



Influence of Supplemental Vitamin D₃ on Production Performance of Aged White Leghorn Layer Breeders and Their Progeny

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ABSTRACT : An experiment was conducted to elucidate the effect of graded levels of vitamin D₃ in White Leghorn (WL) layer breeders on egg production, shell quality, hatchability of eggs and juvenile performance of offspring during their late laying period (72-88 wk). White Leghorn breeder females were randomly divided into 5 groups of 50 each and were housed in individual California cages in an open-side housing system. Considering birds in five cages as a replicate, 10 such replicates were randomly allotted to each treatment. A basal diet was formulated containing all the nutrients as recommended for WL layers except vitamin D₃, which served as control. Another, four diets were formulated by supplementing graded levels of feed grade crystalline cholecalciferol to the basal diet that contained 300, 600, 1,200 and 2,400 ICU of vitamin D₃ per kg. Each diet was offered *ad libitum* to one of the above five treatment groups. The egg production, egg weight, daily feed consumption and the feed intake per dozen eggs or kg egg mass of the birds fed diet without any supplemental vitamin D₃ was comparable with those of supplemental groups. Similarly, the level of vitamin in the diet did not have any effect on any of the above parameters. However, the specific gravity of eggs laid by the birds fed the diet without supplemental vitamin D₃ was comparable with either 600 or 2,400 ICU supplemental groups but significantly higher when compared to the 300 and 1,200 ICU groups. The egg-shell breaking strength was significantly lowered in the 600 ICU supplemental groups as compared to the strength of other dietary groups. The Haugh unit, egg shell weight, shell thickness, tibia breaking strength, bone ash and calcium content were not influenced by vitamin D₃ concentration in the diet. Serum Ca concentration was influenced by vitamin D₃ level in the diet. The serum Ca concentration of birds fed either control or the vitamin supplemented diet up to 1200 ICU/kg diet was comparable. However, increasing the concentration of vitamin D₃ to 2,400 ICU/kg diet significantly enhanced the concentration of Ca in the serum, which was significantly higher compared to other dietary groups. The serum concentration of P and protein, however, was not influenced by level of vitamin D₃ in the diet. Neither fertility nor hatchability was influenced by vitamin D₃ concentration in the diet. Feeding a vitamin D₃ deficient diet or supplementation of vitamin to hens did not have any influence on their progeny chicks. It can be concluded that dietary supplementation of vitamin D₃ may not be essential for optimum production, shell quality, hatchability, and juvenile performance of WL breeders during 72 to 88 weeks of age. (**Key Words :** Vitamin D₃, White Leghorn Layer Breeders, Production Performance)

INTRODUCTION

Vitamin D₃ (cholecalciferol) is one of the most important dietary factor responsible for normal growth, egg production, shell quality and reproduction in fowls (Ameenuddin et al., 1982). It is also a required component of the endocrine system of birds and regulates calcium (Ca) and phosphorus (P) homeostasis and bone mineralization. The 1, 25 (OH)₂ D₃ stimulates Ca resorption from bone and re-absorption from glomerular filtrate, induces intestinal epithelium to synthesize calcium binding proteins (CaBP) and increase Ca absorption from the guts (Bar et al., 1972).

This CaBP has also been identified in the uterus of laying hens (Fuller et al., 1976) and responsible for Ca deposition of egg shell at the on set of egg production (Bar and Hurwitz, 1973).

Resorption of bone Ca for egg shell formation in high producing hens is likely the cause of osteoporosis in layers during post peak production (Abe et al., 1982). It has also been suggested that the efficiency of the hydroxylation reactions necessary to convert cholecalciferol into its metabolically active form i.e. 1, 25 (OH)₂ D₃ may be reduced in aged hens (Frost et al., 1990; Elaroussi et al., 1994). Adequate level of 1, 25 (OH)₂ D₃ is required for the regulation of Ca absorption and excretion and initiate the mobilization of Ca from the bone to provide adequate Ca required for egg shell formation (Abe et al., 1982).

