



Productive performance of broiler breeder hens fed 25-hydroxycholecalciferol

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ABSTRACT - An experiment was carried out with the objective of evaluating the addition of 25-hydroxycholecalciferol (25(OH) D₃) in diets of broiler breeder hens. The experiment used Cobb 500 broiler breeder hens and was allotted to a complete randomized design with four treatments and eight replications of twenty females and two males each. The treatments consisted of vitamin premixes with 2,000 and 3,400 IU/kg diet vitamin D₃ as the only source of vitamin or 2,000 IU D₃ plus 35 or 69 mg/t of 25(OH) D₃. Results of this experiment indicated that 25(OH) D₃ had no significant effect on egg production parameters from 32 to 67 weeks. The supplementation of 25(OH) D₃ resulted in better quality egg shells evaluated by the specific gravity at 60 weeks of age, regardless of the dosage. No significant differences were observed for hatchability of broiler breeder fertile eggs at 54 and 64 weeks. At 64 weeks, the hatch residue breakout showed less embryo mortality at the third week for treatments receiving 2,000 UI D₃ in the diet and less embryo mortality at the second week of development from hens aged 67 weeks and supplemented with 2,000IU D₃ and 2,000IU D₃+ 69 mg 25(OH)D₃. It was concluded that the supplementation with 25-hydroxycholecalciferol with cholecalciferol had similar effects as the diets with vitamin D₃ as the only source on the productive performance of broiler breeder hens.

Key Words: 25-hydroxycholecalciferol, breeder hens, hatchability, productive performance, vitamin D

Desempenho produtivo de reprodutoras de frangos de corte sob suplementação com 25-hidroxicolecalciferol

RESUMO - Foi realizado um experimento com o objetivo de avaliar a suplementação de 25-hidroxicolecalciferol (25(OH)D₃) associado à vitamina D₃ em dietas para reprodutoras de frangos de corte. Foram alojadas matrizes Cobb 500 em delineamento completamente casualizado, composto de quatro tratamentos e oito repetições de 20 fêmeas e 2 machos. Os tratamentos experimentais foram constituídos de premixes vitamínicos contendo 2.000 e 3.400 UI/kg ração de vitamina D₃ como única fonte ou 2.000 UI D₃ associado a 35 ou 69 mg/t de 25(OH)D₃. Não houve efeito da suplementação de 25(OH)D₃ sobre a produção de ovos durante o período de 32 a 67 semanas. A qualidade da casca dos ovos, mensurada por meio da gravidade específica, melhorou com a suplementação de 25(OH)D₃ em reprodutoras com 60 semanas de idade, independentemente da dose. Não foi observada diferença significativa na taxa de eclosão de ovos férteis incubados nas 54 e 64 semanas de idade das aves. Às 64 semanas, houve menor mortalidade de embriões na terceira semana entre as aves recebendo a dieta com 2.000 UI D₃, enquanto, às 67 semanas, houve menor mortalidade na primeira semana de embriões provenientes de reprodutoras sob suplementação com 2.000 UI D₃ e 2.000 UI D₃ + 69 mg 25(OH)D₃. A combinação de 25-hidroxicolecalciferol e colecalciferol teve efeito similar ao das dietas com vitamina D₃ como fonte exclusiva sobre o desempenho produtivo de reprodutoras pesadas.

Palavras-chave: 25-hidroxicolecalciferol, eclodibilidade, matrizes pesadas, produção de ovos, vitamina D

Introduction

Alterations in the skeleton of domestic broilers have frequently been observed with meat birds selected for fast growth, which can harm animal welfare and live performance development. Bird skeletal development starts at day one of

incubation with the use of minerals from the eggshell by the embryo (Tuan et al., 1986), a process directly dependant on the presence of nutrients such as calcium, phosphorus and vitamin D in the egg (Bethke et al., 1936; Narbaitz et al., 1980). Therefore, broiler breeder nutrition, besides supporting hen requirements, should supply minerals for the embryo.

