



## Manure production and mineral excretion in laying hens supplemented with lycopene and different mineral sources

[ Produção de dejetos e excreção de minerais por galinhas poedeiras alimentadas com licopeno e diferentes fontes minerais ]

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**Abstract:** The effects of feeding diets containing lycopene and organic or inorganic minerals on commercial laying hens on manure production and characteristics were evaluated. A total of 192 laying hens were distributed into treatment groups using a completely randomized design with a 2 × 3 factorial arrangement (mineral sources × lycopene levels), with four replicates and eight birds per treatment. The diet was supplemented with lycopene at 0, 400, or 800 mg kg<sup>-1</sup> of diet. The diets were as follows: MI, inorganic mineral diet without lycopene; MI+400, inorganic mineral diet with 400 mg of lycopene; MI+800, inorganic mineral diet with 800 mg of lycopene; MO, organic mineral diet without lycopene; MO+400, organic mineral diet with 400 mg of lycopene and MO+800, organic mineral diet with 800 mg of lycopene. The feed intake; daily manure production; and manure contents of dry matter, organic matter, mineral matter, ammoniacal nitrogen, carbon, and macro- and micronutrients; and manure pH were measured. Feed intake was higher in the MO and MO+800 groups than in the MI and MI+800 groups, with no differences in feed intake between the MI+400 and MO+400 groups. However, the feed intake was higher in the MI+400 group than in the MI and MI+800 group. The diet did not influence the daily amount of manure produced. The dry matter percentage in the manure of the MO+800 group was higher than that in all the other groups; the organic and mineral matter contents, including those of nitrogen, phosphorus, calcium, magnesium, iron, and zinc, were highest in the manure of the chickens in the MO+800 group that in the manure of the chickens from the other groups. The diets did not increase the daily amount of manure produced by the hens; however, the chickens in the MO+800 group excrete larger amounts of macro- and micronutrients.

**Keywords:** carotenoids; chelate; excretion; organic minerals; nutrients.

**Resumo:** O objetivo foi avaliar o efeito de dietas contendo licopeno e minerais orgânicos ou inorgânicos na produção e caracterização dos dejetos de galinhas poedeiras comerciais. Foram utilizadas 192 galinhas poedeiras, distribuídas em delineamento inteiramente casualizado com um arranjo fatorial 2 × 3 (fontes de minerais × níveis de licopeno), com quatro repetições e oito aves por tratamento. As inclusões de licopeno foram de 0, 400 e 800 mg kg<sup>-1</sup> de dieta. Os tratamentos foram: MI, dieta com mineral inorgânico sem licopeno; MI+400, dieta com mineral inorgânico e 400 mg de licopeno; MI+800, dieta com mineral inorgânico e 800 mg de licopeno; MO, dieta com mineral orgânico sem licopeno; MO+400, dieta com mineral orgânico e 400 mg de licopeno; MO+800, dieta com mineral orgânico e 800 mg de licopeno. Foi avaliado o consumo de ração, produção diária de



excretas e os teores de matéria seca, matéria orgânica, matéria mineral, pH, nitrogênio amoniacal, carbono e macro e micronutrientes nos dejetos. O consumo de ração aumentou com MO e MO+800 em comparação com MI e MI+800, sem diferenças entre as fontes de minerais no nível de 400 mg de licopeno. No entanto, MI+400 resultou em maior consumo de ração em comparação com MI e MI+800. As dietas não influenciaram a produção diária de excretas. A dieta MO+800 apresentou maiores percentuais de matéria seca, afetando os valores de matéria orgânica e mineral, levando a uma maior excreção de nitrogênio, fósforo, cálcio, magnésio, ferro e zinco. As dietas não aumentaram a produção diária de excretas das galinhas, mas a dieta MO+800 aumentou a excreção de macro e micronutrientes.

**Palavras-chave:** carotenoides; excreção; minerais orgânicos; nutrientes; quelato.

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## 1. Introduction

Poultry nutrition requires the supply of adequate levels of dietary nutrients, particularly minerals. These nutrients and minerals participate in the biochemical processes that are essential for normal poultry growth and development <sup>(1)</sup>. The sources of minerals in the diets of laying hens are generally inorganic; however, diets with organic sources of minerals are also commercially available. The growing global concern about the environmental impact of the manure produced from animal husbandry systems has stimulated the search for organically sourced mineral sources that optimize nutrient use and consequently reduce the amount of minerals excreted <sup>(2)</sup>.

The bioavailability, stability, and solubility of organic minerals are higher than those of the inorganic mineral sources conventionally used in animal feed <sup>(3)</sup>. Organic minerals consist of metal ions bound to amino acids, peptides, or polysaccharide complexes that do not compete for absorption sites <sup>(4)</sup>. Completely or partially replacing inorganic minerals with organic chelates in the diets of laying hen diets positively affects performance and egg quality, even when organic chelates are added in lower amounts than their corresponding inorganic minerals <sup>(3)</sup>. Organic minerals are easily absorbed and may produce antioxidant effects on the tissues of the hen and egg, enhancing oxidative stability during egg storage.

