JAST (Journal of Animal Science and Technology) TITLE PAGE

Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research Article
Article Title (within 20 words without abbreviations)	Feeding of reduced vitamin premix negatively affects laying performance and vitamin contents in chicken eggs
Running Title (within 10 words)	Varying levels of vitamin premix to layer diet
Author	Yong Jun Choi [first_author] 1, Yong Ran Kim2, Eun Jip Kim2, Kwan Eung Kim3, Byoung Ki An4
Affiliation	1 School of Animal Life Convergence Science, Hankyung National University, Anseong 17579, Korea 2 Division of Animal Husbandry, Yonam College, Cheonan 31005, Korea 3 R&D center, Nonghyup Feed, Seoul 05398, Korea 4 Animal Resources Research Center, Konkuk University, Seoul 05029, Korea
ORCID (for more information, please visit https://orcid.org)	Yong Jun Choi (https://orcid.org/0000-0002-5759-4108) Yong Ran Kim (https://orcid.org/0000-0002-5100-1834) Eun Jip Kim (https://orcid.org/0000-0002-6243-0407) Kwan Eung Kim (https://orcid.org/0000-0002-8392-9864) Byoung Ki An (https://orcid.org/0000-0002-3158-2491)
Competing interests	
Funding sources State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	
Acknowledgements	
Availability of data and material	
Authors' contributions Please specify the authors' role using this form.	Conceptualization: Kim KE, An BK Data curation: Kim YR, Kim EJ Formal analysis: Kim YR Methodology: Kim KE, An BK Software: Choi YJ Validation: Kim KE, An BK Investigation: Kim YR Writing - original draft: Choi YJ, An BK Writing - review & editing: Choi YJ, Kim YR, Kim EJ, Kim KE, An BK
Ethics approval and consent to participate	The Institutional Animal Care and Use Committee at Konkuk University approved the techniques and procedures involved in the animal care and handling (KU21140).

CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below

First name, middle initial, last name	Byoung Ki An
Email address – this is where your proofs will be sent	abk7227@hanmail.net
Secondary Email address	
Address	Animal Resources Research Center, Konkuk University, 120 Neungdong-ro, Gwangjin-gu, Seoul 05029, Republic of Korea
Cell phone number	+82-2-450-3665
Office phone number	+82-10-6364-2036
Fax number	

Feeding of reduced vitamin premix negatively affects laying performance and vitamin contents in chicken eggs

Yong Jun Choi[1], Yong Ran Kim[2], Eun Jip Kim[2], Kwan Eung Kim[3], Byoung Ki An[4*]

[1] School of Animal Life Convergence Science, Hankyung National University, Anseong 17579, Korea

[2] Division of Animal Husbandry, Yonam College, Cheonan 31005, Korea

[3] R&D center, Nonghyup Feed, Seoul 05398, Korea

[4] Animal Resources Research Center, Konkuk University, Seoul 05029, Korea

[*]Corresponding author:

Byoung Ki An

Animal Resources Research Center, Konkuk University, 120 Neungdong-ro, Gwangjin-gu, Seoul 05029, Republic of Korea

Tel: +82-2-450-3665

Email: abk7227@hanmail.net

Abstract

This study was conducted to investigate effects of diets with varying levels of vitamin premix on egg production, eggshell quality, and vitamin contents in eggs of commercial laying hens. A total of 144 Hy-line Brown layers at 45 weeks old were randomly divided into four groups (six replicates, 6 birds for each group) and fed diets containing different levels of vitamin premix for eight weeks. Laying hens in the control group received diet containing 100% vitamin premix (0.1% in experimental diet). The other three groups received diet containing 75%, 50% or 0% of vitamin premix compared to control, respectively. Egg production and daily egg mass in layers fed diet without vitamin premix were significantly lower ($p < 0.01$) than those in the control group during the second half of the experiment (four weeks). Linear trend for egg production was determined with increasing dietary vitamin premix levels during the same period. There was no significant difference in feed intake or blood profile among groups. Significant linear and quadratic improvement for eggshell strength and thickness were found with increasing dietary vitamin premix level at 4 and 6 weeks of the experiment ($p < 0.05$). Concentrations of riboflavin and α-tocopherol in eggs obtained from the control group were significantly higher than those of the group fed a diet without vitamin premix ($p < 0.05$). These results suggest that reducing more than 50% or withdrawal of vitamin premix in layer diet did negatively affect egg production or egg qualities as the period of deprivation increased. To produce healthy chicken eggs, it is recommended to feed a diet that contains sufficient levels of vitamin premix.

