BRUNO REIS DE CARVALHO

NUTRITIONAL REQUIREMENTS AND BIOAVAILABILITY OF DIFFERENT SOURCES OF ZINC AND MANGANESE FOR BROILERS

Thesis submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa as partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

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BIOGRAPHY

Bruno Reis de Carvalho, son of Domingos Sávio de Carvalho Laviola and Maria do Carmo Pedrosa Reis de Carvalho, was born in Carangola, Minas Gerais – Brazil on november 22, 1987.

He started undergraduate degree in Animal Science at Universidade Federal de Viçosa in 2007 and obtained Bachelor of Science in Animal Science in July of 2011.

In August of 2011, he started his Master degree with a major in Poultry and Swine nutrition and production at the Universidade Federal de Viçosa. In July of 2013, he obtained Master degree in Animal Science. In August of 2011, he started his PhD program in Animal Science with a major in Poultry and Swine nutrition and production at UFV. On May 4th of 2017, he submitted his dissertation to the thesis committee to obtain the Doctor Scientiae degree in Animal Science.

SUMMARY

ABSTRACT

CARVALHO, Bruno Reis, D.Sc., Universidade Federal de Viçosa. May, 2017. **Nutritional requirements and bioavailability of different sources of zinc and manganese for broilers.** Advisor: Horacio Santiago Rostagno. Co-advisers: Luiz Fernando Teixeira Albino and Melissa Izabel Hannas.

Two experiments were conducted to evaluate the zinc (Zn) and manganese (Mn) requirements for broilers from organic and inorganic sources, as well as the relative bioavailability of these sources. In each trial 510 male Cobb broilers were used. The animals were raised according to strain guidelines from 1 to 7 days receiving 50% supplementation from the tested mineral in a diet with reduced content of traceminerals. At 8 days 10 animals were slaughtered to verify the mineral deposition at the beginning of the experiment. The other animals were weighed and randomly distributed in plastic cages in 10 treatments with 10 replicates of 5 animals each. The treatments consisted of 5 levels of traceminerals tested (0, 19, 38, 57 and 76 mg/kg of Zn in the first experiment and 0, 25, 50, 75 and 100 mg/kg of Mn in the second), provided in an organic or inorganic supplement, where the other minerals were supplied to meet the requirement. The diets used were semi purified to reduce the content of traceminerals to low levels. At 13 days, trays covered with plastic were installed for excreta collection. At 16 days in the Zn experiment and 17 days in the Mn experiment the animals and feds were weighed to determine the final weight, weight gain, feed intake and feed to gain ratio. At 17 days in the Zn experiment and 18 days in the Mn experiment a bird in each cage were slaughtered for determination of minerals in the liver. The results for the Zn experiment indicate that the requirement for higher performance is 70 mg Zn/kg of weight gain (WG) for inorganic supplementation, which can be reduced to 63 mg Zn/kg of WG in organic mineral supplementation. Higher Zn retention was observed when 62 mg/kg of Zn was supplied in inorganic form or 79 mg/kg of organic Zn. Interactions in Mn and iron retention have been observed in supply of inorganic Zn as well as in the deposition of Mn and iron in the liver when organic sources are provided. The relative bioavailability of the organic Zn was estimated to be 125% of the inorganic Zn. For Mn, the requirement determined based on the performance parameters is 166 mg Mn/kg of WG for the Mn supply in the inorganic form and 95mg Mn/kg WG in the organic form. The highest retention of Mn was obtained with the supply of 68.8 and 84.6 mg/kg of Mn in the diet for organic and inorganic sources, with a relative bioavailability estimated at 100%.

RESUMO

CARVALHO, Bruno Reis, D.Sc., Universidade Federal de Viçosa. maio de 2017. **Exigências Nutricionais e biodisponibilidade de diferentes fontes de zinco e manganês para frangos de corte**. Orientador: Horacio Santiago Rostagno. Coorientadores: Luiz Fernando Teixeira Albino e Melissa Izabel Hannas.

Dois experimentos foram conduzidos para avaliar a exigência de zinco (Zn) e manganês (Mn) para frangos de corte a partir de fontes orgânicas e inorgânicas, bem como sua biodisponibilidade relativa (BDR). Em cada ensaio 510 frangos de corte Cobb machos foram utilizados. Os animais foram criados segundo manual da linhagem de 1 a 7 dias recebendo 50% de suplementação do mineral testado, em uma dieta com reduzido teor de microminerais. Aos 8 dias 10 animais foram abatidos para verificar a deposição mineral ao início do experimento. Os demais animais foram pesados e distribuídos aleatoriamente em gaiolas plásticas nos 10 tratamentos com 10 repetições de 5 animais. Os tratamentos foram constituídos de 5 níveis de suplementação dos microminerais testados (0, 19, 38, 57 e 76 mg/kg de Zn no primeiro experimento e 0, 25, 50, 75 e 100 mg/kg de Mn no segundo), fornecidos em um suplemento orgânico ou inorgânico, onde os demais minerais foram fornecidos em quantidade suficiente para atender sua exigência. As dietas utilizadas foram semi purificadas para reduzir o teor de microminerais em níveis mínimos. Aos 13 dias bandejas recobertas com lonas plásticas foram instaladas para coleta de excretas. Aos 16 (Zn) e aos 17 dias (Mn) dias, animais e rações foram pesados para determinação do peso final, ganho de peso, consumo de ração e conversão alimentar. Aos 17 dias no experimento de Zn e aos 18 dias no de Mn uma ave por gaiola foi abatida para determinação de minerais no fígado. Os resultados para o experimento de Zn indicam que a exigência para maior desempenho é de 70 mg de Zn/kg de ganho para suplementação inorgânica, podendo ser reduzido para 63 mg de Zn/kg de ganho na suplementação com minerais orgânicos. Foi observada maior retenção de Zn com 62 mg/kg de Zn inorgânico ou 79 mg/kg de Zn orgânico. Interações na retenção de Mn e Fe foram observadas no fornecimento de Zn na forma inorgânico, bem como na deposição de Mn e Fe no fígado quando fontes orgânicas foram fornecidas. A BDR do Zn orgânico foi estimada em 125% do Zn inorgânico. Para Mn, a exigência com base no desempenho, pode ser estimada em 166 mg Mn/kg de ganho de peso para o fornecimento de Mn inorgânico e 95 mg Mn/kg de Mn orgânico. A maior retenção de Mn foi obtida com 68,8 e de 84,6 mg/kg de Mn para as fontes orgânicas e inorgânicas, sendo a BDR de 100%.

INTRODUCTION

The application of technologies in livestock production has allowed in the last decades the formulation of precise diets, as well as the precise evaluation of the foods used and the nutritional requirements of the animals. The development and commercial application of ingredients processing techniques and the feasible production of amino acids and additives have made it possible to obtain exceptional performance results, following the genetic evolution improvement.

In this way, some areas of animal nutrition are constantly being reformulated to allow maximum weight gain for current genetics, such as amino acid nutrition and energy requirements. These factors allowed broilers to change the feed conversion rate from 3.84 in 1957 to the recent values of 1.73 (Havenstein et al., 2003). This improvement, however, was not accompanied by advances in all segments of nutrition, such as traceminerals nutrition.

Whether the vegetal ingredients used in the feed formulation have a high intake of the mineral withdraw from the soil or the low cost of its supplementation in the diets, few researches have been carried out with these elements. However, soils vary in their content of traceminerals, and cultivars vary in the uptake of minerals from the soil. Consequently, vegetable feedstuffs may contain excess or deficiency in a certain mineral depending on the amount of mineral in the soil, which may imply the reduction of animal productivity (Aletor & Omoradara, 1994).

Zinc deficiency can lead to processes such as alopecia, dermatitis, retarded growth, immunodeficiency, nocturnal blindness and diarrhea (Grider, 2013). Lack of copper can cause abnormal metabolism of bone tissue by altering the formation of the cartilaginous matrix of the bone, which can also cause rupture of veins, cardiac hypertrophy, abnormal mitochondrial structure and reduced levels of ATP. Manganese deficiency also results in inhibition of osteogenesis, in addition to reduction of pancreatic insulin. However, the greatest effect of copper, zinc and manganese deficiency occurs in the overproduction of free radicals, since the enzymes that control this production depend on these minerals (Stipanuk and Caudill, 2013).

Although they are nutritionally important elements, the knowledge about the metabolism of the traceminerals in the animal nutrition and their interactions is insufficient to allow an ideal mineral supplementation without excesses or deficiencies. Several studies with traceminerals supplementation for birds that determine the current requirements in mg / kg of feed (Zinc: Edwards et al., 1959; Lease et al., 1966; and Manganese: Kealy e Sullivan, 1966). These studies were carried out with low performance strains than those currently used, or were intended to study the status of these minerals in specific situations, such as *Eimeria sp*. infections, and did not take into account the interaction between minerals and anti-nutritional factors such as phytate, suggesting that the levels that we establish as requirement of these traceminerals may be incorrect or, at least, outdated.

Improved protocols and reduced analytical costs now allow more complex work to be developed, establishing nutritional requirements not only for maximum growth and efficiency, but also for optimum enzyme activity, less free radical production, and even to define the amount of mineral that improve characteristics in final product like meat quality.

Another point of great importance is the opportunity to use new sources of traceminerals, which have less interaction at intestinal lumen and are absorbed more effectively. Minerals chelated to organic molecules, or simply organic minerals, that can be supplemented in smaller quantity with less environmental impact without compromising the performance.

Thus, the objective of this work was to determine the nutritional requirement of zinc and manganese and to determine the bioavailability of organic sources of these minerals.

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CHAPTER 1

NUTRITIONAL REQUIREMENT OF DIFFERENT SOURCES OF ZINC FOR BROILERS

ABSTRACT

The objective of the current study was to update the nutritional requirement of zinc values for broilers of two sources of zinc. To this, 510 male Cobb broilers from 1 to 17 days-old were used. The animals were housed (1-7d) following Cobb guidelines receiving a corn-soybean meal diet with low basal trace mineral content (33 mg/kg of basal zinc analyzed) and supplemented with 20 mg/kg of zinc (50% of NRC 1994 recommendations) as ZnSO4 to reduce body stores. At 8d-old, 10 birds were slaughtered to collect liver samples to evaluate initial mineral deposition and the remaining birds were weighed and randomly assigned to 10 treatments with 10 replicates of 5 animals each. The treatments consisted of five levels of zinc supplemented (0; 19; 38; 57 and 76 mg/kg) from two sources (ZnSO4, with Fe, Cu and Mn supplemented in as sulphates and Se as selenite, and Bioplex Zn, with Fe, Cu, Mn and Se supplemented as chelates). The animals were housed in plastic cages with plastic feeders and received demineralized water and feed at libitum. The experimental diets were semi purified (13mg/kg of basal zinc analyzed), with 30% of corn and contained cellulose, phytic acid and phytase to approach to a practical diet. At 16d-old, the birds and feeds were weighed to evaluate the final weight (BW), weight gain (WG), feed intake (FI) and feed conversion (F:G). At 17 d-old, one bird for cage was slaughtered for determination of minerals in the liver. The results for the zinc experiment indicate that the requirement for higher performance is 70 mg Zn/kg of WG for inorganic supplementation, which can be reduced to 63 mg Zn/kg of WG in organic mineral supplementation. Higher zinc retention was observed when 62 mg/kg of zinc was supplied in inorganic form or 79mg/kg of zinc in organic form. Interactions in manganese and iron retention have been observed in supply of inorganic zinc as well as in the deposition of manganese and iron in the liver when organic sources are provided. RBA of the organic zinc was estimated to be 125% of the inorganic zinc.

Key words: Zinc, requirements, bioavailability, traceminerals, broilers.

1. Introduction

To reach the maximum potential genetic performance results of broilers chicks, all nutrients must be met accurately, being necessary to add traceminerals supplements to the corn-soybean meal diets. Due this reason, nutritional requirements must be well determined and constantly updated and the source of nutrient must be taken into account.

The nutritional significance of zinc resides in its essential participation in more than 200 metalloenzymes indispensable to animal metabolism (Stipanuk and Caudill, 2013). These enzymes play a fundamental role in animal development, bone growth, sexual maturity, reproduction and especially into the antioxidant defense system (Grider, 2013). The loss or malfunction of any of these enzymes can result in greater impact on animal performance.

The NRC (1994) stats that zinc requirements for broilers is 40 mg for each kg of diet. The value was established based on the studies of Morrison and Sarett (1958), O'Dell et al. (1958) and Roberson and Shaible (1958) using semi purified diets. The Council reports a performance of 2,088 grs of body weight at 42 days and 3,717 grs of feed intake, resulting in a 70 mg of zinc intake for each kilo of weight gain. Recent studies (Rostagno et al., 2017) report a 42-day weight of 3,218 grs and 5,083 grs in feed intake, reaching 63 mg of zinc for kg of weight gain if NRC (1994) requirement was used. These values represent a 10% reduction in zinc requirement for animals with 55% higher performance and demonstrate a need to update the zinc requirements.

The use of mineral sources bounded to organic molecules has shown effective results in improving the efficiency of zinc utilization. This kind of source may increase zinc retention by reducing the amount of this mineral in excreta (Burrell et al., 2004), thus having a lower polluting effect on the environment. Due to this factor, It is estimated that the requirement of zinc when supplied by organic sources is 55% lower comparing to inorganic sources (Rostagno et al., 2017), but this estimate still needs practical validation.

2. Objective

The aim of this project was to determine zinc requirements for broilers from organic and inorganic sources, as well as to determine zinc bioavailability from organic source.

3. Materials and Methods

The experiment was conducted in the Poultry Farm of the Animal Science Department at Universidade Federal de Viçosa from July to August 2016. All the procedures follow the standards of the National Council of Animal Experimentation Control – CONCEA and was previously approved by the Ethics Committee of Animal Production, CEUP – UFV (Register number 111/2014).

3.1 Animals

Five hundred and ten Cobb male chicks were utilized in this experiment. The birds were housed from the first to seventh day of age in brooders following the strain guidelines recommendations. A corn-soybean meal diet was formulated (Table 1) to meet or exceed the nutritional requirements for this age (Rostagno et al., 2011) except for the trace mineral content.

Table 1. Proximate and calculated/analyzed composition of the start diet used in the period of 1 to 7 d of age.

