Journal of Basic and Applied Research International

Volume 30, Issue 1, Page 41-50, 2024; Article no.JOBARI.12039
ISSN: 2395-3438 (P), ISSN: 2395-3446 (O)

Evaluation of Designer Chicken Eggs Enriched with Vitamin D3 and Zinc

P. S. Megha [a++*], V. Ramnath [b#] and K. V. Vishnudas [c++]

[a] Department of Veterinary Physiology, Poyyara House, Naduvilkara, Vatanappally, Thrissur, Kerala, 680 614, India.
[b] Department of Veterinary Physiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, 680651, India.
[c] Department of Animal Science, College of Agriculture and Life Sciences, Texas A and M University, College Station, Texas, 77840, USA.

Authors' contributions

This work was carried out in collaboration between all authors. Author PSM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors VR and KKV managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.56557/JOBARI/2024/v30i18658

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.ikprress.org/review-history/12039

Original Research Article

Received: 13/02/2024
Accepted: 17/04/2024
Published: 22/04/2024

ABSTRACT

Designer poultry eggs can be produced in various ways. In the present study chicken eggs were enriched with vitamin D3 and zinc by UVB (Ultra Violet Blue) light exposure (3 h/day) and dietary zinc supplementation (75 mg/kg) respectively. The study was conducted in thirty-two crossbred (White Leghorn N strain and Desi) layer birds. The trial was commenced from 29 weeks of age and conducted for 12 weeks. The current research work pointed out the enriching influence of UVB radiation and dietary zinc supplementation on concentration of vitamin D3 and zinc in eggs. Vitamin D3 and zinc concentrations in egg and serum were significantly higher ($p<0.01$) compared to the control group. UVB exposure and zinc supplementation also had a

---

[++] Ph.D Scholar;
[#] Professor and Head;
**Corresponding author: E-mail: meghaps4748@gmail.com, meghaps7@gmail.com;*

significant ($p<0.01$) impact on serum calcium and phosphorus levels. However, Liver enzyme activities of birds were not influenced either by UVB light exposure or by zinc supplementation.
The dietary supplementation of hens with zinc is an effective approach to supply consumers with foods from animal sources that are enriched with zinc. Current findings showed that feeding of zinc supplemented diet to chickens offered a promising alternative to fortify egg with zinc. Furthermore, UVB light exposure would be a better choice for enhancing vitamin D3 content of eggs and to produce designer eggs enriched with vitamin D3. It increased the serum vitamin D3 level which was indicative of its transport from skin to ovaries after synthesis.
The use of UVB exposure and zinc supplementation as an easy, cheap and safe procedure for producing dual enriched designer eggs with vitamin D3 and zinc is recommended as this would offer additional health benefits to consumers.

Keywords: Designer eggs; vitamin D3; zinc supplementation; layer chicken; UVB exposure.

1. INTRODUCTION

Designer eggs refer to eggs whose contents have been modified from regular eggs to align with consumer preferences or current market demands. Various efforts are underway to adjust the composition of eggs by incorporating beneficial ingredients or minimizing harmful components. This adjustment of egg nutrients can be achieved through nutritional adjustments in the feed of laying hens [1]. The popularity of these enhanced eggs has grown as consumers increasingly prioritize the health benefits of designer eggs, and they are willing to pay a premium for high-quality poultry products.

Vitamin D_3 plays a crucial role in various metabolic functions in the body, including normal bone development, the absorption of calcium phosphate, and immune system activation. Increased public awareness of the harmful effects of sun exposure and the widespread use of skin protection measures have inadvertently impacted vitamin D3 levels. Apart from fish and milk, eggs, especially egg yolk, are recognized as a natural source of both vitamin D3 and 25-hydroxyvitamin D3 (25(OH) D3) [2]. It is widely acknowledged that enhancing the content of cholecalciferol and 25-hydroxycholecalciferol in egg yolk can be achieved by adjusting the cholecalciferol content in poultry feed. An effective alternative for increasing vitamin D levels in eggs involves exposing laying hens to UVB light [3].

Zinc is a vital micronutrient essential for numerous enzymatic processes, including those involved in carbohydrate, nucleic acid, and protein metabolism, with over 300 enzymes reliant on its presence. While many plant-based foods, particularly cereal grains and legumes, contain significant levels of zinc, its bioavailability is hindered by the presence of phytates. Enhancing the zinc content of chicken eggs can be achieved through straightforward and cost-effective dietary methods, such as supplementing the hens' feed with zinc compounds, particularly organic complexes like zinc methionine [4] or inorganic sources such as zinc sulfate, zinc carbonate, and zinc oxide. In light of these considerations, the current study aims to produce eggs enriched with both vitamin D3 and zinc. Throughout the study, the concentrations of vitamin D3 in eggs and serum, zinc content in eggs and serum, as well as serum levels of calcium, phosphorus, and liver enzymes, were monitored.

