The effects of *in ovo* injected vitamin D_3 sources on the eggshell temperature and early posthatch performance of Ross 708 broilers[1, 2, 3]

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ABSTRACT The effects of *in ovo* injected vitamin D_3 source on eggshell temperature (**ET**) and performance of broilers through 14 D of age (**doa**) were investigated. Eggs from a 35-wk-old commercial Ross 708 broiler breeder flock were set in a single-stage incubator with 4 treatments representing each of 12 incubator tray levels (blocks). At 432 h of incubation (**hoi**), noninjected and diluent-injected (50 μL) groups were control treatment groups. Vitamin treatments in the commercial diluent were as follows: 2.4 μg of vitamin D_3 (**D_3**) or 25-hydroxylcholecalciferol (**25OHD_3**). After injection, ET readings were recorded (435, 441, 453, 459, and 465 hoi) by infrared thermometry. Hatchability, hatchling BW, and percentage of male and female hatchlings were determined at 502 hoi. Equal numbers of male and female chicks were placed in each pen and grown out for 14 doa. On a per-pen basis, BW was recorded after hatching at day 7 and 14 doa, and BW gain, average daily BW gain, feed intake (**FI**), and feed conversion ratio (**FCR**) were calculated between 0 to 14 doa. The ET of eggs significantly fluctuated during the post-injection time period; however, the type of vitamin D_3 source injected did not affect ET. Nevertheless, the injection of 25OHD_3 resulted in a lower late embryo mortality than the diluent and D_3 injection treatments. In addition, birds that received 25OHD_3 had a lower FI and FCR than birds in all other treatments. In conclusion, the *in ovo* injection of 25OHD_3 has the potential to improve early posthatch broiler performance without affecting ET.

Key words: 25-hydroxylcholecalciferol, broiler performance, eggshell temperature, *in ovo* injection

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INTRODUCTION

Vitamin D_3 (**D_3**) is a prehormone and is involved in biological and metabolic processes, including calcium homeostasis, intestinal absorption of calcium and phosphorus, muscle development, regulation of the immune response, and bone formation in chickens (Soares et al., 1995; Rama-Rao et al., 2006; 2009; Morris et al., 2014; Vignale et al., 2015). After intestinal absorption, D_3 requires 2 metabolic steps to become a biologically active form (Soares et al., 1995). In hepatic cells, D_3 is converted to 25-hydroxylcholecalciferol (**25OHD_3**) by 25-hydroxylase (Atencio et al., 2005) and is later hydroxylated to 1, 25-dihydroxylcholecalciferol [**1,25-$(OH)_2D_3$**] by 1 α-hydroxylase in renal cells (Shanmugasundaram and Selvaraj, 2012). Vitamin D is deposited in the form of 25OHD_3 into the yolk because it is more stable, and unlike D_3, it bypasses liver hydroxylation (Rovegno et al., 2012). In the serum, 25OHD_3 is less toxic at higher levels than 1,25-$(OH)_2D_3$ (Zanuzzi et al., 2012). Furthermore, injection of 2 μg of 25OHD_3 to vitamin D–deficient embryos at 14 D of incubation (**doi**) has been shown to result in increased hatchability, muscle weight, and bone mineralization in broiler embryos (Narbaitz and Tsang 1989).

Factors that are required for proper incubation include temperature, humidity, ventilation, and turning. Of those, temperature is the most critical factor for optimal

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embryonic development and successful incubation (Romanoff, 1960). Eggshell temperature (**ET**) is highly correlated with embryo heat production (Lourens et al., 2006). In addition, an increase in ET is associated with decreases in relative embryo weight and yolk-free BW (Ipek et al., 2014); a lower ET is associated with increased hatchling BW and chick quality (Zhai et al., 2011). In comparison with birds having an ET between 37.8 and 38.2°C, the feed conversion ratio (**FCR**) of broilers that experienced a low (33.3-36.7°C) or high (38.9-40.0°C) ET has been shown to be higher between wk 4 and 6 after hatching (Ipek et al., 2015). Various vitamin D_3 sources can modulate metabolism and have a potential to subsequently affect ET, which may positively affect broiler performance. The effects of various forms of vitamin D_3 on ET have not been previously reported. Therefore, the objective of the present study was to determine the effects of D_3 and $25OHD_3$ on ET and the posthatch performance of Ross 708 broiler chickens.