Ingredients	%	Nutrient	Calculated values
Corn	53.335	Metabolizable energy (kcal/kg)	3135
Soybean Meal (45%)	30.000	Crude protein (%)	22.41
Corn Gluten (60%)	6.880	Calcium (%)	0.828
Soybean oil	3.700	Phytic phosphorus	0.247
Starch	1.585	Available phosphorus (%)	0.391
Potassium phosphate	0.955	Sodium (%)	0.223
Calcium carbonate	1.700	Chloride (%)	0.272
Sodium Chloride	0.530	Potassium (%)	0.987
Vitamins supplement [A]	0.150	Magnesium (%)	0.185
Lysine HCl (79%)	0.303	Copper (mg/kg)	15
Methionine (99%)	0.197	Iron (mg/kg)	217
Threonine (98%)	0.016	Manganese (mg/kg)	73
L-Arginine (98.5%)	0.006	Zinc (mg/kg)	47
Colin Chloride (60%)	0.375	Selenium (mg/kg)	0.48
Coccidiostatic	0.055	Lysine (%)	1.174
Avilamicin	0.003	Methionine + Cysteine (%)	0.846
BHT	0.010	Threonine (%)	0.763
Mineral Supplement [B]	0.100	EN:TN Ratio	49.51
		Electrolytic Balance	273

[A] Vitamin supplement containing per kg of ration: Vit. A – 9,750 IU; Vit. D3 – 2,470 IU; Vit. E – 36.6 IU; Vit. B1 – 2.60 mg; Vit. B2 – 6.50 mg; Vit. B6 – 3.64 mg; Vit. B12 – 0.015 mg; Pantotenic acid – 13.0 g; Biotin – 0.091 mg; Vit. K3 – 1.95 mg; Folic acid – 0.91 mg; Nicotinic acid – 39.0 mg.

[B] The mineral supplement was added following NRC (1994) recommendations for traceminerals, except for zinc (50% of NRC recommendations) (Table 2).

The trace minerals amount in this diet was calculated to meet 100% of NRC (1994) requirements, except for zinc supplemented in 50% of NRC (1994) requirement (Table

2). At 8d-old, 500 animals with 169,6±1,6g of initial body were weighed and randomly distributed in 10 treatments and 10 replications with 5 animals per pen. Each pen was considered as an experimental unit. Extra ten animals were slaughtered to collect liver samples in a pool to evaluate initial trace mineral concentrations.

Table 2. Calculated and analyzed values for Basal diets and supplements used in Zn trial.

SAMPLE	n	Zn (mg/kg)		Fe (mg/kg)		Mn (mg/kg)		Cu (mg/kg)	
		Calc	Anal	Calc	Anal	Calc	Anal	Calc	Anal
Prestart basal diet	5	27	33	137	179	13	14	7	6
Prestart supplement [A]	5	20	26	80	86	60	60	8	7
Prestart diet (+supplement)	9	47	59	217	269	73	71	15	14
Experimental basal diet	24	13	12	27	30	7	4	2	3
Inorganic suppl. (0%Zn) [B]	5	0	0.5	80	76	60	72	12	12
Inorganic suppl. (100% Zn) [B]	5	76	84	80	79	60	66	12	12
Organic suppl. (0% Zn) [B]	5	0	0.8	80	71	60	67	12	12
Organic suppl.(100% Zn) [B]	5	76	82	80	80	60	71	12	12

[A] Inclusion 1kg/ton

[B] Inclusion 2kg/ton

3.2 Diets

A semi purified basal diet was formulated based on casein, albumin, isolated soybean protein and corn, starch and dextrose (Table 3) to meet or exceed Rostagno et al. (2011) recommendations for initial phase. The diet was formulated to obtain minimum amounts of trace minerals. Mineral supplements (Table 4) were added to the diet with 0 or 76 mg/kg of zinc in an organic (Bioplex TM) or inorganic (Sulphate) sources. These diets were mixed in proportions to reach 5 different levels of the tested mineral from both sources.

3.3 Treatments

The experimental treatments (Table 4) consists in 5 levels of zinc and two different sources, an organic (Bioplex TM) and an inorganic (Sulphate) source. The zinc levels were calculated using levels up and below the NRC (1994) recommendation for zinc. The other minerals (copper, iron, manganese, selenium and iodine) were kept in the recent recommendations as suggested by the NRC (1994).

3.4 Housing

The weighted birds were transferred to an experimental room and housed in 0.35 × 0.50 m plastic cages equipped with nipple type drinkers and tubular plastic feeders to provide water and feed *ad libitum*. The water provided to the animals during the experimental period was demineralized to ensure any external contamination. The temperature was maintained constant by heat lamps and curtains according to the range of thermal comfort for this age measured by datalogger installed in the room.

3.5 Parameters Evaluated

During 9 days of experimental period, the performance parameters evaluated was average final body weight, average weight gain, average feed intake, feed-to-gain ratio, and mortality. At 13 d-old (5 days of trial) trays covered with plastic sheeting was installed to allow total excreta collection to evaluate the trace mineral balance. The mineral retention was calculated by the difference between the intake and the excretion of each mineral and the mineral apparent absorption was calculated using Ammerman (1995) equations. The collections were performed over 4 consecutive days until the end of experimental period. Excretas were frozen and homogenized after each collection and an aliquot was taken and dried for further analyses.

Table 3. Proximate and nutritional composition of semi purified diets used in experimental period (8-16 days).

Ingredient	%	Nutrient	Calculated Composition
Corn	30.00	Met. energy (kcal/kg)	3128
Casein	4.00	Crude protein (%)	22.58
Albumin	12.00	Calcium (%)	0.850
Isolated Soy Protein	4.00	Phytic phosphorus	0.161
Starch	13.00	Available P(%)	0.400
Dextrose	13.30	Sodium (%)	0.372
Broken Rice	8.00	Chloride (%)	0.485
Soybean oil	2.00	Potassium (%)	0.885
Cellulose	4.00	Magnesium (%)	0.126
Calcium Bicarbonate	1.695	Copper (mg/kg)	11
Potassium Phosphate	1.485	Iron (mg/kg)	110
Magnesium Chloride	0.650	Manganese (mg/kg)	64
Potassium Chloride	0.468	Zinc (mg/kg) [B]	12-88
Amino acids supplement [A]	0.030	Selenium (mg/kg)	0.52
Mineral Supplement [B]	0.200	Lysine (%)	1.254
Vitamin Supplement [C]	0.150	Met + Cys (%)	0.913
Sodium Phytate	1.020	Threonine (%)	0.831
Phytase	0.010	EN:TN Ratio	50.07
Other ingredients [D]	0.620	Electrolytic Balance	251

[A] Amino acids: Lysine HCL (79%) – 0.030; L-Arginine (98.5%) - 0.275; L-Glycine (98.5%) - 0.400; Alanine (99%) - 0.850; Glutamic acid (100%) - 2.000. [B] Added according each experimental treatment (Table 4).

[C] Vitamin supplement containing per kg of ration: Vit. A – 9,750 IU; Vit. D3 – 2,470 IU; Vit. E – 36.6 IU; Vit. B1 – 2.60 mg; Vit. B2 – 6.50 mg; Vit. B6 – 3.64 mg; Vit. B12 – 0.015 mg; Pantotenic acid – 13.0 g; Biotin – 0.091 mg; Vit. K3 – 1.95 mg; Folic acid – 0.91 mg; Nicotinic acid – 39.0 mg.

[D] Colin Chloride (60%) - 0.375; Coccidiostatic - 0.055; Avilamicin - 0.010; Antioxidant (BHT) - 0.030

At 16 d-old, the animals and feeds were weighed to calculate average final body weight, average weight gain, average feed intake and feed-to-gain ratio. At 17 d-old, one animal per cage was selected. These animals were slaughtered by cervical dislocation for samples collection to evaluate the concentration of each mineral in the tissues.

Table 4. Calculated and Analyzed values of trace minerals for the semi purified diet in mg/kg.

Source	Zn Lvl	n	Mineral							
			Zn (mg/kg)		Fe (mg/kg)		Mn (mg/kg)		Cu (mg/kg)	
			Calcul.[1]	Analy.[2]	Calcul.[1]	Analy.[2]	Calcul.[1]	Analy.[2]	Calcul.[1]	Analy.[2]
Inorganic	0	10	12	15	110	156	64	64	11	10
	19	10	31	34	110	159	64	66	11	10
	38	10	50	48	110	153	64	67	11	10
	57	10	69	66	110	146	64	62	11	10
	76	10	88	72	110	165	64	59	11	10
Organic	0	10	12	15	110	102	64	102	11	12
	19	10	31	33	110	102	64	99	11	12
	38	10	50	53	110	102	64	101	11	12
	57	10	69	72	110	106	64	100	11	12
	76	10	88	85	110	100	64	95	11	12

[1] Total Calculated values (Basal diet + Supplement).

[2] Analyszed in 5 samples with replicate.

3.6 Mineral Concentration Analysis

Liver samples were lyophilized and ground in a mill to be analyzed, as diets and excreta, following the method 935.13 (AOAC, 2000). An aliquot of 0.6g of diets, tissues and excreta samples were weighed on the analytical balance and added 5ml of 4:1 nitroperchloric acid solution (4 parts of nitric acid and 1 part of perchloric acid). The samples were taken at 200ºC in the digester block and the residue was filtrated on quantitative paper (7.5 µm pore) and completed for 50ml with distillate water. This solution was read in atomic absorption spectrophotometer (GBC Avanta Σ) to obtain the mineral levels. The phosphorus analysis was performed by the colorimetric method (Fiske & Subbarow, 1925). The proximate analyses were performed in the Animal Nutrition Laboratory of the Animal Science Department at the Universidade Federal de Viçosa.

3.7 Statistical Analysis

Data were analyzed using the Statistical Analyses System program – SAS 9.4. An ANOVA test was performed using GLM procedure considering a Nested Design with levels inside the different sources and the pens as the experimental unit. The regression equations were estimate using the orthogonal contrasts by PROC REG procedure. For the mineral requirements, only linear and quadratic responses was considered for the model if statistical significance was observed ($P<0.05$). To mineral deposition in tissues, a Dunnett test is also performed to compare the results with initial mineral values in the beginning of the experiment, using the GLM procedure. The requirements values for organic and inorganic sources was compared for the bioavailability determination.

The estimates of the requirements for each parameter were obtained by the following model:

$$Y_{ikj} = \mu + A_j + B(A)_{ij} + e_{ikj}$$

Where:

Yikj = observation *k* in level *i* of factor Source and level *j* of factor Mineral Level;
μ = overall mean of the experiment;
Aj = effect of level *i* of factor Source;
B(A)ij = effect of level *j* of factor Mineral Level within level *i* of factor Source;
eikj = random error associated with each observation.

4 .Results and discussion

4.1 Performance Results

The requirement of a nutritional element can be described as the amount of this element that can be absorbed, transported and be able to supply all metabolic pathways without any depletive effects (Baker and Ammerman, 1995). Traditionally, the main parameter used to determine requirement is performance (Ammerman, 1995), assuming all basal needs are met when the animal have a normal growth. Due the importance of traceminerals as enzymatic cofactors, in some conditions the requirement to performance can be reached, but the animal can still improve enzyme efficiency with a higher mineral level, showing the need of an ample evaluation to define requirement (Huang et al., 2007).

The performance results are presented in table 5. The increasing zinc levels had a linear and quadratic ($P<0.05$) effect in both sources to body weight (BW) and weight gain (WG) of the broilers chicks receiving the experimental diets from 8 to 16-d of age. To feed intake (FI), a linear and quadratic effect were observed ($P<0.05$) in inorganic source, but only a quadratic effect was significant for organic source. Linear and quadratic effects were also observed to feed to gain ratio (F:G) ($P<0.05$) for inorganic source, and the effect was linear to organic source. There is no significant mortality and due this the data was not presented. The regressions equations are presented in table 6.

These results suggest that the requirement of zinc to achieve higher BW and WG is 50 mg/kg of inorganic zinc and 46 mg/kg of zinc in diet in organic source. The higher FI had been met with 50mg/kg and 38 mg/kg of zinc in inorganic and organic source, respectively. To lower values of F:G, 70 mg/kg of zinc in an inorganic form is required and the ratio can be improved using values greater than 76 mg/kg of organic zinc.

Zinc requirement may vary in a wide range depending on diet type, but in general requirements are determined being based on purified diets (10.6 mg/kg to WG, Emmert and Baker, 1995; 18 mg/kg to BW, Dewar and Downie, 1984), semi purified diets (33 mg/kg to WG, Wedekind and Baker, 1990; 27 mg/kg to WG, Batal et al., 2001; 32 mg/kg to WG, Steinruck and Kirchgessner, 2003) or practical diets trials (84 mg/kg to WG, Huang et al., 2007; 68 mg/kg to WG, Bao et al., 2009, 62 mg/kg to Zn in tibia, Xiugong Liao et al., 2013; 100 mg/kg to footpad integrity, Vieira et al., 2013). The use of semi purified diets with cellulose and sodium phytate may have improved the requirements values to performance comparing other semi purified diets researches.

Table 5. Performance[1] of broiler chickens fed diets with different levels of zinc from organic or inorganic sources from 8 to 16 days old.

Parameter	Source	Zinc Level					Mean	MSE	C.V. (%)[6]	P-Value (Nested Effect)	
		0	19	38	57	76				Linear	Quadratic
BW[2]	Inorganic	452	485	487	490	482	489	11.61	2.42	<.0001	<.0001
	Organic	459	494	474	480	479	477			0.0199	0.0013
	Mean	455	490	480	485	481					
WG[3]	Inorganic	282	312	317	321	316	309	10.97	3.56	<.0001	<.0001
	Organic	289	325	304	310	310	308			0.0144	0.0006
	Mean	286	319	311	316	313					
FI[4]	Inorganic	412	440	445	445	442	437	14.46	3.32	<.0001	0.0001
	Organic	422	452	428	434	428	433			0.6996	0.0156
	Mean	417	446	437	439	435					
F:G[5]	Inorganic	1.461	1.410	1.405	1.388	1.416	1.416	0.045	3.16	0.0015	0.0075
	Organic	1.460	1.393	1.409	1.397	1.383	1.408			0.0011	0.1485
	Mean	1.460	1.402	1.407	1.393	1.400					

[1] Due only two animals died during the experiment, mortality presented low variability with no statistical difference and was disregarded.

[2] BW (Body Weight, g); [3] WG (Weight Gain, g); [4] FI (Feed Intake, g); [5] F:G (Feed-to-gain ratio, g/g); [6] C.V. (Coefficient of Variation, %).

Table 6. Regression equations to performance parameters.

