Impact of Different Levels of Vitamin D3 in Laying Hens Diet on Various Aspects of the Eggs

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Abstract
An experiment was conducted to assess the impact of vitamin D3, on laying hens' performances, egg yolk fatty acid profile (FA), antioxidant compounds and vitamin transfer in the eggs. For that, one hundred twenty, 34 weeks old Lohmann Brown laying hens, were allocated to completely randomized block arrangement of three dietary treatments: T1, control (C) with 2500 IU/kg vitamin D3; T2 with 3000 IU/kg vitamin D3 and T3, with 3200 IU/kg vitamin D3. All diets had identical basal diet structure containing 17.50% crude protein and 2780 kcal/kg, metabolizable energy, being isocaloric and isonitrogenous. Feed intake and feed conversion ratio were significantly ($p<0.05$) lower in T2 and T3 groups compared to T1, without any impact on average egg weight. Egg production was significantly ($p<0.05$) higher only in T2 group, compared with T1 and T3. No significant ($p>0.05$) contribution of vitamin D3 supplementation was observed on total saturated fatty acids (SFA), unsaturated fatty acids (UFA) or monounsaturated fatty acids (MUFA) classes determined in egg yolk. From the sum of *cis* FA, significantly ($p<0.005$) higher was palmitoleic FA in T1 egg yolk compared with T2 and T3 egg yolks. On the other hand, from the total *trans* group, nervonic FA was significantly ($p<0.05$) higher in eggs belonging to higher vitamin D3 supplement groups. Total polyunsaturated fatty acids (PUFA), were significantly ($p<0.05$) higher in T2 and T3 egg yolks compared with T1 egg yolk. However, n-3 increase significantly only in T3 yolks being with 43.92% higher compared with T1 yolk and with 35.51% higher compared with T2 yolk. From the antioxidant compounds, total polyphenol content, total antioxidant capacity, lutein and zeaxanthin, vitamin A and vitamin E registered slightly higher values in T2 and T3 eggs versus T1 eggs, but without significant ($p>0.05$) effect. Vitamin D3 transfer in eggs increased significantly ($p<0.05$) in T3 compared with T1 and T2 eggs, as a main effect of supplemental vitamin D3 in laying hens' diets.

Keywords: antioxidants, cholecalciferol, egg quality, fatty acids, laying hens, vitamin D3.

1. Introduction

Vitamin D3, known as cholecalciferol or „*sunshine vitamin*", is the inactive form of vitamin D that can be ingested through dietary intake or be generated endogenously in the skin of animals and humans exposed to ultraviolet light [1]. In order to be used by the organism, vitamin D3 is converted into its active form, 1,25-dihydroxycholecalciferol (1,25-OH2-D3). This conversion follows a two steps hydroxylation process. First is mediated by the 25-hydroxylase enzyme, which occurs in the liver and hydroxylates cholecalciferol to form 25-hydroxycholecalciferol (25-OH-D). Second hydroxylation of 25-OH-D, is mediated by 1α-hydroxylase enzyme which occurs in the kidney to produce 1,25-dihydroxyvitamin D3 (1,25-OH2-D3), the active form of vitamin D [2,3]. Vitamin D3, the main product of the process, plays a fundamental role in biology, serving as a precursor for the hormone 1,25-OH2-D3 with its most fundamental role in the regulation of body calcium homeostasis [4,5]. Vitamin D deficiency is an unrecognized epidemic among many

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countries in the European states as well in some underdeveloped countries. Low intake of vitamin D has been associated with increased risks of different disease (cancers, cardiovascular disease, multiple sclerosis, rheumatoid arthritis, bone disease osteomalacia) [6]. These problems can be eliminated by consuming an adequate daily amount of vitamin D. There are few natural food products that contain notable amount of vitamin D. One of them is milk however it provides less than 50% of the current daily value. Second important natural food product rich in vitamin D are eggs, however, according to USDA, one egg of 50 g provides only 41 IU vitamin D, which is still not enough to provide adequate daily amount of vitamin D. Since eggs are versatile food or ingredient and are well accepted in most cultures, studies have shown that vitamin D concentration in eggs can be increased by feeding laying hens with diets supplemented with higher vitamin D concentrations, in order to transfer lipid soluble molecules from the diet to eggs [7]. It was proved that Vitamin D3 included at 6000 IU/kg feed to laying hen diets increased vitamin D3 concentration in their egg yolk [8]. Positive responses were also obtained when vitamin D3 and its metabolite 25-OH-D3 were used in laying hens' diets [9-11], but the inclusion level of vitamin D (4000, 8000, 12000, 16000, or 200000 IU of vitamin D3) was more beyond the EU limit recommendations (3000 IU/kg vitamin D3). However, vitamin D3 requirements of layers have not been re-evaluated in recent decades and for that reason vitamin D3 metabolites are starting to be used and great efforts are being made to observe their usefulness in the nutrition of layers, to enhance vitamin D3 transfer in eggs.

