Original Article

Effect of Supplementary Zinc from Organic and Inorganic Sources on Laying Performance and Zinc Deposition in Eggs

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Abstract

The experiment was conducted to investigate the effect of supplementary organic and inorganic zinc on the laying performance and zinc deposition in eggs. One hundred and eighty laying hens (ISA Brown), aged 36 weeks, were randomly divided into 5 treatments with 4 replications of 9 hens each. A corn-soy based diet which was formulated to meet the recommended nutrient requirement especially the level of zinc (60 μg Zn/g diet) was used as the control diet. Both sources of zinc, inorganic (zinc sulfate) and organic (zinc amino acid chelate) were supplemented to provide zinc at levels of 300 and 600 μg/g diet, respectively. Supplementation of both the forms and levels of zinc in hens' diet had no effect on egg production, egg weight, the amount of feed consumed and feed per kg egg (p>0.05). Increasing the zinc level in the diet increased zinc deposition in the egg yolk (p<0.01). Ninety-nine percentage of the zinc was deposited in the egg yolk. A very low level of zinc was deposited in the egg white and the level seemed to be constant. No significant difference in the level of zinc deposition in the yolk was found between the organic and inorganic form of zinc. The forms and the levels of zinc supplemented also did not affect the quality of egg.

Keywords : eggs, hens, sources, zinc

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บทคัดย่อ

ผลของการเสริมสังกะสีจากสังกะสีอนินทรีย์และอินทรีย์ต่อสมรรถภาพการผลิตของไก่ไข่และการสะสมสังกะสีในไข่ไก่

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การทดลองครั้งนี้มีวัตถุประสงค์เพื่อศึกษาถึงผลของการเสริมสังกะสีอนินทรีย์และสังกะสีอินทรีย์ในอาหารไก่ไข่ต่อสมรรถภาพการผลิตและปริมาณสังกะสีที่สะสมในไข่ไก่ โดยใช้ไก่ไข่ทดลองจำนวน 180 ตัว อายุ 36 สัปดาห์ แบ่งออกเป็น 5 กลุ่มๆ ละ 4 ซ้ำๆ ละ 9 ตัว ไก่ไข่กลุ่มควบคุมจะได้รับอาหารที่มีปริมาณสังกะสีตามความต้องการสารอาหารคือ 60 ไมโครกรัมต่อกรัมของอาหาร ไก่ไข่ 4 กลุ่มที่เหลือจะได้รับอาหารกลุ่มควบคุมเสริมด้วยสังกะสีอนินทรีย์ (สังกะสีซัลเฟต) และสังกะสีอินทรีย์ (สังกะสีกรดอะมิโนคีเลต) ให้มีสังกะสีในระดับ 300 และ 600 ไมโครกรัมต่อกรัมของอาหาร ตามลำดับ ผลการทดลองพบว่าแหล่งและระดับของการเสริมสังกะสีในอาหารไม่มีผลต่อผลผลิตไข่ น้ำหนักไข่ ปริมาณอาหารที่กินและปริมาณอาหารที่ใช้ต่อไข่ 1 กิโลกรัม ($p>0.05$) การเพิ่มปริมาณสังกะสีในอาหารทำให้การสะสมสังกะสีในไข่เพิ่มขึ้น ($p<0.01$) โดยที่ร้อยละ 99 อยู่ในไข่แดง ขณะที่ในไข่ขาวมีปริมาณน้อยมากและค่อนข้างคงที่ ไม่พบความแตกต่างของปริมาณสังกะสีที่สะสมในไข่แดงระหว่างการใช้สังกะสีอนินทรีย์และอินทรีย์ นอกจากนั้นแหล่งและระดับของสังกะสีที่เสริมไม่มีผลต่อคุณภาพของไข่ไก่

