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Combining Maternal and Post-Hatch Dietary 25-Hydroxycholecalciferol Supplementation on Broiler Chicken Growth Performance and Carcass Characteristics

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Abstract: Dietary inclusion of the vitamin D3 (D3) metabolite, 25-hydroxycholecalciferol (25OHD3), was demonstrated to improve broiler growth performance and breast meat yield. To assess the effect of combined maternal (MDIET) and post-hatch (PDIET) dietary 25OHD3 inclusion on broiler growth performance and carcass characteristics, a randomized complete block design experiment with a 2 × 2 factorial treatment structure was conducted. From 25 to 38 weeks of age, broiler breeder hens were provided with 1 of 2 MDIET formulated to contain: 5000 IU D3 (MCTL), or 2240 IU of D3 + 2760 IU of 25OHD3 per kg of feed (M25OHD3). Their chick offspring (n = 448; 224 per MDIET) hatched from eggs collected from 37 to 38 weeks of age were reared in 16 replicate pens with 7 birds per pen and fed 1 of 2 PDIET in 3 phases up to day 40 formulated to contain: 5000 IU of D3 per kg of feed (PCTL), or 2240 IU of D3 + 2760 IU of 25OHD3 per kg of feed (P25OHD3). No additive or synergistic effects of combining 25OHD3 inclusion in MDIET and PDIET were observed. Broilers from 25OHD3-fed hens (M25OHD3) were heavier on day 40 than those from hens fed only D3 (MCTL; 2.911 vs. 2.834 kg; p = 0.040). Tender weight (123 vs. 117 g) and yield (5.63 vs. 5.44%) were greater in the M25OHD3 broilers than the MCTL broilers (p = 0.006). Broilers fed 25OHD3 (P25OHD3) tended to have heavier breasts (637 vs. 615 g; p = 0.050), bone-in wings (215 vs. 210 g; p = 0.070), and boneless thighs (279 vs. 270 g; p = 0.078) compared with those fed only D3 (PCTL). Neither MDIET nor PDIET altered the severity of Wooden Breast and White Striping ($p \geq 0.106$). Overall, including 25OHD3 in either the maternal or broiler diet increased broiler meat yield.

Keywords: 25-hydroxycholecalciferol; broiler chicken; carcass characteristics; broiler breeder; vitamin D

1. Introduction

Broiler industry entities continuously work to improve the overall efficiency with which broilers are grown to yield heavier carcass parts using genetic selection and optimization of nutrition strategies. Previous research has demonstrated the importance of vitamin D3 (D3) inclusion in broiler diets to support their bone integrity, prevent leg abnormalities, and promote optimal broiler chicken growth [1–3]. D3 is hydroxylated by the 25-hydroxylase cytochrome P-450 enzyme in the liver and becomes the circulating form of vitamin D called 25-hydroxycholecalciferol (25OHD3) [4]. Replacing D3 with 25OHD3 in broiler diets has improved bone integrity and the growth performance of broiler chickens [5].

In addition to the benefits associated with bone mineralization, previous findings indicated that vitamin D has an important role in broiler chicken muscle growth, as vitamin D receptors (VDR) were identified in chicken myoblasts and muscle tissue [6,7]. Vignale et al. [8] demonstrated that feeding broilers 25OHD3 enhances the expression of muscle VDR and protein synthesis-related myogenic factors. Furthermore, including 25OHD3 in broiler diets improves their circulating vitamin D status, enhances the proliferation of skeletal muscle satellite cells (SC) [9,10], and increases broiler breast meat yield and weight [8,11].

Early improvement of the vitamin D status of broiler chicks through maternal feeding could be a complementary strategy to promote further muscle growth. Improving hatched chick vitamin D status via maternal 25OHD3 supplementation seems to also be dependent on breeder age [12], although previous research demonstrates that 25OHD3 can be effectively transferred to egg from hen diets [13]. However, it is unclear how combining maternal and broiler dietary 25OHD3 supplementation impacts broiler meat yield. Therefore, the objective of this experiment was to evaluate the effect of maternal and post-hatch dietary 25OHD3 supplementation on broiler growth performance and carcass characteristics.

2. Materials and Methods

All procedures regarding live birds were approved by the Auburn University Institutional Animal Care and Use Committee (PRN 2017-3012).