The objective of this study was to determine the impact of three different levels of vitamin D3 (2500, 3000 and 3200 IU vitamin D3/kg feed) in laying hens' diet to evaluate the laying hens' performances, egg yolk fatty acids quality, antioxidant compounds, trace elements and vitamins transfer into the eggs.

2. Materials and methods

Ethical consideration, birds' management, housing and experimental diets

Animal use and care were conducted according to the experimental protocol approved by our Ethical Committee according to Directive 2010/63/EU. The study was conducted in the experimental halls of the National Research & Development Institute of Animal Nutrition and Biology. For that, 126 Lohmann Brown laying hens at 32 weeks of age were randomly distributed in 32 pens, each one with 4 birds. Each cage (pen) was considered an experimental unit. The birds were housed in an experimental hall equipped with Big Dutchman cages dimensioned according to the sanitary-veterinary norms regarding the minimum standards for the protection of laying hens. The environmental conditions: average temperature/total period 19.19 ± 1.34°C; humidity 63.88 ± 2.14%, ventilation 6.36 ± 0.44% and CO_2 emission 1496, were controlled with a Viper Touch computer. The basic structure of diets was identically (corn, wheat, sunflower oil, soybean meal), for all groups except for premix structure where vitamin D3 concentration level was different. The treatments consisted of 3 different diets, as follows: T1, control with 2500 IU/kg vitamin D3; T2 with 3000 IU/kg vitamin D3 and T3, with 3200 IU/kg vitamin D3. The diets were characterized by 2780 kcal/kg metabolizable energy; 17.5% crude protein; 4.39% crude fiber. The feed compounds were formulated using specialized formulation HYBRIMIN Futter 5 software, to meet the nutritional requirements of Lohmann Brown laying hens. The all diets were isocaloric and isonitrogenous and fed *ad libitum* during the 8 experimental weeks. The ingredients and calculated nutrient analysis of the experimental diets is shown in Table 1. The primary chemical composition and the antioxidant compounds determined in the feed used for the laying hens are presented in Table 2.

Calculation of laying hens' production performance

Egg production and mortality were recorded daily. Eggs produced over 8 consecutive weeks were measured in grams and recorded daily. The given feed as well as unconsumed feed were weighed and discarded daily at same hour. Egg weight (g), was determined daily, by weighing each egg individually. Average daily feed intake (FI, g feed/hen/day) was calculated using formula (1); feed conversion ratio (FCR, kg of feed/kg of egg) was calculated using formula (2); egg production (%) was calculated using formula (3).

FI (g) = Amount of feed given (g) − Amount of feed unconsumed (1)

FCR = Feed intake / Egg production (2)

Egg production = Number of eggs per day×100 (3)

Table 1. Diet structure and calculated nutritional composition of the standard feed

Ingredients	% as feed basis	Calculated nutritional composition	
		Metabolizable energy, kcal/kg	2780
Corn, %	39.24	Crude protein, %	17.50
Wheat, %	20.00	Ether extractives, %	3.55
Soybean meal, %	26.59	Crude fiber, %	4.39
Vegetal sunflower oil, %	2.32	Calcium, %	3.90
DL–Methionine, %	0.17	Total Phosphorus, %	0.69
Choline, %	0.05	Available Phosphorus, %	0.38
Calcium carbonate, %	8.83	L-lysine-HCL, %	0.87
Monocalcium phosphate, %	1.40	DL-Methionine, %	0.40
Chlorine, %	0.40	Meth. + Cyst, %	0.70
Premix, %	1.00	Threonine, %	0.67
Total, %	100	Tryptophan, %	0.20

T1, T2 and T3 have identical basal diet structure. The premix contained: 1350000 IU/kg vitamin A; 2700 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; *T1-2500 IU/kg vitamin D3; **T2–3000 IU/kg vitamin D3; ***T3 - 3200 IU/kg vitamin D3