คำสำคัญ : ไข่ไก่ ไก่ไข่ แหล่ง สังกะสี

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Introduction

Zinc is one of several micronutrients that have attracted increased attention because of their important role in maintaining health and nutrients condition. It is essential for physiologic and metabolic functions such as physical growth, immuno-competence, reproductive function, and neurobehavioural development (Hotz and Brown, 2004). Zinc is widely available in food sources but its bioavailability from different foods is highly variable. Many plant foods, especially cereal grains and legumes, have a high content of zinc but the bioavailability is low due to the presence of phytate. The richest food sources of zinc are animal products, particularly the organs and flesh of beef, pork and shellfish (Brown and Wuehler, 2000; Hotz and Brown, 2004). These foods do not contain phytate. As a result, they are particularly good sources of absorbable zinc but they tend to be expensive. Therefore, the production of zinc-enriched food at an affordable price should be the most effective way to contribute a zinc source for consumers.

Hens' eggs are one of the common foodstuffs with a concentrated source of nutrients. Egg protein is highly digestible and is recognized as a reference protein due to its completed amino acids profiles (Watson, 2002). It is relatively inexpensive and naturally free of phytate. However, it has a slightly lower zinc content than organs and all kinds of meat (Brown and Wuehler, 2000). The total zinc content of eggs from hens consuming normal laying rations is generally 10-11 µg Zn/g egg (Puwastien et al., 1999; Watson, 2002). Some researchers have reported that the zinc content of hens' eggs can be increased by supplementing zinc in laying hens' diet (Stahl et al., 1988; Mabe et al., 2003; Guo et al., 2006). Furthermore, some information has suggested that some form of organic zinc, such as zinc amino acid chelates or complexes can improve the bioavailability of minerals for animals (Spears, 1989; Wedekind et al., 1992). However, excess zinc can produce toxic effects. In most studies reviewed

(NRC, 1980), no any adverse effects occurred when dietary zinc concentration was not over than 600 μg Zn/g diet. Therefore, the zinc level used in this study was not higher than this safety level.

The present study was conducted to determine the effect of supplementing organic and inorganic zinc on laying performance and zinc deposition in eggs.

Materials and Method

Experimental Diets and Animals: One hundred and eighty laying hens (ISA Brown), aged 20 weeks, were purchased from Charoen Pokphand, Co. Ltd. All the hens were fed with an ordinary commercial diet until the experiment started at the age of 36 weeks. The laying hens were randomly divided into 5 treatments with 4 replications of 9 hens each, three hens per cage (40x40x 35 cm). A corn-soy based diet formulated to meet the recommended requirement (Leeson and Summers, 1997) was used as the control diet. Zinc level in basal diet was analyzed (35 μg/g) and zinc sulfate at the level of 25 μgZn/g diet was added to fulfill hen's requirement. Therefore, zinc level in control diet was totally 60 μg/g diet. Both sources of zinc, inorganic (zinc sulfate) and organic (zinc amino acid chelate) were supplemented to provide zinc at levels of 300 and 600 μg/g diet, respectively. Thus 4 treatments were performed. The ingredients and calculated nutrient composition of the basal diet are shown in Table 1. The experimental diets were prepared and presented in a mash form. The diet and water were provided *ad libitum* throughout the experimental period (60 days). The experimental diets were fed twice a day at 08.00 h and 13.00 h. All hens were reared in an evaporative cooling system with a lighting programme, 16 hours per day. The temperature in the hen house was recorded every day at 13.00 h ranging

Table 1 Ingredient composition and calculated nutrient content of basal diet

Ingredients	Composition (kg/100 kg)
Corn	59.43
Ricebran	4.30
Soybean meal	24.90
Limestone	8.80
Monodicalciumphosphate	1.80
Salt	0.30
DL-Methionine	0.11
Choline	0.11
Premix (vitamins and trace minerals)*	0.25
Total	100
Calculated nutrient content	
Protein (kg/100 kg)	17.02
Crude fat (kg/100 kg)	3.10
Crude fibre (kg/100 kg)	2.69
Metabolized energy (ME) Mcal/kg	2.75

