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Effects of Different Dietary Vitamin Combinations on the Egg Quality and Vitamin deposition in the Whole Egg of Laying Hens

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ABSTRACT

The experiment was conducted to evaluate the effects of different dietary vitamin combinations on the egg quality and vitamin concentrations in the eggs of commercial laying hens. A total of 1,800 25-week-old Lohman pink-shell hens were randomly assigned to four dietary vitamin treatments as follows: NRC(1994) level, NRC (1994) level with Hy.D® (25-hydroxy-cholecalciferol), Local level (current average industry level in China) and OVN® level (optimum vitamin nutrition level), with 10 replicates per treatment and 45 layers per replicate. Hens were housed in commercial laying cages with three birds per cage and given *ad libitum* access to feed. Results showed the hens that received the fortified vitamin levels in the OVN® treatment had a significantly ($p<0.05$) lower number of cracked (.47%) and dirty eggs (.27%), and increased egg deposition of vitamin B_{12}, folic acid, vitamin A, vitamin D, 25-OH-D_3, vitamin E, vitamin B_1, biotin and pantothenate ($p<0.05$). Treatments had no significant effect on egg-shape index, egg specific gravity, Haugh units and eggshell thickness. Hens fed the NRC-Hy.D® combination also experienced a significant decrease in cracked and dirty eggs (.70% and .44%, respectively) and an increased deposition of 25-OH-D_3 in comparison with the NRC treatment. Results of the present study suggest that that the Local treatment was able to improve egg quality parameters of laying hens, but resulted in more cracked and dirty eggs. OVN® reduced the number of cracked eggs and dirty eggs, and improved the deposition of several vitamins in eggs. With the addition of Hy.D®, eggshell strength and 25-OH-D_3 deposition in eggs were also improved, and cracked and dirty egg rates declined.

INTRODUCTION

Vitamins are essential nutrients found in foods. While their requirements are small, they perform specific and vital functions essential for health maintenance. Vitamin deficiencies may lead to a series of diseases in humans. Vitamin A deficiency is the most frequent cause of blindness among pre-school children (Underwood, 1998), abortion of pregnant women and newborn mortality (Radhika *et al*., 2002) in developing countries. Vitamin D deficiency is a common problem during the winter in Europe, because of the restricted ultraviolet light exposure (Scharla, 1998). A less severe vitamin D deficiency can result in postmenopausal osteoporosis (Lips *et al*., 2001) and may also increase the risk of initiation and progression of prostate cancer (Tuohimaa *et al*., 2001). Folate deficiency can result in an increase in plasma homocysteine concentrations, which is linked to an increased risk for cardiovascular disease (Boushey *et al*., 1995; Refsum *et al*., 1998), Alzheimer's disease (Morris, 2003), and osteoporosis (Mclean *et al*., 2004). There is an increased awareness of the importance of this

B-vitamin due to the fact that supplemental folic acid has been shown to reduce a woman's risk for having a baby with a neural tube defect (Czeizel & Dudas, 1992; Scott, 1999). Therefore considerable attention has been given to the development of strategies to increase human vitamin intake.

Poultry products contribute significantly to the vitamin intake of consumers. Eggs are one of the most common daily foods, and naturally contain most of the recognized vitamins (vitamin A, vitamin D, vitamin E and B-vitamins), except for vitamin C. Improvements in egg nutritional value may have direct positive implications for daily nutrient intake and consequently for human health (Nys & Sauveur, 2004). Vitamin-enriched eggs are attractive as a vehicle that can provide consumers with compounds that may be beneficial to health or to over come nutritional imbalances. Like many other nutrients, vitamin levels incorporated into feed directly influence the deposition of vitamins in eggs (Naber & Squires, 1993). Vitamin concentration is influenced by genetics, egg production rate and, similarly to fatty acids, it varies with the composition of the hens' diet (Naber, 1993; Leeson & Caston, 2003). With a moderate enrichment of vitamins in accordance with the recommendations for fortification of foods, eggs could play an important role as a functional food. Numerous relevant data related to egg vitamin enrichment through nutrition strategies are now presented, with special attention paid to fat-soluble vitamins E, A, D, but also to vitamin B_{12} and folic acid. Naber (1993) summarized his findings, classifying the transfer efficiency of vitamins from the diet of hens to eggs, which was very high for vitamin A; high for riboflavin, pantothenic acid, biotin, and vitamin B_{12}; medium for vitamin D_3 and E; and low for vitamin K, thiamine and folacin; however, the combined effects of vitamin premix on vitamin deposition in eggs are rarely reported. Therefore, the aim of the present study was to investigate the possibility of enhancing vitamin accumulation in eggs through their supplementation in commercial laying hen diets and to determine the effects of these vitamins on egg quality parameters.

