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Effects of Dietary Trace Mineral Sources and Levels Fed to Layers in Their Second Laying Cycle on the Quality of Eggs Stored at Different Temperatures and for Different Periods[1]

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ABSTRACT

This study aimed at evaluating the effects of trace mineral levels and sources supplemented to diets fed to semi-heavy layers in their second laying cycle on the quality of eggs stored for 14 days at different temperatures. The experimental diets consisted of the inclusion of inorganic trace minerals (T1 - control: 100% ITM) and five supplementation levels of organic trace minerals (carboaminophopho chelates) (110, 100, 90, 80, and 70% OTM). Trace mineral inclusion levels (mg/kg feed) were: T1: control - 100% ITM: Zn (54), Fe (54), Mn (72), Cu (10), I (0.61) Se (0.3); T2 - 110% OTM: Zn (59.4), Fe (59.4), Mn (79.2), Cu (11.88), I (1.21) Se (0.59); T3 - 100%: OTM: Zn (54), Fe (54), Mn (72), Cu (10.8), I (1.10) Se (0.54); T4 - 90% OTM: Zn (48.6), Fe (48.6), Mn (64.8), Cu (9.72), I (0.99) Se (0.49); T5 - 80% OTM: Zn (43.2), Fe (43.2), Mn (57.6), Cu (8.64), I (0.88), Se (0.43); T6 - 70% OTM: Zn (37.8), Fe (37.8), Mn (50.4), Cu (7.56), I (0.77) Se (0.38). A completely randomized experimental design in a split-plot arrangement with 60 treatments of four replicates each was applied. The combination of six diets versus storage temperature (room or under refrigeration) was randomized in plots, whereas the sub-plots consisted of storage times (0, 3, 7, 10, and 14 days). Data were submitted to analysis of variance of a model in slip-plots in time using the software package SAS (2000) at 5% probability level. It was concluded that 70% OTM supplementation can be used with no damage to egg quality, independently from storage temperature or time. The quality of refrigerated eggs stored up to 14 days is better than those stored at room temperature.

INTRODUCTION

Egg quality is directly related to layer nutrition. Adequate layer nutrition requires the supply of a balanced feed that supplies its nutritional requirements, both in terms of quantity and quality. Minerals are must be properly supplied because they participate in body biochemical process and eggshell formation, and are essential for the quality of the produced eggs (Mabe et al., 2003; Leeson & Summers, 2001; Vicenzi, 1996).

Special attention has been currently given to the supplementation of layer diets with organic or chelated trace minerals. According to the Association of American Feed Control Officials (AAFCO, 2001), organic trace minerals are metal ions chemically bound to an organic molecule, producing structures with unique stability characteristics that are provide high mineral bioavailability to animals.

Reddy et al. (1992) reported that organic forms of trace minerals or chelated trace minerals increase mineral bioavailability, providing several benefits to poultry, including better internal egg quality. Some studies performed to determine the bioavailability of organic trace mineral sources showed that the tested organic forms were more bioavailable than the inorganic forms (Ammerman et al., 1998; Aoyagi & Baker, 1993; Spears et al., 1992; Baker et al., 1991). However, the results of studies on the effect of organic trace minerals on the quality of stored eggs are few and controversial. Scatolini (2007) compared a dietary mineral supplement, containing manganese, zinc, selenium, iron, and copper from inorganic and organic sources, with manganese, zinc, and selenium fed individually or combined, and did not find any effect of organic trace minerals on egg weight, Haugh units, or yolk index of eggs stored for 14 days at room temperature. Correia et al. (2000) fed organic selenium to layers and did not observe significant differences in the quality of eggs stored at room temperature for different periods. On the other hand, Rutz et al. (2006) verified that the replacement of inorganic by organic trace minerals increased egg shelf life. Franco & Sakamoto, (2005) reported that selenium supplementation in layer diets increased the concentration of this trace mineral in the eggs, which in turn allowed maintaining internal egg quality during different storage periods.

