

1,25-Dihydroxyvitamin D₃-glycoside of herbal origin in diets for laying Japanese quails

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ABSTRACT

The aim of the study was to evaluate supplementation with 1,25-dihydroxyvitamin-D₃-glycoside (1,25(OH)₂D₃) of plant origin in diets for laying Japanese quails from 36 to 45 weeks of age. It was used a randomized block design, with five treatments: 0.0; 0.25; 0.50; 0.75 and 1.00 µg of 1,25(OH)₂D₃ kg⁻¹ diet; with six replicates and six birds per experimental unit. There were no significant effects (p>0.05) of 1,25(OH)₂D₃ on the productive traits: feed intake, egg production per day and per housed bird, commercial egg production, average egg weight, egg mass, feed conversion per dozen eggs and egg mass. The addition of 0.75 µg of 1,25(OH)₂D₃ kg⁻¹ feed provided higher shell thickness and percentage ash in the eggshells (p<0.05). The weights and respective relative percentages of heart, liver, gizzard, pancreas, intestinal length and mineral excretion were not affected by the vitamin. It is concluded that the supplement 1,25(OH)₂D₃ kg⁻¹ of plant origin in diets for Japanese quail does not affect productivity, excreta and biometrics of organs of the digestive system; however, improves the quality of the eggs.

Key words: biomolecule, *Coturnix coturnix japonica*, eggs, *Solanum glaucophyllum*

1,25-Dihidroxivitamina D₃-glicosídeo de origem herbal nas rações para codornas japonesas em postura

RESUMO

Objetivou-se avaliar a suplementação com 1,25-dihidroxivitamina D₃-glicosídeo (1,25(OH)₂D₃) de origem vegetal nas rações para codornas japonesas em postura de 36 a 45 semanas de idade. Foi utilizado delineamento em blocos casualizados, com cinco tratamentos: 0,0; 0,25; 0,50; 0,75 e 1,00 µg de 1,25(OH)₂D₃ kg⁻¹ de ração; com seis repetições e seis aves por unidade experimental. Não houve efeito significativo (p>0,05) do 1,25(OH)₂D₃ sobre as características produtivas: consumo de ração, produção de ovos por ave dia e por ave alojada, ovos comercializáveis, peso médio do ovo, massa de ovos, conversão alimentar por dúzia e por massa de ovos. A adição de 0,75 µg de 1,25(OH)₂D₃ kg⁻¹ de ração proporcionou maior espessura e percentual de cinzas nas cascas dos ovos (p<0,05). Os pesos e os respectivos percentuais relativos do coração, fígado, moela e pâncreas, o comprimento intestinal e a excreção mineral não foram influenciados pela vitamina. Conclui-se que o suplemento de 1,25(OH)₂D₃ de origem vegetal nas rações para codornas japonesas não afeta a produtividade, as excretas e a biometria de órgãos do aparelho digestório, todavia, melhora a qualidade dos ovos.

Palavras-chave: biomolécula, *Coturnix coturnix japonica*, ovos, *Solanum glaucophyllum*

INTRODUCTION

The exploitation of quails in Brazil has grown up in the last years. The headcount of quails had 15.47 million of heads in 2017, an increment of 12.0% in the herd, in relation to the value registered in 2016. In addition, egg production was 4.6% higher and had 278 million of dozens eggs (IBGE, 2018).

Among quail species, the *Coturnix coturnix japonica* with use in egg production is the more outstanding. Its commercial use has begun by countless factors, such as the small space requirement, rapid cycle of production, early sexual maturity,

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high production efficiency, high resistance to disease, low initial investment to production and quick capital return (Rosa et al., 2011).

Studies on the nutrition of Japanese quails focus in specific themes, such as protein and energy requirements (Costa et al., 2010; Silva et al., 2018). However, it is necessary to investigate other diet constituents, such as minerals and vitamins with the purpose of obtaining balanced food programs, better productive results, as well as reduce costs with food.

Nowadays, metabolites formed with vitamin D₃ (25-hydroxycholecalciferol – 25(OH)D₃; 1,25-dihydroxycholecalciferol – 1,25(OH)₂D₃) are commercially available for use in animal feeding. The purpose of their use refers to the vitamin availability in a more active form, in order to reduce energy costs with vitamin metabolization and have the consequent increase in the organism efficiency (Garcia et al., 2013; Souza and Vieites, 2014).

