Effects of the in ovo injection of vitamin D₃ and 25-hydroxyvitamin D₃ in Ross 708 broilers subsequently challenged with coccidiosis. I. performance, meat yield and intestinal lesion incidence

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ABSTRACT

Effects of the in ovo administration of vitamin D₃ (D₃) and 25-hydroxyvitamin D₃ (25OHD₃) on broiler intestinal lesion incidence, performance and breast meat yield after a coccidiosis challenge were investigated. On each of 10 incubator tray levels, 10 Ross 708 broiler hatching eggs were randomly assigned to each of the following 5 in ovo injection treatments administrated at 18 d of incubation (doi): 1) noninjected; 2) diluent; diluent containing either 3) 2.4 µg D₃ (D₃), 4) 2.4 µg 25OHD₃ (25OHD₃), or 5) 2.4 µg D₃ + 2.4 µg 25OHD₃ (D₃+25OHD₃). A 50 µL solution volume was injected into each egg using an Inovoject multi-egg injector. Four male chicks were randomly assigned to each of 80 battery cages in each of 2 rooms. Half of the treatment-replicate cages (8) in each room were challenged with a 20× live coccidial vaccine at 14 d of age (doa). One randomly selected bird from each of 4 treatment-replicate cages was scored for coccidiosis lesions before and 2 wk after challenge. Mean BW, BW gain (BWG), feed intake, and feed conversion ratio were determined for all birds from 0 to 14, 15 to 28, and 29 to 41 doa. Carcass weight, and the absolute and relative (% of carcass weight) weights of carcass parts were determined in 3 birds per treatment-replicate cage at 42 doa. Hatchability of live embryonated injected eggs and hatch residue were not affected by treatment. Across challenge treatment, birds in the 25OHD₃ treatment group experienced an increase in BWG between 29 and 41 doa when compared to the D₃ or diluent-injected birds. Furthermore, pectoralis major muscle percentage tended (P = 0.059) to increase in birds belonging to the 25OHD₃ treatment in comparison to birds in the D₃ or diluent-injected treatments. These results indicate that regardless of challenge treatment, 2.4 µg of 25OHD₃ may increase the BWG and breast meat yield of birds relative to those that only received an injection of commercial diluent.

Key words: vitamin D source, in ovo injection, coccidiosis, broiler performance, breast meat yield

INTRODUCTION

Coccidiosis is the major parasitic disease affecting poultry and results in severe economic loss due to severe reductions in feed utilization and BW gain (Ritzi et al., 2014). Increased oxidative stress has been reported in birds infected by coccidiosis (Georgieva et al., 2006) which can lead to a reduction in the fat-soluble vitamin status, including that of vitamin D (Lee et al., 2018). Vitamin D₃ is mainly absorbed via diffusion into enterocytes residing in the duodenum and upper jejunum (Borel, 2003), and is facilitated by the formation of aggregates called micelles, along with other lipophilic food components. These are then transported to the liver as portomicrons (Elaroussi et al., 1994; Cooke and Haddad, 1989). Vitamin D₃ must undergo 2 sequential hydroxylation steps to become active. The first hydroxylation occurs through 25-hydroxylase activity in liver microsomes and mitochondria. This first hydroxylation produces 25-hydroxycholecalciferol (25OHD₃). Later, 25OHD₃ is hydroxylated to 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) by 1α-hydroxylase in the kidney (Henry, 1980).
Vitamin D₃ sources are capable of accelerating calcium (Ca) absorption through increased calbindin activity (Bikle and Munson, 1985). Calbindin is involved in intestinal intracellular Ca transport and its expression occurs in the intestine and kidney. Calbindin activity in chickens is regulated by 1,25(OH)₂ D₃ (Hall and Norman, 1990; Ferrari et al., 1992). In comparison to vitamin D₃ sources on the physiological attributes of performance during a coccidiosis infection are well understood, the influence of the in ovo injection of various vitamin D₃ sources on the physiological attributes of broilers subjected to a coccidiosis infection have to-date not been investigated. Therefore, the objective of this study was to determine the effects of the in ovo administration of D₃ and its metabolite, 25OHD₃, on the incidence of intestinal lesions, and the performance and breast meat yield of broilers after a coccidiosis challenge.

