Relative bioavailability of manganese in relation to proteinate and sulfate sources for broiler chickens from one to 20 d of age

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ABSTRACT The objective of this study was to evaluate the relative bioavailability (**RB**) of manganese (**Mn**) proteinate compared to Mn sulfate for broilers fed a diet based on corn and soybean meal for 20 d. The diets of 1,350 male Cobb broilers were supplemented with 0, 35, 70, 105, or 140 mg of Mn/kg of feed in the form of Mn sulfate or Mn proteinate. Weight gain, feed intake, feed conversion, bone strength, and Mn concentration in the tibia and liver, as well as the concentration of type I collagen in the tibia, were evaluated. No differences were observed for performance variables ($P > 0.05$) or for type I collage concentration in broiler tibia ($P > 0.05$), regardless of the source and level of supplementation used. Relative bioavailability was determined using bone strength values and Mn concentration in the tibia and liver, assuming Mn sulfate as the standard source (100%) by the slope-ratio method. The RB of Mn proteinate based on bone strength was 111%, based on liver Mn concentration was 128%, and based on tibia Mn concentration was 105%. Manganese proteinate was more bioavailable than Mn sulfate; it can be an important source of supplementation to improve bone quality in broilers.

Key words: relative bioavailability, mineral, manganese proteinate, broiler

INTRODUCTION

Manganese (**Mn**) is an essential micromineral that participates in important functions in animals, especially in the organic bone matrix (Cupertino et al., 2005). In addition, Mn has functions in reproduction, the immune system, the free radical defense mechanism, hemostasis, and, along with vitamin K, blood coagulation (Aschner and Aschner, 2005). It also has functions in various biochemical processes as an activator of some metalloenzymes such as pyruvate carboxylase, superoxide dismutase, and glycosyltransferase (Suttle, 2010).

Sources for micromineral supplementation of broiler feed are usually derived from inorganic compounds such as chlorides, oxides, sulfates, carbonates, and phosphates (Araújo et al., 2008). Inorganic salts usually have low bioavailability, which 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).

On the other hand, chelated minerals are formed by minerals attaching to some type of carrier, such as amino acids, proteins, or polysaccharides. The chelate formed is normally a ring structure with the bivalent or multivalent mineral held strongly or weakly through covalent bonds and without an electric charge (Leeson and Summers, 2005). In complex form, and not as free inorganic ions, minerals reduce solubilization and excretion losses before absorption, thereby increasing their bioavailability (Abdallah et al., 2009).

Some authors have already observed the higher bioavailability of chelated minerals, when compared to inorganic minerals (Li et al., 2005; Bai et al., 2012). Liao et al. (2019) concluded that Mn chelates with moderate and high chelation strength present greater intestinal absorption than the inorganic source.

Industrial chelates usually use free amino acids to complex with bivalent minerals. However, there are other types of chelates such as proteinate, which is the result of the chelation of a soluble salt with partially

hydrolyzed amino acids and proteins (Association of American Feed Control Officials, 1999) and which may increase bioavailability and consequently reduce mineral excretion.

The objective of this study was to determine the relative bioavailability (**RB**) of Mn in the form of proteinate (**ProtMn**) in relation to sulfate (**SulfMn**) and compare the concentration of type I collagen in the tibia of broilers fed one or the other sources of Mn (ProtMn or SulfMn).

MATERIALS AND METHODS

The experimental procedures described in this research were approved by the Comissão de Ética no Uso de Animais (**CEUA**; Committee for Ethics in the Use of Animals) under the protocol no. 197/2016.

General Procedures

A total of 1,350 Cobb 500 broilers in the one- to 20-days-old phase were used. The initial phase was studied because the mineral requirements in this phase are fundamental for the bone and muscle formation of broilers. Chickens were housed 25 per experimental box (12 birds/m^2), with a pendular drinking sources and a tubular feeder. The birds received 24 h of artificial light (250 W heating lamp) until 14 d of age, after which they received natural light until the end of the experiment. Feed and water were freely available, and the water contained about 0.003 mg of Mn/L. The basal diet was based on corn and soybean meal (Table 1) and formulated to meet all broiler requirements except for Mn. The National Research Council (NRC, 1994) recommended level of Mn for optimal growth of broiler chickens is 60 mg/kg. However, Mwangi et al. (2019) recommended level is 12 mg/kg.

