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EFFECTS OF DIETARY THIAMINE SUPPLEMENTATION ON PERFORMANCE, EGG QUALITY, AND ANTIOXIDANT-RELATED ENZYMES IN CHINESE EGG-LAYING DUCKS

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ABSTRACT

Thiamine, or vitamin B1, is an important water soluble vitamin in poultry nutrition due to its central role in the metabolism. Thus an experiment was designed to investigate the effects of dietary thiamine supplementation on performance, egg quality, and antioxidant-related enzymes in laying ducks. In total, 900 Longyan pullets 22wk-old with similar body weight were randomly assigned to six treatments (twenty five ducks per replicate pens and six replicates per treatment). The control group was fed a corn-soybean meal basal diet containing 0.93 mg thiamine/kg, while the other treatment groups were fed basal diets supplemented with 1.0, 2.0, 3.0, 4.0, or 5.0 mg thiamine/kg. The experimental data were analysed by one-way ANOVA as a single factor design. Dietary thiamine did not change egg production, egg weight, egg mass, FCR, shape index, Haugh unit, yolk color, egg composition, eggshell quality, and concentrations of antioxidant-related enzymes, but it elevated (linearly; $P< 0.02$) the lipid peroxidation. These findings indicate that corn-soybean meal diet could cover thiamine needs for Longyan egg-laying ducks at 22 to 42 wk of age.

Key words: Thiamine, laying ducks, lipid peroxidation.

INTRODUCTION

Avian species are unable to produce thiamine (vitamin B1; water soluble vitamin) (Weber, 2009). Thus, in birds, diet is the main source of thiamine. Thiamine is a component of thiamine pyrophosphate, which participates in oxidative decarboxylation of pyruvic acid and α-ketoglutaric acid. These reactions generate acetyl-coenzyme A (CoA) and succinyl-CoA, which are involved in carbohydrates, proteins, and lipids metabolism (Hamano *et al.,* 1999, Haas, 1988).

Feeding diet deficient in thiamine to birds leads to loss of appetite, low hatchability, low carcass yield, impaired carbohydrates, proteins and lipids metabolism and high mortality rate (Weber, 2009; Charles *et al.,* 1972). In juvenile fish and young grass carp fish, deficiency of thiamine depress growth performance, immune system, and increases oxidative damage as a result of increasing protein and lipid oxidation (Li *et al.,* 2014; Wen *et al,*. 2015; 2016). Effects of dietary thiamine supplementation on productive performance of meat-type birds have been demonstrated (Olkowski and Classen, 1996; 1999; Geyer *et al.,* 2000), but previous studies that investigated the effects of dietary thiamine supplementation in egg-layer birds are old and did not show its effects on laying performance or egg quality (Polin *et al.,* 1963). Moreover, NRC (1994) did not define thiamine requirements for egg-type ducks. To the best of our knowledge, no study has been designed to investigate the effects of dietary thiamine supplementation on performance, egg quality and antioxidant-related enzymes in egg-laying birds. Therefore, the main objective of this study was to evaluate the effects of dietary thiamine supplementation on performance, egg quality and oxidative status in egg-laying ducks.

MATERIALS AND METHODS

Animals and diets: All of the experimental procedures were accepted by the Animal Care and Use Committee of Guangdong Academy of Agriculture Science, China. A total of 900 Longyan ducks (a typical breed of egg-laying ducks in South China) with similar body weights (1.4±0.01 kg) were randomly assigned to six dietary treatments with six replicate pens in each, as described by Fouad *et al.* (2016a) during 22 - 42 wks of age. The same basal diet, in mash form (Table 1), was supplemented with 0.0 (control), 1.0, 2.0, 3.0, 4.0 and 5.0 mg thiamine in the form of thiamine mononitrate (98.0%, Jiangxi Tianxi Pharmaceutical Co. Ltd, Jiangxi, China) then pelleted and offered to the birds. The control diet was prepared to cover the nutritional requirements of Longyan egg-laying ducks (Fouad *et al.,* 2016b), with the exception of thiamine supplementation. Fresh water was available ad libitum, while feed was offered twice daily with an average of 160 g/bird/d without residue. The light program and housing system that have been applied were reported by (Xia *et al.,* 2017).

Performance and egg quality: Feed consumption, egg production, and feed conversion ratio (FCR) were recorded and calculated as described by Fouad *et al.* (2017). Three eggs were collected three time at random from each replicate during the experimental period to determine egg quality (egg shape index, Haugh unit, yolk color, egg composition, and eggshell thickness) as described by Ruan *et al.* (2015).

Blood and liver indices: At 42 wk of age, two ducks from each replicate were randomly chosen and sacrificed to collect blood (from the wing vein) and liver samples, as described by Ruan *et al.* (2015). The levels of total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) in plasma and liver were estimated with commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China; Fouad *et al.,* 2016c). Each sample was tested in duplicate.