*Vitamin and trace mineral premix per kilogram of diet provided: vitamin A 10,000 IU; vitamin D_3 2,000 IU; vitamin E 20 IU; vitamin K_3 2 mg; vitamin B_1 2 mg; vitamin B_2 5 mg; vitamin B_6 5 mg; vitamin B_{12} 10 mg; niacin 30 mg; panthothenic acid 10 mg; folic acid 0.74 mg; zinc 25 mg; copper 10 mg; iron 120 mg; manganese 60 mg; selenium 0.2 mg.

from 27 to 29°C during the experimental period.

Sample Collection and Preparation: Egg production and egg weight were recorded daily and feed consumption was recorded weekly. After 8 weeks, ten eggs per replication were collected. The eggs were rinsed with deionized water before the preparation of composite samples. Five eggs of each replication (from 20 individual replications) were prepared as egg yolk and egg white. Thus, 20 composite samples of egg yolk and 20 composite samples of egg white were obtained. The samples were thoroughly homogenized with a blender at low speed and then were lyophilized. The lyophilized samples were packed in sealed aluminum foil bags and kept in the refrigerator at -20°C until analysis for the zinc content.

Another set of composite samples of five eggs each were combined and prepared as a composite sample of the whole egg. Thus, 20 composite samples of whole egg were obtained. Main nutrients, i.e., protein, fat, ash and cholesterol, were measured in the lyophilized composite samples of the whole eggs.

Chemical analysis: Lyophilized composite samples of egg yolk and egg white were analyzed for their zinc content as followed; 0.5 g of the lyophilized sample was digested by wet digestion using 5 ml of concentrated nitric acid (65%) and 1 ml of perchloric acid (78%). Digestion took place in a closed Teflon vessel at 100°C until a clear solution (about 6 h) was obtained. Each composite sample was analyzed in duplicate. The zinc content in the digested samples was determined by an inductively coupled plasma optical emission spectrometer (Perkin Elmer: Optima 4200 DV, USA) (Clegg et al., 1981).

The lyophilized composite sample of whole eggs was analyzed for main nutrients and cholesterol, in duplicate. Protein was determined by Kjeldahl method using conversion factor of 6.65. Total fat was determined by acid digestion prior to continuous extraction using petroleum ether in Soxtec system. Ash was determined by dried ash in a muffle furnace at 550°C. Moisture content was determined by drying the sample in a hot air oven at 100°C until constant weight was obtained. Cholesterol was determined by gas-liquid chromatography (AOAC, 1990).

Statistical analysis: All dependent variables were performed as a completely randomized design to determine the effect of treatment groups. The dependent variables, production performance and zinc concentration in yolks and whites, were analyzed using a one-way analysis of variance. The difference between treatments was determined by Duncan's New Multiple Range test at the level of $p<0.05$ (SAS, 1985).

Results

The effects of the level and form of zinc on the performance of laying hens are shown in Table 2. Supplementation of zinc in hen diet at levels of 300 to 600 μg Zn/g had no effect on egg production, egg weight, the amount of feed consumed and feed per kg egg. There was no significant difference in all parameters among treatments.

The production performance of hens fed with supplemented inorganic zinc was no different from those of hens fed with supplemented organic zinc. The egg production of hens fed diets supplemented with 300 to 600 μg Zn/g diet and the control diet ranged from 91 to 95%, and the average weight of eggs ranged from 61 to 63 g, respectively. Feed intake and feed per kg egg of hens from all treatments ranged from 110 to 113 g/d and 1.90 to 1.97, respectively.