2. MATERIALS AND METHODS

2.1 Experimental Design

The birds were randomly assigned to two treatment groups in a fully randomized experimental setup, with each group comprising four replicates, each containing four birds as follows:

The birds received vaccinations against diseases according to the established protocol at the farm. Upon reaching 28 weeks of age (average body weight of 1.28±0.06 Kg), the animals were transferred to well-ventilated cages within the animal house attached to the Department of Veterinary Physiology of the College of Veterinary and Animal Sciences, Mannuthy, KVASU India. They were maintained at an ambient temperature (24.5 ± 0.5˚C) and relative humidity (60-80%). A photoperiod of 16 hours per day was maintained throughout this period [6].

2.2 Exposure to UVB Light

“Birds in treatment group (T2) were subjected to UVB (280-315 nm) light exposure using UVB

tubes (M/s Philips India Ltd., Hyderabad) for 3 hours daily at specific intervals: 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 recorded as 76 µW/cm2, as indicated by the manufacturer" [7]. To ensure optimal exposure, a 60cm long, 240 V, 50 Hz, and 36 W UVB tube equipped with a heat protection reflector was positioned in the lower front section of the cages, targeting the featherless skin of the birds' feet and legs, particularly during feeding period. Additionally, an opaque paper board was placed from the feeder level to the cage top to shield the birds' eyes and combs from any harmful UV radiation. Furthermore, an opaque board was positioned between the irradiated and non-exposed groups to prevent any unintended radiation exposure.

2.3 Experimental Diet

The zinc content in the control diet was formulated in accordance with BIS 2007 standards, while the T2 treatment group received a diet supplemented with inorganic zinc sulphate at a concentration of 75 mg/kg of mash diet [7] while the total daily feed allotment was divided into four portions, each given just before the lighting schedule to ensure that UV radiation reached the featherless skin of the birds' feet and legs while they were standing and pecking at the feed. Throughout the 84-day experimental period, water was provided ad libitum.

2.4 Estimation of Vitamin D_3 in Egg Using LC-MS

Chemicals and solvents of analytical grade were used in this study. Petroleum ether, diethyl ether, ethanol, n-Hexane, ascorbic acid, potassium hydroxide and sodium sulphate, were procured from M/s Sigma Aldrich India Pvt. Ltd. and M/s Merck Specialities Pvt. Ltd. The HighPerformance Liquid Chromatography (HPLC) grade solvents used for Liquid Chromatography and Mass Spectrometry (LC-MS) were procured from M/s Merck Specialities Pvt. Ltd. Water for LC-MS was obtained from ultra-pure water purification system of M/s Millipore, France. Vitamin D_3 standard was obtained from M/s Sigma Aldrich, Sweden.

Stock solution of cholecalciferol (vitamin D_3) was prepared in amber coloured volumetric flasks by dissolving 20 mg of vitamin D_3 in 100 ml of HPLC grade n-Hexane. For the quantification and recovery tests, composite working standard solutions of 125, 250, 500, 750 and 1500 µg/mL were prepared by diluting the stock solution

Table 1. Experimental layout

No	Treatment	No of birds
T1	Control with standard layer diet (BIS [5])	4X4
T2	Zn enriched diet + UVB light exposed	4X4

Table 2. Percent ingredient composition of experimental diet

Sl. No.	Feed ingredients	Percentage
1	Yellow maize	56.50
2	De-oiled rice bran	6.50
3	Soya bean meal	27.50
4	Calcite powder	7.50
5	Dicalcium phosphate	1.50
6	Salt	0.50
Total		100.00
Feed Supplements (g/100 kg feed)		
1	L-Lysine	100
2	DL-Methionine	100
3	Vitamin premix	50
4	[5]Toxin binder	100
5	Choline chloride	100
6	Trace mineral mixture	100
7	Liver tonic powder	25
Total		575

with suitable quantities of n- Hexane. A mixture of HPLC grade methanol with 0.1% formic acid (pH 3.0) and distilled water with 0.1% formic acid (pH 2.83) at 95: 5 was used as mobile phase. Saponification, extraction and final clean-up of the sample was carried out according to the procedures of Megha and Ramnath [8].