MATERIALS AND METHODS

Treatment Layout and Solution Preparation

The trial protocol was approved by the Institutional Animal Care and Use Committee of Mississippi State University. Fertile eggs were collected from 35-wk-old commercial Ross 708 broiler breeder hens and stored at 18°C for 1 D before set. A total of 30 eggs were randomly set in each of 4 preassigned treatment groups on 12 incubator tray levels (blocks) in a Jamesway model PS 500 setter unit (Jamesway Incubator Company Inc., Cambridge, Ontario, Canada) set at 37.5°C dry bulb and 29°C wet bulb temperatures. Treatment group placement was randomized on each level to avoid positional effects in the incubator. Incubator air temperature and relative humidity were monitored in 3 locations within the incubator every 15 min using HOBO ZW Series wireless data loggers (Onset Computer Corporation, Bourne, MA). At 18 doi, 50-μL solution volumes of prespecified treatments were injected using an Inovoject M (Zoetis Animal Health Co., Parsippany, NJ) *in ovo* injection machine. Previous findings in our laboratory revealed that 2.4 μg of vitamin D_3 sources were sufficient to result in a positive effect on hatching chick quality with no negative impact on hatchability of injected live embryonated eggs and hatchling BW (unpublished data). Therefore, at 432 hoi, eggs were subjected to one of the following treatments: (1) noninjected; (2) diluent-injected (control; 50 μL of commercial diluent); (3) D_3-injected (50 μL of commercial diluent containing 2.4 μg D_3/egg), and (4) $25OHD_3$-injected (50 μL of commercial diluent containing 2.4 μg $25OHD_3$/egg) groups. The prespecified concentrations of D_3 (ROVIMIX D_3 500; DSM Nutritional Products Inc., Parsippany, NJ) or $25OHD_3$ (ROVIMIX Hy-D 1.25%; DSM Nutritional Products Inc., Parsippany, NJ) were dissolved in distilled water. For treatment application, the diluent (commercial MD vaccine diluent; Merial Co., Duluth, GA) in each injector infusion bag (400 mL total volume) was removed and replaced with sterile water containing either D_3 (15.3 mL) or $25OHD_3$ (3.8 mL).

Experimental Design and Data Collection

Mean set egg weight for each replicate tray was obtained, and all eggs were candled at 288 and 432 hoi. Before injection, from 357 to 429 hoi, the ET of 5 eggs in each treatment group on each replicate tray was recorded twice daily using an infrared thermometer (Thermoscan Braun, Inc., Kronberg, Germany) In accordance with the procedure described by Olojede et al. (2016). The ET of eggs in the preassigned treatment groups was measured to ensure that there were no positional effects within the incubator on ET. The ET was recorded at the egg equator by a single observer positioned in the incubator with the door closed. The ET recordings were made after the incubation temperature became stable, which was approximately 10 min after the observer's entrance into the incubator. The ET readings of 5 eggs in each treatment group on each replicate tray were recorded at 435, 441, 453, 459, and 465 hoi, which were time periods after injection until the first egg was externally pipped. Furthermore, percentage egg weight loss (**PEWL**) between 0 to 432 hoi was determined on a batch tray weight basis. The PEWL of the eggs was determined to ensure that eggshell porosity was not significantly different among all eggs in the different treatment groups before treatment administration. At 432 hoi, 48 eggs were injected with Coomassie Brilliant Blue G-250 (colloidal) dye for embryo staging analysis as described by Sokale et al. (2017). At that time, the remaining eggs were not injected with dye but were injected with their prespecified treatment. After injection, the remaining eggs were transferred to hatchling basket sections within a separate hatcher unit (Jamesway Incubator Company Inc., Cambridge, Ontario, Canada) that corresponded with their positioning in the setter. All chicks were pulled from the hatcher at 502 hoi (hatch). At hatch, all chicks were feather sexed, and the percentage of male and female hatchlings in each treatment group was determined. In addition, for each replicate treatment group, hatchability of fertile eggs, mean hatchling BW, and hatch residue analysis were determined in accordance with the procedures described by Ernst et al. (2004); total mortality rate was determined by adding the percentages of late embryo and hatchling mortalities. For posthatch evaluation, 9 male and 9 female chicks were placed in each of 48 floor pens (12 replicate pens per treatment). Floor pens contained used litter top dressed with fresh wood shavings and were 1.22 m × 0.914 m (1.12 m^2) in dimension, which allowed for a 0.062-m^2/bird stocking density. Throughout 14 D after hatching, birds were allowed *ad libitum* access to water and feed and were provided a Mississippi State University basal corn–soybean starter diet (Table 1) and were brooded and fed in accordance with Ross 708 guidelines (Aviagen, 2015). At days 7 and 14, the mean chick BW and feed intake (**FI**) were determined for each