Table 2. Chemical composition of the compound feed

Items	T1	T2	T3
Nutrients			
Crude protein, %	19.05	19.08	19.09
Crude fat, %	3.52	3.89	3.75
Crude fiber, %	3.99	4.04	4.69
Antioxidant compounds			
Total polyphenol content, mg. equiv. gallic acid	2.31	1.86	2.25
Antioxidant capacity, mM equiv. Trolox	7.97	7.31	8.63
Trance minerals			
Calcium, %	3.48	3.42	3.47
Phosphorus, %	0.65	0.65	0.63
Copper, ppm	11.97	9.68	7.74
Iron, ppm	366.21	385.22	455.73
Manganese, ppm	130.11	156.53	161.33
Zinc, ppm	96.41	97.94	100.87

T1 (2500 IU/vitamin D3 kg feed), T2 (3000 IU/ vitamin D3 kg feed) T3 (3200 IU/ vitamin D3 kg feed).

Sampling. Samples of diets (about 500 g/treatment) were collected for proximate chemical composition determinations according to Association of Official Analytical Chemists, official methods of analysis standardized methods. After 8 experimental weeks, at the end of the experiment 18 eggs/group were randomly collected and from the 6 pooled yolks (3 eggs/sample) we determined the impact of supplemental vitamin D3 in laying hens' diets on eggs fatty acids, antioxidant compounds and vitamins transfer into the eggs.

Chemical analysis. The basic chemical composition analyses were determined on samples dried at 65°C. Crude protein by Kjeldahl method, according to standard SR EN ISO 5983-2:2009 (Kjeltec 2300 Analyzer Unit, FOSS Analytical, Den-mark). Crude fat was determined by extraction in organic solvents according to standard SR EN ISO 6492:2001 (Soxtec 2055 – Foss Tecator, Sweden). For crude fiber the method with intermediary filtration was used according to standard SR EN ISO 6865:2002 (Fibertec 2010 System – Foss Tecator, Sweden), as described elsewhere [12]. All assays were performed according to the Regulation (CE) nr. 152/2009.

Egg yolk fatty acids determination. The pooled yolk samples were dried at 65°C in order to determine the fatty acid profile, by using the fatty acid methyl ester (FAME) gas chromatography according to ISO/TS 17764–2 (2008). After the fatty acids were extracted from the total lipid were converted to their methyl esters by transesterification in methanol containing 3% concentrated sulfuric acid at 80°C for 4 h by using a Perkin Elmer-Clarus 500 chromatograph as described previously [13]. The average amount of each FA was used to calculate the sum of the total saturated (SFA), unsaturated (UFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids.

Determination of antioxidant compounds. The estimations of total phenolic content (Folin Ciocalteu method) and total antioxidant capacity (phosphomolybdenum method) of the samples were performed according to the methods described by [14]. Lutein and zeaxanthin concentration were determined using a high-performance liquid chromatograph Perkin Elmer 200 series, with a UV detector and a C18 reversed-phase column as presented by [15].

Vitamin's determination was performed according to the method described in EC Regulation no 152/2009, using a high-performance liquid chromatograph with PDA-UV detector and detection at 292 nm. Trace elements determination for calcium (Ca), phosphorus (P), iron (Fe), manganese (Mn) and zinc (Zn) methods, by flame atomic absorption spectrometry are presented elsewhere [16].

Statistical analysis. One-way analysis of variance (ANOVA), using Stat View for Windows (SAS, version 6.0), was carried out to determine the effects of supplemental vitamin D3 on laying hens' performances, fatty acid composition, antioxidant compounds and vitamins of eggs. Significance between individual means was identified using the Tukey's multiple range tests. Mean differences were considered significant at $p<0.05$. The graphical representation of vitamins transfer into the eggs was generated using Sigma Plot V.11 software (San Jose, CA, USA).