2.1. Broiler Breeder Hens

Commercial Ross 708 broiler breeder pullets (n = 350; Aviagen Group, Huntsville, AL, USA) were reared up to 21 weeks of age in a rearing facility kept with an average of 8 light h per d. Temperature was kept at 29 °C and gradually reduced to 21 through rearing according to the birds' comfort. Vaccination against Marek's disease was performed in ovo, and vaccination for *Eimeria* and reovirus was performed at the hatchery. During the rearing period, pullets were vaccinated against Newcastle disease, bronchitis, reovirus, and chicken infectious anemia, according to commercial recommendations. Starter (1 to 3 week; 2910 kcal/kg, 18% CP; fed ad libitum), developer (4 to 21 week; 2820 kcal/kg, 15.4% CP, 1.07% Ca; using skip-a-day feeding program), and pre-breeder (21 to 25 week; 2820 kcal/kg, 14.7% CP, 1.5% Ca; fed daily) diets without 25OHD3 were fed from week 1 to 21. At 21-week-of-age, broiler breeder hens were allotted in groups of 35 per pen (n = 10) at a stocking density of 0.22 m^2 per bird along with three Yield Plus roosters (Aviagen Group) in each pen.

At week 21, the hen photoperiod was changed to 15.25 light hours per day (19 lux) and a target temperature of 20 °C was maintained. After week 25, breeder hens and roosters were fed 1 of 2 corn-soybean meal-based maternal treatment diets (MDIET; Table 1) in meal form formulated to contain: 5000 IU of D3 per kg of feed (MCTL); or 2240 IU of D3 (Rovimix D3 500; DSM Nutritional Products Inc., Parsippany, NJ, USA) + 2760 IU of 25OHD3 (69 μg Rovimix Hy-D; DSM Nutritional Products Inc.) per kg of feed (M25OHD3; MCTL n = 5 pens; M25OHD3 n = 5 pens). Clean water was always provided ad libitum. Hens from both maternal groups were fed the same restricted amounts of feed between 110 to 170 g per bird per day according to egg production and BW. No differences among MDIET were observed for broiler breeder egg production, specific gravity, eggshell quality, egg weight, fertility, hatchability, or hatch of fertile eggs through 40-weeks-of-age ($p > 0.10$; Sweeney and Wilson, University of Georgia, unpublished data).

Table 1. Composition of broiler breeder hen laying diet.

Ingredients, %	Broiler Breeder Hen Diet [1]	
	MCTL	**M25OHD3**
Coarse ground corn	60.39	60.39
Soybean meal	17.00	17.00
Wheat middlings	7.00	7.00
Oats	4.00	4.00
Soybean oil [2]	1.30	1.30
Limestone (fine)	3.80	3.80
Oyster shell (coarse)	3.80	3.80
Dicalcium Phosphate	1.70	1.70
Salt	0.30	0.30
DL-Methionine	0.19	0.19
L-Threonine	0.04	0.04
Choline chloride 60%	0.10	0.10
Trace mineral premix [3]	0.10	0.10
Vitamin premix [4]	0.03	0.03
D3 premix [5]	0.25	-
25OHD3 premix [6]	-	0.25
Total	100.00	100.00
Calculated nutrients		
Vitamin D3, IU per kg of diet	5000.0	2240.0
25-Hydroxycholecalciferol, IU per kg	-	2760.0
Crude protein, %	14.8	14.8
Ca, %	3.4	3.4
P, %	0.6	0.6
Available P, %	0.3	0.3
ME, kcal per kg of diet	2735.0	2735.0
Digestible lysine, %	0.6	0.6
Digestible methionine, %	0.4	0.4
Analyzed Nutrients [7]		
Vitamin D3, IU per kg of diet	4413.0	1760.0
25-Hydroxycholecalciferol, IU per kg	-	1732.0

[1] Fed to breeder hens and roosters starting week 25. MCTL = maternal control diet; M25OHD3 = maternal 25-hydroxycholecalciferol diet (vitamin D contents below [5,6]); [2] Soybean oil included in mixer; [3] Supplied per kg of diet: Mn, 109 mg as Mn sulfate; Zn, 90 mg as Zn sulfate; Fe, 27 mg as ferrous sulfate; Cu 7 mg as basic Cu chloride; I, 1.3 mg as ethylenediamine hydroiodide; Se, 0.3 mg as sodium selenite; [4] Supplied per kg of diet: vitamin A, 11,000 IU; vitamin D3, 2240 IU; vitamin E, 63 mg; vitamin K, 4.2 mg; thiamine, 3 mg; riboflavin, 12 mg; pyridoxin, 4.2 mg; cobalamin, 0.03 mg; niacin, 55 mg; pantothenic acid, 18.3 mg; folic acid, 2.8 mg; biotin, 0.46 mg; [5] Supplied per kg of diet: 2760 IU of D3 in addition to 2240 IU already provided in the D3 premix; [6] Supplied per kg of diet: 69 µg of 25-hydroxycholecalciferol equivalent to 2760 IU; [7] Analyzed by DSM Nutritional Products TMAS laboratory, Belvidere, NJ, USA.

