p-ISSN 1410-5659 e-ISSN 2621-5144
Published by:
Unpad Press
Jurnal Ilmu Ternak Universitas Padjadjaran, Juni 2023, 23(1):60-69
DOI: 10.24198/jit.v23i1.46272
Available online at http://jurnal.unpad.ac.id/jurnalilmuternak

Effects of 1,25-Dihydroxyvitamin D3 Supplementation on The Performance of 60-Week-Old Layers

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Abstract

The Effects of 1,25-Dihydroxyvitamin D3 supplementation on egg production, percent hen house, percent hen day, mortality rate and egg size on 60-week-old laying hens were studied. There were 5 treatment groups of layer hens where Treatment 1 served as control supplemented with commercial feed without vitamin D3, Treatment 2 as positive control supplemented with commercial feed and vitamin D3 HyD, which was already used by the poultry farm, Treatment 3 supplemented with commercial feed and Active D at an inclusion rate of 75 ppm per ton of feeds, Treatment 4 supplemented with commercial feed and Active D at an inclusion rate of 90 ppm per ton of feeds and Treatment 5 supplemented with commercial feed and Active D at an inclusion rate of 110 ppm per ton of feeds. A total of 11,027 Lohmann chickens were used for the study. The vitamin D3 were mixed with normal basal diet. The effects of vitamin D3 showed significant results in egg production, percent hen house, percent hen day, mortality rate and egg size. In summary, the vitamin D3 supplementation had increased in laying performance and egg size quality of the old laying hens.

Keywords: Lohmann chickens, Vitamin D3, Laying performance, Egg size

Efek Suplementasi 1,25-Dihidroksivitamin D3 terhadap Performa Ayam Petelur Umur 60 Minggu

Abstrak

Penelitian ini bertujuan untuk mempelajari efek suplementasi 1,25 dihidroksi vitamin D3 terhadap produksi telur, tingkat kematian dan ukuran telur pada ayam petelur umur 60 minggu. Terdapat 5 kelompok perlakuan ayam petelur yaitu Perlakuan 1 sebagai kontrol, pakan komersial tanpa vitamin D3, Perlakuan 2 sebagai kontrol positif ,pakan komersial dan vitamin D3 HyD yang sudah digunakan oleh peternakan unggas, Perlakuan 3 pakan komersial dan Active D pada tingkat inklusi 75 ppm per ton pakan, Perlakuan 4 pakan komersial dan Active D pada tingkat inklusi 90 ppm per ton pakan dan Perlakuan 5 pakan komersial dan Active D pada tingkat inklusi sebesar 110 ppm per ton pakan. Sebanyak 11.027 ekor ayam Lohmann digunakan dalam penelitian ini. Vitamin D3 dicampur dengan diet basal normal. Efek vitamin D3 menunjukkan hasil yang signifikan pada produksi telur, persen kandang ayam, persen hari ayam, tingkat kematian dan ukuran telur. Kesimpulannya, suplementasi vitamin D3 telah meningkatkan performa petelur dan kualitas ukuran telur ayam petelur tua.

Kata Kunci : Ayam Lohmann, Vitamin D3, Performa bertelur, Ukuran telur

Introduction

The ability of birds to perform up to their optimal genetic potential is dependent on the vitamin content of their diets being at its optimal level. The vitamin requirements that were defined some decades ago did not consider the genetically superior birds that are raised now, which have greater growth, egg output, and improved feed efficiency. Vitamin D3 is essential for sustaining the production parameters in chickens, particularly in elderly laying hens. Vitamin D3 can be produced in the chicken body through the conversion of 7-dehydrocholesterol when exposed to UV radiation between 290 and 315 nm (Barnkob *et al*., 2020). Modern layer flocks, on the other hand, are almost exclusively housed indoors and hence, not exposed to direct sunlight. Thus, their major source of vitamin D3 is diet (Świątkiewicz *et al*., 2017). Vitamin D3 must undergo two biochemical transformations before it can be used in an active form (Christakos *et al*., 2010). In the first instance, 1,25-hydroxyvitamin D3 is formed in the liver, which is the main circulating form of vitamin D3 (25OHD). The 25OHD is then transformed to its bioactive form, known as 1,25-dihydroxyvitamin D3, which occurs mostly in the kidney (1,25OHD).