The experiment was conducted using a completely randomized design. Nine treatments were defined by the basal diet supplemented with 0 (control group), 35, 70, 105, or 140 mg Mn/kg in the form of 26.5% SulfMn and the same inclusions in the form of 18.3% ProtMn with 6 repetitions each. The analyzed Mn concentrations in the experimental diets are presented in Table 2.

To estimate bioavailability, 9 treatments and 6 replicates were used: one control without Mn supplementation, 4 with SulfMn supplementation, and 4 with ProtMn supplementation (Table 2). To determine the percentage of red birefringence in the tibia, 3 treatments and 6 replicates were used: one control treatment without Mn supplementation, and 2 others with the maximum Mn supplementation (140.0 mg Mn/kg) for each of the 2 evaluated sources (ProtMn and SulfMn).

Variables Studied

Weight gain and feed intake per repetition were measured at 7-day intervals to determine weight gain, feed intake, and feed conversion throughout the 20-day period.

Table 1. Basal feed composition.

Ingredients	Basal feed (%)	Mn (mg/kg)
Corn	57.671	5.3 (<1 0[5])
Soybean meal 46% CP	31.600	31.9 (25.0[5])
Meat and bone meal	6.000	1.5 (12.0[5])
Vegetable oil	3.200	
Limestone	0.350	
Salt	0.350	
DL-methionine	0.330	
L-lysine	0.190	
L-threonine	0.070	
Mineral supplement[1]	0.050	
Vitamin supplement[2]	0.050	
Choline chloride	0.025	
Anticoccidial[3]	0.050	
Growth promoter[4]	0.004	
Inert	0.060	
Calculated composition		
Metabolizable energy kcal/kg	3,050	
Crude protein %	21.8	
Calcium %	1.02	
Available phosphorus %	0.45	
Manganese mg/kg	13.25	
Lysine dig. %	1.16	
Methionine dig. %	0.62	
Met + Cys dig. %	0.90	

[1]Content/kg: Co, 100 mg; Se, 200 mg; Zn, 50 g; Cu, 6,000 mg; Fe, 50 mg; I, 1,000 mg.
[2]Content/kg: vit. A, 10,000,000 UI; vit. B1, 1,500 mg; vit. B12, 15,000 μg; vit. B2, 5,000 mg; vit. B6, 2,000 mg; vit. D3, 2,000,000 UI; vit. E, 13,000 UI; vit. K3, 2,500 mg; biotin, 100 mg; niacin, 33 g; folic acid, 800 mg; pantothenic acid, 10 g.
[3]Coxistac-salinomycin
[4]Surmax-avilamycin
[5]Analyzed manganese content.

At 20 d of age, 6 birds from each treatment (one per repetition) were randomly selected and euthanized by cervical dislocation to obtain left and right tibia, left femur, and liver with gallbladder.

After removal of all soft tissue, the left tibias were dried in a forced ventilation oven at 60°C for 72 h followed by fat extraction by immersion in petroleum ether for 3 d. The bones were subsequently dried for 12 h in an oven at 105°C and then burned in a muffle furnace at 600°C for 6 h (AOAC, 2010). Samples of ash bones, feed, and liver with gallbladder were lyophilized, and after acid digestion, they were used to determine the percentage of Mn using an atomic absorption spectrophotometer (Perkin Elmer—Analyst 100) (AOAC, 2010).

Table 2. Concentration of Mn analyzed in the experimental diets.

Mn source	Mn added mg/kg	Mn analyzed mg/kg
Control	0	12.2
SufMn[1]	35.0	48.3
	70.0	82.0
	105.0	117.0
	140.0	150.0
ProtMn[2]	35.0	46.5
	70.0	80.0
	105.0	115.0
	140.0	154.0

[1]Mn sulfate 26.5%.
[2]Mn proteinate 18.3%, from partially hydrolyzed soybean meal (Yes).