Alternative sources of vitamin D₃, such as 1.25(OH)₂D₃ and 25(OH)D₃, may benefit broiler skeletal development as well as eggshell quality since this vitamin is directly involved in Ca and P homeostasis (Norman, 1986; 1987). The form 1.25(OH)₂D₃ has activity tenfold greater than vitamin D₃ itself. However, its supplementation as the only source of vitamin D has demonstrated a reduced transference into eggs (Hart et al., 1986; Narbaitz & Fragiskos, 1984; Narbaitz et al., 1987; Hart et al., 1986). The form 25(OH)D₃ has activity twofold greater than vitamin D₃ and presents favorable characteristics in terms of absorption by the intestinal cells when compared to cholecalciferol (Bar et al., 1980; Teergarden et al., 1997; Teergarden et al., 2000). The metabolite 25(OH)D₃ also has higher rates of transference into eggs, which lead to reduced incidences of progeny skeletal disorders and better immunity and hatchability parameters (Edwards Jr., 1990; Aslam, 1998). Therefore, this form of vitamin D should have an important concomitant effect for breeders as well as for the resulting embryo.

This study was carried out with the objective of evaluating live performance responses related to egg production and hatchability parameters using broiler breeder hens fed diets supplemented with 25-hydroxicolecalciferol and cholecalciferol.

Material and Methods

Six hundred and forty female Cobb 500 and 64 Cobb male broiler breeders were placed in a completely randomized design with 4 treatments and 8 replications of 20 females and 2 males per pen (2.5×2.0 m). An extra amount of 20 males were raised separately to replace sexually inactive or dead males. Each pen had six nests, a female tubular feeder and one chain male feeder, and nipple drinkers. The treatments consisted of experimental feeds with 2,000 or 3,400 UI/kg as only sources of vitamin D₃ or 2,000 UI D₃ associated with 35 or 69 mg/Ton of 25(OH) D₃. The feeds were formulated with corn, soybean meal and wheat bran as macro ingredients (Table 1). The experiment lasted while the broiler breeders were from 32 to 67 weeks of age and the lighting and feeds were supplied following Cobb Breeders recommendations (Cobb, 2005) without drinking water restriction. The environmental temperature was maintained within the animal comfort zone using foggers and tunnel ventilation.

Weekly egg production, hatching eggs, and eggs with defects were obtained through daily collections and were expressed in percentage. Defective eggs were considered those with physical deformities and those bearing cracks.

Double yolk and floor eggs were added up to total production, but were not incubated. Egg specific gravity was evaluated at 35, 40, 50, 56, 60 and 67 weeks of age using eggs accumulated from two consecutive days after individual weighing. Saline solutions with densities from 1,050 to 1,095 with graded increases of 0.005 g/mL were used.

Hatchability was evaluated at early, mid and late egg production. Therefore, eggs were collected at 57, 64 and 67 weeks of age. Embryo mortality was also observed during these periods. Eggs were incubated in a single stage incubator using 90 eggs per replication. Temperature was controlled according to McQuoid (2000). At the end of incubation, eggs that did not hatch were broken to perform embryo diagnosis with classification of eggs as infertile or dead embryos. A visual estimation of the age at death was carefully performed and embryo mortality was separated as early, mid or late dead (1 to 7 days; 8 to 14 days; 15 to 21 days). The percentage of hatching chicks considered improper for placement as well as pips were

Table 1 - Basal feed composition supplied to broiler breeders from 32 to 67 weeks of age

Ingredient	%
Corn	57.60
Soybean meal	20.70
Wheat bran	9.900
Soybean fat	2.002
Oyster shells	3.500
Limestone	2.854
Dicalcium phosphate	1.790
Salt	0.142
Sodium bicarbonate 99%	0.342
Potassium carbonate 98%	0.122
DL-methionine 99%	0.370
L-threonine 98%	0.560
Choline chloride 60%	0.096
Premix ¹	0.488
Nutrients and energy, %	
AMEn, kcal/kg	2,800
Crude protein	15.88
Total lysine	0.69
Total sulfur aminoacids	0.60
Total threonine	0.56
Calcium	2.90
Availabel Phosphorus	0.45
Sodium	0.17
Chlorine	0.15
Potassium	0.77
Dietary electrolyte balance, mEq/kg	230
Linoleic acid	2.50
Choline mg/kg	1,500

AMEn: metabolizable energy corrected for nitrogen retention

¹ Bacitracin methylen disalicylate (55 g/t); chlortetracycline (180 g/t); ethoxiquin (100 g/t); calcium and sodium aluminosilicate (3 kg/t).

calculated. The difference between total eggs set and infertile eggs allowed the calculation of the percent hatchability of fertile eggs.