2.2. Broiler Husbandry

Fertile eggs were collected from both MDIET group pens during the weeks 37 and 38, stored at 18.5 °C, and incubated at 37.5 °C with a relative humidity of 53% in Natureform incubators (Natureform Inc., Jacksonville, FL, USA), rotated 45° every hour, transferred to a hatcher machine at incubation day 18, and then hatched at a temperature of 37 °C. Male (n = 112) and female (n = 112) broiler chicks from each MDIET were allotted to group pens (n = 64; n = 8 replicate pens of each sex per treatment) at a stocking density of 0.36 m^2 per bird (n = 7 per pen). The broiler house temperature was reduced from 32 °C to 20 °C, according to the birds' comfort, and an average relative humidity of 50.3% was kept through the experiment. A lighting program consisted of 23 light h at 30 lux during the first 7 days, and then 18 h of light at 10 lux was maintained until day 40. Broilers from each MDIET were fed ad libitum one of two corn-soybean meal-based broiler post-hatch treatment diets formulated to contain (PDIET; Table 2): 5000 IU of D3 (Rovimix D3 500; DSM Nutritional Products Inc.) per kg of feed (PCTL), or 2240 IU of D3 + 2760 IU of 25OHD3 (P25OHD3; 69 µg Rovimix Hy-D; DSM Nutritional Products Inc.) per kg of feed.

Thus, a total of four factorially arranged treatments were obtained to assess the interaction of MDIET × PDIET (n = 16 pens per treatment; n = 8 per sex). Broiler chickens were fed a crumbled starter diet from day 0 to 14, a pelleted grower diet from day 15 to 28, and a pelleted finisher diet from day 29 to 40 (Table 2). Feed samples from all maternal and broiler diets were analyzed by DSM Nutritional Products TMAS Laboratory (Belvidere, NJ, USA) for D3 contents using the AOAC official method: 2011.12 [14] and HPLC and mass spectroscopy procedure for 25OHD3 [15]. The analyzed D3 and 25OHD3 concentrations for broiler breeder and broiler feed from each phase are shown in Tables 1 and 2, respectively. There was more variation among the formulated and as-fed concentrations of D3 and 25OHD3 than expected. Though the as-fed concentrations were consistently lower than those formulated, they did not differ sufficiently to undermine the overall interpretation of the results.

Table 2. Composition of post-hatch broiler diets for starter, grower, and finisher periods.

Ingredients, %	Broiler Post-Hatch Diet [1]					
	Starter (Day 0 to 14)		Grower (Day 15 to 28)		Finisher (Day 29 to 40)	
	PCTL	**P25OHD3**	**PCTL**	**P25OHD3**	**PCTL**	**P25OHD3**
Ground corn	50.96	50.96	53.80	53.80	61.43	61.43
Soybean meal	38.50	38.50	33.20	33.20	24.50	24.50
Dried distiller grains	3.00	3.00	4.60	4.60	6.20	6.20
Soybean oil [2]	2.50	2.50	3.00	3.00	3.00	3.00
Dicalcium phosphate	1.80	1.80	2.50	2.50	2.00	2.00
Limestone (fine)	1.50	1.50	1.20	1.20	1.20	1.20
Salt	0.40	0.40	0.40	0.40	0.40	0.40
DL-Methionine	0.35	0.35	0.28	0.28	0.32	0.32
L-Lysine	0.15	0.15	0.20	0.20	0.10	0.10
L-Threonine	0.04	0.04	0.02	0.02	0.05	0.05
Choline chloride 60%	0.10	0.10	0.10	0.10	0.10	0.10
Trace mineral premix [3]	0.10	0.10	0.10	0.10	0.10	0.10
No-D3 vitamin premix [4]	0.05	0.05	0.05	0.05	0.05	0.05
D3 premix [5]	0.50	-	0.50	-	0.50	-
25OHD3 premix [6]	-	0.50	-	0.50	-	0.50
Amprolium (coccidiostat)	0.05	0.05	0.05	0.05	0.05	0.05
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutrients						
Vitamin D3, IU per kg	5000.0	2240.0	5000.0	2240.0	5000.0	2240.0
25-Hydroxycholecalciferol, IU per kg	-	2760.0	-	2760.0	-	2760.0
Crude protein, %	23.4	23.4	22.5	22.5	18.2	18.2
Ca, %	1.0	1.0	1.0	1.0	0.9	0.9
Available P, %	0.5	0.5	0.5	0.5	0.4	0.4
ME, kcal per kg of diet	2940.0	2940.0	3000.0	3000.0	3080.0	3080.0
Digestible lysine, %	1.2	1.2	1.1	1.1	0.9	0.9
Digestible methionine, %	0.6	0.6	0.5	0.5	0.5	0.5
Analyzed nutrients [7]						
Vitamin D3, IU per kg	4390.0	2144.0	3794.0	2033.0	3728.0	1803.0
25-Hydroxycholecalciferol, IU per kg	-	1978.0	-	2222.0	-	2092.0