2.4.1 Linearity of vitamin D_3 concentration versus time curve

Squared correlation coefficient is being defined as linearity, which was calculated from the standard curve using Microsoft excel. Ascending standards of vitamin D_3 @ 125, 250, 500, 750, 1500 µg/mL in n-Hexane were analysed using LCMS for the construction of calibration plots. From the chromatograph, peak area was calculated for each dilution, and concentration was plotted against peak area. The reproducibility of the result was verified at least thrice with each concentration of vitamin D_3. The regression was done using MS-Excel® and regression equation was found out.

2.5 Efficiency

Efficiency was calculated by using the following formula; N = 16 $(tR/Wb)^2$. (N = Efficiency, tR = Retention time, Wb = Peak Width).

2.6 Estimation of Zinc Content of Egg

At weekly intervals, one egg was randomly chosen from each treatment group, and its raw contents underwent microwave acid digestion to determine the zinc content. The digested samples were then analysed for zinc using the standard method of Atomic Absorption Spectrometry [9]. The PinAAcle 900H Series AAS facilitated a straightforward automated measurement of zinc, utilizing a hollow cathode lamp with a wavelength of 213.86 nm, an energy level of 40%, and a lamp current of 15 mA.

2.7 Blood Sample Collection and Estimation of Minerals and Liver Enzymes

"Blood samples were collected from the wing vein and analysis of serum for zinc (ppm) and calcium (mg/dL) were done using Atomic Absorption Spectrometry. The serum samples were analyzed for calcium by Atomic Absorption Spectrometry standard method" [9]. The PinAAcle 900H Series AAS enabled a simple automated measurement of Ca using hollow cathode lamp of wavelength 422.67 nm, energy level 66% and lamp current of 10 mA. Serum phosphorous level (mg/dL) and activity of serum enzymes like Alanine aminotransferase (ALT: IU/L), Alkaline phosphatase (ALP: IU/L) and Gamma glutamyl transferase (GGT: IU/L) were quantified by semi-automated biochemical analyser using commercial kits supplied by M/s Agappe diagnostics Ltd, Maharashtra. Serum vitamin D_3 was determined by a conventional spectrophotometric method [10].

3. RESULTS AND DISCUSSION

3.1 Vitamin D_3 Content of Eggs

A good linearity of the method was observed with a correlation coefficient of 0.9999 between the concentration range of 125 µg/ mL to 1500 µg/ mL for vitamin D_3, (Fig. 1).

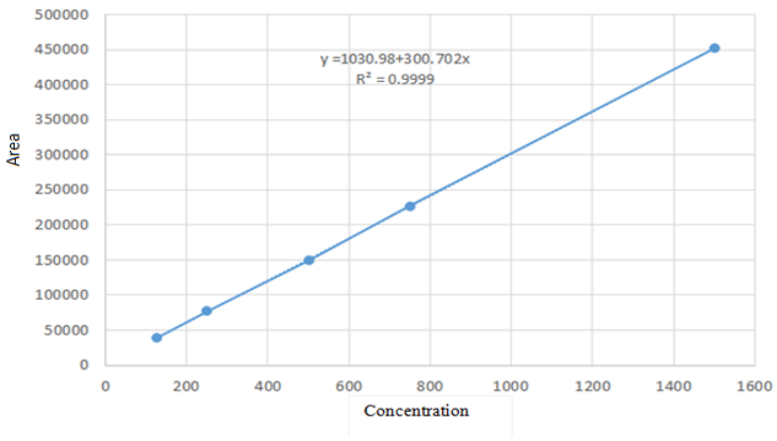

Fig. 1. Linearity of vitamin D_3 concentration versus area curve

Ascending concentrations of vitamin D_3: 125, 250, 500, 750 and 1500 µg/ mL were analysed using LC-MS and the peaks with area were calculated. Linear regression analysis was done using MS-Excel® and the regression equation was found out with regression coefficient (R^2) of 0.9999. Concentration of vitamin D_3 in egg yolk was quantified using the regression equation :Y = mX + C (Y. = peak area; C = Y intercept; m = Slope and X= Concentration (µg/ml)). Here 300.702 is the slope and c is the intercept on y axis. Where, R^2 is regression coefficient. The concentration of vitamin D_3 in egg yolk samples was quantified using the regression equation: Peak area = 300.702 x (Concentration in µg/mL) +1030.98. Unknown egg yolk concentrations were found using the above regression equation. The method validation was done as follows, by calculating the precision, accuracy and sensitivity of the method. The efficiency was determined as mentioned in 3.8.4.6 and it was found to be 5.21N.