The organic minerals and phenolic compounds in plant extracts and essential oils act synergistically, being more efficient when used together than when used alone <sup>(5,6)</sup>. Lycopene is a red carotenoid capable of scavenging peroxide and oxygen radicals through its two unconjugated double bonds <sup>(7)</sup>. This structural characteristic increases the antioxidant effects, protects lipoprotein membranes from oxidative damage, and contributes to the immune system and overall health of birds <sup>(7)</sup>. In this context, antioxidants contribute to proper digestive system functioning by strengthening the antioxidant response to oxidative stress in enterocytes, preserving intestinal morphometry and health <sup>(8)</sup>. Food digestibility can be increased by ensuring intestinal integrity in poultry, which reduces the amount of waste produced and nutrients excreted, which helps minimize environmental contamination and decreases the volume of waste requiring treatment <sup>(2)</sup>.

Studies on the effects of layer nutrition with antioxidants or organic minerals have primarily focused on productivity and egg quality; however, studies are lacking on the quantity of excreta produced, the physicochemical characteristics of the excreta, or the relationships of excreta quantity and characteristics with dietary composition <sup>(2)</sup>. We hypothesized that (1) the including lycopene in the diet would reduce daily manure output, potentially due to increased digestive efficiency or antioxidant action; (2) chickens fed diets supplemented with organic minerals would excrete fewer minerals; and (3) combining lycopene and organic minerals in chicken feed would

increase nutrient use. Thus, the aim in this study was to assess the effects of feeding diets containing lycopene with organic or inorganic minerals on manure production and composition in commercial laying hens.

## 2. Material and methods

The animal experiments were approved and conducted in accordance with the guidelines of the Animal Use Ethics Committee of the State University of Mato Grosso do Sul (protocol N° 018/2013).

A total of 192 Dekalb Brown laying hens (semiheavy layers) aged 58 weeks were used. The hens were fed an experimental diet for 112 days (four cycles of 28 days each). The diets were supplemented with 0, 400, or 800 mg lycopene kg<sup>-1</sup> diet, and two sources of minerals were used: organic and inorganic. A completely randomized experimental design was applied with a 2 × 3 factorial arrangement (mineral source × lycopene level) and four replicates of eight birds per treatment. The treatments are listed in Table 1.

**Table 1.** Experimental diets with different mineral sources and lycopene proportions.

Mineral source	Lycopene (mg kg <sup>-1</sup> diet)	Abbreviation
Inorganic	0	IM
Inorganic	400	IM+400
Inorganic	800	IM+800
Organic	0	OM
Organic	400	OM+400
Organic	800	OM+800

The minerals added to the diet in organic form were copper, iron, manganese, zinc (metal–amino acid chelates), and selenium (selenium yeast). The supplementation levels differed between the inorganic and organic mineral sources. All diets (Table 2) were formulated to meet the nutrient requirements of the hens according to the Dekalb Brown Management Guide <sup>(9)</sup> and Rostagno et al. <sup>(10)</sup>. The lycopene levels were determined based on a study conducted by Olson et al. <sup>(11)</sup>.

**Table 2.** Ingredients and calculated composition of experimental diets

Ingredients (% diet)	IM	IM+400	IM+800	OM	OM+400	OM+800
Corn grain	61.49	61.49	61.49	61.49	61.49	61.49
Soybean meal (45% protein)	25.27	25.27	25.27	25.27	25.27	25.27
Soybean oil	1.25	1.25	1.25	1.25	1.25	1.25
Calcitic lime	8.27	8.27	8.27	8.27	8.27	8.27
Dicalcium phosphate	2.08	2.08	2.08	2.08	2.08	2.08
L-Lysine-HCl	0.11	0.11	0.11	0.11	0.11	0.11
DL-Methionine	0.17	0.17	0.17	0.17	0.17	0.17
Sodium chloride	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin–mineral supplement*	0.10	0.10	0.10	0.30	0.30	0.30
Inert	0.90	0.60	0.20	0.70	0.40	0.00
Lycopene supplement <sup>††</sup>	0.00	0.30	0.70	0.00	0.30	0.70
Butylated hydroxytoluene	0.01	0.01	0.01	0.01	0.01	0.01
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition						
Metabolizable energy (kcal kg <sup>-1</sup> )	2,800	2,800	2,800	2,800	2,800	2,800
Crude protein (%)	17.0	17.0	17.0	17.0	17.0	17.0
Digestible methionine + cystine (%)	0.65	0.65	0.65	0.65	0.65	0.65
Digestible lysine (%)	0.85	0.85	0.85	0.85	0.85	0.85
Calcium (%)	4.10	4.10	4.10	4.10	4.10	4.10
Available phosphorus (%)	0.48	0.48	0.48	0.48	0.48	0.48
Linoleic acid (%)	1.79	1.79	1.79	1.79	1.79	1.79

