5 MnO sources with 35%, 25%, 45-55%, 30%, and 40% purities, respectively; MnSO4 was also used (800 mg/kg of feed) in 1 treatment group as a reference standard with 100% bioavailability (Table 1).

The guide for care and use of laboratory animals was followed, and the project was approved by the animal experimentation ethics committee (CETEA) of the Federal University of Minas Gerais, (protocol number 111/2009).

Determination of Mineral relative bioavailability value (RBV) and apparent ileal availability of minerals (AIAM)

The RBV was determined using basal diet as the standard source by slope ratio comparisons (Littell *et al.* 1995; Littell *et al.* 1997). Variable responses to each source were used to calculate RBV of Mn content of diets and tibia bone. Based on diets and tibia bone Mn, RBV were calculated as equation (1):

RBV= (Tibia Mn–Y intercept) × 100 / (slope of regression line relating diet or tibia Mn×intake Mn) equation (1)

Differences between sources were determined by differences in their respective regression coefficients.

For determining a biological apparent ilea digestibility of basal diet with mineral sources, chromic oxide was used (Cr_2O_3; 0.3 percent in all diets) as an indigestible marker.

Whole ileal digesta were individually collected, and measured for Cr_2O_3 and mineral concentrations. Apparent ideal mineral digestibility (AIAM) in experimental diets was calculated using equation (2) (Kadim and Moughan, 1997; Kadim *et al.* 2002).

AIAM= ((feed Mn/Cr_2O_3%) – (excreta Mn/excreta Cr_2O_3)) / (feed Mn/Cr_2O_3%) equation (2)

Collection and processing of samples

In order to determine the RBV food intake and total droppings, output was measured quantitatively per cage over 4 consecutive days during the 3rd week of the trial (21 to 42 d). The droppings were collected daily, dried overnight at 80 ˚C in a forced-draft oven and subsequently the collections from each pen were combined for analysis. At the end of the trial (d 42), all surviving chicks were killed by intracranial injection of sodium pentobarbitone and their small intestine were immediately exposed. The contents of the lower ileum were expressed into plastic containers by gentle flushing with distilled water. The ileum was defined as that portion of the small intestine extending from the vitelline diverticulum to a point 40 mm proximal to the ileocaecal junction. The ileum was divided into 2 halves and the digesta were collected from the lower half towards the ileocaecal junction. The digesta samples were frozen immediately after collection. Dried droppings and ileal digesta samples were ground to pass through a 0·5-mm sieve and stored at –4 ˚C until required for chemical analysis**.** The Mn concentrations from different sources were measured by flame atomic absorption spectroscopy (Spectro AA, VARIAN) as described by (AOAC, 1990).

Tibia bone characteristics

Tibia bone characteristics were measured according to the methods described by Zhang and Coon (1997) and Park *et al.* (2003). Briefly, all the bones were first weighed in the presence of air, then reweighed while suspended in water at room temperature. Bone volume was calculated with the assumption that the specific gravity of water is 1 g/cm^3 at room temperature. For the fresh bone preparation, breaking strength was first measured, and bones were then dried at 100 ˚C for 24 h and weighed again. For measuring bone ash content, bones were oven-dried at 105 ˚C for 24 h and ashed in a muffle furnace at 600 ˚C for 6 h. The ash percentage was determined relative to the dry weight of the tibia. The Seedor index is the value obtained by dividing the bone weight by its length (Seedor *et al.* 1991; Seedor, 1993; Seedor, 1995).

Table 1 The calculated composition and nutrient content of experimental diets fed to broilers chickens (42 days)

Ingredients	Basal diet (g/kg)
Yellow corn	510.00
Soybean meal 44% CP	280.00
Wheat	140.00
Soy oil	18.50
Dicalcium phosphate	18.00
Mineral oyster shell	8.300
Sodium chloride	1.800
DL-methionine	2.800
L-lysine HCI	2.500
L-threonine	0.900
Choline chloride	0.500
Vitamin premix*	2.500
Mineral premix*	2.500
Calculated values	
Metabolizable energy (MJ/Kg)	12.97
Protein (%)	19.00
Digestible Met + Cys (%)	0.850
Digestible lysine (%)	0.970
Calcium (%)	0.950
Available phosphorus (%)	0.450
Mn mg/kg	1200
Zn mg/kg	1100
Sodium (%)	0.150
Chloride (%)	0.220
Potassium (%)	0.870
(Na+K) - Cl (meq/kg)	231.23

