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 It appears that withdrawn of vitamin premix, especially riboflavin, negatively affects laying performance [11].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.

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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 $p < 0.05$.

252 **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

253 [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
254 containing 0.05% vitamin premix; T3, a group received a diet without any vitamin premix.

255 [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 $p < 0.05$.