\*Composition per kg of diet: retinol (vitamin A), 7,000 IU; cholecalciferol (vitamin D3), 1,600 IU;  $\alpha$ -tocopherol acetate (vitamin E), 8 IU; menadione (vitamin K3), 1.0 mg; nicotinic acid, 20 mg; pantothenic acid, 7 mg; pyridoxine (vitamin B6), 1.0 mg; cyano-cobalamin (vitamin B12), 0.010 mg; biotin (vitamin H), 0.02 mg; copper (copper proteinate or copper sulfate), 10 mg; iron (iron glycinate or ferrous sulphate), 50 mg; calcium iodate, 0.83 mg; manganese (manganese chelate or manganese sulfate), 65 mg; selenium (selenium yeast or sodium selenite), 0.30 mg; zinc (zinc glycinate or zinc sulfate), 60 mg. <sup>†</sup> Commercial products based on pure lycopene and tomato powder. IM, diet with inorganic minerals (25% copper, 28% iron, 31% manganese, 45% selenium, and 35% zinc); IM+400, diet with inorganic minerals and 400 mg kg<sup>-1</sup> lycopene; IM+800, diet with inorganic minerals and 800 mg lycopene kg<sup>-1</sup> diet; OM, diet with organic minerals (10% copper, 6% iron, 8% manganese, 0.2% selenium, and 10% zinc); OM+400, diet with organic minerals and 400 mg lycopene kg<sup>-1</sup> diet; OM+800, diet with organic minerals and 800 mg lycopene kg<sup>-1</sup> diet.

The hens were housed in pairs in galvanized wire cages with four compartments, each measuring 25 × 40 × 45 cm, in a conventional laying house roofed with fiber cement tiles. The hens were maintained on a 17-hour light (natural + artificial):7-hour dark cycle. The maximum and minimum temperatures and mean relative humidity inside the laying house during the experimental period were 33.4°C, 21.8°C, and 63.45%, respectively.

The total manure was collected daily on the last two days of each cycle, totaling eight collections, with different days considered as blocks in the ANOVA. Preweighed plastic bags were placed under the cages and removed after 24 h. Daily manure production was calculated by dividing the total production (g) by the number of birds in each cage. Data were obtained from six treatments, four replicates, and eight days of collection, totaling 192 data points.

Three aliquots ( $\pm$ 500 g) of the obtained manure were collected for analysis. The total solids, pH, and ammoniacal nitrogen content of the fresh samples were analyzed. Another aliquot was predried in a forced-air oven at 65 °C for 72 h, ground to a fine powder in a knife mill, and used for quantifying the dry matter, mineral matter, Kjeldahl nitrogen, macronutrient (potassium, calcium, and magnesium), and micronutrient (iron, manganese, copper, and zinc) contents, as described in <sup>(12)</sup>. The nitroperchloric digestion method was used for extracting macronutrients and micronutrients.

The data were tested for normality, and only normally distributed data were subjected to ANOVA to verify the effects of the factors studied (isolated or interactions). The variables were independently analyzed when no interactions were found. Tukey's test ( $p < 0.05$ ) was performed for comparing the means using R software. The variables studied were subjected to ANOVA using the following statistical model:

$$\hat{Y}_{ijk} = \mu + b_i + N_j + T_k + (N*T)_{jk} + e_{ijk}$$

where  $\hat{Y}_{ijk}$  is the observed value of the dependent variable;  $\mu$  is the overall average;  $b_i$  is the block effect (days of fecal collection);  $N_j$  is the fixed effect of the lycopene level ( $j = 0, 400, \text{ or } 800 \text{ mg kg}^{-1}$ );  $T_k$  is the fixed effect of the mineral source (where  $k$  is organic or inorganic);  $(N*T)_{jk}$  is the effect of the interaction between lycopene level and mineral source;  $e_{ijk}$  is the random error associated with each observation.

### 3. Results and discussion

Adding lycopene with organic minerals to the diet did not influence ( $p > 0.05$ ) feed intake at the tested doses (Table 3). However, the feed intake of the IM+400 diet was higher ( $p < 0.05$ ) than that of the IM and IM+800 diets. The intake of the OM and OM+800 diets was higher ( $p > 0.05$ ) than that of the IM diet, whereas diet intake did not differ between the IM+400 or OM+400 diets. This outcome is supported by the findings of other studies on dietary supplementation with OM<sup>(1,13)</sup>, which also reported no substantial differences in feed intake between supplementing with OM and IM. The feed intake of Japanese quail was higher when lycopene was added to the diet using inorganic minerals at  $200 \text{ mg.kg}^{-1}$  of feed compared to the control group. Lycopene supplementation effectively mitigated the adverse effects of heat stress by restoring the antioxidant status of the quails.<sup>(14)</sup>

The serum concentrations of antioxidant vitamins and minerals in quail decrease under heat stress<sup>(14)</sup>. Therefore, the antioxidant action of lycopene was stronger when fed in diets containing IM, as IM is less bioavailable than OM. Pozzo et al.<sup>(15)</sup> that the feed intake of chickens was lower when fed  $500 \text{ mg}$  of lycopene  $\text{kg}^{-1}$  of feed compared with controls, similar to the findings of this study for doses exceeding  $400 \text{ mg lycopene kg}^{-1}$  feed.