Statistical analysis: One way ANOVA of SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) was used to analyze all data as a single factor design. The linear and quadratic effects of thiamine among treatments were examined using a contrast statement. Significant differences among treatments using Tukey post hoc test were examined and a significance level of $P<0.05$ was considered.

RESULTS AND DISCUSSION

Productive performance and egg quality: Dietary thiamine supplementation did not significantly affect egg production, egg weight, egg mass, FCR, or egg quality as present in Table 2 and 3. Our experiment was not the first experiment to evaluate the impacts of dietary thiamine supplementation on performance and egg quality in egg-laying ducks, but it is the first experiment to be conducted to specifically investigate the influences of dietary thiamine supplementation in egg-layer birds. Therefore, it is not possible to compare our findings with previously published studies. Earlier studies by Polin *et al.* (1963) with egg-type birds did not consider its effects on laying performance or egg quality. They demonstrated that feeding laying hen diets containing 0.68 mg thiamine/kg maximized hatchability. However, feeding laying hens diet contained 1.5 mg riboflavin/kg (diets deficient in riboflavin) resulted in a reduction in egg production and signs of riboflavin deficiency were noticed. Inclusion of 3.6 mg riboflavin/kg in their diets normalized egg production, while feeding laying hens diets with or without supplemental pantothenic acid up to 3.6 mg/kg had no impact on laying performance (Bootwalla and Harms, 1990; 1991). Also, in laying hens, increasing folic acid level up to 100 mg/kg did not affect laying performance (Tactacan *et al*., 2012).

Table 1. Composition of the basal diet and nutrient levels

Ingredients	Value (%)
Corn	31.90
Corn starch	20.10
Puffing soybean meal	27.85
Distillers dried grains with soluble	8.80
Limestone	8.62
Calcium hydrogen phosphate	1.27
DL-Methionine	0.118
L-lysineHCl	0.085
Sodium chloride	0.3
Premix[1]	1.0
Total	100.0
Nutritional value	
AME (Kcal/kg)	2500
Crude protein (%)	17.0
Calcium (%)	3.6
Total P(%)	0.59
Available Phosphorus(%)	0.35
Total Lysine (%)	0.90
Total Methionine (%)	0.40
Thiamine (mg/kg)	0.93

[1] Supplied per kilogram of diet: retinylpalmitate, 12000 IU; cholecalciferol, 2000 IU; DL-α-tocopheryl acetate, 38 mg; menadione sodium bisulphite, 1.0 mg; thiamine mononitrate, 0.0mg; riboflavin, 9.6 mg; pyridoxine hydrochloride, 6.0 mg cobalamin, 0.03 mg; chloride choline, 500 mg; nicotinic acid, 25 mg; calcium-D-pantothenate, 28.5 mg; folic acid, 0.6 mg; biotin, 0.15 mg; Fe, 50 mg; Cu, 10 mg;Mn, 90 mg Zn, 90 mg; I, 0.5 mg; Se, 0.4 mg.

In broiler chickens, Olkowski and Classen (1996) noted that increasing concentrations of thiamine from 0.0 to 32 mg/kg diet did not alter growth performance. Also, Geyer *et al.* (2000) found that adding 1.5 mg thiamine/kg from different sources during starter phase did not result in significant differences in average daily gain, feed consumption, or FCR in broiler chickens, while Olkowski and Classen (1999) recorded that increasing thiamine concentration from 0.0 to 32 mg /kg in broiler breeder's diets improved feed efficiency of their offspring during starter phase. In turkeys, Sullivan *et al.* (1967) observed that increasing level of thiamine than 1.2 mg/kg diet had no effect on growth performance, but formulating diets containing 0.8 mg thiamine/kg impaired growth performance, whereas feeding them diets prepared to contain 0.4 mg thiamine/kg resulted in exhibition of thiamine deficiency signs and 100% mortality rate. Previous studies showed that preparing the basal diet to contain 1.0 mg thiamine/kg or more did not lead to a significant change in productive performance of turkeys or broiler chickens (Sullivan *et al.,* 1967; Olkowski and Classen, 1996; Geyer *et al.,* 2000). In our experiment, the concentration of thiamine in the basal diet was 0.93 mg/kg, which corresponds to concentration of 0.93 mg thiamine/kg, is sufficient to cover thiamine requirements for productive performance and egg quality in laying ducks. May this explain why laying performance and egg quality did not affect by increasing thiamine levels in our experiment.

