Original Article

Effects of Vitamin D_3 and Calcium on Productive Performance, Egg quality and Vitamin D_3 Content in Egg of Second Production Cycle Hens

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Abstract

This experiment was conducted to investigate the effect of supplementary vitamin D_3 on productive performance, internal egg quality, eggshell quality, apparent calcium digestibility and vitamin D_3 content in egg of second production cycle hens fed on different levels of calcium. A total of 140 second cycle brown-egg laying hens, aged 98 weeks, were randomly divided into 5 treatments with 4 replicates. The treatments consisted of diets containing 3.5%Ca and 2,000 IU of vitamin D_3/kg of feed (control), 3.5%Ca and 6,000 IU of vitamin D_3/kg of feed (treatment 2), 3.75%Ca and 2,000 IU of vitamin D_3/kg of feed (treatment 3), 3.75%Ca and 6,000 IU of vitamin D_3/kg of feed (treatment 4), and 4.0%Ca and 2,000 IU of vitamin D_3/kg of feed (treatment 5). All diets were fed for 8 weeks. Thereafter, apparent calcium digestibility was studied for 5 days. There was no significant difference in productive performance and internal egg quality among the dietary treatments (p>0.05). Thickness of eggshell increased with the addition of 6,000 IU vitamin D_3 in the diet containing 3.5%Ca or with the supplementation of calcium higher than 3.5% in the diet (p<0.05). The hens fed on 4.0%Ca in the diet produced eggs with greater specific gravity than the 3.5%Ca groups (p<0.05). Apparent calcium digestibility was not affected by the dietary treatments (p>0.05). Vitamin D_3 content in egg yolks increased with the content of vitamin D_3 in the diet (p<0.01). In conclusion, second production cycle brown-egg laying hens require dietary calcium higher than 3.5% or the addition of 6,000 IU vitamin D_3 in diet containing 3.5%Ca for eggshell quality improvement. Vitamin D_3 content in egg yolks can be increased at least 2.5 times by adding 6000 IU of vitamin D_3/kg feed to hens feed.

Keywords: calcium, eggshell quality, hens, performance, vitamin D_3

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Introduction

Many nutritional factors are involved in eggshell quality of laying hens. Among those factors, calcium and vitamin D_3 appear to be the most relevant and important. Calcium plays an important role in eggshell formation and maintenance of eggshell quality. The shell of egg is composed primarily of calcium carbonate; Ca makes up about 38% of the eggshell (Leeson and Summers, 2001; McDowell, 2003). Functions of vitamin D_3 are associated with calcium. Vitamin D_3 is essential for intestinal uptake and subsequent calcium metabolism. The active form of vitamin D_3 promotes the synthesis of Ca-binding protein which is involved in the calcium transportation in the gut and probably in the uterus. The requirement for vitamin D_3 is increased if the supply of calcium is not at an optimum level (Bar et al., 1992; Leeson and Summers, 2001; Sreenivasaiah, 2006; Bar et al., 2008). In aged hens, eggshell quality is reduced due to disorders related to calcium and vitamin D metabolism. Eggshell deposition is not enough to prevent a decline in eggshell quality when egg size is larger. A large number of eggs with poor shell quality are produced from older hens (Roland, 1980; Abe et al., 1982; Albatshan et al., 1994; Bar et al., 2002). This problem can be ameliorated by forced molting. During the molt the reproductive system is allowed a complete rest from laying and the bird builds up its body reserves of nutrients. There are evidences that layers improve their calcium and Vitamin D metabolism before starting a second laying cycle (Albatshan et al., 1994; Bar et al, 2001; Sreenivasaiah, 2006; Nascimento et al, 2014). However, the beneficial effect of calcium and Vitamin D on eggshell quality does not remain after a long period. In a study, Arpasova et al. (2010) indicated that some eggshell qualitative parameters and dietary calcium utilization for shell formation decreased while the number of cycles increased. Therefore, supplementing calcium and vitamin D_3 to a diet might be a good strategy to maintain eggshell quality of second cycle laying hens. Limited research information is available on calcium and vitamin D_3 requirements of repeat cycle brown-egg laying hens. Zapata and Gernat (1995) observed that increasing the level of Ca from 3.0 to 3.5% significantly increased specific gravity of egg produced by molted white leghorn hens from 1.073 to 1.075. Cufadar et al. (2011) suggested that molted brown laying hens should be fed 3.6%Ca in the diet to maintain eggshell quality. Recently, Nascimento et al. (2014) have recommended that the Ca level for second cycle white egg layers should be approximately 4%. In older brown egg layers, Safaa et al. (2008) reported that more than 3.5%Ca in the diet was required to maintain egg production and eggshell quality. Vitamin D for poultry must be in the form of vitamin D_3 (NRC, 1994). Some studies reported that shell quality increased when vitamin D_3 or its metabolites were supplemented in laying hen diets (Frost and Roland, 1990; Tsang and Grunder, 1993). However, excessive vitamin D_3 can produce adverse effects. The maximum tolerance of poultry to vitamin D_3 is 4 to 10 times of the minimum requirement (NRC, 1994). Therefore, the vitamin D_3 content used in this study was 3 times of the CP brown layer nutrient recommendation and this level was not toxic for hens (Mattila et al., 2004). In addition, many researches showed that vitamin D content in egg could be increased by supplementing laying hen diets with vitamin D (Mattila et al., 1999; Mattila et al., 2003; Yao et al., 2013; Browning and Cowieson, 2014). In the present study, the objective was to evaluate the effect of the additional vitamin D_3 on productive performance, internal egg quality, eggshell quality, apparent calcium digestibility and vitamin D_3 content in egg of second cycle laying hens fed various levels of calcium.

