VARIOUS DIETARY LEVELS OF INORGANIC VITAMIN D ON INTERNAL AND EXTERNAL PHYSICAL QUALITY PARAMETERS OF LAYING HEN EGGS

G.M. Cornescu[1*], P.A. Vlaicu[1], A. Oancea[1], A.E. Untea[1], T.D. Panaite[1], O. Avram[2]

[1]National Research & Development Institute for Animal Nutrition and Biology, Balotesti, Ilfov, Romania
[2]S.C. Aviputna S.R.L., Golești, Romania

Abstract

The study investigated the effect of different dietary levels of inorganic vitamin D3 on the laying hens performances, internal and external physical parameters of eggs, instrumental color measurements and self-life. The experiment was conducted on 114 Lohmann Brown laying hens, (38 laying hens/group; 34 weeks age) with an average initial body of 1850 g for 8 weeks trial period. Three different levels of vitamin D3 inorganic form were taken into consideration: Diet 1 (Control, 2500 UI/kg premix), Diet 2 (3000 UI/kg premix) and Diet 3 (3200 UI/kg premix). All internal and external egg quality parameters were measured at the begging, after 4 and 8 experimental weeks, including instrumental color measurements. We also determined the self-life of eggs stored for 28 days at room temperature. The internal and external physical parameters measured during the experiment were: egg white and yolk pH, egg weight height, yolk color, Haugh unit, breaking shell strength, eggshell thickness, yolk height, yolk diameter, and yolk index. We observed significant differences for feed intake and feed conversion ratio in the first four experimental weeks. Diet 1 had the highest feed intake compared with diet 2 and diet 3. During the entire experimental period, diet 2 had constantly the lowest feed conversion ratio, compared to diet 1 and diet 3. At the end of the trial (8 weeks) diet 3 group presented eggs with significantly higher weight compared with diet 1 and diet 2. For egg quality parameters, a significant ($P<0.05$) eggshell thickness was noticed at diet 2 (0.42 mm) compared to diet 1 group (0.39mm) during the first 4 weeks of feeding, while during the 8 weeks of feeding no difference could be notice between groups (0.39 mm). The breaking shell strength was higher in diet 3 (5.21 kgf) and diet 2 (5.08 kgf) compared to diet 1 group (4.83kgf), without statistically significance during the first 4 weeks, with similar trend after 8 weeks of feeding. Albumen pH increased significantly after first 4 weeks of feeding, with significant modification on Haugh units ($p<0.0001$) between the groups.

Keywords: egg, inorganic, physical parameters, vitamin D3, laying hens

INTRODUCTION

Known as "sunshine vitamin" vitamin D is usually synthesized within the skin exposed to ultraviolet radiation and refers to both vitamin D2 and vitamin D3 [1]. The main role of vitamin D is related to calcium absorption and bone health, its deficiency increasing the risk of developing rickets or osteomalacia [2]. Vitamin D is a fat-soluble vitamin essential for the proper metabolism of calcium (Ca) and phosphorus (P), and the maintenance of normal skeletal integrity in animals [3]. Vitamin D is also responsible for normal growth, egg and shell quality, and reproduction in laying hen, and it is of particular importance when hens are raised indoors [4]. There are some foods from animal origin considered as naturally rich in vitamin D, such as oily fish and egg yolks [5]. Vitamin D enrichment eggs can be achieved either by increasing sunlight exposure (inexpensive way) or by its supplementation into poultry diet (inorganic or organic sources) [6]. Although a 2014

*Corresponding author: gabriela_cornescu@yahoo.com
The manuscript was received: 08.10.2021
Accepted for publication: 12.01.2022

EFSA nutritional surveys in 14 European countries showed that vitamin D intake is below the upper safe limit supplementing vitamin D into laying hens diet it is limited by EU regulations, 3000 IU/kg D3 being considered maximum content that is safe for the target animals, consumer, user and the environment [7]. The common level of vitamin D3 in layer diets is about 2200 IU/kg, while the commercial egg breeders' guides recommend 2.500 IU/kg in diets, and 3000 to 5000 IU/kg is recommended by commercial producers [8]. Cashman [9] considers being rational to study both conventional vitamin D3 and his metabolite (25-OH-D3) sources transfer efficiency of commercial vitamin D inclusion levels (1500 IU/kg feed) and the maximum EU recommended vitamin D inclusion level. Vitamin D fortification of eggs it is seen as an affordable and easiest option to reduce vitamin D deficiency throughout the world, but this depends greatly of every country food standard policy [10]. Reports from the literature showed differential results. Mattila [11] showed that supplementation of hen diets with 6000 and 15000 IU of D3/kg has increase egg yolk vitamin D3 content with no negative effects on hen production or egg quality in a 48-week feeding experiment. Also, Park [12], in a 30-days experiment, diets containing 4000, 8000, 12000, 16000, or 200000 IU of vitamin D3 showed no significant differences in eggshell strength, Haugh unit, feed intake, feed conversion, or egg yolk color. Moreover, in another experiment, 40000 IU of D3/kg of diet was shown to be well tolerated by laying hens, but 200000 IU of D3/kg of diet decreased egg weight, shell quality, feed consumption, and fertility [13].