Vitamin D3 is associated with mineral homeostasis, according to research (Chang *et al*., 2005). The amount of calcium supplements that should be given to laying chickens is directly related to the age of the animal being fed (An *et al*., 2016). 1,25-dihydroxyvitamin D3, also known as 1,25-dihydroxycholecalciferol, is the biologically active form of vitamin D found in dietary supplements (Kappeli *et al*., 2010). The supplementation of phytobiotics or probiotics in layers will not only be able to help improve the performance but also to provide an alternative and a more "natural" way for poultry farmers in giving layer pullets adequate supportin termss of its growth and laying performance (Medina *et al*., 2022). Productive traits and immunity in laying hens decrease sharply during the late phase of laying due to aging, which negatively affects the performance, metabolism, and hormonal status (Attia *et al.*, 2020). The sharp decline of laying performance and eggshell quality is a common problem in late period laying hens (Jing *et al.*, 2022). Production performance and egg quality in 60-week-old layers may be improved by supplementing them with the biologically active form, which avoids the hydroxylation process in the kidneys and liver.

Chickens start laying eggs at 18-19 weeks of age. They lay eggs continuously until they reach 72-78 weeks of age (Biggs, 2022). In a study conducted by Damerow (2021), it was determined that hens could live for up to 12 years or even longer, but their behavior and appearance might change over time. The feet and legs of an older hen will become scalier and thicker making the chickens tend to be less active and walk seldomly. The performance of an older hen typically declines, and the number of eggs produces is low (Damerow, 2021).

The main objective of this study was to examine the effect of varying dietary doses of vitamin D3 on the performance and egg size of Lohmann laying chickens until 68 weeks of age, since there are limited studies of vitamin D3 on poultry layers that are 60 weeks old and older. The study was conducted because most of the problems encountered in a commercial poultry farm are due to poor performance concerning older hens.

Materials and Methods

Ethics statement

The protocols and procedures of this experimental study were performed after the approval of the Institutional Animal Care and Use Committee (IACUC) of the College of Veterinary Medicine, University of the Philippines Los Baños, and the Institutional Biosafety Committee.

Dietary Supplement

Phytobiotics used in this experiment are an innovative herbal formulation that contains natural vitamin D glycosides and functional triterpenes, namely Active D. These substances work together synergistically to provide metabolic support for the animal, enabling optimal performance and strength. The inclusion rate was based on the recommendations of the manufacturer, and it ranges from 75 ppm to 110 ppm per ton of feed. Layers were fed with commercial feed (Table 1) every 4 am and 1 pm every day. Feeds were placed on the feeding troughs manually and mixed in between feeding times using a hand brush to reorient bigger feed particles that settled at the bottom of the feeder. Water was available ad libitum in PCV pipes cut in half that served as drinking troughs. The water supply was from a nearby spring and disinfected with chlorine.

Experimental Design

A total of eleven thousand twenty-seven (11,027) 60-week-olds by San Miguel Corporation were used for the trial. Lohmann hens Laying Hens were allocated for the experiment which came from one flock. The hens were divided into five treatment groups (Table 2). The treatment groups were distributed into 5 rows of cages. The dietary supplements were mixed with their current formulation in feed, and it was given to the hens through feed intake.

Parameters

Eggs were collected from the nesting boxes and total number and weight were recorded daily. The experiment was carried out for 8 weeks. The production performance was measured based on the egg production, egg size, the total number of hens that were housed at the start of the laying cycle, and the total number of eggs laid throughout the period of the time. The variables were calculated as follows:

a. Egg production

$$= \frac{egg\ production\ on\ a\ day}{7\ days} \times 100$$

b. HDEP (Hen day egg production)

$$= \frac{total\ number\ of\ eggs\ produced\ on\ a\ day}{total\ number\ of\ hen\ days\ in\ the\ same\ period} \times 100$$

c. HHEP (Hen house egg production)

$$= \frac{total\ number\ of\ eggs\ laid\ on\ a\ day}{total\ number\ of\ hens\ housed\ at\ the\ beginning\ of\ laying\ period} \times 100$$

d. Mortality

$$= \frac{Dead\ birds}{Total\ number\ of\ birds\ at\ the\ beginning\ of\ the\ experiment} \times 100$$

e. Egg size

$$= \frac{total\ number\ of\ eggs\ laid}{egg\ size\ produced} \times 100$$

Statistical analysis.