The right tibias of the broilers of the control treatments with higher inclusion of Mn for both sources (140 mg Mn/kg) were analyzed for the concentration of type I collagen in bone. After soft tissue removal, the tibial epiphysis was cut and demineralized in 10% EDTA for 12 d. The demineralized fragments were processed for histology after fixation in neutral buffered formalin and embedding in paraffin. Serial sections (5 μm) at 3 different depths were stained with the *Picro-Sirius Red* technique and subsequently photographed and analyzed in bright field and polarized light (Junqueira et al., 1978). Quantification of the mature bone matrix (collagen type I) was performed using *ImageJ* software, considering only the birefringence of the red fibers, and an average of the 3 depths was calculated for each repetition.

After soft tissue removal, left femur samples were mechanically tested using an EMIC model DL 3000 universal test machine (Belo Horizonte, Brazil) with load applied at a velocity of 5 mm/min and cell load of 2000 N in a 3-point flexion test with the central region of the bone (diaphysis) selected for load application.

Statistical Analysis

Normality and homogeneity of variances were evaluated by Shapiro-Wilk and Levene tests, respectively. Contrast was performed between the control treatment and the means of the treatments supplemented with Mn. The results of the percentage red birefringence in the tibia were analyzed by ANOVA, with dietary treatments being the main factor. Mn RB was determined using SulfMn as the standard source by multiple linear regression and the slope-ratio method (Littell et al., 1995). Responses were considered significant when $P < 0.05$. All statistical analyses were performed using R software (R Core Team, 2017).

RESULTS

Broiler performance was not affected ($P > 0.05$) by Mn source or supplement level (Table 3). Bone strength, tibial Mn concentration, and liver Mn concentration increased with Mn supplementation ($P < 0.05$), regardless of the source and level used ($P < 0.05$).

These variables were more sensitive to changes in Mn in the diet and were used to perform the slope-ratio method, comparing the RB of the 2 sources of supplementation (Figure 1), with SulfMn being considered the standard source (100%).

The slope of the line for ProtMn was higher than that for SulfMn for the 3 variables tested ($P < 0.05$), demonstrating greater bioavailability of ProtMn. RB of Mn for ProtMn based on bone strength was 111%, based on liver Mn concentration 128%, and based on tibial Mn concentration 105%, compared to SulfMn ($P < 0.05$). The mean superiority of Mn RB for ProtMn in relation to SulfMn was 15%.

No difference ($P > 0.05$) was observed between the control treatments and the inclusion of Mn in the form of SulfMn or ProtMn (140.0 mg Mn/kg), in relation to the percentage of red fibers (collagen type I) in the tibial epiphysis (Table 4).

DISCUSSION

Supplementation did not alter performance results, as also reported by Brooks et al. (2012) who also observed no differences in broiler performance with Mn supplementation, regardless of source (SulfMn and Mn propionate) and level (0, 20, 100, and 500 mg of Mn/kg) for the period of 7 to 21 d of age. Similarly, Ghosh et al. (2016) found no differences in broiler performance with different levels of Mn supplementation in diet. As in the present study, these authors considered that the concentration of Mn in the basal diet (12.2 mg Mn/kg of feed) was sufficient to maintain the proper development of the birds without showing signs of deficiency.

On the other hand, Jasek et al. (2019) observed an improvement in feed conversion with an inclusion of 40 mg Mn/kg of feed in the form of Mn hydroxychloride in the final rearing period. However, for the initial phase from one to 20 d, as in the present study, they also did not observe any differences in performance with the inclusion of Mn at different levels (40, 80, 120, and 160 mg Mn/kg of feed).

Table 3. Effects of Mn level and dietary source on performance of broilers one to 20 d of age, bone quality, and liver Mn concentration in 20-day-old broilers.