**MATERIAL AND METHODS**

**Experiment Design and Egg Incubation**

This study was conducted according to a protocol (IACUC # 17-406) approved by the Institutional Animal Care and Use Committee of Mississippi State University. Fifty eggs were assigned to each of 5 preassigned treatment groups (trays) on each of 10 incubator tray levels (replicate blocks) in a single stage Chick Master Incubator (Chick Master Incubator Company, Medina, OH) set at 37.5°C dry bulb and 29°C wet bulb temperatures. The same incubator served as both a setter and hatcher unit. Positional effects were removed by randomizing all treatments between each incubator tray. The same incubator was used as both a setter and hatcher unit. Positional effects were removed by randomizing all treatments between each incubator tray. The position of the trays for each respective treatment group was determined as those mortalities that occurred between 18 doi (432 h of incubation) and 21 doi (502 h of incubation) prior to pip, during the pipping process, after the pipping process, and immediately after complete emergence from the shell. All chicks were feather-sexed to select for male broilers in their prespecified treatment, and then male chicks from each replicate basket were pooled within their respective treatment group. Four male chicks were randomly selected from each pooled treatment group, and were weighed and placed in each of 8 replicate isolated wire-floored battery cages belonging to each treatment group in each of 2 separate rooms of a light-controlled research facility (320 total birds). Each battery cage measured 0.76 m × 0.46 m (0.35 m²). All birds received ad libitum access to water and a Mississippi State University basal corn-soybean diet formulated to meet Ross 708 commercial guidelines (Aviagen, 2014) throughout the 41 d of age (doa) period (Fatemi et al., 2021a; Table 1).

**Growth Performance**

All birds were fed a starter diet from 0 to 14 doa, a grower diet from 15 to 28 doa, and a finisher diet from 29 to 41 doa. The BW, BW gain, average daily gain (ADG), feed intake (FI), and average daily FI (ADFI) of the birds on a pen basis were determined in each dietary phase. Percentage mortality and feed conversion ratio (FCR; g feed/g gain) adjusted for bird mortality, were calculated for the same time periods.

**Challenge, and Lesion Score and Oocyst Counts**

At 14 doa, the chicks that belonged to the diluent, D₃, 25OHD₃, and D₃ + 25OHD₃ treatment groups were challenged by oral gavage with a 20 × dose of a commercial coccidial vaccine containing live oocysts of *Eimeria acervulina, maxima, mivati*, and *tenella* (Coccivac-B52, Intervet Inc. Omaha, NE), that was diluted in 1 mL of distilled water. Coccidial lesions from *E. acervulina* and
maxima in the duodenum, jejunum, and ileum, and overall lesion incidence in the ceca were determined at 14 and 28 doa as described by Johnson and Reid (1970). Fecal samples from each of the 8 replicate cages in each treatment group of each room that belonged to the diluent, D3, 25OHD3, and D3 + 25OHD3 treatment groups were collected for oocyst count analysis at 7 and 14 d post-coccidiosis challenge (21 and 28 doa, respectively). The sporulated oocysts were counted in each 1.0 mL of solution using the hemocytometer method described by Holdsworth et al. (2004). Fecal samples from 4 replicate cages in each treatment group of each room were also randomly collected at 21 and 28 doa from the noninjected and unchallenged treatment group for oocyst count analysis for comparative purpose in order to confirm success of the coccidiosis challenge.

Processing

The birds that remained in each pen (approximately 47 birds/treatment) were processed at 42 doa according to the method described by Wang et al. (2018). Weights of the whole carcass, and carcass parts including the pectoralis (P) major and P. minor muscles, and leg, thigh, and wing were determined. Parts yields were calculated as percentages of cold carcass weight.