Vitamin D₃ has various physiological roles in the metabolism of calcium (Ca) and phosphorus (P), including maintenance of the concentration of these minerals in blood, stimulation of intestinal absorption, renal reabsorption and incorporation of these minerals in the bone matrix. In production birds, this vitamin is essential to maintain egg production, shell development, and Ca homeostasis (Sepúlveda and Rosales, 2014; Świątkiewicz et al., 2017).

Innumerable positive results were observed for broilers (Guerra et al., 2014; Souza et al., 2013; Świątkiewicz et al., 2017). However, there is still a lack of information about the effects of 1,25(OH)₂D₃ on laying birds, especially Japanese quails, thus justifying the accomplishment of the present study.

The purpose of this study was to evaluate the zootechnical performance, egg quality, biometrics of organs from the digestive system and excreta of laying Japanese quails feed with diets containing 1,25-dihydroxyvitamin D₃-glycoside of plant origin.

MATERIAL AND METHODS

The experiment was conducted in Viçosa, Minas Gerais State, Brazil, with the duration of 63 days (three periods of 21 days). The Project was registered in the Ethics Committee for Use of Production Animal – CEUAP, n° 82/2013. The experiment had 180 quails (*Coturnix coturnix japonica*), from 36 to 45 weeks of age.

Experimental delineation constituted of randomized blocks (DBC), with five treatments (0.0; 0.25; 0.50; 0.75 and 1.00 µg of 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) kg⁻¹ diet); with six repetitions of six birds per experimental unit. Bird weight (light and heavy weight) was used as the criterion for forming blocks. Animal density per experimental unit was 141.66 cm² per bird.

Rations was formulated according to the recommendations of Rostagno et al. (2011). The commercial product was used as source of active vitamin D₃ of plant origin – *Solanum glaucophyllum* (10 ppm of 1,25(OH)₂D₃ kg⁻¹), and it was included in diets in substitution to the inert material (sand) (Table 1).

Temperature (minimum and maximum) in the barn were registered once per day at 4:00 PM, and air relative humidity was registered twice per day at 8:00 AM and 04:00 PM by using a digital thermo-hygrometer placed in the center of barn at bird height. Provision of light had 17 hours daily and was controlled by an automatic timer, which allowed turn on and off the lights during the night period, in accordance with the procedure adopted in commercial farms.

The average weight of quails was 210 ± 0,016 grams (36 weeks of age) in the beginning of the experiment, and it was 215 ± 0,014 grams (45 weeks of age) at the end of the experiment. Water and rations were ad libitum during the whole experimental period. Surplus food and waste food were weighed and discounted from the quantity of the initially weighed feed that was provided in the different periods. At the end of each period of 21 days, it was carried out the division of the amount of feed consumed by the number of birds in each treatment and by the number of days, and it is expressed in grams of consumed feed bird⁻¹ day⁻¹. In case of poultry mortality during the experimental period, average consumption was corrected.

The average of egg production was obtained by collecting daily the number of produced eggs, including broking eggs, cracked eggs and un-typical eggs (soft-shelled eggs and eggs not in shell), and it is expressed in percentage divided by the average of birds from the period (egg bird⁻¹ day⁻¹) and divided by the average of housed birds in the beginning of the experiment (egg housed bird⁻¹). The relation of intact eggs produced was expressed in percentage for each treatment, which corresponds to the commercial egg production.

All intact eggs produced in each repetition were weighed during the last three days in each period (19th, 20th and 21st day), in order to obtain the average weight that was multiplied by the total number of eggs produced in the experimental period, thus obtaining total egg mass. This mass was divided by the total number of birds per day, and it is expressed in grams of egg day⁻¹. Feed conversion per dozen eggs was determined by the total consumption of feed in kilograms divided by the dozen eggs produced (kg dozen⁻¹), and the conversion per egg mass was determined by the total consumption of feed (kg) divided by the egg mass in kilograms (kg kg⁻¹).