Data gathered were submitted for statistical analysis using IBM® SPSS® Statistics Software v27 (International Business Machines Corporation, USA). Statistical differences between treatment groups were considered significant with $p<0.05$. The data obtained in the study were subjected to Shapiro-Wilk and Levene's tests. Egg production values, HDEP%, mortality rates were analyzed using ANOVA with post hoc Tukey's HSD test while HHEP% were analyzed using Welch's ANOVA with post hoc Games-Howell test. Pearson Chi-Square test was used to check the equality of proportions of egg sizes.

Results and Discussion

Calcium is an important part of making the quality of eggshells good. The eggshell is mostly made of calcium carbonate wherein calcium makes up about 38% of the eggshell (Gautron *et al*., 2021). The findings of this study are comparable with those found in the study of Adhikari *et al*. (2020) which stated that if there is no vitamin D3 in the diet at all, eggs that are produced would either have very thin shells or none at all. In the study of Jing *et al*. (2022), it is stated that vitamin D3 had a positive effect on eggshell quality, which is similar to the results of the present study since the layers fed with various vitamin D3 inclusion produced decent eggshell quality.

Egg production

Treatment 2 was found to be significantly different compared to other treatments throughout the first month of egg production as seen in Table 4. Meanwhile, T4 had the lowest egg mean production of all the treatments. It was obviously shown that the treatment with 90 ppm Active D had the lowest egg production in overall data, as well as during the second month, whereas other treatments were not statistically different from one another.

The egg production evidently showed an increase in yield in the first month of the trial, however, it dropped during the second month. After the experimentation has concluded, it was observed that there was a significant difference among the treatments. In summary, Treatment 2 fed with vitamin D3 HyD showed statistically significant difference among treatment levels, while Treatment 4 had the lowest mean egg production.

In terms of Active D inclusion rates, Treatment 3 had the highest monthly and overall mean egg production. The results are comparable with the study of Chen *et al*. (2021) which stated that vitamin D3 supplementation has a beneficial impact on egg production especially with aged laying hens. It would be converted from 25-hydroxy vitamin D3 in the liver into bioactive form which is 1,25-dihydroxyvitamin D3 in the kidney (Christakos *et al*., 2010) thus, no further metabolism in the body.

Treatment 4 had the lowest egg production among different treatments. The concentration of the mentioned treatment level was 90 ppm per ton of feeds, but it did not show any improvement in performance even it had a high dose of vitamin D3. The results are similar with what Li *et al*. (2021) had found. They stated that high dose of vitamin D3 had no significant positive effect in the performance of layers in terms of egg production despite the various inclusion rates. To support this, a study of Persia *et al*. (2013) concluded that an increase in the dietary concentration of vitamin D3 had no apparent adverse effect on hen performance. Ca and P utilization were increased by different forms of vitamin D in the diets (Adhikari *et al*., 2020)

Percent HDEP and percent HHEP

Treatment 2 was significantly different compared to other treatment levels throughout the first month of hen day production, whereas the Active D treatment with 90 ppm had the lowest mean hen day production. T4 showed the lowest mean percent hen day production for both the second month and overall data, whereas other treatments did not show any significant differences as seen on Table 5.

The HDEP and HHEP clearly demonstrated that supplementation had led to an increase in egg production. There was an increase in production during the first month of the trial, but it gradually decreased during the second month. As seen on Table 6, in the first month of hen house production, T2 was statistically different compared to other treatments, whereas T4 had the lowest mean hen house production. For the second month, the control and T2 showed significant differences with respect to T3, T4, and 110 ppm Active D. Overall, the T4 had the lowest mean percent hen house production among the various treatment levels, while T1 and T2 were significantly different from 90 ppm Active D and T5.

In terms of the Active D inclusions, Treatment 3 had the highest percent hen house and hen day while Treatment 4 and Treatment 5 achieved an acceptable range (based on farm protocol) of 80% production. The standard hen day production of 68 weeks old Lohmann hens is 83.7%. However, the highest hen day production in the study was 83.49%, which is significantly lower compared to the standard percentage. This could be due to mismanagement and hot weather since the trial was conducted during early summer. The results are comparable with the study of do-Nascimento *et al*. (2014) which reported that vitamin D3 is essential to maintain laying performance in aged hens. The treatments with vitamin D3 in basal diet had no significant differences in terms of performance. The results are similar to a study by Wen *et al*. (2019) where they have found no significant difference in laying performance among the various levels of vitamins D3 supplementation in the diet.