Independently from nutrition, egg quality starts to decrease immediately after lay. These changes can be delayed, but not completely prevented (Theron et al., 2003; Rose, 1997).

Internal egg quality includes the presence of a dense albumen layer, which depends of the protein ovomucin (Stevens, 1996). Storage time and conditions seem to be the most important factors affecting albumen quality, and after three to four days of storage, albumen quality decreases rapidly (Strong et al., 1987), and continuously (Moreng & Avens, 1990).

An important factor that aids the conservation internal egg quality is refrigeration (Carvalho et al., 2003; Seleim & El-Prince, 2000; Souza et al., 1997). Maximum shelf life of egg at environmental temperature, preserving its internal quality, ranges from four (Ahn et al., 1981) to 15 days (Oliveira, 2000) after lay. Nevertheless, the period of fresh egg consumption is established as three weeks (Cepero et al., 1995), generating discussions on the establishment of storage periods for egg at room temperature and under refrigeration.

Egg quality loss is associated mainly to water and carbon dioxide losses during storage, which are proportional to environmental temperature increase (Leandro et al., 2005). Santos (2005), Barbosa et al. (2004), and Véras et al. (1999) evaluated the effects of environmental temperature and storage time on internal egg quality, and observed that egg weight and Haugh units changes as a function of storage time and temperature.

The objective of the present study was to evaluate the effects of the supplementation of inorganic and organic trace minerals to layer diets in the second laying period on the quality of eggs stored at different temperatures for different periods.

MATERIAL AND METHODS

The experiment was carried out at the Research and Development Unit of the Agency of Agribusiness Technology (Agência Paulista de Tecnologia dos Agronegócios - APTA) of the Department of Agriculture (Secretaria de Agricultura and Abastecimento - SAA) of the state of São Paulo.

Eggs from commercial layers with 99 weeks of age at the end of the experimental period were used. Layers were housed in a layer house with battery cages places in double layer at both sides of a central aisle. The galvanized iron cages were 1.00m long x 45cm deep x 40cm high. Each cage had two internal compartments for five birds each, therefore housing ten birds per cage.

Nipple drinkers and wood trough feeders were placed in front of the cages. Feed was offered ad libitum and supplied in the morning and in the afternoon. A lighting program of 17 hours of light was adopted. Environmental temperature was daily recorded using a maximum-minimum thermometer located in the center of the house.

Birds were fed for 112 days with feeds containing equal protein, energy, and amino acid levels, and different zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), iodine (I), and selenium (Se) level.

The inorganic trace mineral supplement contained (T1 - 100% ITM) 54, 54, 72, 10, 0.61, and 0.30g/kg of Zn, Fe, Mn, Cu, I, and Se, respectively, and the organic trace mineral supplement (carboaminophospho-chelate) contained 30, 30, 40, 6, 0.61, and 0.3g/kg de of, Fe, Mn, Cu, I, and Se, respectively. Both inorganic and organic trace mineral supplements were manufactured by Tortuga Companhia Zootécnica Agrária, for research purposes, and its guaranteed levels aimed at supplying the recommended nutritional requirements of layers (Table 1).

A level of 0.10% of inorganic trace mineral supplement was added to the feed (100% ITM) and in order to obtain similar trace mineral concentration, 0.18% of organic trace mineral supplement were added, obtaining 100% OTM in the feed. Based on this value, OTM inclusion levels were calculated as 0.198, 0.162, 0.144, and 0.126%, corresponding to the treatments of 110, 90, 80, and 70% dietary OTM inclusion, respectively.

The dietary levels of each trace mineral as a function of treatment are presented in Table 2.

Table 1 - Composition of the trace mineral supplements.

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

Table 2 - Trace mineral supplementation according to treatment and dietary inclusion levels.

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

ITM = inorganic trace minerals; OTM = organic trace minerals.