Table 1. Feed composition of the experimental diets from 0 to 14 D of age (**doa**).

Item	%
Ingredient	
Yellow corn	53.23
Soybean meal	38.23
Animal fat	2.60
Dicalcium phosphate	2.23
Limestone	1.27
Salt	0.34
Choline chloride 60%	1.00
Lysine	0.28
DL-Methionine	0.37
L-threonine	0.15
Premix[1]	0.25
Coccidiostat[2]	0.05
BMD[3]	0.05
Total	100
Calculated nutrients	
Crude protein	23
Calcium	0.96
Available phosphorus	0.48
AME (Kcal/kg)	3,000
Digestible methionine (%)	0.51
Digestible lysine (%)	1.28
Digestible threonine (%)	0.86
Digestible TSAA (%)	0.95
Sodium (%)	0.16
Choline (%)	0.16

Abbreviations: AME, Apparent metabolizable energy; TSAA, Total sulfur amino acid.

[1]The broiler premix provided the following per kilogram of diet: vitamin A (retinyl acetate), 10,000 IU; cholecalciferol, 4,000 IU; vitamin E (DL-α-tocopheryl acetate), 50 IU; vitamin K, 4.0 mg; thiamine mononitrate (B_1), 4.0 mg; riboflavin (B_2), 10 mg; pyridoxine HCl (B_6), 5.0 mg; vitamin B_{12} (cobalamin), 0.02 mg; D-pantothenic acid, 15 mg; folic acid, 0.2 mg; niacin, 65 mg;biotin, 1.65 mg; iodine (ethylene diamine dihydroiodide), 1.65 mg; Mn ($MnSO_4H_2O$), 120 mg; Cu, 20 mg; Zn, 100 mg, Se, 0.3 mg; Fe ($FeSO_4.7H_2O$), 800 mg.

[2]Decocx (Zoetis Canada Inc., Kirkland, QC, Canada).

[3]Bacitracin methylene disalicylate (BMD 110; Zoetis, Parsippany, NJ): containing 55 mg of BMD per kg.

pen, and average daily BW gain (**ADG**), FCR, and mortality rate were determined for the 0- to 14-D period.

Statistical Analyses

A randomized complete block design was applied for the entire incubation and 2-wk posthatch periods, and a repeated-measures ANOVA was used to analyzed all data. A 4 × 4 (treatment × observation) factorial arrangement was used for the preinjection ET data, and a 4 × 5 (treatment × observation) factorial arrangement was used for the postinjection ET data. Incubator tray level was the blocking factor, with all *in ovo* injection treatments randomly represented on each of 6 levels (blocks). The Jamesway trays were experimental units, and time period and treatment were fixed effects and block was considered a random effect using the procedure for linear mixed models (PROC MIXED) of SAS©, version 9.4 (SAS Institute Inc., Cary, NC) by the following mixed-effects model used for ET data:

$$Y_{ijk} = \mu + B_i + T_j + H_k + (TH)_{jk} + E_{ijk}$$

where μ was the population mean, B_i was incubator levels (i = 1 to 6), T_j was treatment (j = 1 to 4), H_k time and date of ET observations (k = 1 to 4 for preinjection and 5 for postinjection), and E_{ijk} was the residual error.

Table 2. Eggshell temperature of noninjected, diluent-injected, vitamin D_3 (**D_3**)–injected, and 25-hydroxylcholecalciferol (**25OHD_3**)–injected Ross 708 broilers at 435, 441, 453, 459, and 465 h of incubation (**hoi**).