Twelve samples from each treatment groups were selected randomly and the samples were separated by reverse phase affinity elution using mobile phase. The MS detector was set with vapourizer temperature 40˚C and pressure at 42-45 bar. The ions observed through single ion monitoring (SIM) method at m/z ratio of 385.41 which was specific for vitamin D_3. The UV-VIS detection was carried out at 265 nm wavelength. The extracted egg yolk samples of vitamin D_3 were analyzed and the representative chromatograms from each treatment groups were shown in the Fig. 2 and 3 respectively. The data acquisition time for each sample was set for 13 minutes. The retention time for control sample was 10.35 minutes while the retention time for samples ranged from 10.30 to 10.48 minutes. Chromatogram of control and test samples are shown in the Figures (Fig 2 and Fig 3) given below (Table 3).

The effect of UVB light treatment was highly significant ($p<0.01$) for vitamin D_3 concentration

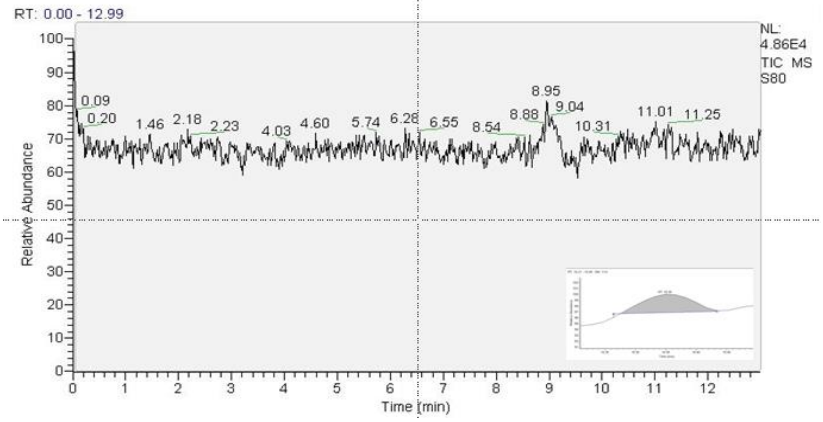

Fig. 2. Chromatogram of control sample

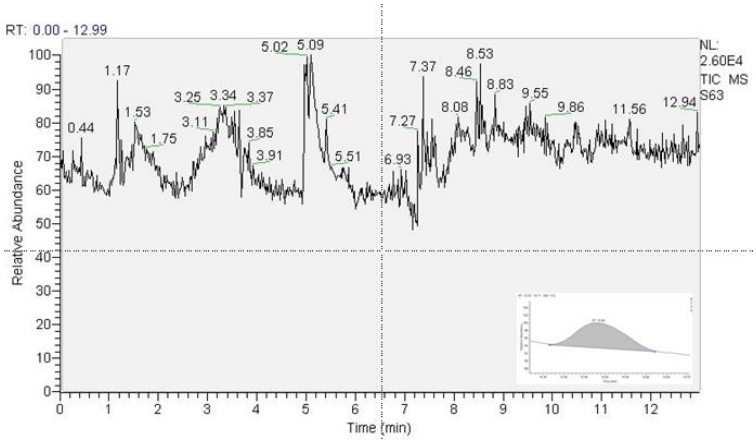

Fig. 3. Chromatogram of treatment group

Table 3. Chromatogram results of control and test samples

Sample Groups	Retention time (minute)	Area of the peak (min x mAu)	Height of the peak (mAu)
T1 (Fig: 2)	10.35	6292.98	1082.72
T2 (Fig: 3)	10.48	20565.74	1599.55

Table 4. Mean values of retention time, area, height and amount of treatment groups

Treatment groups	Mean value of Area (min*mAu)	Mean value of Height (mAu)	Mean value of amount (µg/10 g egg yolk)	
	Mean ± S.D.	Mean ± S.D.	Mean ± S.E.	p-value
T1	6434.38±2069.75	930.18±374.98	17.92^{a}±1.98	0.000**
T2	22187.46±2204.02	1671.52±632.12	70.35^{b}±2.11	

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

in egg samples during the experimental period. The concentration of vitamin D_3 in samples of T1 were significantly lower than in treatment group T2. Treatment groups exposed to UVB light treatment showed higher amount of vitamin D_3 in egg samples compared to unexposed groups (Table 4).