Feed supplementation with lycopene and organic mineral did not influence ( $p > 0.05$ ) daily fresh manure production (Table 3). The amount of manure produced by the hens was similar to that previously reported<sup>(2)</sup>, in which rosemary oil was used as an antioxidant with organic and inorganic mineral sources, resulting in daily manure production of  $112 \text{ g}$  per bird. The tested diets did not affect the amount of manure produced, which is relevant because increased excreta production is undesirable. Higher quantities of excreta require increased labor for handling, including collection, transport, and disposal, which in turn raises waste treatment costs.

Significant interaction effects were observed between the mineral source and lycopene level. The manure from hens fed OM+800 had higher contents ( $p < 0.05$ ) of dry, organic, and mineral matter than manure from birds fed IM+800. The lycopene doses did not influence ( $p > 0.05$ ) the dry, organic, or mineral matter contents of the manure from birds fed inorganic minerals (Table 3). In contrast, the manure from hens fed OM+800 had higher ( $p < 0.05$ ) dry and organic matter contents than that from hens fed OM or OM+400. The mineral matter content in the manure of hens fed OM+800 was higher than that in the manure of hens fed IM+800.

**Table 3.** Feed intake and manure production (g bird<sup>-1</sup> day<sup>-1</sup>), manure fractions (dry matter basis, %) of laying hens fed diets supplemented with inorganic or organic minerals and different lycopene doses.

Variable	MS	LYC (mg kg <sup>-1</sup> )			Mean	CV (%)	ANOVA ( <i>p</i> -value)		
		0	400	800			MS	LYC	MS × LYC
Feed intake (g)	Inorganic	94.88 <sup>bB</sup>	102.69 <sup>A</sup>	92.23 <sup>bB</sup>	96.60	5.81	0.003	0.020	0.015
	Organic	100.56 <sup>a</sup>	101.56	102.22 <sup>a</sup>	101.45				
	Mean	97.72	102.13	97.23					
Manure (g)	Inorganic	106.04	105.67	106.41	106.04	11.94	>0.05	>0.05	>0.05
	Organic	94.84	110.67	113.80	106.44				
	Mean	100.44	108.17	110.10					
Dry matter (%)	Inorganic	18.63	19.51	18.19 <sup>b</sup>	18.78	10.93	0.015	>0.05	0.003
	Organic	19.21 <sup>B</sup>	17.82 <sup>B</sup>	21.98 <sup>aA</sup>	19.67				
	Mean	18.92	18.67	20.09					
Organic matter (%)	Inorganic	13.87	14.85	13.82 <sup>b</sup>	14.18	11.23	>0.05	>0.05	0.005
	Organic	14.52 <sup>B</sup>	13.65 <sup>B</sup>	16.73 <sup>aA</sup>	14.97				
	Mean	14.19	14.25	15.28					
Mineral matter (%)	Inorganic	4.76	4.66	4.37 <sup>b</sup>	4.60	11.49	>0.05	>0.05	0.003
	Organic	4.69 <sup>AB</sup>	4.17 <sup>B</sup>	5.25 <sup>aA</sup>	4.70				
	Mean	4.72	4.42	4.81					

Means within columns followed by different lowercase letters and means within rows followed by different uppercase letters significantly differ according to Tukey's test ( $p < 0.05$ ). LYC, lycopene; CV, coefficient of variation; ANOVA, analysis of variance; MS, mineral source.

The dry matter and organic matter contents were similar to previously reported <sup>(2)</sup> average values of 22% and 16%, respectively. However, the dry matter and organic matter contents did not differ with antioxidant dose or mineral source in the prior study <sup>(2)</sup>. The dry matter content in manure varies with diet, amount of water consumption, environmental conditions, and timing of waste collection <sup>(16)</sup>. However, the dry matter content was higher in the manure of hens fed higher lycopene dose with organic minerals than in the other treatments, which affected fractions in addition to the excreted minerals.

The mineral content in the manure of hens fed OM+800 was higher than that of hens fed IM+800; the same was not observed with the control diet, which promoted higher feed intake. Liver lesions were reported in chickens at a dose of 500 mg of lycopene per kilogram of feed <sup>(15)</sup>, suggesting impaired protein absorption and immune system degradation due to spleen and cloacal bursa lesions; however, mineral excretion levels were not evaluated. Carotenoid antioxidants may act as pro-oxidants at high concentrations <sup>(15)</sup>. Thus, a high lycopene dose of 800 mg kg<sup>-1</sup> feed may have acted as a pro-oxidant and, in conjunction with the presence of higher-bioavailability trace minerals, increased the excretion of solids and minerals. Supplementing feed with organic minerals typically leads to reduced mineral excretion because more of these minerals are absorbed by the animal, as organic minerals and amino acids share the same absorption pathways, which reduces the competition for absorption sites with inorganic minerals <sup>(3)</sup>. However, excretion depends on the amount of OM in the diet when used; high OM doses may lead to increased mineral excretion <sup>(17)</sup>.