CP: crude protein.
* Provided per kilogram of diet: vitamin A: 44000 IU; vitamin D_3: 17000 IU; vitamin E: 440 mg; vitamin K_3: 40 mg; vitamin B_{12}: 70 mg; vitamin B_1: 65 mg; vitamin B_2: 32 mg; Pantothenic acid: 49 mg; Niacin: 122 mg; vitamin B6: 65 mg; Biotin: 22 mg and Choline chloride: 27 mg.
** Minerals: Mn (MnO): 99.20 mg; Zn (ZnO): 85 mg; Fe ($FeSO_4$): 50 mg; Cu (Cu $SuSO_4$): 10 mg; Se (Na_2SeO_3): 0.2 mg; I (KI) 13 mg and Co: 250 mg.

The Seedor index is a measure of bone density, in another word the higher the Seedor index value, the denser the bone.

Statistical analysis

The data were analyzed by the General Linear Models (GLM) procedure (SAS, 2004). Duncan's multiple range test was used to compare each experimental group with the control group of means ($P<0.05$).

RESULTS AND DISCUSSION

The purity of Mn concentrations from sources contained 25 to 55% for oxide, respectively, whereas the sulfate source had 30.5% Mn. Although, the oxide source of 3 had a higher concentration of Mn compared to other sources. Examining analysis of Mn sources using flame atomic absorption spectroscopy method indicated a lower degree of purity for all oxide and sulfate mineral sources, respectively (Table 2). Thus, presenting less concentration of Mn in each source could affect Mn bioavailability. Nevertheless, these differences in Mn concentrations may be related to low solubility, also decreased availability, and more excreted.

Data presented those concentrations of manganese from different sources in diets fed to broilers chickens had significant effects on biological apparent ileal digestibility values dietary minerals (g/100 g) in different sources (Table 3), whereas, manganese oxide source 3 had relatively significantly higher Mn availability ($P<0.05$). However, manganese oxide sources 1 and 2 were significantly lower than other sources. The availability of Mn for utilization varies between inorganic Mn sources including MnO and MnSO4. In terms of experiment results, in contrast to the Baker and Halpin (1987) study, bioavailability of Mn from MnSO4 in the current experiment was lower than MnO sources. This can be attributed to the chemical form in which the Mn occurs.

Estimates of the relative bioavailability were obtained by the slope ratios of linear regression equations (Table 4). When the regression slope for sulfate source was set equal to 100 %, relative bioavailability of 145 ± 5.21, 27.27 ± 3.98, 181.82 ± 5.03, 36.36 ± 7.72, and 181.82 ± 0.81 were obtained for dietary Mn concentrations of different Mn sources, respectively. The means of relative bioavailability values of Mn oxide sources ranged from 27.27 to 181.82 and were significantly different.

Table 2 Analyzed Mn concentrations from different sources

Manganese sources	Reported Mn concentration*	Analyzed Mn concentration**
Manganese oxide 1	35	19.55
Manganese oxide 2	25	17.10
Manganese oxide 3	45-55	40.50
Manganese oxide 4	30	24.69
Manganese oxide 5	40	29.71
Manganese sulfate	30.5	26.60

* By producer company and ** In our laboratory.

Table 3 Bioavailability and concentrations of manganese from different sources in diets fed to broilers chickens (as-fed basis)

Manganese sources	Added Mn, (mg/kg)	Analyzed dietary Mn, (mg/kg)	Apparent ileal availability[1] (g/100g)
Control diet	0.00	159.5	0.36^{cd}
Manganese oxide 1	400	210.0	0.27^{de}
	800	232.3	0.19^{e}
Manganese oxide 2	400	203.0	0.26^{de}
	800	209.8	0.25^{e}
Manganese oxide 3	400	234.5	0.42^{bc}
	800	738.4	0.58^{a}
Manganese oxide 4	400	303.6	0.45^{b}
	800	337.8	0.28^{de}
Manganese oxide 5	800	458.5	0.37^{bcd}
Manganese sulfate	800	402.0	0.43^{b}
SEM	-	-	0.08
P-value	-	-	0.002

[1]Apparent ileal availability of minerals (AIAM)= ((feed Mn/Cr_2O_3 %) - (excreta Mn/excreta Cr_2O_3)) / (feed Mn/Cr_2O_3 %).
SEM: standard error of the means.