3. Results and discussion

Laying hen performance was compared among the dietary treatments (Table 3). The hens fed the diet supplemented with 2500 IU of vitamin D3 (T1) had significantly higher ($p= 0.0106$) FI compared to hens fed the diet supplemented with 3000 IU and 3200 IU of vitamin D3. Hens fed the diet T2 had significantly lower FCR than T1 and T3, but both T2 and T3 had significantly lower ($p=0.0001$) FCR than control (T1). However, there was no difference for egg weight ($p=0.1211$) between the groups. Egg production was significantly higher ($p=0.0001$) in T2 group, which had 3000IU vitamin D3/kg feed supplement, compared with T1 (2500 IU/kg feed) and T3 (3200 IU/kg feed). Recently, Wen [17] reported that hens fed diets with three different levels (500 IU, 1500 IU and 3000 IU) of vitamin D3/kg feed supplementation showed a significant increase in egg weight and a significant decrease of FI and FCR compared with the un-supplemented group. Akbari [18] reported that additional 3300 IU of vitamin D3/kg did not show any egg production improvement in aged Lohmann LSL layers (72- to 81-week-old). Other studies [19, 20] have reported no significant differences at all in laying performance among different level of vitamin D3 supplementation in laying hen's diet.

Table 3. Effect of vitamin D3 supplementation on laying hens' performances

Item	T1	T2	T3	SEM	P
Feed intake (FI), g/day/hen	117.19[a]	115.14[b]	115.78[b]	0.286	0.0106
Feed conversion ratio (FCR), kg feed/kg egg	1.95[a]	1.83[c]	1.90[b]	0.008	<0.0001
Egg weight, g	64.54	64.39	64.89	0.104	0.1211
Egg production, %	94.84[b]	97.70[a]	94.89[b]	0.271	<0.0001

[a,b] means marked with a different superscript letter within each column are significantly different
T1 (2500 IU/vitamin D3 kg feed), T2 (3000 IU/ vitamin D3 kg feed) T3 (3200 IU/ vitamin D3 kg feed).

Table 4 shows the effect of vitamin D3 on egg yolk fatty acids composition. We observed that the FA profile was not the same in all groups. The modifications were found in the total SFA, where palmitic acid was significantly (p=0.0239) higher in T1 yolks compared with T3, while stearic acid was significantly (p=0.0147) higher in T2 and T3 yolks compared with T1 egg yolks.

Table 4. Effect of vitamin D3 supplementation on egg yolk fatty acids

Items	T1	T2	T3	SEM	p
	g/100 g total FAME				
SFA	37.03	36.92	36.02	0.409	0.5872
Myristic C14:0	0.222	0.250	0.226	0.019	0.8439
Pentadecanoic C15:0	0.052	0.006	0.061	0.005	0.7895
Palmitic C16:0	25.92[a]	24.79[ab]	23.32[b]	0.427	0.0239
Heptadecanoic C17:0	0.156	0.156	0.155	0.003	0.9958
Stearic C18:0	10.67[b]	11.68[a]	12.25[a]	0.253	0.0147
UFA	62.95	62.96	63.82	0.406	0.6516
MUFA	36.61	35.19	35.93	0.348	0.2733
cis	35.96[a]	34.37[b]	35.30[a]	0.255	0.0101
Myristioleic C14:1	0.037	0.031	0.035	0.004	0.8938
Palmitoleic C16:1	2.931[a]	2.609[b]	2.305[c]	0.088	0.0014
Oleic C18:1	32.99	31.73	32.96	0.338	0.2322
trans	0.340[b]	0.471[a]	0.387[b]	0.010	0.0350
Erucic C22:1n9	0.092	0.111	0.073	0.007	0.0727
Nervonic C24:1n9	0.248[b]	0.360[a]	0.350[a]	0.020	0.0141
PUFA	26.34[b]	27.78[a]	27.89[a]	0.273	0.0144
n-6	25.09	26.39	25.75	0.214	0.0249
Pentadecenoic C15:1	0.153[ab]	0.228[a]	0.143[b]	0.016	0.0396
Heptadecenoic C17:1	0.172[a]	0.190[a]	0.060[b]	0.021	0.0051
Linoleic C18:2n6	19.52[a]	19.96[a]	18.49[b]	0.206	0.0005
Linolenic γ C18:3n6	0.135[a]	0.133[a]	0.123[b]	0.002	0.0056
Eicosadienoic C20:2n6	0.155[b]	0.168[b]	0.199[a]	0.007	0.0078
Eicosatrienoic C20:3n6	0.258[b]	0.318[ab]	0.375[a]	0.020	0.0380
Arachidonic C20:4n6	3.915[c]	4.425[b]	5.123[a]	0.170	0.0012
Docosatetraenoic C22:4n6	1.118[b]	1.403[a]	1.438[a]	0.058	0.0274
n-3	1.20[b]	1.38[b]	2.14[a]	0.123	<0.0001
Linolenic α C18:3n3	0.266	0.244	0.292	0.009	0.1067
Eicosatrienoic C20:3n3	0.225	0.263	0.274	0.017	0.0204
Docosapentaenoic C22:5n3	0.065[c]	0.082[b]	0.110[a]	0.006	0.0004
Docosahexaenoic C22:6n3	0.686[b]	0.790[b]	1.399[a]	0.098	<0.0001
Others	0.055	0.212	0.161	-	-
n-6/n-3	20.29[a]	19.36[a]	12.03[b]	1.172	<0.0001