Materials and Methods

Animals and experimental diets: All experimental procedures used in this experiment were approved by the Animal Ethics Committee of Chulalongkorn University. One hundred and forty 98-week-old commercial brown-egg laying hens (CP Brown) in their second production cycle were divided into five treatments. Twenty-eight birds were assigned to each treatment consisting of four replicates. The treatments consisted of diets containing 3.5%Ca and 2,000 IU of vitamin D_3/kg of feed (control), 3.5%Ca and 6,000 IU of vitamin D_3/kg of feed (treatment 2), 3.75%Ca and 2,000 IU of vitamin D_3/kg of feed (treatment 3), 3.75%Ca and 6,000 IU of vitamin D_3/kg of feed (treatment 4), and 4.0%Ca and 2,000 IU of vitamin D_3/kg of feed (treatment 5). A control diet was formulated to meet the calcium and vitamin D_3 requirements of CP brown layer. Vitamin D_3 was supplemented in two lower calcium level treatments (treatments 2 and 4). The basal or control diet was based on corn and soybean meal. The ingredients and calculated nutrient composition of the basal diet are shown in Table 1. All experimental diets were fed for 8 weeks. At the end of the 8th week, chromic oxide (0.25%) was added to the diets to conduct a calcium digestibility study. These diets were fed for 5 days. All experimental diets were prepared and presented in a mash form. The diet and water were provided ad libitum throughout the experimental period. The experimental diets were fed twice a day at 08.00 h and 13.00 h. All hens were reared in an evaporative cooling system with a lighting program, 16 h per day. Temperature in the hen house was recorded daily during the experimental period.

Data collection and chemical analyses: The number of total eggs were recorded and weighed daily by replicate. Feed intake was measured weekly on a replicate. Egg production, average egg weight and average daily feed intake were calculated from these data. At the end of feeding period, sixteen eggs from each treatment were randomly collected to measure internal egg quality, eggshell quality and vitamin D_3 content. The internal egg quality was determined by Haugh unit and percentage of albumen and yolk. The Haugh unit measurement was performed by using a standard tripod micrometer which measured the albumen height in millimeters. This device was adjusted manually for egg weight and displayed HU directly. The albumen and yolk were separated and weighed. Then, the percentage of albumen and yolk were calculated. The eggshell quality was determined by shell thickness and specific gravity of egg. The shell thickness was measured at three different parts (aircell, equator and sharp end) by micrometer. Egg specific gravity was determined by using NaCl solutions made of incremental concentration of 0.004 in the range from 1.070 to 1.110. The vitamin D_3 content was determined in freeze-dried egg yolk using the method of the Association of German Agricultural Analytic and Research Institutes (VDLUFA, 2007). For the calcium digestibility study, fecal content of each replicate was collected in approximately the same amount at the same time of the day, during the last 3 days. Chromic oxide of diets and feces were measured by the method described by Fenton and Fenton (1979). Dry matter and calcium content of diets and feces were determined by the methods of AOAC (2005).