MATERIALS AND METHOD

This animal experiment was conducted in accordance with guidelines approved by Animal Health and Care Committee of Sichuan Agricultural University and was performed in accordance with recommendations of the China Council on Animal Care as specified in the Guide to the Care and Use of Experimental Animals.

Animals and Housing

A total of 1,800 25-week-old Lohmanpink-shell commercial laying hens were randomly assigned to four dietary treatments, with 10 replicates per treatment and 45 layers per replicate. All hens were housed in commercial laying cages with three birds per cage (40×35×40cm) according to a randomized complete block design at the Animal Nutrition Research Centre of Sichuan Agricultural University for a 39-wk trial. Hens received 16 h/d of manipulated lighting and ventilation at a natural ambient temperature. Feed and water were provided *ad libitum* throughout the experiment.

Experimental design and diets

The single factorial design consisted of four vitamin levels (as shown in Table 1): NRC (1994) level, NRC (1994) level with Hy.D® (25-hydroxy-cholecalciferol), Local level (the current average industry level in China) and OVN® level (optimum vitamin nutrition level), the supplemental level of each of them in basal diet was 400 mg/kg. The basal diet was a corn-soybean meal-rapeseed meal layer diet formulated according to the recommendations of NRC (1994) (Table 2). Vitamins were supplied by DSM (DSM China Ltd Corp, Shanghai, P. R. China).

Table 1 - Composition and content of the experimental vitamin premixes added to the layer diet.

Ingredients (- / kg diet)	Vitamin content			
	Local	OVN®	NRC+Hy.D®	NRC
Vitamin A, IU	8520	12500	3000	3000
Vitamin D_3, IU	2400	2500	300	300
25-OH-D_3, mg	—	0.035	0.035	—
Vitamin E, IU	18.50	30.00	5.00	5.00
Vitamin K_3, mg	1.43	2.00	0.50	0.50
Vitamin B_1, mg	—	1.50	0.70	0.70
Vitamin B_2, mg	3.30	6.30	2.50	2.50
Vitamin B_6, mg	0.715	3.80	2.50	2.50
Vitamin B_{12}, mg	—	0.0188	0.004	0.004
Niacin, mg	24.50	40.00	10.00	10.00
Pantothenic acid, mg	8.90	11.30	2.00	2.00
Folic acid, mg	0.50	1.00	0.25	0.25
Biotin, mg	—	0.125	0.10	0.10
Vitamin C, mg	—	100.00	—	—

Data collection

The number of dirty and cracked eggs were recorded daily by replicate and determined on the basis of each replicate weekly. Every four weeks of the trial, 10 eggs per replicate were randomly collected for the measurement of egg-shape index (ESI), egg specific gravity (ESG), eggshell strength (ESS), eggshell

thickness (EST) and Haugh unit (HU). Egg specific gravity was determined by using the saline flotation method as described by Hempe *et al*. (1988). Haugh unit was calculated using the formula of Eisen *et al*. (1962) based on the height of albumen determined by a micrometer and egg weight. The eggshell strength and thickness were determined using the eggshell strength meter and eggshell thickness gauge (Fujihira Corp., Tokyo, Japan) respectively. After the last eggshell quality measurements were taken, the entire contents of eggs were collected and homogenized on the basis of each replicate. Samples were frozen and then freeze-dried to constant weight. Subsamples were reground and then allowed to equilibrate with ambient moisture at room temperature. Samples were sent to Analytical Research Center of DSM Nutritional Products Ltd (Switzerland) to determine the content for all vitamins using HPLC.

Table 2 - Ingredients and nutrient composition of the basal diet fed to laying hens.