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

In order to study the individual effects of trace mineral sources and storage temperature and time, a completely randomized experimental design in a split-plot arrangement, consisting of 60 treatments with four replicates each, was applied. Treatments consisted of the combination of inorganic trace minerals and organic trace mineral levels (100% ITM, 110, 100, 90, 80 and 70%OTM) versus two storage temperatures (room temperature and refrigeration) randomized in the plots, and the five storage times (0, 3, 7, 10, and 14 days) were used as subplots.

At the end of the experimental period, 240 egg were collected, out of which 48 were used as controls and analyzed two hours after collection. The 192 remaining eggs (96 belonging to the two other plots) were identified, placed on carton trays, and stored either at environmental temperature or placed in a fridge. At 3, 7, 10, and 14 days of storage, 24 eggs per temperature (4 eggs per trace mineral source) were submitted to external and internal egg quality analyses.

The following parameters were evaluated: egg weight (EW), specific gravity (SG), yolk (Y %), albumen (A%) and eggshell (ES%) percentages, yolk index (YI), and Haugh units (HU).

Specific egg gravity was determined by immersing the eggs in saline solution with densities ranging between 1,060 and 1,100, with 0.005 variations. Gravity was checked using a densimeter.

In order to determine Haugh units, eggs were broken on a glass plate, and the albumen was measured in triplicate in the median region, between the external

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

Ingredients (%)		OTM supplementation level (%)[1]				
	100% ITM	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
Soapstock	1.00	1.00	1.00	1.00	1.00	1.00
Calcitic limestone	7.73	7.73	7.73	7.73	7.73	7.73
Mineral supp.	0.100*	0.198**	0.180**	0.162**	0.144**	0.126**
Vitamin supp. (***)	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
Available 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
Zinc, mg/kg feed	81.48	86.87	81.47	76.07	70.68	65.28
Fe, mg/kg feed	121.14	126.5	121.11	115.71	110.32	104.93
Mn, mg/kg feed	89.35	96.54	89.34	82.15	74.95	67.75
Cu, mg/kg feed	16.72	18.6	17.52	16.44	15.36	14.28
Se, mg/kg feed	0.45	0.74	0.69	0.64	0.58	0.53

*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/kg feed: Vit A 7,520,000 IU, Vit. D3 1,816,000 IU, Vit. E 8400 mg, Vit. K3 1,280 mg, Vit. B1 1,340 mg, Vit. B2 3,000 mg, Vit. B6 1,660 mg, Vit B12 8,000 mg, nicotinic acid 20,000 mg, calcium pantothenate 8,000 mg, folic acid 300 mg, biotin 40 mg.

edge and the yolk, using a micrometer. The average of the three measurements was applied to the equation of 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 the difference from whole egg, yolk, and dry eggshell weight. Albumen percentage was determined as the ratio between albumen weight and egg weight multiplied by 100.

Yolk percentage was calculated as the ratio between yolk weight and egg weight multiplied by 100. Yolk quality was evaluated by measuring yolk height (YH) and yolk width (YWd), which were used to calculate the yolk index (YI): YI = YH/YWd.

Eggshells were washed under running water, dried at room temperature for 48h, and then weighed in digital scale. Eggshell percentage was calculated as the ratio between egg weight and dry eggshell weight multiplied by 100. Eggshell thickness was determined in three different regions using a special micrometer (Mitutoyo), with 0.01mm precision. These determinations were performed according to Souza et al. (1984).

The effects of diets, storage temperature and storage time on the studied parameters were evaluated by analysis of variance using a slip-plot in time model, and considering possible significant interactions among the factors included in the model. The effects of storage temperature on the parameters were compared by the F test, and of diets by the test of Tukey, both at 5% probability level. The effect of time on the parameters was estimated by polynomial equations, considering the significant results obtained in the regression analysis. All statistical analyses were processed using Statistical Analysis System (SAS, 2000).

