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Effect of Organic Mineral Supplementation on The Egg Quality of Semi-Heavy Layers in Their Second Cycle of Lay[1]

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ABSTRACT

This study was carried out to evaluate the effects of dietary trace mineral levels and sources on egg quality parameters of second-cycle semi-heavy layers. A number of 360 72-week-old layers were submitted to forced molting. Upon return of lay (83 weeks of age), birds were distributed according to a completely randomized experimental design of six treatments with six replicates of 10 birds each. The control treatment consisted of 0.10% dietary supplementation of trace minerals from inorganic sources, which was proportionally replaced by five levels (110, 100, 90, 80, 70%) of an organic trace mineral supplement containing 30, 30, 40, 6, 0.61, and 0.3 g/kg product of Zn, Fe, Mn, Cu, I, and Se, respectively. All diets contained equal protein, energy, and amino acid levels. Every 28 days of the experimental period (112 days) four eggs per replicate were collected for egg quality evaluation. The following parameters were evaluated: specific gravity, yolk, albumen and eggshell percentages, yolk index, Haugh units, and eggshell thickness and breaking strength. One sample per replicate, consisting of the pool of the yolks of three eggs collected at the end of each experimental period, was used to assess protein and mineral (Ca, P, Cu, Fe, Mn, and Zn) contents. The results were submitted to ANOVA, and means to the test of Tukey at 5% significance level. The evaluated trace mineral levels and sources did not influence any of the studied egg quality parameters. It was concluded that reducing organic trace mineral supplementation in up to 70% relative to 100% inorganic trace mineral supplementation does not affect egg parameters and therefore, can be applied to the diet of semi-heavy layers in their second cycle of lay.

INTRODUCTION

Forced molting of layers has been increasingly used in Brazil as it results in high egg production and large eggs, which consequently demands the adjustment of dietary nutritional levels. Despite the intensive use of a second laying cycle, there is little information on the nutritional levels recommended for layers in this phase; in general, the nutritional requirements of layers at the end of the first cycle of lay are applied with slight reduction of the nutritional levels.

Improving mineral bioavailability by using organic sources may be an option to adjust nutritional levels. Both large and trace minerals added to poultry diets traditionally derive from inorganic sources, such as oxides, sulfates, chlorides, carbonates, and phosphates. In practice, excessive levels of minerals, such as Zn, Cu, Fe, and Mn, have been used to prevent deficiencies (Bertechini, 2003) caused by possible interactions among these minerals and other dietary components, rendering them unavailable to the bird's digestive system (Mabe *et al.*, 2003).

Organic trace minerals are compounds consisting of metal ions bound to organic substances, including amino acids, peptides, or polysaccharide complexes, providing these ions with high biological availability, stability, and solubility (Vieira, 2004). As a function of these characteristics, these minerals are more bioavailable, and are readily transported and absorbed in the intestines. In addition, they are more stable and biochemically protected against unfavorable reactions with other dietary components, which may reduce their absorption rates (Close, 1998).

Some studies conducted to determine the bioavailability of organic trace mineral sources showed that these were more available that the inorganic sources (Ammerman *et al*.,1998; Aoyagi & Baker, 1993; Spears *et al*., 1992; Baker *et al*., 1991).

Lundeen (2001) added organic trace minerals to the diet of 40- to 60-week-old layers and observed higher eggshell percentage, stronger eggshells, and lower number of cracked eggs. A significant reduction of eggshell defects and higher eggshell breaking strength were observed by Moreng *et al*. (1992) when layer diets were supplemented with organic zinc as compared with its inorganic source.

Layer nutrition, in addition to affecting physical egg quality (size, percentages of the egg components, eggshell strength), may influence egg chemical composition (Franco & Sakamoto, 2005). The egg consists of four main components: eggshell membrane, eggshell, yolk, and albumen, with the three latter accounting for 10, 30, and 60% of total egg weight, respectively.