In the 16th and 17th day in each period, all intact eggs collected were submitted to the analysis of specific gravity, with immersion and evaluation in saline solutions of NaCl, with density varying from 1.055 to 1.100 g cm⁻³, with intervals of 0.005 g cm⁻³ among them. Density was measured by a densimeter (Incoterm-OM-5565®).

The yolk weight, albumen, and eggshell were evaluated with four random eggs from each repetition separated in the last two consecutive days (20th and 21st day) of each period. Eggs were individually weighed in precision scale of ± 0.001 g. After weighing eggs, the eggs were broken, and the yolk weight of each egg was registered. The respective shell was washed and air dried, and, posteriorly, weighed. The albumen weight was obtained between the difference in the egg weight and yolk weight summed by the shell weight. Percentage values of egg components were also calculated. The measure of shell thickness (obtained from component analysis) was accomplished using a digital pachymeter (Mitutoyo® 0-150mm, precision 0.001 mm). Shells were measured in three different points of the equatorial area for obtention of the thickness average. Determination of shell ashes followed the methodology described in Detmann et al. (2012).

In the end of the experimental period of 63 days, one bird per repetition was weighed, sacrificed by bleeding and organs of the digestive system (heart, liver, gizzard, pancreas and intestine) were removed and immediately weighed. Intestine length was measured with the assistance of meter rule, quantifying from the beginning of duodenum to the cloaca.

In the digestibility assay, four repetitions of each treatment were chosen randomly, and birds were housed in cages having two-floors. On the floor of cages, it was put a tray of galvanized metal sheets, feeding bowl was positioned in the frontal part of the cage, and a drinker was set in the back of the cage. Birds were submitted to a period of

Table 1. Composition of experimental diets on natural matter.

Ingredients (kg)	Treatments (μg of 1,25(OH) ₂ D ₃ kg ⁻¹ diets)				
	0.00	0.25	0.50	0.75	1.00
Corn	56.990	56.990	56.990	56.990	56.990
Soybean meal	32.553	32.553	32.553	32.553	32.553
Soybean oil	1.274	1.274	1.274	1.274	1.274
Limestone	6.802	6.802	6.802	6.802	6.802
Dicalcium phosphate	1.071	1.071	1.071	1.071	1.071
Salt	0.323	0.323	0.323	0.323	0.323
Lysine-HCl	0.193	0.193	0.193	0.193	0.193
DL-methionine	0.372	0.372	0.372	0.372	0.372
L-tryptophan	0.013	0.013	0.013	0.013	0.013
L-arginine	0.049	0.049	0.049	0.049	0.049
Vitamin premix ¹	0.100	0.100	0.100	0.100	0.100
Mineral premix ²	0.050	0.050	0.050	0.050	0.050
Choline chloride	0.100	0.100	0.100	0.100	0.100
Antioxidant ³	0.010	0.010	0.010	0.010	0.010
Source of 1,25-dihydroxyvitamin-D ₃ ⁴	0.000	0.0025	0.005	0.0075	0.010
Inert material ⁵	0.100	0.098	0.095	0.093	0.090
Total (kg)	100.00	100.00	100.00	100.00	100.00
Calculus of compositions					
Crude protein (%)	19.70	19.70	19.70	19.70	19.70
Metabolizable energy (kcal kg ⁻¹)	2,800	2,800	2,800	2,800	2,800
Calcium	2.922	2.922	2.922	2.922	2.922
Available phosphorus (%)	0.304	0.304	0.304	0.304	0.304
Sodium (%)	0.146	0.146	0.146	0.146	0.146
Digestible lysine (%)	1.097	1.097	1.097	1.097	1.097
Digestible Methionine+ Cystine (%)	0.900	0.900	0.900	0.900	0.900
Digestible Threonine (%)	0.665	0.665	0.665	0.665	0.665
Digestible Tryptophan (%)	0.230	0.230	0.230	0.230	0.230
Digestible Arginine (%)	1.273	1.273	1.273	1.273	1.273
Digestible Valine (%)	0.829	0.829	0.829	0.829	0.829
Digestible Isoleucine (%)	0.762	0.762	0.762	0.762	0.762
Digestible Glycine+ Serine (%)	1.634	1.619	1.619	1.619	1.634
Digestible Leucine (%)	1.537	1.537	1.537	1.537	1.537
Linoleic acid (%)	2.048	2.048	2.048	2.048	2.048
Crude fiber (%)	2.711	2.711	2.711	2.711	2.711