RESULTS AND DISCUSSION

The temperature of eggs stored under refrigeration was 7ºC, whereas minimum and maximum temperatures of eggs stored at room temperature were 21 and 24ºC, respectively.

There was no effect of diets on the quality of eggs stored for 14 days at room temperature or under refrigeration. This may be explained by the possible excessive supplementation of trace minerals. The levels of the studied trace minerals, including those obtained from the organic source may have been higher than those required to optimize egg quality. According to Bertechini (2003), the trace mineral inclusion levels in layer diets used in Brazil are higher than the birds' requirements. This occurs because there are few studies on layer trace mineral requirements: only 1.5% of the studies carried out in the last 15 years focused on this subject, possibly due to the low relative cost of these nutrients (Leeson, 2003).

The few studies in literature relative to the effects of dietary supplementation of organic trace minerals on the quality of eggs stored at different temperatures and for different periods present controversial results. This may be explained not only by the possible excessive trace mineral supplementation, but also to the utilization of different organic sources and different inclusion levels. Scatolini (2007) evaluated supplements with inorganic and organic Mn, Zn, Se, Fe, and Cu, and Mn, Zn and Se tested individually and/or combined, on the quality of eggs stored for 14 days at environmental temperature. He observed that organic trace minerals allowed maintaining egg weight during the experimental period, and eggs from layers fed a combination of organic Mn and Zn loss less weight ($p<0.05$) the eggs of layers fed organic Zn and Se, but were not different from the other treatments. The treatment with organic Mn presented the worst Haugh unit results, and was different only from the treatment containing the combination of organic Mn and Se. There was no influence of treatments on yolk index. Correia et al. (2000) fed layers with feeds supplemented or not with organic selenium and did not observe any effect on the external or internal egg quality of eggs stored at environmental temperature for up to 21 days, which is consistent with the results of the present study.

There was a significant effect of the interaction trace mineral source x temperature x storage time on Haugh units. The regression equations are shown in Table 4.

When eggs were stored under refrigeration there were no significant differences in Haugh units during the 14-d storage period, independently from trace mineral sources or level. The treatment with 70% organic trace mineral supplementation presented similar results as the other treatments during the entire storage period. However, Haugh units of eggs stored at room temperature were linearly reduced ($p<0.05$) with storage time, independently of trace mineral sources or levels, and the lowest values were observed at 14 days of storage. Haugh units decreased faster in eggs of hens supplemented with 100% inorganic trace minerals, with the lowest value obtained at seven days of storage. However, the 0.62 coefficient of determination calculated for this treatment shows a wide data dispersion. The other treatments presented similar reduction of the Haugh units during the storage period, and the supplementation of trace mineral sources and

Table 4 - Regression equations of Haugh units as a function of trace mineral sources and levels of eggs stored for 14 days at room temperature or under refrigeration.

Trace mineral source	Temperature	Equations	R^2
100 % ITM[1]	Room temperature	Y = 79.86 - 3.33X	0.62
	Refrigeration	NS*	
110 % OTM[2]	Room temperature	Y = 71.34 - 2.67X	0.79
	Refrigeration	NS	
100 % OTM	Room temperature	Y = 82.03 - 2.92X	0.86
	Refrigeration	NS	
90 % OTM	Room temperature	Y = 80.81 - 2.71X	0.76
	Refrigeration	NS	
80 % OTM	Room temperature	Y = 79.20 - 2.75X	0.85
	Refrigeration	NS	
70 % OTM	Room temperature	Y = 80.12 - 2.86X	0.79
	Refrigeration	NS	

1 - ITM = inorganic trace minerals. 2 - OTM = organic trace minerals. *NS = non significant (p>0.05).

levels did not attenuate the reduction of Haugh units during storage of eggs stored at room temperature.