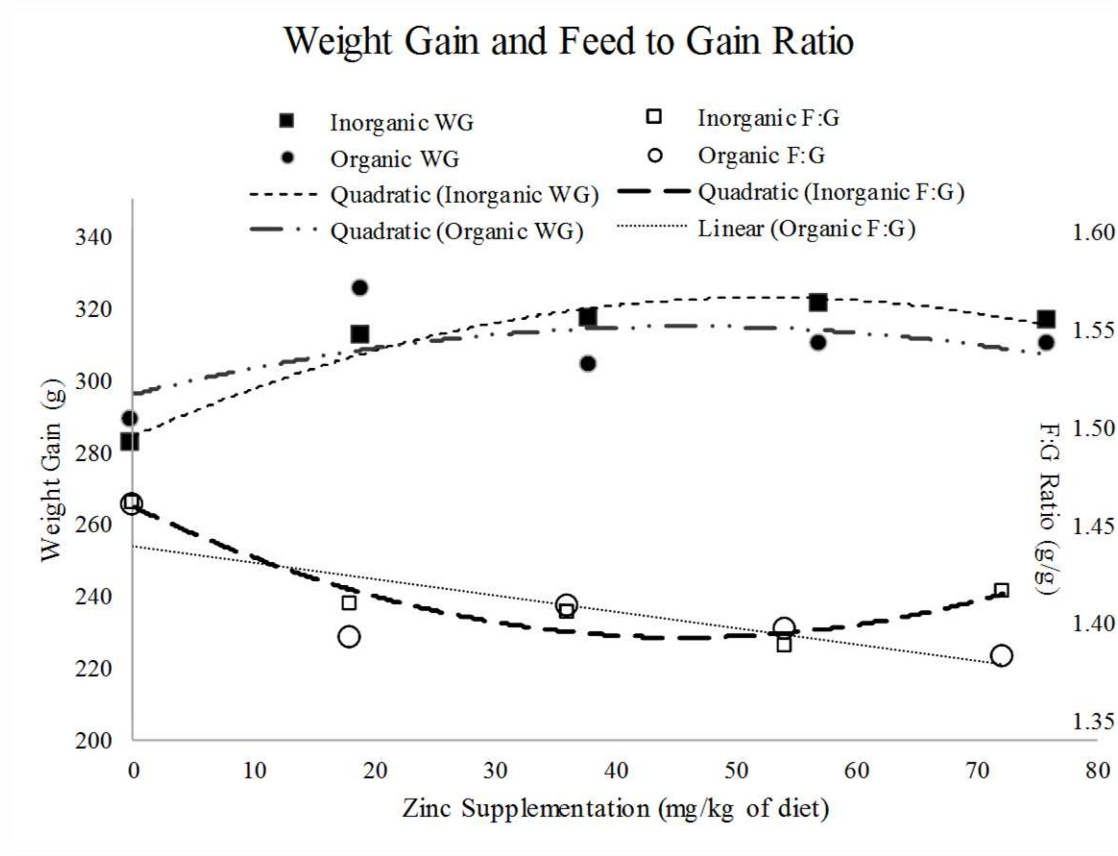
Parameter	Source	Contrast	Regression Equations	Optimal Level	P-Value	R^2
BW[1] (g)	Inorganic	Linear	$Y=0.3341x+466.13$	>76.00	<0.0001	0.4407
		Quadratic	$Y=-0.016x^2 +1.5866x+454.33$	49.58	<0.0001	0.9294
	Organic	Linear	$Y=0.1458x+471.65$	>76.00	0.0199	0.1065
		Quadratic	$Y=-0.009x^2+0.8306+465.14$	46.14	0.0013	0.3446
WG[2] (g)	Inorganic	Linear	$Y=0.3625x + 295.13$	>76.00	<.0001	0.5970
		Quadratic	$Y=-0.0157x^2 + 1.555x + 283.79$	49.52	<.0001	0.9595
	Organic	Linear	$Y=0.1438x + 302.08$	>76.00	0.0144	0.1083
		Quadratic	$Y=-0.0091x^2 + 0.8343x + 295.52$	46.14	0.0006	0.3231
FI[3] (g)	Inorganic	Linear	$Y=0.3463x + 423.73$	>76.00	<.0001	0.5370
		Quadratic	$Y=-0.0136x^2 + 1.3798x + 413.91$	50.73	<.0001	0.9445
	Organic	Quadratic	$Y=-0.0085x^2+0.6389x+425.50$	37.58	0.0156	0.2432
F:G[4] (g/g)	Inorganic	Linear	$Y=-0.0005x + 1.4384$	>76.00	0.0017	0.6122
		Quadratic	$Y=0.00002- 0.0028x + 1.4592$	70.00	0.0075	0.9363
	Organic	Linear	$Y=-0.000727x+1.4347$	>76.00	0.0011	0.4229

[1] BW (Body Weight, g); [2] WG (Weight Gain, g); [3] FI (Feed Intake, g); [4] F:G (Feed-to-gain ratio, g/g)

The zinc values found to BW and WG give a maximum WG of 322 and 315 grams for inorganic and organic sources. Using this zinc values in FI equations, the amount of diet used to reach these WG are 449 grams of feed to inorganic and 437 grams to organic sources. So, the requirement expressed in mg of zinc to kg of gain to inorganic and organic sources are 70 and 63 mg/kg WG.

The NRC (1994) recommendation for zinc is 40 mg for kg of diet, different of 50 and 46 mg/ kg of diet presented in this study. But the NRC requirement calculated based on mg of zinc to kilo of weight gain is the same to the results found inorganic source, whereas organic source is almost 10% less required. However, these results take on count only the supplemented zinc, disregard the absorption of each source by the animal.

Graphic 1. Weight gain and feed to gain ratio of broiler chickens fed diets with increasing levels of zinc from organic or inorganic sources from 8 to 16 days old.



4.2 Mineral Balance Results

The results for zinc balance are shown in table 17. Increasing zinc levels improved significantly ($P<0.05$) the zinc intake in animals receiving organic and inorganic trace minerals supplements, with ascending linear and quadratic response. An ascending linear response was also observed in zinc excretion when the animals receive one of these sources. The difference between the zinc intake and excretion was considered the zinc retention and was higher when the zinc levels are raised, with significant linear and quadratic responses. Zinc apparent absorption was calculated as a percentage of retained zinc of the zinc consumed (Ammerman and Baker, 1995) and the increase in zinc levels had a linear effect in this parameter, to inorganic and organic source. The significant regression equations for each parameter in each source can be observed in table 8.

Graphic 2. Zinc retention in broiler chickens fed diets with ascending levels of zinc from organic or inorganic sources from 8 to 16 days old.

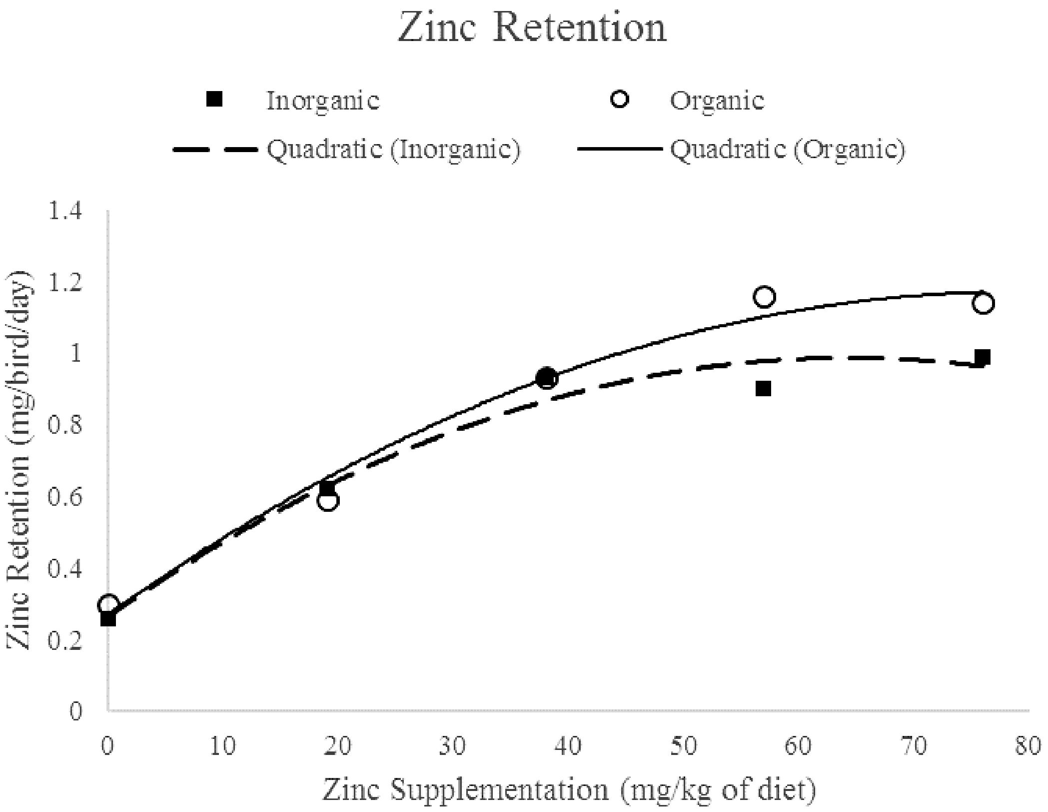


Table 7. Zinc Balance in broiler chickens fed with diets supplemented with different levels of zinc from organic or inorganic sources.

Parameter	Source	Zinc Level					Mean	MSE	C.V. (%)	P-Value (Nested Effect)	
		0	19	38	57	76				Linear	Quadratic
Zn Intake (mg/kg/bird/day)	Inorganic	0.59	1.37	1.99	2.69	2.92	1.91	0.12	6.01	<.0001	<.0001
	Organic	0.59	1.32	2.17	2.93	3.39	2.08			<.0001	<.0001
	Mean	0.59	1.34	2.08	2.81	3.15					
Zn Excretion (mg/kg/bird/day)	Inorganic	0.32	0.74	1.06	1.79	1.93	1.17	0.16	13.73	<.0001	0.3935
	Organic	0.29	0.72	1.24	1.76	2.24	1.25			<.0001	0.5919
	Mean	0.31	0.73	1.15	1.78	2.09					
Zn Retention (mg/kg/bird/day)	Inorganic	0.26	0.62	0.93	0.90	0.99	0.74	0.18	23.23	<.0001	0.0001
	Organic	0.30	0.59	0.93	1.16	1.14	0.83			<.0001	0.0009
	Mean	0.28	0.61	0.93	1.03	1.07					
Zn Apparent Absorption (%)	Inorganic	45%	46%	47%	34%	34%	41%	7.27	17.44	<.0001	0.1026
	Organic	50%	45%	43%	40%	34%	42%			<.0001	0.8170
	Mean	48%	45%	45%	37%	34%					

Table 8. Regression equations to zinc balance parameters.

Parameter	Source	Contrast	Regression Equations	Optimal Level	P-Value	R²
Zn Intake (mg/kg/bird/day)	Inorganic	Linear	$Y=0.0315x+0.7123$	>76.00	<0.0001	0.9747
		Quadratic	$Y=0.0002x^2+0.0471x+0.5654$	117.75	<0.0001	0.9950
	Organic	Linear	$Y=0.038x+0.6352$	>76.00	<0.0001	0.9912
		Quadratic	$Y=0.00013x^2+0.0475x+0.544$	182.69	<0.0001	0.9966
Zn Excretion (mg/kg/bird/day)	Inorganic	Linear	$Y=0.022x + 0.3181$	>76.00	<0.0001	0.9691
	Organic	Linear	$Y=0.026x+0.2634$	>76.00	<0.0001	0.9993
Zn Retention (mg/kg/bird/day)	Inorganic	Linear	$Y=0.0091x + 0.3948$	>76.00	<.0001	0.8205
		Quadratic	$Y=0.00018x^2 + 0.0222x + 0.2704$	61.67	0.0001	0.9704
	Organic	Linear	$Y=0.0119x+0.3718$	>76.00	<.0001	0.9149
		Quadratic	$Y=0.000146x^2+0.023x+0.2661$	78.77	0.0009	0.9837
Zn Apparent Absorption (%)	Inorganic	Linear	$Y=0.182x + 47.82$	>76.00	<.0001	0.9695
	Organic	Linear	$Y=0.2035x+49.95$	>76.00	<.0001	0.6613

Considering that the retained zinc was the portion of the zinc that was truly absorbed, the amount of zinc that promote the highest retention is 62 mg/kg to inorganic source and 79 mg/kg when organic source is supplemented, corresponding respectively to 0.955 and 1.172 mg/kg/bird/day of retained zinc. Using the 0.955 mg/kg/bird/day as a standard in the equation of organic zinc retention, the amount of organic zinc required to retain this same 0.955 mg/kg is 40 mg/kg of zinc in the diet, 22 mg (or 45%) less than the amount of zinc to reach the same retention by inorganic source (62 mg/kg).

This difference can be explained by the absorption process of each source. The inorganic source of zinc (zinc sulphate; Zn_2SO_4) is disassociate in gastric pH and zinc goes to the gut as Zn^{2+} and will be transported to the enterocyte by DMT1, ZIP and ZnT family transporters (Gunshin et al., 1997). When plasmatic concentration of zinc is enough to reach all the metabolic needs, the Zn^{2+} in cytosol will be bound to metallothionein and zincosomes to be disposed by gut desquamation (Stipanuk and Caudill, 2013) and the number of zinc transporters will be reduced (Henriques et al., 2003). However, this regulation system will be activated only in the presence of Zn^{2+}. When zinc is supplied in chelated organic sources (as proteinates), the absorption is guaranteed by peptides and amino acid transporters in brush border and basolateral membrane, not been able to activate the regulatory mechanism in the gut (Ashmead, 2012) and can be readily complexed in enzyme molecules (Ashmead, 1992).

The down regulation on zinc absorption in fact can affect the metabolism of other minerals. Divalent Metal Transporter 1 (DMT1) was initial described as Fe^{2+} transporter in brush border, can also transport Co^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+} e Zn^{2+} (Gunshin et al., 1997), though is regulated by iron in cytosol. ZnT10, a self-regulated zinc transporter of ZnT family attached to basolateral membrane, was describe by Da Silva et al. (2016) as a manganese transporter. Metallothionein is an intracellular zinc binder regulated by zinc concentration, can also link to copper and disable its utilization (Leone et at., 1985). These physiological processes may cause interactions in mineral absorption and can be responsible to tracemineral antagonisms. Due this fact, manganese, iron and copper balance was also evaluated and are presented in tables 9, 11 and 13. The significant regressions equations are presented in tables 10, 12 and 14.

To manganese balance parameters, the increasing zinc levels has significant ($P<0.05$) quadratic effects in manganese intake for inorganic and organic sources and manganese excretion to organic source. A decrease in manganese retention and balance was observed when the animals receive the inorganic source supplement with increasing zinc levels and a linear and quadratic effect was observed.

The iron balance parameter results can be observed in table 11. To iron intake, the increased zinc levels had a significant ($P<0.05$) linear effect to inorganic source and a quadratic effect to organic source. A quadratic effect to inorganic source and a linear effect to organic source was also observed ($P<0.05$) to iron excretion. A linear and quadratic effect can be observed ($P<0.05$) in iron retention when inorganic sources of trace minerals were supplemented with increasing zinc levels. In iron apparent absorption, quadratic effect in inorganic source and linear effect in organic source were significant ($P<0.05$). The regression equation to these data can be observed in table 12.

In tables 13 and 14 are presented the results of copper balance parameters and the regression equations to copper balance in organic and inorganic trace mineral supplementations with increasing zinc levels. The supply of increasing zinc levels in inorganic and organic sources had no significant effect ($P<0.05$) on copper intake, retention or balance. The copper excretion increase when animals receive higher levels of zinc in organic source, with significantly ($P<0.05$) linear and quadratic responses.

Table 9. Manganese Balance in broiler chickens fed with diets supplemented with different levels of zinc from organic or inorganic sources.

