Enrichment of Vitamins D_3, K and Iron in Eggs of Laying Hens

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ABSTRACT : An experiment was conducted to produce eggs enriched with vitamins D_3, K and iron in eggs. Six hundred 97-wk-old ISA Brown force molted hens were allocated to completely randomized block arrangement of six dietary treatments: T1; control (C), T2; C+4,000 IU vitamin D_3+2.5 mg vitamin K+100 ppm Fe, T3; C+8,000 IU vitamin D_3+5.0 mg vitamin K+100 ppm Fe, T4; C+12,000 IU vitamin D_3+7.5 mg vitamin K+100 ppm Fe, T5; C+16,000 IU vitamin D_3+10.0 mg vitamin K+100 ppm Fe, T6; C+20,000 IU vitamin D_3 +12.5 mg vitamin K+100 ppm Fe. Fe was supplemented with Fe-methionine. Each treatment consisted of five replicates of ten cages with two birds per cage. Egg production and egg weight were highest in T2 and incidence of soft and broken egg was highest in T6. Haugh unit was not different among treatments although it tended to be increased as dietary vitamins D_3 and K increased. Eggshell strength was not different among treatment. Concentrations of vitamin D_3 and K in egg yolk increased and plateaued approximately 20 days after feeding supplemented diets. The level of these vitamins peaked at 12,000 IU/kg vitamin D_3 and 7.5 mg/kg vitamin K supplementation and then decreased at the higher than these supplementation levels. The peak concentrations of vitamin D_3 and vitamin K were 4.6 times and 4.8 times greater than the control, respectively. Supplementary Fe also increased Fe content in egg yolk. It is concluded that vitamin D_3 and K in eggs can be effectively enriched by proper supplementation time and level of these vitamins. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 2 : 226-229)***

Key Words : Egg, Vitamin D_3, Vitamin K, Iron, Fe-methionine

INTRODUCTION

In the past, poultry nutritionists have been interested in establishing nutrient requirements of poultry to support maximum performance of laying hens. Recently, however, nutritionists have been interested in enriching or altering the amounts of certain nutrients in poultry products such as carcass and eggs in relation with increased consumer's interest in the nutritive value of foods. It has been well known that the nutritive composition of eggs is changed by the nutritional composition of diet. In general, the transfer efficiency of dietary fat-soluble vitamins to egg is lower than that of water-soluble vitamins (Naber, 1993). The level of vitamin D in eggs was increased considerably in response to the increase of dietary level of this vitamin (Bethke et al., 1936,1937; Naber, 1979; Mattila et al., 2004). Although there is no available data on transfer of dietary vitamin K to eggs, Griminger and Brubacher (1966) demonstrated a marked reduction in the prothrombin time in plasma of chicks hatched from eggs laid by hens given ten times more dietary vitamin K_1 than the control. This was an indirect proof that dietary vitamin K_1 transferred to the egg. Transfer efficiency of dietary iron to egg is known to be low (Naber, 1979) but a preliminary study showed that supplementation of iron at the level of 100ppm by Fe-methionine chelate effectively enriched iron content in egg yolk (Paik, 2001; Paik et al.,2004).

Vitamin A enriched eggs are in the market under various brand names but vitamin D_3 and K enriched eggs are not available. In order to produce vitamin D_3, K and iron enriched eggs, a study was conducted to determine the effects of days and levels of supplementation of vitamin D_3 and K and predetermined level of iron on their concentration in eggs of laying hens.

MATERIALS AND METHODS

Six hundred 87 wk-old ISA Brown molted laying hens were allotted to one of six treatments. One hundred birds were assigned to each treatment consisted of five replicates of ten cages (2 birds per cage). Treatments are shown in Table 1. The basal diet (Table 2) was considered the control and contained 2,000 IU vitamin D_3 and 0.5 mg vitamin K. Source of vitamin K was menadione sodium bisulfate. Iron was supplemented as Fe-methionine chelate. Experimental diets were fed for 30 days. Egg production and egg weight were recorded daily and feed consumption was recorded weekly. Birds were subjected to 14 h of light per day. Water and feed were available for *ad libitum* consumption. Fifty eggs from each treatment were collected weekly at random to measure egg quality such as eggshell strength, egg yolk color and Haugh units. Haugh unit was calculated using the HU formula (Eisen et al., 1962) based on the height of albumen determined by a micrometer (Model S-8400, AMES, Waltham, MA 02254, USA). Egg yolk color was measured using Roche color fan. Eggshell strength was measured by Compression Test Cell of Texture Systems