As depicted in Table 3, the concentration of zinc in egg yolks increased as dietary zinc concentration in the diet increased from 60 to 600 μg Zn/g diet. The zinc content of egg yolk from hens fed the supplemented zinc at a level of 600 μg Zn/g diet was significantly higher than the control fed group ($p<0.05$). There was no significant difference in the zinc content of egg yolk between hens fed diets supplemented with inorganic and organic zinc. Hens fed with the highest zinc level diet (600 μg/g) produced the highest zinc concentration egg yolks (43 μg/g); while hens fed with the control diet contained the lowest zinc levels (60 μg/g) produced the

Table 2 Production performance of laying hens fed with different forms and levels of supplemented zinc for 8 weeks(1)

Parameters	control	Inorganic Zn 300	Inorganic Zn 600	Organic Zn 300	Organic Zn 600	*P-value*
Egg production (%)	92.9 ± 2.1	92.0 ± 4.8	95.1 ± 1.2	92.1 ± 1.3	90.6 ± 5.9	0.5268
Egg weight (g/egg)	62.5 ± 0.7	62.6 ± 1.1	62.3 ± 0.9	62.9 ± 1.3	60.6 ± 1.9	0.1307
Feed intake (g/d)	111.0 ± 5.5	111.9 ± 2.0	112.9 ± 2.9	111.6 ± 4.2	110.3 ± 1.7	0.8752
Feed/kg egg	1.93 ± 0.1	1.95 ± 0.1	1.90 ± 0.1	1.93 ± 0.1	1.97 ± 0.1	0.3028

(1)mean±SD

Table 3 The concentrations of zinc in egg yolk and egg white

Parameters	control	Inorganic Zn 300	Inorganic Zn 600	Organic Zn 300	Organic Zn 600	*P-value*
Zn in egg yolk						
Concentration (µg/g)(1)	39.5 ± 1.7^{a}	41.8 ± 1.4^{a,b}	43.7 ± 1.6^{b,c}	41.2 ± 1.4^{a,b}	43.4 ± 1.6^{b,c}	0.0076
Yolk/whole egg (%)	98.7	99.3	98.6	98.6	98.9	
Zn in egg white						
Concentration (µg/g)(1)	0.20 ± 0.07	0.22 ± 0.03	0.24 ± 0.08	0.25 ± 0.07	0.20 ± 0.07	0.77
White/whole egg (%)	1.3	1.3	1.4	1.4	1.1	

(1)mean±SD

$^{a-c}$Means in the row with different superscripts are significantly different ($p<0.05$)

Table 4 Main nutrients and cholesterol levels in whole eggs

Nutrients (unit) per 100 g fresh weight	control	Inorganic Zn 300	Organic Zn 600	Organic Zn 300	Organic Zn 600
Moisture (g)	76.3	76.5	76.4	76.8	76.4
Protein (g)	13.1	13.1	13.1	12.9	13.0
Fat (g)	8.85	8.67	8.49	8.66	8.69
Ash (g)	0.87	0.84	0.86	0.84	0.83
Cholesterol (mg)	385.8	371.6	392.1	384.0	379.8

lowest zinc concentration egg yolks (39.5 µg/g). Zinc concentration in the whites of eggs from all studied groups was less than 0.3 µg/g.

Supplementation of zinc in the hens' diet did not affect the main nutrients and cholesterol in eggs of the current study. The data presented in Table 4 shows similar levels of protein, fat, ash, moisture and cholesterol in eggs obtained from hens fed the diets supplemented with various levels and forms of zinc and those fed the control diet.