Parameter	Source	Zinc Level					Mean	MSE	C.V. (%)	P-Value (Nested Effect)	
		0	19	38	57	76				Linear	Quadratic
Mn Intake (mg/kg/bird/day)	Inorganic	2.50	2.65	2.78	2.53	2.39	2.57	0.17	5.39	0.0590	<.0001
	Organic	3.99	3.95	4.14	4.07	3.78	3.99			0.0985	0.0008
	Mean	3.24	3.30	3.46	3.30	3.09					
Mn Excretion (mg/kg/bird/day)	Inorganic	1.99	2.16	2.06	2.27	2.15	2.13	0.24	9.43	0.0773	0.3195
	Organic	2.88	3.15	3.05	3.07	2.91	3.01			0.9573	0.0137
	Mean	2.43	2.65	2.56	2.67	2.53					
Mn Retention (mg/kg/bird/day)	Inorganic	0.51	0.49	0.72	0.26	0.24	0.44	0.28	40.27	0.0077	0.0475
	Organic	1.11	0.80	1.09	1.00	0.87	0.97			0.3358	0.9764
	Mean	0.81	0.65	0.90	0.63	0.56					
Mn Balance (%)	Inorganic	20%	18%	26%	10%	10%	17%	8.11	39.48	0.0004	0.0470
	Organic	28%	20%	26%	25%	23%	24%			0.5147	0.6101
	Mean	24%	19%	26%	17%	17%					

Table 10. Regression equations to manganese balance parameters.

Parameter	Source	Contrast	Regression Equations	Optimal Level	P-Value	R^2
Mn Intake (mg/kg/bird/day)	Inorganic	Quadratic	$Y=-0.0002x^2+0.0471x+0.5654$	117.75	<0.0001	0.8656
	Organic	Quadratic	$Y=-0.000144x^2+0.0094x+3.9405$	32.64	0.0008	0.6505
Mn Excretion (mg/kg/bird/day)	Organic	Quadratic	$Y=0.0001427x^2+0.0108x+2.9104$	37.75	0.0137	0.9882
Mn Retention (mg/kg/bird/day)	Inorganic	Linear	$Y=-0.0041x + 0.598$	>76.00	0.0077	0.3750
		Quadratic	$Y=0.00013x^2 + 0.0061x + 0.5011$	23.46	0.0475	0.5900
Mn Apparent Absorption (%)	Inorganic	Linear	$Y=-0.1570x + 22.80$	>76.00	0.0004	0.4153
		Quadratic	$Y=0.00382x^2 + 0.1334x + 20.045$	17.46	0.0470	0.5666

Table 11. Iron Balance in broiler chickens fed with diets supplemented with different levels of zinc from organic or inorganic sources.

Parameter	Source	Zinc Level					Mean	MSE	C.V. (%)	P-Value (Nested Effect)	
		0	19	38	57	76				Linear	Quadratic
Fe Intake (mg/kg/bird/day)	Inorganic	6.09	6.40	6.35	5.95	6.68	6.29	0.30	5.69	0.0130	0.1634
	Organic	3.99	4.07	4.18	4.31	3.98	4.11			0.4490	0.0288
	Mean	5.04	5.23	5.26	5.13	5.33					
Fe Excretion (mg/kg/bird/day)	Inorganic	3.69	4.00	3.90	3.96	3.55	3.82	0.35	11.77	0.3534	0.0002
	Organic	1.91	2.09	2.21	2.28	2.20	2.14			0.0337	0.1773
	Mean	2.80	3.05	3.05	3.12	2.87					
Fe Retention (mg/kg/bird/day)	Inorganic	2.40	2.39	2.45	2.00	3.14	2.47	0.41	18.63	0.0108	0.0005
	Organic	2.08	1.97	1.97	2.04	1.79	1.97			0.2013	0.6707
	Mean	2.24	2.18	2.21	2.02	2.46					
Fe Apparent Absorption (%)	Inorganic	39%	37%	39%	34%	47%	39%	6.94	15.96	0.1252	0.0032
	Organic	52%	49%	47%	47%	45%	48%			0.0230	0.6215
	Mean	46%	43%	43%	40%	46%					

Table 12. Regression equations to iron balance parameters.

Parameter	Source	Contrast	Regression Equations	Optimal Level	P-Value	R^2
Fe Intake (mg/kg/bird/day)	Inorganic	Linear	$Y=0.0039x+6.143$	>76.00	0.0130	0.1648
	Organic	Quadratic	$Y=-0.00015x^2+0.013x+3.950$	43.33	0.0288	0.6744
Fe Excretion (mg/kg/bird/day)	Inorganic	Quadratic	$Y=-0.000254x^2 + 0.0176x + 3.700$	34.65	0.0002	0.8588
	Organic	Linear	$Y=0.00398x+1.985$	>76.00	0.0337	0.7102
Fe Retention (mg/kg/bird/day)	Inorganic	Linear	$Y=0.00567x + 2.259$	>76.00	0.0108	0.1744
		Quadratic	$Y=0.000351x^2 -0.021x + 2.512$	29.91	0.0005	0.5103
Fe Apparent Absorption (%)	Inorganic	Quadratic	$Y=0.00493x^2 - 0.318x + 40.486$	32.25	0.0032	0.5893
	Organic	Linear	$Y=-0.0845x+51.087$	>76.00	0.0230	0.9143

Table 13. Copper Balance in broiler chickens fed with diets supplemented with different levels of zinc from organic or inorganic sources.

Parameter	Source	Zinc Level					Mean	MSE	C.V. (%)	P-Value (Nested Effect)	
		0	19	38	57	76				Linear	Quadratic
Cu Intake (mg/kg/bird/day)	Inorganic	0.390	0.402	0.415	0.408	0.405	0.404	0.024	5.38	0.1699	0.0544
	Organic	0.470	0.479	0.491	0.488	0.478	0.481			0.2606	0.0859
	Mean	0.430	0.440	0.453	0.448	0.441					
Cu Excretion (mg/kg/bird/day)	Inorganic	0.258	0.293	0.274	0.295	0.279	0.280	0.029	9.38	0.0752	0.0834
	Organic	0.315	0.353	0.354	0.363	0.356	0.348			0.0033	0.0193
	Mean	0.287	0.323	0.314	0.329	0.318					
Cu Retention (mg/kg/bird/day)	Inorganic	0.132	0.110	0.140	0.112	0.126	0.124	0.035	27.57	0.7352	0.7031
	Organic	0.155	0.126	0.137	0.126	0.122	0.133			0.0988	0.5205
	Mean	0.143	0.118	0.139	0.119	0.124					
Cu Apparent Absorption (%)	Inorganic	34%	27%	34%	28%	31%	31%	7.19	24.76	0.4330	0.3693
	Organic	33%	26%	28%	26%	26%	28%			0.0339	0.2497
	Mean	33%	27%	31%	27%	28%					

Table 14. Regression equations to copper balance parameters.

Parameter	Source	Contrast	Regression Equations	Optimal Level	P-Value	R^2
Cu Excretion (mg/kg/bird/day)	Organic	Linear	$Y=0.000467x+0.33$	>76.00	0.0033	0.5883
		Quadratic	$Y=-0.000016x^2+0.0017x+0.3181$	53.13	0.0193	0.9221

The difference in feed intake observed in performance parameters due the improvement of weight gain can be also observed in manganese and iron intake to organic and inorganic supplementation. In manganese balance for organic supplementation, the quadratic variation in feed intake with higher level on 33 mg/kg of zinc was followed by a quadratic variation on manganese excretion (higher level on 38mg/kg of zinc), and the retention was keep constant. The supplementation of inorganic sources of traceminerals by increasing levels of zinc did not affect manganese excretion, but had a quadratic effect in manganese retention and apparent absorption. At low levels of zinc 23 and 17 mg/kg, the manganese retention was 0.573 mg/day and 21% of apparent absorption. At the level of higher inorganic zinc retention (62 mg/kg), the manganese retention was 0.380 mg/day and apparent absorption fall to 13.6%. In synthesis, 2.7-fold increase in zinc levels reduce 35% the manganese retention (or 1/-0.13).

The opposite pattern was observed to iron retention. Higher values to iron retention and apparent absorption can be reach by supplying 30 and 32 mg/kg of zinc, respectively, and it correspond to 2.20 mg/day of iron retention and 35% of iron apparent absorption. When 62 mg/kg of zinc is supplied, this value up to 2.60 mg/day and 40% of absorption, an increase of 18% of iron retention and 14% on iron absorption when zinc is increase twice (1/+0.09).

Surprisingly, a quadratic effect on copper excretion was observed when organic sources of traceminerals was utilized with increasing levels of zinc. The supplementation of 53 mg/kg of zinc had the higher copper excretion of 0.363 mg/day and this level fall to 0.362 mg/day when 62 mg/kg was supplied. This reduction corresponds to 0.28% in copper excretion when zinc level was increased 17% above 52 mg/kg (1/-0.016), but did not have any effect on copper retention and absorption.

Traceminerals, beyond the self-interactions, are subject to interactions with macro minerals. The excess of calcium and phosphorus could be solubilized in the stomach by low pH and this precipitate may form absorbable complex with trace minerals in duodenum, improving the mineral losses (Li et al., 2004). Taking in consideration the role of trace mineral in several enzymes related to bone growth, an evaluation of calcium and phosphorus balance when organic and inorganic source are supplemented is required and can be observed in tables 15 and 17, with significantly regression equation presented in tables 16 and 18.

Table 15. Calcium Balance in broiler chickens fed with diets supplemented with different levels of zinc from organic or inorganic sources.

Parameter	Source	Zinc Level					Mean	MSE	C.V. (%)	P-Value (Nested Effect)	
		0	19	38	57	76				Linear	Quadratic
Ca Intake (mg/kg/bird/day)	Inorganic	252.43	254.20	264.62	258.90	255.14	284.80	0.013	5.40	0.4666	0.1008
	Organic	249.70	251.22	262.04	260.35	246.56	283.44			0.8342	0.0092
	Mean	251.07	252.71	263.33	259.63	250.85					
Ca Excretion (mg/kg/bird/day)	Inorganic	63.29	64.29	60.65	65.60	61.95	84.56	0.008	14.67	0.8499	0.9925
	Organic	57.43	59.91	58.52	59.71	58.21	80.28			0.8937	0.5727
	Mean	60.36	62.10	59.59	62.66	60.08					
Ca Retention (mg/kg/bird/day)	Inorganic	189.14	189.92	203.97	193.30	193.19	200.24	0.015	6.02	0.4619	0.1568
	Organic	192.28	191.30	203.51	200.64	188.35	203.16			0.9082	0.0475
	Mean	190.71	190.61	203.74	196.97	190.77					
Ca Apparent Absorption (%)	Inorganic	75%	75%	77%	75%	76%	75%	3.59	4.72	0.6966	0.6003
	Organic	77%	76%	78%	77%	76%	77%			0.9407	0.7290
	Mean	76%	75%	77%	76%	76%					

Table 16. Regression equations to calcium balance parameters.

Parameter	Source	Contrast	Regression Equations	Optimal Level	P-Value	R^2
Ca Intake	Organic	Quadratic	$Y=-0.00853x^2 + 0.6633x + 247.244$	38.88	0.0092	0.7167
Ca Retention	Organic	Quadratic	$y=-0.00737x^2 + 0.5688x + 189.499$	38.59	0.0475	0.6009

Table 17. Phosphorus Balance in broiler chickens fed with diets supplemented with different levels of zinc from organic or inorganic sources.

Parameter	Source	Zinc Level					Mean	MSE	C.V. (%)	P-Value (Nested Effect)	
		0	19	38	57	76				Linear	Quadratic
P Intake (mg/kg/bird/day)	Inorganic	273.89	287.99	292.00	289.48	280.66	257.06	0.001	5.41	0.4006	0.0115
	Organic	276.71	289.10	283.74	288.83	278.82	253.97			0.8459	0.0451
	Mean	275.30	288.54	287.87	289.15	279.74					
P Excretion (mg/kg/bird/day)	Inorganic	80.03	87.03	81.34	90.54	83.84	63.15	0.001	13.23	0.2734	0.4397
	Organic	75.32	81.66	84.42	80.38	79.63	58.76			0.7835	0.2474
	Mean	77.68	84.34	82.88	85.46	81.74					
P Retention (mg/kg/bird/day)	Inorganic	193.85	200.96	210.65	198.94	196.81	193.90	0.002	9.01	0.8693	0.4860
	Organic	201.39	207.44	199.31	208.45	199.20	195.22			0.9563	0.4874
	Mean	197.62	204.20	204.98	203.70	198.00					
P Apparent Absorption (%)	Inorganic	71%	70%	72%	69%	70%	70%	4.00	5.64	0.4952	0.8351
	Organic	73%	72%	70%	72%	71%	72%			0.5946	0.3550
	Mean	72%	71%	71%	70%	71%					

Table 18. Regression equations to phosphorus balance parameters.

Parameter	Source	Contrast	Regression Equations	Optimal Level	P-Value	R^2
P Intake	Inorganic	Quadratic	$Y=-0.0104x^2 + 0.8667x + 274.31$	41.79	0.0227	0.8656
	Organic	Quadratic	$Y=-0.00679x^2+0.5371x +277.74$	39.55	0.1265	0.6716

The increasing of zinc levels of the diet didn’t affect significantly ($P<0.05$) the calcium excretion or balance presented in table 15, but a quadratic effect can be observed in calcium intake and retention when organic source of trace minerals was supplied by increasing levels of zinc and regressions equations for calcium balance parameters are shown in table 16.

There was no significant effect ($P<0.05$) to phosphorus excretion, retention or balance either (table 17). To phosphorus intake, a quadratic effect can be observed supplying inorganic or organic sources of trace minerals with increasing zinc levels and regressions equations for phosphorus intake are presented in table 18.

The difference in mineral intake can be related once again to the performance results. However, the quadratic effect in calcium intake of organic source of traceminerals was followed by a quadratic effect on calcium retention, with a higher intake and retention at 39 mg/kg of zinc, with 200 mg/day of calcium retained, highlighting the importance of maintain tracemineral supplementation in accurate levels.

After absorption, the mineral uptake from basolateral membrane will be delivered in liver to packed into metalloproteins to storage (metallothionein, ceruloplasmin) or transported to target tissues (albumin, ferritin) (Stipanuk and Caudill, 2013) regardless this fact, between cell uptake and plasma transportation, part of the organic chelated tracemineral will be hydrolyzed and presented as inorganic molecules. The amount of organic tracemineral coming directly from an ingested chelate to plasma is difficult to quantify and may vary from situation to situation (Ashmed, 2012), but all the plasmatic mineral will be regulated by pathways in liver. In this way, an evaluation of minerals in liver is essential to understand tracemineral nutrition.

4.3 Mineral deposition

Ashmead (2012) describe the liver role in storage mineral chelates. The non-hydrolyzed amount of chelated minerals that get in the liver is “ready to use” in metalloproteins where the mineral is required, as superoxide dismutase, collagenase and alkaline phosphatase. This is probably the reason of organic chelated minerals had higher biological value than inorganic molecules. Part of the chelated molecules can also be used as amino acid sources and will be incorporated in proteins with metal bounded. In an excess situation, regulatory pathways will be activated to reduce plasmatic levels of circulating minerals. High levels of zinc induce the metallothionein transcription (Leone et al., 1985), as the same path the highest levels of copper activate ceruloplasmin production. This tracemineral excess will be transported to the pancreatic duct and launched in intestinal lumen and can be reabsorbed if necessary. In the absence of an intestinal regulatory step for organic tracemineral supplementation, this pathway in liver can be an important adjust to keep mineral concentration in physiological levels.