A high mineral matter content in manure indicates high contents of macro- and micronutrients, which can compromise soil, air, and water quality if disposed without treatment. This manure can be reused as organic fertilizer after composting, which is a low-cost solid waste treatment method that transforms those minerals into compounds that are available for plant uptake <sup>(18)</sup>. Anaerobic digestion is another method of treating manure, which produces biofertilizers and energy through producing biogas <sup>(19)</sup>.

The interaction of mineral source and lycopene level exerted significant effects ( $p < 0.05$ ) on the pH of the manure (Table 4). The pH of the manure was lower in hens fed organic mineral diets without lycopene supplementation. The manure pH was lowest in manure from hens supplemented with 400 mg of lycopene, regardless of mineral source (OM+400 and IM+400). The pH of manure is directly associated with diet composition and feed use efficiency, reflecting the nutrient content in the manure. pH may also be influenced by storage conditions and duration between collection and analysis.

**Table 4.** Ammoniacal nitrogen, pH, and macronutrient levels in manure from laying hens fed diets supplemented with inorganic or organic minerals and different levels of lycopene.

Variable	Mineral source	LYC (mg kg <sup>-1</sup> )			Mean	CV (%)	ANOVA ( <i>p</i> -value)		
		0	400	800			MS	LYC	MS × LYC
pH	Inorganic	5.91 <sup>aA</sup>	5.52 <sup>B</sup>	5.87 <sup>A</sup>	5.77	2.93	>0.05	<0.001	0.010
	Organic	5.65 <sup>bAB</sup>	5.61 <sup>B</sup>	5.82 <sup>A</sup>	5.69				
	Mean	5.78	5.56	5.85					
Ammoniacal nitrogen (mg L <sup>-1</sup> excreta)	Inorganic	394	373	373	380	16.16	>0.05	>0.05	>0.05
	Organic	383	361	344	363				
	Mean	389	367	359					
Total carbon (g bird <sup>-1</sup> day <sup>-1</sup> )	Inorganic	8.53	9.11	8.46	8.70	30.40	>0.05	>0.05	>0.05
	Organic	7.91	8.61	10.90	9.14				
	Mean	8.22	8.86	9.68					
Total nitrogen (g bird <sup>-1</sup> day <sup>-1</sup> )	Inorganic	1.31	1.35	1.28 <sup>b</sup>	1.32	19.92	>0.05	>0.05	0.05
	Organic	1.16 <sup>B</sup>	1.35 <sup>B</sup>	1.77 <sup>aA</sup>	1.43				
	Mean	1.23	1.35	1.53					
Total phosphorus (g bird <sup>-1</sup> day <sup>-1</sup> )	Inorganic	0.69	0.70	0.68 <sup>b</sup>	0.69	20.64	>0.05	>0.05	0.048
	Organic	0.62 <sup>B</sup>	0.65 <sup>B</sup>	0.84 <sup>aA</sup>	0.70				
	Mean	0.65	0.68	0.76					
Potassium (g bird <sup>-1</sup> day <sup>-1</sup> )	Inorganic	0.27	0.28	0.27	0.27	23.86	>0.05	>0.05	>0.05
	Organic	0.24	0.25	0.33	0.27				
	Mean	0.25	0.26	0.30					
Calcium (g bird <sup>-1</sup> day <sup>-1</sup> )	Inorganic	1.63	1.58	1.60 <sup>b</sup>	1.60	20.91	>0.05	0.031	0.025
	Organic	1.44 <sup>B</sup>	1.43 <sup>B</sup>	2.02 <sup>aA</sup>	1.63				
	Mean	1.54	1.51	1.81					
Magnesium (g bird <sup>-1</sup> day <sup>-1</sup> )	Inorganic	0.10	0.10	0.09 <sup>b</sup>	0.10	18.05	>0.05	>0.05	0.004
	Organic	0.10 <sup>B</sup>	0.10 <sup>B</sup>	0.13 <sup>aA</sup>	0.11				
	Mean	0.10	0.10	0.11					

Means within columns followed by different lowercase letters and means within rows followed by different uppercase letters significantly differ according to Tukey's test ( $p < 0.05$ ). CV, coefficient of variation; ANOVA, analysis of variance; MS, mineral source; LYC, lycopene.

