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Effect of cholecalciferol (D_3) replacement with 1alpha-hydroxycholecalciferol on broiler breeder hen's performance

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Abstract

This experiment was carried out to compare the effects of 1α-hydroxy cholecalciferol replacement with cholecalciferol on broiler breeder hen's performance. 288 Ross"308" broiler breeder hens at 57 weeks of age were allocated to a randomized complete design with six treatments and four replication of twelve females and 1 male each. Treatments include: 3500 IU/kg vitamin D_3 (T1), 3340 (T2), 3300 (T3), 3260 and 3180 IU D_3 (T5). The incomplete levels of the vitamin D_3 in T2, T3, T4 and T5 supplemented by adding 1α(OH)D_3 to the diets at levels 10, 12.5, 15 and 20 gr/ton, respectively and treatment with no D_3 supplementation. Results showed, compared to lower levels of the same metabolites and also as compared with the hens fed D_3, significant reduction in egg production when 20 g/Ton 1α(OH)D_3 was added in diet; however, no differences were observed in egg weight, egg specific gravity, hatchability, early, middle and late embryo mortality and piped egg, plasma calcium and phosphorous concentration and tibia ash between treatments supplemented with combination of vitamin D_3 and 1α(OH)D_3 as compared with D_3. The hens fed diet without supplement vitamin D showed significant decrease in egg production and egg mass, egg specific gravity, hatchability and significant increase in feed conversion ratio and early and late embryonic mortality, without any effect on other traits. In conclusion, replacement of 1α(OH)D_3 with D_3 in broiler breeder diets have no beneficial effect on egg production, egg shell quality and hatchability. It seems, that hens are able to metabolize sufficient 1, 25$(OH)_2D_3$ from dietary vitamin D_3 to meet requirement.

Key words: 1α-hydroxycholecalciferol, Broiler breeder, Vitamin D

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Introduction

In poultry industry eggshell quality is one of the most important problems, influencing economic profitability of egg production and egg hatchability (Roque and Soares, 1994). The successful development of chicken embryos is depend on a robust eggshell for mechanical protection, protection from infection, prevention of water loss and as a primary source of calcium for the embryonic skeleton (Hunton, 2005). Maternal nutrition has a crucial role in subsequent development and hatching of avian embryos. If nutritional deficiencies occur during the formation of the egg, it can have significant repercussions on the developing embryo (Moran, 2007). In this regard, vitamin D content of the egg is an important factor in the calcium metabolism of the developing embryo and feeding the hen inadequate vitamin D results in reduced eggshell quality and hatchability (Wilson, 1997). The form of 1, $25(OH)_2D_3$ has activity ten-fold greater than vitamin D_3 itself. However, its supplementation as the only source of vitamin D has demonstrated with reduced hatchability (Sunde et al., 1978; Henry and Norman, 1978; Abdulrahim et al., 1979). Hart et al. (1986) showed that both 1, $25(OH)_2D_3$ and $25(OH)D_3$ are transported from the hen to the egg and that the ratio of 1, $25(OH)_2D_3$ to $25(OHD)_3$ in the yolk is the same as that in the hen's plasma. 1, $25(OH)_2D_3$ is present in very low concentrations in the plasma as it is cleared quickly from circulation due to its high toxicity at relatively low concentrations and this is why very little $1,25(OH)_2D_3$ is appeared in the egg (such low and sufficient quantities can be used to support embryonic development). In contrast, the metabolite as $25(OH)D_3$ has higher absorption and longer half-life, may be reflection of high rate of transference into eggs (Bar et al., 1980). As the hen ages, the number of egg produced declines along with a reduction in eggshell quality. These changes are attributable at least in part through reduced populations of estrogen receptor in kidney and shell gland, diminished activity of 25-hydroxycholecalciferol1α- hydroxylase, an enzyme involved in calcium homeostasis (Elaroussi et al., 1993). However, attempts to improve eggshell quality with several vitamin D_3 metabolites have yielded inconsistent results (Beneficial results: Soares et al., 1988; Tsang et al., 1990 a,b; Adverse results: Roland and Harms, 1976; Harrms et al., 1988). One of the reasons for the negative effects obtained is related to overdosing. 1α-hydroxycholecalciferol is also known as $1\alpha(OH)D_3$, alfacalcidol and 1α-OH vitamin D_3. $1\alpha(OH)D_3$, a synthetic derivative of cholecalciferol which lacks the hydroxyl group on carbon-25. The $1\alpha(OH)D_3$ derivative, once absorbed from the diet, is probably hydroxylated at carbon-25 in the liver to become $1,25(OH)_2D_3$ (Hausler et al., 1973) and has been shown to be very potent source of vitamin D activity for growing

chickens (Edwards et al., 2002). No results have been reported on the effect of $1\alpha(OH)D_3$ when supplemented to broiler breeder diets.