The aim of this study was to evaluate the effect of dietary supplementation with different levels of inorganic vitamin D3 sources on laying hens' performance, external and internal physical quality egg parameters, shelf life and instrumental color.

MATERIAL AND METHOD

The experiment was conducted within the experimental halls of the National Research and Development Institute of Animal Biology and Nutrition (Romania) according to a protocol approved by the Commission of Ethics of the institute. The experiment respected the animal welfare principles stipulated by the EC Directive 63/2010/EEC. An 8-week study with 114 Lohmann Brown laying hens, 34 weeks initial age (2 hens/cage; 19 cages/group; 38 hens/group) with an average initial body of 1850 g were randomly allotted in 3 groups (Diet 1, Diet 2 and Diet 3). They were housed in Big Dutchman battery dimensioned according to the sanitary-veterinary norms and designated for digestibility trials. The average daily feed intake (g CF/layer/day), feed conversion ratio (kg CF/kg egg), and egg intensity (%); average weight/egg (g) were registered. The microclimate was computer controlled (temperature 19.00±1.00°C and humidity 64.00 %). Feed and water were provided ad libitum. A single factorial design consisted of three vitamin levels was experiment structured (diet 1 - 2500 IU/kg feed; diet 2 - 3000 IU/kg feed; diet 3 - 3200 IU/kg feed). The basic structure of diets was identically for all groups except for premix structure where vitamin D3 concentration level was different. The diets were characterized by 2780 kcal/kg metabolizable energy (ME); 17.50% crude protein (CP); 4.39% crude fiber (CF). The basal diet was a corn-wheat-soybean meal diet formulated according to the recommendations of Lohmann Brown hybrid management guide. The basal diet was formulated (Table 1) using Hybrimin Futter 5 software. The all diets were isocaloric and isonitrogenous.

Table 1 Ingredients and calculated nutrient analysis of the diet

Ingredients, % as feed basis	C
Corn, %	39.24
Wheat, %	20.00
Soybean meal, %	26.59
Vegetal oil, %	2.32
DL–Methionine, %	0.17
Choline, %	0.05
Calcium carbonate, %	8.83
Monocalcium phosphate, %	1.40
Salt, %	0.40
Premix**, %	1.00
Total, %	100

Nutrient analysis (calculated)	
Dry matter, %	87.11
Metabolizable energy, kcal/kg	2780
Crude protein, %	17.50
Ether extractives, %	3.55
Crude fiber, %	4.39
Calcium, %	3.90
Total phosphorus, %	0.69
Available Ph, %	0.38
Sodium, %	0.17
Chlorine, %	0.30
Lysine, %	0.87
Methionine, %	0.40
Meth + Cyst, %	0.70
Threonine, %	0.67
Tryptophan, %	0.20

Note: Diet 1, Diet 2 and Diet 3 have identical basal diet structure

*Group Diet 1, per kg premix contained: 1350000 IU/kg vitamin A; 250000 IU/kg vitamin D3; 2500 IU/kg vitamin E; 200 mg/kg vitamin K; 200 mg/kg vitamin B1; 480 mg/kg vitamin B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 300 mg/kg vitamin B6; 4 mg/kg vitamin B7; 100 mg/kg vitamin B9; 1.8 mg/kg vitamin B12; 2500 mg/kg vitamin C; 7190 mg/kg manganese; 6000 mg/kg iron; 600 mg/kg copper; 6000 mg/kg zinc; 50 mg/kg cobalt; 114 mg/kg iodine; 18 mg/kg selenium;

** Group Diet 2 - 300000 IU/kg vitamin D3;

***Group Diet 3 - 320000 IU/kg vitamin D3

Hen Performance and Egg Sampling

Feed intake (FI, g feed/hen), laying percentage (%), egg weight (g), and feed conversion rate (FCR, kg of feed/kg of egg) data were recorded and calculated daily. The bioproductive performances were reported every two weeks to show the effect of vitamin D3 supplementation over time during the 8 experimental weeks.

We selected 18 eggs before the actual trial started to have a comparison of vitamin D3 effect supplement over 8 weeks. From these eggs we determined all quality parameters. From eggs produced over 8 consecutive weeks we collected after 4 and 8 respectively weeks, 18 eggs per group for egg quality assessment.