[a,b] means marked with a different superscript letter within each column are significantly different. T1=2500 IU vitamin D3 kg feed; T2=3000 IU vitamin D3 kg feed; T3=3200 IU vitamin D3 kg feed.

No modifications were found in total UFA and MUFA. However, from the *cis* MUFA, palmitoleic acid was significantly (p=0.0014) higher in T1 compared with T2 and T3 yolks. On the other hand, from the *trans* MUFA, nervonic acid was significantly (p=0.0141) higher in T2 and T1 yolks versus T1. Total PUFAs were significantly higher (p=0.0144) in T2 and T3

compared with T1 egg yolk. However, no significant alterations for total n-6, while the total n-3 were significantly (p=0.0001) higher in T3 egg yolks compared with T1 and T2 yolks. This significant difference was given especially by the docosahexaenoic acid in T3, which was almost two times higher compared with the T1 and T2. Interestingly, are the modifications that we found in some FA, especially considering the fact that in the diet only vitamin D3 supplement was different the basal diet remaining identical. In other studies, not find any other papers reporting the effects of vitamin D supplementation on laying hens' egg yolk FA. We can only assume that because laying hens have high lipogenic activity, could be responsive to dietary vitamin D3 via altering nutrient partitioning in the intestine.

The effect of supplemental vitamin D3 in laying hens' diet on the antioxidant compounds is presented in Table 5. It was observed that the total polyphenols content, antioxidant capacity as well as lutein and zeaxanthin in eggs, were not significantly (p>0.05) impacted by the different dietary vitamin D3 levels added. However, zinc concentration, increased significantly (p=0.0001) in T2 and T3 eggs compared with T1, while iron and phosphorus concentration increased (p=0.0001) only in T3 eggs compared with T1 and T2 eggs. Copper concentration decreased (p=0.0001) in both T2 and T3 eggs compared with T1, while manganese concentration decreased (p=0.0017) only in T3 eggs compared with T1 and T2. Calcium concentration had close values among the groups, while phosphorus concentration increased significantly (p=0.0003) in T3 eggs. From our data, it was clear to observe vitamin D3 supplement had no effect on FA composition [10]. Contrary, Turgut [21] reported some significant alterations of egg yolk FA acids when laying hens were fed vitamin D combined with fat sources. This effect reported by Turgut [21] was expected knowing that manipulating laying hens feed by adding different sources of fat, modifies the egg yolk FA profile [22]. It has been reported that neither higher dose (12000 and 15000 IU/kg feed) of vitamin D did not affect the fatty acid composition [7, 8]. Currently we could a zinc, iron – copper, manganese antagonism. In groups T2 and T3 where zinc and iron concentration increased, copper and manganese decreased. In line with our results, it was reported that iron concentration in yolk was 159.2 mg/kg, when laying hens were fed only with trace mineral supplements [23]. Same authors reported that zinc concentration in egg yolk, ranged between 62.10–72.50 mg/kg, which is still lower compared with our values. Similar increase of phosphorus in eggs of hens supplemented with 3000 or 9000 IU vitamin D2/kg was also reported recently by Adhikari [24]. However, the increased bioavailability of zinc and iron in products is beneficial for human health [25].

From this data, it was clear that vitamin D3 significantly impacted the trance minerals determined in the eggs. These modifications, as previously reported [26] could be caused by the higher bioavailability of organic minerals which are probably related to the different absorption mechanism such as peptide or amino acid uptake in the intestine. However, it is unclear if this effect is due to the enrichment with vitamin D3, or due to the laying hen's metabolism.