¹Composition kg⁻¹ of product: Vit. A: 12.000.000 U.I., Vit. D₃: 3.600.000 U.I., Vit. E: 3.500 U.I., Nicotinic Acid: 40.000 mg, Pantothenic acid: 12.000 mg, Vit. B₁₂: 20.000 mg, Vit. B₂: 8.000 mg, Vit. B₆: 5.000 mg, Vit. K: 3.000 mg, Vit. B₅: 2.500 mg, Folic acid: 1.500mg, Biotin: 200 mg, Vehicle q.s.p.: 1.000 g. ²Composition kg⁻¹ of product: Manganese: 160 g, Iron: 100 g, Zinc: 100 g, Copper: 20 g, Cobalt: 2 g, Iodine: 2 g, Selenium: 150 mg, Vehicle q.s.p.: 1000 g. ³Butil-hydroxy-toluene, 99%. ⁴Commercial product, source of active vitamin D₃ of plant origin – *Solanum glaucophyllum*, containing 10 ppm of 1,25(OH)₂D₃ kg⁻¹ product. ⁵Sand.

environmental adaptation of two days; afterwards, excreta were collected twice per day for three consecutive days and stored in a freezer.

The sample collected was unfrozen, homogenized, and weighed; aliquots were put in an oven of forced cooling for 72 hours at 55 °C in order to pre-drying. Subsequently, samples were ground in a mill of type pulverizer (Fritsch Pulverisett 14[®], mesh sieve of 0.5 mm, speed of 6,000 rpm) for posterior analysis. When determining dry matter, the samples previously dried were weighed and were put in an oven at 105 °C for 16 hours. Ash quantification followed the methodology of Detmann et al. (2012). Based in the laboratory results, it was calculated the percentages of dry matter and ashes.

Data obtained were submitted to variance and regression analysis at 5% of probability. The statistical model used was as follows: $Y_{ij} = \mu + b_j + n_i + \varepsilon_{ij}$, where: Y_{ij} is value observed in the experimental unit of the j^{th} block which received the i^{th} treatment; μ = general average; b_j = effect of block j ; 1; 2; n_i = effect of 1,25(OH)₂D₃ supplementation i ; $i = 0.0$; 0.25; 0.50; 0.75 and 1.00 μg of 1,25(OH)₂D₃; ε_{ij} = random error associated with each observation. The choice of Student-Newman-Kewls test (SNK) followed the recommendation of Sampaio (2010), considering the coefficient of variation and the number of averages to be compared. Analysis were performed in the software SISVAR, version 5.6 (Build 86), which does statistical analysis and plan experiments (Ferreira, 2014).

RESULTS AND DISCUSSION

Averages of minimum and maximum temperatures and air relative humidity in the morning and afternoon, that was registered in the interior of the barn, were 16.0 \pm 1.90 °C, 26.4 \pm 1.73 °C, 64.6 \pm 7.87% and 81.9 \pm 3.58%, respectively. The range of thermal comfort of adult quails is between 18 and 28°C and relative humidity between 65 and 70% (Oide, 2013; Guimarães et al., 2014). Thus, it can be inferred that birds were in thermal comfort during the experimental period.

There were no effects ($P > 0.05$) of 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) on productive traits: feed consumption, egg production per bird per day and per housed bird, commercial eggs, average egg weight, egg mass, feed conversion per dozen eggs and per egg mass, and viability of birds (Table 2). Possibly, birds were able to metabolize enough 1,25(OH)₂D₃ from dietetic cholecalciferol, and/or use additional quantity of this metabolite in the maintenance of productive performance. Similarly, Frost and Roland (1990) evaluated the supplementation of 1 α -hydroxycholecalciferol (1 α (OH)D₃) and 1,25(OH)₂D₃ (0; 0.75; 1.50; 3.50; and 4.50 μg kg⁻¹) in feed for laying hens (HyLine W36), with 53 weeks of age, and also did not observe difference in productive variables and in the quality of the egg shell ($P > 0.05$).