Keywords: Vitamin premix, Laying performance, Egg quality, Riboflavin, Laying hen

INTRODUCTION

Vitamins are organic compounds required in minute amounts for normal metabolism and production in animals [1]. Because vitamins cannot be synthesized by host animals to meet their requirements, they must be supplied through diets [2]. Most feedstuffs used for poultry diet are rich in energy and protein but lack sufficient amounts of micro element or certain vitamins [3]. In addition, the availability of different vitamins in feedstuffs varies considerably [4]. Vitamin analysis in feedstuffs is time consuming and costly. Thus, vitamin premix is usually supplemented at recommended levels. Normally vitamin premix contains 12 essential vitamins for poultry to meet recommended levels of vitamins and ensure normal function and productivity [5].

Vitamin premix needs to be applied in adequate amounts without deficiency or adverse effects on productivity [6]. Recently, the supply and price of vitamins are changing rapidly in the global market. Thus, attention is focused on applying appropriate levels of vitamin premix into poultry diets because vitamins are among the most expensive feed ingredients. Some studies have determined effects of vitamin premix removal on growth performance of broiler chicks. Chickens fed diets lacking vitamin premix exhibit reduced weight gain, feed efficiency, and survival rate [7]. On the contrary, Moravej et al. [8] have reported that reduction or withdrawal of vitamin premix in broiler diets does not affect growth performance during the finishing period. Such discrepancy might be due to differences in raw material compositions of basal diet and duration of withdrawal. Furthermore, only limited information is available on dietary effects of reduction or withdrawal of vitamin premix on egg production, egg qualities, and nutritional quality of eggs in commercial layers. Thus, this study was conducted to determine effects of absence or reduction of vitamin premix in corn soybean meal-based layer diet on egg production, egg quality, and some vitamin contents in chicken eggs.

MATERIALS AND METHODS

Animals, diets, and management

A total of 144 45-week-old Hy-line brown layers were randomly assigned to four groups (36 layers for each group) so that egg production was similar for each group. They were fed diets with different levels of vitamin premix during eight weeks. Treatments consisted of four experimental diets containing 1,000 mg, 750 mg, 500 mg, or 0 mg vitamin premix/kg of diet. Laying hens in the control group received diet with 100% vitamin premix (0.1% in experimental diet). The other two groups received a diet containing 75% or 50% of vitamin premix compared to control, respectively. The fourth group of layers received the basal diet without supplemental vitamin premix. Layers were housed in wire cages. Each replicate comprised six cages with six birds in each cage. The formula and chemical compositions of experimental diets are shown in Table 1. All diets were formulated to meet and exceed the nutrients requirements of NRC (1994). Experimental diets and water were provided for *ad libitum* consumption. Vitamin contents in premix are described in Table 2. A room temperature of 22 ± 3°C and an artificial lighting of 16 h were maintained throughout the entire experimental period. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Konkuk University (KU21140).

Egg production

Diets were freshly added every day and feed intake was recorded weekly by replicate. Eggs were collected at a fixed time every day. The number and weight of eggs laid were recorded every day during the experimental period. Abnormal eggs were excluded from egg weight measurement. Egg mass was calculated as hen-day egg production multiplied by the average egg weight.