Table 5. Effect of vitamin D3 supplementation on egg antioxidant compounds and trance minerals

Items	T1	T2	T3	SEM	P
		Antioxidant compounds			
Polyphenols, mg/g GAE	0.75	0.76	0.76	0.016	0.9375
Antioxidant capacity, mM AAE	1.17	1.23	1.35	0.034	0.0693
Lutein + Zeaxanthin, mg/kg	5.83	5.66	6.25	0.139	0.2171
		Trance minerals			
Zinc, mg/kg	71.03^{b}	73.40^{a}	74.29^{a}	0.399	0.0001
Iron, mg/kg	143.4^{b}	144.5^{b}	151.9^{a}	1.078	<0.0001
Calcium, mg/kg	3.62	3.69	3.69	0.002	0.2695
Phosphorus, mg/kg	1.14^{b}	1.12^{b}	1.20^{a}	0.011	0.0003
Copper, mg/kg	2.79^{a}	2.37^{b}	1.98^{c}	0.087	<0.0001
Manganese, mg/kg	1.57^{a}	1.50^{a}	0.83^{b}	0.108	0.0017

^{a,b} means marked with a different superscript letter within each column are significantly different

The impact of supplemental vitamin D3 on vitamins concentration deposited in the eggs is presented in Figure 1. Vitamin A (retinoic acid), was not significantly increased from T1 (6.40 mg/kg) to T2 (6.83 mg/kg) or T3 (7.40 mg/kg) even if the increment was with about 13.51% in T3 eggs. Retinoic acid plays an important role in the maintenance of integrity of epithelial tissue, being involved in several processes such as vision, reproduction, bone development, immune response, cell differentiation and proliferation.

Further, vitamin E increased from 79.35 mg/kg (T1) and 80.34 mg/kg (T2) to 88.52 mg/kg (T3), but this increment is not statistically sustained ($p>0.2020$). However, this increment is beneficial because tocopherols play a fundamental role in the normal functioning of the organism, because of its great antioxidant power against the degenerative action of free radicals and cellular oxidation prevention. Vitamin D3 (cholecalciferol) which controls principally, calcium and phosphorus metabolism, bone mineralization and egg formation, was significantly impacted ($p=0.0042$) in our study. In eggs from T1 (2500 IU/kg feed) the concentration of vitamin D3 in eggs was 75.18 mg/kg while in T2 (3000 IU/kg feed) the concentration was 77.16 mg/kg. The significant increase (98.74 mg/kg) was noted in T3 (3200 IU/kg feed) which was with 23.86% higher than T1 and with 21.86% higher than T2. Significant transfer of vitamin D3 into the eggs was also reported by Yao [10] during a 40 weeks experiment. However, they reported only 11 to 14% transfer from diets supplemented with 9700, 17200, 15000, or 24700 IU vitamin D3 to eggs compared with control (2200 IU vitamin D3/kg feed). Significant impact of vitamin D3 transfer into the eggs was reported by Duffy [27] when hens were fed with 3000 IU vitamin D3/kg compared with control. The transfer of vitamin D3 from the feed into egg yolk was very efficient when laying hens diets were supplemented with 11200 to 12000 IU vitamin D3/kg feed [7, 8]. However, vitamin D can be toxic if administered in high doses which exceed the maximum safe dietary level of 1000 mg/ kg (40000 IU). Nevertheless, as it was recently reported [28] there is a need to reduce the gap between population dietary vitamin D intakes and vitamin D dietary requirements. Previously, studies were conducted to explore the antagonism between vitamin A and vitamin D in rats, [29]. They concluded that there is a clear antagonism at the molecular level and can this also account for the reduced toxicity of vitamin D caused by high levels of vitamin A and vice versa.

This aspect was also speculated by other authors and stated that there is a potential antagonism mechanism at molecular level between the two fat-soluble vitamins (A and D) [30]. The interactions between the vitamin A and D can be expressed in various manners if different indicators are considered. In this study, vitamin A could be responsible for improved contents of lipid, while dietary vitamin D significantly increased content cholecalciferol in the eggs. Nevertheless, their antagonistic implications need further studies and attention, especially in poultry, as there are no studies reporting this effect to our knowledge.