Table 4 Regression coefficient and relative bioavailability value (RBV) of manganese sources

Manganese sources	Regression coefficient (slope±SE)	Relative bioavailability value[1] (±SE)	P-value
Manganese oxide 1	0.016±0.05	145±5.21	0.007
Manganese oxide 2	0.003±0.06	27.27±3.98	0.002
Manganese oxide 3	0.02±0.035	181.82±5.03	0.003
Manganese oxide 4	0.004±0.07	36.36±7.72	0.0027
Manganese oxide 5	0.02±0.01	181.82±0.81	0.02
Manganese sulfate	0.011±0.02	100±1.62	0.02

[1] RBV= (Mn sources–Y intercept) ×100 / (slope of regression line relating diet or Mn sources×Mn intake).

These results agreed with the study of Luo (1989) claiming that among several inorganic manganese sources used in broiler diets, manganese oxide would be lower in bioavailability in comparison to the other sources. Another way, these differences between relative bioavailability values of sources would be depended on the chemical or physical form Mn sources. Besides certain components of the sources can interfere with their absorption or relative bioavailability.

Otherwise, Mn contents in bird's manure are far more than nitrogen. Hence, Mn in poultry manure is far more than crop requirements, causing accumulation in the soil, especially in regions of intensive poultry production (Meijer and Kroger, 1973; Hahn, and Baker, 1993; Mohanna and Nys, 1998). The less soluble compounds, such as carbonates and oxides, have lower relative biological availabilities (Fly *et al.* 1989; Wedekind *et al.* 1992; Ammerman *et al.* 1998).

Also, Mn element have specific physiological and biochemical functions in the body and, as a result, their deficiency leads to reduced production and reproductive performance and maybe impaired fat and carbohydrate metabolism and immune response (Underwood and Suttle, 1999).

There were significant ($P<0.05$) effects of manganese sources on the apparent retention of Mn (Table 5). The apparent Mn retention and retained Mn of oxide form of sources 4 and 3, were significantly higher than Mn oxide sources 5, 1, 2, also Mn sulfate, and control diet. Our experiment showed that Mn retention and retained Mn of sulfate sources were significantly lower than oxide sources ($P<0.05$). This may be mostly reflected in the trends observed for apparent Mn retention. Mn content of each source can form complexes with multivalent anions to produce insoluble salts, thereby reducing the availability of some Mn sources and showing significant differences.

Table 5 Mean apparent Mn retention (%) and apparent retained Mn content (mg/kg) of manganese sources

Manganese sources	Added Mn (mg/kg)	Apparent Mn retention (%)*	Apparent retained Mn content (mg/kg)**
Control diet	0	89.60^{cd}	2.49^{cd}
Manganese oxide 1	800	74.51^{cd}	3.82^{c}
Manganese oxide 2	800	116.21^{c}	3.75^{c}
Manganese oxide 3	800	169.11^{b}	7.75^{b}
Manganese oxide 4	800	355.54^{a}	14.29^{a}
Manganese oxide 5	800	57.21^{d}	0.91^{d}
Manganese sulfate	800	74.51^{cd}	1.54^{cd}
SEM		37.21	1.87
P-value		< 0.0001	< 0.0001
R^2		0.89	0.86

* Apparent mineral retention %= (diet mineral×feed intake) - (excreta mineral×total excreta) × 100 / mineral diet × feed intake.
** Apparent retained mineral content (mg/kg)= diet mineral (mg/kg) × apparent mineral retention %.
SEM: standard error of the means.