The results for the mineral deposition in liver of broiler chicks receiving diets with increasing zinc levels supplied by an inorganic or organic trace mineral source, as the values for initial mineral deposition on liver, are presented in table 19. The increase on zinc levels of the diets enhance the deposition of zinc in the liver with linear and quadratic responses for organic and inorganic sources and the equations can be viewed in table 20.

The highest level of zinc deposited is reach when 67 mg/kg of diet is supplied in both sources, corresponding to 80 mg of zinc for kg of liver to inorganic source and 87 mg to organic source. The value of 67 mg/kg is close the optimal levels to performance results (63 and 70 mg/kg) and around 80 mg/kg of zinc in liver seems to be the physiological level of this mineral whereas the initial value of 87 mg/kg in liver was observed.

It can be also noted a difference in levels 0, 19 and 38 mg/kg of zinc in inorganic source can be observed in comparison to the zinc in liver of the animals at day 8 by Dunnett test. This difference to organic source is only significant in levels 0 and 19 mg/kg of zinc, indicating the organic sources can maintain values near the physiological conditions easily than inorganic sources.

To manganese and iron deposition, significant linear and quadratic responses ($P<0.05$) can be observed when organic sources with increasing levels of zinc are supplied. Levels of 40 and 47 mg/kg of zinc reach the lower levels of manganese and iron in the liver, respectively. This data suggests an after-absorption antagonism can occur in liver when zinc levels vary to deficiency to above requirement.

The storage of mineral in liver is an efficient manner to prevent mineral toxicity by high intake and absorption or any depletion situation that may occur, like infections, lower amount of minerals supplied or improved demand and the point when the mineral start to be storage can be an indicator of the point of the requirement is reached.

Table 19. Mineral deposition in liver (kg) in broiler chickens fed with diets supplemented with different levels of zinc from organic or inorganic sources in dry matter basis.

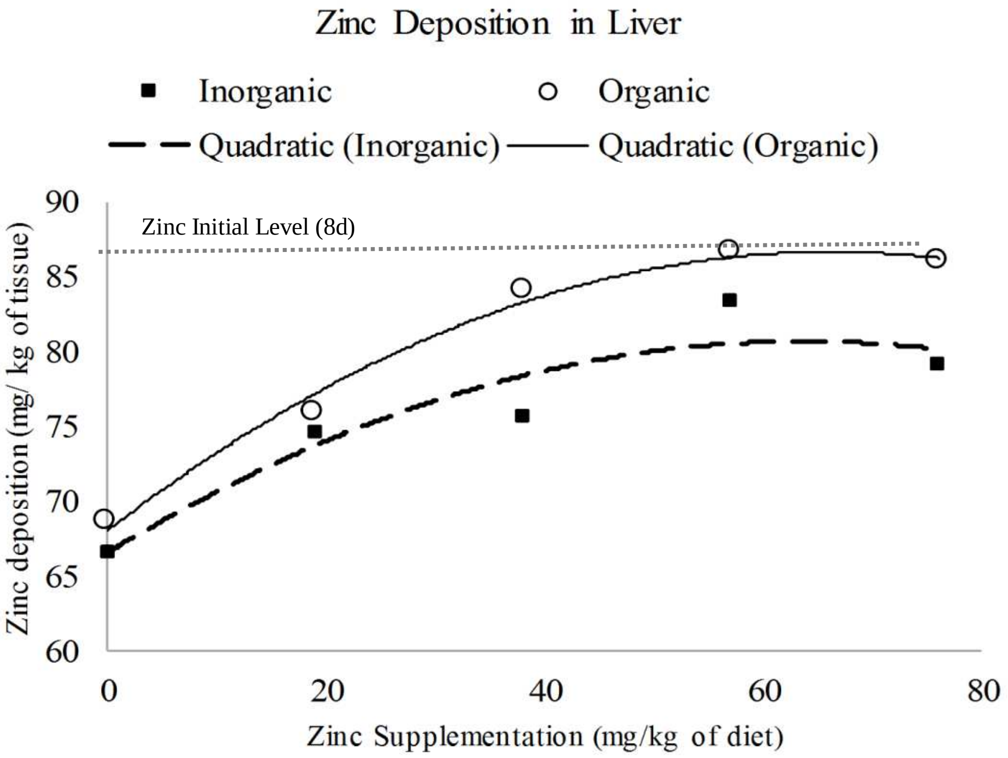
Parameter	Initial (8 d-old)	Source	Zinc Level					Mean	MSE	C.V. (%)	P-Value	
			0	19	38	57	76				Linear	Quadratic
Zn (mg/kg)	87.75	Inorganic	66.59*	74.61*	75.62*	83.30	81.40	75.87	6.17	7.91	<.0001	0.0233
		Organic	68.60*	75.83*	84.06	86.56	85.95	80.20			<.0001	0.0045
		Mean	67.60	75.22	79.84	84.93	82.58					
Mn (mg/kg)	9.61	Inorganic	8.72	8.54	8.70	8.51	9.32	8.76	8.09	38.37	0.8718	0.3409
		Organic	10.83	10.00	9.39	9.80	10.71	10.15			0.0443	0.0022
		Mean	9.77	9.27	9.04	9.15	10.01					
Fe (mg/kg)	363.16	Inorganic	397.76	436.79	393.91	438.91	383.65	410.20	178.00	61.80	0.7585	0.5790
		Organic	395.63	309.13	221.13	257.98	279.43	292.66			0.0083	0.0100
		Mean	396.69	372.96	307.52	348.44	331.54					
Cu (mg/kg)	13.38	Inorganic	16.64	12.90	12.77	15.44	12.86	14.12	134.30	172.47	0.8592	0.1886
		Organic	13.90	12.78	12.47	12.84	12.59	12.92			0.0982	0.2464
		Mean	15.27	12.84	12.62	14.14	12.73					
Ca (mg/kg)	166.29	Inorganic	171.82	152.70	164.30	175.95	151.17	163.19	21.52	13.59	0.4045	0.6590
		Organic	160.42	163.45	155.98	153.93	155.12	157.78			0.4017	0.9925
		Mean	166.12	158.07	160.14	164.94	153.15					
P (g/kg)		Inorganic	11.87	11.76	11.84	11.71	11.61	11.76	4.00	5.64	0.4952	0.8351
		Organic	12.10	12.00	11.70	11.77	11.76	11.87			0.5946	0.3550
		Mean	11.99	11.88	11.77	11.74	11.68					

*Significantly different from Initial values (8d-old) by Dunnett test (P<0.05).

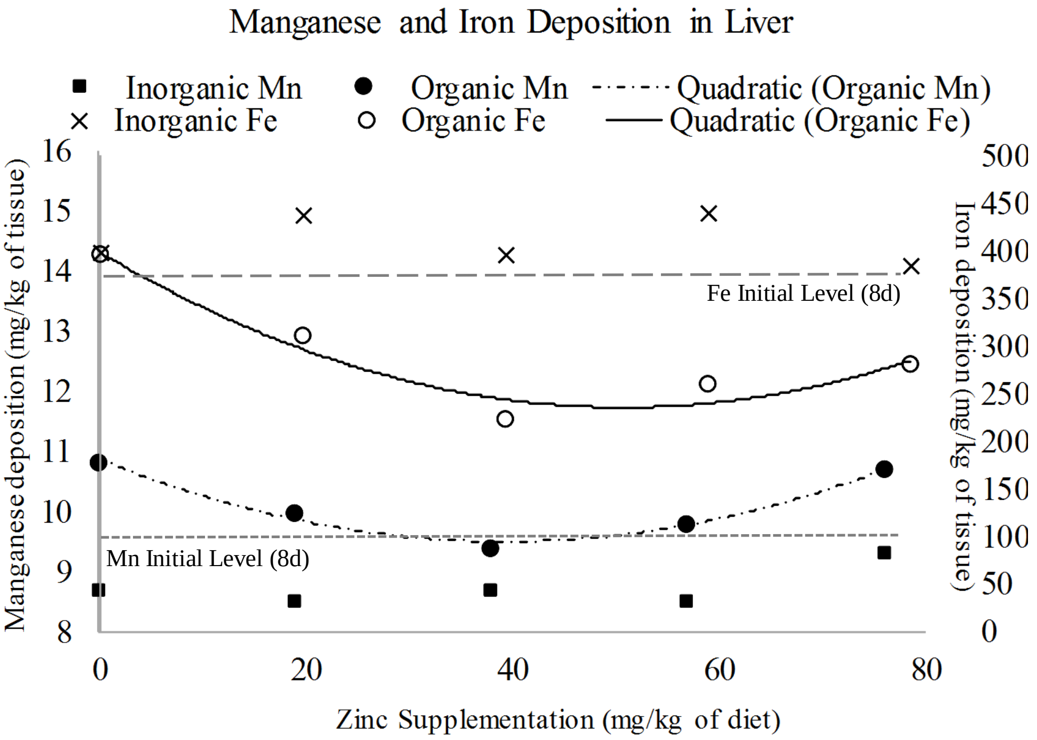
Table 20. Regression equations to mineral deposition in liver.

Parameter	Source	Contrast	Regression Equations	Optimal Level	P-Value	R^2
Zn (mg/kg)	Inorganic	Linear	$Y=0.1786x+69.08$	>76.00	<.0001	0.7469
		Quadratic	$Y=-0.0035x^2+0.4425x+66.57$	63.21	0.0233	0.8897
	Organic	Linear	$Y=0.2391x+71.114$	>76.00	<.0001	0.8526
		Quadratic	$Y=-0.0042x^2+0.5611x+68.055$	66.80	0.0045	0.9879
Mn (mg/kg)	Organic	Linear	$Y=-0.0037x+10.36$	>76.00	0.0443	0.3186
		Quadratic	$Y=0.00093x^2-0.074x+11.03$	39.78	0.0022	0.7603
Fe (mg/kg)	Organic	Linear	$Y=-1.3347x+346.85$	>76.00	0.0083	0.4629
		Quadratic	$Y=0.0715x^2-6.772x+398.49$	47.36	0.0100	0.9404

Graphic 3. Liver deposition of zinc in broiler chickens fed with diets supplemented with different levels of zinc from organic or inorganic sources.



Graphic 4. Liver deposition of manganese and iron in broiler chickens fed with diets supplemented with different levels of zinc from organic or inorganic sources.



4.4 Relative Bioavailability

Bioavailability can be defined as a portion of nutrient intake that will be absorbed, transported and used to supply metabolic requirements (Matsui et al., 1996). This process is followed by higher performance results in poultry production and growth response has been used as primary criterium to determine bioavailability, but mineral absorption may provide a more accurate value (Ammerman, 1995). The relative bioavailability (RBA) is the percentage of nutrient source available comparing to a standard source and can be calculated comparing slope ratios when both sources had a linear response (Littell et al., 1995). In this study, the absorption parameters that presented significant ($P<0.05$) linear effects in both sources was Zinc retention, Zinc Apparent Absorption and Zinc in Liver. A compilation of this responses is presented in table 21.

The estimated bioavailability of organic source of zinc is 125% on average. This value represents improvement on zinc absorption when organic source is used and is lower than reported by Ao et al. (2006) of 183% based in performance and 157% based on zinc deposition in tibia. However, using the average data to zinc retention on liver to calculated relative bioavailability reach in 66% of bioavailability, demonstrating the importance in determine a standard parameter to evaluate bioavailability.

Table 21. Linear response slopes and bioavailability of sources.

Parameter	Inorganic Equation Slope	Organic Equation Slope	BioAv.(%)
Zn Retention	0.0091	0.0119	131%
Zn Apparent Absorption	-0.182	-0.2035	112%
Zn Deposition in Liver	0.1786	0.2391	134%
Mean			125%

5 Conclusions

The nutritional requirement of zinc is 70 mg/kg of weight gain when supplied by an inorganic source, but this value can be reduced to 63mg/kg of weight gain when an organic source is utilized without performance losses. The absorption antagonisms in inorganic source was calculated as 1:-0.13 to Zn:Mn and 1:0.09 to Zn:Fe based in retention results. Relative Bioavailability of Zinc organic source is 125% of inorganic source calculated by zinc absorption capacity.

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CHAPTER 2

NUTRITIONAL REQUIREMENT AND BIOAVAILABILITY OF DIFFERENT SOURCES OF MANGANESE FOR BROILERS

ABSTRACT

The objective of the current study was to update the nutritional requirement of manganese values for broilers and determine the relative bioavailability (RBA) of two sources of manganese. To this, 510 male Cobb broilers from 1 to 18 days-old were used. The animals were housed (1-7d) following Cobb guidelines receiving a corn-soybean meal diet with low basal trace mineral content (13 mg/kg of basal manganese analyzed) and supplemented with 30 mg/kg of manganese (50% of NRC 1994 recommendations) as MnSO4 to reduce body stores. At 8d-old, 10 birds were slaughtered to collect liver samples to evaluate initial mineral deposition and the remaining birds were weighed and randomly assigned to 10 treatments with 10 replicates of 5 animals each. The treatments consisted of five levels of manganese supplemented (0; 25; 50; 75 and 100 mg/kg) from two sources (MnSO4, with Fe, Cu, Zn and Se supplemented in as sulphates, and Bioplex Mn, with Fe, Cu, Zn and Se supplemented as chelates). The animals were housed in plastic cages with plastic feeders and received demineralized water and feed at libitum. The experimental diets were semipurified (5 mg/kg of basal manganese analyzed), with 30% of corn and contained cellulose, phytic acid and phytase to approach to a practical diet. On 17 d-old, the birds and feeds were weighed to evaluate the final weight (BW), weight gain (WG), feed intake (FI) and feed conversion (F:G). At 18 d-old, a bird for cage was slaughtered for determination of minerals in the liver. The results indicate that the manganese requirement for higher performance is 166 mg Mn/kg of weight gain for inorganic supplementation, which can be reduced to 95 mg Mn/kg of weight gain in organic mineral supplementation. Both sources have an ascending linear retention in the gut and the highest deposition on liver is reach with supplying 69 mg/kg of diet of inorganic manganese or 85mg/kg of diet of organic manganese. The inorganic source supplementation reduces zinc and copper absorption and increases manganese, copper and iron excretion. RBA of the organic manganese was estimated to be 100% of the inorganic manganese.

Key words: Manganese, requirements, bioavailability, traceminerals, broiler.

1. Introduction

Manganese is an essential element in animal nutrition as part of several enzymatic processes. This essentiality is due manganese ability in interchange its valence state (Mn^{2+}, Mn^{3+} and Mn^{7+}) what makes it an important cofactor in catalytic reactions (Stipanuk and Cauldill, 2013). Metalloenzymes like arginase, superoxide dismutase and pyruvate carboxylase had a manganese constituent allowing then

It is fundamental understand the role of manganese in body to establish the requirement accurately to avoids deficiency or excess symptoms. It became a problem in poultry production knowing that practical diets can reduce the bioavailability of some sources of manganese (Halpin and Baker, 1986). The actual requirement of manganese for broilers is 60 mg/kg of diet, defined by NRC (1994) based in Kealy and Sullivan (1966). The Council at the time presents at 42 d-old 3,717 grs of feed intake and 2,088 grs of body weight. Calculating the manganese intake by kilogram of weight gain the requirement was 107 mg/kg WG. The recent data (Rostagno et al., 2017) set forth 5,083 grs in feed intake and weight of 3,218 grs at 42-day and, reaching 95 mg of manganese for kg of weight gain. The use of the NRC (1994) manganese recommendations in the actual broiler strains represent 11% less manganese supply for animals with performance 55% higher.