[1] Starter (crumble), day 0 to 14; grower (pellet), day 15 to 28; finisher (pellet), day 29 to 40. PCTL = post-hatch control diet; P25OHD3 = post-hatch 25-hydroxycholecalciferol diet (vitamin D contents below [5,6]); [2] Sprayed 1.5% post-pellet in starter; sprayed 2% post-pellet in grower and finisher; [3] Supplied per kg of diet: Mn, 109 mg as Mn sulfate; Zn, 90 mg as Zn sulfate; Fe, 27 mg as ferrous sulfate; Cu 7 mg as basic Cu chloride; I, 1.3 mg as ethylenediamine hydroiodide; Se, 0.3 mg as sodium selenite; [4] Supplied per kg of diet: vitamin A, 12,474 IU; vitamin E, 100 mg; vitamin K, 4 mg; thiamine, 3 mg; riboflavin, 8 mg; pyridoxin, 4 mg; cobalamin, 0.03 mg; niacin, 60 mg; pantothenic acid, 15 mg; folic acid, 2.5 mg; biotin, 0.4 mg; [5] Supplied per kg of diet: 5000 IU of vitamin D_3; [6] Supplied per kg of diet: 69 µg of 25-hydroxycholecalciferol equivalent to 2760 IU; [7] Analyzed by DSM Nutritional Products TMAS laboratory, Belvidere, NJ, USA.

2.3. Broiler Growth Performance and Carcass Characteristics

Birds were individually weighed and residual feed weights were recorded to determine the mortality corrected (using chick days) body weight (MCBW), fasted empty live BW (ELBW) at day 40, body weight gain (MCBWG), feed intake (MCFI), and feed conversion ratio (MFCR; MCFI/MCBWG; feed:gain). These data were determined for the starter (day 0 to 14), grower (day 15 to 28), finisher (day 29 to 40), and overall (day 0 to 40) periods. Broiler chickens were fasted for 8 h prior to being weighed at day 40 and processed to measure the weights of the hot and cold carcasses without giblets (HWOG and CWOG, respectively), skinless breasts, tenders, abdominal fat pads, thighs (boneless and skinless), drums (bone-in), and wings (bone-in). Average weights of these parts and their yields as proportions (%) of CWOG were also calculated. Breast fillets were scored for Wooden Breast (WB) and White Striping (WS) meat quality defects using a palpation and visual evaluation system, respectively. A four-point scale (0 = normal; 1 = mild; 2 = moderate; 3 = severe) for WB and WS was used. WB and WS scores of 0 were assigned to fillets considered normal and that exhibited complete absence of the defect. Score 1, the breasts were mildly affected (up to 1/3 of the fillet was affected), Score 2, the breasts were moderately affected (up to 2/3 of the fillet was affected), and Score 3, the fillets were severely affected (whole fillet was affected). All breast fillets were evaluated and scored by one trained and experienced evaluator.

2.4. Statistical Analysis

A 2-way analysis of variance analysis was performed using a generalized linear mixed model (GLIMMIX) procedure with the Statistical Analysis Software (SAS) version 9.4 (SAS Institute Inc., Cary, NC, USA) where MDIET, PDIET, and MDIET $\times$ PDIET were the main effects. Pen (n = 64; 16 replicate pens per treatment) served as experimental unit. Pairwise mean comparisons were performed using the PDIFF option of SAS. Proportional data analysis was performed using events/possible syntax with a binomial distribution and all data were analyzed using an R-side covariance structure in SAS. Significant differences between means were declared when $p \leq 0.05$. Tendencies for differences among treatment means were declared when $0.0501 < p \leq 0.10$.

3. Results and Discussion

3.1. Combining Maternal and Post-Hatch 25-Hydrocholecalciferol Supplementation

3.1.1. Growth Performance

No interaction between the MDIET and PDIET was observed for broiler MCBW, MCBWG, or MCFCR for any of the feeding periods evaluated ($p \geq 0.297$, Table 3). However, P25OHD3-fed broilers from the breeders fed 25OHD3 (M25OHD3:P25OHD3) tended to consume more feed during the finisher period (d 29 to 40) compared with those from MCTL-fed hens (p = 0.088), though their overall (d 0 to 40) feed intake was not affected (p = 0.727).

Table 3. Effect of combined maternal and post-hatch dietary 25-hydroxycholecalciferol inclusion on broiler growth performance.