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Table 1. Dietary treatments, per kg diet

Treatment	Vitamin D_3, IU	Vitamin K, mg	Iron, mg as Fe-methionine
1 (control)	2,000	0.5	0
2	4,000	2.5	100
3	8,000	5	100
4	12,000	7.5	100
5	16,000	10	100
6	20,000	12.5	100

Table 2. Formula of the basal (control) diet

Ingredients	Percentage
Corn	63.50
Corn gluten	4.12
Defatted rice bran	2.00
Wheat bran	6.42
Soybean meal	14.04
Limestone	8.26
Defluorinated phosphate	1.25
Layer premix[1]	0.41
Total	100.00
Composition, calculated	
ME (kcal/kg)	2,739
Crude protein (%)	15.37
Lysine (%)	0.57
Meth and cyst (%)	0.47
Calcium (%)	3.62
Phosphorus-available (%)	0.35

[1] Provide per kg of diet: 0.11%; Magnesium, 420 mg; Nonphytate phosphrous, 0.21%; Potassium, 0.13%; Sodium, 0.13%; Iodine, 0.029 mg; Iron, 38 mg; Manganese, 17 mg; Selenium, 0.05 mg; Zinc, 29 mg; vitamin A, 2,500 IU; vitamin D_3, 2,000 IU; vitamin E, 4 IU; vitamin K, 0.5 mg; vitamin B_{12}, 0.004 mg; Biotin 0.08 mg; Choline 875 mg; Folacin, 0.21 mg; Niacin, 8.3 mg; Panthothenic acid 1.7 mg; Pyridoxine, 2.1 mg; Riboflavin, 2.1 mg; Thiamin, 0.6 mg.

(Model T2100C, Food Technology Corp., Rockville, MD 20852 USA). Another 20 egg samples per treatment were collected to measure contents of Fe and vitamins D_3 and K at day 0, 2, 4, 6, 8, 10, 15, 20, 25, 30. Out of 20 eggs, 5 eggs were boiled and served for Fe analysis individually. The rest 15 eggs were divided into 3 groups of 5 eggs each. Egg yolks of five eggs were pooled and served for the analysis of vitamins. Iron content of egg yolk was analyzed by the method of AOAC (1997) using ICP (Inductively Coupled Plasma Spectroscopy, Jovon Yvon, JY-24, France).

Table 3. HPLC Condition for vitamin D_3 and K assay

	Vitamin D_3	Vitamin K
Model	Gilson 305 system (France)	
Column	Hypersil-ODS 3 μm×150 mm	
Column temperature	Ambient	
Mobile phase	methanol:water (94:6, v/v)	100% methanol
Detection wavelength	264 nm	270 nm
Flow rate	1.0 ml/min	
Run time	15 min	
Injection volume	20 μl	

Vitamin D_3 and K of egg yolk were analyzed by the method of Ueda (1987) using HPLC (Gilson 305 system, France). The results were expressed per 100 g fresh egg base. HPLC condition for analysis of vitamin D_3 and K is shown in Table 3. Cholecalciferol and menadione sodium bisulfite were used to make standard solutions. The results obtained from experiments were analyzed by ANOVA using GLM procedure of SAS® (SAS Institute, 1985). Significant differences among treatment means were determined using Duncan's multiple range test at $p<0.05$.

RESULT AND DISCUSSION

The effect of dietary vitamins D_3 and K and Fe on the performance of laying hens is shown in Table 4.

Egg production for hens fed supplementary vitamins D_3 and K and Fe was not significantly different from that of the control group although egg production for hens fed vitamins D_3 and K was highest in T2 and lowest in T4. Egg weight of T2 and T4 was higher than that of the control group. Supplementation of vitamins D_3 and K and Fe did not affect feed intake, feed efficiency and eggshell strength but T6 produced highest soft and broken egg. Haugh unit was highest in control and lowest in T6 and there was a tendency that Haugh unit decreases as dietary supplementation of vitamin D_3 and K increases. Egg yolk color index increased as the level of vitamin supplementation increased. These results indicate that excessive supply of vitamin D_3 and K do not improve the performance of laying hens. Shen (1981) reported that the amount of dietary vitamin D_3 did not affect egg weight.