It has been supposed that chelated sources could reach the requirement amount of the mineral in the body in a faster and readily available way, reducing the mineral supplemented and reducing losses to environment. Therefore, besides the updating the requirements values to manganese, it is also necessary establish the bioavailability of this source to meet the requirements.

2. Objective

The aim of this project was to determine manganese requirements for broilers from organic and inorganic sources, as well as to determine zinc bioavailability from organic source.

3. Materials and Methods

The experiment was conducted in the Poultry Farm of the Animal Science Department at Universidade Federal de Viçosa from September to October 2016. All the procedures follow the standards of the National Council of Animal Experimentation

Control – CONCEA and were previously approved by the Ethics Committee of Animal Production, CEUP – UFV (Register number 111/2014).

3.1 Animal**s**

Five hundred and ten Cobb male chicks were utilized in this experiment. The birds were housed from the first to seventh day of age in brooders following the strain guidelines recommendations. A corn-soybean meal diet was formulated (Table 1) to meet or exceed the nutritional requirements for this age (Rostagno et al., 2011) except for the trace mineral content.

Table 1. Proximate and calculated/analyzed composition of the start diet used in the period of 1 to 7 d of age.

Ingredients	%	Nutrient	Calculated values
Corn	53.335	Metabolizable energy (kcal/kg)	3135
Soybean Meal (45%)	30.000	Crude protein (%)	22.41
Corn Gluten (60%)	6.880	Calcium (%)	0.828
Soybean oil	3.700	Phytic phosphorus	0.247
Starch	1.585	Available phosphorus (%)	0.391
Potassium phosphate	0.955	Sodium (%)	0.223
Calcium carbonate	1.700	Chloride (%)	0.272
Sodium Chloride	0.530	Potassium (%)	0.987
Vitamins supplement [A]	0.150	Magnesium (%)	0.185
Lysine HCl (79%)	0.303	Copper (mg/kg)	15
Methionine (99%)	0.197	Iron (mg/kg)	217
Threonine (98%)	0.016	Manganese (mg/kg)	43
L-Arginine (98.5%)	0.006	Zinc (mg/kg)	67
Colin Chloride (60%)	0.375	Selenium (mg/kg)	0.48
Coccidiostatic	0.055	Lysine (%)	1.174
Avilamicin	0.003	Methionine + Cysteine (%)	0.846
BHT	0.010	Threonine (%)	0.763
Mineral Supplement [B]	0.100	EN:TN Ratio	49.51
		Electrolytic Balance	273

[A] Vitamin supplement containing per kg of ration: Vit. A – 9,750 IU; Vit. D3 – 2,470 IU; Vit. E – 36.6 IU; Vit. B1 – 2.60 mg; Vit. B2 – 6.50 mg; Vit. B6 – 3.64 mg; Vit. B12 – 0.015 mg; Pantotenic acid – 13.0 g; Biotin – 0.091 mg; Vit. K3 – 1.95 mg; Folic acid – 0.91 mg; Nicotinic acid – 39.0 mg.

[B] The mineral supplement was added following NRC (1994) recommendations for traceminerals, except for manganese (50% of NRC recommendations) (Table 2).

Table 2. Calculated and analyzed values for basal diets and supplements used in Mn trial.

SAMPLE	n	Mn (mg/kg)		Fe (mg/kg)		Zn (mg/kg)		Cu (mg/kg)	
		Calc	Anal	Calc	Anal	Calc	Anal	Calc	Anal
Prestart basal diet	15	13	20	137	108	27	34	7	7
Prestart supplement [A]	5	30	29	80	41	40	35	8	5
Prestart diet (+supplement)	10	43	44	217	141	67	65	15	10
Experimental basal diet	12	5	6	32	29	12	18	3	2
Inorganic suppl. (0% Mn) [B]	5	0	1	80	82	40	53	10	7
Inorganic suppl. (100% Mn) [B]	5	100	93	80	64	40	55	10	6
Organic suppl. (0% Mn) [B]	5	0	1	80	82	40	39	10	11
Organic suppl. (100% Mn) [B]	5	100	93	80	76	40	31	10	10

[A] Inclusion 1kg/ton
[B] Inclusion 2kg/ton

The trace minerals amount in diets were calculated to meet 100% of NRC (1994) requirements, except for manganese supplemented in 50% of NRC (1994) requirement (Table 2). At 8d-old, 500 animals with 174.6±1g initial body were weighed and randomly distributed into one of 10 treatments and 10 replications and 5 animals per pen. Each pen was considered as an experimental unit. Extra ten animals were slaughtered to collect samples in a pool to evaluate initial trace mineral concentrations.

3.2 Diets

A semi purified basal diet was formulated based on casein, albumin, isolated soybean protein and corn, starch and dextrose (Table 3) to meet or exceed Rostagno et al. (2011) recommendations for initial phase. The diet was formulated to obtain minimum amounts of trace minerals while meet or exceed the requirements for the other nutrients. Mineral supplements (Table 4) were added to this diet with 0 or 100 mg/kg of manganese in an organic (Bioplex TM) or inorganic (Sulphate) sources. These diets were mixed in a proportion to reach 5 different levels of the tested mineral from both sources.

3.3 Treatments

The experimental treatments (Table 4) consists in 5 levels of manganese and two different sources, an organic (Bioplex TM) and an inorganic (Sulphate) source. manganese levels were calculated using levels up and below the NRC (1994) recommendation for manganese. The other minerals (copper, iron, zinc, selenium and iodine) were kept in the actual recommendations as suggested by the NRC (1994).

3.4 Housing

The weighted birds were transferred to an experimental room and housed in 0.35 × 0.50 m plastic cages equipped with nipple type drinkers and tubular plastic feeders to provide water and feed *ad libitum*. The water provided to the animals during the experimental period was demineralized to ensure any external contamination. The temperature was maintained constant by heat lamps and curtains according to the range of thermal comfort for this age measured by datalogger installed in the room.

3.5 Parameters Evaluated

During 10 days of experimental period, the performance parameters evaluated was average final body weight, average weight gain, average feed intake, feed-to-gain ratio, and mortality. At 13 d-old (5 days of trial) trays covered with plastic sheeting was installed to allow total excreta collection to evaluate the trace mineral balance. The mineral balance was calculated by the difference between the intake and the excretion of each mineral. The collections were performed over 5 consecutive days until the end of experimental period. Excretas were frozen and homogenized after each collection and an aliquot was taken and dried for further analyses.

At 17 d-old, the animals and feeds were weighed to calculate average final body weight, average weight gain, average feed intake and feed-to-gain ratio. At 18 d-old, one animal per cage was selected to slaughter. The animals were slaughtered by cervical dislocation for samples collection to evaluate the concentration of each mineral in the tissues.

Table 3. Proximate and nutritional composition of semipurified diets used in experimental period (8-17 days).

Ingredient	%	Nutrient	Calculated Composition
Corn	30.00	Met. energy (kcal/kg)	3128
Casein	4.00	Crude protein (%)	22.58
Albumin	12.00	Calcium (%)	0.850
Isolated Soy Protein	4.00	Phytic phosphorus	0.161
Starch	13.00	Available P (%)	0.400
Dextrose	13.30	Sodium (%)	0.372
Broken Rice	8.00	Chloride (%)	0.485
Soybean oil	2.00	Potassium (%)	0.885
Cellulose	4.00	Magnesium (%)	0.126
Calcium Bicarbonate	1.695	Copper (mg/kg)	11
Potassium Phosphate	1.485	Iron (mg/kg)	110
Magnesium Chloride	0.650	Manganese (mg/kg) [B]	5-105
Potassium Chloride	0.468	Zinc (mg/kg)	72
Amino acids supplement [A]	0.030	Selenium (mg/kg)	0.52
Mineral Supplement [B]	0.200	Lysine (%)	1.254
Vitamin Supplement [C]	0.150	Met + Cys (%)	0.913
Sodium Phytate	1.020	Threonine (%)	0.831
Phytase	0.010	EN:TN Ratio	50.07
Other ingredients [D]	0.620	Electrolytic Balance	251

[A] Amino acids: Lysine HCL (79%) – 0.030; L-Arginine (98.5%) - 0.275; L-Glycine (98.5%) - 0.400; Alanine (99%) - 0.850; Glutamic acid (100%) - 2.000. [B] Added according each experimental treatment (Table 4).

[C] Vitamin supplement containing per kg of ration: Vit. A – 9,750 IU; Vit. D3 – 2,470 IU; Vit. E – 36.6 IU; Vit. B1 – 2.60 mg; Vit. B2 – 6.50 mg; Vit. B6 – 3.64 mg; Vit. B12 – 0.015 mg; Pantotenic acid – 13.0 g; Biotin – 0.091 mg; Vit. K3 – 1.95 mg; Folic acid – 0.91 mg; Nicotinic acid – 39.0 mg. [D] Colin Chloride (60%) - 0.375; Coccidiostatic - 0.055; Avilamicin - 0.010; Antioxidant (BHT) - 0.030

Table 4. Calculated and Analyzed values of minerals for the semi purified diet in mg/kg.

Source	Mn Lvl	n	Mineral							
			Manganese		Iron		Zinc		Copper	
			Calcul.[1]	Analy.[2]	Calcul.[1]	Analy.[2]	Calcul.[1]	Analy.[2]	Calcul.[1]	Analy.[2]
Inorganic	0	10	5	6	110	113	72	67	11	6
	19	10	30	27	110	103	72	73	11	5
	38	10	55	53	110	109	72	66	11	6
	57	10	80	73	110	113	72	73	11	6
	76	10	105	96	110	97	72	69	11	8
Organic	0	10	5	6	110	108	72	51	11	12
	19	10	30	27	110	104	72	51	11	13
	38	10	55	49	110	105	72	56	11	12
	57	10	80	70	110	107	72	53	11	13
	76	10	105	93	110	111	72	52	11	12

[1] Total Calculated values (Basal diet + Supplement).

[2] Analyszed in 5 samples with replicate.

3.6 Mineral Concentration Analysis

Liver samples were lyophilized and ground in a mill to be analyzed, as diets and excreta, following the method 935.13 (AOAC, 2000). Tibia without the adjacent cartilage and free of muscular tissue, breast and liver samples were lyophilized and ground in a mill. An aliquot of 0.6g of diets, tissues and excreta samples were weighed on the analytical balance and added 5ml of 4:1 nitroperchloric acid solution (4 parts of nitric acid and 1 part of perchloric acid). The samples were taken at 200ºC in the digester block and the residue was filtrated on quantitative paper (7.5 µm pore) and completed for 50ml with distillate water. This solution was read in atomic absorption spectrophotometer (GBC Avanta Σ) to obtain the mineral levels. The phosphorus analysis was performed by the colorimetric method (Fiske & Subbarow, 1925). The proximate analyses were performed in the Animal Nutrition Laboratory of the Animal Science Department at the Universidade Federal de Viçosa.

3.7 Statistical Analysis

Data was analyzed using the Statistical Analyses System program – SAS 9.4. An ANOVA test was performed using GLM procedure considering a Nested Design with levels inside the different sources and the pens as the experimental unit. The regression equations were estimate using the orthogonal contrasts by PROC REG procedure. For the mineral requirements, only linear and quadratic responses was considered for the model if statistical significance was observed ($P<0.05$). To mineral deposition in tissues, a Dunnett test is also performed to compare the results with initial mineral values in the beginning of the experiment, using the GLM procedure. The requirements values for organic and inorganic sources was compared for the bioavailability determination.

The estimates of the requirements for each parameter were obtained by the model:

$$Y_{ikj} = \mu + A_j + B(A)_{ij} + e_{ikj}$$

Where:

Yikj = observation *k* in level *i* of factor Source and level *j* of factor Mn Level;

μ = overall mean of the experiment;

Aj = effect of level *i* of factor Source;

B(A)ij = effect of level *j* of factor Mineral Level within level *i* of factor Source;

eikj = random error associated with each observation.

4. Results and Discussion

4.1. Performance Results

The most difficult step in establish a trace mineral requirement is define a parameter which is sensitive to manganese deficiency without provide changes in mineral metabolism. Footpad integrity, leg deformity, incidence and severity of perosis were been reported (Henry, 1995) as good measurements of manganese requirement, as several researches reports no difference in growth performance (Olgun, 2016; Brooks et al., 2012; Li et al., 2005) even in high doses (Yan and Waldroup, 2006; Sunder, 2006). However, the manganese participation on essential enzymes for bird growth as arginase, pyruvate carboxylase and glycosyltransferase (Stipanuk and Caudill, 2013) leads to expect performance responses.

The performance results are showed in table 5. No mortality rate or clear deficiency symptoms was noted is this research. The increasing manganese levels had a significantly ($P<0.05$) linear effect in body weight (BW) and weight gain (WG) of animals receiving inorganic or organic sources, suggesting that higher levels of manganese may are required. The linear effect was also significant ($P<0.05$) to feed intake (FI) and feed to gain ratio (F:G) when manganese levels was increased and supplied in an inorganic form. The organic form had a significant ($P<0.05$) quadratic response in F:G, and the level of 70.22 mg/kg of manganese is required to lower values of F:G, as showed in table 6.

The lower feed to gain ratio obtained with the 70.22 mg of Mn/kg of diet in organic source is 1.360 and it represent 95 mg of Mn / kg of WG. Using this response in the linear equation for inorganic source, the level of inorganic manganese to reach 1.360 in F:G is 121.80 mg/kg of diet (or 166 mg of manganese/kg of WG), although this level was nor tested.

Few publications reports improve in WG when manganese levels were tested. Pierce et al. (2009) found highest BW by using 90 mg/kg in diet of organic and inorganic manganese, corresponding to 153.0 (organic) and 159.3 (inorganic) mg/ kg of WG. The same value was found by Neeraj (2014) when manganese was supplied concurrently with 400mg of ascorbic acid. However, the absorption rate of these sources of manganese have to be evaluated.

Table 5. Performance[1] of broiler chickens fed diets with different levels of manganese from organic or inorganic sources from 8 to 17 days old.