Mortality rate

There were no significant findings regarding mortality between the first and second months of the study as seen on Table 7. To summarize, it was clear that 90 ppm has the lowest mean mortality rate among the various treatment levels, while T2 had the highest mortality rate.

For the mortality rate, there was no statistically significant difference among the five treatments in monthly data. However, it has revealed that there was a significant difference among different treatment groups in overall data. In summary, it was concluded that Treatment 2 was statistically different among other treatments, while Treatment 4 had the lowest mortality rate. Unfortunately, there were no references that correlates vitamin D3 supplementation with an increase in mortality, hence, the mortality rate may be due to poor management and the age of the animal

Egg size

Regarding the size of the eggs, Treatment 2 produced a decent size of eggs for both the first and second month. As seen in Table 8-10, the control produced eggs that were significantly smaller than those produced by any of the other treatments, and it had the highest rate of defective eggs.

In terms of egg size, Treatment 2 produced mostly medium, large, and jumbo eggs while Treatment 1 produced smaller eggs with soft-shelled and defective eggs. The quality of an eggshell depends on a laying hen's diet in many ways. Calcium and vitamin D3 seem to be the most important and relevant factors.

Table 1. Nutrient composition of layer basal diet

Ingredients	Concentration (%)
Maize	64.63
Soybean meal	23.30
Corn gluten meal	3.00
Wheat barn	1.80
Soybean oil	2.50
Dicalcium phosphate	1.92
Limestone	1.25
Premix	1.00
Salt	0.25
L-Lysine	1.22
DL-Methionine	0.25
ME (Kcal/kg)	2800.00
CP (%)	18.70
Ash (%)	10.28
Crude fat (%)	4.72
Crude fiber (%)	4.45
Calcium (%)	3.68
Total phosphorus (%)	6.05

Note: ME = Metabolizable Energy, CP = Crude Protein

Table 2. The dietary treatments of the study

Treatment	Description	Number of layer hens
Treatment 1	Basal Diet	1,738
Treatment 2	Basal Diet + 300 ppm Vitamin D3 HyD	3,996
Treatment 3	Basal Diet +75 ppm Active-D-concentration	1,771
Treatment 4	Basal Diet +90 ppm Active-D-concentration	1,758
Treatment 5	Basal Diet +110 ppm Active-D-concentration	1,764

Table 3. Specifications of the egg (USDA, 2000)

Size	Weight (in grams)
Jumbo	70 and above
Extra large	65-69
Large	60-64
Medium	55-59
Small	50-54
Peewee	40-49
Reject	<40

Table 4. Egg production of layer supplemented with 1,25-dihydroxyvitamin D3

Egg production (%)	T1	T2	T3	T4	T5
1st month	85.72±0.49[b]	87.74±0.98[a]	84.38±0.59[bc]	82.55±0.66[d]	83.81±0.52[cd]
2nd month	83.39±0.92[a]	81.94±4.41[a]	81.64±1.10[a]	79.18±1.15[b]	81.99±0.67[a]
Overall	84.56±1.42[a]	84.84±4.28[a]	83.01±1.68[a]	80.87±2.00[b]	82.90±1.12[a]

Note: All data are presented as means ± SD. Means in the same row followed by different superscripts shows a significant difference (P<0.05). T1 = Basal diet, T2 = Basal diet + 300 ppm vitamin D3 HyD, T3 = Basal diet + 75 ppm Active D-concentration, T4 = Basal diet + 90 ppm Active D-concentration, T5 = Basal diet + 110 ppm Active D-concentration

Table 5. Hen day egg production (HDEP) of layer supplemented with 1,25-dihydroxyvitamin D3

HDEP (%)	T1	T2	T3	T4	T5
1st month	85.87±0.50[b]	88.04±0.98[a]	84.57±0.55[bc]	82.65±0.62[d]	84.03±0.636[c]
2nd month	83.49±0.89[a]	82.21±4.44[a]	81.82±1.13[a]	79.28±1.21[b]	82.13±0.61[a]
Overall	84.68±1.43[a]	85.12±4.31[a]	83.19±1.68[a]	80.96±2.00[b]	83.08±1.16[a]

Note: All data are presented as means ± SD. Means in the same row followed by different superscripts shows a significant difference (P<0.05). T1 = Basal diet, T2 = Basal diet + 300 ppm vitamin D3 HyD, T3 = Basal diet + 75 ppm Active D-concentration, T4 = Basal diet + 90 ppm Active D-concentration, T5 = Basal diet + 110 ppm Active D-concentration