Table 4. Effect of supplemental vitamins D_3 and K and Fe on performance and egg quality of laying hens

Parameters	Treatment[1]						SEM
	T1	T2	T3	T4	T5	T6	
Hen-day egg production (%)	74.4^{abc}	76.8^{a}	74.5^{abc}	72.1^{c}	75.3^{ab}	73.6^{bc}	6.76
Egg weight (g)	69.6^{bc}	70.9^{a}	70.3^{ab}	70.3^{abc}	70.5^{a}	69.6^{c}	1.21
Feed intake (g/hen/day)	134.4	135.7	132.3	130.2	134.9	134.4	3.48
Feed conversion (feed/egg mass)	2.56	2.47	2.49	2.55	2.50	2.57	0.09
Soft, broken egg (%)	4.70^{b}	5.04^{b}	5.40^{b}	4.82^{b}	4.13^{b}	8.41^{a}	0.97
Eggshell strength (kg/cm^2)	0.65	0.66	0.65	0.68	0.68	0.65	0.05
Haugh unit	79.7^{a}	78.3^{ab}	78.4^{ab}	78.0^{ab}	77.7^{bc}	76.0^{c}	0.41
Eggyolk color	10.1^{ab}	10.2^{ab}	10.0^{b}	10.2^{ab}	10.3^{ab}	10.5^{a}	0.73

[a-d] Values in the same row with no common superscripts are significantly different ($p<0.05$). [1] Refer to Table 1.

Table 5. Effect of supplemental vitamins D_3 and K and Fe on the contents of vitamin D_3 and K and Fe in egg

Items	Day	Treatments[1]						SEM
		T1	T2	T3	T4	T5	T6	
	0	35.6^{abc}	36.8^{ab}	36.4^{ab}	33.2^{c}	34.5^{bc}	37.6^{a}	0.56
	2	36.0^{d}	45.6^{c}	70.9^{a}	52.8^{b}	18.8^{e}	19.4^{e}	0.98
	4	39.8^{c}	48.4^{c}	111.7^{a}	87.6^{b}	18.8^{d}	19.9^{d}	3.53
	6	44.9^{d}	92.4^{b}	84.1^{bc}	101.7^{a}	76.8^{c}	84.2^{bc}	1.92
Vitamin D_3	8	48.7^{d}	99.2^{b}	74.0^{c}	106.5^{b}	135.7^{a}	127.1^{a}	3.76
(IU/100 g egg)	10	43.4^{d}	76.9^{c}	112.9^{b}	183.9^{a}	123.3^{b}	144.9^{b}	5.22
	15	50.8^{e}	106.1^{d}	130.1^{c}	206.4^{a}	151.1^{b}	140.7^{bc}	4.09
	20	49.6^{d}	172.4^{c}	205.6^{b}	241.2^{a}	151.1^{c}	151.2^{c}	4.98
	25	55.7^{e}	167.9^{c}	219.1^{b}	250.1^{a}	153.0^{cd}	137.7^{d}	4.26
	30	53.8^{d}	165.1^{c}	227.1^{b}	247.6^{a}	147.3^{c}	154.2^{c}	4.53
Average, total		45.8^{d}	101.1^{c}	127.2^{b}	151.1^{a}	101.0^{c}	101.7^{c}	3.73
Average, 20-30		53.1^{e}	168.5^{c}	217.3^{b}	246.3^{a}	150.5^{d}	147.7^{d}	4.60
	0	9.3^{c}	11.1^{a}	11.0^{a}	9.8^{bc}	10.9^{a}	10.1^{b}	0.15
	2	11.1^{c}	35.0^{a}	34.2^{a}	11.0^{c}	17.9^{b}	10.6^{c}	0.45
	4	9.1^{e}	30.9^{b}	34.1^{a}	12.7^{d}	18.3^{c}	17.6^{c}	0.35
	6	12.4^{b}	29.7^{a}	30.6^{a}	34.0^{a}	33.3^{a}	34.1^{a}	1.88
Vitamin K	8	11.6^{b}	28.7^{a}	33.6^{a}	37.4^{a}	37.6^{a}	36.7^{a}	2.28
(μg/100 g egg)	10	12.8^{b}	33.0^{a}	34.7^{a}	38.0^{a}	37.6^{a}	35.5^{a}	1.76
	15	11.6^{c}	34.0^{b}	36.2^{b}	45.3^{a}	34.7^{b}	38.1^{ab}	1.90
	20	11.1^{d}	35.7^{c}	43.1^{b}	52.9^{a}	39.5^{bc}	38.6^{bc}	1.37
	25	11.2^{d}	35.9^{c}	45.8^{b}	53.0^{a}	38.6^{c}	38.9^{c}	1.45
	30	11.0^{d}	38.6^{c}	46.1^{b}	53.0^{a}	38.5^{c}	38.8^{c}	1.30
Average, total		11.1^{c}	31.3^{b}	34.9^{a}	34.7^{a}	30.7^{b}	29.9^{b}	1.47
Average, 20-30		11.1^{d}	36.7^{c}	45.0^{b}	53.0^{a}	38.9^{c}	38.8^{c}	1.37
	0	1.93	1.87	1.89	2.00	2.01	1.85	0.06
	2	1.89	1.91	1.86	1.92	1.89	1.93	0.06
	4	1.95	2.04	2.03	1.98	1.98	1.89	0.08
	6	1.95	2.10	1.99	2.03	1.98	1.93	0.05
Fe	8	2.10	2.20	2.02	1.97	2.10	2.02	0.07
(mg/100 g egg)	10	2.03^{b}	2.35^{a}	2.28^{ab}	2.17^{ab}	2.20^{ab}	2.32^{a}	0.08
	15	1.97^{b}	2.17^{ab}	2.10^{ab}	2.38^{a}	2.32^{a}	2.17^{ab}	0.07
	20	2.08^{b}	2.37^{a}	2.23^{ab}	2.28^{ab}	2.22^{ab}	2.13^{ab}	0.08
	25	2.04^{b}	2.34^{a}	2.42^{a}	2.36^{a}	2.31^{ab}	2.31^{ab}	0.08
	30	2.07^{b}	2.35^{a}	2.36^{a}	2.29^{ab}	2.33^{a}	2.28^{ab}	0.07
Average, total		2.00^{c}	2.17^{a}	2.12^{ab}	2.13^{ab}	2.13^{ab}	2.08^{b}	0.07
Average, 20-30		2.06^{b}	2.35^{a}	2.34^{a}	2.31^{a}	2.29^{a}	2.24^{a}	0.08