Table 1 Ingredient composition and calculated nutrient composition of basal diet

Ingredient	Amount (kg/100kg)
Corn	54.66
Soybean meal	22.55
Rice bran	12.00
Limestone	8.28
Monodicalciumphosphate	1.33
Lard	0.33
Sodiumbicarbonate	0.24
Salt	0.18
Choline Chloride	0.11
DL-Methionine	0.11
Vitamin Premix*	0.10
Mineral Premix**	0.10
Antioxidant	0.01
Calculated nutrient content	
Crude Protein (%)	16.30
ME (Kcal/kg)	2750
Crude Fat	4.35
Crude Fiber	3.70
Calcium	3.50
Available Phosphorus	0.40
Vitamin D_3 (IU/kg)	2000

*Vitamin premix per kilogram of diet provided 10,000 IU vitamin A; 2,000 IU vitamin D3; 20 IU vitamin E; 2 mg vitamin K3; 2 mg vitamin B1; 5 mg vitamin B2; 5 mg vitamin B6; 10 mg vitamin B12; 30 mg niacin; 10 mg panthothenic acid; and 0.74 mg folic acid.
**Mineral premix per kilogram of diet provided 25 mg zinc, 10 mg copper, 120 mg iron, 60 mg manganese, and 0.2 mg selenium.

Calculation and statistical analysis: The apparent calcium digestibility was calculated using the following formula:

Apparent Ca digestibility = [1 - (% Chromic oxide in diet x % calcium in feces)/% Chromic oxide in feces x % calcium in diet)] x 100

All dependent variables were performed as a completely randomized design to determine the effect of treatment groups. The dependent variables, production performance egg quality and calcium digestibility were analyzed by using a one-way analysis of variance. Difference between treatments was determined by Duncan's New Multiple Range test at the level of $p<0.05$.

Results

The effects of supplementary vitamin D_3 on the productive performance and internal egg quality of second production cycle laying hens fed different levels of calcium are shown in Table 2. The dietary treatments had no effect ($p>0.05$) on the amount of feed consumed, egg weight and egg production. The average of feed intake, egg weight and egg production of hens from all treatments ranged from 110.9 to 111.8 g/d, 63.4 to 65.9 g and 72.8 to 74.6%, respectively. The Haugh units and percentage of albumen and yolk were not influenced by the dietary treatments ($p>0.05$).

The eggshell quality and apparent calcium digestibility are shown in Table 3. The eggshell quality parameters were affected by the dietary calcium levels or vitamin D_3 supplementation. The eggshell thickness was lowest in eggs from hens fed the control diet ($p<0.05$). The thickness of eggshell increased with the addition of 6,000 IU vitamin D_3 containing 3.5%Ca or with the increase in calcium higher than 3.5% in the diet. The hens fed 4.0%Ca in the diet produced eggs with greater specific gravity than the 3.5%Ca groups ($p<0.05$). There was no significant difference in the apparent calcium digestibility among the treatment groups ($p>0.05$).

As depicted in figure 1, the vitamin D_3 content in egg yolks increased with the content of vitamin D_3 in the diet ($p<0.01$). There was no significant difference in the vitamin D_3 content of egg yolk among the treatments with the same level of vitamin D_3 in the diet.

The hens fed on the higher vitamin D_3 level (6,000 IU/kg feed) produced higher vitamin D_3 content in egg yolks (3360-3527 IU/kg), while the hens fed on the lower vitamin D_3 level (2,000 IU/kg feed) produced lower vitamin D_3 content in egg yolks (1324-1495 IU/kg).