Statistical Analysis

The experimental design was a randomized complete block for both the hatch and rearing periods. Incubator level in the setter and hatcher served as the unit of treatment replication for the hatch data, and battery cage as the unit of treatment replication for the performance, meat yield, and coccidiosis lesion scoring data. Room was the blocking factor for the grow out phase of the experiment. The noninjected control group was not subjected to a coccidiosis challenge at 14 doa, as were the 4 in ovo-injected treatment groups. Therefore, there were

### Table 1

**Feed composition of the experimental diets from 0 to 41 d of age (doa).**

| Feed composition          | Commercial diet |
|---------------------------|-----------------|
| **Starter (0−14 doa)**    |                 |
| **Ingredient (%)**        | **Pct**         |
| Yellow corn               | 53.23           |
| Soybean meal              | 38.23           |
| Animal fat                | 2.6             |
| Dicalcium phosphate       | 2.23            |
| Limestone                 | 1.27            |
| Salt                      | 0.34            |
| Choline chloride 60%      | 0.10            |
| Lysine                    | 0.28            |
| DL-methionine             | 0.37            |
| L-threonine               | 0.15            |
| Premix                    | 0.25            |
| BMD²                      | 0.05            |
| **Total**                 | **100**         |

Calculated nutrients

| Crude protein             | 23              |
| Calcium                   | 0.96            |
| Available phosphorus      | 0.48            |
| Apparent metabolizable energy (AME; Kcal/kg) | 3,000 |
| Digestible methionine     | 0.51            |
| Digestible lysine         | 1.28            |
| Digestible threonine      | 0.86            |
| Digestible total sulfur amino acids (TSAA) | 0.95 |
| Sodium                    | 0.16            |
| Choline                   | 0.16            |

**Grower (15−28 doa)**

| Item                      | **Pct**         |
|---------------------------|-----------------|
| Yellow corn               | 57.13           |
| Soybean meal              | 34.8            |
| Animal fat                | 3.5             |
| Dicalcium phosphate       | 2               |
| Limestone                 | 1.17            |
| Salt                      | 0.34            |
| Choline chloride 60%      | 0.10            |
| Lysine                    | 0.21            |
| DL-methionine             | 0.32            |
| L-threonine               | 0.16            |
| Premix                    | 0.25            |
| BMD²                      | 0.05            |
| **Total**                 | **100**         |

Calculated nutrients

| Crude protein             | 21.5            |
| Calcium                   | 0.87            |
| Available phosphorus      | 0.435           |
| AME (Kcal/kg)             | 3,100           |
| Digestible methionine     | 0.47            |
| Digestible lysine         | 1.15            |
| Digestible threonine      | 0.77            |
| Digestible TSAA           | 0.87            |
| Sodium                    | 0.16            |
| Choline                   | 0.16            |

**Finisher (29−45 doa)**

| Item                      | **Pct**         |
|---------------------------|-----------------|
| Yellow corn               | 54.23           |
| Soybean meal              | 38.23           |
| Animal fat                | 2.5             |
| Dicalcium phosphate       | 2.23            |
| Limestone                 | 1.27            |
| Salt                      | 0.34            |
| Choline chloride 60%      | 0.10            |
| Lysine                    | 0.28            |
| DL-methionine             | 0.37            |
| L-threonine               | 0.15            |
| Premix                    | 0.25            |
| BMD²                      | 0.05            |
| **Total**                 | **100**         |

Calculated nutrients

(continued)

### Table 1 (Continued)

| Feed composition          | Commercial diet |
|---------------------------|-----------------|
| Crude protein             | 19.5            |
| Calcium                   | 0.78            |
| Available phosphorus      | 0.39            |
| AME (Kcal/kg)             | 3,200           |
| Digestible methionine     | 0.43            |
| Digestible lysine         | 1.02            |
| Digestible threonine      | 0.68            |
| Digestible TSAA           | 0.8             |
| Sodium                    | 0.16            |
| Choline                   | 0.16            |

¹The broiler premix provided per kilogram of diet: vitamin A (retinyl acetate), 10,000 IU; cholecalciferol, 250 IU; vitamin E (DL-α-tocopheryl acetate), 50 IU; vitamin K, 4.0 mg; thiamine mononitrate (B1), 4.0 mg; riboflavin (B2), 10 mg; pyridoxine HCL (B6), 5.0 mg; vitamin B12 (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 (MnSO4.H2O), 120 mg; Cu, 20 mg; Zn, 100 mg; Se, 0.3 mg; Fe (FeSO4.7H2O), 800 mg.

