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Effect of the Inclusion of Organic Copper, Manganese, And Zinc in The Diet of Layers on Mineral Excretion, Egg Production, and Eggshell Quality

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ABSTRACT

This study aimed at evaluating the replacement of inorganic copper, manganese, and zinc sources by organic sources in the diet of laying hens during the second laying cycle in trace mineral excretion, egg production, and eggshell quality. Two hundred and fifty 100-week-old Dekalb hens were distributed according to a completely randomized design into five treatments with five replicates of ten birds each. The control treatment consisted of a basal diet with all trace minerals in the inorganic form. The other treatments consisted of a basal diet with a mixture of the minerals copper, manganese, and zinc in the organic form with concentrations of 100%, 90%, 80%, and 70% of the levels of inclusion of inorganic mineral sources in the control treatment. Trace mineral excretion was determined in five layers per treatment by the method of total excreta collection. Excreta trace mineral contents were determined by atomic absorption spectrophotometry. Egg production and eggshell quality were determined by the mass of the eggs and the egg specific gravity, respectively. For all trace minerals examined, the dietary supplementation with organic sources reduced trace mineral excretion compared with the control group, even at 70% inclusion level, without compromising egg production or eggshell quality. The replacement of the inorganic trace mineral sources by organics source effectively reduced the excretion of copper, manganese, and zinc by laying hens in the second laying cycle.

INTRODUCTION

Trace minerals are involved in the animals' metabolism as catalytic agents present in all metabolic reactions, and therefore, they are essential for growth, development, and production (Aksu *et al.*, 2012).

Copper is part of blood proteins and it is related to iron metabolism and absorption, oxygen metabolism, collagen and elastin synthesis, bone formation (Uauy *et al.*, 1998), feathers development and coloring (Scheideler, 2008). Manganese and zinc are enzymes cofactors involved in the synthesis of mucopolysaccharides and carbonate, which are essential for eggshell formation and quality (Swiątkiewicz & Koreleski, 2008).

The main feedstuffs commonly included in layer diets present marginal mineral deficiency. In order to overcome such deficiencies, diets are commonly supplemented with inorganic sources, such as oxide, carbonate, chloride, or sulfate salts. However, due to the pH changes that naturally occur in the digestive tract of poultry, there may be antagonism and interactions among trace minerals, as well as with other compounds added with the aim of stabilizing the molecule, forming insoluble compounds, and preventing their absorption by the body (Aksu *et al.*, 2012).

In order to prevent that trace mineral deficiency due to lack of absorption, they are usually added to the diets at levels higher than the animals actual requirements (Aksu *et al.*, 2012), which contributes to increase mineral excretion and may cause environmental pollution (Mohana & Nys, 1998).

Nutritional changes and precise estimates of the specific requirements for each production stage and the use of more bioavailable trace mineral sources are strategies that can contribute to reduce environmental pollution. Organic trace mineral sources may be an alternative to inorganic sources. Chelated or organic trace minerals do not sufferionic dissociation in the acidic gastric pH, remaining electrically neutral and protected from chemical reactions with other molecules in the intestinal lumen, which optimizes their absorption and increases their bioavailability relative to inorganic sources (Swiątkiewicz *et al.*, 2014), with consequent improvement in performance (Nollet *et al.*, 2007).

Several authors reported egg quality and performance maintenance or improvement with the addition organic trace minerals in the diet of laying hens (Fernandes *et al.*, 2008; Saldanha *et al.*, 2009; Maciel *et al.*, 2010; Gheisari *et al.*, 2011; Sun *et al.*, 2012; Figueiredo Júnior *et al.*, 2013). However, few studies have evaluated their mineral excretion.

Therefore, this study aimed at evaluating the effects of different dietary supplementation levels of organic copper, manganese and zinc on trace mineral excretion, egg production, and eggshell quality of layers in their second laying cycle.

MATERIAL AND METHODS

The project was approved by the Ethics Committee on the Use of Animals (CEUA) under protocol number 131/10.