Egg quality

Egg quality was determined bi-weekly during the post-molting experiment (at 2, 4, and 6 week of experiment). Five eggs from each replicate were collected, weighed, and stored overnight at room temperature (20°C) for subsequent analyses. The breaking strength of each sampled egg was measured using a DET-6000 digital egg tester (Nabel Co., Ltd., Kyoto, Japan). Eggshell thickness without shell membrane was measured using a micrometer (Digimatic micrometer, series 547-360, Mitutoyo, Japan). Egg yolk color was determined by comparison with a Roche yolk color fan (Hoffman-La Roche, Basel, Switzerland). Albumen height was also measured using the DET-6000 digital egg tester (Nabel Co., Ltd., Kyoto, Japan). Haugh unit calculation was then performed.

Blood profiles

At the end of the experiment, one bird was randomly selected for each replicate and blood was drawn from a wing vein. Serum was then obtained after a gentle centrifugation (2,000 x *g* for 15 min). Serum samples were stored at -20°C until analysis. Levels of serum albumin, globulin, total cholesterol, and triacylglycerol were measured using an automatic blood analyzer (Labospect 008AS, Hitachi, Japan). Glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) levels in serum samples were measured according to a colorimetric method as previously described [9].

Analyses of riboflavin and α-tocopherol

All eggs laid on the last day of the experiment were collected. Two eggs obtained from each replicate were broken. Egg yolk was separated from the respective egg white and homogenized using a food blender for α-tocopherol analysis. Another two eggs also were broken and whole eggs were used for riboflavin analysis. An HPLC (Nexera 40 series UHPLC, Shimadzu, Tokyo, Japan) equipped with a diode array detector (DAD), a autosampler, a dual pump, and a YMC C30 column (150 × 4.6 mm, 3 µm; YMC, Wilmington, NC, USA) was used for α-tocopherol analysis as previously described [10]. Samples were scanned (180–800 nm) with a 0.05 min (1 s) response time at a detection wavelength of 295 nm. An HPLC (Nanospace SI-2, OSAKA SODA, Tokyo, Japan) equipped with an autosampler, a pump, a UV detector, and a PDA detector system was used for riboflavin analysis.

Statistical analysis

Data were analyzed by the GLM procedure of SAS with cage lot (six adjacent cages) as experimental unit for evaluating egg production, egg quality, and vitamin contents in eggs. Individual layers were considered as unit for blood profiles. Orthogonal polynomial contrasts were used to determine linear and quadratic effects of dietary vitamin premix levels on responses measured. Statistical significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

Laying performances of hens fed diets with varying levels of vitamin premix are presented in Table 3. There was no significant difference in egg production or daily egg mass among groups during the first four weeks of experiment. Egg production and daily egg mass in the control group were significantly higher ($p < 0.01$) than those in hens fed diet without vitamin premix during the second half of the experiment (the second four weeks). A linear trend for egg production with increasing dietary vitamin premix levels was found during the same period. Egg weight and feed intake were not affected by dietary treatment.

As shown in Table 4, diets with varying levels of vitamin premix did not influence any egg quality parameters when measured at 2 weeks of the experiment. A linear trend for yolk color with increasing dietary vitamin premix level was found during the same period. Eggshell strength and thickness for hens fed diet without vitamin premix were significantly lower ($p < 0.05$) than those for hens in the other three groups at 4 and 6 weeks of the experiment. Significant linear and quadratic improvement for eggshell strength and thickness with increasing dietary vitamin premix levels ($p < 0.05$) were found. However, Haugh unit was not affected by dietary treatment.