Concomitant, at the end of the trial, we collected another batch of 54 eggs (18 eggs per group), labelled according to date of production and deposited them in a room at constant temperature (18-19°C), for 28 days, to test the influence of supplemental vitamin D3 on shelf life external and internal quality parameters of eggs. Relative humidity was regulated at 50 to 60% for all samples.

Evaluation of external and internal egg quality

The external and internal quality parameters of the eggs were determined on each egg component. Measurements for egg weight, albumen, yolk and eggshell weight with a Kerm scales, 0.001 precision; color intensity was determined with Egg Analyzer TM; eggshell breaking strength with an Egg Force Reader, Sanovo engineering A/S, Denmark; albumen and yolk pH with a portable pH meter Five Go F2-Food kit with LE 427IP67, Sensor Mettler Tolledo and albumen height was determined using stage micrometer manually.

Haugh unit with was determined using the following formula:

$$HU=100 \times \log (H - 1.7W0.37 + 7.6) \qquad (1)$$

Where:

H: Albumen height

W: Egg weight;

The yolk index, which is an egg quality indicator that shows that as egg deterioration progresses and in the same time the yolk index score lowers because the vitelline membrane loosens therefore its strength decreases over time, was calculated by dividing the yolk height by the yolk diameter of the egg broken onto a flat surface using the following formula:

$$YI=YH/YD \qquad (2)$$

Where:

YI: Yolk index

YH: yolk height

YD: yolk diameter

Instrumental Color Determination

Yolk color analyses were performed after the method described elsewhere [14], using a Konica Minolta CR-400 (Holdings Inc., Tokyo, Japan) colorimeter. For that, the colorimeter was calibrated using the standard white ceramic reference (illuminate C).

The average of 18 measurements per group in three different points (54 measurements/ group) on the on the yolk surface were recorded for lightness (L*), redness (a*) and yellowness (b*) according to the CIE Lab (Commission Internationale de l'Eclairage, Vienna, Austria) trichromatic system. In the CIE system L* represent dark to light on a scale of 0 to 100; a* represents green to red on a scale of -60 to +60; and b* represents blue to yellow on a scale of -60 to +60.

Statistical analysis

The analytical data were compared by variance analysis (ANOVA) using Stat View for Windows (SAS, version 6.0). One-way ANOVA was used to compare the laying hen's performance, external and internal physical quality egg parameters, shelf life and instrumental color. The difference between the means was considered significant at P<0.05. Significant differences at 5% among treatment means were evaluated using Tukey's multiple range tests.

RESULTS AND DISCUSSIONS

The higher value for feed intake registered significant differences (p<0.0104) at group fed with Diet1 compared to the Diet2 after two weeks of feeding. FCR, was significantly lower (p<0.002) in group fed Diet2, compared with Diet1 and Diet3, while same group registered significantly higher (p<0.0123) laying percentage. No significant effect was noted for egg weight.

After 4 weeks of feeding, group Diet1 had significantly higher (p<0.0003) FI compared with Diet2 and Diet3. FCR was significantly higher (p<0.0002) in both Diet1 and Diet3 compared with Diet2, while in the same group laying percentage was significantly higher (p<0.0472).

Egg weight was not affected by any of dietary treatments after four weeks feeding supplemental vitamin D3. Convergingly, after six and respectively eight weeks of experiment, FI was without modifications between the groups. FCR was still significantly higher in D1 group compared with Diet 2 and Diet 3 after six weeks, while after eight weeks the difference was maintained only for Diet 1 vs Diet 2.

Laying percentage, maintained his higher value in Diet 2 group, when compared with Diet 1 and Diet 3, while for egg weight, Diet 3 was significantly higher (p<0.0001) compared with Diet 1 and Diet 2, in the last four experimental weeks.

Some studies, reported adverse effect of vitamin D3 supplementation in laying hens' diets. Persia et al. [15] reported that FI was higher in group supplemented with higher concentration of vitamin D3, when compared to group with lower supplemental concentration. The increased egg weight in Diet 3 group, which contained 3200IU vitamin D3/kg diet, are similar with some studies which showed similar responses [10] when 15000IU vitamin D3/kg diets was used.

Even though effort is made to enrich eggs with vitamin D, a concentration of 200000 IU of vitamin D3/kg diet have previously been reported to reduce egg weights [13]. However, Adhikari et al. [16] reported that, there was no difference in FI, egg production and egg weight between hens fed diets supplemented with different levels of vitamin D3. Similarly, Akbari et al. [17] reported that additional supplementation of 3300IU of vitamin D3/kg did not show any egg production improvement in aged Lohmann LSL layers (72- to 81-week-old), whereas significantly increased egg weight.