²Bacitracin methylene disalicylate (BMD 110; Zoetis, Parsippany, NJ): containing 55 mg of BMD per kg.
### Table 2

| In ovo injection treatment | N  | Egg weight (g) | PEWL (%) | Hatchling BW (%) | Late embryo mortality (%) | Pip embryo mortality (%) | Post-pip embryo mortality (%) | Hatchling mortality (%) |
|----------------------------|----|----------------|----------|-----------------|--------------------------|-------------------------|---------------------------|------------------------|
| Noninjected                | 10 | 60.8           | 6.7      | 96.3            | 42.9                     | 1.1                     | 1.7                       | 0                      |
| Diluent                    | 10 | 60.9           | 6.4      | 92.3            | 43.2                     | 3.7                     | 1.3                       | 0                      |
| D3                        | 10 | 61.1           | 6.3      | 97.3            | 43.5                     | 2.3                     | 0                         | 1.1                    |
| 25OHD3                     | 10 | 61.4           | 6.4      | 94.9            | 43.2                     | 2.9                     | 1.8                       | 0.6                    |
| D3 + 25OHD3                | 10 | 61.8           | 7.0      | 95.5            | 43.0                     | 2.5                     | 1.9                       | 0.2                   |

Pooled SEM: 0.30, 0.26, 1.75, 0.22, 1.93, 0.73, 0.29, 1.65

**P-value:** 0.171, 0.243, 0.304, 0.427, 0.747, 0.340, 0.445, 0.555

1. Mortality between 18 doa (432 h of incubation) and 21 doa (502 h of incubation).
2. Mortality during the pipping process at 21 doa.
3. Mortality immediately after complete emergence of hatchlings from the shell at 21 doa.

**RESULTS**

No significant treatment differences ($P > 0.05$) were observed for egg weight, 0 to 12 doi PEWL, HI, hatching BW, and hatch residue analysis (Table 2). There were also no significant ($P > 0.05$) treatment effects on the broiler performance variables of the coccidiosis-challenged broilers in the 0 to 14, 15 to 28, and 0 to 41 doa intervals (Table 3). However, coccidiosis-challenged birds injected in ovo with 2.4 $\mu g$ of 25OHD$_3$ had a higher ($P > 0.05$) BWG and ADG between 29 and 41 doa in comparison to those injected with diluent or D$_3$ alone (Table 3).

No coccidiosis lesions ($P > 0.05$) were observed in all intestinal sections before challenge at 14 doa. There were also no lesions in the ceca and no *E. acervulina* lesions in the ileum at 28 doa. Furthermore, at 28 doa, there were no significant ($P > 0.05$) treatment differences for *E. acervulina* and *maxima* lesion scores in the duodenum and jejunum, and there were no significant ($P > 0.05$) treatment differences for *E. acervulina* lesion scores in the ileum (Table 4). No coccidia oocysts were observed ($P > 0.05$) in the fecal samples taken from the noninjected and unchallenged birds at 21 (7 d post-coccidiosis challenge) and 28 doa (14 d post-coccidiosis challenge). Conversely, in challenged birds, fecal oocyst counts ranged from 93 to 121 per g of feces at 21 doa and from 59 to 81 per g of feces at 28 doa, depending on in ovo injection treatment (Figure 1). Nevertheless, there were no significant ($P > 0.05$) in ovo injection treatment differences for the fecal coccidia oocyst counts at both 7 and 14 d post-coccidiosis challenge (Figure 1).

Absolute carcass weight and the relative weights of the P. major and P. minor muscles, as well as the breast, wings, legs, thighs, and abdominal fat pad process parts
### Table 3. Effects of treatment (noninjected; diluent-injected; injected with 2.4 μg of vitamin D$_3$ (D$_3$) or 25-hydroxycholecalciferol (25OHD$_3$); and 2.4 μL of D$_3$ and 25OHD$_3$) on BW, BW gain (BWG), average daily gain (ADG), feed intake (FI), average daily feed intake (ADFI), and total mortality through 41 d of age (doa).