Table 6 Effect of dietary manganese source and concentration on blood minerals (mg/dL)

Manganese sources	Added Mn, mg/kg	Ca	P	Mn
Control diet	0	12.81^{b}	9.37	0.30^{bcd}
Manganese oxide 1	400	13.46^{ab}	9.42	0.26^{cd}
	800	13.80^{ab}	9.30	0.22^{d}
Manganese oxide 2	400	15.55^{ab}	8.87	0.26^{cd}
	800	14.51^{ab}	9.60	0.27^{cd}
Manganese oxide 3	400	13.21^{b}	9.15	0.30^{bcd}
	800	15.82^{ab}	9.02	0.33^{abc}
Manganese oxide 4	400	17.86^{a}	9.12	0.39^{ab}
	800	16.19^{ab}	9.37	0.34^{abc}
Manganese oxide 5	800	15.51^{ab}	9.00	0.39^{ab}
Manganese sulfate	800	13.07^{b}	8.97	0.43^{a}
SEM		2.77	2.77	0.07
P-value		0.25	0.25	0.003

SEM: standard error of the means.

Furthermore, few studies have investigated the impact of inorganic Mn sources supplemented to broiler diets on the ileal digestibility or retention of Mn, or tended to be contradictory (Khakpour *et al.* 2019). Although the proportion of most trace elements absorbed in the intestine is rather low, homoeostasis of elements such as Mn is regulated via endogenous secretions (Sebastian *et al.* 1996; Sandstrom, 1997; Saima *et al.* 2009; Rutherfurd *et al.* 2012).

Significant differences (P<0.05) were observed between treatments in Mn contents of blood sera on d 35 to 42 of the experiment (Table 6). Therefore, mean Ca concentrations of birds fed Mn oxide 4 source were significantly higher than those fed Mn sulfate or basal diet. Also, mean Mn concentrations of the group fed Mn sulfate source is significantly higher than those fed Mn oxide or the basal diet (P<0.05).

The effect of different sources of dietary Mn, Ca, P, on ash concentrations, diameter, and length of tibia bone are presented in Table 7. The Mn concentrations in tibia bone obtained from Mn sulfate source were significantly higher than all oxide sources (P<0.05).

These results agreed with Yan and Waldroup (2006) that reported the significant effects of Mn source on Mn content of tibia bone, in contrast to the other study (Wong-Valle *et al.* 1988; Henry *et al.* 1989; Smith *et al.* 1995). Moreover, Mn sources did not affect the ash, diameter, and length of tibia bone.

Tibia bone Mn concentrations responded linearly to Mn intake in the 35 to 42 days of birds age (Table 8). The relative bioavailability values from Mn oxide sources were 22.28 to 142.85% and for Ca within the range 50.18 to 76.75% (P>0.05). The birds fed source 2 and 5 Mn oxide forms had 43 and 23% higher levels of tibia Mn than those fed the sulfate form, respectively. However, these differences for bioavailability values for the reason that inorganic minerals are present in chyme as ions that interact with other dietary constituents to form differences available or less unavailable complexes for absorption. Because the inorganic complexes formed by various sources have different characteristics or functions that might affect their absorption in the bird gut.

Table 7 Effect of dietary manganese source and concentration on tibia manganese, Ca, P and ash concentrations of 35-42 d old broiler chickens

Manganese sources	Added Mn, mg/kg	Ca	P	Mn	Ash %	Diameter (mm)	Length (cm)
Control diet	0	19.80	8.79	4.08^{ab}	51.40	7.02	94.54
Manganese oxide 1	800	18.61	7.74	3.48^{b}	48.78	7.91	95.48
Manganese oxide 2	800	18.65	7.82	4.88^{ab}	50.32	7.27	94.27
Manganese oxide 3	800	18.68	8.64	4.59^{ab}	47.98	7.57	95.22
Manganese oxide 4	800	19.08	8.63	4.71^{ab}	48.76	6.64	96.07
Manganese oxide 5	800	20.08	8.48	2.33^{b}	47.93	7.30	93.39
Manganese sulfate	800	19.98	8.37	7.12^{a}	48.02	7.07	96.35
SEM		2.06	0.77	1.79	2.26	0.67	2.98
P-value		0.92	0.55	0.13	0.43	0.41	0.89

SEM: standard error of the means.