Discussion

Increasing the dietary calcium levels and vitamin D_3 supplementation did not affect the feed intake, egg weight and egg production of the laying hens in the current study. Several published researches also reported that no additional improvement in productive performance or internal egg quality were obtained when dietary calcium was increased to more than 3.5% (Keshavarz et al., 1993; Roland and Bryant, 1994; Castillo et al., 2004). In addition, Bar et al. (2002) found that egg production, egg weight and feed intake were not affected when the calcium content of the diet was increased from 3.6 to very high level of 4.9% in Lohmann Brown hens aged 66 to 78 weeks. However, Safaa et al. (2008) indicated that an increase in calcium level from 3.5 to 4% of the diet improved some performance parameters, including egg production, egg mass, and feed conversion ratio in Lohmann Brown laying hens aged 58 to 73 weeks. The reason for the disparity among researchers might be due to the differences in age, number of cycle, rate of egg production and nutrient content of the diet used. For vitamin D_3 supplementation, it did not affect the productive performance and internal egg quality of the hens receiving 3.5 and 3.75% of calcium in the diet. This finding agrees with the report of Mattila et al. (2004), who found that vitamin D_3 supplements (6,000 IU/kg feed) had no effects on production traits compared with the control diet (2,500 IU/kg feed) for groups of laying hens (20 to 67 weeks of age) receiving 3.69% of calcium in the diet. Browning and Cowieson (2014) also reported no difference in layer performance or egg quality with higher levels of vitamin D_3 (up to 10,000 IU/kg feed). From this study, it is revealed that increasing calcium levels and supplementing vitamin D_3 cannot improve animal productive performance and internal egg quality. This is probably because 3.5% of calcium and 2,000 IU/kg feed of vitamin D_3 in the diet are adequate for maintaining productive performance and internal egg quality of second production cycle laying hens.

Table 2 Productive performance and internal egg quality as influenced by dietary Ca and vitamin D_3 in second production cycle hens

Item	Ca 3.5%	Ca 3.5% + Vit D_3	Ca 3.75%	Ca 3.75% + Vit D_3	Ca 4%	SEM	*p*-value
Performance							
Egg production (%)	73.21	73.66	74.11	72.77	74.55	2.316	0.825
Egg weight (g)	64.40	64.21	64.95	65.89	63.43	2.884	0.805
Feed intake (g/d)	110.9	111.8	111.1	111.4	110.9	1.779	0.951
Internal egg quality							
Haugh unit	79.84	81.13	80.44	82.85	83.42	3.382	0.525
Percentage of albumen (%)	64.42	62.08	61.19	61.66	60.06	2.928	0.349
Percentage of yolk (%)	25.53	23.75	24.95	24.56	24.07	1.3542	0.397

Table 3 Eggshell quality and apparent calcium digestibility as influenced by dietary Ca and vitamin D_3 in second production cycle hens

Item	Ca 3.5%	Ca 3.5% + Vit D_3	Ca 3.75%	Ca 3.75% + Vit D_3	Ca 4%	SEM	*p*-value
Eggshell quality							
Eggshell thickness (mm)	0.335^{a}	0.370^{b}	0.361^{b}	0.373^{b}	0.379^{b}	0.031	0.011
Egg specific gravity	1.092^{a}	1.093^{a}	1.095^{ab}	1.097^{ab}	1.099^{b}	0.006	0.047
Apparent calcium digestibility (%)	51.25	40.69	51.28	43.46	48.44	8.667	0.348

^{a,b} means in a row with different superscripts are significantly different (p<0.05).