Ingredient and composition	Amount (%)
Corn	66.08
Soybean meal	20.86
Rapeseed meal	3.00
Calcium Carbonate	7.82
CaHPO4	1.13
Salt	0.37
Choline Chloride	0.10
DL-Methionine	0.10
Vitamin premix[1]	0.04
Mineral premix[2]	0.50
Calculated nutrient composition	
ME, MJ/kg	11.29
Crude protein,%	16.00
Calcium,%	3.50
Non-phytate phosphorus,%	0.32
Methionine,%	0.35
Lysine,%	0.75
Methionine + Cystine, %	0.65

1 - Vitamin premix (supplied per kilogram of diet) as shown in Table 1. 2 - Mineral premix (supplied per kilogram of diet): Iron ($FeSO_4 7H_2O$), 60mg; Copper ($CuSO_4 5H_2O$), 8.0mg; Manganese ($MnSO_4 H_2O$), 60mg; Zinc ($ZnSO_4 7H_2O$), 80mg; Iodine (KI), 0.35mg; Selenium (Na_2SeO_3), 0.3mg.

Statistical analyses

Data were analyzed by one-way analysis of variance using ANOVA procedure of SPSS (SPSS11.0, 2001) and significant differences among treatment means were compared using the least significant difference test. Statements of statistical significance were based on a probability of ($p<0.05$).

RESULTS

Egg quality parameters, cracked egg and dirty egg of laying hens

As shown in Table 3, the effects of different dietary vitamin levels exhibited did not result in significant differences in the egg quality parameters measured, such as ESI, ESG, HU and EST ($p>0.05$); however, ESS in the Local group (4.33 kg/cm^2) was significantly higher than the other treatments (4.06, 4.07, 3.92 kg/cm^2 for OVN®, NRC with Hy.D®, and NRC level, respectively) ($p<0.05$). CEP and DEP in the vitamin fortified group (OVN®) was significantly reduced ($p<0.05$) as compared to other treatments. As for NRC with Hy.D®, with the addition of Hy.D® in the diet, CEP and DEP significantly declined. Furthermore, ESS was significantly improved ($p<0.05$), as compared with NRC or Local level diet.

Vitamin concentration in whole eggs

Results of vitamin concentration in whole eggs were presented in Table 4. Concentrations of vitamin B_{12}, vitamin A, vitamin E, vitamin B_1 and pantothenate in whole egg in the OVN® group were all significantly higher in comparison with Local, NRC with Hy.D® and NRC groups ($p<0.05$). Folic acid and vitamin D concentrations for OVN® and Local levels were both significantly higher than NRC with Hy.D® and NRC levels ($p<0.05$), while no significant difference was observed between them ($p>0.05$). Vitamin B_2 concentrations were similar among the four dietary treatments with no significant difference ($p>0.05$). 25-OH-D_3 concentration for the OVN® level was

Table 3 - Effects of dietary treatment on ESI, ESG, ESS, HU, EST, CEP and DEP of laying hens.

Treatment	Egg quality						
	ESI[1]	ESG[2] (g/cm^3)	ESS[3] (kg/cm^2)	HU[4]	EST[5] (mm)	CEP[6] (%)	DEP[7] (%)
Local	1.33	1.092	4.33c	79.59	0.37	1.01b	0.59c
OVN®	1.34	1.089	4.06b	78.19	0.36	0.47a	0.27a
NRC+Hy.D®	1.34	1.090	4.07b	77.93	0.37	0.70ab	0.44b
NRC	1.34	1.088	3.92a	78.91	0.36	1.46c	0.65c
SEM	0.009	0.001	0.060	0.793	0.005	0.190	0.066
P-Value	0.300	0.031	<0.001	0.171	0.269	<0.001	<0.001

a-c - Within a column, means with no common superscript letters are significantly different($p< 0.05$). 1 - Egg-shape index. 2 - Egg specific gravity. 3 - Egg-shell strength. 4 - Haugh unit. 5 - Eggshell thickness. 6 - Cracked egg percent. 7 - Dirty egg percent.

Table 4 - Effects of dietary treatment on the vitamin deposition in eggs of laying hens.