Table 8 Relative bioavailability values of manganese sources based on slop ratio of tibia bone manganese concentrations

Manganese sources	Added Mn, mg/kg	Relative bioavailability values[1]	
		Ca	Mn
Manganese oxide 1	800	57.16	48.57
Manganese oxide 2	800	76.75	142.85
Manganese oxide 3	800	54.65	22.28
Manganese oxide 4	800	61.57	65.71
Manganese oxide 5	800	50.18	123.00
Manganese sulfate	800	100	100

[1] RBV= (tibia Mn–Y intercept) × 100 / (slope of regression line relating diet or tibia Mn×Mn intake).

Table 9 Relative bioavailability value (RBV) of tibia bone Mn on broiler chickens

Manganese sources	RBV Mn diet	RBV Mn tibia bone
Manganese oxide 1	15.55±0.73^{d}	3.30±0.12^{e}
Manganese oxide 2	82.21±3.67^{a}	4.83±0.003^{b}
Manganese oxide 3	13.02±0.49^{d}	4.36±0.06^{d}
Manganese oxide 4	61.70±2.86^{b}	4.57±0.01^{c}
Manganese oxide 5	12.56±0.61^{d}	2.31±0.0002^{f}
Manganese sulfate	22.78±1.11^{c}	7.04±0.001^{a}
SEM	5.12	0.63
P-value	< 0.0001	< 0.0001

SEM: standard error of the means.

The relative bioavailability value of Mn oxide relative to Mn sulfate obtained in our study, apart from oxide sources of 2 and 5, agrees with the other reports. In this regard, we can refer to the study of Black *et al.* (1984) and Wong-Valle *et al.* (1988) in which they reported bioavailability of 65.6, 81.9, 91.3, 75.0, and 70.3% for different Mn oxide sources compared to Mn sulfate based on multiple linear regressions on bone Mn concentration for broiler chicks (Ammerman *et al.* 1998). The reason for this difference in bioavailability can be attributed to higher Mn bioavailability of 2 and 5 oxide sources compared to sulfate form that may be associated with lower levels of interacting minerals such as Ca and Fe in these sources. Though bioavailability responses to these sources did not show the same trend for all indices measured.

According to results, retention of Mn in diet and tibia bone was significantly different between Mn sources (P>0.05).

In the case of, retention of Mn element, in tibia, there was more sensitivity to Mn sources. Tibia Mn has come to be recognized as the variable of choice in the calculation of relative bioavailability value (Cook, 1973; Ranhotra *et al.* 1976). These variable responses on each source were used to calculate RBV of Mn content of diets and tibia bones. Based on diets and tibia bone Mn, RBV were calculated as follows:

RBV= (tibia Mn–Y intercept) × 100 / (slope of regression line relating diet or tibia Mn×Mn intake)

Utilizing this method, the RBV of Mn sources were significantly different (Table 9) (based on reference diet=100%). These differences may be depended on physico-chemical properties of Mn sources such as the cation exchange capacity and compound absorptive properties (Van Der Klis *et al.* 1993; Bach Knudsen, 2001).

CONCLUSION

In conclusion, the results of the present study clearly demonstrated that Mn concentrations from Mn oxide sources were that were examining analysis by using flame atomic absorption spectroscopy method in our laboratory lower degree of purity. Thus, this could be contributed to reduced relative bioavailability values, absorption, and also, Mn retention. Furthermore, there were indigestible that could be from altered mineral balance caused by physiological changes such as decreased Mn bioavailability and will increase dietary Mn requirements, thence, there must be Mn supplemented addition to poultry diets. Therefore, our data indicated that different inorganic Mn oxide sources have differences regarding the relative bioavailability values, retention, or absorption in the ileum of birds. Nevertheless, these aforementioned differences of Mn concentrations and relative bioavailability values may be related to low solubility, physiological and biochemical functions, or chemical form in which the Mn occurs.

ACKNOWLEDGEMENT

The authors wish to acknowledge Sepahan Daneh Parsian Co. for funds in support of this research.