The samples and standards for analysis were arranged in accordance with the methods described by Mattila et al. [11]. Mobile phase used for the detection of vitamin D_3 consisted of a mixture of HPLC grade methanol with 0.1% formic acid (pH 3.0) and distilled water with 0.1% formic acid (pH 2.83) at 95: 5. The C18 column temperature was held constant at 40°C and the results were monitored at 265 nm wavelength as described by Kumar et al. [12] with slight modification in the flow rate as 0.5 mL/min. Goren et al., [13] and time window period of 13 min.

Since an optimal purification procedure was used and the diode array system was chosen for peak purity analysis, the quantification of vitamin D_3 was reliable and accurate. The reason for the variation in results reported by various authors probably laid on extraction clean up procedures used in solid phase extraction, which were sensitive to changes in the elution conditions (Mattila et al., 1992). The present study indicated that UVB exposure at 3h/day at a distance of 20 cm on featherless body parts could increase the vitamin D_3 content.

Vitamin D3, synthesized in the skin under UVB exposure, binds with vitamin D binding protein and then transfers to eggs after binding with specific plasma proteins produced by the liver, ultimately reaching the ovary. In the ovary, it binds with yolk protein Fraser and Emtage, [14] for storage. The current findings indicate that exposure to UVB at a distance of 20 cm with an intensity of 76 µW/cm2 effectively increased the vitamin D content in egg yolk. These results align with those of Schutkowski et al. [3], who showed that both UVB light exposure and dietary vitamin D3 supplementation could enhance the vitamin D3 content in eggs, with UVB irradiation proving more effective than dietary supplementation alone. The highest levels of vitamin D3 in eggs were achieved through a combination of UVB exposure and dietary vitamin D3 supplementation. Wei et al. [15] concluded that UVB supplementation using LEDs brought a significant ($p<0.01$) increase in yolk $1,25(OH)_2D_3$ concentration during the later laying stage of hens.

3.2 Zinc Content in Eggs

The effect of zinc feeding was highly significant ($p<0.01$) for zinc concentration range in eggs. It was noticed that hens fed the diet enriched with inorganic zinc sulphate showed higher zinc level in eggs compared to other group under conventional feed. Supplemented zinc resulted in significant difference in zinc content of eggs and it was clearly noticed that egg zinc content increased significantly ($p<0.01$) by increasing the zinc level to 75 mg/Kg diet (Table 5).

The results of the current study are consistent with those of Stahl et al. [4], who observed a 57-95% increase in zinc levels in eggs when hens were fed diets containing elevated zinc levels at a rate of 1861 mg/kg. This gradual increase in egg zinc content due to dietary supplementation may be attributed to the production of vitellogenin, a trace mineral transport protein. Vitellogenin facilitates the transfer of zinc from liver reserves to the egg yolk [16].

Table 5. Mean (±S.E.) value of zinc concentration [mg/L (ppm)] in eggs of different treatment groups

Treatment group	No of samples	Mean value of amount (ppm) Mean±S.E.	p-value	F value
T1	12	33.36^{a}±0.89		
T2	12	40.55^{b}±0.59	0.000**	31.798

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

Table 6. Mean (±S.E.) value of serum mineral concentration in treatment groups

Treatment group	No of samples	Zn (ppm) Mean±S.E.	Ca (mg/dL) Mean±S.E.	P (mg/dL) Mean±S.E.	Vitamin D3 (ng/mL) Mean±S.E.
T1	12	2.54^{a}±0.06	8.65^{a}±0.15	3.77^{a}±0.20	5.25^{a}±0.68
T2	12	3.09^{b}±0.14	16.11^{b}±0.22	4.39^{b}±0.32	13.38^{b}±0.41
p value		0.000**	0.000**	0.000**	0.000**

*Means bearing same superscript within a column do not differ significantly (p<0.01); ** Highly significant (p<0.01)*

According to Cousins [17], over-supplementation of zinc through the diet could lead to increased permeability of the absorptive membrane, allowing zinc to enter cells and bind indiscriminately to cellular proteins and other molecules. Therefore, for the current study, an optimal dietary supplementation level of 75mg/kg was chosen, resulting in the production of zinc-enriched eggs. The recommended zinc requirement for layer birds (21-45 weeks) by BIS 2007 is 60 mg/kg.

Studies by Plaimast et al. [18] and Aghaei et al. [19] have demonstrated a strong positive correlation between both dietary zinc levels and the zinc content of egg yolks. However, Skrivan et al. [20] observed no significant variations in the zinc content of egg yolks, whites, and shells when 80 mg of zinc was added to the basal diet of hens. They attributed this lack of enrichment to the antagonistic effects of zinc and copper.