Diet had no effect ( $p>0.05$ ) on the ammoniacal nitrogen content in the manure (Table 4). A high ammoniacal nitrogen content is high may result in alkaline manure, although this was not observed in this study (pH 5.52–5.91). This lack of effect on ammoniacal nitrogen content in the manure could be attributed to the short storage time, which did not enable the proliferation of microorganisms that break down nitrogen compounds into ammonium ions or free ammonia, depending on the pH of the medium. pH correction may be necessary if anaerobic digestion is used for treating this manure, as methanogenic bacteria are sensitive, with an optimal pH range of 6.7 to 7.5 <sup>(20)</sup>. Poultry manure differs from that of other livestock because ammoniacal nitrogen derives from the degradation of uric acid rather than urea in poultry. The hydrolysis rate of uric acid depends on the manure storage conditions; thus, the concentration of ammoniacal nitrogen in poultry manure substantially differs from that of other animals <sup>(21)</sup>.

The organic carbon and potassium contents in the manure were not influenced ( $p>0.05$ ) by the experimental diet, with mean excretion values of 8.92 and 0.27 g bird<sup>-1</sup> day<sup>-1</sup>, respectively. However, the manure differed in total nitrogen, phosphorus, calcium, and magnesium contents despite the diets having the same nutrient levels, which was likely due to the interaction between the mineral source and lycopene level. The hens fed the OM+800 diet had higher levels of macronutrients in their manure than those in the other groups.

The total nitrogen and phosphorus contents in the manure from the hens fed the OM+800 diet were 27% and 20% higher, respectively, than those of hens fed other diets supplemented with organic minerals. Overall, the macro- and micronutrient levels in the OM+800 group were markedly different than those the other diets, likely due to increased manure dry matter and mineral concentrations.

The manganese and copper levels in the manure were not influenced by the mineral source or lycopene level, with mean excretion levels of 6.01 and 1.07 mg bird<sup>-1</sup> day<sup>-1</sup>, respectively (Table 5). The iron and zinc levels in the manure from the hens fed inorganic mineral diets were similar, regardless of the lycopene supplementation level. The iron and zinc levels in the manure of the hens in the OM+800 group were higher than those of the hens in the OM or OM+400 group (Table 5).

**Table 5.** Micronutrient content of manure from laying hens fed diets supplemented with inorganic or organic minerals and different levels of lycopene.

Micronutrient	Mineral source	LYC (mg kg <sup>-1</sup> )			Mean	CV (%)	ANOVA ( <i>p</i> -value)		
		0	400	800			MS	LYC	MS × LYC
Iron (mg bird <sup>-1</sup> day <sup>-1</sup> )	Inorganic	34.79	33.35	33.44 <sup>b</sup>	33.86	18.84	>0.05	>0.05	0.028
	Organic	32.22 <sup>B</sup>	31.88 <sup>B</sup>	42.65 <sup>aA</sup>	35.58				
	Mean	33.51	32.62	38.05					
Manganese (mg bird <sup>-1</sup> day <sup>-1</sup> )	Inorganic	5.53	6.17	5.50	5.74	22.80	>0.05	>0.05	>0.05
	Organic	5.54	5.86	7.48	6.29				
	Mean	5.54	6.01	6.49					
Copper (mg bird <sup>-1</sup> day <sup>-1</sup> )	Inorganic	0.99	0.90	0.88	0.92	38.65	0.015	>0.05	>0.05
	Organic	1.10	1.26	1.32	1.23				
	Mean	1.04	1.08	1.10					
Zinc (mg bird <sup>-1</sup> day <sup>-1</sup> )	Inorganic	8.68	8.82	7.97 <sup>b</sup>	8.49	17.95	>0.05	>0.05	0.001
	Organic	7.89 <sup>B</sup>	8.88 <sup>B</sup>	11.44 <sup>aA</sup>	9.40				
	Mean	8.28	8.85	9.70					

Means within columns followed by different lowercase letters and means within rows followed by different uppercase letters significantly differ according to Tukey's test ( $p < 0.05$ ). CV, coefficient of variation; ANOVA, analysis of variance; MS, mineral source; LYC, lycopene.

The coefficients of variation were high for some macro- and micronutrients such as organic carbon and copper, whose levels did not significantly differ between treatments ( $p>0.05$ ). The physicochemical properties of poultry waste, including feces, urine, feed, feathers, and broken eggs, typically widely vary owing to the differences in the levels of macro- and micronutrients. In contrast to our results, the copper and manganese levels were lower in the manure of laying hens fed organic mineral diets than in the manure of those fed inorganic mineral diets in other studies, indicating increased mineral bioavailability<sup>(22, 23)</sup>.

The high bioavailability of organic minerals trace promotes enzyme activity and increases mineral retention in poultry and therefore reduces mineral excretion, even when used at lower concentrations than inorganic trace minerals in the feed<sup>(24)</sup>. These effects may be explained by organic minerals being absorbed through not only the normal ion absorption mechanisms in the intestine but also the same pathways as the organic molecules with which they are complexed, minimizing competition among minerals for carriers and increasing their availability<sup>(25)</sup>.