These different results may be attributed also to age of laying hens. Vitamin D3 and his metabolites may have better effects on egg weight in older laying hens.

It must be taken into consideration, that our study, is conducted in European Union, were currently we are limited at maximum dosage level of vitamin D3, which makes difficult comparing our results with other studies developed under different regulations. Even though, all the results are comparable considering the fact that they have been conducted following different effects in laying hens' diets.

From the egg quality physical parameters (Table 3), determined initially (prior to trial start) after 4 and 8 weeks respectively, egg weight significantly (p<0.0001) increased during the feeding trial, especially after 4 weeks in Diet 3. As expected, the albumen weight was significantly (p<0.0002) higher in the same group after 4 weeks, but also the difference was maintained till the end of the trial. Yolk weight was significantly higher (p<000.1) after 8 weeks in both Diet 2 and Diet 3, compared with initially analysed eggs and those after 4 weeks. No effect (p<0.3438) was found for eggshell weight. After 4 weeks feeding the same vitamin Diet 3 supplement, albumen pH significantly decreased, but at the end of the trial, the values for all groups increased considerably.

Table 2 The influence of vitamin D3 supplementation on laying hens performances

Item	Diet 1	Diet 2	Diet 3	SEM	P
After two weeks					
Feed intake, g/day	119.37^{a}	115.00^{b}	117.38a^{b}	0.610	0.0104
Feed conversion ratio, kg feed/kg egg	2.04^{a}	1.89^{b}	2.02^{a}	0.018	0.0002
Laying percentage, %	94.87^{b}	97.82^{a}	93.45^{b}	0.018	0.0123
Egg weight, g	63.08	62.56	62.62	0.018	0.4058
After four weeks					
Feed intake, g/day	117.65^{a}	114.34^{b}	112.42^{b}	0.018	0.0003
Feed conversion ratio, kg feed/kg egg	1.88^{a}	1.82b	1.84^{ab}	0.018	0.0472
Laying percentage, %	97.25^{a}	97.82^{a}	94.25^{b}	0.018	0.0008
Egg weight, g	64.72	64.38	64.91	0.018	0.0610
After six weeks					
Feed intake, g/day	115.32	115.05	115.82	0.018	0.8958
Feed conversion ratio, kg feed/kg egg	1.99^{a}	1.82^{b}	1.85^{b}	0.018	0.0001
Laying percentage, %	91.21^{b}	97.42^{a}	95.73^{a}	0.018	0.0002
Egg weight, g	64.66^{b}	64.93^{b}	65.64^{a}	0.018	<0.0001
After eight weeks					
Feed intake, g/day	116.81	116.66	115.49	0.018	0.2768
Feed conversion ratio, kg feed/kg egg	1.92^{a}	1.83^{b}	1.89a^{b}	0.018	0.0104
Laying percentage, %	95.97^{a}	97.62^{a}	92.04^{b}	0.018	<0.0001
Egg weight, g	65.52^{b}	65.57^{b}	66.09^{a}	0.018	0.0189

^{a,b} means marked with a different superscript letter within each column are significantly different.
Diet 1 – 250000IU/kg vitamin D3; Diet 2 - 300000 IU/kg vitamin D3; Group Diet 3 - 320000 IU/kg vitamin D3. SEM – standard error of the mean. P- significance

Table 3 External and internal egg quality physical parameters

Item	Initial	After 4 weeks			After 8 weeks			SEM	P
		Diet 1	Diet 2	Diet 3	Diet 1	Diet 2	Diet 3		
Egg weight, g	58.51^{d}	64.08^{b}	62.02^{c}	64.87^{a}	62.55^{c}	62.51^{c}	63.25^{c}	0.806	<0.0001
Albumen weight, g	37.04^{c}	40.62^{a}	38.67^{b}	40.98^{a}	39.88^{b}	40.11^{b}	41.54^{a}	0.851	0.0002
Yolk weight, g	13.57^{c}	15.75^{b}	15.21^{b}	15.62^{b}	15.22^{b}	16.19^{a}	16.24^{a}	0.220	<0.0001
Shell weight, g	8.20	7.12	8.17	8.18	7.85	7.70	7.63	0.348	0.3438
Yolk pH	6.73^{a}	5.37^{b}	5.42^{b}	5.38^{b}	5.36^{b}	5.35^{b}	5.45^{b}	0.049	<0.0001
Shell thickness	0.43	0.39	0.45	0.42	0.39	0.39	0.39	0.018	0.0950
Breaking strength, kgf	4.90	4.82	5.53	5.29	4.53	4.99	4.60	0.309	0.2526
Yolk Height,mm	17.47^{b}	16.17^{b}	18.11^{a}	19.00^{a}	17.13^{b}	16.54^{b}	17.33^{b}	0.253	0.0004
Yolk diameter	36.97^{c}	39.95^{b}	39.45^{b}	40.46^{a}	39.68^{b}	41.29^{a}	39.05^{b}	0.386	<0.0001
Yolk index	0.47^{a}	0.41^{b}	0.45^{a}	0.47^{a}	0.43^{b}	0.40^{b}	0.44^{a}	0.006	<0.0001