All obtained responses were analyzed following the GLM procedure of SAS 8.2 (2001). Embryo diagnosis data, which were expressed in percentage, were submitted to arc sin transformation and the variables that presented statistical differences by the F test were further submitted to Tukey test ($P < 0.05$).

Results and Discussion

Results for hen day egg production (total, hatching, and eggs with defects) did not differ statistically between treatments ($P > 0.6564$; $P > 0.6363$ and $P > 0.1525$, respectively), which indicated that there was no influence of the sources of vitamin D on these parameters. As demonstrated in Table 2, there were no differences in total eggs produced per hen from 32 to 67 weeks of age.

When evaluated on a weekly basis, an increase in egg weight ($P < 0.05$) was observed in parallel with a reduction in specific gravity ($P < 0.05$) (Table 3). There were no significant effects of the supplementation vitamin D ($P > 0.05$) source on these responses on the overall period. Exceptions to this trend were observed at 35 and 40 weeks of age when interactions occurred, but also at 60 weeks when single effects were observed. At 35 weeks, birds fed 3,400 IU D₃ produced eggs with higher specific gravity compared to those fed 2,000 IU D₃ or 2,000 D₃ + 69 mg 25(OH)D₃. At 40 weeks, birds fed 2,000 + 35 mg 25(OH)D₃ produced eggs with higher specific

Table 2 - Total eggs and total hatching eggs¹ produced per hen from 32 to 67 weeks of age

Item	Total	Hatching
2,000 D ₃	162.1	158.7
3,400 D ₃	163.7	160.2
2,000 D ₃ + 35 mg 25 OH D ₃	167.4	163.6
2,000 D ₃ + 69 mg 25 OH D ₃	163.5	159.0
PROB	0.6302	0.6672
Mean	164.2	160.6
CV, %	5.12	5.11

¹ Devoid of cracks and shell failures.

gravity in comparison to those fed 2,000 D₃ + 69 mg 25(OH)D₃. At 60 weeks, birds fed 25-(OH)D₃ produced eggs with higher specific gravity.

Supplementation with both sources of vitamin D did not show significant effects on egg hatchability at 57 and 64 weeks. On the week 67, however, there was higher hatchability with eggs from breeders fed the lower doses of vitamin D₃ ($P < 0.05$) (Table 4).

Incubated eggs from hens fed 2,000 IU D₃ at 64 weeks of age demonstrated lower late embryo mortality and a reduced number of pips. At 67 weeks of age, however, fertile eggs from birds fed 3,400 IU D₃ and 2,000 + 35 mg 25(OH)D₃ showed lower early embryo mortality compared to those from hens fed 2,000 IU D₃. Incubated eggs from hens fed 2,000 IU D₃ at 64 weeks presented lower late embryo mortality and lower number of pips. At 67 weeks, fertile eggs from hens fed 3,400 IU D₃ and 2,000 + 35 mg 25(OH)D₃ presented lower early embryo mortality compared to eggs from hens fed 2,000 IU D₃ (Table 5).

