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Influence of direct ultraviolet blue (UVB) light exposure on serum levels of vitamin D_3, calcium and phosphorus in layer chickens

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Abstract
The body needs vitamin D_3 for a number of metabolic processes, such as healthy bone development, calcium phosphate absorption, and immune system stimulation. This study investigated the impact of UVB exposure (3 hours per day) on the levels of calcium, phosphorus, and vitamin D_3 in the serum of crossbred (White Leghorn N strain and Desi) layer chickens from 29 to 40 weeks of age. In order to achieve optimal UVB light exposure on the featherless skin of bird feet, a 60 cm long, 240 V, 50 Hz, and 36 W UVB tube outfitted with a heat protection reflector was positioned in the lower front area of the cages at a distance of 20 cm. The mean vitamin D_3 concentration in the serum of UVB exposed birds was 13.38±0.41 ng/mL which was significantly higher (p<0.01) than that of the unexposed group of birds with a mean serum vitamin D_3 concentration of 5.25±0.68 ng/mL. At the same time, UVB exposure also had a significant (p<0.01) impact on serum calcium and phosphorus levels which were 16.13±0.16 mg/dL and 5.22±0.36 mg/dL respectively. However, during the experimental period, the serum calcium and phosphorus levels of the control birds were considerably (p<0.01) lower than those of the UVB exposed birds. In a nutshell, the serum levels of calcium, phosphorus, and vitamin D_3 in layer chickens are significantly influenced by UVB exposure.

Keywords: Ultraviolet blue (UVB) light, vitamin D_3, calcium, phosphorus, layer chicken

Introduction
Vitamin D_3 is synthesised in the skin from 7-dehydrocholesterol by the action of sunlight. Under UV rays between 280-320 nm convert 7-dehydrocholesterol to cholecalciferol (vitamin D_3). After being translocated into circulation, vitamin D_3 undergoes hydroxylation in the liver and kidney respectively. Vitamin D_3 helps in the absorption of calcium from the intestine and enhances the responsiveness of bone to parathyroid hormone. Available calcium (Ca) and phosphorus (P) are regarded as the most crucial minerals in laying hens' diets because of their demonstrative engagement in bone metabolism, eggshell quality and improvements in production performance.

Although vitamin D_3 can be endogenously produced in the skin under sunlight, exposure to ultraviolet light also poses a chance of skin cancer. Public consciousness of the adverse effects of sun exposure, seasonal changes in ultraviolet blue (UVB) light exposure and increased intensive rearing of birds lead to an unintended deficiency of vitamin D_3. Excess intake of vitamin D_3 causes the excessive absorption of calcium from the diet or its resorption from the bone and it can be toxic. Hence maximum limits are defined worldwide for the supplementation of cholecalciferol in animal feeds. Therefore, a promising alternative to increase the quantity of vitamin D_3 would be UVB light exposure to laying hens (Schutkowski *et al.*, 2013) [5].

Materials and Methods
Experimental layout
The experiment was conducted using thirty-two numbers of 28 weeks old crossbred (White Leghorn N Strain X Desi) layer birds procured from All India Coordinated Research Projects (AICRP) on Poultry for Eggs, Mannuthy, KVASU. The experiment was approved by the Institutional Animal Ethics Committee (IAEC) of the College of Veterinary and Animal Sciences, Mannuthy. The birds were randomly distributed in a completely randomized experimental design and they were placed into two treatment groups, each with four replicates having four birds in each replicate. The treatment groups were as follows:

Table 1: Experimental layout

No	Treatment	No of birds
T1	Control with standard layer diet (BIS 2007)	4 x 4
T2	UVB light exposed + Standard layer diet	4 x 4

Experimental Birds, Housing and Management

All birds were fed with a standard layer diet and raised under standard managemental conditions up to 28 weeks of age at AICRP on Poultry for Eggs Mannuthy, KVASU. The birds were immunized against diseases as per standard protocol followed on the farm. On completion of 28 weeks of age, the birds (average body weight of 1.28±0.06 Kg) were brought and housed in well ventilated cages of animal house attached to the Department of Veterinary Physiology. They were kept at 24.5 ± 0.5˚C ambient temperature and at relative humidity ranging from 60-80 per cent. A photoperiod of 16 h per day was ensured throughout the period. For the treatment group the total quantity of feed assigned per day was divided into 4 parts and each part was fed just before the lighting schedule to ensure that the UV radiation falls on the featherless skin of feet and legs while all of them were pecking the feed in standing position. Water was provided *ad libitum* throughout the experimental period (84 days).