Nascimento et al. (2014) evaluated the effect on the performance and quality of eggs of the Hy-Line W36 hens with 80 weeks of age with the use of different sources of Vitamin D (cholecalciferol, 25(OH)₂D₃ and 1,25(OH)₂D₃), by supplying 2000 UI of vitamin D₃. Authors concluded that the use of D₃ and 25(OH)₂D₃ improved the performance and quality of eggs. They also explained the different results obtained with the use of 1,25(OH)₂D₃, which can be attributed to short half-life of this metabolite (from 4 to 6 hours) in the organism, when compared with 25(OH)₂D₃ (from 2 to 3 weeks). Thus, the use of 1,25(OH)₂D₃ can be affected by the lack of body supply.

It is important to point out in this study that 1,25(OH)₂D₃ had herbal origin, from *Solanum glaucophyllum* (SG), with pharmacokinetic properties different from the synthetic form of metabolites studied by Frost and Roland (1990) and Nascimento et al. (2014), such as absorption and slower release in the organism (Bachmann et al., 2013). Such pattern of release can be attributed to the fact that 1,25(OH)₂D₃ is cleaved by glycosidases or passes by modifications in the Ring-A, thus requiring bacterial populations with ubiquitous enzymes, which can be presented in different quantities and in intestine segments. Then, there will be the release of this metabolite in the organism only after the occurrence of these processes (Zimmerman et al., 2015).

Cumulative mortality had 5.42% (0.60% per week), and viability was not influenced by supplementation of active vitamin D₃ in diets. Soares (2013) reported as normal the mortality of 0.30% per week or 12.0% accumulated for quails from six to 48 weeks of age, in lots with no health problems, well-managed, and adequate nutrition.

In terms of egg quality, there was 1,25(OH)₂D₃ influence in the egg weight, in albumen (g and %), in shell thickness and ash percentage of shell (Table 3). The eggs with higher weight and albumen quantity were from birds fed with 0.25 µg of 1,25(OH)₂D₃ kg⁻¹ diets.

In this study, the addition of 0.75 µg of 1,25(OH)₂D₃ in rations was favorable to egg quality, characterized by a higher

thickness (p<0.05) and ash percentage of shells (p<0.05). With the increase of age, laying birds diminish progressively the liver ability to transform vitamin D₃ in 25(OH)₂D₃. Reduction in the hydroxylation of vitamin D₃ by the liver or kidney results in inadequate production of 1,25(OH)₂D₃, which acts in the absorption of calcium and phosphorus for bone and shell development (Carvalho and Fernandes, 2013). Possibly, the birds evaluated showed a higher activity of the enzyme 1α-hydroxylase in kidney and plasma concentrations of 1,25(OH)₂D₃, and a higher activity of calbindin in duodenum and uterus, since laying hens that produce eggs with normal shells show higher activity of this enzyme and constituents (Salvador et al., 2009).

Fuller et al. (2005) evaluated calcium (2.5; 3.0 and 3.5%) and 1,25(OH)₂D₃ (0.0; 5.0 and 10 g of dry leaves of *Solanum glaucophyllum* – SG kg⁻¹ diet) in the second production cycle (from 70 to 90 weeks of age) of the laying hen Leghorn White. The supply of SG did not affect egg production; however, it increased shell resistance and specific gravity of eggs.

Specific gravity of eggs was not influenced by the additional use of 1,25(OH)₂D₃. Similarly, Safamehr et al. (2013) studied different sources and levels of calcium and cholecalciferol (3,000 and 5,000 UI kg⁻¹) in the diet of the laying hen Hy-Line W36. They verified an improvement in shell thickness of eggs by means of an increase in the quantity of vitamin D₃, but with no effects on the bird performance and specific gravity of eggs. Overall, the higher value of specific gravity is related to a thicker eggshell, which is a desirable trait by industries processors of eggs.

The supply of vitamin D₃ in higher levels than the level recommended to birds promote the delay in growth, bristling hairs, poluris, dehydration, movement discoordination, and weakness in legs (Souza and Vieites, 2014). It was not observed any toxicity symptoms in Japanese quails fed with the evaluated quantities of the metabolite 1,25(OH)₂D₃.