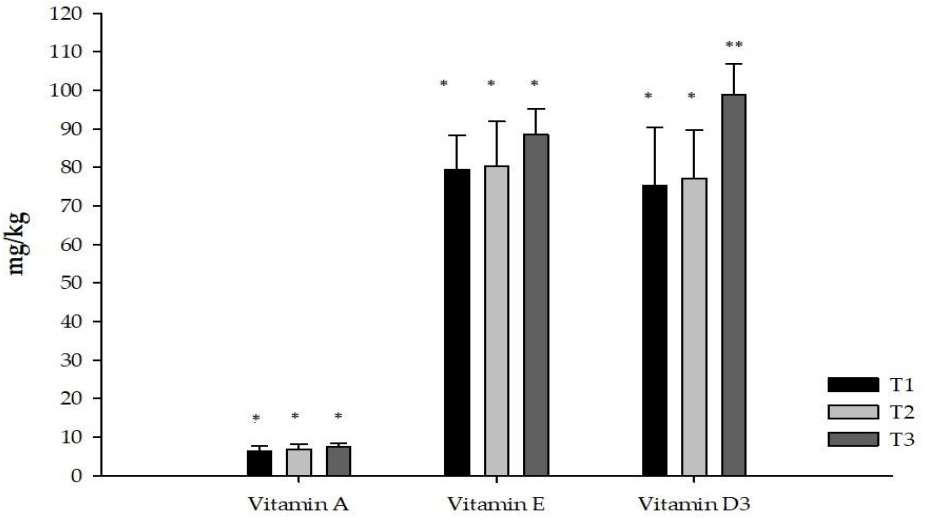

Figure 1. Impact of different dietary levels of vitamin D3 supplement in laying hens' diet on vitamins transfer into the eggs. * no difference; **significant difference

4. Conclusions

Feeding diets supplemented with vitamin D3 could result in an increase in vitamin D3 in eggs, as well as some trace minerals. Since eggs are such a versatile food or ingredient and are well accepted in most cultures, production of a high vitamin D3 egg may provide a unique opportunity to enhance human vitamin D consumption without altering food consumption. However, the lack of new amendments at European level to allow the use of higher doses of vitamin D3 in laying hen feed limits the production of large-scale vitamin D3-enriched eggs to cover daily needs for consumers.

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References

1. Zhang, R., and Naughton, D. P., Vitamin D in health and disease: current perspectives, Nutrition journal, 2010, 9(1), 1-13.
2. Norman, A. W., From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health, The American Journal of Clinical Nutrition, 2008, 88(2), 491S-499S.
3. Morris, A., Shanmugasundaram, R., Lilburn, M. S., and Selvaraj, R. K., 25-Hydroxycholecalciferol supplementation improves growth performance and decreases inflammation during an experimental lipopolysaccharide injection, Poultry science, 2014, 93(8), 1951-1956.
4. Holick, M. F., Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease, Am J Clin Nutr. 2004, 80(6 Suppl):1678S-88S.
5. Holick, M. F., Vitamin D: A millenium perspective, J Cell Biochem. 2003, 1, 88(2), 296-307.
6. Pilz, S. et al., Vitamin D, cardiovascular disease and mortality. Clinical endocrinology, 2011, 75(5), 575-584.
7. Mattila, P., Rokka, T., Könkö, K., Valaja, J., Rossow, L., Ryhänen, E. L., Effect of cholecalciferol-enriched hen feed on egg quality, Journal of agricultural and food chemistry, 2003, 51(1), 283-287.
8. Mattila, P., Valaja, J., Rossow, L., Venäläinen, E., and Tupasela, T., Effect of vitamin D2-and D3-enriched diets on egg vitamin D content, production, and bird condition during an entire production period, Poultry science, 2004, 83(3), 433-440.
9. Park, S. W., Namkung, H., Ahn, H. J., Paik, I. K., Enrichment of vitamins D3, K and iron in eggs of laying hens, Asian-australasian journal of animal sciences, 2005, 18(2), 226-229.
10. Yao, B. et al., The incidence of post-transplantation diabetes mellitus during follow-up in kidney transplant recipients and relationship to Fok1 vitamin D receptor polymorphism. In Transplantation proceedings, 2013, 45(1), 194-196.
11. Browning, L. C., and Cowieson, A. J., Vitamin D fortification of eggs for human health, Journal of the Science of Food and Agriculture, 2014, 94(7), 1389-1396.
12. Vlaicu, P. A., Panaite, T. D., Voicu, I., Turcu, R. P., Olteanu, M., and Ropota, M., Determining the feeding value of some food industry by-products, Scientific Papers: Animal Science and Biotechnologies, 2018, 51(1), 62-69.
13. Turcu, R. P., Olteanu, M., Criste, R. D., Panaite, T. D., Ropotă, M., Vlaicu, P. A., & Drăgotoiu, D., Grapeseed meal used as natural antioxidant in high fatty acid diets for Hubbard broilers, Brazilian Journal of Poultry Science, 2019, 21.
14. Untea, A. E., Varzaru, I., Panaite, T. D., Gavris, T., Lupu, A., & Ropota, M., The effects of dietary inclusion of bilberry and walnut leaves in laying hens' diets on the antioxidant properties of eggs, Animals, 2020, 10(2), 191.
15. Varzaru, I., Untea, A. E., Van, I., Determination of bioactive compounds with benefic potential on health in several medicinal plants, Romanian Biotechnological Letters, 2015, 20(5), 10773-10783.
16. Untea, A., Criste, R., Vladescu, L., Development and validation of a microwave digestion–FAAS procedure for Cu, Mn and Zn determination in liver, Revista de Chimie, 2012, 63(4), 341-346.
17. Wen, J., Livingston, K. A., & Persia, M. E., Effect of high concentrations of dietary vitamin D3 on pullet and laying hen performance, skeleton health, eggshell quality, and yolk vitamin D3 content when fed to W36 laying hens from day of hatch until 68 wk of age, Poultry science, 2019, 98(12), 6713-6720.
18. Akbari Moghaddam Kakhki, R., Heuthorst, T., Wornath-Vanhumbeck, A., Neijat, M., Kiarie, E., Medullary bone attributes in aged Lohmann LSL-lite layers fed different levels of calcium and top-dressed 25-hydroxy vitamin D3, Canadian Journal of Animal Science, 2018, 99(1), 138-149.
19. Persia, M. E., Higgins, M., Wang, T., Trample, D., Bobeck, E. A., Effects of long-term supplementation of