REFERENCES

Ammerman C.B., Henrty P.R. and Miles R.D. (1998). Supplemental organically-bound mineral compounds in livestock nutrition. Pp. 67-91 in Recent Advances in Animal Nutrition. P.C. Garnsworthy and J. Wiseman, Eds. Nottingham University Press, Nottingham, United Kingdom.

AOAC. (1990). Official Methods of Analysis. Vol. I. 15th Ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Bach Knudsen K.E. (2001). The nutritional significance of ''dietary fiber'' analysis. *Anim. Feed Sci. Technol*. **90,** 3-20.

Baker D.H. and Halpin K.M. (1987). Research note: Efficacy of a manganese-protein chelate compared with that of manganese sulfate for chicks. *Poult. Sci*. **66,** 1561-1563.

Bao Y.M., Choct M., Iji P.A. and Bruerton K. (2007). Effect of organically complexes copper, iron, manganese, and zinc on broiler performance, mineral excretion, and accumulation in tissues. *J. Appl. Poult. Res*. **16,** 448-455.

Bertinchamps A.J., Miller S.T. and Cotzias G.C. (1966). Interdependence of routes excreting manganese. *American. J. Physiol.* **211,** 217-225.

Black J.R., Ammerman C.B., Henry P.R. and Milles 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.

Cook D.A. (1973). Availability of magnesium: Balance studies in rats with various inorganic magnesium salts. *J. Nutr*. **103,** 1305-1312.

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

Hahn J.D. and Baker D.H. (1993). Growth and plasma zinc responses of young pigs fed pharmacological levels of zinc. *J. Anim. Sci*. **71,** 3020-3028.

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

Kadim I.T. and Moughan P.J. (1997). Development of an ilea digestibility assay for the growing chiken- effect of the imposition of a fasting period and the nature of the test diet. *Brezilian Poult. Sci*. **38,** 285-290.

Kadim I.T., Moughan P.J. and Ravindran V. (2002). Ileal amino acid digestibility assay for the growing meat chicken comparison of ileal and excreta amino acid digestibility in the chicken. *Brezilian Poult. Sci.* **43(4),** 587-597.

Keen C.L., Ensunsa J.L., Watson M.H., Baly D.L., Donovan S.M., Monaco M.H. and Clegg M.S. (1999). Nutritional aspects of manganese from experimental studies. *Neurotoxicology*. **20,** 213-224.

Khakpour F., Janmohammadi H., Kianfar R. and Sahraei M. (2019). Evaluation of chemical characteristics and effects of different manganese sources on kinetics of manganese absorption and performance of broiler chickens. *Iranian J. Appl. Anim. Sci*. **9(3),** 463-471.

Littell R.C., Henry P.R., Lewis A.J. and Ammerman C.B. (1997). Estimate of relative bioavailability of nutrients using SAS procedures. *J. Anim. Sci*. **75,** 2672-2683.

Littell R.C., Lewis A.J. and Henry P.R. (1995). Statistical evaluation of bioavailability assays. Pp. 5-35 in Bioavailability of Nutrients for Animals. C.B. Ammerman, Ed. Academic Press, San Diego, California.

Lonnerdal B., Keen C.L., Bell J.G. and Sandstrom B. (1987). Manganese uptake and retention. Pp. 7-20 in Nutritional Bioavailability of Manganese. C. Kies, Ed. American Chemical Society, Anaheim, California.

Luo X.G. (1989). Studies on manganese requirement and its bioavailability in practical diets for broiler chicks. Ph D. Thesis. Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.

Meijer H. and Kroger H. (1973). Kupferfütterung beim Schwein. *Uebers. Tierernaehr*. **1,** 4-9.

Mohanna A.C. and Nys Y. (1998). Influence of age, sex and cross on body concentrations of trace elements (zinc, iron, copper and manganese) in chickens. *Brezilian Poult. Sci.* **39,** 536-543.

Moomaw E.W., Angerhofer A., Moussatche P., Ozarowski A., Garciarubio I. and Richards N.G.J. (2009). Metal dependence of oxalate decarboxylase activity. *Biochemistry*. **48,** 6116-6125.