Results from broilers regarding effects of vitamin premix withdrawal on growth performance are conflicting. Chickens fed diets lacking vitamin premix exhibit reduced weight gain, feed efficiency, and survival rate in some studies [7]. Conversely, Moravej et al. [8] did not find any significant effects of reduction or withdrawal of vitamin premix on growth performance during the finishing period. Information is limited concerning effects of reduction or withdrawal of vitamin premix on egg production and egg quality measurements in commercial layers. It appears that withdrawn of vitamin premix, especially riboflavin, negatively affects laying performance [11]. Long-term riboflavin deficiency can reduce egg production, egg weight, and body weight of layers [12]. Our results revealed that reduction more than 50% or withdrawal of vitamin premix in layer diet did negatively affect egg production and egg qualities as the period of deprivation increased. Most vitamins are not stable. They can undergo significant deterioration during storage [5]. Vitamin availability in plant feedstuffs is often very low [13]. The calculated riboflavin value of basal diet used in this study appears to barely meet the riboflavin requirement [3,5]. Considering these, a part of the decline in laying performance might be due to insufficient supply of riboflavin.

Blood profiles of hens fed diets with varying levels of vitamin premix are presented in Table 5. There were no significant differences in concentrations of albumin, globulin, total cholesterol, or triacylglycerol among groups. A linear trend for serum albumin with increasing dietary vitamin premix level was found. Activities of serum GOT and GPT as indicatives of tissue damages were not affected by dietary treatment.

In most studies, blood biochemistry and immune criteria of poultry were not affected when vitamin premix was reduced or removed from experimental diets. For example, Deyhim and Teeter [7] did not find any significant effects of reducing vitamin premix on humoral immune response. In addition, removal of vitamin premix in broiler diets had no adverse effect on immunocompetence or antibody titer production over a relatively short period [14]. In the present study, relative weights of thymus and bursa of Fabricius were not influenced by dietary treatment (data not shown). Studies about long-term effects of vitamin premix withdrawal on immune criteria and blood biochemistry in commercial laying hens are limited. The possibility of impaired immune index should not be precluded. Further studies are needed to clarify effects of diets containing various levels of vitamin premix on immune criteria of commercial haying hens.

Contents of riboflavin and α-tocopherol obtained from hens fed diets containing varying levels of vitamin premix are presented in Table 6. The level of α-tocopherol of eggs obtained from the control group was significantly higher than those of groups with vitamin premix reduction of more than 50% or withdrawal of vitamin premix ($p < 0.001$). A significant linear increment for egg α-tocopherol with increasing dietary vitamin premix level was found. The level of riboflavin of eggs obtained from control group was significantly higher than that of the group fed diet without vitamin premix ($p < 0.05$). Similarly, a linear trend for riboflavin content with increasing dietary vitamin premix level was found.

Naber and Squires [11] have reported that riboflavin levels are dropped within several days in egg from layers fed diets without riboflavin or all supplemental vitamins. Another study has also found that egg riboflavin concentration is rapidly decreased after feeding diets without riboflavin supplement [12]. These studies have suggested that riboflavin concentration in egg albumen can be used to assess nutritional status of laying hens [11,12]. In the present study, the riboflavin content of eggs was affected by dietary levels of vitamin premix, in agreement with those results. As expected, levels of α-tocopherol in experimental diets were reflected in concentrations of this vitamin in egg obtained each group. Scheideler et al. [15] have reported that egg yolk α-tocopherol is increased linearly with vitamin E supplementation, although dietary levels are not different from those in the present study. Chicken eggs are one of the most common daily foods. They contain most of the essential vitamins except for vitamin C [16]. Vitamin E is a natural fat-soluble antioxidant that tends to bring about storage stability of eggs [17]. Eggs low in riboflavin and α-tocopherol are less attractive to consumers who consider their health.

CONCLUSION

Overall, reducing more than 50% or withdrawal of vitamin premix in layer diet did negatively affect egg production or egg qualities. From results of the present study, it is recommended to feed a layer diet containing sufficient levels of vitamin premix to produce healthy chicken eggs with a long shelf life.

REFERENCES

1. McDowell LR. Vitamins in Animal and Human Nutrition. 2nd ed. Ames. Iowa State University Press; 2000.

2. Larbier M, Leclercq B. Nutrition and Feeding of Poultry. Nottingham University Press; 1994.

3. NRC [National Research Council]. Nutrients requirements of poultry. 9th ed. Washington, DC. National Academic Press; 1994.