Table 6. Hen house egg production (HHEP) of layer supplemented with 1,25-dihydroxyvitamin D3

HHEP (%)	T1	T2	T3	T4	T5
1st month	85.42±0.22[b]	88.04±0.49[a]	84.51±0.30[c]	82.57±0.20[d]	83.72±0.21[c]
2nd month	84.59±0.48[a]	84.61±1.09[a]	83.07±0.62[b]	81.22±0.64[c]	82.73±0.44[bc]
Overall	85.01±0.56[a]	86.32±4.28[a]	83.79±0.89[ab]	81.89±0.84[c]	83.22±0.62[b]

Note: All data are presented as means ± SD. Means in the same row followed by different superscripts shows asignificant difference (P<0.05). T1 = Basal diet, T2 = Basal diet + 300 ppm vitamin D3 HyD, T3 = Basal diet + 75 ppm Active D-concentration, T4 = Basal diet + 90 ppm Active D-concentration, T5 = Basal diet + 110 ppm Active D-concentration

Table 7. Mortality rate of layer supplemented with 1,25-dihydroxyvitamin D3

Mortality rate (%)	T1	T2	T3	T4	T5
1st month	0.17±0.08	0.33±0.15	0.23±0.08	0.11±0.10	0.26±0.16
2nd month	0.13±0.07	0.32±0.09	0.21±0.07	0.13±0.11	0.17±0.10
Overall	0.15±0.07[bc]	0.33±0.11[a]	0.22±0.07[a]	0.12±0.10[c]	0.21±0.13[ab]

Note: All data are presented as means ± SD. Means in the same row followed by different superscripts shows a significant difference (P<0.05). T1 = Basal diet, T2 = Basal diet + 300 ppm vitamin D3 HyD, T3 = Basal diet + 75 ppm Active D-concentration, T4 = Basal diet + 90 ppm Active D-concentration, T5 = Basal diet + 110 ppm Active D-concentration

Table 8. Percentage (%) of eggs by treatment and size of layer supplemented with 1,25-dihydroxyvitamin D3 first month (4 weeks)

	Percentage (%) of eggs by treatment and size												
	RJ	PW	PL	ST	SK	M	L	XL	JB	B	B	L	DE
T1	0.35	0.87	1.04	6.02	23.31	23.37	28.22	5.90	1.16	4.92	2.08	1.45	1.33
T2	0.25	0.54	0.69	6.02	9.93	25.23	39.48	9.95	2.44	2.27	1.04	1.19	0.96
T3	0.40	0.86	0.63	5.38	22.39	20.96	33.96	7.62	1.37	3.84	0.69	0.63	1.26
T4	0.23	0.11	0.97	4.56	22.12	23.55	30.39	7.87	1.54	5.64	0.63	0.97	1.43
T5	0.12	0.35	0.98	4.15	20.02	22.45	34.51	7.33	1.96	4.85	0.58	0.98	1.73
Pearson Chi-Square	3.29	13.77	3.64	12.07	263.44	14.08	188.66	31.4	15.66	52.09	27.60	6.30	6.41
p-value	0.511	0.008	0.457	0.017	<0.01	0.007	<0.01	<0.01	0.004	<0.01	<0.01	0.178	0.171

Note: RJ - Reject (Misshapen eggs or underweight), PW – Pewee, PL – Pullets, ST – Small, SK- Small-Medium, M – Medium, L – Large, XL - Extra Large JB – Jumbo, B, B and L - (Soft shelled egg), DE - Dirty Egg. T1 = Basal diet, T2 = Basal diet + 300 ppm vitamin D3 HyD, T3 = Basal diet + 75 ppm Active D-concentration, T4 = Basal diet + 90 ppm Active D-concentration, T5 = Basal diet + 110 ppm Active D-concentration

Table 9. Percentage (%) of eggs by treatment and size of layer supplemented with 1,25-dihydroxyvitamin D3 second month (4 weeks)