The yolk contains 12 to 16% protein and 1.0 to 2.0% minerals, whereas the albumen contains 10% and 0.6 to 0.9%, respectively (Madrid *et al*., 1996). The yolk consists of approximately 50% solids, and it is a solution of particles in a protein suspension with high mineral content, particularly of iron and phosphorus, and its composition may be influenced by the diet (Rose, 1997).

According to Cao *et al*. (1996), layers have high dietary iron requirements as each egg contains approximately 1.5 mg iron in the yolk, which represents 25% of the iron reserves available in the liver. Paik (2001) observed an increase in yolk iron content when layers were fed an iron-methionine complex as compared to those fed inorganic iron.

Selenium metabolism in poultry has been frequently studied in the last 30 years, but it is still not clear if selenium is incorporated in the eggshell and/or yolk and if this depends on dietary selenium supply. According to Franco & Sakamoto (2005), selenium supplementation in layer feeds results in higher egg selenium content, favoring higher selenium ingestion by egg consumers. In addition, higher egg selenium content allows the maintenance of egg internal quality during storage.

The objective of the present study was to evaluate the effects of detary mineral supplement sources and levels on the internal and external egg quality of semi-heavy layers in their second cycle of lay.

MATERIAL AND METHODS

The experiment was carried out at the Unit of Research and Development of Brotas of the Agency of Agribusiness Technology of the Agriculture Department of the State of São Paulo for 112 days (4 cycles of 28 days).

A number of 360 Hy-Line Brown semi-heavy layers with 72 weeks of age at the end of the first cycle of lay was used. Birds were submitted for forced molting using the conventional method - 14 days of fasting and then feeding ground corn for 28 days. At 83 weeks of age, after lay was resumed, birds were distributed into the experimental treatments.

Birds were housed in a layer house with two double and overlapping batteries of cages and a central aisle. Galvanized iron cages were 1.00 m long x 45 cm deep x 40 cm high. Each cage had two internal compartments, with a capacity of five birds each, therefore allowing 10 birds per cage to be housed. Each cage was considered an experimental unit.

The cages were equipped with nipple drinkers and wood trough feeders placed along the front of the cages. Feed was offered ad libitum in the morning and in the afternoon. A lighting program of 17 hours of light daily was adopted. Daily environmental temperature was recorded using a maximum-minimum temperature thermometer located in the center of the house.

A completely randomized experimental design with six treatments with six replicates of 10 birds each was applied. The experimental treatments consisted of the dietary inclusion of 100% inorganic mineral supplement (IM), or organic mineral (OM) supplement included at 110, 100, 90, 80, or 70% of IM levels. Both inorganic and organic supplements were formulated with Zn, Mn, Cu, Fe, Se, and I.

In the experimental diet of the 100% IM treatment, 0.10% of the mineral supplement was added. In order to supply the same mineral concentration as the 0.10% inorganic mineral supplementation, 0.18% organic mineral supplement was added to the feed, corresponding to the 100% OM treatment. The other OM inclusion levels were calculated based on this value, and the inclusion of 0.198, 0.162, 0.144, and 0.126% OM supplement corresponded to the treatments 110, 90, 80, and 70% OM, respectively. Mineral supplements were formulated according to the recommendations of the company Tortuga®, with guaranteed levels to supply the nutritional requirements of layers at the end of lay. Table 1 shows the composition of the trace mineral supplements, and Table 2 their inclusion levels in the experimental diets.

The formulation of the experimental diets (Table 3), based on corn and soybean meal, was adapted from Rostagno *et al*. (2000). Diets were different only as to Zn, Fe, Mn, Cu, I, and Se levels.

At the end of each experimental period of 28 days, four eggs from each experimental unit were collected to evaluate the following parameters: specific gravity, yolk, albumen and eggshell percentages, yolk index, Haugh units, and eggshell thickness and breaking strength.