3.3 Level of Serum Minerals and Liver Enzymes

Zinc and calcium concentration in the serum was significantly higher (p<0.01) for birds in treatment group 2 when compared to control group. While the phosphorus concentration was not significantly different between the treatment groups. The vitamin D_3 concentration in serum of birds exposed to UVB was significantly higher (p<0.01) throughout the experimental period compared to control group. Concentration of serum vitamin D_3 was seen lower in non-exposed groups whereas, serum vitamin D_3 concentrations showed tendency to rise upon UVB treatment.

In the current study, the mean serum zinc concentration in the group supplemented with zinc and exposed to UVB was significantly lower (p <0.01) compared to the control group. These findings align with those of Kaya et al. (2001), who noted that only dietary zinc supplementation influenced plasma zinc concentration, with the highest recorded level being 4.39 µg/mL in the group fed 100 mg of zinc/kg. Conversely, plasma zinc levels declined significantly after feeding doses exceeding 100 mg Zn/kg, possibly due to the homeostatic mechanisms regulating zinc absorption, distribution, metabolism, and excretion in tissues.

In the current study, the serum calcium levels of birds were significantly influenced by both UVB treatment and zinc supplementation (p <0.01), as indicated in Table 6. UVB exposure led to a significant increase in serum calcium levels in birds, consistent with the findings of de Matoes [21], who highlighted the health and welfare benefits of UVB wavelengths by supporting the endogenous synthesis of vitamin D. Vitamin D is well-established to play a crucial role in calcium absorption, metabolism, and excretion.

Idowu et al. [22] found that incorporating either inorganic or chelated zinc into diets improved calcium utilization. Similarly, Bahakaim et al. [23] reported that increasing zinc levels in diets significantly elevated plasma calcium levels, with hens receiving 100 mg/kg zinc methionine diets exhibiting the highest plasma calcium levels. In broiler breeder males and females aged 58 to 66 weeks, Al-Daraji and Amen [24] observed that zinc supplementation led to a significant increase in blood plasma calcium levels.

In present study serum phosphorus levels of birds were significantly ($p<0.01$) influenced by UVB exposure and zinc supplementation.

Wei et al. [15] observed a significant increase in serum phosphorus levels with longer UVB-LED exposure durations, noting a synergistic effect between serum phosphorus and calcium levels in laying hens exposed to UVB. These findings were corroborated by the present study, which also showed higher serum phosphorus levels in birds under UVB exposure without additional zinc supplementation in the feed. In contrast to our findings, Schutkowski et al. [3] reported that plasma inorganic phosphate concentration was unaffected by both UVB exposure and dietary vitamin D3 supplementation. The highest plasma phosphorus levels were observed in the control group, which received the recommended level of inorganic zinc (50mg/kg diet) without additional supplementation [23].

The serum vitamin D_3 levels were significantly higher ($p <0.01$) in the group exposed to UVB light compared to the non-exposed groups. Similarly, Schutkowski et al. (2013) observed that UVB radiation and dietary vitamin D_3 supplementation in hens increased plasma $25(OH)D_3$ concentrations. In the present study, serum vitamin D_3 concentrations tended to increase upon UVB exposure. However, Fraser and Emtage (1976) found that the ratio of 25-hydroxycholecalciferol to cholecalciferol in serum was approximately 3:1, suggesting that yolk preferentially accumulated cholecalciferol over $25(OH)D_3$.

3.4 Liver Enzyme Activity of Birds

The effect of UVB light treatment and Zn feeding triggered insignificant change in liver enzyme status of birds during the experimental period. Status of liver enzymes activity in serum was estimated to rule out the side effects of UVB exposure and zinc supplementation in birds. In the present study non -significant changes in the activity of various liver enzymes were recorded.

Several studies have demonstrated that increasing zinc supplementation in diets leads to its accumulation in the liver of broiler chickens, with liver zinc levels ranging from 117 to 143 ppm at 4 weeks of age [25]. Similarly, Sandoval et al. [26] observed a significant increase in liver zinc deposition ($p < 0.01$) with higher dietary zinc levels. Diaz et al. [27] highlighted the utility of measuring plasma enzyme activities, particularly ALT, LDH, and GDH, for diagnosing liver damage in birds. In their study, White Leghorn laying hens (UCD-003 strain) consistently exhibited significantly elevated plasma liver enzyme levels, indicating extensive hepatic lesions, while plasma ALT activity averaged 1.0 ± 1.0 IU/L in normal birds. The results of the present study closely resembled this reported value.