^{a,b,c} means marked with a different superscript letter within each column are significantly different.
Diet 1 – 250000IU/kg vitamin D3; Diet 2 - 300000 IU/kg vitamin D3; Group Diet 3 - 320000 IU/kg vitamin D3. SEM – standard error of the mean. P- significance

A drastic alteration was also found in HU after 8 weeks of feeding, compared with those initially analysed. Shell thickness and breaking strength of the eggs, were not affected by the supplement. Yolk height, in Diet 2 registered significantly higher values after 4 experimental weeks, while conversing, same group registered a drastic decrease after 8 weeks of feeding 3000IU/kg feed vitamin D3 supplement compared with those Diet 1 and Diet 3. On the other hand, while yolk diameter increased after 8 weeks, yolk index decreased when compared with those from initial stage. Our results have shown that hens fed diets with different vitamin D3 supplementation showed a significant alteration of some parameters, while others, and were significantly improved. These changes were accompanied by decreasing HU parameter after 8 feeding weeks. Literature data is very controversial about the effects of vitamin D3 supplementation, on different egg quality parameters. In the current study, the addition of different levels of vitamin D3, did not improve all egg quality parameters measured. This is in agreement with other studies. Mattila et al. [10, 18] reported that Haugh unit, were not significantly affected by vitamin D3 or D2 in the diets during the 48-wk experimental period. On the basis of previous studies [15], improvement in egg quality parameters was not expected in hen fed vitamin D3 dietary treatments. Egg strength in laying hens from other study fed with 1500 IU and 3000 IU/kg dietary vitamin D3 was significantly greater than that of hens fed 500 IU vitamin D3/kg of diet. It was assumed that no effect of different forms and levels of vitamin D on egg quality parameters may be due to inclusion of adequate levels of Ca, available P, and vitamin D3 in the control diet [16]. Recently Attia et al. [8] reported substantial effects of vitamin D3 supplementation on shape index, and shell quality traits, when 2000 IU/kg vitamin D3 was used. Moreover, yolk percentage was reduced at 1000 IU/kg vitamin D3 and Ca supplementation, but increased the yolk index, while contradictory, albumen percentage decreased in the same group.

When comparing the HU mirror of albumen quality with albumen pH, showed a considerable increase in Diet2 and Diet3 after 4 weeks of feeding 3000IU and 3200 IU vitamin D3, with lower pH values. But, in contrast, after 8 experimental weeks, HU decreased considerably, while pH values in albumen increased considerable. Attia et al. [8] reported that 2000 IU/kg vitamin D3 increased the HU compared with 1000 IU/kg vitamin D3 and Ca supplementation, in older laying hens (72 weeks of age). On the other hand, diets containing 4000, 8000, 12000, 16000, or 20000 IU of vitamin D3 showed no significant differences in Haugh unit [11]. From Figure 2, it can be observed that albumen height decreases, as expected after 8 weeks for all groups regardless the vitamin D3 level concentration. At 4 weeks the collected egg samples registered significant differences ($P<0.05$) between group Diet1 (5.23±1.39 mm) and Diet2 (7.07 ±0.95 mm). This characteristic is determined by the stability of the protein chains in albumen [19]. Albumen quality is not only an important indicator for maintaining egg freshness, but it is also important for the egg breaking industry because albumen and yolk have different markets [20]. In another experiment, it was reported that 40000 IU of vitamin D3/kg of diet was shown to be well tolerated by laying hens, but 200000 IU of vitamin D3/kg of diet decreased some egg quality parameters [12].