4. NRC [National Research Council]. Nutrients requirements of poultry. 8th ed. Washington, DC. National Academic Press; 1984.

5. Korean Feeding Standard for Poultry. 4th ed. National Institute of Animal Science, Rural Development Administration; 2017.

6. Ao T, Paul MA, Pescatore AJ, Macalintal LM, et al. Growth performance and bone characteristics of broiler chickens fed corn-soy diet supplemented with different levels of vitamin premix and sources of mineral premix. J Appl Anim Nutr. 2019;7:1-5.

7. Deyhim F, Teeter RG. Dietary vitamin and/or trace mineral premix effects on performance, humoral mediated immunity, and carcass composition of broilers during thermoneutral and high ambient temperature distress. J Appl Poult Sci. 1993;2:347-55.

8. Moravej H, Alahyari SM, Shivazad M. Effects of the reduction or withdrawal of the vitamin premix from the diet on chicken performance and meat quality. Braz J Poult Sci. 2012;14:239-43.

9. An BK, Jung JH, Oh ST, Kang CW, Lee KW, Lee SR. Effects of diets with graded levels of canola meal on the growth performance, meat quality, relative organ weights, and blood characteristics of broiler chickens. Braz J Poult Sci. 2016;18:351-6.

10. Ko EY, Saini RK, Keum YS, An BK. Age of laying hens significantly influences the content of nutritionally vital lipophilic compounds in eggs. Foods. 2021;10-22, doi:10.3390/foods10010022.

11. Naber EC, Squires MW. Research note: Early detection of a vitamin permix in layer diets by egg albumen riboflavin analysis. Poult Sci. 1993;72:1989-93.

12. Squire MW, Naber EC. Vitamin profiles of eggs as indicators of nutritional status in the laying hen: Riboflavin study. Poult Sci. 1993;72:483-94.

13. Summers JD, Leeson S. Choline, niacin and thiamine supplementation of canola and soybean protein diet fed to broilers to 6 w k of age. Can J Anim Sci. 1985;65:217-20.

14. Khajali F, Khoshoei EA, Moghaddam AKZ. Effect of vitamin and trace mineral withdrawal from finisher diets on growth performance and immunocompetence of broiler chickens. Br Poult Sci. 2006;47:159-62.

15. Scheideler SE, Weber P, Monsalve D. Supplemental vitamin E and selenium effects on egg production, egg quality, egg deposition of α-tocopherol and selenium. J Appl Poult Sci. 2010;19:354-60.

16. Zang H, Zhang K, Ding X, Bai S, Hernandez JM, Yao B. Effects of different dietary vitamin combinations on the egg quality and vitamin deposition in the whole egg of laying hens. Braz J Poult Sci. 2011;13:189-96.

17. Panda AK, Cherian G. Role of vitamin E in counteracting oxidative stress in poultry. J Poult Res. 2014;51:109-17.

Table 1. Formula and chemical compositions of experimental diets[1)]

tems	Control	T1	T2	T3
Ingredients, %				
Corn	54.69	54.72	54.74	54.79
Soybean meal, 45%	23.68	23.68	23.68	23.68
Rapeseed meal	1.10	1.10	1.10	1.10
Rice bran	2.00	2.00	2.00	2.00
DDGS	4.00	4.00	4.00	4.00
Animal fat	2.10	2.10	2.10	2.10
Lysine-HCl, 78%	0.10	0.10	0.10	0.10
DL-methionine, 99%	0.18	0.18	0.18	0.18
Tricalcium phosphate	1.52	1.52	1.52	1.52
Limestone	9.98	9.98	9.98	9.98
Salt	0.25	0.25	0.25	0.25
Vitamin premix[2)]	0.10	0.075	0.05	-
Mineral premix[3)]	0.10	0.10	0.10	0.10
Choline-Cl, 50%	0.10	0.10	0.10	0.10
$NaHCO_3$	0.10	0.10	0.10	0.10
Total	100.0	100.0	100.0	
Calculated values[4)]				
CP, %	16.50	16.50	16.50	16.50
Ca, %	4.10	4.10	4.10	4.10
Avail. P, %	0.43	0.43	0.43	0.43
Total Lys, %	0.92	0.92	0.92	0.92
Total TSAA, %	0.74	0.74	0.74	0.74
AME_n, kcal/kg	2,750	2,750	2,750	2,750