Intact eggs were identified, individually weighed in a digital-precision scale (0.01 g), and submitted to specific gravity assessment using the method of egg immersion in saline solution. Nine solutions, with densities between 1.060 a 1.100, with graded variation of 0.005 between each solution, were prepared. Specific gravities were determined using a densitometer.

Table 1 - Composition of the trace mineral supplements.

Source	Trace minerals (mg/kg)					
	Zn	Fe	Mn	Cu	I	Se
Inorganic trace minerals	54.00	54.00	72.00	10.00	0.61	0.30
Organic trace minerals	30.00	30.00	40.00	6.00	0.61	0.30

Table 2 - Amount of trace minerals in the feed.

Treatments	Feed inclusion (%)	Trace minerals (mg/kg)					
		Zn	Fe	Mn	Cu	I	Se
100% IM	0.100	54.00	54.00	72.00	10.00	0.61	0.30
110% OM	0.198	59.40	59.40	79.20	11.88	1.21	0.59
100% OM	0.180	54.00	54.00	72.00	10.80	1.10	0.54
90% OM	0.162	48.60	48.60	64.80	9.72	0.99	0.49
80% OM	0.144	43.20	43.20	57.60	8.64	0.88	0.43
70% OM	0.126	37.80	37.80	50.40	7.56	0.77	0.38

Table 3 - Ingredient and calculated compositions of the experimental diets of semi-heavy layers in their second cycle of lay supplemented with different trace mineral sources and levels.

Ingredients (%)	OM supplementation level (%)[1]					
	100 IM	110	100	90	80	70
Ground corn	65.41	65.32	65.33	65.35	65.37	65.39
Soybean meal 45%	20.24	20.24	20.24	20.24	20.24	20.24
Wheat midds	3.66	3.66	3.66	3.66	3.66	3.66
Dicalcium phosphate	1.29	1.29	1.29	1.29	1.29	1.29
Soybean soapstock	1.00	1.00	1.00	1.00	1.00	1.00
Limestone	7.73	7.73	7.73	7.73	7.73	7.73
Mineral suppl	0.100*	0.198**	0.180**	0.162**	0.144**	0.126**
Vitamin suppl (***)	0.10	0.10	0.10	0.10	0.10	0.10
Salt (NaCl)	0.35	0.35	0.35	0.35	0.35	0.35
DL-methionine	0.12	0.12	0.12	0.12	0.12	0.12
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition						
ME (kcal/kg feed)	2790	2790	2790	2790	2790	2790
Crude protein (%)	15.50	15.50	15.50	15.50	15.50	15.50
Calcium (%)	3.50	3.50	3.50	3.50	3.50	3.50
Avail. phosphorus (%)	0.34	0.34	0.34	0.34	0.34	0.34
Methionine (%)	0.35	0.35	0.35	0.35	0.35	0.35
Methionine + cystine (%)	0.64	0.64	0.64	0.64	0.64	0.64
Lysine (%)	0.74	0.74	0.74	0.74	0.74	0.74

*Inorganic trace mineral supplement, g/kg product: zinc 54g, iron 54g, manganese 72g, copper 10g, iodine 0.61g, selenium 0.30g. **Organic trace mineral supplement, g/kg product: zinc 30g, iron 30g, manganese 40g, copper 6g, iodine 0.61g, selenium 0.30g. ***Vitamin supplement, composition per kg product: Vit A 7,520,000 IU, Vit. D3 1,816,000 IU, Vit. E 8400 mg, Vit. K3 1280 mg, Vit. B1 1340 mg, Vit. B2 3000 mg, Vit. B6 1660 mg, Vit B12 8.000 mg, nicotinic acid 20,000 mg , calcium pantothenate 8000 mg, folic acid 300 mg, biotin 40 mg.

Eggshell breaking strength was assessed in the intact egg using a specific cell coupled to a Texture Analyzer TA.XT plus with Cyl Stainless 2-mm probe code P/2, which recorded the strength (kgf) required to break the eggshell.