Up to 100% of the inorganic minerals were replaced with organic minerals in this study. Poultry may not fully absorb high amounts of available minerals, resulting in the partial use of organic minerals. This may explain the results of this study. The total replacement of inorganic Zn with an organic source resulted in similar excreted levels of Zn; excretion decreased only when poultry was supplemented with lower concentrations of organic Zn (70%, 80%, and 90%)<sup>(22)</sup>. Some organic minerals such as copper, iron, zinc, selenium, and manganese function as cofactors for antioxidant enzymes. These minerals synergistically act with lycopene to enhance the antioxidant activities in birds, potentially because increases in antioxidant functions lead to increased feed intake, which in turn increases mineral excretion.

Whether the differences in the macro- and micronutrient contents between the groups were due to the use of organic or inorganic mineral sources, different levels of lycopene supplementation, or their interactions could not be determined because the residue samples contained materials in addition to feces and urine. The aim in this study was to evaluate the quantity and characteristics of the excreta of laying hens fed different diets. The results indicated differences between the groups. These differences increased when the data were extrapolated to commercial facilities containing thousands of birds. These results can guide decision making on the reuse and treatment of manure waste.

## 4. Conclusion

The mineral source and lycopene dose in chicken feed did not increase the amount of manure produced. However, the levels of dry matter in the manure were higher when chickens were fed a diet supplemented with 800 mg lycopene and organic minerals than in the other groups, which increased the excretion of minerals such as nitrogen, phosphorus, calcium, magnesium, iron, and zinc. The level of lycopene supplementation in the feed for laying hens still requires optimization, focusing on the absorption and excretion of minerals to prevent waste and ensure that animal production is benefited.

### Conflict of interest statement

The authors declare that they have no competing financial interests or personal relationships that may have influenced the work reported in this study.

### Data availability statement

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

### Author contributions

Conceptualization: Garcia, E. R. M., Santos, T. M. B.; Data curation: Xavier, C. A. N., Cruz, F. K.; Formal analysis: Xavier, C. A. N., Garcia, E. R. M.; Methodology: Xavier, C. A. N.; Project administration: Garcia, E. R. M.; Supervision: Xavier, C. A. N., Cruz, F. K.; Writing – original draft: Xavier, C. A. N., Leite, B. K. V.; Writing – review & editing: Leite, B. K. V.

### Generative AI use statement

The authors did not use generative Artificial Intelligence tools or technologies in the creation or editing of any part of this manuscript.

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### References

1. Stefanello C, Santos TC, Murakami AE, Martins EN, Carneiro TC. Productive performance, eggshell quality, and eggshell ultrastructure of laying hens fed diets supplemented with organic trace minerals. *Poultry Science*. 2014; 93:104–113. <http://dx.doi.org/10.3382/ps.2013-03190>
2. Carvalho KCN, Xavier CAN, Silva AA, Garcia ERM, Sanches DS, Kiefer C, Santos TMB. Qualitative and quantitative characterization of waste layers fed diets containing mineral sources and rosemary oil levels. *Brazilian Animal Science*. 2024; v.25, 77674E. doi: <http://dx.doi.org/10.1590/1809-6891v25e-77674E>
3. Qiu JL, Zhou Q, Zhu JM, Lu XT, Liu B, Yu DY, Lin G, Ao T, Xu JM. Organic trace minerals improve eggshell quality by improving the eggshell ultrastructure of laying hens during the late laying period. *Poultry Science*. 2020; 99:1483–1490. <https://doi.org/10.1016/j.psj.2019.11.006>
4. Kratzer FH, Vohra P. 1986-Chelates in nutrition. 1st ed. Florida: CRC Press. 178p. <https://doi.org/10.1201/9781351070508>
5. Stef DS, Gergen I. Effect of mineral-enriched diet and medicinal herbs on Fe, Mn, Zn, and Cu uptake in chicken. *Chemistry Central Journal*. 2012; v.6(19). <http://journal.chemistrycentral.com/content/6/1/19>
6. Garcia ERM, Chaves NRB, Oliveira CAL, Kiefer C, Melo EP. Performance and egg quality of laying hens fed with mineral sources and rosemary oil. *Anais da Academia Brasileira de Ciências*. 2019; v.91, e20180516. doi: <https://doi.org/10.1590/0001-3765201820180516>
7. Yunitasari F, Jayanegara A, Ulupi N. Performance, Egg Quality, and Immunity of Laying Hens due to Natural Carotenoid Supplementation: A Meta-Analysis. *Food Sci. Anim. Resour*. 2023; 43(2):282-304. <https://doi.org/10.5851/kosfa.2022.e76>
8. Toniazzo G, Ranna LF, Andrade TS, Pereira MFC, Henz EM, Gris B, Carvalho PLO, Rohloff Júnior N, Eyng C, Nunes RN. Mistura de óleos essenciais na dieta de frangos de corte sobre o desempenho, saúde intestinal e atividade de enzimas antioxidantes. *Semina: Ciências Agrárias*. 2025; v. 46, n. 4, p. 1005-1024. doi: <https://doi.org/10.5433/1679-0359.2025v46n4p1005>
9. Granja Planalto. Manual de manejo das poedeiras Dekalb Brown. 2009. Uberlândia: Côrtes.
10. Rostagno HS, Albino LFT, Donzele JL, Gomes PC, Oliveira RF, Lopes DC, Ferreira AS, Barreto SLT, Euclides RF. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 3ª ed. 2011. Viçosa, MG: UFV, DZO. 252 p.
11. Olson JB, Ward NE, Koutsos EA. Lycopene incorporation into egg yolk and effects on laying hen immune function. *Poultry Science*. 2008; 87:2573–2580. doi: <https://doi.org/10.3382/ps.2008-00072>
12. APHA. Standard Methods for the Examination of Water and Wastewater. 2017. (23rd ed.). Washington DC: American Public Health Association.
13. Bakhshalinejad R, Torrey S, Kiarie EG. Comparative efficacy of hydroxychloride and organic sources of zinc, copper, and manganese on egg production and concentration of trace minerals in eggs, plasma, and excreta in female broiler breeders from 42 to 63 weeks of age. *Poultry Science*. 2024; 103:103522. <https://doi.org/10.1016/j.psj.2024.103522>
14. Sahin K, Onderci M, Sahin N, Gursu MF, Khachikd F, Kucuke O. Effects of lycopene supplementation on antioxidant status, oxidative stress, performance and carcass characteristics in heat-stressed Japanese quail. *Journal of Thermal Biology*. 2006; 31, 307–312. doi: <https://doi.org/10.1016/j.jtherbio.2005.12.006>
15. Pozzo L, Tarantola M, Biasibetti E, Capucchio MT, Pagella M, Mellia E, Bergagna S, Gennero MS, Strazzullo G, Schiavone A. Adverse effects in broiler chickens fed a high lycopene concentration supplemented diet. *Canadian journal of animal science*. 2013; 93: 23124. doi: <https://doi.org/10.4141/CJAS2012-081>
16. Farias RM, Orrico Junior MAP, Orrico ACA, Garcia RG, Centurion SR, Fernandes ARM. 2012; Biodigestão anaeróbia de dejetos de poedeiras coletados após diferentes períodos de acúmulo. *Ciência Rural*, v.42, n.6, p.1089-1094.