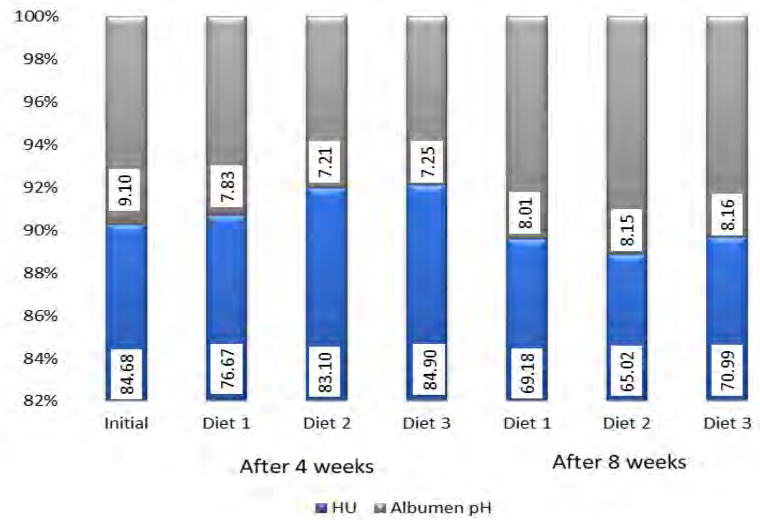

Figure 1 Influence of vitamin D3 supplement on egg HU and albumen pH

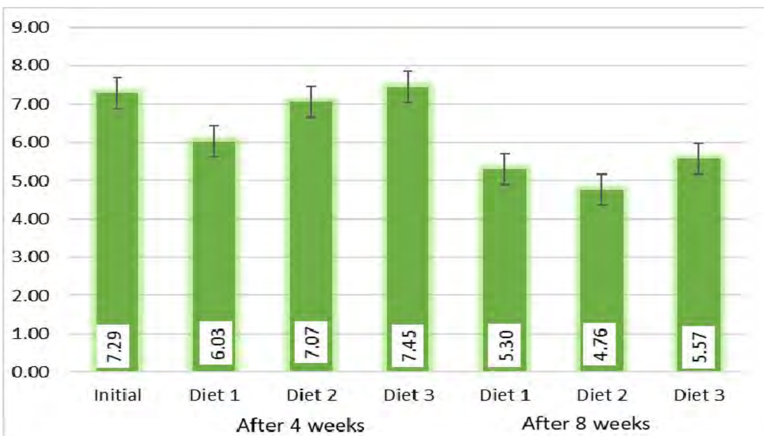

Figure 2 Influence of vitamin D3 supplementation on albumen height (mm)

The effect of diets on Yolk Fan score and instrumental colour determination are shown in Table 4. There were differences among diet and period ($p<0.0001$). Yolks from hens fed with diets prior to trial starting, had significantly higher colour, and compared with those from Diet 3 group. After 4 weeks of continue feeding with supplemental vitamin D3, yolk colour decreased with 2.30% in Diet 1 (2500 IU/kg), 7.75% in Diet 2 (3000 IU/kg) and with 16.14% in Diet 3 (3200 IU/kg). After 8 weeks of feeding experimental diets, the Yolk Fan colour, was even lower than after first 4 weeks. As expected, lightness parameter (L*) measured with chromameter indicated higher values ($p<0.0001$) after 4 and 8 weeks respectively, because Yolk Fan is directly correlated with higher luminosity of the yolks. Similarly, a* values which represent redness of egg yolks on a scale from -60 (green) to +60 (red), decreased significantly ($p<0.0001$), while the b* values, represented by the yellowness of the yolks, on a scale from -60 (blue) to +60 (yellow), was maintained in range values at the end of the trial, except for Diet 1, which had the highest value and Diet 2 which had the lowest value after first 4 weeks. Persia et al. [15] showed that Yolk Fan color score was not different among treatments, fed with 2200 IU/kg, 9700 IU/kg 17200 IU/kg or 24000IU/kg vitamin D3, but the 102200 IU vitamin D3 produced eggs with significantly increased egg yolks of hens aged 58 weeks.

Table 4 Influence of vitamin D3 supplement on instrumental color of egg yolk

Item	Initial	After 4 weeks			After 8 weeks			SEM	P
		Diet 1	Diet 2	Diet 3	Diet 1	Diet 2	Diet 3		
Yolk fan	4.77[a]	4.66[a]	4.40[a]	4.00[b]	3.00[c]	3.11[c]	3.16[c]	0.111	<0.0001
L	39.37[c]	40.05[c]	41.015[b]	42.68[b]	46.40[a]	44.81[a]	45.43[a]	0.585	<0.0001
a	0.85[a]	0.48[b]	-0.02[d]	0.27[c]	-0.42[e]	-0.15[d]	-0.26[de]	0.069	<0.0001
b	12.91[b]	14.60[a]	11.24[c]	12.65[b]	13.65[b]	13.43[b]	13.23[b]	0.495	<0.0001

[a,b,c,d,e] means marked with a different superscript letter within each column are significantly different.
Diet 1 – 250000IU/kg vitamin D3; Diet 2 - 300000 IU/kg vitamin D3; Group Diet 3 - 320000 IU/kg vitamin D3. SEM – standard error of the mean. P- significance