These results indicate that refrigeration reduces the adverse effect of storage time on egg quality, independently of trace mineral sources or levels fed to the hens, and consequently, increase the shelf life of fresh eggs. Although the importance of storing eggs under refrigeration, as their internal quality is preserved, is already well-documented in scientific literature, this is not a common practice among egg suppliers. According to Leandro et al. (2005), 92% of the eggs in the Brazilian market are sold at room temperature, and no refrigeration is used from farm to retail.

Significant effects of the interaction storage time x temperature were observed on yolk index, Haugh units, and egg specific gravity. The regression equations and mean results are presented in Tables 5 and 6, respectively.

There was a linear negative effect of time (days) on Haugh units and specific gravity at both temperatures and on yolk index only in eggs stored at room temperature (Table 5).

Specific gravity decreased with storage time at both temperatures. However, on day 7 of storage, egg stored at room temperature presented significantly higher values (Table 6). Equal yolk index values were observed at both temperatures until day 3 of storage, after which egg stored under refrigeration presented better yolk index. Haugh units de eggs stored at room temperature linearly decreased, and significant differences between temperatures were observed after day 3.

Therefore, temperature and storage time considerably influenced yolk index and Haugh units, with eggs stored under refrigeration presenting the best results.

The obtained Haugh unit results are consistent with those of Oliveira (2006), Barbosa et al. (2004), and Véras

Table 5 - Regression equations of yolk index, Haugh units, and specific gravity evaluated on days 0, 3, 7, 10, and 14 of storage at room temperature or under refrigeration.

Parameter	Source of variation	Equations	R^2
Yolk index	Time/room temp.	Y = 0.427 - 0.0058X	0.92
	Time/refrigeration	NS*	-
Haugh units	Time/room temp.	Y = 78.93 - 2.83X	0.88
	Time/refrigeration	Y = 80.78 - 0.667X	0.63
Specific gravity (g/ml)	Time/room temp.	Y = 1.083 - 0.001089X	0.95
	Time/refrigeration	Y = 1.083 - 0.001148X	0.93

*NS: non significant (p>0.05).

Table 6 - Mean specific gravity, yolk index and Haugh unit results of eggs stored for 0, 3, 7, 10, and 14 days at room temperature or under refrigeration.

Parameter	Temperature	Storage time (days)				
		0	3	7	10	14
Specific gravity	Room	1.082	1.082	1.077*	1.072	1.068
	Refrigeration	1.085	1.080	1.073	1.074	1.068
Yolk index	Room	0.42	0.42	0.38*	0.38*	0.34*
	Refrigeration	0.42	0.42	0.42	0.44	0.43
Haugh units	Room	82.23	69.43*	50.41*	57.11*	39.23*
	Refrigeration	84.36	75.81	73.80	73.75	73.48

*Significant by the F test at 5% probability for means in the same column for each parameter.

et al. (1999), who observed that increasing storage periods, both at room temperature and refrigeration, cause a reduction in Haugh units, with worse results for eggs stored at room temperature. Jones & Musgrove (2005) analyzed storage time of eggs maintained at 4 ºC and verified that Haugh units significantly decreased after the second week of storage.

Abdallah et al. (1993) reported that egg specific gravity was directly related to eggshell percentage and breaking strength and that it can be used as an indirect method to determine eggshell quality. According to Sauver (1993), egg specific gravity is linearly reduced, in approximately 0.0016 units per day, at room temperature (15 to 22 ºC). However, in the present study, egg specific gravity decreased independently from storage temperature, with no effect on external egg quality. Similar results were obtained by other authors (Jordão Filho et al., 2006; Carvalho et al., 2003), who observed that storage temperature and time influenced internal egg quality, but had no effect on their external quality.

There was an isolated effect ($p<0.05$) of storage time on the parameters specific gravity, albumen, yolk, and eggshell percentages, yolk index, and Haugh units. There was a linear effect on specific gravity, albumen percentage, yolk index, and Haugh units, which decreased ($p<0.05$) along the 14 days of storage, whereas yolk percentage increased ($p<0.05$). There was a quadratic effect on eggshell percentage (Table 7).