Treatment	Eggshell temperature (°C)
Noninjected	38.07
Diluent[1]	37.94
Vitamin D_3[2]	38.04
25OHD_3[3]	37.99
Hour	
435 hoi	38.01[b]
441 hoi	38.15[a]
453 hoi	37.89[c]
459 hoi	37.99[b]
465 hoi	38.02[b]
Pooled SEM	0.048
P-values	
Treatment	0.169
Hour	<0.0001
Hour*treatment	0.994

[a–c]Means within Temperature column and hour category with no common superscript differ significantly ($P \leq 0.05$).

[1]Eggs injected with 50 μL of commercial diluent at 432 hoi.

[2]Eggs injected with 50 μL of commercial diluent containing vitamin D_3 at 2.4 μg/egg at 432 hoi.

[3]Eggs injected with 50 μL of commercial diluent containing 25OHD_3 at 2.4 μg/egg at 432 hoi.

For analysis at hatch, egg weight was included as a covariate for hatchling BW. The hatch data were analyzed as one-way ANOVA using the procedure for linear mixed models by the following mixed-effects model used for hatch data:

$$Y_i = \mu + I_i + T_i + E_i$$

where μ was the population mean, I_i was incubator level (block) (i = 1 to 6), T_i was treatment (i = 1 to 4), and E_i was the residual error.

For broiler performance analysis, the experimental unit was the individual pen and a group of 4 treatments was represented in each block. Block was the blocking factor that *in ovo* injection treatments are randomly represented in each of the 12 blocks. The performance data were analyzed as one-way ANOVA using the procedure for linear mixed models (PROC MIXED) of SAS, version 9.4. Pairwise differences between means were considered significant at $P \leq 0.05$.

$$Y_i = \mu + B_i + T_i + E_i$$

where μ was the population mean, B_i was block (i = 1 to 6), T_i was treatment (i = 1 to 4), and E_i was the residual error.

RESULTS AND DISCUSSION

Eggshell Temperature

There was no significant main or interactive effect due to treatment for ET from 15 to 18 doi (data not shown). These data were analyzed to ensure that there were no positional effects in the incubator on the ET of eggs among

Table 3. Hatch parameters in noninjected, diluent-injected, vitamin D_3 (D_3)–injected, and 25-hydroxylcholecalciferol ($25OHD_3$)–injected Ross 708 broilers.

Treatment	n[1]	Female	Male	PEWL[2] %	Hatchability[3]	Hatchling BW, g
Noninjected	12	47.22	52.78	12.32	94.14	42.86
Diluent[4]	12	47.85	52.15	12.91	90.68	45.03
D_3[5]	12	52.06	47.94	12.28	91.84	43.99
$25OHD_3$[6]	12	47.89	52.11	13.08	93.96	43.91
Pooled SEM		3.308	3.338	0.466	1.474	0.875
P-value		0.680	0.680	0.332	0.291	0.397
Covariate egg weight		-	-	-	-	0.014

Abbreviation: PEWL, percentage egg weight loss.
[1]Incubator tray level was the unit of replication.
[2]Percentage of egg weight loss from day 0 to 432 h of incubation (**hoi**).
[3]Hatchability of injected live embryonated eggs deemed alive via candling immediately before *in ovo* injection at 432 hoi.
[4]Eggs injected with 50 μL of commercial diluent at 432 hoi.
[5]Eggs injected with 50 μL of commercial diluent containing vitamin D_3 at 2.4 μg/egg at 432 hoi.
[6]Eggs injected with 50 μL of commercial diluent containing $25OHD_3$ at 2.4 μg/egg at 432 hoi.

the different treatment groups. There was also no significant main or interactive effect due to treatment for ET from time of injection until hatch. Nevertheless, there was a significant ($P < 0.0001$) effect due to time on ET (Table 2). ET was highest when it was recorded at 441 hoi in comparison with all other time periods, and it was lowest when it was recorded at 453 hoi. In addition, ET was higher when it was recorded at 465, 459, and 435 hoi in comparison with that recorded at 453 hoi.