Similar to the current study's results, Idowu et al. [22] investigated the effects of various zinc sources in the diets of laying birds over a 10-week trial and found that serum ALT activity did not differ among the different zinc sources. Conversely, Al-Daraji and Amen [24] reported a significant ($p < 0.05$) increase in plasma alkaline phosphatase (ALP) activity in broiler breeders with dietary zinc supplementation. The present study's findings indicate that neither UVB exposure nor zinc supplementation resulted in hepatic damage, as all estimated serum biochemical parameters in the exposed and supplemented groups were statistically comparable to those of the control group.

The current research helped to assess the efficacy of zinc supplementation and UVB irradiation in enriching egg and serum contents of the same.

Table 7. Mean (±S.E.) value of liver enzymes (IU/L) in different treatment groups

Treatment group	No of samples	ALP (IU/L)	ALT/ SGPT (IU/L)	GGT (IU/L)
		Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
T1	12	432.35±11.59	1.43±0.10	10.31±0.33
T2	12	430.19±8.23	1.30±0.09	10.56±0.47
p value		0.898	0.591	0.780

The effect of UVB light treatment was highly significant ($p<0.01$) for the concentration of vitamin D_3 in eggs and serum from 29 to 40 weeks of age. Hence it can be concluded that UVB light exposure would be a better choice for enhancing vitamin D_3 content of eggs and to produce designer eggs enriched with vitamin D_3. At the same time UVB exposure increases the serum vitamin D_3 level which was indicative of its transport from skin to ovaries after synthesis.

Serum vitamin D3, zinc, calcium and phosphorus levels of birds were significantly affected ($p<0.01$) by UVB light exposure and dietary zinc supplementation. The effect of UVB exposure and zinc supplementation through diet was non-significant for serum liver enzyme activity of birds in all groups irrespective of treatments

The concentration of zinc in egg and serum of birds supplemented with zinc diet was significantly higher ($p<0.01$) than that of the supplemented group [28].

4. CONCLUSIONS

The dietary supplementation of hens with zinc is an effective approach to supply consumers with foods from animal sources that are enriched with zinc. Current findings showed that feeding of zinc supplemented diet to chickens offered a promising alternative to fortify egg with zinc. Furthermore, UVB light exposure would be a better choice for enhancing vitamin D_3 content of eggs and to produce designer eggs enriched with vitamin D_3. It increased the serum vitamin D_3 level which was indicative of its transport from skin to ovaries after synthesis.

ETHICAL APPROVAL

Approval for the experiment was obtained from the Institutional Animal Ethics Committee (IAEC) of the College of Veterinary and Animal Sciences, Mannuthy, KVASU (Kerala veterinary and Animal sciences University).

ACKNOWLEDGEMENT

The authors are thankful to Central Instruments laboratory, Kerala Veterinary and Animal Sciences University for providing the facilities needed for carrying out the research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Singh VP, Sachan N.. Designer eggs: A smart approach for health conscious persons. Poult. Plann. 2010;11: 21-23.
2. Browning LC, Cowieson AJ. Vitamin D fortification of eggs for human health. J. Sci. Food Agric. 2014;94:1389-1396.
3. Schutkowski A, Kramer J, Kluge H, Hirche, F, Krombholz A, Theumer T, Stangl GI. UVB exposure of farm animals: study on a food-based strategy to bridge the gap between current vitamin D intakes and dietary targets. PloS One. 2013;8:e69418.
4. Stahl JL, Cook ME, Greger JL. Zinc, iron, and copper contents of eggs from hens fed varying levels of zinc. J. Food Compos. Anal. 1988;1: 309-315.
5. BIS [Bureau of Indian Standards]. Poultry feed specification. (5th Ed.). Bureau of Indian Standards, New Delhi. 2007;3-5.
6. Megha PS, Ramnath V, Vishnudas KV. Effect of vitamin D3Enrichment by Ultra Violet Blue (UVB) radiation and dietary zinc supplementation on egg production and feed consumption in layer birds. Arch. Curr. Res. Int. 2024;2:102-109.
7. Megha PS, Ramnath V. Influence of direct Ultra Violet Blue (UVB) light exposure on serum levels of vitamin D3, calcium and phosphorus in layer chickens. The Pharma Innovation Journal. 2023;12(1):16-19
8. Megha PS, Ramnath V. Production of vitamin D3 Enriched Designer Chicken Eggs by Direct Ultra Violet Blue (UVB) Light Exposure. J. Anim. Res. 2021;11:977-982. Available:https://doi.org/10.30954/2277-940X.06.2021.4
9. AOAC [Association of Official Analytical Chemists]. Official methods of analysis. (20th Ed.). Washington DC. 2016;587.
10. Megha PS, Ramnath V. Influence of direct ultra violet blue (UVB) light exposure on serum levels of vitamin D3, calcium and phosphorus in layer chickens. Pharma Innov. 2023;12:16–19. Available:https://doi.org/10.22271/tpi.2023.v12.i1a.17939
11. Mattila P, Piironen V, Backman C, Asunmaa A, Uusi-Rauva E, Koivistoinen, P.. Determination of vitamin D_3 in egg yolk by high-performance liquid chromatography with diode array detection. J. Food. Compos. Anal. 1992;5: 281-290.