Discussion

The production performance of laying hens; egg production, egg weight, feed intake and feed per kg egg, was not affected by the supplementation of either inorganic or organic forms of zinc at levels of 300 and 600 µg/g in the diet which is supported by Stahl et al.(1988). They concluded from two experiments that egg production and egg weight were not affected with a supplement of inorganic zinc from zinc sulfate of 257 µg/g diet. Guo et al. (2006) also reported no effect on feed intake, egg production, egg weight and feed per kg egg in laying hens fed diets supplemented with inorganic zinc (zinc sulfate) and organic zinc (zinc amino acid complex) at a level of 190 μg Zn/g diet. In this study, although zinc level in the diet increased to 600 μg/g, there was also no adverse effect on production performance. Previous studies have demonstrated that high zinc supplementation (3,000 to 20,000 μg Zn/g diet) could reduce the feed consumption and egg production of hens due to the anorexic effect of high zinc levels (Kim and Patterson, 2005; Palafox and Elodie, 1980). However, the purpose of using high zinc diet in those studies was not to study the improvement of production performance but for evaluate the effect of dietary zinc on reducing ammonia volatilization and molting. Nevertheless, in this study it revealed that supplementing 300 and 600 μgZn/g diet could not improve performance of animal. This is probably due to the zinc level in control diet is adequate for highest production in normal production condition.

An increase the amount of zinc deposition in egg yolk was observed with increasing levels of zinc in the diet. Egg yolk obtained from hens fed 600 μg Zn/g diet of both inorganic and organic sources, contained about 10.3% more zinc whereas hens fed with 300 μg Zn/g diet gave about 5.1% more zinc than egg yolk from hens fed the control diet. These findings are in agreement with previous studies (Stahl et al., 1988; Mabe et al., 2003; Guo et al., 2006). The increase in yolk zinc content probably involved the production of vitellogenin, a trace mineral transporting protein. Vitellogenin may be increased when zinc concentration increases because of the transfer of zinc which is stored in the liver to the yolk of the egg (Richards, 1997). However, the percentage increase of zinc in yolk in this study was lower than Stahl et al. (1988). It can increase 25% by increasing the level of zinc sulfate from 27 to 238 μg/g diet. The difference in the increasing percentage of zinc in eggs might be due to the amount of zinc present in the control diet or the amount of ligands in the diet. Zinc absorption is interfered with the presence of dietary ligands such as phytate, which form an insoluble complex with zinc (Camara and Amaro, 2003). Moreover, the present results also show no advantage in zinc deposition in yolk at the same level of organic over inorganic form which is similar to the result supported by Ammerman et al. (1995) but in contrast with Wedekind et al. (1992) and Guo et al. (2006). The same result of zinc deposition in yolk from both sources may relate to the absorption process of zinc. Hoadley et al. (1987) demonstrated that when the luminal zinc concentration was high, zinc was transport through non-mediated process in the brush border over a major route, a carrier-mediated process. Thus, the absorbable zinc from inorganic source might be greater and was not different from organic source, which was absorbed via the pathway of a low molecular weight peptide. Moreover, yolk might not be the main tissue to deposit zinc. A preferential accumulation of zinc was found in the liver and kidney (Verheyen et al., 1988). Unfortunately, zinc deposition in liver and kidney were not determined in the current study.

The deposition of zinc in egg yolk in the present study was about 99% of the total zinc in the whole egg. This is due to the fact that zinc is required for the development of the avian embryo (Richards, 1997). Therefore, egg yolk is a major source of zinc in eggs. Unfortunately, no information on the zinc content of egg white is available when zinc was supplemented in the laying hens' diets. However, the result in this study indicated that the zinc content in egg white was very low (0.2 μg/g egg) and seemed to be constant when both forms and levels were supplemented.

Moreover, the levels of main nutrients (protein and fat) and cholesterol in eggs were also analyzed. They were nearly the same values in all treatment groups. The levels of these components were within the normal range of general eggs, 12.9-13.1 g for protein, 8.5-8.9 g for fat and 370-390 mg for cholesterol per 100 g of whole egg content (Puwastien et al., 1999; Watson, 2002). This implies that the forms and the levels of zinc supplemented in this experiment did not affect the quality of egg. Therefore, the quality of egg obtained from hens fed diets supplemented with inorganic and organic forms of zinc at a level of 600 μg Zn/g diet can be a good source of zinc. It can contribute 21%, 16% and 13% of the recommended levels of the Thai Dietary Reference Intake for children age 4-5, 6-8 and 9-12 years, respectively (NDMoPH, 2003).

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