Oxidative status: Dietary thiamine supplementation had no effects on total antioxidant capacity, superoxide dismutase, and glutathione peroxidase in plasma or liver of laying ducks, while MDA in plasma was elevated with increasing thiamine levels (Table 4). The activity of antioxidant enzymes including SOD and GSH-Px declined and MDA level elevated by feeding young grass carp fish (*Ctenopharyngodonidella*) purified diets deficient in thiamine, but increasing thiamine concentration did not change antioxidant enzymes activities or MDA level compared with the optimal level in their diets (Wen *et al.,* 2015, 2016). However, in chickens, thiamine increased oxygen consumption rate and accelerated lipid oxidation (Hamano *et al*., 1999). Increasing oxygen consumption rate and activating lipid oxidation generate more reactive oxygen species which leads to increase MDA, the end product of lipid peroxidation, as a result of oxidative damage to cell membranes (Rey *et al*., 2010; Mujahid *et al*., 2009). Similar results have been reported in an earlier study by Hu *et al*. (1995) who found that lipid peroxidation (MAD) was elevated by increasing thiamine level. This may explain why increasing thiamine level increased MDA level and did not affect laying performance and egg quality in our experiment

Table 2. Effects of dietary thiamine supplementation on the performance of laying ducks

Item	Dietary thiamine (mg/kg)						SEM[1]	*P-value*		
	0.93	1.93	2.93	3.93	4.93	5.93		Level	Linear	Quadratic
Egg production, %	78.3	80.5	79.7	81.1	80.3	80.5	0.8	0.9	0.5	0.6
Egg weight, g	66.0	65.8	65.4	66.3	65.4	65.3	0.2	0.7	0.4	0.7
Egg mass, g	51.6	52.9	52.1	53.7	52.6	52.6	0.6	0.9	0.7	0.6
FCR	2.88	2.81	2.85	2.77	2.85	2.83	0.03	0.9	0.7	0.6

[1]Feed conversion ratio, g of feed/g of egg mass. [2]Standard error of means, n = 6.

Table 3. Effects of dietary thiamine supplementation on egg quality

Item	Dietary thiamine (mg/kg)							*P-value*		
	0.93	1.93	2.93	3.93	4.93	5.93	SEM[1]	Level	Linear	Quadratic
Egg shape index	73.5	73.5	73.9	74.2	74.2	73.6	0.1	0.6	0.4	0.1
Haugh units	75.0	74.9	74.5	74.6	74.9	74.6	0.3	0.9	0.7	0.7
Yolk colour	4.1	4.0	4.2	3.9	4.1	4.0	0.04	0.3	0.3	0.4
Yolk weight, g	20.5	20.4	20.8	20.7	20.5	20.5	0.2	0.9	0.9	0.4
Yolk,%	31.4	31.1	31.2	31.3	31.5	31.0	0.2	0.9	0.9	0.9
Albumin weight, g	39.1	39.3	39.0	39.1	39.9	39.2	0.2	0.9	0.9	0.9
Albumin,%	59.5	59.7	59.6	59.3	59.5	59.5	0.1	0.9	0.8	0.8
Eggshell thickness, mm	0.320	0.322	0.324	0.323	0.318	0.314	0.002	0.7	0.3	0.4
Eggshell strength, N	3.9	3.7	3.8	3.7	3.7	3.9	0.1	0.8	0.6	0.8
Eggshell weight, g	6.08	6.10	6.07	6.12	6.01	5.92	0.03	0.5	0.1	0.2
Eggshell, %	9.31	9.23	9.33	9.23	9.18	9.01	0.04	0.4	0.056	0.4

[1]Standard error of means, n = 18.

Conclusion: In conclusion, the basal diet that contained 0.93 mg thiamine/kg was sufficient to maximize laying performance and egg quality without affecting antioxidant defense system in Longyang laying ducks from 22 to 42 wk of age.

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Table 4. Effects of dietary thiamine supplementation on antioxidant indices in plasma and liver of laying ducks

Item	Dietary thiamine (mg/kg)						SEM[1]	*P-value*		
	0.93	1.93	2.93	3.93	4.93	5.93		Level	Linear	Quadratic
Plasma										
T-AOC, U/mL	17.7	19.7	18.3	19.3	18.6	18.5	0.6	0.9	0.9	0.6
T-SOD, U/mL	93.5	90.4	90.7	97.0	89.3	101.0	1.7	0.3	0.2	0.3
GSH-Px, U/L	321.1	344.7	310.8	314.8	315.3	385.2	9.8	0.2	0.2	0.1
MDA,nmol/mL	7.8	12.5	10.1	10.9	12.2	13.2	0.6	0.05	0.02	0.7
Liver										
T-AOC, U/mg prot	1.2	1.1	1.3	1.2	1.0	1.2	0.03	0.5	0.6	0.9
T-SOD, U/mg prot	254.8	251.9	261.9	242.9	246.1	241.5	2.6	0.2	0.1	0.5
GSH-PxU/mg prot	69.7	66.5	75.3	70.2	67.6	79.3	2.0	0.4	0.3	0.5
MDA,nmol/mg prot	0.96	1.25	0.98	1.15	1.26	1.23	0.04	0.1	0.1	0.8

T-AOC= total antioxidant capability; T-SOD= total superoxide dismutase; GSH-Px = glutathione peroxidase; MDA=malondialdehyde.
[1]Standard error of means, n = 12.

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