Variable [1]	Dietary Treatment Combination [2]				SEM	*p*-Value
	MCT: PCTL	MCTL: P25OHD3	M25OHD3: PCTL	M25OHD3: P25OHD3		
d 0 BW, g	42	41	42	41	0.3	0.429
d 14 BW, g	433	423	426	425	5	0.432
d 28 BW, g	1558	1543	1560	1583	19	0.297
d 40 ELBW, g	2840	2828	2868	2953	38	0.190

Table 3. *Cont.*

Variable [1]	Dietary Treatment Combination [2]				SEM	*p*-Value
	MCT: PCTL	**MCTL: P25OHD3**	**M25OHD3: PCTL**	**M25OHD3: P25OHD3**		
Starter (d 0 to 14)						
MCFI, g	536	531	527	529	8	0.709
MCBWG, g	386	378	381	377	6	0.767
MCFCR	1.3922	1.4047	1.3851	1.4053	0.02	0.816
Grower (d 15 to 28)						
MCFI, g	1599	1572	1600	1598	21	0.545
MCBWG, g	1121	1109	1115	1125	19	0.564
MCFCR	1.4282	1.4186	1.4283	1.4237	0.01	0.796
Finisher (d 29 to 40)						
MCFI, g	2218 [y]	2178 [y]	2243 [xy]	2300 [x]	29	0.088
MCBWG, g	1277	1271	1282	1341	26	0.205
MCFCR	1.7402	1.7165	1.7233	1.7205	0.02	0.509
Overall (d 0 to 40)						
MCFI, g	4276	4213	4245	4233	73	0.727
MCBWG, g	2739	2719	2704	2733	51	0.629
MCFCR	1.5626	1.5506	1.5650	1.5508	0.01	0.896

[1] BW = average live body weight; MCFI = mortality corrected feed intake (amount fed—residual); MCBWG = mortality corrected body weight gain (final BW—initial BW); MCFCR = mortality corrected feed conversion ratio (MCFI/MCBWG); [2] MCTL = maternal control (5000 IU of D3 per kg of laying hen breeder feed); M25OHD3 = maternal 25OHD3 (2760 IU of 25OHD3 + 2240 of D3 per kg of laying hen breeder feed); PCTL = post-hatch control (5000 IU of D3 per kg of broiler chicken feed); P25OHD3 = post-hatch 25OHD3 (2760 IU of 25OHD3 + 2240 of D3 per kg of broiler chicken feed); [x,y] Means within row with different superscripts differ at $0.0501 \leq p \leq 0.10$).

3.1.2. Carcass Characteristics

Combining maternal and post-hatch dietary treatments did not impact the weight and yields of HWOG, CWOG and carcass parts ($p \geq 0.113$; Table 4). As illustrated in Figures 1 and 2, most broilers were affected with Wooden Breast myopathy and White Striping. However, the mean scores (Table 4) and severity for both meat quality defects, Wooden Breast (Figure 1A) and White Striping (Figure 1B), were not altered by the interaction of MDIET and PDIET ($p \geq 0.302$ and $p \geq 0.600$, respectively).

Table 4. Effect of combined maternal and post-hatch dietary 25-hydroxycholecalciferol inclusion on broiler carcass characteristics at day 40 post-hatch.

Variable [1]	Dietary Treatment Combination [2]				SEM	*p*-Value
	MCTL: PCTL	**MCTL: P25OHD3**	**M25OHD: PCTL**	**M25OHD3: P25OHD3**		
HWOG, g	2146	2155	2145	2206	31	0.399
CWOG, g	2144	2159	2148	2211	31	0.428
Abdominal fat pads, g	34	32	33	34	1	0.403
Breast (boneless, skinless), g	613	627	618	646	11	0.508
Tenders, g	117	117	121	125	2	0.297
Wings (bone-in), g	209	211	210	219	3	0.251
Thighs (boneless), g	272	277	269	280	5	0.496
Drums (bone-in), g	244	247	246	253	4	0.592
CWOG/HWOG, %	99.7	99.4	99.6	100.0	0.2	0.113
HWOG/ELBW, %	75.6	76.3	74.8	74.7	0.4	0.338
CWOG/ELBW, %	75.5	76.4	74.9	74.9	0.4	0.246

Table 4. *Cont.*

Variable [1]	Dietary Treatment Combination [2]				SEM	*p*-Value
	MCTL: PCTL	MCTL: P25OHD3	M25OHD: PCTL	M25OHD3: P25OHD3		
Abdominal fat pads/CWOG, %	1.6	1.5	1.5	1.5	0.04	0.574
Breasts/CWOG, %	28.6	29.1	28.8	29.2	0.2	0.995
Tenders/CWOG, %	5.5	5.4	5.6	5.6	0.1	0.612
Wings/CWOG, %	9.8	9.8	9.8	9.9	0.1	0.478
Thighs/CWOG, %	12.7	12.8	12.5	12.7	0.1	0.899
Drums/CWOG, %	11.4	11.4	11.5	11.4	0.1	0.712
Mean Wooden Breast score [3]	2.0	2.1	2.1	2.2	0.1	0.990
Mean White Striping score [3]	1.1	1.1	1.1	1.1	0.1	0.627

