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Effects of vitamin D addition for laying hens in the final third of production on egg quality

Efeitos da adição de vitamina D para poedeiras no terço final da produção sobre a qualidade dos ovos

Efectos de la adición con vitamina D para gallinas ponedoras en el último tercio de producción sobre la calidad del huevo

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Abstract
This study determined whether supplementing the diet of laying hens in the final third of laying with vitamin D improves bird health and egg performance and quality. The hens were separated into four groups with six repetitions each and five animals per repetition to test three doses of vitamin D supplementation (50, 100, and 150 mg/kg). The vitamin D supplementation had no effect on the hens performance; however, when we individually evaluated each repetition, we found that the birds of T100 and T150 showed significant increases (P<0.001) in the percentage of the laying rate. Regarding egg quality, the T150 group presented a better result of shell resistance, and the T150 I had a better range of colors and color "a". The yolk percentage was higher in the supplemented groups than the control (T0). On day 21, serum cholesterol levels were lower in supplemented birds groups than in control. At 42 days, the highest vitamin D supplementation for T150 birds resulted in a higher serum albumin concentration, whereas birds in the T100 group had a higher protein concentration. The supplementation had positive and timely effects on the birds' metabolism, reflected in the higher laying rate and the better egg quality.
Keywords: Laying hens; Vitamin D; Poultry; Eggs.

Resumo
Este estudo determinou se a suplementação da dieta de poedeiras no terço final da postura com vitamina D melhora a saúde das aves e o desempenho e a qualidade dos ovos. As galinhas foram separadas em quatro grupos com seis repetições cada e cinco animais por repetição para testar três doses de suplementos de vitamina D (50, 100 e 150 mg/kg). A suplementação de vitamina D não teve efeito sobre o desempenho das galinhas; no entanto, quando

avaliamos cada repetição individualmente, verificamos que as aves do T100 e T150 apresentaram aumentos significativos (P<0,001) na porcentagem de taxa de postura. Em relação à qualidade dos ovos, o grupo T150 apresentou melhor resultado de resistência da casca, e o grupo T150 apresentou melhor variação de cores e cor "a". A porcentagem de gema foi maior nos grupos suplementados do que no controle (T0). No dia 21, os níveis séricos de colesterol foram menores nos grupos de aves suplementados do que no controle. Aos 42 dias, a maior suplementação de vitamina D para as aves T150 resultou em maior concentração de albumina sérica, enquanto as aves do grupo T100 apresentaram maior concentração de proteína. A suplementação teve efeitos positivos e oportunos sobre o metabolismo das aves, refletindo em maior taxa de postura e melhor qualidade dos ovos.
Palavras-chave: Galinhas poedeiras; Vitamina D; Aves; Ovos.

Resumen
Este estudio determinó si complementar la dieta de las gallinas ponedoras en el tercio final de la puesta con vitamina D mejora la salud de las aves y el rendimiento y la calidad de los huevos. Las gallinas se separaron en cuatro grupos con seis repeticiones cada uno y cinco animales por repetición para probar tres dosis de suplementos de vitamina D (50, 100 y 150 mg/kg). La suplementación con vitamina D no tuvo ningún efecto sobre el rendimiento de las gallinas; sin embargo, cuando evaluamos individualmente cada repetición, encontramos que las aves de T100 y T150 mostraron incrementos significativos (P<0.001) en el porcentaje de la tasa de puesta. En cuanto a la calidad del huevo, el grupo T150 presentó un mejor resultado de resistencia de la cáscara, y el T150 tuve una mejor gama de colores y color "a". El porcentaje de yema fue mayor en los grupos suplementados que en el control (T0). El día 21, los niveles de colesterol sérico fueron más bajos en los grupos de aves suplementados que en el control. A los 42 días, la mayor suplementación de vitamina D para las aves T150 resultó en una mayor concentración de albúmina sérica, mientras que las aves del grupo T100 tuvieron una mayor concentración de proteínas. La suplementación tuvo efectos positivos y oportunos sobre el metabolismo de las aves, reflejados en una mayor tasa de puesta y una mejor calidad de los huevos.
Palabras clave: Gallinas ponedoras; Vitamina D; Aves de corral; Huevos.

1. Introduction

Egg production in Brazil carries substantial economic importance, generating income for producers and allied enterprises and providing several jobs directly and indirectly. To achieve success in the commercial egg production sector, it is necessary to achieve excellent zootechnical indexes of egg performance and quality. The age of laying birds is a determining factor for performance and internal and external egg quality (Faria et al., 1999). Egg quality is a matter of concern not only for commercial farms but also for merchants and consumers; this is because, in addition to the economic aspects associated with loss of the product, defects in quality can mean risks to public health (Alleoni & Antunes, 2001).