Table 3 - Egg weight and egg specific gravity of broiler breeders supplemented with cholecalciferol and 25-hydroxycholecalciferol

Week	2,000 D ₃	3,400 D ₃	2,000 D ₃ + 35 mg 25(OH)D ₃	2,000 D ₃ + 69 mg 25(OH)D ₃	Mean
Egg weight, g					
35	64.0 ± 0.34	63.4 ± 0.43	63.7 ± 0.40	63.3 ± 0.34	63.6 ± 0.19A
40	66.1 ± 0.36	65.9 ± 0.46	66.0 ± 0.42	65.8 ± 0.36	66.0 ± 0.20B
50	69.3 ± 0.42	69.3 ± 0.53	69.7 ± 0.48	69.4 ± 0.42	69.4 ± 0.23C
56	69.5 ± 0.48	70.1 ± 0.61	69.7 ± 0.55	70.3 ± 0.48	69.9 ± 0.26C
60	69.8 ± 0.60	70.8 ± 0.75	69.9 ± 0.69	70.1 ± 0.60	70.1 ± 0.33C
67	71.4 ± 0.45	72.1 ± 0.57	70.7 ± 0.52	72.2 ± 0.45	71.6 ± 0.25D
Mean	68.4 ± 0.32	68.6 ± 0.40	68.3 ± 0.37	68.5 ± 0.32	
Egg specific gravity, g/mL					
35	1.083 ± 0.44a	1.085 ± 0.44b	1.084 ± 0.44ab	1.083 ± 0.44a	1.084 ± 0.22A
40	1.083 ± 0.34ab	1.083 ± 0.34ab	1.084 ± 0.34b	1.082 ± 0.34a	1.083 ± 0.17A
56	1.078 ± 0.41	1.078 ± 0.51	1.078 ± 0.51	1.078 ± 0.51	1.078 ± 0.25B
60	1.074 ± 0.43ab	1.073 ± 0.43a	1.076 ± 0.43b	1.075 ± 0.43b	1.075 ± 0.21C
67	1.074 ± 0.42	1.074 ± 0.57	1.073 ± 0.57	1.074 ± 0.57	1.074 ± 0.28C
Mean	1.079 ± 0.30	1.079 ± 0.30	1.079 ± 0.30	1.079 ± 0.30	

Means followed by the same capital letters within a column did not differ ($P > 0.05$) by Bonferroni test. Means followed by small letters within a row did not differ ($P > 0.05$) by Tukey test.

Table 4 - Hatchability of eggs from broiler breeders supplemented with cholecalciferol and 25-hydroxicolecalciferol

Item	Week, % hatching		
	57	64	67
2,000 D ₃	79.61	69.86	90.10b
3,400 D ₃	70.34	67.22	82.25a
2,000 D ₃ + 35 mg 25 (OH)D ₃	76.94	73.80	89.51ab
2,000 D ₃ + 69 mg 25 (OH)D ₃	73.99	56.8	84.92ab
PROB	0.246	0.077	0.027
Mean	75.22	66.94	86.69
CV %	12.64	20.61	7.26

Means followed by the same letters within a column did not differ ($P>0.05$) by Tukey test.

Cholecalciferol is the routinely used form of vitamin D supplemented in broiler breeder feeds. The 25(OH)D₃ form presents higher polarity compared to the usual D₃, therefore it is expected to have a higher absorption plateau and it is also expected to lead to higher intestinal Ca absorption. Research conducted in the past has demonstrated that supplementation with 25(OH)D₃ led to improvements in egg shells from commercial layers (Charles et al., 1978; Jackson & Jhong, 1998; Koreleski & Swiatkiewicz, 2005) and reductions in losses during egg storage and processing (Mcloughlin & Soares, 1976). However, in the present

study, breeder hens failed to demonstrate improvements when 25 (OH)D₃ was added to feeds already containing 2,000 IU vitamin D₃. Some results in the literature have produced similar outcomes such as those reported by Roland & Harms (1976), Abdulrahim et al. (1978), Cohen et al. (1978) and Hamilton (1980).

An improved conversion into vitamin D is obtained when birds are fed 25 (OH) D₃, therefore, supplementing breeder hen feeds with this compound was expected to improve eggshell quality (Soares et al., 1976). In the present study, however, using 25 (OH)D₃ did not lead to improvements when eggshell quality was evaluated using specific gravity measurements, with the exception of data from weeks 40 and 60, which were better. Specific gravity is a measurement, which has the advantage of not destroying the eggs when measuring eggshell quality; however, it is not very accurate when minimal differences are expected. Previous results evaluating supplementation of 25 (OH) D₃ have also demonstrated a limitation in obtaining advantage when eggshell quality was assessed using specific gravity (Roland & Harms, 1976; Keshavarz, 1996; Keshavarz, 2003).