[1] ELBW = empty live body weight on day 40; HWOG = hot carcass weight without giblets; CWOG = chilled carcass weight without giblets; [2] MCTL = maternal control (5000 IU of D3 per kg of laying hen breeder feed); M25OHD3 = maternal 25OHD3 (2760 IU of 25OHD3 + 2240 of D3 per kg of laying hen breeder feed); PCTL = post-hatch control (5000 IU of D3 per kg of broiler chicken feed); P25OHD3 = post-hatch 25OHD3 (2760 IU of 25OHD3 + 2240 of D3 per kg of broiler chicken feed); [3] Wooden Breast and White Striping scores determined with a palpation and visual scoring system, respectively: Score 0 = normal; Score 1 = mild; Score 2 = moderate; Score 3 = severe.

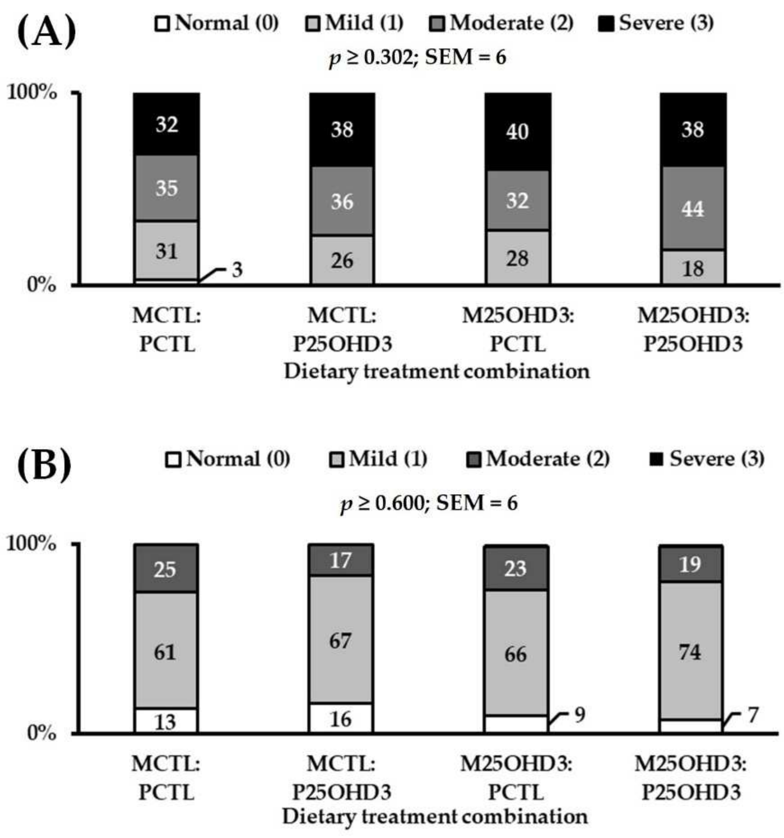

Figure 1. Effect of combining maternal and post-hatch 25-hydroxycholecalciferol supplementation on broiler Wooden Breast (**A**) and White Striping (**B**) score incidence and severity. n = 16 pens per dietary MDIET × PDIET treatment. CTL diets were formulated to provide 5000 IU of D3 per kg of feed for either the breeder hen (MCTL) or broiler (PCTL) control diets, while 25OHD3 diets were formulated to provide 2760 IU of 25OHD3 + 2240 IU of D3 per kg of feed for either the breeder hen (M25OHD3) or the broiler (P25OHD3) diets.