In order to determine Haugh units eggs were broken on a flat glass surfaces, and three measurements of albumen height were made used a micrometer in the median region between the external edge and the egg yolk. The average of the obtained values was used in the formula proposed by Card & Nesheim (1978): $HU = 100.\log (H + 7.57 - 1.7 W^{0.37})$, where: H = albumen height (mm) and W = egg weight (g). Albumen weight was calculated as result of intact egg weight minus yolk and dry eggshell weights. Albumen percentage was determined by the ratio between albumen and egg weights multiplied by 100.

Yolk percentage was calculated as the ratio between yolk and egg weights multiplied by 100. Yolk quality was assessed by measuring yolk height (YH) and yolk width (YW), and yolk index (YI), was calculated as the ration between those parameters as YI = YH/YW.

Eggshells were washed under running water, dried in an oven at 60 ºC for 48 hours, and then weighed in a digital scale. Eggshell percentage was calculated as the ration between egg weight and dried eggshell weight. Eggshell thickness was determined in three different regions using a special micrometer, of the brand Mitutoyo, with 0.01-mm precision. These determinations were carried out according to the description of Souza *et al*. (1984).

Yolk protein and mineral (P, Ca, Cu, Fe, Mn, and Zn) analyses were carried out in the eggs collected at the end of the experimental period. Eighteen eggs were used per treatment. Each sample consisted of a pool of three eggs per experimental unit, totaling six replicates per treatment.

After the separation of the albumen, the three yolks were homogenized and placed in an oven at 60 ºC for 96 hours. After this period, protein content was analyzed by determining total nitrogen content using the method of Kjeldahl according to the A.O.A.C. (1995), and calculating protein percentage by multiplying mean values of nitrogen percentage by 6.25. Mineral contents (P, Ca, Cu, Fe, Mn, and Zn) were determined according to the norms of the A.O.A.C. (1995).

The results were compared by analysis of variance for balanced data, and means were compared by the test of Tukey, using Sisvar statistical package as described by Ferreira (2000). A 5% significance level was considered.

RESULTS AND DISCUSSION

Table 4 presents the mean egg quality results of semi-heavy layers in their second cycle of lay fed diets with different trace mineral (Zn, Fe, Mn, Cu, I, and Se) sources and levels. Internal egg quality, represented by the parameters yolk percentage, yolk index, albumen percentage, and Haugh units, was not significantly influenced by the treatments.

These results may be explained by the excessive supplementation levels of the evaluated trace minerals. The studied trace minerals levels, including those obtained by organic sources, may have been higher than those required to optimize egg quality. According to Bertechini (2003), trace mineral inclusion levels in layer diets currently applied in the Brazilian market are higher than those required by the birds. As in the present experiment the inclusion of organic trace minerals was calculated relative to the level of inclusion of inorganic trace minerals, the organic sources supplied higher levels than those required.

Many research studies have been carried out on

Table 4 - Mean egg quality results of semi-heavy layers in their second cycle of lay fed an inorganic trace mineral supplement (IM) or different levels or an organic trace mineral supplement (OM) (Zn, Fe, Mn, Cu, I, and Se).

Parameters	IM (%)	OM (%)					CV(%)*
	100	110	100	90	80	70	
Yolk (%)	24.99	24.62	24.48	24.24	24.67	24.67	3.59
Yolk index	0.441	0.442	0.443	0.447	0.443	0.443	2.02
Albumen (%)	65.92	66.24	66.30	66.62	66.03	66.36	1.41
Haugh units	86.50	87.80	86.75	86.80	87.63	88.03	3.71
Eggshell (%)	9.08ab	9.15ab	9.22ab	9.14ab	9.29a	8.96b	2.78
Eggshell thickness (mm)	0.396	0.398	0.403	0.408	0.405	0.397	2.91
SG**	1.085ab	1.085ab	1.086ab	1.085ab	1.087a	1.084b	0.16
Breaking strength (gF)	2622	2673	2699	2776	2657	2766	5.31

*CV (%) = Coefficient of variation. ** SG = specific gravity. Means followed by different letter in the same row are different by the test of Tukey ($p<0.05$).

the dietary supplementation of inorganic and organic trace minerals, tested either individually or in association. However, literature results relative to the addition of different mineral sources to commercial layer diets are still controversial. The lack of better and significant results in terms of egg quality may be explained not only due to the possible excessive supplementation of trace minerals, but also due to differences in organic sources and levels used in these studies.