The objective of this study was to evaluate the effects of $1\alpha(OH)D_3$ supplementation in broiler breeders diets and their subsequent egg production, egg quality, hatchability, plasma calcium and phosphorus level and bone ash.

Materials and Methods

Broiler breeders were obtained from a local company, Guilan, Iran. Two hundred and eighty eight female and twenty four male broiler breeder were used in a randomly complete design with 6 treatments and 4 replication of 12 females and 1male per pen. Treatments consisted of experimental diets with 3500 IU/kg vitamin D_3 (T1; DSM Swiss), 3340 (T2), 3300 (T3), 3260 (T4) and 3180 IU D_3 (T5). The incomplete levels of the vitamin D_3 in T2, T3, T4 and T5 supplemented by adding $1\alpha(OH)D_3$ (Vitamin Derivatives Inc., Georgia, USA) to the diets at levels 10, 12.5, 15 and 20 gr/ton, respectively and one additional group was fed no supplemental D_3 (T6) . All treatments assumed to contain a sum of 3500 Iu/kg vitamin D, except for the treatment (T6). The basal diet formulated (Table 1) to meet all nutrient requirements of broiler breeder hens according to Ross Breeders Management Guide (2006). No D_3 source was used in the vitamin premix, and no animal by-product was used in the corn, soybean meal based diet to guarantee that no unintentional vitamin D activity was present in the experimental diets. The adaptation period to experimental diets after allotment lasted 21days. The birds were fed the experimental diets for 14 wks (57 to 70 wks of age) during the fall and winter. A 3 weeks pre experiment was conducted to record egg production and egg weight. The means of these traits for different treatments were shown similar at the start of the experiment ($P > 0.05$). Eggs were collected daily throughout the duration of the experiment and stored at 17°C. Only nest eggs that were not dirty, misshapen, broken, cracked, excessively small or double-yolked were classified as settable eggs. Eggs collected during 7 d were grouped and set in the incubator by treatment and pen. At the end of incubation, eggs that did not hatch were broken to perform embryo diagnosis with classification of eggs as infertile or dead embryos and piped (beak penetrated eggshell but chick did not emerge). Embryo mortality was separated as early (1 to 10 days), mid (11 to 17 days) or late dead (18 to 21 days). The differences between total eggs set and infertile eggs allowed the calculation of the percent hatchability of fertile eggs. Egg specific gravity and egg weight were measured on all eggs produced for two consecutive days. Egg specific gravity was determined using salt solutions varying in specific

Table1. Composition and calculated analysis of experimental diets

Ingredient	g/kg
Corn	695.3
Soybean meal	200
Limestone	56.67
Oyster shell	20
Mono Phosphate	10.67
Vegetable oil	3.3
NaCl	3.3
Sodium Bicarbonate	1.0
Vitamin premix[1]	2.5
Mineral premix[2]	2.5
DL-Methionine	0.5
Choline chloride	2.0
Toxin- binder	2.0
Sel-plex	0.26
Calculated composition	
ME (MJ/ kg)	11.7
Crude protein (g/kg)	150
Calcium (g/kg)	31
Available phosphorus (g/kg)	3.5
Lysine (g/kg)	5.8
Methionine (g/kg)	2.8

[1]Vitamin mix provided the following: (2.5 kilogram of diet): vitamin A, 11000000 IU; Vitamin E, 100000 IU; K3, 5000mg; Vitamin B1, 3000 mg; vitamin B2, 12000mg, niacin, 15000mg; pantothenic acid,55000mg; pyridoxine, 4000mg; folic acid, 2000 mg; vitamin B12, 30 mg; biotin, 250 mg; antioxidant, 2500 mg.