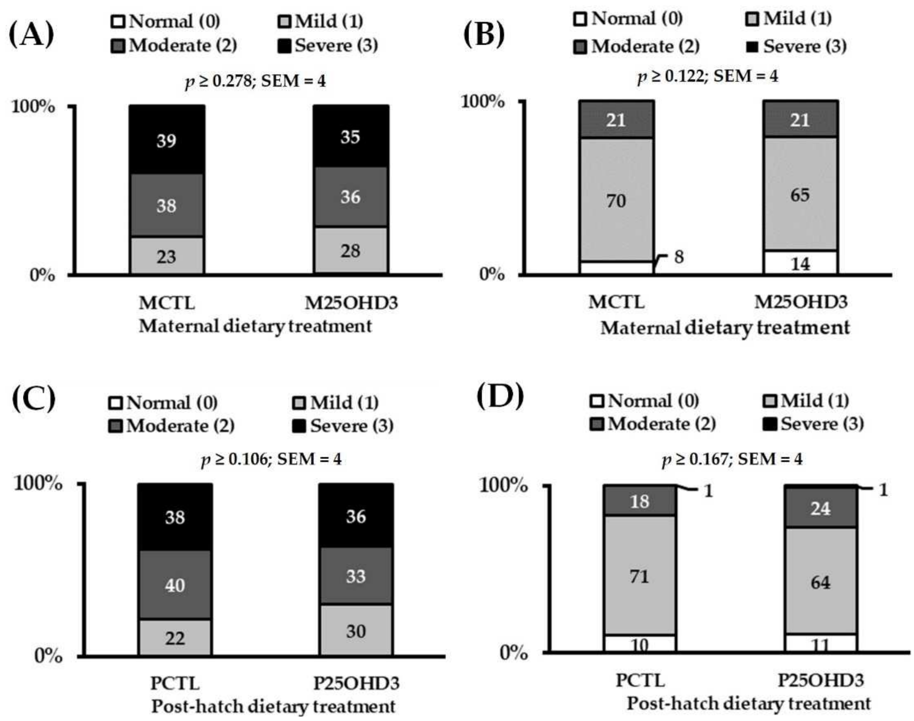

Figure 2. Effects of maternal (**A**,**B**) and post-hatch (**C**,**D**) dietary 25-hydroxycholecalciferol inclusion on broiler Wooden Breast (**A**,**C**) and White Striping (**B**,**D**) score incidence and severity. n = 32 pens per dietary main effect treatment (MDIET or PDIET). CTL diets were formulated to provide 5000 IU of D3 per kg of feed for either the breeder hen (MCTL) or broiler diets (PCTL), while 25OHD3 diets were formulated to provide 2760 IU of 25OHD3 + 2240 IU of D3 per kg of feed for either the breeder hen (M25OHD3) or the broiler diet (P25OHD3).

In the present study, no additive or synergistic effects of combined material and post-hatch 25OHD3 supplementation on broiler carcass characteristics were observed ($p \geq 0.113$; Table 4). Interestingly, a similar maternal and post-hatch factorial experiment describing the effect of combined 25OHD3 and canthaxanthin on broiler progeny performance demonstrated reported greater BWG, lower FCR, and higher carcass and breast yields of the broiler progeny when either feeding the breeder hens with 25OHD3 + canthaxanthin, or feeding broilers the same ingredient combinations [16]. These researchers also observed a synergistic effect of breeder and broiler supplementation on growth performance variables ($p \leq 0.032$), carcass, and breast yields ($p \leq 0.036$) when eggs were collected at breeder ages of week 35, 45, and 62. However, performance and carcass traits responses were attributed to feeding a combination of 25OHD3 + canthaxanthin in addition to basal concentrations of D3 in feed, and not when partially replacing D3 with 25OHD3, as in the current experiment.

3.2. Maternal 25-Hydroxycholecalciferol Supplementation

3.2.1. Growth Performance

Table 5 contains the results of growth performance of broilers from breeder hen vitamin D supplementation (MDIET). In this experiment, M25OHD3 dietary treatment did not affect hatched chick BW (41 vs. 41 g; p = 0.546). Saunders-Blades and Korver [12] reported lower chick hatched BW when derived from young (21 to 33 week-old) and older (46 to 48 and 61 to 63 week-old) D3-fed hens compared with those from 25OHD3-fed hens. Though overall day 0 to 40 MCFI and MCFCR were unaltered by maternal vitamin D supplementation ($p \geq 0.684$), broilers from the M25OHD3-fed breeders consumed more feed during the finisher period (d 29 to 40; p = 0.011) and were heavier at day 40 compared with those from MCTL-fed hens (2911 vs. 2834 g; p = 0.040). Overall, the literature would suggest that improvement of hen vitamin D status by increasing dietary vitamin D is most beneficial to the offspring of very young (<32 week) and older (>40 week) broiler breeders [12,17]. The lack of consistent responses regarding the impact of maternal vitamin D supplementation and its impact on the growth performance of broiler offspring suggests that there is much more to learn in this area.

Table 5. Effect of maternal dietary 25-hydroxycholecalciferol inclusion on broiler growth performance.