Sources of organic trace minerals used individually and/or associated did not have any influence, considering the average obtained during the entire experimental period, on Haugh units (Scatolini, 2007; Sechinato, 2003) or yolk index (Scatolini, 2007). Correia *et al*. (2000) evaluated the supplementation of organic selenium in layer diets and did not find significant differences in Haugh units, yolk and albumen percentages, or albumen height.

On the other hand, Xavier *et al*. (2004) observed better egg quality in semi-heavy layers in their second cycle of lay, and concluded that the inclusion of Se, Zn, and Mn as an organic complex is beneficial in this phase. Rutz *et al*. (2006) evaluated the eggs of Isa Brown layers in their first cycle of lay and fed organic selenium (particularly selenomethionine), and observed a trend of better yolk and albumen weights, and a consistent improvement of albumen quality, as evaluated by Haugh units, indicating a positive effect of organic selenium on the absorption and/or protection of fat-soluble vitamins. Layers in their second cycle of lay and supplemented with organic trace minerals (Se, Zn e Mn) tended to have higher yolk and albumen weights.

Despite the lack of significant differences among the evaluated trace mineral sources in the present study, organic trace mineral level significantly influenced egg specific gravity and eggshell percentage, with the 80% organic trace mineral level promoting higher eggshell percentage and specific gravity values as compared to the level of 70%. However, these treatments were not significantly different as compared to the others (Table 4).

The results obtained in the present study are consistent with the findings of Scatolini (2007), Albuquerque (2004), Sechinato (2003), and Mabe *et al*. (2003), who used diets supplemented with individual and/or associated organic trace minerals and inorganic trace minerals and did not observed any treatment effect on external egg quality.

Organic selenium supplementation was evaluated by Correia *et al*. (2000), who did not observe significant effect on egg specific gravity or eggshell thickness. On the other hand, Paton & Cantor (2000) found higher eggshell breaking strength in 80-week-old Babcock layers fed organic selenium.

The supplementation of layer diets with organic Zn and Mn, according to some authors, has shown beneficial effects on external egg quality. Paik (2001) evaluated organic Zn, Cu, and Mn sources in layer diets, and observed higher specific gravity and eggshell percentage in the eggs of layers fed organic trace minerals, and the association between organic Zn and Mn improved eggshell strength. According to that author, Zn influenced the synthesis of the enzyme carbonic anhydrase, which is essential for eggshell formation.

These results are consistent with those of Klecker *et al*. (1997), who observed higher breaking strength in the eggs of hens fed Zn and Mn proteinate replacing 20 to 40% of the inorganic forms present in the tested diets. Lundeen (2001) observed better eggshell quality in the eggs of layers supplemented with chelated Mn and Zn between 20 and 60 weeks of age.

Isa Brown layers supplemented with organic trace minerals (Se, Zn, and Mn) tended to have higher eggshell weight (Rutz *et al*., 2006). According to the authors, the supplementation of layer diets with organic trace minerals improves

Eggshell quality, provided organic Mn and Zn are added.

Mean protein percentage and mineral composition of the egg yolk are presented in Table 5.

The use of inorganic trace mineral sources as compared to organic sources, in the same or different concentrations, did not influence yolk protein content or mineral composition. The results obtained with the supplementation of 70% organic trace minerals were statistically similar to the treatments with 100% inorganic trace minerals or 110, 100, 90, and 80% de organic trace minerals, suggesting that the studied levels of trace minerals, including those of organic sources, may be higher than those required by the birds.