In this experiment, 250 Dekalb White layers with 100 weeks of age and in the second laying cycle, derived from a commercial farm, were used. Birds were housed in group cages (five birds per cage) in a conventional open-sided houses, at Glória experimental farm of the Federal University of Uberlândia (UFU), located in Uberlândia, state of Minas Gerais, Brazil. Water was provided *ad libitum* and each bird consumed, on average, 106 grams of feed daily. A lighting program of 17 hours of natural and artificial light was applied throughout the experimental period.

Birds were distributed according to a completely randomized experimental design, including five treatments with five replicates of ten birds each.

The basal diet was formulated based on sorghum and soybean meal (Table 1).

The inorganic mineral (ITM) treatment, considered as control, consisted of the basal diet and all trace minerals in the form of salts, as provided in the mineral premix. The other treatments consisted of the inclusion in the basal diet of the addition of a mixture of organic trace minerals (copper, iron, manganese and iodine, chelated with amino acid and partially hydrolyzed proteins) replacing 100% (100% OTM), 90% (90%OTM), 80% (80% OTM), or 70% (70%OTM) of the inorganic mineral source. The dietary inclusion levels and the calculated values of the trace elements present in the diets are shown in Table 1.

After eight weeks of feeding the experimental diets, all intact eggs from the five treatments were used to determine egg production as egg mass (EM, g/bird/day) and eggshell quality. Eggshell quality was determined as egg specific gravity by immersion of the eggs in salt solutions with different densities (1070, 1075, 1080, 1085 and 1090), as proposed by Hamilton (1982).

Mineral excretion was evaluated by total excreta collection in one layer per replicate (five birds per treatment), which was randomly selected and housed in individual cages. Trays lined with plastic canvas were fitted under to cages to allow excreta collection. Iron oxide at 1% was added to the diets to identify the first and last day of the 5-day period of collection. Excreta were collected twice a day, placed in duly identified plastic bags, and stored in a freezer. After thawing at room temperature, excreta were homogenized. Samples dried in a forced-ventilation oven at 55ºC for 72 hours, and then ground in a conventional mill. Samples weighing approximately one gram per replicate were placed in porcelain crucibles and burnt in a muffle at 600 ° C for two hours. The ashes removed from the muffle were cooled in a desiccator, weighed, and diluted in 20 mL hydrochloric acid at 50%. This solution was filtered through paper filter and transferred to a volumetric flask, to which distilled water was added to reach 100 mL volume. Copper, manganese, and zinc levels in the solutions read in an atomic absorption spectrophotometer. All analyses were performed in triplicate and in accordance with the methods of the Brazilian Compendium of Animal Nutrition (Sindirações, 2009).

Table 1 – Ingredients, calculated nutritional levels of the basal diet, and copper, manganese, and zinc inclusion levels and calculated values in the experimental diets.

Ingredients (%)	Composition				
Sorghum 8.8 %CP	64.28				
Soybean meal 46.5 %CP	22.39				
Limestone	9.12				
Dicalcium phosphate	1.62				
Soybean oil	1.68				
Vitaminpremix[1]	0.40				
Salt	0.35				
DL-Methionine	0.103				
Mineral Premix[2]	0.05				
Carophyll Yellow pigment	0.004				
Carophyll Red pigment	0.0030				
TOTAL	100.00				
Nutrients (%)	Calculated Values				
Crude Protein	16.00				
Calcium	4.00				
Available Phosphorus	0.40				
Sodium	0.17				
Available Lysine	0.70				
Available Methionine	0.39				
Available Methionine+Cystine	0.61				
Available Threonine	0.54				
Available Tryptophan	0.18				
Available Arginine	0.95				
Metabolizable Energy (kcal)	2700				
Trace Mineral Inclusion Levels					
Trace minerals (mg/kg)	ITM*	100% OTM**	90% OTM	80% OTM	70% OTM
Cu	9.00	9.00	8.10	7.20	6.30
Mn	60.00	60.00	54.00	48.00	42.00
Zn	60.00	60.00	54.00	48.00	42.00
Calculated Values					
Cu	18.66	18.66	17.76	16.86	15.96
Mn	76.01	76.01	70.01	64.01	58.01
Zn	85.25	85.25	79.25	73.25	67.25