There is little data regarding the effect of vitamin D3 on instrumental color of the egg yolk, even though color is an important attribute influencing the appearance and overall acceptability of the products. These modifications on L*, a* and b* parameters, were also observed in others studies [14, 21], and it was concluded that they are modified when carotenoids are added as dietary supplements. As we reported previously shelf life of eggs is defined as the period from egg collection to their consumption, during which time the product is in a satisfactory state of quality in terms of chemical, physical, microbiological and sensory attributes [20, 22]. Shelf life of eggs is a very important aspect, especially when eggs are enriched with different nutrients. In our study, vitamin D3 supplementation at different levels was not a significant contributor for any of the egg quality physical parameters stored for 28 days at room temperature (Table 5).

Table 5 Effect of vitamin D supplementation on shelf life and instrumental colour of eggs after 28 days storage time at room temperature (19°C)

Item	Diet 1	Diet 2	Diet 3	SEM	P
Egg weight, g	63.02	61.57	63.46	0.164	0.7924
Albumen weight, g	39.93	38.23	39.26	1.098	0.8332
Yolk weight, g	17.03	15.82	17.42	0.302	0.0704
Shell weight, g	7.53	7.51	7.95	0.096	0.1023
Albumen pH	8.42	8.38	8.49	0.020	0.0532
Yolk pH	5.57	5.62	5.62	0.035	0.8228
Yolk height	3.81	3.52	4.07	0.161	0.4842
Haugh Unit	52.90	50.30	54.66	1.813	0.6624
Shell thickness, mm	0.42	0.40	0.40	0.005	0.2014
Breaking strength, kgf	4.88	5.10	5.21	0.197	0.7948
Yolk height, mm	11.17	11.00	11.84	0.370	0.6745
Yolk diameter	45.47	39.18	45.20	1.906	0.3310
Yolk index	0.25	0.22	0.26	0.011	0.3271
Instrumental colour measurements					
Yolk Fan Color	4.08	4.00	3.92	0.114	0.8539
Lightness (L*)	47.43	47.81	46.53	0.272	0.1438
Redness (a*)	0.03	0.03	0.02	0.044	0.3195
Yellowness (b*)	19.12	18.81	20.02	0.488	0.1557

Diet 1 – 250000IU/kg vitamin D3; Diet 2 - 300000 IU/kg vitamin D3; Group Diet 3 - 320000 IU/kg vitamin D3. SEM – standard error of the mean. P- significance

CONCLUSIONS

This experiment has demonstrated that increasing the vitamin D content in laying hens' (34 to 42 weeks of age) diet could improve eggs external and internal physical parameters but also proves that further experiments using higher vitamin D inclusion levels above 3000 IU/kg/feed the maximum EU recommended could have advantages on internal and external egg properties.

ACKNOWLEDGEMENTS

This study was conducted within project P_40_441 - GALIM PLUS -” Development of innovative feeding solutions for gallinaceans, with the purpose of producing affordable foods with improved nutritional qualities”, funding contract 144/13.10.2016. Project co-funded by the European Union from the European Fund for Regional Development, through the Operational Program Competitiveness 2014-2020. The content of this material does not necessarily express the official position of the European Union or the Government of Romania.

REFERENCES

[1]. Borradale D. & Kimlin M: Vitamin D in health and disease: an insight into traditional functions and new roles for the sunshine vitamin, Nutr Res Rev 22, 2009, p 118–136.

[2]. Holick MF, Binkley NC, Bischoff-Ferrari HA et al. Evaluation, treatment, and prevention of vitamin D deficiency:an Endocrine Society clinical practice guideline, J. Clin. Endocrinol. Metab. 96, 2011, p 1911–1930.

[3]. Garcia, A. F. Q. M., Murakami, A. E., do Amaral Duarte, C. R., Rojas, I. C. O., Picoli, K. P., & Puzotti, M. M. (2013). Use of vitamin D3 and its metabolites in broiler chicken feed on performance, bone parameters and meat quality. Asian-Australasian journal of animal sciences, 26(3), 408.

[4]. Mattila, P., Rokka, T., Könkö, K., Valaja, J., Rossow, L., & Ryhänen, E. L. (2003). Effect of cholecalciferol-enriched hen feed on egg quality. Journal of agricultural and food chemistry, 51(1), 283-287.

[5]. Schmid A, & Walther B.: Natural vitamin D content in animal products. Adv Nutr 4,2013, p 453–462.

[6]. Kuhn J., Schutkowski A., Kluge H. et al. Free-range farming: a natural alternative to produce vitamin D-enriched eggs. Nutrition 30, 2014, p 481–484.