Weight and respective relative percentages of heart, liver, gizzard, pancreas and intestinal length of Japanese

Table 2. Performance of laying Japanese quails feed with diets containing 1,25-dihydroxyvitamin-D₃-glycoside of plant origin.

Variable	µg of 1,25-dihydroxyvitamin-D ₃ kg ⁻¹ diet					CV %	P-value
	0.00	0.25	0.50	0.75	1.00		
FI ¹ (g bird ⁻¹ day ⁻¹) ^{ns}	28.61	29.25	28.80	28.67	28.50	2.92	0.5819
EP ² per day (%) ^{ns}	84.28	89.98	88.46	85.97	83.73	7.55	0.4193
EP ² per housed bird (%) ^{ns}	84.17	89.95	88.36	85.94	83.64	7.69	0.4313
Commercializable EP ² (%) ^{ns}	98.82	99.35	99.00	99.07	99.35	0.77	0.7108
Egg weight (g) ^{ns}	12.37	12.55	12.22	12.40	12.35	3.07	0.6783
EM ³ (g egg bird ⁻¹ day ⁻¹) ^{ns}	10.42	11.26	10.80	10.65	10.33	6.73	0.2155
FC ⁴ per MO ³ (kg kg ⁻¹) ^{ns}	2.74	2.58	2.63	2.68	2.71	5.43	0.3228
FC ⁴ per dozen eggs (kg feed dozen ⁻¹) ^{ns}	0.407	0.388	0.386	0.399	0.401	6.26	0.5352
Viability (%) ^{ns}	91.67	95.83	93.75	97.92	93.75	0.09	0.9311

¹FI = feed intake; ²EP = egg production; ³EM = egg mass; ⁴FC = feed conversion. CV = coefficient of variation; ns = non-significant values (P>0,05).

Table 3. Egg quality of laying Japanese quails feed with diets containing 1,25-dihydroxyvitamin-D₃-glycoside of plant origin.

Variable	µg of 1,25-dihydroxyvitamin-D ₃ kg ⁻¹ diet					CV %	P-value
	0.00	0.25	0.50	0.75	1.00		
Egg weight (g)*	12.54ab	12.73a	12.29b	12.58ab	12.46ab	2.01	0.0703
Albumen weight (g)*	7.64b	7.95a	7.59b	7.78ab	7.66b	2.44	0.0186
Shell weight (g) ^{ns}	1.00	1.03	0.98	1.04	1.00	3.51	0.0591
Yolk weight (g) ^{ns}	3.83	3.82	3.74	3.77	3.81	3.68	0.7730
Albumen (%) [*]	61.00b	62.20a	61.70ab	61.82ab	61.50ab	1.32	0.0402
Yolk (%) ^{ns}	31.00	29.80	30.35	29.95	30.50	2.83	0.2207
Egg Shell (%) ^{ns}	8.00	8.00	7.95	8.23	8.00	3.08	0.3284
ST ¹ (mm)*	0.1767ab	0.1717ab	0.1683b	0.1833a	0.1750ab	4.69	0.0464
SG ² (g cm ⁻³) ^{ns}	1.075	1.074	1.074	1.075	1.073	0.13	0.3516
DM ³ shells (%) ^{ns}	97.64	97.67	97.70	97.72	97.59	0.14	0.4906
Shell ashes (%) [*]	90.26b	90.44ab	90.42ab	91.14a	90.93ab	0.55	0.0240

¹ST = Shell thickness; ²SG = specific gravity; ³DM = dry matter; CV = Coefficient of variation; ns = non-significant values (P>0,05). *Averages followed by the same letter in the line do not differ between them by Student-Newman-Keuls test (SNK), at 5% of probability.

Table 4. Biometrics of organs from the digestive system of laying Japanese quails feed with diets containing 1,25-dihydroxyvitamin-D₃-glycoside of plant origin.