With advancing hen age, the eggs become larger, entailing distribution of a constant amount of shell around a larger egg surface, resulting in thinner shells, resulting in egg breakage and diminished quality (Trindade et al., 2007). Calcium is the primary element in bones and shells. During the pre-laying period, calcium is absorbed in the intestine by the action of the hormone calcitriol, a hormonal form of vitamin D3 (Vieites et al., 2017). Because this hormone is derived from vitamin D, changes in the bird's calcium metabolism as it prepares to enter the production cycle are primarily mediated by vitamin D and its metabolism (Salvador et al., 2009).

Light is necessary for endogenous production of vitamin D. Birds kept in low light, as in production systems in closed sheds, must be supplemented with vitamin D (Hester et al., 2011). The hormone 1,25 (OH) 2D3 plays an essential role in the metabolism of calcium and phosphorus, facilitating the absorption of these minerals and bone mobilization and mineralization (Garcia et al., 2013).

Therefore, vitamin D supplements in the chicken diet are justified because metabolic disorders are more common during the final third of production. This is the period when egg size increases, requiring more calcium. We hypothesized that calcium supplementation would directly affect the birds' metabolism, increasing serum calcium and consequently improving the quality of eggshells and indirectly improving health and performance. The objective of this study was to determine whether the addition of vitamin D to the diet of laying hens in the final third of production would improve bird health, productive efficiency, and egg quality.

2. Methodology

This research had an exploratory and quantitative nature (Pereira et al., 2018). The project was approved by the Ethics Committee for Animal Well-Being at the State University of Santa Catarina-UDESC, under protocol nº 6427070619.

2.1 Vitamin D

The vitamin D used in this study was a commercial product of plant origin, containing the bioactive form of 1,25-dihydroxyvitamin D3-glycoside (PANBONIS 10PPM®, Technofeed, SP, Brazil).

2.2 Experiment location and period

The experiment was carried out in the poultry sector of the University of the State of Santa Catarina - UDESC Oeste, in the municipality of Chapecó, state of Santa Catarina, Brazil. Before the beginning of the experiment, as the laying rate was evaluated for 5 days, with a rate of 69.5 ± 3.46% being identified. The experiment lasted 42 days, divided into two productive cycles of 21 days each.

2.3 Experimental design

According to the nutritional requirements of laying hens (Table 1), the diet used was formulated based on corn and soybean meal, described in the Brazilian tables for poultry and swine (Rostagno et al., 2011). This basal diet was formulated using a premix that contained vitamin D among the ingredients due to the demands of laying hens (2500 IU/kg of feed). Therefore, the supplementary purpose of vitamin D to the basal diet was to provide a greater contribution of vitamin D to the birds in the final third of the production cycle.

Table 1: Ingredients and chemical composition of the basic diet offered to chickens.

Ingredients	%
Corn	65.70
Soybean meal. 45 %	21.80
Calcitic limestone	8.90
Soybean oil	1.10
Dicalcium phosphate	1.50
DL-Methonine 98%	0.20
NaCl (table salt)	0.50
Premix*	0.30
TOTAL	**100.00**
Values calculated according to the proximate composition (Rostagno, 2011)	
Metabolizable Energy (Kcal/Kg)	2.848
Crude protein (%)	15.67
Calcium (%)	3.87
Available phosphorus (%)	0.37
Digestible Lysine (%)	0.68
Digestible methionine (%)	0.42
Digestible methionine + cystine (%)	0.65
Sodium	0.23

* Product composition (kg): vit. At 7,000,000 IU; vit. D3 4,000,000 IU; vit. E 5000 mg; vit. K 1200 mg; vit. B1 360 mg; vit. B2 2000 mg; vit. B6 700 mg; vit. B12 7000mcg; niacin 7500 mg; biotin 30 mg; pantothenic acid 6000 mg; folic acid 300 mg; iron 1 1000 mg; copper 3000 mg; iodine 204 mg; chlorine 360 mg; to promote growth and efficacy, Feed 20 mg; coccidiostatic 100 g; antifungal 2000 mg; antioxidant 10 mg; magnesium 50 g; sulfur 40 g; energy and protein vehicle (q. s. p.) 1,000 g. Source: Authors.