[1)] Control, a group received a diet containing 0.10% vitamin premix; T1, a group received a diet containing 0.075% vitamin premix; T2, a group received a diet containing 0.05% vitamin premix; T3, a group received a diet without any vitamin premix.

[2)] Vitamin mixture provided the following nutrients per kg of control diet: vitamin A, 10,000 IU; vitamin D_3, 3,500 IU; vitamin E, 10 mg; vitamin K_3, 3 mg; vitamin B_1, 2 mg; vitamin B_2, 6 mg; vitamin B_6, 4 mg; vitamin B_{12}, 0.0235 mg; pantothenic acid, 10 mg; folic acid, 0.82 mg; nicotinic acid, 30 mg.

[3)] Mineral mixtures provided the following nutrients per kg of diets: Mn, 25 mg; Zn, 50 mg; Fe, 60 mg; Cu, 10 mg; Co, 0.15 mg; Se, 0.10 mg.

[4)] Calculated values are based on raw materials.

Table 2. Specification of vitamin premix used in this study

Vitamins	Contents, per kg
Vitamin A, IU	10,000,000
Vitamin D_3, IU	3,500,000
Vitamin E, ppm	10,000
Vitamin K_3, ppm	3,000
Thiamin, ppm	2,000
Riboflavin, ppm	6,000
Niacin, ppm	30,000
Pantothenic acid, ppm	10,000
Vitamin B_6, ppm	4,000
Vitamin B_{12}, ppb	23.5
Biotin, ppm	120
Folic acid, ppm	820

Table 3. Egg productivity of laying hens subjected to diets containing varying levels of vitamin premix[1),2)]

	Dietary treatments				SEM	P values		
	Control	T1	T2	T3		Linear	Quadratic	ANOVA
1~4 weeks								
Egg production, %	86.0	81.6	83.0	82.2	2.718	0.320	0.562	0.648
Egg weight, g/egg	61.0	61.6	60.2	62.6	0.892	0.433	0.359	0.281
Daily egg mass	52.3	50.3	50.1	50.7	1.703	0.426	0.529	0.763
Feed intake, g/bird/d	113.8	114.0	112.2	113.3	0.944	0.447	0.877	0.525
5~8 weeks								
Egg production, %	80.0^{a}	76.4^{ab}	78.5^{a}	65.7^{b}	2.833	0.005	0.092	0.006
Egg weight, g/egg	61.9	61.0	60.5	60.4	0.848	0.192	0.827	0.604
Daily egg mass	49.5^{a}	46.7^{ab}	47.5^{a}	39.8^{b}	1.965	0.005	0.168	0.010
Feed intake, g/bird/d	102.2	94.4	102.7	91.8	3.400	0.125	0.779	0.078

[1)] Control, a group received a diet containing 0.10% vitamin premix; T1, a group received a diet containing 0.075% vitamin premix; T2, a group received a diet containing 0.05% vitamin premix; T3, a group received a diet without any vitamin premix.

[2)] Data are presented as least square of mean of six replicates with six birds per replicate.

[a,b] Mean values with different superscripts within the same row differ significantly at $p < 0.05$.