Parameter	Source	Manganese Level					Mean	MSE	C.V. (%)	P-Value (Nested Effect)	
		0	25	50	75	100				Linear	Quadratic
BW[2]	Inorganic	526.08	542.12	547.20	554.46	551.96	544.36	19.70	3.61	0.0015	0.1459
	Organic	527.04	553.30	549.12	540.44	556.40	545.26			0.0221	0.2771
	Mean	526.56	547.71	548.16	547.45	554.18					
WG[3]	Inorganic	351.22	367.32	372.36	379.62	377.08	369.52	19.57	5.29	0.0015	0.1374
	Organic	352.18	378.44	374.26	365.60	381.56	370.41			0.0196	0.2889
	Mean	351.70	372.88	373.31	372.61	379.32					
FI[4]	Inorganic	498.85	510.44	516.32	518.80	520.08	512.90	23.99	4.69	0.0392	0.4002
	Organic	498.32	521.36	504.68	505.92	518.68	509.79			0.2889	0.9272
	Mean	498.59	515.90	510.50	512.36	519.38					
F:G[5]	Inorganic	1.421	1.391	1.389	1.366	1.381	1.390	0.0393	2.84	0.0096	0.1341
	Organic	1.417	1.377	1.349	1.384	1.360	1.377			0.0070	0.0475
	Mean	1.419	1.384	1.369	1.375	1.370					

[1] Due only two animals died during the experiment, mortality presented low variability with no statistical difference and was disregarded.

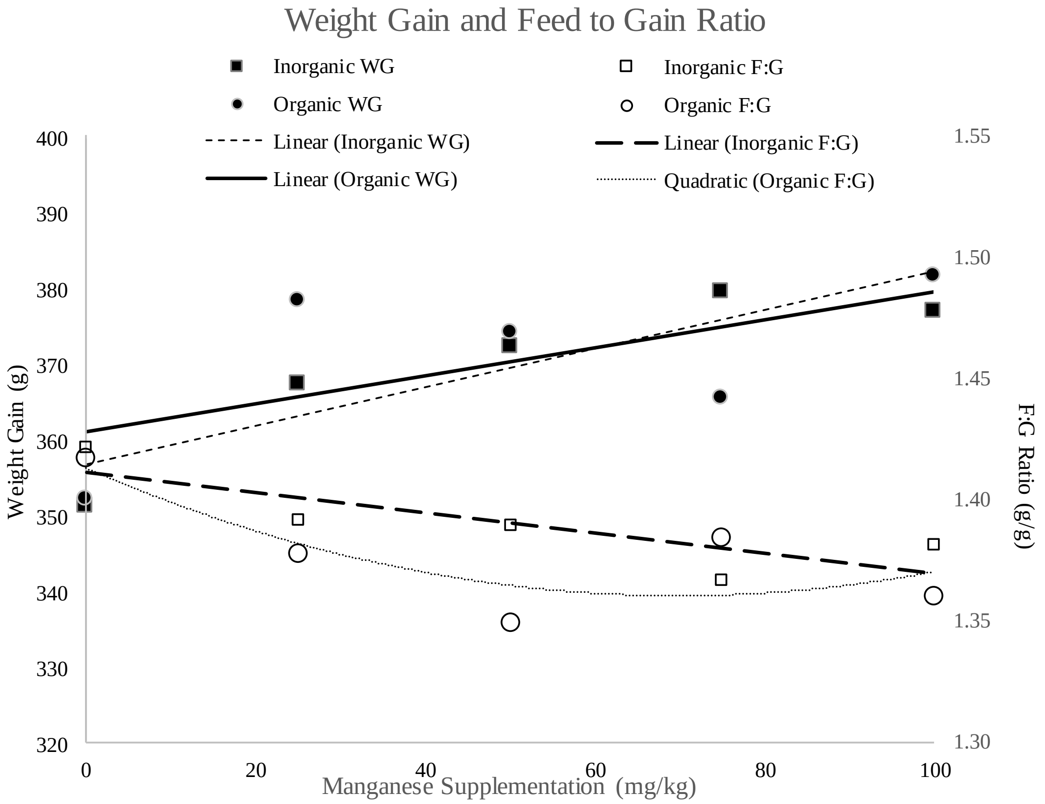
[2] BW (Body Weight, g); [3] WG (Weight Gain, g); [4] FI (Feed Intake, g); [5] F:G (Feed-to-gain ratio, g/g).

Table 6. Regression equations to performance parameters.

Parameter	Source	Contrast	Regression Equations	Optimal Level	P-Value	R^2
BW[1] (g)	Inorganic	Linear	$Y=0.2576x+531.46$	>100	0.0015	0.8104
	Organic	Linear	$Y=0.1836x+536.04$	>100	0.0221	0.3763
WG[2] (g)	Inorganic	Linear	$Y=0.2564x+356.6$	>100	0.0015	0.8085
	Organic	Linear	$Y=0.1860x+361.1$	>100	0.0196	0.3772
FI[3] (g)	Inorganic	Linear	$Y=0.2008x+502.84$	>100	0.0392	0.8566
F:G[4] (g/g)	Inorganic	Linear	$Y=0.000416x+1.4105$	>100	0.0096	0.6809
	Organic	Linear	$Y=0.0004336x+1.3991$	>100	0.0070	0.4207
		Quadratic	$Y=0.00001068x^2-0.0015x+1.4125$	70.22	0.0475	0.6576

[1] BW (Body Weight, g); [2] WG (Weight Gain, g); [3] FI (Feed Intake, g); [4] F:G (Feed-to-gain ratio, g/g)

Graphic 1. Weight gain and feed to gain ratio of broiler chickens fed diets with ascending levels of manganese from organic or inorganic sources from 8 to 17 days old.



6.1 Mineral Balance Results

The results for manganese, zinc, iron and copper balance are shown in tables 7, 9, 11 and 13. The significant regressions equations are presented in tables 8, 10, 12 and 14.

An ascending linear and a quadratic effect was significantly ($P<0.05$) observed in manganese intake when supplied by inorganic sources, with a 188 mg/kg as a maximum level. When organic source was supplemented, an ascending linear response was observed. A significant ($P<0.05$) linear increase in manganese excretion and manganese retention are also observed with an increase in manganese levels supplementation in organic or inorganic sources, as well manganese balance to organic supplementation. The regression equations are present in table 8.

The linear increase in manganese retention suggest an improve in performance may be recorded if higher levels of manganese were used. The apparent absorption rate seen in this study was low and can be linearly increased by using organic source of manganese. This agree with Brooks et al. (2012) that manganese is normally poor absorbed and several minerals (Ca and P) and phytate can reduce Mn bioavailability.

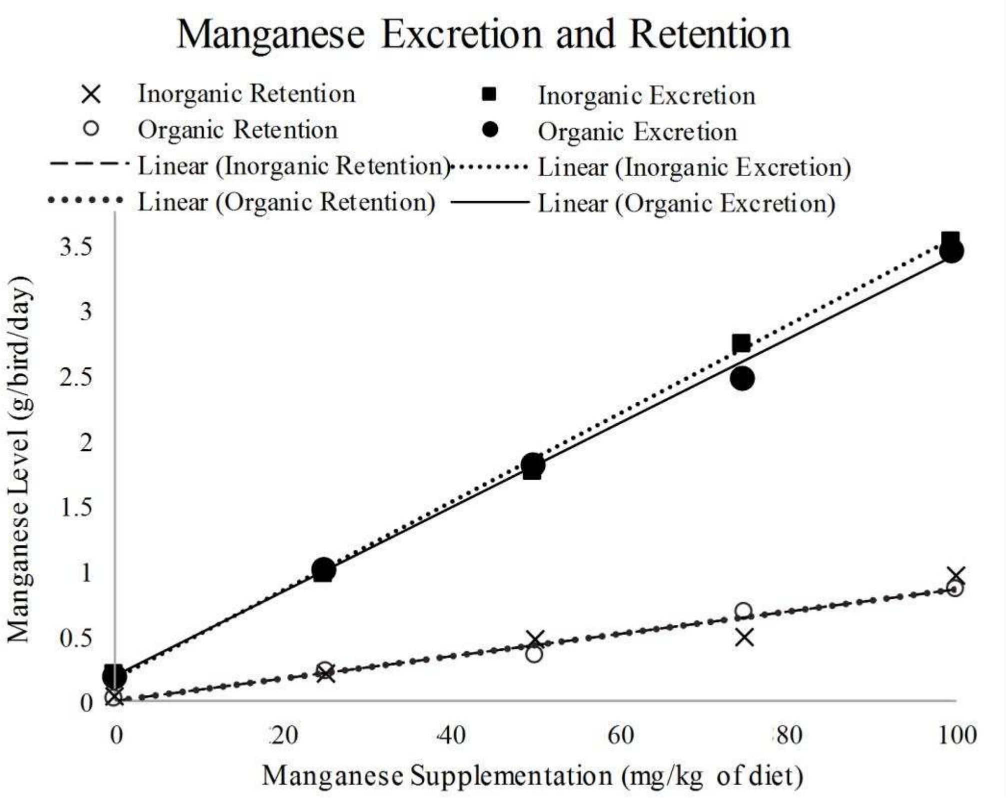
Table 7. Manganese Balance in broiler chickens fed with diets supplemented with different levels of manganese from organic or inorganic sources.

Parameter	Source	Manganese Level					Mean	MSE	C.V. (%)	P-Value (Nested Effect)	
		0	25	50	75	100				Linear	Quadratic
Mn Intake (mg/kg/bird/day)	Inorganic	0.26	1.20	2.26	3.27	4.54	2.31	0.1581	6.94	<0.0001	0.0012
	Organic	0.23	1.26	2.20	3.20	4.37	2.25			<0.0001	0.0631
	Mean	0.24	1.23	2.23	3.23	4.46					
Mn Excretion (mg/kg/bird/day)	Inorganic	0.22	0.99	1.79	2.77	3.57	1.87	0.2387	12.95	<0.0001	0.3880
	Organic	0.20	1.03	1.84	2.52	3.50	1.82			<0.0001	0.5394
	Mean	0.21	1.01	1.82	2.64	3.54					
Mn Retention (mg/kg/bird/day)	Inorganic	0.04	0.21	0.47	0.50	0.97	0.44	0.1826	41.96	<0.0001	0.0812
	Organic	0.03	0.23	0.35	0.68	0.87	0.43			<0.0001	0.4017
	Mean	0.03	0.22	0.41	0.59	0.92					
Mn Apparent Absorption (%)	Inorganic	16%	18%	21%	15%	21%	18%	7.974	44.65	0.3058	0.9843
	Organic	12%	18%	16%	21%	20%	17%			0.0140	0.3745
	Mean	14%	18%	18%	18%	21%					

Table 8. Regression equations to manganese balance parameters.

Parameter	Source	Contrast	Regression Equations	Optimal Level	P-Value	R²
Mn Intake (mg/kg/bird/day)	Inorganic	Linear	$Y=0.056x+0.1776$	>100	<0.0001	0.9972
		Quadratic	$Y=-0.000124x^2+0.0466x+0.2670$	187.90	0.0012	0.9995
	Organic	Linear	$Y=-0.0538x+0.2082$	>100	<0.0001	0.9986
Mn Excretion (mg/kg/bird/day)	Inorganic	Linear	$Y=0.04467x+0.170$	>100	<0.0001	0.9983
	Organic	Linear	$Y=0.0425x+0.204$	>100	<0.0001	0.9975
Mn Retention (mg/kg/bird/day)	Inorganic	Linear	$Y=-0.0113x+0.0092$	>100	<0.0001	0.9277
	Organic	Linear	$Y=-0.01125x+0.004$	>100	<0.0001	0.9810
Mn Apparent Absorption (%)	Organic	Linear	$Y=-0.1052x+13.409$	>100	0.0140	0.7051

Graphic 2. Manganese excretion and retention in broiler chickens fed diets with ascending levels of manganese from organic or inorganic sources from 8 to 17 d-old.



Zinc balance results for broilers fed with increasing levels of manganese in organic or inorganic sources are presented in table 9. No significant difference ($P<0.05$) was observed to organic source supplementation. The increased manganese levels in inorganic form increase linearly zinc intake and zinc excretion, but also reduce the zinc balance ($P<0.05$) in a linear response. The linear equations are presented in table 10.

In table 11 are presented the result for iron balance parameters with the supplementation of increased manganese levels. Iron intake and excretion are increased by the supplementation of manganese from inorganic source with linear significance ($P<0.05$). In table 12 the linear equations are presented.

The copper balance parameters are presented in table 13. No significant difference ($P<0.05$) was observed to organic source supplementation. The copper excretion was increased ($P<0.05$) in a linear response when inorganic source of manganese was supplied. Due this fact, the copper apparent absorption to inorganic supplementation had a significantly ($P<0.05$) linear decline. These equations can be observed in table 14

Table 9. Zinc Balance in broiler chickens fed with diets supplemented with different levels of manganese from organic or inorganic sources.

Parameter	Source	Manganese Level					Mean	MSE	C.V. (%)	P-Value (Nested Effect)	
		0	25	50	75	100				Linear	Quadratic
Zn Intake (mg/kg/bird/day)	Inorganic	3.24	3.39	3.37	3.44	3.45	3.38	0.1667	5.70	0.0063	0.3691
	Organic	2.41	2.53	2.46	2.46	2.50	2.47			0.4770	0.6456
	Mean	2.82	2.96	2.91	2.95	2.98					
Zn Excretion (mg/kg/bird/day)	Inorganic	2.03	2.07	2.14	2.29	2.29	2.16	0.2308	11.90	0.0017	0.9945
	Organic	1.62	1.70	1.80	1.69	1.76	1.72			0.2695	0.4322
	Mean	1.83	1.88	1.97	1.99	2.03					
Zn Retention (mg/kg/bird/day)	Inorganic	1.21	1.32	1.22	1.15	1.16	1.21	0.1934	19.64	0.1454	0.4438
	Organic	0.78	0.82	0.66	0.77	0.74	0.76			0.4966	0.5952
	Mean	1.00	1.07	0.94	0.96	0.95					
Zn Apparent Absorption (%)	Inorganic	37%	39%	36%	33%	34%	36%	6.51	19.56	0.0435	0.6245
	Organic	32%	33%	27%	31%	30%	31%			0.3048	0.4594
	Mean	35%	36%	32%	32%	32%					

Table 10. Regression equations to zinc balance parameters.

Parameter	Source	Contrast	Regression Equations	P-Value	R²
Zn Intake (mg/kg/bird/day)	Inorganic	Linear	Y=0.0245x+3.2834	0.0063	0.7811
Zn Excretion (mg/kg/bird/day)	Inorganic	Linear	Y=0.0039x+2.0145	0.0017	0.9263
Zn Apparent Absorption (%)	Inorganic	Linear	Y=-0.07016x+38.68	0.0435	0.6316

Table 11. Iron Balance in broiler chickens fed with diets supplemented with different levels of manganese from organic or inorganic sources.

Parameter	Source	Manganese Level					Mean	MSE	C.V. (%)	P-Value (Nested Effect)	
		0	25	50	75	100				Linear	Quadratic
Fe Intake (mg/kg/bird/day)	Inorganic	5.73	5.98	5.95	6.07	6.09	5.97	0.3364	5.62	0.0168	0.4352
	Organic	5.84	6.13	5.97	5.97	6.07	5.99			0.3655	0.5682
	Mean	5.78	6.06	5.96	6.02	6.08					
Fe Excretion (mg/kg/bird/day)	Inorganic	3.24	3.33	3.31	3.61	3.66	3.43	0.4889	14.31	0.0226	0.7046
	Organic	3.30	3.32	3.54	3.27	3.58	3.40			0.2997	0.8794
	Mean	3.27	3.32	3.43	3.44	3.62					
Fe Retention (mg/kg/bird/day)	Inorganic	2.49	2.65	2.64	2.46	2.43	2.54	0.4431	17.27	0.4804	0.3147
	Organic	2.54	2.81	2.43	2.70	2.49	2.59			0.6512	0.5571
	Mean	2.52	2.73	2.53	2.58	2.46					
Fe Apparent Absorption (%)	Inorganic	44%	44%	44%	41%	40%	43%	7.31	17.03	0.1379	0.4081
	Organic	43%	46%	41%	45%	41%	43%			0.4929	0.6493
	Mean	44%	45%	43%	43%	40%					

Table 12. Regression equations to iron balance parameters.