[2]Trace mineral mix provided the following: (2.5 kilogram of diet): manganese, 120000 mg; iron, 50000 mg; zinc, 100000 mg; copper, 10000 mg; iodine, 2000 mg; selenium, 300 mg; cobalt, 500 mg.

gravity from 1.05 to 1.100 in increments of 0.005 units on a biweekly period of the experiment (Hamilton, 1982). At the end of 14 weeks experimental period, blood samples were obtained from the brachial veins of two hens per pen; the plasma was separated by centrifugation blood for 10 min at 3000 rpm and saved for determination of plasma calcium and phosphorous. The plasma calcium and phosphorus were measured on auto analyzer using commercial kits. Two birds from each experimental groups were killed by cervical dislocation and left tibia obtained from each hen at the end of the experiment. Tibia ash was measured according to method described by the Hall et al. (2003).

Data were analyzed using the general linear models procedure of SAS software (SAS Institute, 2002). The means of differences were determined using Duncan's multiple range test.

Results and Discussion

Production performance characteristics of breeder hens are shown in Table 2. Inclusion of $1\alpha(OH)D_3$ to the diet had no adverse effect on egg production of broiler breeders except for T_5. The hens fed diet containing 20 gr/ton $1\alpha(OH)D_3$(T5) had a lower egg production, egg mass and higher feed conversion ratio than those fed lower levels of the $1\alpha(OH)D_3$ and 3500 IU/Kg

of vitamin D (T1) ($P < 0.05$). On the other hand, the group fed the vitamin D_3 deficient basal diet (T_6) significantly depressed egg production and egg mass as compared to other treatments ($P < 0.05$). The best feed conversion ratio value was recorded for T_4 while T_5 and T_6 appeared with the worst feed conversion ratio. Weekly egg production for 57 to 70 wks of age is shown in Figure 1. After 2 weeks of experiment significant decreases ($P < 0.05$) were observed in egg production for T_5 and T_6, and continued until the end of the experiment. Egg production was similar in T_1, T_2, T_3 and T_4 till 64 week of ages. After 64 week of age, a rapid decrease in egg production was recorded in birds fed 3500 IU vitamin D_3 (T_1) as compared to those fed $1\alpha(OH)D_3$. Colecalciferol deficiency in the diet of laying hen affects estrogen metabolism (Tsang and Grunder, 1984), in particular, the sulphate pathway that resulting in the reduced conversion of estradiol- 17 β- 3 sulphate ($E_2\beta$-3S) to estradiol-17α- 3 sulphate (E2α-3S), and could partially explain the drop in egg production under these condition. This result is consistent with the observations of Abdulrahim et al. (1979). Shen et al. (1981) reported that withdrawing of supplemental D_3 led to reduced egg production and egg shell quality. Soares et al. (1983) found that the toxic level of lα-hydroxyvitamin D_3 for the laying hen without vitamin D_3 supplementation was 6.8 µg/kg diet. Tsang et al. (1990b) observed 20% lower egg production in laying hens fed on diet 7 µg $1,25(OH)_2D_3$ as compared to the control. Harms et al. (1988) found a significant reduction in egg production by feeding 4 µg/kg $1,25(OH)_2D_3$ to the diet of 68-wk-old laying hens. In an experiment that was conducted by Abdulrahim et al. (1979), $1\alpha(OH)D_3$ and $1,25(OH)_2D_3$ were fed to laying hens at 3 and 9 µg/kg, but they failed to maintain good hatchability at either level. Egg production increased when 3 µg/kg of both D metabolites were supplemented in the diet, but was reduced at the 9 µg/kg level. In contrast, Soares et al. (1988) did not observe differences in egg production, egg weight and egg mass with hens receiving $1\alpha(OH)D_3$ as compared to vitamin D_3. This result is inconsistent with finding of Harms et al. (1990) and Frost et al. (1990) who did not observe differences in egg production by addition of $1,25(OH)_2D_3$ and $1\alpha(OH)D_3$ to a basal diet containing vitamin D_3 (2200 IU/Kg). The reason for the discrepancy among investigators might be due to differences in the strain, age, nutrient specifications of the diets used, levels of D metabolites used in the experimental diets, duration of the studies, exposure to UV light and presence of animal byproducts containing vitamin D (Atencio et al., 2006).