Yolk index and albumen percentage, parameters related to internal egg quality, significantly worsened along storage time. Yolk percentage was inversely proportional to albumen percentage. This occurs because, at lay, there is an osmotic pressure gradient between the albumen and the yolk that progressively increases as water is transferred from the albumen to the yolk (Silversides & Budgell, 2004; Sauveur, 1993), weakening the vitelline membrane (Moreng & Avéns, 1990). In the beginning, this transference is slow (10mg/day at 10 ºC). However, transference rate depends on temperature: at 30ºC the transference occurs in 30 days, but at 10ºC, it occurs in 120 days (Sauveur, 1993). The migration of water to the yolk is related to the large protein molecules that enter the yolk by osmosis (Barbosa et al., 2004). Environmental temperature is one of the main factors that cause egg moisture loss as storage time increases (Stadelman & Cotterill, 1995). During the storage period, yolk moisture content ranges between 46 and 59%, depending on storage time and conditions (Ordónez, 2005; Souza-Soares & Siewerdt, 2005). Carvalho et al. (2003) found higher yolk percentage in eggs stored for more than nine days, whereas in the present study, there was a linear increase in yolk percentage with storage time.

Haugh units and egg specific gravity values significantly changed during storage. This is explained by the fact that, immediately after lay, the egg still does not have the egg chamber and higher albumen height, and consequently, higher Haugh unit values, and better egg quality (Carbó, 1987). As the egg cools, its content retracts and air enters the eggshell pores, creating the egg chamber, located in the widest end of the egg. The chamber continues to increase in size due to water loss to the external environment during storage, and consequently, egg specific gravity is reduced (Oliveira, 2006).

Similar results were obtained by Oliveira (2006), Vêras et al. (1999), and Brugalli et al. (1998), who evaluated the internal quality of eggs stored in two different environments (natural and refrigerated) for different periods, and observed a reduction in Haugh units and specific gravity as storage time increased.

There was a quadratic increase in eggshell percentage during storage at room temperature. The highest value was obtained on day 10 of storage. This was probably due to the higher ($p>0.05$) egg weight loss in the first 10 days of storage, as eggshell weight remains constant. The egg and the albumen loose weight during storage to moisture and CO_2 loss through the eggshell (Silversides & Budgell 2004; Solomon, 1991). Silversides & Scott (2001) observed an increase in eggshell percentage after the third day of storage in eggs stored at room

Table 7 - Effect of storage time on the external and internal egg quality of eggs of second-laying cycle semi-heavy layers fed diets supplemented with different trace mineral sources and levels.

Param.	Storage time (days) 0	3	7	10	14	Mean	CV (%)	Equations	R2
YI (1)	0.42	0.42	0.40	0.41	0.38	0.41	7.28	Y=0.42-0.00263T	0.92
Y (%)	24.18	24.93	25.25	26.12	25.52	25.52	9.07	Y=24.15+0.200T	0.97
A (%)	66.94	65.87	65.47	64.39	63.63	65.26	4.01	Y=66.82-0.229T	0.97
HU	83.30	72.62	66.93	62.11	56.35	68.26	15.00	Y=80.69-1.82T	0.88
ES (%)	8.88	9.20	9.28	9.49	9.27	9.22	1.031	$Y=8.89+0.11T-0.0058T^2$	0.91
SG	1.083	1.081	1.075	1.073	1.068	1.076	0.49	Y=1.08-0.00109T	0.98

1 - YI = yolk index, Y (%) = yolk percentage; A (%) = albumen percentage, HU = Haugh units, ES (%) = eggshell percentage, SG = specific gravity.

temperature for 10 days. Santos (2005) found higher eggshell percentage in eggs stored at room temperature for more than 14 days. On the other hand, Oliveira (2006) did not observe any variation in eggshell percentage of eggs stored at room temperature for 30 days.