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Table 1. Ingredients and nutrient composition (%) of basal diet

Ingredients	Composition (%)
Yellow maize	60.00
Soybean meal	12.50
Sunflower cake	13.50
Deoiled rice bran	3.30
Oyster shell grit	8.96
Dicalcium phosphate	0.96
Common salt	0.50
DL-Methionine	0.05
Lysine	0.05
Trace mineral and vitamins ^a	0.18
Nutrient composition (%)	
Crude protein*	18.05
Calcium*	3.81
Phosphorus*	0.71
Lysine**	0.71
Methionine**	0.31
ME (kcal/kg)**	2,580

^aSupplies per kg diet: Vitamin A, 16,500 IU; vitamin E, 12 mg; vitamin K, 2 mg; vitamin B₁, 1.2 mg; vitamin B₂, 10 mg; vitamin B₆, 2.4 mg; vitamin B₁₂, 12 µg; niacin, 18 mg; pantothenic acid, 12 mg; Mn, 50 mg; Zn, 112.5 mg; Fe, 60 mg; Cu, 10 mg; I, 1.2 mg.

* Analyzed value.

** Calculated value.

The elucidation of metabolism of vitamin D₃ and the role of this vitamin in Ca and P metabolism and egg shell quality has been one of the phenomena of nutritional research over the last three decades. Investigations into the ability of the older hens to metabolize or respond to vitamin D₃ have shown that shell quality and bone strength deteriorates more rapidly (Bar and Hurwitz, 1987). However, supplementation of vitamin D₃ to the deficient diets alleviated the decline in productivity and shell quality (Newman and Leeson, 1997).

Birds can synthesize cholecalciferol from cholesterol when they receive adequate sunlight. Vitamin D₃ insufficiency may be a common problem when birds reared in environmentally controlled house. However, no informations in literature are available whether there is a need to supply vitamin D₃ in the diet of layer in tropical country like India, where the birds are reared in cages with open side housing system. Therefore, the present study was conducted to elucidate the effect of graded level of vitamin D₃ in aged White Leghorn (WL) breeders on egg production, shell quality, hatchability of eggs and juvenile performance of offspring during their late laying period.

MATERIALS AND METHODS

Birds and management

The White Leghorn breeders of 72 weeks of age were randomly divided into 5 groups of 50 each and were housed in individual California cages in open side housing system. Considering birds in five cages as a replicate, 10 such

replicates were randomly allotted to each treatment. A continuous 16-h light per day was provided using incandescent bulbs. All the birds were maintained under uniform managemental conditions throughout the experimental period. The temperature of the house varied between 36-38°C during the entire experimental period.

Experimental diets

A basal diet was formulated containing all the nutrients as recommended for WL layers except vitamin D₃, which served as control (Table 1). Another, four diets were formulated by supplementing graded levels of feed grade crystalline cholecalciferol (vitamin D₃, Dulphar Interfran, Mumbai, India) to the basal diet that contained 300, 600, 1,200 and 2,400 ICU of vitamin D₃ per kg. To ensure proper mixing of cholecalciferol in diets, the vitamin was dissolved in 100 ml of propylene glycol-ethanol solution (95 ml propylene glycol and 5 ml ethanol). A separate premix of cholecalciferol was prepared by mixing the above solution with finely ground maize and finally the vitamin premix was mixed with respective experimental feed. Each diet was provided *ad libitum* to one of the above five groups, from 72 to 88 weeks of age.

Response criterion

Body weight, egg production and egg weight : Individual body weight of the bird was recorded at the beginning and end of the experiment. Egg production on individual basis was recorded daily and percent hen day egg production (HDEP) was calculated. All the eggs laid during the last three consecutive days of every 28 day period, were collected to measure the egg weight.

Egg shell quality : Fifteen eggs were randomly chosen from each treatment from the eggs laid during the last three consecutive days of each 28-day period to determine the specific gravity (Densitometer, Mettler-Toledo, ISO-14001, Switzerland), shell weight, shell thickness and shell breaking strength (Universal Testing Machine, EZ test, 120891-04, Japan). The cleaned egg-shells, dried for twenty-four hours, were weighed and expressed as % of whole egg. The shell thickness was measured at three different locations (middle, broad and narrow end) using a micrometer gauge (Mitutoyo Code, 7027, Japan) and mean value was calculated.