The level of $1\alpha(OH)D_3$ supplementation had no significant effect on egg weight and egg specific gravity. Egg weight and egg specific gravity were not affected by different method of D metabolites inclusion in this experiment (Table 2). However, a significance reduction in egg specific gravity was observed between the treatment devoid of vitamin D_3 (T_6) and the other

Table 2. Effect of treatments on productive performance of broiler breeder hens form 57 to 70 wks of age (Means±SEM)

	Treatment[1]					
Parameter[2]	T1	T2	T3	T4	T5	T6
EP (%)	61.94±1.29^{a}	59.87±1.13^{a}	60.91±1.32^{a}	62.43±1.19^{a}	57.12±1.33^{b}	51.88±1.31^{c}
EW (g)	68.64±0.12^{a}	69.00±0.16^{a}	68.96±0.15^{a}	69.05±0.14^{a}	68.93±0.13^{a}	68.96±0.16^{a}
EM (g)	42.50±0.87^{a}	41.25±0.73^{ab}	41.97±0.89^{ab}	43.11±0.82^{a}	39.39±0.92^{c}	35.72±0.87^{d}
FC (g:g)	3.75±0.09^{c}	3.83±0.06^{c}	3.80±0.09^{c}	3.69±0.08^{c}	4.07±0.10^{b}	4.51±0.12^{a}
ESG (g/cm3)	1.0741±0.0006^{a}	1.0734±0.0007	1.0733±0.0007^{a}	1.0733± .0005^{a}	1.729±0.0007^{a}	1.0698±0.0008^{b}

[1]Row with different superscripts are significantly differ ($P < 0.05$)
[2]EP: Egg Production; EW: Egg Weight; EM: Egg Mass; FC: Feed Conversion; ESG: Egg Specific Gravity

Table 3. The effect of treatments on hatchability and embryonic mortality in different days of incubation period (Means ±SEM)

	Treatment[1]					
Parameter2	T1	T2	T3	T4	T5	T6
FH (%)	93.67±0.86^{a}	93.25±0.85^{a}	92.02±0.86^{a}	92.68±0.87^{a}	92.80±0.97^{a}	86.16±1.45^{b}
EEM (%)	2.84±0.52^{b}	3.23±0.40^{b}	4.47±0.69^{b}	3.68±0.55^{b}	3.98±0.70^{b}	7.82±1.19^{a}
MEM (%)	0.98±0.24^{a}	1.81±0/39^{a}	1.19±0.30^{a}	1.09±0.29^{a}	1.81±0.38^{a}	1.98±0.29^{a}
LEM (%) (g:g)	1.99±0.44^{b}	1.23±0.40^{b}	1.83±0.49^{b}	1.74±0.29^{b}	1.18±0.33^{b}	3.39±0.56^{a}
PE (%)	0.62±0.20^{a}	0.58±0.19^{a}	0.61±0.25^{a}	1.00±0.37^{a}	0.27±0.13^{a}	0.79±0.55^{a}

[1]Row with different superscripts are significantly differ ($P < 0.05$)
[2]FH: Fertile hatchability; EEM: Early Embryo Mortality; MEM: Middle Embryo Mortality; LEM: Late Embryo Mortality; PE: Piped Egg

Table 4. Effect of treatments on Ca and P levels of plasma and tibia ash (Means±SEM)

	Parameters[1]		
Treatments	Ca (mg/dl)	P (mg/dl)	Tibia ash (%)
T_1	23.56±1.08	6.72±0.62	71.02±0.81
T_2	23.22±1.69	6.44± 0.60	71.27±0.64
T_3	24.98±1.01	8.55±0.49	70.95±0.60
T_4	23.83±1.32	7.86±0.95	71.52±0.53
T_5	23.88±1.47	7.77±1.07	70.45±0.76
T_6	21.51±2.36	6.05±0.67	69.43±0.41

five treatments. In studies conducted by Keshavarz (2003) and Atencio et al. (2006) no beneficial effect of supplementing $25(OH)D_3$ as compared to D_3 were found in egg weight and egg specific gravity in the experiments using laying hen and broiler breeder respectively. The result of this experiment is consistent with the findings of Harms et al. (1988), who showed that addition of 1, 25-dihydroxycholecalciferol at 4 µg/kg to a basal diet containing vitamin D_3 (2200 IU/kg) resulted in no beneficial effect in egg specific gravity. The presence of $1\alpha(OH)D_3$ did not appeared with significant effect on egg weight and egg specific gravity, that is in agreement with Harms et al. (1990) who showed the addition of the $l\alpha(OH)D_3$ or the $l,25(OH)_2D_3$ metabolite did not affect egg weight and egg specific gravity. Tsang et al. (1990 a,b) showed that calcitriol added to at the optimal level of 5 µg/kg a diet without supplemental vitamin D_3 improve egg shell quality.