Exposure to UVB light

Birds of treatment group were exposed to UVB (280-315 nm) light (M/s Philips India Ltd., Hyderabad) for 3h daily @ 8.00-8.30 a.m., 11.00 a.m.-12.00 p.m., 2.00-3.00 p.m. and 4.30-5.00 p.m. The UVB radiation dosage at a distance of 20 cm was 76 µW/cm^2. A 60 cm long, 240 V, 50 Hz and 36 W UVB tube equipped with heat protection reflector (Figure 1) was placed in the lower front part of the cages as shown in Figure 2, so as to ensure an optimal UVB light exposure on the featherless skin of feet and legs of birds, especially during the feeding time. An opaque paper board was placed from the level of feeder to the top of the cage in order to prevent any harmful UV radiation falling on eyes and combs (Megha and Ramnath, 2021) [3]. An opaque board was placed between the irradiated and non-exposed groups to block any incidental radiation.

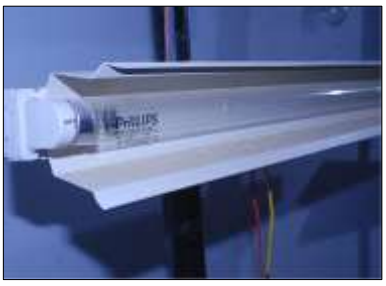

Fig 1: UVB tube equipped with heat protection reflector

Fig 2: UVB exposure to featherless

Blood sample collection and analysis

Blood samples were collected once every month from the wing vein under aseptic conditions using a 26-gauge hypodermic needle and syringe. After holding the collected blood samples in vials for 30 min at room temperature, they were subjected to serum separation by centrifuging at 3000 rpm for 15 minutes. Serum samples were stored in Eppendorf vial at -20 ˚C till further estimations.

Estimation of serum vitamin D_3

Serum vitamin D_3 was determined by a conventional spectrophotometric method. Extraction of vitamin D_3 from serum was done by following the method of Terpinene *et al*. (2003) [9]. To 0.5 mL of serum, added 350 µl of Methanol and 2-propanol in the ratio of 80:20 (v/v). The contents were mixed in a vortex mixer for 60s. Vitamin D_3 was extracted by mixing with 1 mL of n-Hexane followed by centrifugation at 10,000 rpm for 10 min. The upper organic phase was transferred to a conical tube and dried using a speed vacuum concentrator (MSVQ-20, M/s Operon Co., Ltd., Korea). The residue was dissolved in the appropriate volume of n-Hexane. Different concentrations of vitamin D_3 standards (5, 10, 20, 25, 100, 1000 ng/mL) were also prepared in n-Hexane. The results were monitored spectrophotometrically at 265 nm in multimode micro/ ELISA plate reader (Varioskan Flash, M/s Thermo Fisher Scientific, Finland).

Estimation of serum calcium and phosphorus levels

Analysis of serum for calcium (mg/dL) was done using Atomic Absorption Spectrometry (M/s PerkinElmer, USA). The serum samples were analysed for calcium by Atomic Absorption Spectrometry standard method. The Pinnacles 900H Series AAS enabled a simple automated measurement of Ca using a hollow cathode lamp of wavelength 422.67 nm, energy level 66% and lamp current of 10 mA. The calibration curve was plotted with standards ranging from 0 to 100 ppm with a calibration equation nonlinear through zero. Slit width of 0.7 nm ensured accurate analysis. The flame atomizer with a temperature of 2400 ˚C was set for the measurement. Fuel and oxidant gases were acetylene (2.5 L/min) and air (10 L/min) respectively. Serum phosphorous level (mg/dL) was quantified by semi-automated biochemical analyser (Master T (M/s Hospital, Italy) using commercial kits supplied by M/s Agappe Diagnostics Ltd, Maharashtra.

Statistical analysis

Results were expressed as mean (±S.E.). The statistical significance of the difference or relation between the two treatments was analysed by CRD using the software Statistical Product and Services (SPSS) version 24.0 and the differences were considered statistically significant at 5% level ($p<0.05$).

Results and Discussion

Serum vitamin D_3 level

Vitamin D_3 extracted serum samples were analysed (Table 2 (b)) by the spectrophotometric method in a multimode micro plate reader at 265 nm. The vitamin D_3 concentration in serum of birds exposed to UVB was significantly higher ($p<0.01$) throughout the experimental period compared to the unexposed group.