Vitamins	Unit	Treatment				SEM	P-Value
		Local	OVN®	NRC+Hy.D®	NRC		
Vitamin B_{12}	µg/kg	4.81a	41.43c	18.10b	18.85b	1.03	<0.001
Folic acid	mg/kg	4.34b	4.50b	2.53a	2.35a	0.14	<0.001
Vitamin A	IU/kg	25375.00b	31875.00c	20950.00a	22375.00ab	1573.38	<0.001
Vitamin D_3	IU/kg	1075.00b	1175.00b	0.00a	0.00a	85.39	<0.001
25-OH-D_3	µg/kg	26.33b	33.30bc	34.63c	12.82a	3.36	<0.001
Vitamin E	mg/kg	80.73b	112.75c	33.40a	32.75a	6.52	<0.001
Vitamin B_1	mg/kg	1.75a	2.40b	2.03a	1.88a	0.14	0.003
Vitamin B_2	mg/kg	15.34	15.88	13.93	14.94	1.00	0.298
Vitamin B_6	mg/kg	LOD	LOD	LOD	LOD		
Vitamin K_3	mg/kg	LOD	LOD	LOD	LOD		
Biotin	µg/kg	525.75a	871.50b	1115.00c	1072.50c	56.97	<0.001
Niacin	mg/kg	9.99a	13.33ab	18.85c	17.38bc	2.20	0.007
Pantothenate	mg/kg	89.93b	98.53c	40.63a	41.78a	3.27	<0.001

LOD - Means below detection limit <LOD. a-c - Within a row, means with no common superscript letters are significantly different($p< 0.05$).

significantly higher than for the NRC level ($p<0.05$), but no significant difference was observed as compared with Local level or NRC level with Hy.D®. As for NRC level with Hy.D® and NRC level, there was no significant difference for most of the vitamins, except for 25-OH-D_3 concentrations, which improved in the NRC level with Hy.D® group. Vitamin B_6 and vitamin K concentrations in eggs were not determined because they were below the detection limit.

DISCUSSION

As compared to the Local level, NRC level with Hy.D® and NRC level, the fortified vitamins in the OVN® diet significantly decreased CEP and DEP. Hens receiving only NRC vitamins had an extremely high CEP and DEP, but this was reduced with the addition of Hy.D®. Furthermore, the ESS was significantly improved, suggesting the inability of regular vitamin D to support maximal shell quality in eggs. The results of the present study were in accordance with the report of Soto-Salanova & Hernandez (2004) and Soto-Salanova & Molinero (2005). It was clear that for NRC level with Hy.D® and NRC level, the only difference between them was Hy.D® inclusion, and no other dietary difference. The OVN® diet still contained vitamin C except for Hy.D®. Hy.D® is a metabolite of vitamin D_3, 25-OH-D_3 which is the most abundant circulating form of vitamin D and plasma levels of the metabolite gives a good indication of the vitamin D status of the chick (Haussler & Rasmussen, 1972). Positive effects of 25-OH-D_3 on egg production and quality have been reported and summarized by Soares *et al*., (1995). Fritts & Waldroup (2003) showed that at comparable levels of potency, 25-OH-D_3 was more efficacious than vitamin D_3 in terms of promoting bone and shell formation. Keshavarz (1996) reported that diets containing 6.25 µg vitamin D_3/kg feed increased the incidence of cracked eggs and eggshell deformations. Poor shell quality is normally a result of poor calcium, phosphorus and vitamin D metabolism. Birds receiving Hy.D® were able to maintain their plasma 25-OH-D_3 at a higher level, thus helping to maintain their bone integrity, egg production and eggshell quality.

In addition to the observed changes in eggshell strength, vitamin C may play some role in improving bone properties and eggshell formation (Sergeev *et al*., 1990; Orban *et al*., 1993). Vitamin C is required for the conversion of vitamin D into its metabolite form calcitrol, which is essential for calcium regulation and the calcification process (Thornton, 1970). Vitamin C is an essential cofactor in the formation of collagen and of the extracellular matrix (Newman & Leeson, 1997). This improvement may be attributed to increased calcium absorption, or possibly to the role that vitamin C plays in the development of bone tissue.