Serum bio-chemical studies : At the end of experimental period, 5 ml of blood was collected from brachial vein from 10 birds in each dietary treatment. Subsequently serum was separated and the levels of Ca (AOAC, 1990), inorganic P (Fiske and Subba row, 1925) and protein (Doumas et al., 1971) in serum were analysed.

Hatchability and performance of progeny

Hatchability of eggs laid by WL layers fed diet with or

Table 2. Influence of vitamin D₃ supplementation on production performance of White Leghorn layer breeders

Attributes	Levels of vitamin D ₃ (ICU per kg diet)					SEM
	0	300	600	1,200	2,400	
Hen housed egg production (%)	61.25	60.90	62.13	63.05	62.06	1.03
Egg weight (g)	55.12	56.00	55.57	54.35	55.77	0.22
Feed intake (g/day)	98.09	99.02	98.86	97.48	98.02	1.02
FCR* (g/12 eggs)	1,968.68	1,960.50	2,039.21	2,030.82	1,982.10	36.82
FCR*/kg egg mass	2.822	2.714	2.872	2.874	2.730	0.08
Specific gravity	1.073 ^a	1.065 ^b	1.070 ^{ab}	1.065 ^b	1.069 ^{ab}	0.008
Egg breaking strength (Newton)	31.44 ^a	31.25 ^a	27.48 ^b	30.98 ^a	31.12 ^a	0.47
Haugh unit	60.59	62.54	60.40	62.66	63.03	1.35
Shell weight (%)	9.19	8.81	8.85	8.95	8.89	0.19
Shell thickness (mm)	0.364	0.366	0.369	0.359	0.363	0.003
Tibia breaking strength (Newton)	54.82	55.64	54.20	56.24	55.58	1.24
Bone ash (%)	48.90	48.20	49.15	49.20	48.88	0.64
Bone calcium (%)	39.70	40.20	39.42	40.05	39.30	0.45
Ca (mg/dl)	20.09 ^b	20.26 ^b	20.62 ^b	20.42 ^b	22.27 ^a	0.28
P (mg/dl)	7.10	7.18	7.02	7.06	6.98	0.14
Protein (mg/dl)	4.51	4.98	4.07	5.14	5.95	0.27

^{a,b} Means with different superscripts in a row differ significantly ($p < 0.05$).

* FCR: Feed conversion ratio.

without vitamin D₃ supplementation was evaluated at 86 weeks of age. All the hens in each treatment were inseminated with pooled semen from males of the same age. Eggs were collected through the 3rd to 8th day following insemination and incubated to determine the fertility and hatchability. The chicks hatched were individually weighed to record day old body weight. The chicks were wing banded and reared to 14 days of age in stainless steel battery brooders under uniform feeding and managerial condition to determine the survivability and body weight gain.

Bone mineralization

Six birds from each treatment were selected at random and sacrificed by cervical dislocation at the end of experiment. Both the tibiae were freed from soft tissue and diaphysis, defatted by soaking in petroleum ether for 48 h and dried at 100°C for 12 h. The right and left tibiae were used for determination of bone ash and bone strength, respectively. Dried bone samples were ashed at 600±30°C for 12 h for estimation of bone ash and Ca (AOAC, 1990). Breaking strength on the left tibia was determined by universal testing machine (EZ test, 120891-04, Shimadzu-Japan).

Statistical analysis

Data were subjected to statistical analysis under completely randomized design employing one-way analysis of variance (Snedecor and Cochran, 1989). The means of different treatments were compared with Duncan multiple range tests (Duncan, 1955). Significance was considered at $p < 0.05$ level.