Table 2(a): Absorbance and calculated concentrations (ng/mL) of vitamin D_3 Standards

Concentration of standards (ng/mL)	OD at 265 nm	Calculated concentration
Blank	3.7539	0
5	3.76568	6.035
10	3.76921	10.952
20	3.78093	28.971
25	3.7829	32.172
100	3.81676	91.691
1000	4.2219	1000.618

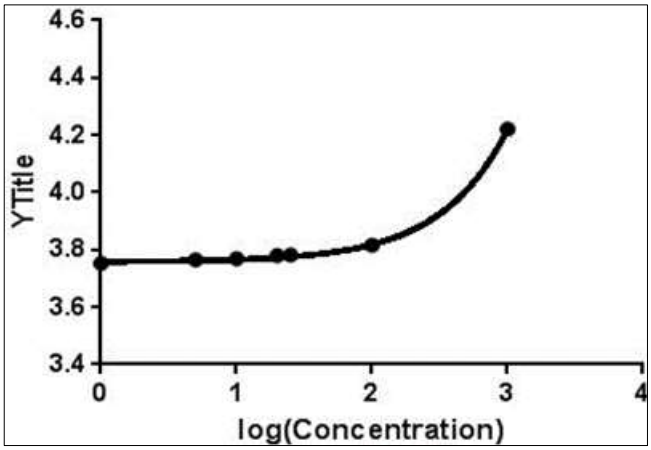

Fig 3: Calibration curve of vitamin D_3 with different standards (ng/mL)

Table 2(b): Mean (±S.E.) value of serum vitamin D_3 (ng/mL) in treatment groups

Treatment group	Mean value of vitamin D_3 (ng/mL)	p-value	F value
	Mean ± S.E.		
T1	5.25^{a}±0.68	0.000**	87.70
T2	13.38^{b}±0.41		

Means bearing different superscript within a column differ significantly (p<0.05)
* Significant (p<0.05)
** Highly significant (p<0.01)

The serum level of vitamin D_3 was significantly different (p<0.01) in UVB exposed group (13.38±0.41 ng/mL) and non-exposed group (5.25±0.68 ng/m/L). The serum vitamin D_3 level in exposed group was almost 2.5 times higher than that of the control group.

Similarly, Schutkowski *et al.* (2013) [5] reported that UVB radiation and dietary vitamin D_3 supplementation in hens increased the plasma 25(OH)D_3 concentration. The 1,25-dihydroxylated metabolite is considered to be the most active form of vitamin D**,** derivatives in stimulating Ca and P absorption (Sedrani, 1984) [6] and in Ca mobilization from the bone.

Hughes *et al.* (1924) [2] conducted a trial during the winter season where young laying hens were found that ultraviolet light treatment had brought about marked beneficial effects on egg production and a reversal of leg weakness.

Tian *et al.* (1994) [8] studied the kinetic aspects of cutaneous synthesis and translocation of vitamin D_3 in chicken skin. The concentrations of 7-dehydrocholesterol in different skin areas like leg skin and back skin were 3524 ± 937 ng/ cm^2 and 120 ± 62 ng/ cm^2 respectively. They also evaluated the circulating concentration of vitamin D_3, in response to UVB radiation and found that a fourfold rise in the concentration of vitamin D_3 could be achieved only after 30 h radiation. This suggested the time required for the translocation of vitamin D_3 from skin to circulation.

Serum calcium level

Results of calcium levels in serum of birds are given in Table 3 (b).

Table 3 (a): Absorbance and calculated concentrations of calcium standards

Concentration of standard (mg/L)	OD at 422.67 nm (Abs)	Calculated concentration (mg/L)
Blank	0.00	0.00
3.0	0.2046	2.937
6.0	0.4073	6.181
10.0	0.6114	9.891
15.0	0.8570	15.189
50.0	1.6422	47.385
100.0	2.0530	105.918

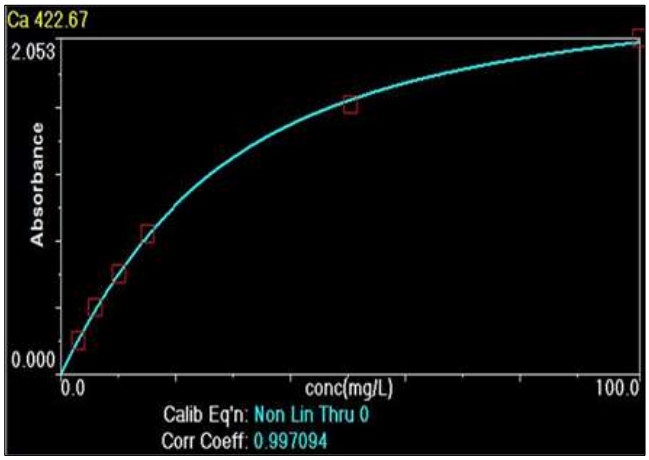

Fig 4: Calibration curve of calcium with different standards

Table 3 (b): Mean (±S.E.) value of serum calcium (mg/dL) concentration in treatment groups

Treatment group	Mean value of calcium (mg/dL)	p-value	F value
	Mean ± S.E.		
T1	8.65^{a}±0.15	0.000**	568.56
T2	16.13^{b}±0.16		

Means bearing different superscript within a column differ significantly (p<0.05)
* Significant (p<0.05)
** Highly significant (p<0.01)

In the present study, The UVB treatment brought about significant (p<0.01) changes in serum calcium level of birds. The calcium level was significantly higher (16.13±0.16 mg/dL) for UVB exposed group compared to the control group and the findings were in full agreement with the reports of de Matoes (2008) [1] who found that UVB wavelengths offered health and welfare benefits through supporting the endogenous synthesis of vitamin D_3.