The presence of cholecalciferol in eggs is very important to support the embryo Ca metabolism during incubation.

Table 5 - Embryo mortality from incubating eggs of breeder hens supplemented with cholecalciferol and 25-hydroxicolecalciferol

Item	57 weeks					
	1-7	8-14	15-21	Pips	Malformed	Culls
2,000 D ₃	7.62	1.80	5.00	1.25	1.00	2.30
3,400 D ₃	6.25	2.00	5.75	1.75	0.00	1.60
2,000 D ₃ + 35 mg 25 (OH) D ₃	7.71	2.60	6.12	2.12	0.00	1.60
2,000 D ₃ + 69 mg 25 (OH) D ₃	6.62	1.71	3.75	1.12	1.00	1.50
PROB	0.798	0.906	0.135	0.586	0.580	0.398
Mean	7.05	2.02	5.15	1.56	0.5	1.77
CV, %	20.94	11.39	10.24	11.15	2.66	10.75
Item	64 weeks					
	1-7	8-14	15-21	Pips	Malformed	Culls
2,000 D ₃	10.87	1.75	5.62b	0.875a	1.00	1.75
3,400 D ₃	12.12	1.33	10.12a	2.00ab	1.00	3.00
2,000 D ₃ + 35 mg 25 (OH) D ₃	8.25	1.66	8.37ab	2.25ab	1.00	1.83
2,000 D ₃ + 69 mg 25 (OH) D ₃	14.50	1.00	10.12a	3.25b	1.00	2.6
PROB	0.064	0.720	0.030	0.021	0.847	0.787
Mean	11.43	1.43	8.56	2.09	1.00	2.29
CV, %	18.79	8.18	14.87	12.68	3.96	12.95
Item	67 weeks					
	1-7	8-14	15-21	Pips	Malformed	Culls
2,000 D ₃	6.37b	2.00	2.75	0.75	0.00	1.75
3,400 D ₃	2.87a	1.60	2.50	0.50	1.00	1.00
2,000 D ₃ + 35 mg 25 (OH) D ₃	2.87a	0.00	1.87	0.37	1.00	1.00
2,000 D ₃ + 69 mg 25 (OH) D ₃	5.37ab	1.25	2.87	0.62	0.00	1.50
PROB	0.012	0.059	0.765	0.754	0.580	0.644
Mean	4.37	1.21	2.50	0.56	0.50	1.31
CV, %	16.79	7.69	12.94	6.75	2.66	4.20

Means followed by the same letters within a column did not differ ($P>0.05$) by Tukey test. Data analyzed after arc sin transformation.

Therefore, excess or deficiency of this vitamin can lead to reduced hatchability, which can be specially related to late embryo mortality (Narbaitz et al, 1987; Narbaitz & Tsang, 1989). The result reported in this study with higher hatchability from eggs supplemented with 2,000 IU D₃ as compared to those fed 3,400 IU D₃ or 2,000 IU D₃ + 69 mg 25(OH)D₃ opposes the results from Atencio et al. (2006), which demonstrated that higher doses of vitamin D₃ led to increased hatchability. Ameenuddin et al. (1982) and Soares et al. (1995) affirmed that 25(OH)D₃ is the hydroxylated form of vitamin D₃ with the highest ability to be transported into eggs. Therefore, it is capable of supporting high hatchability and embryo development when fed as the only source of vitamin D. Consequently, the high late embryo mortality found in the present study with eggs from breeders at 64 weeks of age and fed 25(OH)D₃ lacks former published support data and is left to be explained.

Conclusions

There were no effects of the associations of 25-hydroxycholecalciferol on breeder performance and incubation results compared to vitamin D₃ as the only source of supplementation at 2,000 IU. Improvements in egg shell quality, however, were observed at mid and end of production when 25(OH)D₃ was supplemented.

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