Table 4. Egg quality of laying hens subjected to diets containing varying levels of vitamin premix[1),2)]

	Dietary treatments				SEM	P values		
	Control	T1	T2	T3		Linear	Quadratic	ANOVA
2 weeks								
Eggshell strength, kg/cm^2	3.9	3.9	3.8	3.7	0.199	0.500	0.694	0.884
Eggshell thickness, mm	47.5	47.1	45.7	45.7	0.795	0.083	0.867	0.293
Haugh unit	88.7	86.4	85.3	86.7	0.669	0.294	0.378	0564
Yolk color	7.8	7.2	7.3	7.2	0.160	0.014	0.197	0.052
4 weeks								
Eggshell strength, kg/cm^2	4.0^{a}	4.1^{a}	3.8^{a}	2.9^{b}	0.232	0.003	0.014	0.004
Eggshell thickness, mm	46.7^{a}	47.5^{a}	46.6^{a}	41.1^{b}	0.885	<0.001	<0.001	<0.001
Haugh unit	90.5	90.0	89.1	89.6	1.277	0.518	0.830	0.888
Yolk color	8.5	8.1	8.1	8.0	0.154	0.013	0.482	0.086
6 weeks								
Eggshell strength, kg/cm^2	3.9^{a}	4.0^{a}	3.9^{a}	2.8^{b}	0.258	0.010	0.026	0.010
Eggshell thickness, mm	45.5^{a}	46.7^{a}	46.1^{a}	41.3^{b}	0.787	0.003	<0.001	<0.001
Haugh unit	89.6	93.2	90.1	89.7	1.305	0.945	0.073	0.199
Yolk color	8.5	8.5	8.1	7.9	0.169	0.020	0.293	0.060

[1)] Control, a group received a diet containing 0.10% vitamin premix; T1, a group received a diet containing 0.075% vitamin premix; T2, a group received a diet containing 0.05% vitamin premix; T3, a group received a diet without any vitamin premix.

[2)] Data are presented as least square of mean of six replicates with six birds per replicate.

[a,b] Mean values with different superscripts within the same row differ significantly at $p < 0.05$.

Table 5. Blood profiles of laying hens subjected to diets with varying levels of vitamin premix[1),2)]

	Dietary treatments				SEM	P values		
	Control	T1	T2	T3		Linear	Quadratic	ANOVA
Albumin, g/*dL*	2.28	2.08	2.13	1.90	0.089	0.019	0.708	0.063
Globulin, g/*dL*	2.35	2.38	2.58	2.58	0.118	0.138	0.752	0.394
Total cholesterol, mg/*dL*	51.5	54.8	59.0	64.0	5.604	0.133	0.659	0.454
Triacylglycerol, mg/*dL*	467.0	531.8	532.5	453.3	55.594	0.958	0.228	0.646
GOT, U/*L*	221.8	205.3	207.8	213.5	18.330	0.708	0.578	0.922
GPT, U/*L*	5.08	5.23	5.20	5.10	0.120	0.787	0.334	0.781

[1)] Control, a group received a diet containing 0.10% vitamin premix; T1, a group received a diet containing 0.075% vitamin premix; T2, a group received a diet containing 0.05% vitamin premix; T3, a group received a diet without any vitamin premix.

[2)] Data are presented as least square of mean of six birds per treatment.

Table 6. Vitamin contents in eggs of laying hens subjected to diets containing varying levels of vitamin premix[1),2)]

	Dietary treatments				SEM	P values		
	Control	T1	T2	T3		Linear	Quadratic	ANOVA
α-tocopherol, mg/100g egg yolk	3.716[a]	3.326[ab]	2.922[bc]	2.570[c]	0.130	<0.001	0.292	<0.001
Riboflavin, mg/100g whole egg	0.156[a]	0.137[ab]	0.113[ab]	0.100[b]	0.012	0.003	0.685	0.021

[1)] Control, a group received a diet containing 0.10% vitamin premix; T1, a group received a diet containing 0.075% vitamin premix; T2, a group received a diet containing 0.05% vitamin premix; T3, a group received a diet without any vitamin premix.

[2)] Data are presented as least square of mean of six eggs per treatment.

[a,b,c] Mean values with different superscripts within the same row differ significantly at $p < 0.05$.