Most reports about the effect of vitamin on egg quality focus on fat-soluble vitamins, while a few reports are on water-soluble vitamins, which also present different results. Keshavarz (2003) reported that supplementing vitamin D or 25-OH-D_3 in the diet did not influence specific gravity of eggs from hens from 49 to 65 wk of age. Mattila *et al*. (2004) reported that no major differences in Haugh units, eggshell strength or specific gravity were observed between the control and vitamin D_2- or D_3-enriched diets. On the other hand, Atencio *et al*. (2006) reported that eggs from hens fed the lowest vitamin D_3 levels had lower specific gravity than those from hens fed the highest vitamin D_3 levels. Mendonca *et al*. (2002) and Mori *et al*. (2003) reported

that the eggshell index, specific gravity, shell thickness and albumen quality of eggs obtained from hens fed supplemental vitamin A or vitamin A and E did not differ from those laid by hens fed the basal diet. Puth pongsiriporn *et al*. (2001) showed that dietary vitamin E and C supplementation had no significant effect on eggshell or Haugh units. Radwan*et al*. (2008) reported that the addition of vitamin E (100 or 200 mg/kg diet) in laying hens diets did not significantly affect eggshell thickness, egg shape index or Haugh units. Sahin*et al*. (2002), however, reported that egg specific gravity, eggshell thickness and Haugh unit were positively influenced by vitamin E supplementation in Japanese quails. Alteration of dietary vitamin level had minor effects on egg quality parameters in terms of the egg shape index, specific gravity, shell thickness and Haugh unit in the present study, which was in agreement with most of reports discussed above.

Vitamin concentration in the hen diet is the most important factor in determining vitamin content in the egg. As vitamin levels in the diet are increased, there is an increase in vitamin levels deposited in the albumen and/or yolk. Supplementation with increasing amounts of dietary vitamins for OVN® level produced significant improvement in egg vitamin concentrations in this present study, which is consistent with the report of Perez-Vendrell*et al*. (2003), who conducted a study in layers using fortified vitamin levels or those commonly used in Spain. For most of the vitamins there were increased accumulations in the egg in response to higher dietary supplementation. Some promising results have been highlighted by Leeson & Caston (2003) for the potential transfer efficiency of some vitamins from hen diets to the egg. These authors studied the effect of supplementing the diet with two vitamin premixes (the regular level and enriched level), and results showed that the concentration of vitamin B_{12} in enriched eggs significantly increased from 36 to over 100% DRI in response to an 11-fold increase in diet vitamin supplementation. There was also meaningful egg enrichment for vitamin D_3 and E, and the level of pantothenic acid was doubled, while vitamin K and biotin levels in modified eggs were not significantly different to those observed in regular eggs.

In the present study, Local level and OVN® level significantly improved the concentrations of vitamin A, vitamin D_3, vitamin E, folic acid and pantothenic acid in eggs as compared to NRC level with Hy.D® and NRC level. Also the OVN® level produced significantly higher levels of vitamin A, E and pantothenic acid than the Local level. These results were in agreement with other investigators who reported the improvement of certain vitamin deposition in eggs when the hen diet was supplemented with a high dose of vitamin A (Squires & Naber, 1993; Jiang *et al*., 1994; Surai *et al*., 1998), vitamin D3 (Mattila *et al*., 1999; Mattila *et al*., 2003; Mattila *et al*., 2004), vitamin E (Frigg *et al*., 1992; Jiang *et al*., 1994; Qi & Sim, 1998; Galobart *et al*., 2002; Grobas *et al*., 2002), folic acid (House *et al*., 2002; Hebert *et al*., 2005; Tactacan *et al*., 2010) or pantothenic acid (Leeson & Caston, 2003). Mendonca *et al*., (2002) reported that progressive increases in the incorporation of retinol into egg yolk was verified when vitamin A was supplemented to the basal diet; egg yolk retinol content increased linearly as dietary vitamin A increased. Mori *et al*. (2003) reported yolk retinol concentration was enhanced by added vitamin A, from 24.6 IU/g for eggs from the control group, to 33.6 and 37.7 IU/g of yolk when hens were fed 15,000 and 30,000 IU/kg of diet. Yolk α-tocopherol was significantly increased by dietary tocopherol supplementation, ranging from 10.9 µg/g (control group) to 160.6, 264.1, and 383.2 µg/g of yolk, respectively, when 200, 400 and 600 mg/kg of ration were added, respectively. Jiang *et al*. (1994) reported that egg yolk α-tocopherol level linearly increased as dietary tocopherol increased. Pal *et al*. (2002) also showed that using 110 *vs.* 55 IU vitamin E /kg diet doubled egg vitamin E content. Dickson *et al*. (2010) concluded that eggs of laying hens were consistently enriched with folate by the dietary supplementation with 4 mg of folic acid/kg of diet throughout the production cycle. Hoey*et al*. (2009) and Bunchasak & Kachana (2009) reported that it was possible to use synthetic folate at high doses (16 and 10 mg/kg diet) to produce novel eggs enriched with natural folates.