| In ovo injection treatment | N | BW (g) | BWG (g) | ADG (g) | FI (g) | ADFI (g) | FCR (g/g) |
|----------------------------|---|--------|---------|---------|-------|---------|----------|
| Noninjected$^1$            | 8 | 427    | 385     | 27.5    | 496   | 35.4    | 1.29     |
| Diluent$^2$                | 8 | 410    | 368     | 26.3    | 485   | 34.6    | 1.34     |
| D$_3$                      | 8 | 405    | 363     | 25.9    | 488   | 34.8    | 1.35     |
| 25OHD$_3$                  | 8 | 421    | 378     | 27.0    | 497   | 35.5    | 1.32     |
| D$_3$+25OHD$_3$            | 8 | 419    | 376     | 26.9    | 513   | 36.6    | 1.37     |
| Pooled SEM                 |   | 9.5    | 14.2    | 1.01    | 15.2  | 1.08    | 0.042    |
| P-value                    |   | 0.508  | 0.489   | 0.490   | 0.332 | 0.330   | 0.288    |

| Grower (15−28 doa)         | BW (g) | BWG (g) | ADG (g) | FI (g) | ADFI (g) | FCR (g/g) |
|----------------------------|--------|---------|---------|-------|---------|----------|
| Diluent                    | 8      | 1,390   | 980     | 70.0  | 1,623   | 116      | 1.67     |
| D$_3$                      | 8      | 1,398   | 993     | 70.9  | 1,746   | 122      | 1.63     |
| 25OHD$_3$                  | 8      | 1,469   | 1048    | 74.8  | 1,702   | 125      | 1.74     |
| D$_3$+25OHD$_3$            | 8      | 1,431   | 1014    | 72.5  | 1,750   | 125      | 1.74     |
| Pooled SEM                 | 31.5   | 28.1    | 2.00    | 67.2  | 4.8     | 0.081    |
| P-value                    | 0.282  | 0.353   | 0.353   | 0.513 | 0.512   | 0.518    |

| Finisher (29−41 doa)       | BW (g) | BWG (g) | ADG (g) | FI (g) | ADFI (g) | FCR (g/g) |
|----------------------------|--------|---------|---------|-------|---------|----------|
| Diluent                    | 8      | 2,925   | 2,881   | 70.3  | 4977    | 226      | 1.70     |
| D$_3$                      | 8      | 2,943   | 2,900   | 70.7  | 5188    | 227      | 1.76     |
| 25OHD$_3$                  | 8      | 3,096   | 3,053   | 74.5  | 5232    | 233      | 1.68     |
| D$_3$+25OHD$_3$            | 8      | 3,054   | 3,011   | 73.4  | 5304    | 234      | 1.74     |
| Pooled SEM                 | 80.4   | 83.0    | 1.43    | 145.5 | 5.0     | 0.063    |
| P-value                    | 0.098  | 0.100   | 0.100   | 0.431 | 0.578   | 0.741    |

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1. Treatment means within the same column within effect with no common superscripts are significantly different ($P < 0.05$).
2. Eggs that were not injected with diluent and that were also not challenged with coccidiosis at 14 doa.
3. Eggs injected with 50 μL of commercial diluent at d 18 of incubation (doi) and the subsequent coccidiosis challenge of chicks at 14 doa.
4. Eggs injected with 25OHD$_3$ at 18 doi and the subsequent coccidiosis challenge of chicks at 14 doa.
5. Eggs injected with of 50 μL of commercial diluent containing 2.4 μg of D$_3$ and 2.4 μg of 25OHD$_3$ at 18 doi and the subsequent coccidiosis challenge of chicks at 14 doa.

### Table 4. Effects of treatment (noninjected; diluent-injected; injected with 2.4 μg of vitamin D$_3$ (D$_3$) or 25-hydroxycholecalciferol (25OHD$_3$); and 2.4 μL of D$_3$ and 25OHD$_3$) on *Eimeria acervulina* and *maxima* lesion scores in the duodenum (D), jejunum (J), and ileum (I), and overall lesion scores in the ceca at 28 d of age (doa).

| In ovo injection treatment | N | Acervulina-D$^1$ | Maxima-D$^2$ | Acervulina-J$^3$ | Maxima-J$^4$ | Acervulina-I$^5$ | Maxima-I$^6$ | Ceca$^7$ |
|----------------------------|---|-----------------|--------------|-----------------|--------------|-----------------|--------------|---------|
| Diluent                    | 8 | 2.50            | 0            | 0.13            | 2.88         | -               | 0.25         | -       |
| D$_3$                      | 8 | 3.00            | 0.13         | 0.25            | 2.38         | -               | 0.13         | -       |
| 25OHD$_3$                  | 8 | 1.75            | 0.13         | 0.25            | 1.25         | -               | 0.50         | -       |
| D$_3$+25OHD$_3$            | 8 | 1.75            | 0            | 0.13            | 1.75         | -               | 0.38         | -       |
| Pooled SEM                 | 0.695 | 0.096          | 0.201         | 0.628           | -            | 0.194          | -            | 0.566   |
| P-value                    | 0.520 | 0.546          | 0.942         | 0.304           | -            | 0.578          | -            | 0.741   |