The improvement in eggshell quality was observed with the increase in level of calcium or supplementation of vitamin D_3 content in the diet. The eggshell thickness was improved by adding 6,000 IU vitamin D_3 in the diet containing 3.5%Ca or supplementing calcium higher than 3.5% in the diet. This finding indicates that the supplementation of vitamin D_3 into diet has the ability to compensate for the adverse effect of low calcium level (3.5% in feed) on eggshell quality. More vitamin D_3 content might improve the eggshell quality by increasing the active form of vitamin D_3 (1,25-dihydroxycholecalciferol; 1,25$(OH)_2D_3$) production in the kidney. 1,25$(OH)_2D_3$ stimulated the synthesis of calcium-binding protein which is essential for transportation of calcium across the intestinal membrane and may be essential for transportation of calcium for eggshell formation in the shell gland (Bar et al., 1992; Yoshimura et al., 1997; Bar et al., 2008). However, the thickness of eggshell was not changed by supplementing vitamin D_3 content in the group of hen fed on the higher level of calcium (3.75%Ca). Some authors also observed that egg specific gravity and shell thickness were not significantly different between diets containing 1,500-2,500 and 6,000 IU vitamin D_3/kg feed when laying hens were fed on 3.69-3.87%Ca (Yannakopoulos and Morris, 1979; Mattila et al., 2004). However, the group receiving 4.0%Ca in the diet had a significantly greater specific gravity than those receiving 3.5%Ca. This finding is supported by the report of Safaa et al. (2008), who observed improvement in shell thickness (from 0.342 to 0.351 mm) and shell density (82.0 to 83.8 mg/cm2) of eggs in Lohmann Brown laying hens aged 58 to 73 weeks when the calcium level of the diet was increased from 3.5 to 4%. The results also agree with the report of Lichovnikova (2007), who suggested 4.1% of calcium in the diet for maintaining eggshell quality in the last period of the production cycle. The result of calcium digestibility study may support this finding. The second production cycle laying hens fed on different diets had similar apparent calcium digestibility while the amount of feed consumed did not differ among the treatment groups. Therefore, the total amount of calcium absorbed from higher calcium diets should be greater than the lower calcium diets with similar feed intake. The better eggshell quality could be obtained from the higher amount of absorbable calcium. These might be the reason for the higher egg specific gravity when the calcium content of the diet was increased to 4%.

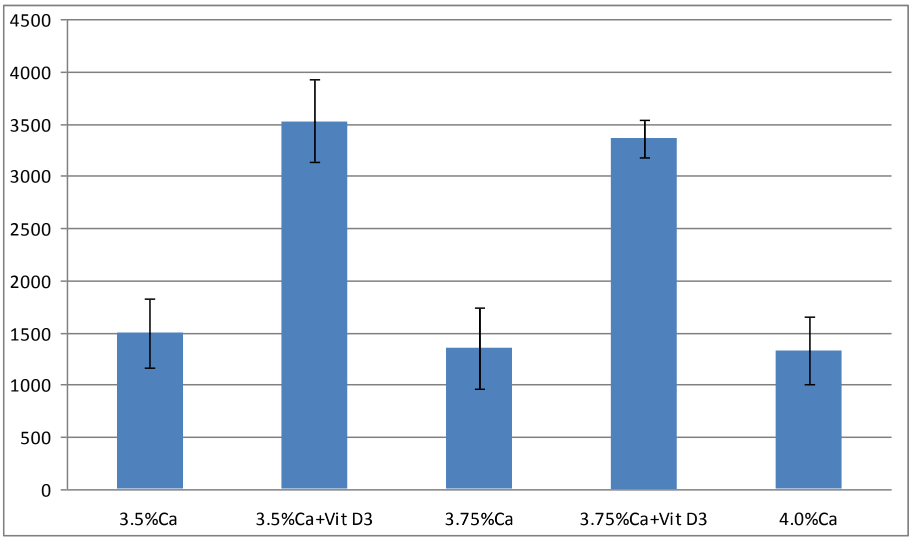

Figure 1 Vitamin D_3 content (IU/kg) in egg yolk as influenced by dietary Ca and vitamin D_3 in second production cycle hens

Vitamin D is found naturally in only a few food sources of human, and eggs are considered to be one of the most important sources of vitamin D. In present study, the vitamin D_3 content in egg yolks could be increased by supplementing hens feed with vitamin D_3. The addition of 6000 IU of vitamin D_3/kg feed to laying hen diets resulted in vitamin D_3-enriched eggs which contained more than 2.5 times of the amount of vitamin D_3 found in those produced from the control group. The capacity of vitamin D deposition in egg yolk was similar to previous studies. Mattila et al. (2004) reported that eggs produced from hens receiving 6000 IU of vitamin D_3/kg feed contained 2.7 to 3.6 times more vitamin D than eggs from hens receiving 2500 IU of vitamin D_3/kg feed. A similar result was also reported by Browning and Cowieson (2014), who observed that the vitamin D content of egg yolk increased (1.6 times) with the addition of vitamin D_3 (5000 IU/kg feed) compared with the lower concentration of vitamin D_3 (2500 IU/kg feed) in the layer feed. It is evident that eggs with higher vitamin D content can be produced by feeding hens with increasing level of vitamin D_3.