Park S.Y., Birkhold S.G., Kubena L.F., Nisbet D.J. and Ricke S.C. (2003). Effect of storage condition on bone breaking strength and bone ash in laying hens at different stages in production cycles. *Poult. Sci*. **82,** 1688-1691.

Ranhotra G.S., Loewe R.J. and Puyat L.V. (1976). Bioavailability of magnesium from wheat flour and various organic and inorganic. *Cereal. Chem.* **53,** 770-777.

Rutherfurd S.M., Chung T.K., Thomas D.V., Zou M.L. and Moughan P.J. (2012). Effect of a novel phytase on growth performance, AME and the availability of minerals and amino acids in a low-phosphorus corn-soybean meal diet for broilers. *Poult. Sci*. **91,** 1118-1127.

Saima M.Z., Khan U., Jabbar M.A., Ija M. and Qadeer M.A. (2009). Efficacy of microbial phytates at different levels on growth performance and mineral availability in broiler chickens. *J. Anim. Plant Sci*. **19,** 58-62.

Sandstrom B. (1997). Bioavailability of zinc. *European J. Clin. Nutr*. **51(1),** 17-19.

SAS Institute. (2004). SAS®/STAT Software, Release 9.4. SAS Institute, Inc., Cary, NC. USA.

Scott M.L., Nesheim M.C. and Young R.J. (1976). Scott's Nutrition of the Chicken, Ithica, New York, USA.

Sebastian S., Touchburn S.P., Chavez E.R. and Lague P.C. (1996). Efficacy of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper and zinc in broiler chickens fed corn-soybean meal diets. *Poult. Sci*. **75,** 729-736.

Seedor J.G. (1993). The biophosphanate alendronate (MK-217) inhibit bone loss due to ovariectomy in rats. *J. Bone. Miner. Res*. **4,** 265-270.

Seedor J.G. (1995). The biophosphanate alendronate (MK-217) inhibit bone loss due to ovariectomy in rats. *J. Bone. Miner. Res*. **4,** 265-270.

Seedor J.G., Quarruccio H.A. and Thompson D.D. (1991). The bisphosphonate alendronate (MK-217) inhibits bone loss due to ovariectomy in rats. *J. Bone. Miner. Res*. **6,** 339-346.

Smith M.O., Sherman I.L., Miller L.C. and Robbins K.R. (1995). Relative biological availability of manganese from manganese protein ate, manganese sulfate, and manganese monoxide in broilers reared at elevated temperatures. *Poult. Sci*. **74,** 702-707.

Southern L.L., Baker D.H. and Halpin K.M. (1987). Manganese homeostasis in the chick. Pp. 35-45 in Nutritional Bioavailability of Manganese. C. Kies, Ed. American Chemical Society, Anaheim, California.

Suzuki H. and Wada O. (1981). Role of liver lysosomes in uptake and biliary excretion of manganese in mice. *Environ. Res.* **26,** 521-528.

Underwood E.J. (1977). Trace Elements in Human and Animal Nutrition. Academic Press, New York, USA.

Underwood E.J. and Suttle N. (1999). The Mineral Nutrition of Livestock. CAB International, Wallingford, United Kingdom.

Van Der Klis J.D., Verstegen M.W.A. and Van Voorst A. (1993). Effect of a soluble polysaccharide (carboxy methyl cellulose) on the absorption of minerals from the gastrointestinal tract of broilers. *British Poult. Sci.* **34,** 985-997.

Wedekind K.J., Hortin A.E. and Baker D.H. (1992). Methodology for assessing zinc bioavailability: Efficacy estimates for zinc-methionine, zinc sulfate, and zinc oxide. *J. Anim. Sci*. **70,** 178-187.

Wong-Valle J., Ammerman C.B., Henry P.R. and Miles R.D. (1988). Bioavailability of Mn as feed grade Mn oxides for chicks. *Poult. Sci.* **68,** 1368-1373.

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

Zhang B. and Coon C.N. (1997). The relationship of calcium intake, source, size, solubility *in vitro* and *in vivo*, and gizzard limestone retention in laying hens. *Poult. Sci.* **76,** 1698-1701.