ET is influenced by embryonic heat production, and changes in ET have been shown to be associated with changes in hatchling quality and posthatch broiler performance (Molenaar et al., 2011; Ipek et al., 2015). Increases in ET beyond standard levels (ET between 38.9 and 40.0°C) during the second half of incubation have resulted in a decreased hatchability and hatchling weight and increased residual yolk sac weight in early posthatch broilers (Ipek et al., 2015). At the same time period of incubation, ET at 38.9°C or greater also has resulted in a higher FCR in 6-wk-old broiler chickens and has led to increases in the incidences of metabolic diseases such as ascites (Molenaar et al., 2011). These results indicate that high ET is linked to reduced hatchling quality and posthatch broiler production and an increased susceptibility to metabolic diseases. According to Zhai et al. (2011), the *in ovo* injection of 120 μL of different carbohydrate sources or saline that were stored at cool temperatures before being injected into embryonated eggs at 18 doi resulted in a lower ET at 22 h after injection than dry-punch controls. However, in the present study, the solution that was injected at room temperature did not affect ET at any time period observed ($P = 0.169$). There were significant differences in the ET observed at various time periods examined, but there was no clear pattern for the changes in the ET between the various time periods.

Hatch and Posthatch Variables

PEWL from 0 to 21 doi, hatchability, hatchling BW, female and male percentages, external pip live, external pip dead, and hatchling mortalities did not differ between treatments (Table 3 and 4). However, the *in ovo* injection of $25OHD_3$ resulted in a lower late embryo mortality than the injection of D_3 or diluent (Table 4). Chick BW did not differ between treatments at 7 and 14 D posthatch (Table 5). There were also no significant treatment differences for BW gain from 0 to 14 D of posthatch age. However, birds that received $25OHD_3$ had a lower FI than birds in the other treatment groups, and $25OHD_3$ *in ovo* injected birds had a lower FCR than the birds in the other treatment groups.

Satellite cells play an important role in hypertrophic skeletal muscle growth and myofibrillar protein synthesis in broiler chickens (Moss, 1968; Moss and Leblond, 1971). Dietary supplementation with $25OHD_3$ significantly impacts muscle development by increasing satellite cell activity and size in broiler chickens (Hutton et al., 2014). In addition, supplemental $25OHD_3$ in broiler or broiler breeder diets has resulted in increased intestinal villus length during the embryonic and later posthatch developmental periods (Chou et al., 2009; Ding et al., 2011). Using dietary $25OHD_3$ as a partial or complete replacement for D_3 has been shown to increase "walkability" and bone quality, as well as overall broiler performance (Yarger et al., 1995; Sun et al., 2013). There are 2 possible explanations for these improvements. First, in comparison with D_3, $25OHD_3$ is more efficiently stored in target tissues (Burild et al., 2016). Both vitamin D_3 sources can be stored in several tissues such as adipose, liver, and white and red muscle tissues (Burild et al., 2016). When provided at low levels in the diet (5 μg/feed), $25OHD_3$ is largely stored in the liver. Approximately half the amount in the liver is further stored in adipose and white and red muscle tissue. However, vitamin D_3 is mainly stored in adipose tissue, whereas only small amounts are stored in the liver and muscle tissues for longer periods. When administrated at 18 doi, differences in the storage efficiencies of the 2 forms of vitamin D_3 could be the possible reason for their differential effects on hatchability and bone quality (Bello et al., 2013, 2014), as well as broiler performance (Ebrahimi et al., 2016).

The second reason for the greater effectiveness of $25OHD_3$ is that 1 α-hydroxylase converts $25OHD_3$ to the active form of the hormone, but D_3 does not

Table 4. Effects of noninjected and diluent-injected (50 μL) controls and eggs injected with 2.4 μg/egg of vitamin D_3 (**D_3**) and 25-hydroxycholecalciferol (**25OHD_3**) in 50 μL of diluent in hatch residue at 502 h of incubation (**hoi**).

Treatment	n[1]	EPD[2]	EPL[3]	Late embryo mortality[4]	Dead chicks[5]	Total mortality rate[6]
				(%)		
Noninjected	12	0.67	0.34	4.74[a,b]	0.68	5.42
Diluent[7]	12	0.34	1.10	6.72[a]	1.73	8.45
D_3[8]	12	0.70	0.34	6.12[a]	1.04	7.16
25OHD_3[9]	12	1.11	1.17	1.69[b]	1.98	3.67
P-value		0.638	0.369	0.050	0.508	0.150
SEM		0.563	0.472	1.000	0.674	2.141