We used 120 laying hens of commercial lineage (Rhode Island Red), housed in collective cages, receiving feed and water ad libitum. The birds were separated in a completely randomized design of four treatments with six replicates each, containing five chickens per repetition. We performed an analysis before the laying rate experiment to allocate the birds, aiming to apply treatment with the same laying rate on day 1 of the experiment. Vitamin D supplementation via the basal diet occurred at three levels (50, 100, and 150 mg/kg), with the groups being identified as the control group (T0); birds supplemented with 50 mg/kg (T50); birds supplemented with 100 mg/kg (T100), and birds supplemented with 150 mg/kg (T150).

2.4 Zootechnical performance

Performance in the production phase was evaluated at the end of two 21-day production cycles, in which the following were considered: percentage of egg production, average egg weight, egg mass, feed consumption, and feed conversion (kg of feed/kg of egg produced and kg of feed/dozen of egg produced). The percentage of eggs was obtained by counting and collecting daily eggs from each experimental unit. An analytical balance of precision ± 0.01g (model SHIMADZU BL-3200H) was used for the average egg weight. The egg mass was calculated in the final three days of each production cycle by individually weighing all eggs in each experimental plot (cage), and then the egg mass was calculated using the equation: egg mass = average weight of the eggs (g) x production of the day (%). The feed was stored in buckets, one per repetition of each treatment, with weekly consumption measurement.

2.5 Sample collection

At the end of each productive cycle, eggs were collected (two per repetition) for analysis. The eggs were analyzed on the same day of laying and were considered fresh.

Blood samples were collected on days 1, 21, and 42 of the experimental period. The birds were manually restrained (n = 6 per group), the ulnar vein was accessed using a syringe (3-ml) and needle (25/7). The collected blood was placed in an Eppendorf microtube and subsequently centrifuged to separate the serum and maintained frozen (–20 °C) until analysis.

2.6 Egg analysis

Physicochemical and egg quality analyses were performed on eggs collected on the 21st and 42nd day of the experiment. We measured the shell resistance (kgf) using a texturometer (Model TA. XT plus, Extralab, Brazil). The pH of albumen and yolk was measured using a digital pH meter (Modelo testo 205, Testo, Brazil). Egg specific gravity was measured as per (Freitas et al., 2004). To obtain the percentages of yolk, shell, and albumen, the yolks were separated from the albumen, weighed separately, and the shell was washed to completely remove the albumen and placed to dry at room temperature until weighing. Haugh's unit were calculated using the equation: UH = 100log (average albumen height + 7.57 – 1.7 x egg weight in grams x 0.37) (Haugh, 1937). The yolk index was calculated using the equation: yolk index = yolk height ÷ yolk width. The yolk color was determined using a DSM colorimetric fan and a colorimeter (Model CR400, Konica Minolta Sensing Americas, Inc, USA) that assesses the luminosity (L*), red intensity (a*), and yellow intensity (b*).

2.7 Serum biochemistry

Serum levels of total proteins, albumin, calcium, and cholesterol were measured using semi-automatic BioPlus equipment (Bio-2000) and specific commercial kits. Serum globulin levels were calculated as the difference between levels of total proteins and albumin.

2.8 Statistical analysis

We evaluated the two together for zootechnical performance and egg quality (days 21 and 42); differences in the blood variables were analyzed concerning collection days (days 0, 21, and 42). All data were subjected to normality testing, and when they did not show normal distribution, they were transformed into logarithms. Normally distributed data were compared using one-way analysis of variance followed by precision analyses using the Tukey test. We used the statistical package in R, v.2.15.1 (R Development Core Team 2012). Significant differences between treatments were considered when P ≤ 0.05.

3. Results

3.1 Performance

The performance results of the hens are shown in Table 2 with the averages of the two production cycles. The laying percentage, egg weight, egg mass, feed conversion did not differ between treatments, suggesting that vitamin D supplementation had no impact on performance. Regarding feed consumption, groups T50 and T100 had lower intake than control. However, knowing the laying rate for each repetition before the experiment, we calculated the increase laying rate for all treatments: T0: 1.03%; T50: 1.39%; T100: 4.52%; T150: 10.1%. Statistically, the treatments T100 and T150 were larger than the others (P < 0.001); the birds in the T150 treatment had the greatest increase in the laying rate.

Table 2. Zootechnical performance of chickens supplemented with vitamin D in the diet.