Variable	µg of 1,25-dihydroxyvitamin-D ₃ kg ⁻¹ diet					CV %	P-value
	0.00	0.25	0.50	0.75	1.00		
Live weight (g) ^{ns}	221.66	212.91	212.63	221.74	215.39	5.87	0.5601
ILI ¹ (cm) ^{ns}	57.15	57.75	56.42	57.82	58.25	9.00	0.9767
Heart (g) ^{ns}	2.04	1.88	1.95	2.00	1.83	14.21	0.6799
Liver (g) ^{ns}	5.36	5.02	5.76	5.88	5.71	15.83	0.4473
Gizzard (g) ^{ns}	3.34	3.46	3.57	3.64	3.48	12.67	0.8072
Pancreas (g) ^{ns}	0.58	0.58	0.59	0.64	0.57	30.36	0.9716
Heart (%) ^{ns}	0.92	0.89	0.93	0.90	0.85	13.90	0.7803
Liver (%) ^{ns}	2.42	2.35	2.68	2.66	2.67	14.93	0.1201
Gizzard (%) ^{ns}	1.51	1.64	1.67	1.65	1.62	13.85	0.7437
Pancreas (%) ^{ns}	0.26	0.27	0.27	0.29	0.27	30.29	0.9887

¹IL = Intestinal length; CV = Coefficient of variation; ns = non-significant values (P>0.05).

Table 5. Contents of ashes and dry matter (DM) in the excreta of laying Japanese quails feed with diets containing 1,25-dihydroxyvitamin-D₃-glycoside of plant origin.

Variable	µg of 1,25-dihydroxyvitamin D ₃ kg ⁻¹ diet					CV %	P-value
	0.00	0.25	0.50	0.75	1.00		
Ashes (%) ^{ns}	24.59	24.65	25.92	24.93	24.80	7.69	0.8598
DM (%)*	28.27ab	28.69a	26.58b	28.53a	27.22ab	3.34	0.0237

CV = Coefficient of variation; ns = non-significant (P>0.05). *Averages followed by the same letter in the line do not differ between them by Student-Newman-Keuls test (SNK) at 5% of probability.

quails were not influenced by the active vitamin D₃ (Table 4). Biometric results of organs from the digestive system of quails showed conformity with the results verified in the zootechnical performance, indicating that there was not a physiological involvement of birds. According to Artoni et al. (2014), it is relevant to consider morphological alterations in the digestive system, once they show a deep effect in productive performance in function of influence in utilization of all nutrients.

Weight and the relative percentage of liver did not show alterations with the use of 1,25(OH)₂D₃. Such results become evident, since the differentiation of the studied metabolite refers to the fact that it does not need to pass through the liver; then, it avoids to overload this organ and the energy expenditure with metabolism of vitamin, which permits the increase in the organism efficiency. Kapica and Puzio (2004) reported that liver from broilers with active vitamin D₃ and phytase showed a lower relative weight, when compared to the relative weight from birds fed with standard diet (control).

In the evaluation of excreta, mineral material (ashes) showed no alterations in supplementation until 1.0 µg of 1,25(OH)₂D₃ kg⁻¹ diet (Table 5). Vieites et al. (2015) studied the additional supplying of *Solanum malacoxylon* – SM (0.0; 2.5 and 5.0 g of SM kg⁻¹ diet) for broilers until 21 days of age. Similarly, the authors verified that inclusion of SM did not affect (p>0.05) contents of calcium, magnesium, potassium and sodium in the excreta. However, only the content of phosphorus was altered and the higher value for excreta was verified in 5.0 g of SM kg⁻¹ diet, this is possibly a result that derives from the attempt of birds in maintaining homeostasis.

Excretion of dry matter was higher in the quails group, which received 0.25 µg (28.69%) and 0.75 µg (28.53%) of 1,25(OH)₂D₃ kg⁻¹ diet, respectively. Values found for excreta variables are in conformity with the values reported for Japanese quails from 45 to 57 weeks of age by Costa et al. (2010).

CONCLUSIONS

1. The supply of 1,25-dihydroxyvitamin-D₃-glycoside of plant origin in diets for laying Japanese quails from 36 to 45

weeks of age does not affect productivity, the excreta, and the biometrics of organs from the digestive system.

2. There is an improvement in egg quality, characterized by higher thickness and ash percentage of shell with the addition of 0.75 µg of 1,25(OH)₂D₃ of plant origin in the diets.

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