17. Yenice E, Mizrak C, Gültekin M, Atik Z, Tunca M. Effects of Organic and Inorganic Forms of Manganese, Zinc, Copper, and Chromium on Bioavailability of These Minerals and Calcium in Late-Phase Laying Hens. *Biol Trace Elem Res.* 2015; 167(2):300-7. doi: <https://doi.org/10.1007/s12011-015-0313-8>
18. Orrico Junior MAP, Orrico ACA, Fava AF, Sunada NS, Schwingel AW, Garcia RG, Aspilcueta Borquis RR. 2018; Crude glycerin in co-composting with laying hen manure reduces N losses. *Sci. Agric.*, v.75, n.5, p.361-367. <http://dx.doi.org/10.1590/1678-992X-2016-0478>
19. Schwingel AW, Orrico ACA, Lucas Junior J, Orrico Junior MAP, Aspilcueta Borquis RR, Fava AF. 2019; Laying hen manure in anaerobic Co-Digestion with glycerin containing different glycerol and impurity levels. *Journal of Cleaner Production*, 215, 1437e1444. <https://doi.org/10.1016/j.jclepro.2019.01.125>
20. Kunz A, Steinmetz RLR, Amaral AC. 2019; Fundamentos da digestão anaerobia, purificação do biogas, uso e tratamento do digestato. Concórdia: Sbera: Embrapa Suínos e Aves. 209p.
21. Feilberg A, Sommer S.G. 2013; Ammonia and malodorous gases: sources and abatement technologies. *Animal Manure Recycling*. Wiley: West Sussex, p.153-175. <https://doi.org/10.1002/9781118676677.ch9>
22. Carvalho LSS, Rosa DRV, Litz FH, Fagundes NS, Fernandes EA. 2015; Effect of the inclusion of organic copper, manganese, and zinc in the diet of layers on mineral excretion, egg production, and eggshell quality. *Revista Brasileira de Ciência Avícola*, p.87-92. doi: <https://doi.org/10.1590/1516-635xSpecialIssue>
23. Engin Y, Mizrak C, Gültekin M, Atik Z, Tunca M. 2015; Effects of organic and inorganic forms of manganese, zinc, copper, and chromium on bioavailability of these minerals and calcium in late-phase laying hens. *Biological Trace Element Research*, v.167, p.300-307. doi: <https://doi.org/10.1007/s12011-015-0313-8>
24. Sun Q, Guo Y, Li J, Zhang T, Wen J. 2012; Effects of methionine hydroxy analog chelated cu/mn/zn on laying performance, egg quality, enzyme activity and mineral retention of laying hens. *The Journal of Poultry Science*, v.49, p.20-25. doi: <https://doi.org/10.2141/jpsa.011055>