	Percentage (%) of eggs by treatment and size												
	RJ	PW	PL	ST	SK	M	L	XL	JB	B	B	L	DE
T1	0.22	0.44	0.88	4.53	16.74	28.44	28.44	8.77	1.54	5.26	1.75	1.61	1.39
T2	0.15	0.35	0.84	6.54	8.23	31.91	35.58	11.15	1.19	1.34	1.04	0.79	0.89
T3	0.00	0.30	0.67	4.27	15.04	26.72	33.91	9.81	1.87	3.89	0.82	0.75	1.95
T4	0.08	0.23	0.91	5.68	14.77	23.56	32.20	10.76	2.20	6.14	0.61	0.76	2.12
T5	0.08	0.38	1.44	4.98	18.88	24.09	30.51	10.05	1.13	4.23	0.83	1.21	2.19
Pearson Chi-Square	3.56	1.01	4.80	11.24	91.65	38.74	22.68	5.74	7.77	59.98	10.56	8.17	12.77
p-value	0.469	0.908	0.309	0.024	<0.01	0.007	<0.01	0.219	0.100	<0.01	0.032	0.086	0.012

Note: RJ - Reject (Misshapen eggs or underweight), PW – Pewee, PL – Pullets, ST – Small, SK- Small-Medium, M – Medium, L – Large, XL - Extra Large JB – Jumbo, B, B and L - (Soft shelled egg), DE - Dirty Egg. T1 = Basal diet, T2 = Basal diet + 300 ppm vitamin D3 HyD, T3 = Basal diet + 75 ppm Active D-concentration, T4 = Basal diet + 90 ppm Active D-concentration, T5 = Basal diet + 110 ppm Active D-concentration

Table 10. Percentage (%) of eggs by treatment and size of layer supplemented with 1,25-dihydroxyvitamin D3 overall (8 weeks)

	Percentage (%) of eggs by treatment and size												
	RJ	PW	PL	ST	SK	M	L	XL	JB	B	B	L	DE
T1	0.29	0.68	0.97	5.36	20.41	25.61	28.32	7.17	1.32	5.07	1.94	1.52	1.36
T2	0.21	0.48	0.74	6.20	9.36	27.46	38.18	10.35	2.03	1.96	1.04	1.05	0.94
T3	0.23	0.622	0.65	4.90	19.21	23.46	33.94	8.57	1.59	3.86	0.75	0.68	1.56
T4	0.16	0.16	0.94	5.04	18.97	23.55	31.16	9.11	1.82	5.86	0.62	0.88	1.72
T5	0.10	0.36	1.18	4.51	19.53	23.16	32.78	8.51	1.60	4.58	0.69	1.08	1.93
Pearson Chi-Square	3.23	11.45	6.80	14.57	312.71	32.78	104.78	27.85	6.95	107.56	36.67	11.50	18.35
p-value	0.520	0.022	0.147	0.006	<0.01	<0.01	<0.01	<0.01	0.138	<0.01	<0.01	0.021	0.001

Note: RJ - Reject (Misshapen eggs or underweight), PW – Pewee, PL – Pullets, ST – Small, SK- Small-Medium, M – Medium, L – Large, XL - Extra Large JB – Jumbo, B, B and L - (Soft shelled egg), DE - Dirty Egg. T1 = Basal diet, T2 = Basal diet + 300 ppm vitamin D3 HyD, T3 = Basal diet + 75 ppm Active D-concentration, T4 = Basal diet + 90 ppm Active D-concentration, T5 = Basal diet + 110 ppm Active D-concentration

According to the research of Kakhki, *et al*. (2019) supplementing the diet with vitamin D3 may have beneficial effects on egg weight and mass especially in older laying hens. It is known that Treatment 1 had no vitamin D3 in the basal diet, and it was revealed that it had the highest number of defective eggs. This result is coherent with study of Attia *et al*. (2020) and Zang *et al*., (2011), which concluded that cracked eggshells are associated with vitamin D deficiency in laying hens. Moreover, the results are also comparable to the study of Park & Sohn (2018) wherein the shells of the eggs laid by older hens were concluded to tend to be poorer in quality. Another study by Vlčková *et al*. (2018) supported this claim after they observed that the quality of the eggshell degrades as birds get older.

Conclusions

According to the results of this study, maintaining a consistent intake of vitamin D3 seems to be the most advisable course of action, as doing so, it will contribute to an improvement in both the quantity and quality of the eggs. It is recommended to check the calcium and phosphorus levels of the flock to assess if there is calcium and phosphorus deficiency in the poultry farm. Another recommendation would be to conduct an experimental trial over a longer period and increase the dosage of Active D to determine whether there would be an increase in the performance or quality of the eggs.

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