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1. *Eimeria acervulina* lesion score in the duodenum at 14 d of post-coccidiosis challenge.
2. *Eimeria maxima* lesion score in the duodenum at 14 d of post-coccidiosis challenge.
3. *Eimeria acervulina* lesion score in the jejunum at 14 d of post-coccidiosis challenge.
4. *Eimeria maxima* lesion score in the jejunum at 14 d of post-coccidiosis challenge.
5. *Eimeria maxima* lesion score in the ileum at 14 d of post-coccidiosis challenge.
6. *Eimeria maxima* lesion score in the ileum at 14 d of post-coccidiosis challenge.
7. No coccidiosis lesions were observed.
8. Eggs injected with 50 μL of commercial diluent at d 18 of incubation (doi) and the subsequent coccidiosis challenge of chicks at 14 doa.
9. Eggs injected with 25OHD$_3$ at 18 doi and the subsequent coccidiosis challenge of chicks at 14 doa.
10. Eggs injected with of 50 μL of commercial diluent containing 2.4 μg of D$_3$ and 2.4 μg of 25OHD$_3$ at 18 doi and the subsequent coccidiosis challenge of chicks at 14 doa.
11. Eggs injected with of 50 μL of commercial diluent containing 2.4 μg of D$_3$ and 2.4 μg of 25OHD$_3$ at 18 doi and the subsequent coccidiosis challenge of chicks at 14 doa.
of birds challenged with coccidiosis, were not significantly affected \((P > 0.05)\) by in ovo injection treatment. However, the effects of in ovo injection treatment approached significance for absolute carcass weight \((P = 0.058)\) and relative P. major muscle weight \((P = 0.059)\). Coccidiosis-challenged birds that received 25OHD₃ alone tended to have a higher carcass weight in comparison to those that were injected with diluent or D₃ alone. Additionally, in ovo injection of 25OHD₃ alone tended to increase relative P. major muscle weight when compared to the injection of diluent or D₃ alone (Table 5).

### DISCUSSION

The fat absorption in the small intestine is reduced during a coccidiosis infection (Adams et al., 1996). In addition, liver functionality is reduced in response to severe Eimeria infections (Ali, 1997). Vitamin D₃ is categorized as a fat soluble vitamin whose absorption is facilitated by the formation of micelles and the presence of bile salts (Garrett and Young, 1975). Vitamin D₃ is predominantly converted to 25OHD₃ in the hepatic cells (Booth et al., 1985), with smaller rates of conversion occurring in the intestine, kidney (Norman, 1987), and skin (Hansdottir et al., 2008), in response to 25-hydroxlase. This information indicates that fat soluble vitamin requirements may increase during a coccidiosis infection. Coccidiosis is a parasitic disease, mainly affecting the intestinal tract of many species, including chickens. Subclinical coccidiosis results in decreases in BW and feed intake, and increases in the FCR of broiler chickens (Amerah and Ravindran, 2015). Coccidiosis has also been shown to inhibit small intestine morphological development (Sharma et al., 2015), decrease cellular immunity (Morris et al., 2015), and increase inflammatory responses (Morris et al., 2014) in chickens. A decline in small intestine morphological development in response to a coccidiosis infection is associated with impaired broiler performance (Wang et al., 2019). In addition to this, a lower BWG due to a coccidiosis infection has been linked to an increase in the inflammatory response of broilers (Morris et al., 2014).

Comparison of the fecal oocyst counts between the unchallenged and challenged birds showed that under the housing conditions in this study, fecal oocysts were only observed in those birds that received a coccidiosis vaccine challenge, and that overall counts across injection treatment were reduced between 21 and 28 doa, indicating a lack of oocyst cycling. These current results reflect those of Shanmugasundaram et al. (2019), whose likewise observed similar fecal oocyst counts in turkeys that had received on oral coccidiosis vaccine and were housed in suspended cages. Conversely, Sokale et al. (2016) observed that the fecal oocyst shedding continued when birds were housed in floor pens containing used litter.