Mean yolk percentage and egg weight observed in the present study with 99-week-old Hy-Line Brown layers in their second cycle of lay were 24.61% and 68.62 g, respectively. The observed yolk mineral concentration (Table 5) was higher as compared to some literature studies (Kuit, 1984; Mabe *et al*., 2003) possibly as a function of bird age and strain in the present study. Faria *et al*. (2007) observed that the

Table 5 - Protein content and mineral composition, on dry matter basis, of the egg yolk of semi-heavy layers in their second cycle of lay fed an inorganic trace mineral supplement (IM) or different levels or an organic trace mineral supplement (OM).

Treat.	Protein (%)	P g/kg	Ca g/kg	Cu mg/kg	Fe mg/kg	Mn mg/kg	Zn mg/kg
100 % IM	31.10	12.03	3.00	4.00	154.00	3.00	86.00
110 % OM	31.00	11.42	3.00	3.70	152.00	3.00	81.80
100 % OM	29.60	12.23	3.00	4.00	167.00	3.00	84.50
90 % OM	31.50	11.85	3.00	4.30	160.00	3.00	82.00
80 % OM	31.60	11.62	3.00	4.00	155.00	3.00	83.80
70 % OM	31.10	11.95	3.00	4.00	147.00	3.00	85.30
Mean	31.00	11.85	3.00	4.00	156.00	3.00	83.90
CV* (%)	5.48	6.13	0.00	22.67	12.10	0.00	9.21

* CV (%) = Coefficient of variation.

concentration of solids in the yolk was influenced by strain, with higher values obtained with brown layers. Those authors also reported that the older the bird, the heavier the egg, and the higher the yolk percentage.

In the present study, it was observed that the dietary supplementation of trace minerals, either from organic or inorganic sources, was not sufficient to enrich the egg yolk, as the utilized level of inorganic trace minerals followed the recommendations of the strain's manual, and there was no difference between treatments.

Benites *et al*. (2005) showed that the diet influences yolk protein, fatty acid, and cholesterol contents. As to minerals, the yolk can be enriched with iron.

Bertechini *et al*. (2000) found a linear increase ($p<0.05$) in iron content when a corn and soybean meal diet was supplemented with up to 80 ppm iron (ferrous sulfate). Paik (2001) observed that the use of chelated iron increases in up to 20% yolk iron content.

Mabe *et al*. (2003) supplemented a diet based on corn and soybean meal with inorganic or organic trace minerals (Zn, Mn, and Cu), and observed that, independently of the source, the supplementation of 60, 60, and 10 mg/kg Zn, Mn e Cu, respectively, as compared to the treatment with no supplementation, increased ($p<0.01$) Mn and Zn yolk concentration, whereas copper concentration was not affected.

Skrivan *et al*. (2005) used supplemented inorganic Zn, Fe, and Cu individually or associated in layer diets, and found that the supplementation of the basal diet with iron increased yolk iron concentration in 6.3%. Those authors observed an antagonism between Zn and Cu, and found that Zn deposition in the yolk was significantly lower as copper increased and vice-versa. The enrichment of the eggs with the other elements was marginal (Cu) or absent (Zn).

The lack of increase in yolk mineral concentration in the present study, as opposed to the findings of Skrivan *et al*. (2005), Mabe *et al*. (2003) Bertechini *et al*. (2000), and Paik (2001), may have been a function of the determination of dietary trace mineral levels, which interval may have been insufficient to express the potential effects of those trace minerals on the yolk.

CONCLUSIONS

Under the conditions of the present study, it was concluded that the level of 70% organic trace mineral supplement, as compared to 100% inorganic trace minerals, may be used in the diet of semi-heavy layers in their second cycle of lay with no changes in egg quality or yolk protein level and mineral composition.

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