[1]Composition of vitamin the premix and additives–Guaranteed levels per kg of product: folic acid (min) 125 mcg; pantothenic acid (min) 1,610 mg; biotin (min) 3.75 mg; choline (min) 52.2 g; niacin (min) 5,000 mg; Vit-A (min) 2,000,000 IU; Vit-B1 (min) 50 mg; Vit-B12 (min) 2,500 mcg; Vit-B2 (min) 750 mg; Vit-B6 (min) 425 mg; Vit-D3 575,000IU; Vit-E (min) 3,750IU; Vit-K3 (min) 250 mg; Se (min) 62.5 mg; Halquinol 7,500 mg.

[2]Composition of mineral premix: Guaranteed levels per kg of product: Cu (min) 18 g; Fe (min) 60g; I (min) 2g; Mn (min) 120g; Zn (min) 120g.

ITM* - Inorganic Trace Minerals; OTM** - Organic Trace Minerals.

Data were first tested for residue normality and variance homogeneity using Shapiro-Wilk normality test, and then submitted to variance analysis. The means of the OTM treatments were compared with the control treatment (ITM) by Dunnett's test. The means of the OTM treatments were subjected to linear regression analysis. A 5% significance level was adopted for the statistical tests, using the software program SAS (Statistical Analysis System) version 9.3 (SAS, 2011).

RESULTS

The excretion of copper and manganese was linearly reduced when layers were fed any of the diets supplemented with the organic source compared with the control treatment (Table 2). However, zinc excretion was reduced only when OTM zinc was added at levels lower than 100%. The total replacement of inorganic zinc by the organic source resulted in the same zinc excretion level as the control group.

Table 2 – Excretion (mg/kg) of copper, manganese, and zinc; egg mass (gram of egg/bird/day); and specific gravity of eggs from laying hens in the laying second cycle fed with organic trace minerals.

Trace Mineral Excretion				Egg Production and Eggshell Quality	
Treatments	Copper	Manganese	Zinc	Egg Mass	Specific Gravity
Control ITM	20.6033	254.7825	874.0275	57.5220	1082.5322
OTM 100%	16.4075*	189.5950*	672.6675	60.8320	1083.8908
OTM 90%	16.6500*	163.7250*	579.5125*	61.6471	1083.9302
OTM 80%	13.2825*	136.1600*	572.6400*	58.0202	1083.4908
OTM 70%	13.3250*	129.9850*	489.2275*	61.7211	1081.5640
CV (%)	18.6101	28.0712	20.0101	9.2915	0.1421
P value	<0.0001	<0.0001	0.0084	0.7568	0.1679
Regression	Linear[1]	Linear[2]	Linear[3]	0.7825	0.1114

ITM–Inorganic Trace Minerals; OTM–Organic Trace Minerals.

Means followed by * in the same column differ from the control treatment ($P < 0.05$) by Dunnett´s test.

[1] $Y = 4.1935 + 0.12615x$ ($R^2 = 0.7628$)

[2] $Y = -20.5695 + 2.06395x$ ($R^2 = 0.9451$)

[3] $Y = 104.89825 + 5.57193x$ ($R^2 = 0.92$)

Egg production, represented by egg mass, and eggshell quality, expressed by the shell specific gravity (Table 2), were not influenced by the treatments, even at the lowest inclusion level of the organic trace mineral source.

DISCUSSION

There was no effect of organic trace mineral supplementation on eggs mass or egg specific gravity. However, the reduced copper and manganese excretion obtained with the replacement of the inorganic by the organic source is a clear indication of the increased bioavailability of organic trace mineral sources.