Shanmugasundaram et al. (2019) further reported that 25OHD₃ at a 110 \(\mu g/kg\) level reduced fecal oocyst counts 5 d after a coccidial vaccine challenge in turkeys. The occurrence of fewer oocysts in the feces has been shown to be associated with a decrease in coccidiosis lesion scores and improved broiler performance (Ritzi et al., 2014). Nevertheless, vitamin D-injection

### Table 5. Effects of treatment (noninjected; diluent-injected; injected with 2.4 \(\mu g\) of vitamin D₃ (D₃) or 25-hydroxycholecalciferol (25OHD₃); and 2.4 \(\mu L\) of D₃ and 25OHD₃ on carcass weight and weights of pectoralis major (P-major) and minor (P-minor) muscle, breast, wing, leg, thighs, and abdominal fat pad parts relative to carcass weight at 42 d of age (doa).

| In ovo injection treatment | N | Carcass (kg) | P-major (%) | P-minor (%) | Breast (%) | Wings (%) | Legs (%) | Thighs (%) | Fat (%) |
|---------------------------|---|-------------|-------------|-------------|------------|-----------|---------|-----------|--------|
| Diluent \(^1\)            | 47 | 2,062       | 28.2        | 5.72        | 33.9       | 10.7      | 13.5    | 17.4      | 1.54   |
| D₃ \(^2\)                 | 47 | 2,072       | 28.1        | 5.78        | 33.9       | 10.5      | 13.4    | 17.4      | 1.61   |
| 25OHD₃ \(^3\)             | 47 | 2,168       | 30.0        | 5.85        | 35.9       | 10.6      | 13.4    | 17.3      | 1.55   |
| D₃ + 25OHD₃ \(^4\)        | 47 | 2,153       | 29.2        | 5.64        | 34.8       | 10.3      | 13.1    | 16.7      | 1.58   |
| Pooled SEM                |   | 0.058       | 0.059       | 0.540       | 0.89       | 0.085     | 0.563   | 0.632     | 0.434  |

\(^1\)Eggs injected with 50 \(\mu L\) of commercial diluent at d 18 of incubation (doi) and the subsequent coccidiosis challenge of chicks at 14 doa.

\(^2\)Eggs injected with 50 \(\mu L\) of commercial diluent containing 2.4 \(\mu g\) of vitamin D₃ at 18 doi and the subsequent coccidiosis challenge of chicks at 14 doa.

\(^3\)Eggs injected with 50 \(\mu L\) of commercial diluent containing 2.4 \(\mu g\) of 25OHD₃ at 18 doi and the subsequent coccidiosis challenge of chicks at 14 doa.

\(^4\)Eggs injected with 50 \(\mu L\) of commercial diluent containing 2.4 \(\mu g\) of D₃ and 2.4 \(\mu g\) of 25OHD₃ at 18 doi and the subsequent coccidiosis challenge of chicks at 14 doa.
treatment did not significantly affect fecal oocyst counts at 7 or 14 d post-challenge. More specifically, the in ovo injection of either D3 or 25OHD3 did not affect oocyst shedding at both 7 and 14 doi. The differing results between the current and previous studies could be due to the different methods of 25OHD3 administration (in ovo injection vs. dietary supplementation), differences in the levels of administered of 25OHD3 (5 μg vs. 110 μg), and differences in the species of bird (broilers vs. turkeys) used. Among the various vitamin D3 sources, 25OHD3 has been reported to be the more potent and safer form for chickens, because it has a longer half-life (approximately 15 d) in comparison to the other forms of vitamin D3 (Mawer et al., 1969; Jones et al., 2014). It is also less toxic in comparison to 1,25(OH)2 D3 (Pesti and Shivaprasad, 2010), and does not require liver hydroxylation. In comparison to D3, 25OHD3 is more efficiently absorbed due to its greater polarity (Bar et al., 1980), and at the same level of inclusion, 25OHD3 has been shown to better promote performance (Yarger et al., 1995), protein synthesis, and breast muscle yield (Vignale et al., 2015; Fatemi, 2016) in broilers. Furthermore, dietary 25OHD3 has been reported to increase the BWG of chickens challenged with coccidiosis (Morris et al., 2014; Leyva-Jimenez et al., 2019). In addition, when compared to the in ovo injection of D3 or diluent alone, the in ovo injection of 2.4 of 25OHD3 has been shown to increase the breast meat yield (Fatemi et al., 2021a,b) and improve the live performance (Fatemi et al., 2021a) of Ross 708 broilers. This same treatment has also been shown to comparatively improve small intestine morphology (Fatemi et al., 2021c) and immunity (Fatemi et al., 2021a,c) of the broilers. In the current study, the in ovo injection of 25OHD3 resulted in an increase in the BWG and ADG of Ross 708 broilers when compared to the injection of diluent alone. Therefore, improvements in the performance of the Ross 708 broilers in response to the in ovo injection of 25OHD3 may be due to its moderation of the negative effects caused by coccidiosis.