Treatment	PL (%)	FI (g)	EW (g)	EF (g)	FC: kg/dz	FC: kg/kg
T0	70.25	128 A	67.05	47.04	2.18	2.72
T50	70.47	121 B	70.15	49.24	2.06	2.45
T100	72.63	121 B	66.18	48.04	2.00	2.52
T150	76.36	125 AB	66.50	50.88	1.96	2.46
P	0.285	<0.001	0.704	0.805	0.506	0.180
CV (%)	7.28	2.35	8.75	13.21	12.04	8.23

PL = percentage of laying; FC = feed Intake (g/bird/day EW = egg weight (g); EF = egg flour (g/bird/day); FC – feed conversion. Note: Mean with P ≤ 0.05 were different, illustrated with different letters in the same column. Source: Authors.

3.2 Egg quality

The results of egg quality are shown in Table 3. Specific gravity, yolk index, albumen height, Haugh unit, colors (L and “b”), pH (albumen and yolk) did not differ between treatments (P > 0.05). Regarding shell resistance, supplementation with 150 mg/kg of vitamin D (T150) gave greater resistance than the control group (T0). For the range of colors, the eggs in the T150 group had higher indices than the other groups. The yolk percentage was higher in the supplemented groups (T50, T100, and T150) than T0. The intensity of the color “a” was lower in the eggs of the T150 group than the T0.

3.3 Blood variables

Table 4 shows the results of blood variables related to metabolism. On day 21, only serum cholesterol levels were lower in groups supplemented with vitamin D, T0 compared to T0. At 42 days, total protein levels were higher in the T100 group than T0. There were higher levels of albumin in the group supplemented with 150 mg/kg of vitamin D (T150) than the other groups. Globulin levels were higher in groups T50 and T100 than T0.

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Table 3. Chemical-physical composition of chicken eggs supplemented with vitamin D in the diet.

	T0 (control)	T50 (50 mg/kg)	T100 (100 mg/kg)	T150 (150 mg/kg)	P	CV
Specific gravity	1.07	1.08	1.08	1.07	0.963	2.75
Shell resistance	3517 B	3826 AB	3823 AB	4085 A	0.038	8.74
Yolk index	0.45	0.43	0.38	0.45	0.116	14.6
Albumen height	8.76	7.85	7.47	8.04	0.092	9.78
HU²	90.2	81.9	86.0	87.6	0.071	6.78
Fan	5.37 B	5.35 B	4.95 B	6.10 A	0.014	8.24
Yolk %	24.5 B	27.1 A	27.9 A	27.7 A	<0.001	2.97
L color	59.0	58.0	54.7	60.4	0.087	7.63
a color	-5.53 B	-5.70 AB	-5.86 AB	-6.31 A	0.026	3.44
b color	43.2	42.1	41.9	46.5	0.357	3.07
Albumen pH	8.01	8.07	8.05	8.04	0.951	1.55
Yolk pH	5.92	5.88	5.85	5.86	0.948	1.86

Note: Note: Mean with P ≤ 0.05 were different, illustrated with different letters in the same line. Source: Authors.

Table 4. Serum biochemistry of laying hens supplemented with vitamin D in the diet.

	Calcium (mg/dL)	Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Cholesterol (mg/dL)
Day 0					
T0	46.3	5.00	1.42	3.58	40.6
T50	50.6	5.32	1.85	3.47	45.4
T100	50.4	4.78	1.73	3.05	43.1
T150	48.9	4.90	1.70	3.20	38.0
P	0.92	0.83	0.62	0.58	0.42
CV (%)	5.97	3.89	5.10	6.74	10.7
Day 21					
T0	50.6	6.54	1.80	4.74	59.0 A
T50	43.0	5.52	1.71	3.81	46.4 AB
T100	47.5	6.50	1.83	4.67	41.2 B
T150	46.5	5.63	1.96	3.67	46.7 AB
P	0.54	0.39	0.74	0.57	<0.001
CV (%)	6.74	4.49	3.57	5.41	9.01
Day 42					
T0	46.5	4.20 B	1.53 B	2.67 B	51.8
T50	42.3	5.11 AB	1.49 B	3.62 A	48.2
T100	43.5	5.23 A	1.23 B	4.00 A	46.2
T150	40.6	4.86 AB	2.31 A	2.55 AB	48.4
P	0.21	0.04	< 0.001	< 0.001	0.86
CV (%)	3.74	2.98	3.24	4.29	8.24

Note: Mean with P ≤ 0.05 were different, illustrated with different letters in the same column. Source: Authors.