RESULTS

The egg production and egg weight of the birds fed diet without any supplemental vitamin D₃ was comparable with those of supplemental groups (Table 2). Similarly, the levels of the vitamin in the diet did not have any effect either on egg production or egg weight. The average daily feed intake and the feed consumed per dozen eggs or kg egg mass was comparable among all the dietary groups. However, the specific gravity of eggs laid by the birds fed diet without supplemental vitamin D₃ was comparable with either 600 or 2,400 ICU supplemental groups but significantly higher as compared to 300 and 1,200 ICU groups. The egg -shell breaking strength was significantly lowered in the 600 ICU supplemental groups as compared to the comparable strength of other dietary groups. The Haugh unit, egg shell weight, shell thickness, tibia breaking strength, bone ash and calcium content were not influenced due to vitamin D₃ concentration in the diet.

Serum Ca concentration was influenced by vitamin D₃ levels in the diet. The serum Ca concentration of birds fed either control or the vitamin supplemented diet up to 1200 ICU/kg diet was comparable. However, increasing the concentration of vitamin D₃ to 2,400 ICU/kg diet significantly enhanced the concentration of Ca in the serum, which was significantly higher as compared to other dietary groups. The serum concentration of P and protein, however, was not influenced by increasing levels of vitamin D₃ in the diet.

Neither fertility nor hatchability (TES and FES) was influenced by vitamin D₃ concentration in the diet (Table 3). The fertility and hatchability of eggs from vitamin D₃ deficient diet were comparable with those fed diets supplemented with vitamin D₃ upto 2,400 ICU/kg.

Table 3. Influence of Vitamin D₃ supplementation on hatchability and performance of progeny chicks

Attributes	Levels of vitamin D ₃ (ICU per kg diet)					SEM
	0	300	600	1,200	2,400	
Fertility (%)	83.04	82.44	84.94	85.81	87.25	2.59
Hatchability (%)						
TES*	71.55	69.46	69.83	71.59	72.05	2.68
FES**	84.45	81.54	80.91	85.70	83.49	3.07
Chick weight (g)						
Day old	36.98	37.33	36.36	36.64	38.67	0.52
14 th day	98.80	97.75	98.04	96.98	97.64	1.84

* TES: Total egg set. ** Fertile egg set.

Supplementing vitamin D₃ at 300 ICU/kg diet to meet the NRC (1994) requirement or linearly increasing to 2,400 ICU/kg diet did not reveal any additional advantage in either fertility or hatchability. Feeding vitamin D₃ deficient diet or supplementation of vitamin to dams did not have any influence on their progeny chicks. The day old and 14th day body weight of chicks obtained from dams fed either deficient or supplemental vitamin D₃ was comparable.

DISCUSSION

Majority of the parameters including egg production and egg shell quality were not affected by vitamin D₃ concentrations in the breeder diet. Even without supplemental vitamin D₃, the birds continued to laid eggs without any influence on egg production. The egg weight and shell quality of the eggs of birds fed without vitamin D₃ was also comparable with those of vitamin D₃ supplemental groups. Supplementing vitamin D₃ at 300 ICU/kg diet to meet the NRC (1994) requirement or linearly increasing the concentration to 2,400 ICU/kg did not have any additional advantage on any of the production parameters. Though, egg specific gravity and breaking strength varied due to levels of vitamin D₃ in the diet, no specific trend could be observed. Serum Ca varied significantly due to vitamin D₃ supplementation. Though, serum Ca concentration was highest in the birds fed diet supplemented with 2400ICU/kg, no influenced could be reflected on any of the production parameters. Fertility, hatchability and post-hatch performance of the progeny (14th day post hatch) was also not influenced due to vitamin D₃ levels in the diet.

Contrary to the findings of the present study, Tsang and Grunder (1990) reported poor egg shell quality in WL layers fed diet without supplemental vitamin D₃ during 59-80 wks of age, which were on a diet containing 3.1% Ca and 1,100 ICU/kg supplemental D₃ prior to the experiment. Withdrawing of supplemental D₃ also reduced the egg production drastically (59 wk-73%: 80 wk-3.8%). However, no effect was observed on average daily feed intake. Hatchability was significantly lowered in the birds fed vitamin D₃ deficient diet. In a 13 weeks experiment with 21 weeks old single comb WL pullets, Abdulrahim et al.