In agreement with the results of this study, a coccidiosis challenge has been shown to result in impaired broiler performance (Amerah and Ravindran, 2015; Wang et al., 2019). In addition to its effects on performance, a reduction in breast meat yield of coccidiosis-challenged birds was observed in this study. Wang et al. (2019) reported that reductions in the breast meat yield of coccidiosis-challenged broilers can be linked to decreases in their intestinal villus height to crypt depth ratios (VCR). The small intestine morphological findings observed in studies in which coccidiosis-unchallenged (Fatemi et al., 2021c) and coccidiosis-challenged (unpublished data) birds were used, revealed that the in ovo injection of 25OHD3 increased their VCR in comparison to the in ovo injection of diluent or D3 alone. Thus, the improvement in small intestine morphology might have been a partial reason for the increase in the BWG and breast meat yield of the broilers that received 25OHD3 alone during a coccidiosis challenge. In addition to small intestine morphology, a reduction in meat yield caused by coccidiosis can be due to changes in breast muscle histomorphology. A subclinical Eimeria infection has been observed to result in a decrease in muscle fiber cross-sectional area (Chodová et al., 2018) and an increase in plasma levels of 3-methyl histidine, which is associated with muscle breakdown (Fetterer and Allen, 2001). Dietary 25OHD3 has been shown to increase muscle fiber cross-sectional area (Hutton et al., 2014), which can subsequently result in an increase in breast meat yield (Vignale et al., 2015) in broilers. In chickens, 1α-hydroxylase and 24-hydroxylase are both expressed in high amounts (Shanmugasundaram and Selvaraj, 2012), with 1α-hydroxylase converting 25OHD3 to the active hormone, 1,25(OH)2 D3. Subsequently, 1,25(OH)2 D3 is converted to the inactive form of vitamin D, 24,25-dihydroxyvitamin D3, by 24-hydroxylase (Jones et al., 2012). Jones et al. (2012) further reported that the expression of 1α-hydroxylase remained constant, whereas the expression of 24-hydroxylase was reduced in chicken breast muscle during an inflammatory response. These results indicated that 25OHD3 has a greater impact on breast meat yield in comparison to the in ovo injection of D3 alone.

In conclusion, the impact of the in ovo injection of 2.4 μg of D3 and 25OHD3 on broiler performance and meat yield of Ross 708 broilers before and after a coccidiosis challenge was investigated. Our findings revealed that a coccidiosis challenge resulted in a decrease in broiler performance and to some extent a decrease in breast meat yield. Nevertheless, regardless of challenge treatment, 2.4 μg of 25OHD3 exhibited a potential to increase the BWG and breast meat yield of birds relative to those that only received an injection of commercial diluent or D3 alone. The improvement in breast meat yield and performance observed in response to the in ovo injection of 2.4 μg of 25OHD3 may be due to its longer half-life, the greater expression of 1α-hydroxylase than 24-hydroxylase in breast meat tissue, and improvements in Ross 708 broiler immunity and small intestine morphology during a coccidiosis challenge. Further research is required to determine effects of the in ovo injection of vitamin D3 sources on immunity, small intestine morphology and gene expression of broilers during a coccidiosis challenge.

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DISCLOSURES

There is no conflict of interest.
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