The results of this study corroborate the findings of Engin *et al.* (2015), who described reduction of mineral excretion when inorganic trace mineral sources were replaced by organic minerals in layer diets. Those authors also showed higher bioavailability of copper, manganese, zinc, chromium and calcium, as determined by their serum levels in layers fed with diets containing organic trace mineral sources. Leeson & Caston (2008) observed 66% and 78% reductions in the excretion of zinc and manganese, respectively, and no performance changes when feeding layers with trace-mineral proteinates at 20% of the inorganic TM level, and therefore proposed the supplementation of minimal levels of organic trace minerals as an alternative to reduce trace mineral excretion.

In the study of Gheisari *et al.* (2010), the replacement of inorganic sources of copper, manganese, and zinc by organic sources at levels from 50% to 75% lower than the NRC recommendations were sufficient to maintain the performance and eggshell and albumen quality in layers in their first laying cycle. According to the authors, when the supplementation levels of trace minerals are low or marginal, the substitution of inorganic by organic sources can improve layer performance and reduce the percentage of broken eggs.

The higher bioavailability of organic trace mineral sources compared with inorganic sources promotes high erenzyme activity, as well as higher trace mineral retention (Sun *et al.*, 2012) and lower excretion (Leeson, 2008), even when added to diets at levels below those used for inorganic trace mineral inclusion. This may be explained by the fact that, in addition of the normal ion absorption mechanisms in the intestines, chelated minerals are also absorbed through the same path ways as the organic molecules with which they are complexed, thereby avoiding competition among minerals for the same carrier, and making them more readily transportable and absorbable for subsequent utilization by the body (Kiefer *et al.*, 2005). According to Manangi *et al.* (2012), the use of organic trace minerals, among other benefits, offers the opportunity to reduce the environmental impact caused by the poultry industry.

The maintenance of performance, even at the lower levels of OTM inclusion observed in the present experiment, indicates two important facts. The first is that, due to their greater bioavailability, OTM sources are more efficient to maintain layer production performance and eggshell quality than ITM sources. The second suggests that commercial layer diets current contain excessive amounts of inorganic trace minerals, which is a cause of concern both in terms of egg production and quality, by triggering antagonism between trace minerals and other dietary nutrients, which are equally important for the layer metabolism, and from the environmental point of view, because the excess of trace minerals is excrete in the environment.

Excreta with a high concentration of minerals contaminate the soil, and may result in groundwater contamination (Nollet *et al.*, 2007), especially in intensive poultry production areas (Swiątkiewicz *et al.*, 2014). Soil fertilization with poultry manure usually exceeds zinc (Mohana & Nys, 1998), iron, copper, and manganese plants requirements (Swiątkiewicz *et al.*, 2014), which may cause ground phytotoxicity. Therefore, the use of more bioavailable trace mineral sources is important both for animal production and the environment.

Genetic improvement has improved the production efficiency of layers; however, research on trace mineralsh as not evolved at the same pace. Leeson & Caston (2008) pointed to that the low cost of these ingredients in the total diet composition, less than 0.2%, is responsible for the lack of interest of the scientific community on this subject. The authors also highlighted the lack of knowledge of trace minerals availability in the main feedstuffs as the main obstacle to determine appropriate supplementation levels. In a review about this topic, Asku *et al.* (2012) indicate that the use of organic trace minerals at low levels has become increasingly common, primarily because of their physiological and ecological contribution; however, experimental data on the effects of such low levels on poultry metabolism are still insufficient. Considering the complexity of mineral metabolism, much needs to be investigated in order to determine accurate estimates of trace mineral inclusions appropriate to each stage of the production cycle in order to maintain productivity while reducing environmental impact.

In the present study, the reduced trace mineral excretion and the maintenance of egg production, as determined by egg mass, and of eggshell, as measured by egg specific gravity, even at the lowest organic mineral inclusion levels, indicate that it is possible to reduce the addition of trace minerals in commercial diets without losses to the producer.

CONCLUSION

The total replacement of inorganic trace mineral sources by organic sources in the layer diets was efficient to reduce trace mineral excretion and did not compromise egg production or eggshell quality.

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