(1979) observed significantly lower egg production of the pullets fed the vitamin D₃ deficient basal diets as compared to those fed diets supplemented with 120 to 720 ICU/kg vitamin D₃ in the diet. The egg shell quality was also the poorest in the vitamin D₃ deficient diet. No difference in egg production or shell quality was observed due to variation in the vitamin D₃ (120-720 ICU/kg) content in the diet. Feed consumption, however, was not influenced due to vitamin D₃ levels in the diet. Though egg production, egg weight, shell weight and specific gravity were not influenced due to variation in vitamin D₃ concentration (250, 500 or 2,000 ICU/kg) in the diet, the highest percentage of cracked eggs were observed for hen (54 wk) fed 250 ICU vitamin D₃ per kg diet (Keshavarz, 1996). During a 48-week period study with 20 wk old laying hen, Matilla et al. (2004) reported no variation in production performances by supplementing enhanced levels of vitamin D₃ (6,000 or 15,000 ICU/kg) to a control diet (2,500 ICU/kg). Similarly Park et al. (2005) did not find any difference in egg production in laying hens (87 wk) by supplementing higher levels of vitamin D₃ (4,000 or 8,000 ICU/kg) to diet containing 2,000 ICU/kg of D₃.

Most of the studies conducted on requirement and optimization vitamin D₃ in layers was in either temperate countries or environmentally controlled house where vitamin D₃ insufficiency is a common problem because of restricted ultraviolet light exposure (Scharla, 1998). Probably, this may be the reason why in most of the studies vitamin D₃ deficient diet had adverse effect on egg production and shell quality. Enhancing the vitamin D₃ concentration in the diet beyond the requirement (NRC, 1994) did not found to be beneficial in further increasing the production performances (Keshavarz, 1996; Matilla et al., 2004). However, in tropical countries like India where sunlight is more potent and most of the poultry farming is operated in open sided housing system, vitamin D₃ insufficiency may not be a problem that becomes nutritionally important. The birds might have synthesized enough cholecalciferol through adequate sunlight thereby optimizing calcium metabolism (Shen et al., 1981). This could be one of the probable cause that no influence of dietary supplementation of the vitamin on production

performance of WL breeders could be observed during the experimental period.

In addition to sun-light many other factors influence the vitamin D₃ requirement such as amount and ratio of dietary Ca and P, their availability, species and physiological factors (McDowell, 1989). In the present study, the birds from the beginning of the laying period (18 wks) were on a standard diet containing 3.8% Ca and 0.71% P till 72 weeks of age. The same diet was continued during the experimental period of 16 weeks. Probably, these concentration and proportion of Ca and P in the diet were optimum to meet Ca and P requirement of the breeder diet devoid of vitamin D₃.

Besides the persistence of vitamin D₃ in animals during the period of vitamin D₃ deficiency may be explained by slow turnover rate of vitamin D₃ in certain tissues such as skin and adipose tissue. During the time of deprivation, vitamin D₃ in these tissues is released slowly, thus meeting vitamin D₃ needs of the animal over a long period of time (Miller and Norman, 1984). The birds in the present study had fed diet supplemented with 2400 ICU vitamin D₃ /kg diet prior to the experimental study (20-72 wk). The excess vitamin D₃ fed prior to the experiment might have stored in the body and consequently met the requirement during deficient period, thereby maintaining the optimum performance.

Sign of vitamin D₃ deficiency begin to occur in laying hens in confinement about 1 to 2 months after they are deprived of vitamin D₃ and the first sign of deficiency is thinning of the egg shell and subsequent decline in egg production (McDowell, 1989). However, in the present experiment no symptoms of vitamin D₃ deficiency could be noticed even after 4 months of experimental period in birds fed vitamin D₃ deficient diet. Thus, it can be concluded that dietary supplementation of vitamin D₃ may not be essential for optimum production, shell quality, hatchability, and juvenile performance of WL breeders during 72 to 88 weeks of age.

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