[7]. EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed): Scientific Opinion on the safety and efficacy of vitamin D3 (cholecalciferol) as a feed additive for chickens for fattening, turkeys, other poultry, pigs, piglets (suckling), calves for rearing, calves for fattening, bovines, ovines, equines, fish and other animal species or categories, based on a dossier submitted by DSM. EFSA J 10, 2968, 2012, p 26.

[8]. Attia, Y. A., Al-Harthi, M. A., & Abo El-Maaty, H. M. (2020). Calcium and cholecalciferol levels in late-phase laying hens: effects on productive traits, egg quality, blood biochemistry, and immune responses. Frontiers in Veterinary Science, 7, 389.

[9]. Cashman, K. D., Seamans, K. M., Lucey, A. J., Stocklin, E., Weber, P., Kiely, M., & Hill, T. R.: Relative effectiveness of oral 25-hydroxyvitamin D_3 and vitamin D3 in raising wintertime serum 25-hydroxyvitamin D in older adults. The American Journal of Clinical Nutrition, 95(6), 2012, p 1350–1356.

[10]. Cashman K.D.& Kiel M.: Tackling inadequate vitamin D intakes within the population: fortification of dairy products with vitamin D may not be enough. Endocrine 51, 2016, p 38–46.

[11]. Mattila P., Valaja, J., Rossow, L., Venäläinen, E., & Tupasela, T. (2004). Effect of vitamin D2-and D3-enriched diets on egg vitamin D content, production, and bird condition during an entire production period. Poultry science, 83(3), 433-440.

[12]. Park, S. W., Namkung, H., Ahn, H. J., & Paik, I. K. (2005). Enrichment of vitamins D3, K and iron in eggs of laying hens. Asian-australasian journal of animal sciences, 18(2), 226-229.

[13]. Ameenuddin, S., Sunde, M. L., DeLuca, H. F., & Cook, M. E. (1986). Excessive cholecalciferol in a layer's diet: decline in some aspects of reproductive performance and increased bone mineralisation of progeny. British Poultry Science, 27(4), 671-677.

[14]. Panaite, T. D., S. M. Mădălina Iuga, and P. A. Vlaicu. (2019). Liquid egg products characterization during storage as a response of novel phyto-additives added in hens diet Emirates Journal of Food and Agriculture, 31(4) 304-1.

[15]. Persia, M. E., Higgins, M., Wang, T., Trample, D., Bobeck, E. A. (2013). Effects of long-term supplementation of laying hens with high concentrations of cholecalciferol on performance and egg quality. Poultry Science, 92(11), 2930-2937.

[16]. Adhikari R, White D, House JD, Kim WK.: Effects of additional dosage of vitamin D3, vitamin D2, and 25-hydroxyvitamin D3 on calcium and phosphorus utilization, egg quality and bone mineralization in laying hens. Poult Sci. 99(1), 2020, p 364-373.
[17]. Akbari M. K., Heuthorst T., Mills A., Neijat M., Kiarie E.: Interactive effects of calcium and top-dressed 25-hydroxy vitamin D3 on egg production, egg shell quality, and bones attributes in aged Lohmann LSL-lite layers Poult. Sci., 98, 2018, pp. 1254-1262
[18]. Mattila, P. H., Valkonen, E., & Valaja, J. (2011). Effect of different vitamin D supplementations in poultry feed on vitamin D content of eggs and chicken meat. Journal of agricultural and food chemistry, 59(15), 8298-8303.
[19]. Acker, L., Ternes W.: Physikalisch-chemische Eigenschaftsveränderungen bei der Alterung von Hühnereiern. In Ternes, Acker und Scholtyssek: ‚Ei und Eiprodukte'. Paul Parey Verlag Berlin und Hamburg, ISBN 3-489-63114-5, 1994, 322-328
[20]. Vlaicu, P. A., Panaite, T. D., & Cornescu, G. M. (2021). Shelf life of eggs from hens fed diets rich in polyunsaturated fatty acids and antioxidants under the effect of different storage time and temperatures. Scientific Papers. Series D. Animal Science. Vol. LXIV, No. 1, 487-495.
[21]. Faitarone, A. B. G., Garcia, E. A., Roca, R. O., Andrade, E. N., Vercese, F., & Pelícia, K. (2016). Yolk color and lipid oxidation of the eggs of commercial white layers fed diets supplemented with vegetable oils. Brazilian Journal of Poultry Science, 18, 09-16.
[22]. Vlaicu, P.A., Panaite, T.D. Turcu, R.P. (2021). Enriching laying hens eggs by feeding diets with different fatty acid composition and antioxidants. Scientific Reports, DOI:10.1038/s41598-021-00343-1