12. Kumar S, Chawla D, Tripathi AK. 2015. An Improved and sensitive method for vitamin D_3 estimation by RPHPLC. Pharm. Anal. Acta. 2015;6: 1-6.
13. Goren AC, Bilsel G, Bilsel M. Rapid and simultaneous determination of 25-OH-vitamin D_2 and D_3 in human serum by LC/MS/MS: Validation and uncertainty assessment. J. Chem. Metrl. 2007;1:1-9.
14. Fraser DR, Emtage JS. Vitamin D in the avian egg.Its molecular identity and mechanism of incorporation into yolk. *Biochem.* J. 1976;160: 671-682.
15. Wei Y, Zheng W, Li B, Tong Q, Shi H, Li X. Effects of b-wave ultraviolet supplementation using light-emitting diodes on caged laying hens during the later phase of the laying cycle. Anim. 2020;10:15.
16. Richards MP. Trace mineral metabolism in the avian embryo. Poult. Sci. 1997;76:152-164.
17. Cousins RJ. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiol. Rev. 1985;65: 238-309.
18. Plaimast H, Sirichakwal P, Puwastien P, Kijparkorn S. Effect of supplementary zinc from organic and inorganic sources on laying performance and zinc deposition in eggs. Thai. J. Vet. Med. 2008;38: 47-53.
19. Aghai A, Khosravinia H, Mamuoei M, Azarfar A, Shahriari A.. Effects of dietary supplementation of zinc and α-tocopheryl acetate on performance and zinc concentrations in egg and tissues of Japanese quails. Poult. Sci. J. 2017;5:57-64.
20. Skrivan M, Skrivanova V, Marounek M. Effects of dietary zinc, iron, and copper in layer feed on distribution of these elements in eggs, liver, excreta, soil, and herbage. Poult. Sci. 2005;84:1570-1575.
21. de Matos, R.. Calcium metabolism in birds. *Vet*. Clin. North. Am. Exot. Anim. Pract. 2008;11:59-82.
22. Idowu OMO, Ajuwon RO, Oso AO, Akinloye OA. Effects of zinc supplementation on laying performance, serum chemistry and Zn residue in tibia bone, liver, excreta and egg shell of laying hens. Int. J. Poult. Sci. 2011;10: 225-230.
23. Bahakaim A, Abdel Magied H, Osman S, Omar A, Abdel Malak NY, Ramadan N. Effect of using different levels and sources of zinc in layer's diets on egg zinc enrichment. Egypt. Poult. Sci. J. 2014; 34:39-56.
24. Al-Daraji HJ, Amen MH. Effect of dietary zinc on certain blood traits of broiler breeder chickens. Int. J. Poult. Sci. 2011;10:807-813.
25. Sunder GS, Panda AK, Gopinath NCS, Rao SR, Raju MVLN, Reddy MR, Kumar, CV. Effects of higher levels of zinc supplementation on performance, mineral availability, and immune competence in broiler chickens. J. Appl. Poult. Res. 2008;17:79-86.
26. Sandoval M, Henry PR, Ammerman CB, Miles RD, Littell RC. Relative bioavailability of supplemental inorganic zinc sources for chicks. J. Anim. Sci. 1997;75:3195-3205.
27. Diaz GJ, Squires EJ, Julian RJ. The use of selected plasma enzyme activities for the diagnosis of fatty liver-hemorrhagic syndrome in laying hens. Avian. Dis. 1999; 43:768-773.
28. Megha, P.S., Ramnath, V., Raji, K., Babitha V, Chacko B. Production of zinc enriched designer eggs through dietary supplementation. J. Vet. Anim. Sci. 2021;52:77–80. Available:https://doi.org/10.51966/jvas.2021.52.1.77-80

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.ikprress.org/review-history/12039