In conclusion, during the second production cycle commercial brown-egg strain of laying hens require dietary calcium higher than 3.5% or the addition of 6,000 IU vitamin D_3 in diet containing 3.5%Ca for eggshell quality improvement. Vitamin D_3 content in egg can be increased (>2.5 times) by adding 6000 IU of vitamin D_3/kg feed to hen feed.

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บทคัดย่อ

ผลของวิตามินดีสามและแคลเซียมต่อสมรรถภาพการผลิต คุณภาพไข่และปริมาณวิตามินดีสามในไข่ของไก่ไข่ที่ให้ผลผลิตรอบที่สอง

หทัยรัตน์ พลายมาศ สุวรรณา กิจภากรณ์ ปัญพัสตร์ อิทธิธนาวงษ์*

การทดลองนี้ศึกษาผลของการเสริมวิตามินดีสามต่อสมรรถภาพการผลิต คุณภาพภายในของไข่ คุณภาพเปลือกไข่ การย่อยได้แบบปรากฏของแคลเซียมและปริมาณวิตามินดีสามในไข่ของไก่ไข่ที่ให้ผลผลิตรอบที่สองที่ได้รับปริมาณแคลเซียมในระดับต่างๆ แบ่งไก่ไข่ให้เปลือกไข่สีน้ำตาลที่ให้ผลผลิตรอบสองจำนวนทั้งหมด 140 ตัว อายุ 98 สัปดาห์ ออกเป็น 5 กลุ่มๆละ 4 ซ้ำ อาหารทดลองประกอบด้วยอาหารควบคุมที่มีระดับแคลเซียม 3.5% และวิตามินดีสาม 2,000 IU ต่อกิโลกรัมของอาหาร อาหารที่มีระดับแคลเซียม 3.5% และวิตามินดีสาม 6,000 IU ต่อกิโลกรัมของอาหาร อาหารที่มีระดับแคลเซียม 3.75% และวิตามินดีสาม 2,000 IU ต่อกิโลกรัมของอาหาร อาหารที่มีระดับแคลเซียม 3.75% และวิตามินดีสาม 6,000 IU ต่อกิโลกรัมของอาหาร และอาหารที่มีระดับแคลเซียม 4.0% และวิตามินดีสาม 2,000 IU ต่อกิโลกรัมของอาหาร การทดลองพบว่าสมรรถภาพการผลิตและคุณภาพภายในของไข่แต่ละกลุ่มทดลองไม่แตกต่างกัน ($p>0.05$) การเสริมวิตามินดีสามในอาหารที่มีระดับของแคลเซียม 3.5% หรือการให้แคลเซียมในระดับที่สูงกว่า 3.5% มีผลทำให้เปลือกไข่มีความหนาเพิ่มขึ้น ($p<0.05$) ไก่ไข่ที่ได้รับอาหารที่มีระดับของแคลเซียม 4.0% ให้ผลผลิตไข่ที่มีความถ่วงจำเพาะสูงกว่ากลุ่มที่ได้รับแคลเซียม 3.5% ($p<0.05$) อาหารทดลองที่แตกต่างกันไม่มีผลต่อการย่อยได้แบบปรากฏของแคลเซียม ($p>0.05$) ปริมาณวิตามินดีสามในไข่แดงเพิ่มขึ้นตามปริมาณวิตามินดีสามที่เพิ่มขึ้นในอาหาร ($p>0.01$) จากผลการทดลองสรุปได้ว่าไก่ไข่ให้เปลือกไข่สีน้ำตาลที่ให้ผลผลิตรอบสองต้องการแคลเซียมในอาหารสูงกว่า 3.5% หรือวิตามินดีสามเสริมในปริมาณ 6,000 IU ต่อกิโลกรัมของอาหารที่มีระดับของแคลเซียม 3.5% เพื่อปรับปรุงคุณภาพของเปลือกไข่ ปริมาณวิตามินดีสามในไข่แดงเพิ่มขึ้นอย่างน้อย 2.5 เท่าเมื่อเสริมวิตามินดีสามในอาหารไก่ไข่ 6,000 IU ต่อกิโลกรัมของอาหาร

คำสำคัญ: แคลเซียม คุณภาพเปลือกไข่ ไก่ไข่ สมรรถภาพการผลิต วิตามินดีสาม

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