There was a significant increase in egg 25-OH-D_3 concentration with the addition of Hy.D® to NRC level diet. This result was not consistent with Mattila *et al*. (1999), who reported a strong positive correlation between cholecalciferol content in poultry feed and cholecalciferol ($r = 0.995$) and 25-hydroxycholecalciferol ($r = 0.941$) contents in egg yolk. Ovesen *et al*. (2003) also suggested that vitamin D in eggs is present almost exclusively as 25-hydroxycholecalciferol, which is absorbed better and faster and has greater biological activity than cholecalciferol. Meanwhile, there was also a significant increase of in egg enrichment of vitamin B_1, vitamin B_{12} and biotin in OVN® level, whereas the Local level did not produce any changes. Vitamin B_{12} perhaps showed the best response, and this finding

was in agreement with Squires & Naber (1992), who reported that egg yolk vitamin B_{12} concentration rapidly responded to dietary changes in the levels of this vitamin and was indicative of the vitamin B_{12} status of the hen. Concentration of biotin in egg albumen increased with incremental dietary biotin levels, but egg yolk concentration was stable, and positive relationship between dietary biotin and the amount of biotin in eggs was also observed (Robel, 1991). The concentration of riboflavin in the eggs of hens fed the OVN® level and the Local level was higher than with the NRC level with Hy.D® and NRC level, but no significant effect was observed. Naber & Squires (1993) reported that there was nearly a linear relationship of diet riboflavin to egg riboflavin contents in the range of 1.5 to 5.0 mg/kg of feed. At 2 to 4 times the dietary requirement of the hens, riboflavin deposition in the eggs was limited by the transfer of riboflavin into the ovum (Squires & Naber, 1993). Riboflavin deposition in the eggs was dependent on dietary riboflavin and reached half-maximal values at about 2 mg of supplemental riboflavin (White *et al*., 1986).

Concentrations of vitamin B_6 and vitamin K_3 were not obtained because they were below the detection limit level in the present study. Considering its restricted biological role, very few studies have attempted to enhance its concentration in eggs. Suzuki & Masayuki (1997) studying the increasing number of hemorrhagic diseases of newborn babies in relation to vitamin K deficiency of pregnant women, demonstrated that by feeding hens with high doses of either phylloquinone (vitamin K_1), menaquinone (vitamin K_2) or menadione (vitamin K_3), it was possible to increase the level of both K_1 and K_2 up to 1,908 and 240 µg/100 g egg yolk, respectively.

CONCLUSION

In conclusion, results of the present study demonstrated that hens receiving vitamins only at NRC levels presented an extremely high cracked egg and dirty egg rates, but this was reduced with the addition of Hy.D®. The addition of Hy.D® significantly enhanced eggshell strength and 25-OH-D_3 concentration in the egg. Hens receiving the Local vitamin levels produced more cracked eggs, dirty eggs and lower vitamin levels in eggs. Hens receiving OVN® vitamins produced eggs containing higher levels of most vitamins, with the least impact seen for dirty and cracked eggs. Apparently, proper vitamin nutrition has significant implications in gastrointestinal health of hens. This could have positive ramifications in marketing eggs with a higher vitamin level and a better nutritive value. The outcomes of this study will enable layer farmers to use vitamins more effectively for the development of functional eggs to meet the needs of specific individuals.

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