[1] Refer to Table 1.

Haugh units and feed intake. Keshavarz (1996) reported that increasing the vitamin D_3 level from 250 to 2,000 ppm decreased the number of cracked eggs. The result of the laying performance was not clear enough to draw any conclusion about the effects of dietary supplementation of graded levels of vitamins D_3 and K while the main objective of this experiment was to investigate the transfer of dietary vitamins D_3 and K and Fe to eggs.

The effects of vitamins D_3 and K and iron supplementation on their content in eggs are shown in Table 5. Vitamin D_3 content in egg from hens fed vitamin D_3 supplemented diets increased rapidly and reached plateau about 20 days after feeding. The highest level of vitamin D_3 in eggs was found in T4 (12,000 IU D_3/kg diet). Further supplementation of vitamin D_3 beyond T4 level decreased its concentration in eggs. The average concentration of vitamin D_3 in the eggs of T4 laid during 20-30 days after feeding supplemented diets was 4.6 times of that of the control. Bethke et al. (1936) reported that level of vitamin D in eggs increased considerably in response to dietary level but not in direct proportion to diet levels. Mattila et al. (2004) reported that supplementing diets with vitamin D_3 increased egg yolk vitamin D content more effectively than did supplementation with vitamin D_2. For groups of hens receiving 6,000 or 15,000 IU of vitamin D_3 /kg diet for 4 to 44 wks, egg yolk vitamin D_3 content was 11.35 and 29.5 μg/100 g egg yolk, respectively. Relative to the control group receiving 2,500 IU vitamin D_3 /kg diet, these vitamin D_3 concentrations of egg yolk were 3.0 and 7.9 times higher, respectively. Vitamin K content in eggs followed similar trend to that of vitamin D_3. Vitamin K concentration in eggs increased rapidly and reached plateau 20 days after feeding the supplemented diets. The eggs of maximum vitamin K concentration were laid in T4 containing 7.5 mg vitamin K kg of diet. The average concentration of vitamin K in the eggs of T4 laid during 20-30 days after feeding supplemented diets was 4.8 times of that of the control. The similarity of vitamin D_3 and K in their pattern of accumulation in eggs may be due to the common nature of fat-soluble vitamins. Differences in egg iron content appeared after 10 days on experimental diets. Average iron content in eggs from hens fed iron-supplemented diets was 14% higher than that from hens fed unsupplemented diet (T1).

In conclusion, vitamins D_3 and K in eggs can be effectively enriched by feeding diets supplemented with 12,000 IU vitamin D_3 and 7.5 mg vitamin K/kg of diet for 20 days. Compared to the control, the maximum levels achieved by these supplementation were 4.6 times greater for vitamin D_3 and 4.8 times for vitamin K, respectively. Iron concentration in eggs could be also increased up to 14% in average by supplementing 100 ppm Fe by Fe-methionine chelate.

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