Source	Level Mn (mg/kg)	Weight gain (g)	Feed intake (g)	Feed conversion (kg:kg)	Bone resistence (kgf)	Mn in tibia (mg/kg)	Mn in liver (mg/kg)
Control	0	874.0	1,059.0	1.21	13.54	4.52	30.93
MnProt	35	833.0	1,026.0	1.23	14.55	6.48	63.75
	70	862.0	1,063.0	1.22	16.49	6.87	68.95
	105	837.0	1,042.0	1.24	17.66	7.45	76.03
	140	854.0	1,052.0	1.23	17.07	7.78	82.53
MnSO$_4$	35	878.0	1,055.0	1.21	15.14	5.92	57.40
	70	872.0	1,061.0	1.22	16.07	6.62	62.65
	105	861.0	1,045.0	1.22	17.01	7.25	70.00
	140	853.0	1,025.0	1.21	16.90	7.72	74.95
SEM		3.63	4.19	0.002	0.36	0.16	2.75
P value[1]		0.109	0.371	0.088	0.008	<0.001	<0.001

[1]Control vs. mean of treatments with Mn supplementation (t test; $P < 0.05$). Contrast testing was done.
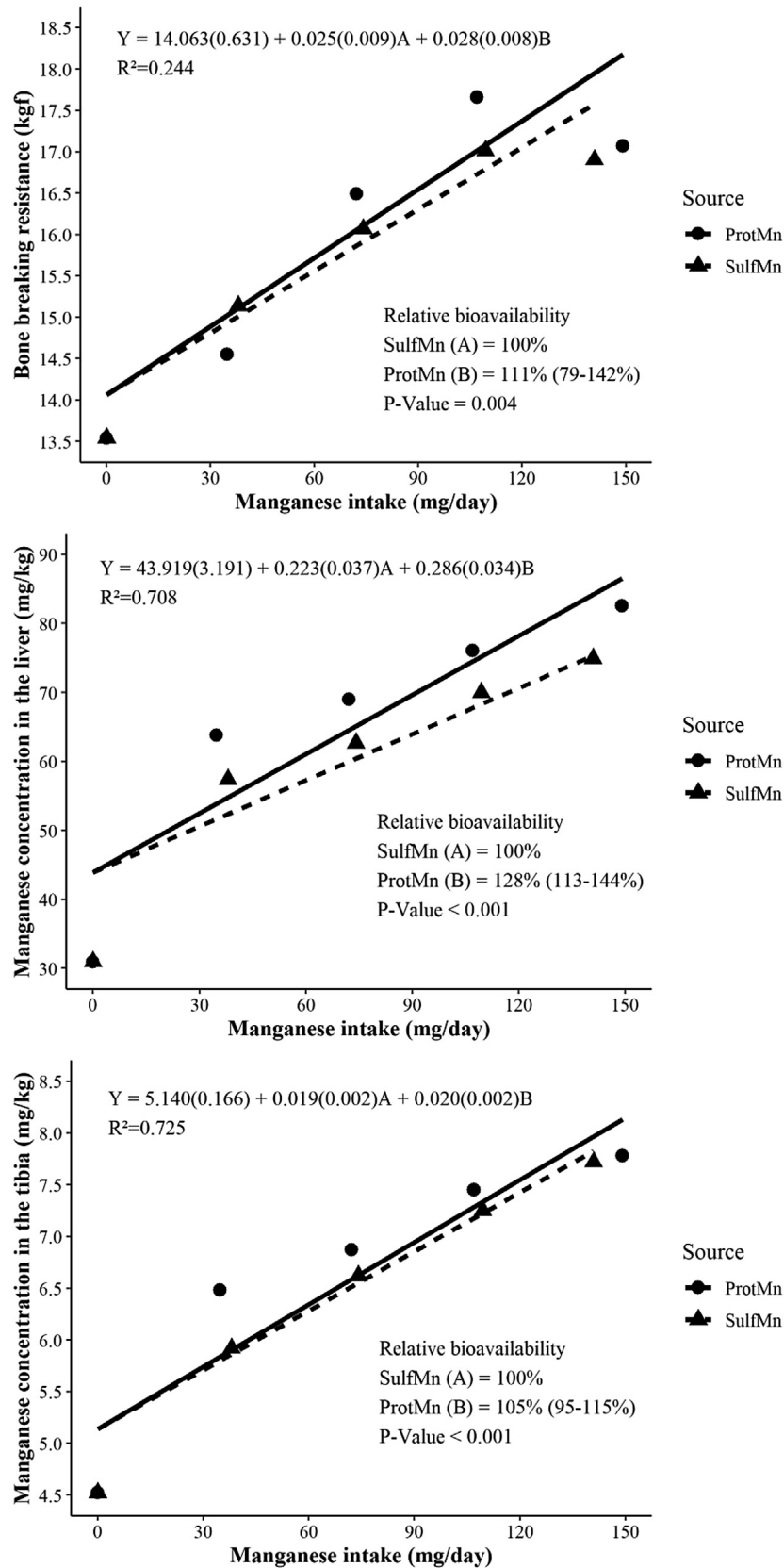
Figure 1. Relative bioavailability (RB), by the slope-ratio method, according to the supplementation sources Mn sulfate (SufMn), as standard source (100%), and Mn proteinate (ProtMn), for the variables of bone strength, liver Mn concentration, and tibia Mn concentration. Values in parentheses indicate the 95% confidence interval.