There was an isolated effect of storage temperature on egg weight and yolk and albumen percentages. Mean results are presented in Table 8.

Table 8 - Effect of temperature during 14 days of storage on the egg quality of eggs of second-laying cycle semi-heavy layers fed diets supplemented with different trace mineral sources and levels.

Temperature	Parameters		
	Egg weight	Yolk percentage	Albumen percentage
Room	66.40 B	25.81 A	64.90 B
Refrigeration	67.59 A	25.22 B	65.62 A
Mean	66.99	25.52	65.26
CV (%)	8.44	9.07	4.01
Probability	0.049	0.01	0.00

A, B Different letter in the same column indicate significant differences by the F test ($p<0.05$).

Eggs stored under refrigeration lost less ($p<0.05$) weight as compared to those stored at room temperature (Table 8). Room temperature is one of the main factors that cause egg moisture reduction as storage time increases (Stadelman & Cotterill, 1995). Weight loss is caused by the reduction in albumen water content, which linearly decreases with storage time, and is significantly higher in eggs stored at room temperature (Santos, 2005). Egg water loss occurs by evaporation and varies as a function of storage time, environmental temperature, air relative humidity, and eggshell porosity.

Jordão Filho et al. (2006) and Oliveira (2006) did not observe any weight loss in eggs stored for 28 days at 5 ºC and 30 days at 6 ºC, respectively. However, significant egg weight losses were observed on day 5 in eggs stored at room temperature (Silversides & Scot, 2001; Oliveira, 2006), and at 15 days of storage 25 ºC, eggs lost 5% of their weight (Oliveira, 2006).

Similarly to the whole egg, albumen percentage was also reduced, whereas yolk percentage increased, and the worst results were observed in eggs stored at room temperature. The higher moisture loss in the albumen of eggs stored at higher temperatures causes the transference of water to the yolk, which weight consequently increases (Oliveira, 2006). According to Brake et al. (1997), reducing storage temperature results in a decrease in the movement of water from the albumen to the yolk. Cepero et al. (1995) concluded that the higher the storage temperature, the higher the reduction in albumen weight and the higher the increase in yolk weight.

The adequate balance of feeds, with consequent reduction of mineral excretion in the environment is an increasing concern of farmers and consumers, and it is becoming one of the most important social movements of all times. The social and environmental actions of a company are relevant factors in the purchase decision of consumers. Organic trace minerals are more bioavailable, and therefore, excreted in lower amounts by poultry. The results obtained in the present study showed that organic trace minerals in the form of carboaminophospho-chelates can be used in lower concentrations in layer feeds without affecting the quality of eggs stored at different for a period up to 14 days. However, the quality of commercially available organic trace mineral products may be different according to manufacturer and source, and therefore, studies must be performed to determine which products better supply the needs of poultry producers.

CONCLUSIONS

Under the conditions of the present study, it was concluded that the supplementation of organic trace minerals in levels lower than those of inorganic sources presented advantages. The lowest level, corresponding to the treatment 70% OTM, containing 37.8, 37.8, 50.4, 7.56, 0.77, and 0.38mg/kg of zinc, iron, manganese, copper, iodine, and selenium, respectively, supplemented to a diet based on corn and soybean meal fed to semi-heavy Hy-line Brown layers in their second laying cycle, can be used in layer feeds as it maintained the quality of eggs stored for 14 days at room temperature or under refrigeration.

Considering storage time and temperature, it was verified that storage time caused higher internal quality losses of eggs maintained at room temperature. This situation is similar to those found in most sales points in Brazil, where storage temperature and humidity are not controlled. This demonstrates that, despite being well-documented in scientific literature, this practice is not applied by suppliers. Some measures, such as the implementation and control of egg storage rules in retail, must be taken to ensure egg quality to the consumers.

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