Wasserman and Taylor (1966) [10] reported that vitamin D_3 enhanced duodenal absorption of calcium in rachitic chicks by stimulating the synthesis or operation of a "carrier" that would facilitate the uphill or downhill transepithelial movement of calcium. The intestinal absorption of calcium was generally depressed in vitamin D_3 deficient animals. The increased production of vitamin D_3 is physiologically required for providing Ca for the mineralization of the eggshell and medullary bone.

Wasserman *et al.* (1982) [11] established the correlation between the stimulation of Ca absorption and the appearance of Ca binding protein (CaBP) in the same duodenal segment.

The calcium absorptive system of the vitamin D_3 treated chicks responded more rapidly to $1,25(OH)_2D_3$. A significant increase in Ca absorption was apparent at 4 h after the 1, $25(OH)_2D_3$ dose. Whereas, no response occurred in the vitamin D deficient chicks. This report supports the proposal that the movement of Ca across the duodenal brush border takes place through facilitated transport and is initiated by $1,25(OH)_2D_3$.

However, reports of Schutkowski et al. (2013) [5] and Wei et al. (2020) did not reveal any significant effects of UVB exposure or dietary vitamin D_3 on plasma concentrations of calcium.

Serum phosphorus level

In the current study serum phosphorus levels of birds were significantly ($p<0.01$) influenced by UVB exposure. The serum phosphorus level was higher for UVB exposed groups under basal diet (5.22±0.36) compared to the control group (Table 4).

Table 4: Mean (±S.E.) value of serum phosphorus (mg/dL) levels in treatment groups

Treatment group	Mean value of phosphorus (mg/dL) Mean ± S.E.	p-value	F value
T1	3.69^a±0.20	0.001**	6.515
T2	5.22^b±0.36		

Means bearing different superscript within a column differ significantly ($p<0.05$)
* Significant ($p<0.05$)
** Highly significant ($p<0.01$)

Wei et al. (2020) reported that serum phosphorus content was significantly increased by UVB-LED exposure duration and they found that the serum phosphorus and calcium levels of laying hens exposed to UVB were synergistic. This report was fully endorsed by the present study results. Serum calcium and phosphorus were differed significantly ($p<0.01$) due to the interaction of calcium to phosphorus ratio and vitamin D_3 levels. Similar results were also observed by Popat *et al.* (2019) [4].

On contrary to the present study, the findings of Schutkowski *et al.* (2013) [5] reported that plasma inorganic phosphate concentration was not affected by UVB exposure and dietary vitamin D_3 supplementation.

Tanaka and De Luca (1973) [7] studied rats that were maintained on a phosphorus deficient diet and found that $1,25(OH)_2$ vitamin D_3 levels increased even in thyroparathyroidectomised rats. While rats maintained on low calcium diet produced 1,25-dihydroxyvitamin D_3, but lost this ability within 48 h after thyroparathyroidectomy. However, by providing calcium gluconate glucose solution through drinking water, the level of vitamin D_3 could be retained to normal, while reducing the level of elevated serum inorganic phosphorus. They reported that in thyroparathyroidectomised rats, 1,25-dihydroxyvitamin D_3 correlated with serum phosphorus value below 7-8 mg/100 mL.

Conclusion

The present experiment was designed to study the influence of ultraviolet blue (UVB) radiation on vitamin D_3, calcium and phosphorus levels of layer chickens. Vitamin D_3, calcium and phosphorus are the most important nutrients required for optimum production performance and maintenance of the eggshell quality of laying hens. It can be concluded that UVB light exposure increases the serum vitamin D_3 level which was indicative of its transport from skin to ovaries after synthesis and it would be a better choice for enhancing vitamin D_3 content in eggs of layer chicken. Vitamin D_3 (cholecalciferol) is also a required component of the endocrine system of birds and regulates Ca and P homeostasis and bone mineralization. Hence, UVB exposure also had a significant ($p<0.01$) impact on serum calcium and phosphorus levels of layer chicken which specifies the interaction of these minerals with vitamin D_3.

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