Table 4. Quantitative analysis of collagen by red birefringence in the epiphysis region of the tibia of the control groups (without Mn supplementation) and with the inclusion of maximum Mn for the sources SufMn and ProtMn (140.0 mg Mn/kg).

Treatments	Area (% red birefringence)
Control	4.01
140 mg de Mn/kg SufMn	3.68
140 mg de Mn/kg ProtMn	3.65
SEM	0.245
P value	0.836

Bones are fundamental for the occurrence of vertebrate muscle growth (Araújo et al., 2012), so bone parameters should also be taken into account when evaluating the bioavailability of minerals for broilers. In the present experiment, bone strength and Mn concentration in the tibia, as well as Mn concentration in the liver, were altered, and their values increased with Mn supplementation in the diet ($P < 0.05$). These results are in agreement with Mwangi et al. (2019), who also observed an increase in Mn concentration in the tibia and liver with Mn supplementation in the diet, regardless of the supplementation source used (sulfate and proteinate).

According to Sauveur (1984), the need for Mn for normal skeletal development is related to its role in the biosynthesis of proteoglycans present in the organic matrix of bone. Moreover, within the cell, Mn is directed toward mitochondria, thus being very abundant in mitochondria-rich tissues such as the liver (Kato, 1963). For this reason, bone and liver Mn concentration parameters are more sensitive to nutritional changes in Mn and can be used for bioavailability analysis (Berta et al., 2004; Sakomura et al., 2014). According to Henry et al. (1989) and Miles et al. (2003), the accumulation of microminerals in target tissues during dietary supplementation has been shown to be an appropriate criterion for estimating the RB of Mn.

Comparing the sources of Mn supplementation in the diet, the results for bone strength revealed that Mn concentrations in the tibia and liver showed higher RB for ProtMn than for SulfMn. These results are in agreement with several authors who consider chelated sources to be more bioavailable than inorganic sources (Bao et al., 2007; El-Husseiny et al., 2012; Baloch et al., 2017).

Regarding bone strength, ProtMn was 11% more bioavailable than SulfMn. This parameter is very important considering the large losses in slaughterhouses and poultry farms due to bone fragility of broilers, which has been increasing due to high growth rates (Coto et al., 2008).

Evaluating the RB of Mn in relation to tibial Mn concentration, Smith et al. (1995) observed 125% RB for ProtMn in relation to SulfMn (100%). Similarly, Brooks et al. (2012) also observed higher RB of Mn when a chelated source was used (139%) compared to SulfMn (100%), for Mn concentration in the tibia.

Bioavailability can be understood as the amount of mineral that is ingested, absorbed, transferred to its site of action, and transformed into its physiologically active form, supplying its demand in target tissues (Cozzolino, 1997). Therefore, ProtMn was a better source for broiler chickens than Mn sulfate in relation to the bioavailability of this mineral for bone analysis.

Osteoblasts synthesize the bone matrix, which basically consists of type I collagen, noncollagen proteins, and hydroxyapatite. Using polarized light, the bone matrix presents a characteristic birefringence, which allows the quantification of type I collagen (Junqueira et al., 1978; Roach, 1992); these fibers are considered more mature, thicker, and more organized. It was possible to observe that the inclusion or not of Mn, regardless of the source used, did not change the amount of type I collagen, that is, the amount of mature bone present in the tibia of broilers.

From the results presented, it can be concluded that Mn proteinate is more bioavailable than Mn sulfate for broilers from one to 20 d of age, with a 15% higher average RB.

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