4. Discussion

Vitamin D supplementation in chickens in the final third of laying improves shell resistance and yolk percentage, two indications of improved egg quality. However, adding more vitamin D to the chickens' diet does not increase serum calcium levels in the blood, nor does it improve the productive efficiency of the birds. Our main finding was the increase in globulins at the serum level, a vital protein fraction in building an effective immune response. A limitation to our conclusions was that we could not measure levels of vitamin D on day 0. Doing so would allow us to determine whether the birds had normal vitamin D levels or were deficient.

Laying hens from the control treatment had higher feed intake. According to Ribeiro et al. (2013), performance characteristics can be impaired in the absence or deficiency of vitamin D in laying hens; however, when chickens receive recommended levels of vitamin D in the diet, performance characteristics are not influenced, which we believe was due to the high standard deviation in the laying rate of birds on day 0; that is, the laying rate ranged from 66.04% to 72.96%. The higher feed consumption of the T0 birds consequently increased the consumption of vitamin D; however, when we separately evaluated each repetition and calculated the percentage increase in the laying rate, we found that the egg production increased significantly in all cages/repetitions with birds supplemented with 100 or 150 mg/kg of vitamin D.

We observed greater shell resistance in the group with greater inclusion of vitamin D (T150). Soares et al. (1995) reported that the metabolite 25 (OH) D3 was 2.5 to 4.5 times more active than cholecalciferol; therefore, it is of great value in preventing bone problems and improving eggshell thickness. In addition, vitamin D is essential for maintaining egg production, shell formation, and calcium homeostasis. Carvalho and Fernandes (2013) also concluded that the mechanism of egg shell formation is a dynamic process that depends on several factors and that the understanding and correct handling of these are critical.

Regarding the range of colors, the highest values of yellow were found in the T150 group, different from the red color "a," which was lower in the eggs of the T150 group. According to Biscaro et al., (2006), yolk pigmentation results from the deposition of carotenoid pigments, with corn as the primary source of these pigments in the laying hens. Synthetic carotenoids such as canthaxanthin can also be used. Yolk color is the internal characteristic most often observed by the consumer, despite being a subjective measure that varies from light yellow to reddish-orange (Harder et al., 2007). We did not expect changes in yolk color, just as we do not know how to explain the mechanisms involved; nevertheless, it is worth mentioning that the result was positive when we consider characteristics desired by consumers.

The yolk percentage was higher in the groups with vitamin D supplementation than the control group, suggesting an effect on the lipid metabolism of this supplementation, which also reduced serum cholesterol levels. Salvador et al. (2009) studied laying hens and concluded that the association of vitamin D in the form of 25 (OH) D3 with 200 ppm of vitamin C increases the percentage of egg yolk. They also observed an improvement in the feed conversion. The authors do not explain which mechanisms are related to this positive increase in the yolk percentage; however, we believe it is an indirect effect that deserves to be elucidated in future studies by our research group.

The higher concentration of total protein in the blood of birds supplemented with 100 mg/kg of vitamin D is a consequence of higher serum levels of globulins in birds from the T50 and T100 groups. According to Schmidt et al. (2007), the increase in plasma proteins before the laying period is induced by estrogens. In layers, higher serum proteins (physiological hyperproteinemia) can be explained by the protein requirement for egg formation, 12.5% protein on average. According to the literature, in addition to the classic effects on calcium and bone homeostasis, the vitamin D receptor is expressed in immune cells (B cells, T cells, and antigen-presenting cells), and these immune cells synthesize the active metabolite of vitamin D. Therefore, vitamin D acts in an autocrine manner, modulating innate and adaptive immune responses (Aranow, 2011). Immunoglobulins are globulins produced by B cells that may have been stimulated by supplementation with vitamin D, which could explain the increase in globulins observed in our study after 42 days of ingestion. However, it is notable that this behavior was observed for T50 and T100 and not for T150; in T150, we observed the greatest increase in the laying rate.

5. Conclusion

Supplementation of 150 mg/kg of vitamin D did alter serum calcium levels; however, it increased shell resistance and yolk percentage. This dose did not affect serum protein metabolism; however, 100 mg/kg stimulated the synthesis of globulins, consequently increasing total serum protein levels. There was an effect of supplementation on the laying rate, as the productive efficiency of the T100 and T150 hens increased by approximately 3.5 and 9%, respectively, when compared to the T0. We conclude that vitamin D supplementation in hens in the final third of laying has potential and can be a viable option, especially to produce better shell resistance.

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Ethics committee

The project was approved by the Ethics Committee on the Use of Animals of the University of the State of Santa Catarina, under protocol number: 6427070619.

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