laying hens with high concentrations of cholecalciferol on performance and egg quality, Poultry Science, 2013, 92(11), 2930-2937.
20. Saunders-Blades, J. L., & Korver, D. R., Effect of hen age and maternal vitamin D source on performance, hatchability, bone mineral density, and progeny in vitro early innate immune function, Poultry science, 2015, 94(6), 1233-1246.
21. Turgut, L., Hayirl, A., Celebi, S., Yoruk, M. A., Gul, M., Karaoglu, M., & Macit, M., The effects of vitamin D supplementation to peak-producing hens fed diets differing in fat source and level on laying performance, metabolic profile, and egg quality, Asian-australasian journal of animal sciences, 2006, 19(8), 1179-1189.
22. Vlaicu, P. A., Panaite, T. D., Turcu, R. P., Enriching laying hens eggs by feeding diets with different fatty acid composition and antioxidants, Scientific Reports, 2021, 11(1), 1-12.
23. Skřivan, M., Skřivanová, 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, Poultry Science, 2005, 84(10), 1570-1575.
24. Adhikari, R., White, D., House, J. D., Kim, W. K., 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, Poultry science, 2020, 99(1), 364-373.
25. Vlaicu, P. A., Untea, A. E., Turcu, R. P., Saracila, M., Panaite, T. D., Cornescu, G. M., Nutritional composition and bioactive compounds of basil, thyme and sage plant additives and their functionality on broiler thigh meat quality. Foods, 2022, 11(8), 1105.
26. Schiavone, A., Barroeta, A. C., (). Egg enrichment with vitamins and trace minerals. In Improving the safety and quality of eggs and egg products. Woodhead Publishing, 2011, pp. 289-320
27. Duffy, S. K. et al., The potential of cholecalciferol and 25-hydroxyvitamin D3 enriched diets in laying hens, to improve egg vitamin D content and antioxidant availability, Innovative Food Science & Emerging Technologies, 2017, 44, 109-116.
28. Clark, A. et al., Egg enrichment with vitamin D: The Sunshine Eggs projects, Nutrition Bulletin, 2021, 46(3), 332-338.
29. Rohde, C. M., Manatt, M., Clagett-Dame, M., & DeLuca, H. F., Vitamin A antagonizes the action of vitamin D in rats, The Journal of nutrition, 1999, 129(12), 2246-2250.
30. Zhang, W., Mai, K., Xu, W., Tan, B., Ai, Q., Liufu, Z., Wang, X., Interaction between vitamins A and D on growth and metabolic responses of abalone *Haliotis discus* hannai, Ino., Journal of Shellfish Research, 2007, 26(1), 51-58.