Parameter	Source	Contrast	Regression Equations	P-Value	R^2
Fe Intake (mg/kg/bird/day)	Inorganic	Linear	$Y=0.00432x+5.802$	0.0168	0.7970
Fe Excretion (mg/kg/bird/day)	Inorganic	Linear	$Y=-0.00597x+3.2032$	0.0226	0.8604

Table 13. Copper Balance in broiler chickens fed with diets supplemented with different levels of manganese from organic or inorganic sources.

Parameter	Source	Manganese Level					Mean	MSE	C.V. (%)	P-Value (Nested Effect)	
		0	25	50	75	100				Linear	Quadratic
Cu Intake (mg/kg/bird/day)	Inorganic	0.355	0.371	0.369	0.377	0.378	0.370	0.0264	5.75	0.0727	0.6102
	Organic	0.535	0.562	0.547	0.547	0.556	0.549			0.2923	0.4080
	Mean	0.445	0.466	0.458	0.462	0.467					
Cu Excretion (mg/kg/bird/day)	Inorganic	0.268	0.269	0.278	0.302	0.311	0.286	0.0426	12.22	0.0078	0.5273
	Organic	0.382	0.420	0.430	0.402	0.429	0.412			0.0741	0.2147
	Mean	0.325	0.345	0.354	0.352	0.370					
Cu Retention (mg/kg/bird/day)	Inorganic	0.087	0.102	0.091	0.074	0.067	0.084	0.0352	31.82	0.0768	0.2828
	Organic	0.153	0.142	0.117	0.145	0.127	0.137			0.1761	0.2723
	Mean	0.120	0.122	0.104	0.110	0.097					
Cu Apparent Absorption (%)	Inorganic	25%	27%	25%	20%	18%	23%	7.778	35.51	0.0078	0.1750
	Organic	29%	25%	21%	27%	23%	25%			0.2017	0.3637
	Mean	27%	26%	23%	23%	20%					

Table 14. Regression equations to copper balance parameters.

Parameter	Source	Contrast	Regression Equations	P-Value	R^2
Cu Excretion (mg/kg/bird/day)	Inorganic	Linear	Y=0.00061x+0.2628	0.0078	0.9094
Cu Apparent Absorption (%)	Inorganic	Linear	Y=--0.11136x+2.0219	0.0078	0.7603

The linear decreasing of zinc apparent absorption, copper apparent absorption and the increase in zinc, copper and iron excretion when inorganic levels of manganese were supplied clearly show the antagonism in this trace minerals absorption. Mn^{2+}, Fe^{2+}, Cu^{2+} and Zn^{2+}, as also Co^{2+} and Ni^{2+} are transported in brush border of enterocytes by the divalent Metal Transporter 1 (DMT1) in duodenum (Gunshin et al., 1997). Mice expressing a functionally impaired DMT1 have reduced intestinal manganese absorption (Bressler et al., 2007). Increase iron intake levels can inhibit manganese uptake (Stipanuk and Caudill, 2013). DMT1 is not the only copper, zinc and iron transporter, but it is the main manganese transporter (Bai et al., 2008), that also can be transported in calcium channels.

Results of calcium balance parameters are presented in table 15. When manganese levels were increased by organic manganese no significant ($P<0.05$) effects were observed. An ascending linear increase was observed in calcium intake and calcium retention by supplying increased levels of inorganic source of manganese with statistical significance ($P<0.05$). The linear regression equations for calcium intake and calcium retention are presented in table 16.

The results for mineral deposition in liver of broilers fed with increasing levels of manganese in organic or inorganic sources are presented in table 17. The comparison with initial values demonstrate a significant reduction ($P<0.01$) on liver zinc for all treatments. The treatments without manganese supplementation were significantly different ($P<0.01$) than initial values. A significant difference ($P<0.05$) between the calcium values with 100 mg/kg inorganic supplementation of manganese and initial amount of calcium is also observed. Evaluating the differences between manganese levels, there is no significant difference ($P<0.05$) to zinc, iron and copper deposition in liver for neither sources. A significate ($P<0.05$) linear decrease can be observed in calcium deposition in the liver to the manganese supplementation in inorganic source. The manganese deposition in liver was significant ($P<0.05$) to inorganic and organic supplementation with ascending linear and quadratic effects. The higher value for manganese in liver can be reach with 68.8 mg/kg of manganese in inorganic source or 84.6 mg/kg in organic source. Regressions equations are presented in table 18.

Table 15. Calcium Balance in broiler chickens fed with diets supplemented with different levels of manganese from organic or inorganic sources.

Parameter	Source	Manganese Level					Mean	MSE	C.V. (%)	P-Value (Nested Effect)	
		0	25	50	75	100				Linear	Quadratic
Ca Intake (mg/kg/bird/day)	Inorganic	279.85	292.17	290.62	296.59	297.64	291.37	15.94	5.63	0.0139	0.4282
	Organic	267.27	280.83	273.29	273.38	277.97	274.55			0.3831	0.5858
	Mean	273.56	286.50	281.95	284.99	287.81					
Ca Excretion (mg/kg/bird/day)	Inorganic	92.20	85.72	92.28	93.27	91.60	91.02	11.41	12.53	0.4885	0.7934
	Organic	93.21	89.05	93.81	85.23	93.90	91.04			0.8446	0.3492
	Mean	92.70	87.39	93.04	89.25	92.75					
Ca Retention (mg/kg/bird/day)	Inorganic	187.65	206.45	198.34	203.32	206.04	200.36	13.42	6.99	0.0190	0.2459
	Organic	174.06	191.78	179.48	188.15	184.07	183.51			0.2304	0.1508
	Mean	180.85	199.11	188.91	195.73	195.06					
Ca Apparent Absorption (%)	Inorganic	67%	71%	68%	69%	69%	69%	4.96	3.36	0.6201	0.4368
	Organic	65%	68%	66%	69%	66%	67%			0.4313	0.1446
	Mean	66%	69%	67%	69%	68%					

Table 16. Regression equations to calcium balance parameters.

		Contrast	Regression Equations	P-Value	R^2
Ca Intake (mg/kg/bird/day)	Inorganic	Linear	Y=-0.21055x+283.2729	0.0092	0.7981
Ca Retention (mg/kg/bird/day)	Inorganic	Linear	y=-0.16878x+193.9449	0.0475	0.4646

Table 17. Mineral deposition in liver of broilers fed with diets supplemented with different levels of manganese in organic or inorganic sources.

Parameter	Initial (8 d-old)	Source	Manganese Level								P-Value	
			0	25	50	75	100	Mean	MSE	C.V.(%)	Linear	Quadratic
Zn (mg/kg)	91.48	Inorganic	74.04*	71.62*	73.79*	71.01*	72.12*	72.51	6.57	9.02	0.5097	0.7924
		Organic	76.38*	72.278	75.63*	70.85*	71.59*	73.34			0.0761	0.7646
		Mean	75.21	71.94	74.71	70.93	71.85					
Mn (mg/kg)	11.06	Inorganic	5.59*	8.66	12.06	11.35	10.53	9.64	2.06	20.97	<.0001	<.0001
		Organic	6.26*	10.04	10.90	11.07	12.01	10.06			<.0001	0.0110
		Mean	5.93	9.35	11.48	11.21	11.27					
Fe (mg/kg)	285.84	Inorganic	331.60	277.83	266.79	343.06	242.98	292.45	85.83	30.53	0.2050	0.9605
		Organic	260.99	305.51	285.46	231.50	270.20	270.73			0.5024	0.6707
		Mean	296.30	291.67	276.13	287.28	256.59					
Cu (mg/kg)	13.12	Inorganic	11.51	11.53	11.36	10.14	10.09	10.93	2.25	20.52	0.0635	0.6572
		Organic	11.37	11.47	11.12	10.21	10.83	11.00			0.2943	0.8402
		Mean	11.44	11.50	11.24	10.17	10.46					
Ca (mg/kg)	151.08	Inorganic	136.29	139.97	141.23	129.71	116.70*	132.78	20.10	15.10	0.0158	0.0554
		Organic	132.86	127.57	142.21	131.35	132.86	133.37			0.8471	0.6144
		Mean	134.57	133.77	141.72	130.53	124.78					

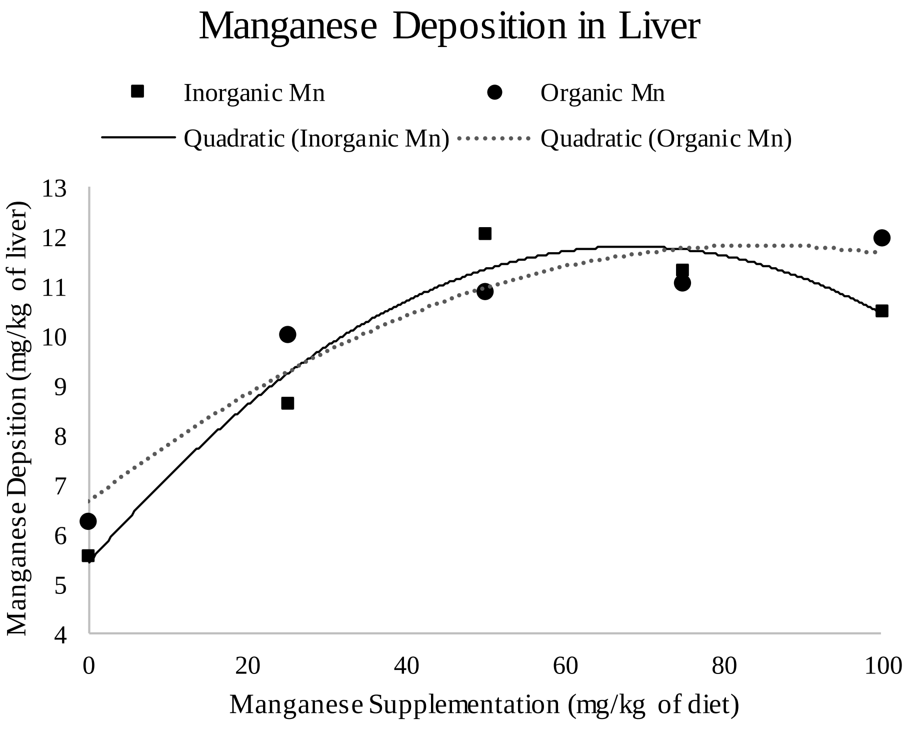
*Significantly different from Initial values (8d-old) by Dunnett test (P<0.05).

Table 18. Regression equations to mineral deposition in liver.

Parameter	Source	Contrast	Regression Equations	Optimal Level	P-Value	R^2
Mn (mg/kg)	Inorganic	Linear	$Y=0.0503x+7.0756$	>100	<.0001	0.5866
		Quadratic	$Y=-0.00134x^2+0.1844x+5.43337$	68.81	<.0001	0.9615
	Organic	Linear	$Y=0.04995x+7.5516$	>100	<.0001	0.7862
		Quadratic	$Y=-0.000723x^2+0.12223x+6.6482$	84.53	0.0110	0.9314
Ca (mg/kg)	Inorganic	Linear	$Y=-0.19777x+142.6669$	>100	0.0158	0.6059

Interactions between calcium and manganese have been extensively studied due its influence on perosis conditions. High levels of calcium intake can precipitate manganese sulphate rendering it unavailable to absorption, but it apparently depends of the calcium source. Wedekind and Baker (1990) found a reduction on tibia manganese in high levels of calcium supplemented as dicalcium phosphate. The excess on calcium supplied by bone meal or calcium phosphate seems to have the same effect (Schaible and Bandemer, 1942). However, Wedekind et al. (1991) reported that excess of calcium by limestone or dicalcium phosphate had no effect on reducing absorption or endogenous excretion of manganese. In fact, Scheideler (1991) demonstrate the excess of phosphorus more than calcium cause depletive effect on manganese utilization and manganese content in bone and liver increased when calcium was increased. More recent research (Ji et al., 2006) shows that the manganese uptake is significantly higher in high calcium levels. Regardless specific evidence, manganese had a high paracellular absorption in tight junctions and the permeability of this junctions are influenced by calcium (Ji et al., 2006), but the way of increased levels of manganese can increase calcium retention and reduce calcium deposition in liver remains unclear and is assumed that it can be related to a higher bone growth.

Graphic 3. Manganese deposition in liver of broiler chickens fed diets with increasing levels of manganese from organic or inorganic sources for 8 to 17 d-old chicks.



After absorption, manganese is transported in association to macroglobulin to liver. In hepatocytes, manganese will be quickly sent to mitochondria to incorporate to superoxide dismutase molecule; linked to transferrin to delivery to target tissue; or stored in lysosomes to further excretion by active transport to bile vesicle, where its concentration can be 150x higher than manganese concentration in plasma (Stipanuk and Caudill, 2013). The fast active transport to bile vesicle makes manganese less toxic than other minerals and easier to archive deficiency, since it cannot be stored for longs periods of time (Grider, 2013). Therefore, manganese evaluation in liver represents the manganese in metalloenzymes, manganese in transit and manganese that will be sent to bile vesicles.

The quadratic response to manganese in liver when organic and inorganic levels of manganese was supplied reach the highest values at 68.8 mg/kg of manganese in inorganic source or 84.6 mg/kg in organic source, both corresponding to 11.8 mg of manganese for kg of liver tissue. The highest value for organic source is probably due the organic manganese did not require transporter pathway and had facilitated utilization of organic manganese in biological systems, being less stored to elimination by bile vesicle.

4.2 Relative Bioavailability

The portion of nutrient absorbed, transported and used to supply metabolic pathways is defined by Matsui et al (1996) as bioavailable nutrient. Traditionally, performance results and growth response has been used as primary criterion to determine bioavailability for poultry, but parameters of mineral absorption may provide a more accurate value (Ammerman, 1995). The relative bioavailability (RBA) is the percentage of nutrient source available in comparison to a standard source and can be calculated comparing slope ratios when both sources had a linear response (Littell et al., 1995). The absorption parameters in this study that meet these criteria are manganese retention and deposition in liver. The bioavailability calculated with slope ratios for these parameters is 100% for manganese organic supplementation.

5. Conclusions

Manganese requirement based on growth performance are higher than 100 mg/kg (estimated as 121.8 mg/kg of diet or 166 mg/kg of WG) by using inorganic manganese source or 70.22 mg of Mn/kg of diet (95 mg of Mn / kg of WG) by organic source. Organic source avoids manganese interactions with copper, zinc and iron and have 100% of bioavailability comparing with inorganic source.

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