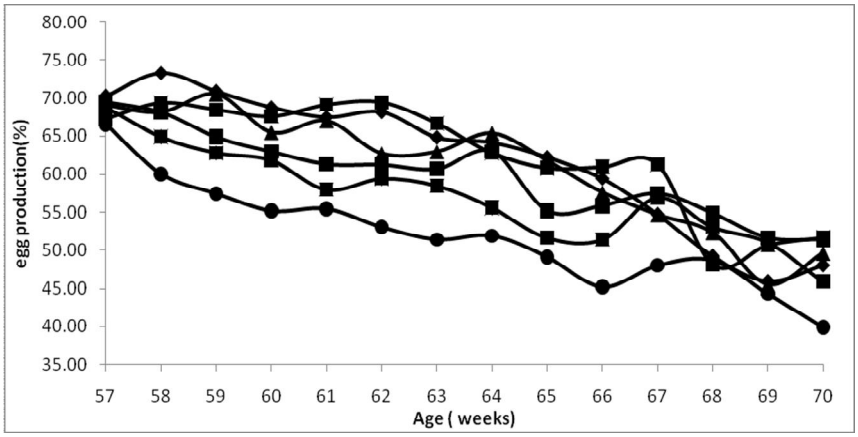

Figure 1. Egg production of hens fed corn-soybean meal diets supplemented with various 1α(OH)D_3 from 57 to 70 wk of age. D source: T1(-◊-), T2(-□-), T3(-Δ-), T4(-x-), T5(*), T6(-●-).

Data obtained for hatchability of fertile eggs and embryonic mortality are presented in Table 3. The results of this experiment indicate that inclusion of 1α(OH)D_3 to the diet had no significant effect on hatchability of fertile eggs and embryonic mortality ($P > 0.05$)l; however, the hens fed diet with no supplemental vitamin D_3 (T6) showed significant decrease in hatchability and significant increase in early and late embryonic mortality ($P < 0.05$). Middle embryo mortality and piped eggs were not affected by experimental treatments ($P > 0.05$).

Vitamin D metabolites are required by the embryo in order to mobilize calcium from the shell, and decreased hatchability in vitamin D-deficient embryos is related to a defect in calcium mobilization from the shell. Abdulrahim et al. (1979) reported that hens fed 25(OH)D3 had normal hatchability and embryo development in the same proportion as hens fed D3. Several studies have reported lower hatchability and high incidence of embryonic mortality when hens fed 1,25(OH)2D3 or 1α(OH)D3 as the only source of dietary vitamin D3 (Sunde et al., 1978; Abdulrahim et al., 1979; Soares et al., 1979). In the study conducted by Sunde et al. (1978) four levels of 1,25(OH)2D3 were fed to laying hens (1, 2, 4 and 8 µg/kg). The levels of 1,25(OH)2D3 and 1α(OH)D3 used by Abdulrhim et al. (1979) were 3 and 9 µg/kg. In a study conducted by Soares et al. (1979) hens consumed diet containing 5 µg/kg 1α(OH)D3. In contrast, Harms et al. (1990) reported that neither 1,25$(OH)_2D_3$ nor 1α(OH)D_3 affected hatchability of egg. However, the diet of the hens in their experiment also contained 2200 IU of vitamin D_3/kg of diet.

As presented in Table 4, the supplementation of 1α(OH)D_3 had no significant effect on tibia ash, blood plasma calcium and phosphorous concentration. Previous reports indicated that vitamin D metabolites had no effect on tibia ash (Harms et al., 1988; Soares et al., 1988;

Keshavarz, 1996 and 2003). It seems that a more sensitive method than bone ash is required to evaluate the possible changes in the status of bone quality caused by supplemental vitamin D metabolites. Roland and Harms (1976) did not observe differences in serum calcium with young or old hens receiving $25(OH)D_3$ as compared to vitamin D_3. In contrast, Harms et al. (1988) revealed that $1,25(OH)_2D_3$ caused an increase in serum phosphorus but calcium level was not affected by $1,25(OH)_2D_3$ supplementation. Tsang and Grunder (1993) showed that replacing D_3 by the optimal concentration of Calcitriol (5 μg/kg diet) had no significant effect on plasma calcium.

Conclusion

In conclusion, results from this study revealed that added $1\alpha(OH)D_3$ to insufficient $1,25(OH)_2 D_3$ broiler breeder hens diet could be applied with beneficial effects on shell quality and production performance. However, long-term inclusion of $1\alpha(OH)D_3$ and its desirable level need more results from farm studies.

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