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).
[1]Incubator tray level was the unit of replication.
[2]External pip dead (chick pipped shell and was dead) at 502 hoi.
[3]External pip live (chick pipped shell and was alive) at 502 hoi.
[4]Dead embryos that were not externally pipped at 502 hoi.
[5]Dead chicks that were found at 502 hoi.
[6]Total mortality rate calculated by adding the percentages of late embryo and hatchling mortalities.
[7]Eggs injected with 50 μL of commercial diluent at 432 hoi.
[8]Eggs injected with 50 μL of commercial diluent containing D_3 at 2.4 μg at 432 hoi.
[9]Eggs injected with 50 μL of commercial diluent containing D_3 at 2.4 μg at 432 hoi.

stimulate 1 α-hydroxylase. The expression of 1 α-hydroxylase occurs in high amounts in the kidney, as well as in the thigh and breast muscles, in chickens (Shanmugasundaram and Selvaraj, 2012). In addition, 1 α-hydroxylase has been isolated from the small intestine, bone, and macrophages of both mammals and chickens (Omdahl et al., 2002; Shanmugasundaram and Selvaraj, 2012). Therefore, birds receiving 25OHD_3 may have a greater potential to ultimately increase nutrient absorption from the gut with a subsequent increase in 1,25-$(OH)_2D_3$, with subsequent increases in protein deposition and muscle formation. In this study, the tissue expression of 1 α-hydroxylase was not examined, but increased 25OHD_3 serum concentrations were observed in embryos that received 25OHD_3 or D_3 rather than commercial diluent alone. Therefore, a lower FCR in broilers that were injected with 25OHD_3 may be due to an elevated serum 25OHD_3 concentration, which subsequently contributes to improved intestinal absorptive capabilities.

In conclusion, the findings observed in this study showed that ET was different but without a definite pattern between the time periods examined. In addition, the use of either source of vitamin D_3 used did not affect embryonic ET. Nevertheless, subsequent effects of *in ovo* injected 25OHD_3 were observed on early posthatch broiler performance. The birds *in ovo* injected with 2.4 μg of 25OHD_3 showed reduced FCR from 0 to 14 doa in comparison with diluent-injected and noninjected control groups. This finding suggests that the *in ovo*

Table 5. Broiler performance observations from 0 to 14 D of age (**doa**) in noninjected, diluent-injected, vitamin D_3 (**D_3**)–injected, and 25-hydroxylcholecalciferol (**25OHD_3**)–injected Ross 708 broilers.

Treatment	n[1]	BW-d7[2]	BW-d14[3]	ADG[4]	BWG[5]	FI[6]	FCR[7]
				g			g/g
Noninjected	12	163.6	428.7	27.82	389.5	514.4[a]	1.32[a]
Diluent[8]	12	163.0	428.6	27.68	387.6	523.2[a]	1.35[a]
D_3[9]	12	163.1	427.3	27.63	386.8	518.9[a]	1.34[a]
25OHD_3[10]	12	163.9	430.4	27.83	389.6	493.4[b]	1.27[b]
Pooled SEM		1.61	4.71	0.086	5.51	5.35	0.016
P-value		0.969	0.949	0.787	0.943	0.002	0.005

[a,b]Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).
Abbreviations: ADG, average daily BW gain; BWG, bodyweight gain; FCR, feed conversion ratio; FI, feed intake.
[1]Pen was the unit of replication.
[2]Mean pen BW at 7 doa.
[3]Mean pen BW at 14 doa.
[4]Mean average daily BW gain from 0 to 14 doa.
[5]BW gain from 0 to 14 doa.
[6]Feed intake from 0 to 14 doa.
[7]Feed conversion ratio which is gram daily feed intake per gram average daily gain from 0 to 14 doa.
[8]Eggs injected with 50 μL of commercial diluent at from 0 to 14 doa.
[9]Eggs injected with 50 μL of commercial diluent containing vitamin D_3 at 2.4 μg/egg at from 0 to 14 doa.
[10]Eggs injected with 50 μL of commercial diluent containing 25OHD_3 at 2.4 μg/egg at from 0 to 14 doa.

injection of $25OHD_3$ at a level of 2.4 μg may be cost-effective in broiler production. Further studies are needed to identify biological and morphological changes that may result in response to the *in ovo* injection of vitamin D_3 sources that are associated with changes in broiler performance and to explore the potential of injecting higher levels of vitamin D_3 sources.

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