Variable [1]	**Maternal Diet** [2]		**SEM**	***p*-Value**
	MCTL	**M25OHD3**		
d 0 BW, g	41	41	0.2	0.546
d 14 BW, g	428	426	4	0.684
d 28 BW, g	1551	1571	13	0.253
d 40 ELBW, g	2834 [b]	2911 [a]	26	0.040
Starter (day 0 to 14)				
MCFI, g	533	528	6	0.529
MCBWG, g	382	379	4	0.626
MCFCR	1.3984	1.3952	0.01	0.844
Grower (day 15 to 28)				
MCFI, g	1585	1599	15	0.518
MCBWG, g	1115	1120	13	0.794
MCFCR	1.4234	1.4260	0.01	0.789
Finisher (day 29 to 40)				
MCFI, g	2198 [b]	2272 [a]	20	0.011
MCBWG, g	1274	1312	18	0.149
MCFCR	1.7284	1.7219	0.01	0.684
Overall (day 0 to 40)				
MCFI, g	4245	4239	51	0.939
MCBWG, g	2729	2718	36	0.828
MCFCR	1.5566	1.5579	0.01	0.876

[1] BW = live body weight; MCFI = mortality corrected feed intake; MCBWG = mortality corrected body weight gain; MCFCR = mortality corrected feed conversion ratio (MCFI/MCBWG); [2] MCTL = maternal control (5000 IU of D3 per kg of laying hen breeder feed); M25OHD3 = maternal 25OHD3 (2760 IU of 25OHD3 + 2240 of D3 per kg of laying breeder hen feed); [a,b] Means within a row with unlike letters are declared different ($p \leq 0.05$).

3.2.2. Carcass Characteristics

Table 6 illustrates the effect of maternal 25OHD3 supplementation (MDIET) on broiler carcass characteristics. Feeding breeder hens 25OHD3 increased their broiler offspring tender weight (123 vs. 117 g; $p = 0.006$) and yield as a proportion of CWOG (5.6 vs. 5.4%) compared with those broilers derived from MCTL-fed hens ($p = 0.002$; Table 6). Even with HWOG and CWOG yield results being different between MDIET broiler groups, these results suggest that maternal dietary 25OHD3 supplementation exerts a positive effect on their broiler offspring ELBW at day 40, and tender yield and weights. Feeding 25OHD3 to breeder hens may increase the metabolite carryover to the egg [13], and have a similar stimulatory effect on early offspring chick skeletal muscle development, as observed in previous broiler dietary experiments [8–11]. Perhaps feeding 25ODH3 to hens influences the embryonic myoblast kinetics, which result in alterations in the secondary skeletal muscle fiber numbers and/or has lasting effects that influence muscle satellite cell function during the post-hatch rearing period. Regardless, understanding how these changes occur will require further investigation. Wooden Breast and White Striping scores ($p \geq 0.451$; Table 6), as well as incidence and severity ($p \geq 0.122$; Figure 2A,B), were unaffected by maternal supplementation of 25OHD3.

Table 6. Effect of maternal dietary 25-hydroxycholecalciferol inclusion on broiler carcass characteristics at day 40 post-hatch.

Variable [1]	Maternal Diet [2]		SEM	*p*-Value
	MCTL	M25OHD3		
HWOG, g	2151	2175	22	0.420
CWOG, g	2151	2180	21	0.348
Abdominal fat pads, g	33	33	1	0.589
Breast (boneless, skinless), g	620	632	8	0.264
Tenders, g	117 [b]	123 [a]	1	0.006
Wings (bone-in), g	210	215	2	0.166
Thighs (boneless), g	274	275	3	0.947
Drums (bone-in), g	245	250	3	0.246
CWOG/HWOG, %	99.6	99.9	0.1	0.151
HWOG/ELBW, %	76.0 [a]	74.7 [b]	0.3	0.006
CWOG/ELBW, %	76.0 [a]	74.9 [b]	0.3	0.011
Abdominal fat pads/CWOG, %	1.5	1.5	0.03	0.891
Breasts/CWOG, %	28.8	29.0	0.2	0.477
Tenders/CWOG, %	5.4 [b]	5.6 [a]	0.0	0.004
Wings/CWOG, %	9.8	9.8	0.1	0.370
Thighs/CWOG, %	12.7	12.6	0.1	0.212
Drums/CWOG, %	11.4	11.5	0.1	0.541
Average Wooden Breast score [3]	2.1	2.1	0.1	0.451
Average White Striping score [3]	1.1	1.1	0.05	0.634

[1] ELBW = empty live body weight on day 40; HWOG = hot carcass weight without giblets; CWOG = chilled carcass weight without giblets; [2] MCTL = maternal control (5000 IU of D3 per kg of laying hen breeder feed); M25OHD3 = maternal 25OHD3 (2760 IU of 25OHD3 + 2240 of D3 per kg of laying breeder hen feed); [3] Wooden Breast and White Striping scores determined with a palpation and visual scoring system, respectively: Score 0 = normal; Score 1 = mild; Score 2 = moderate; Score 3 = severe; [a,b] Means within a row with unlike letters are declared different ($p \leq 0.05$).

3.3. Broiler 25-Hydroxycholecalciferol Supplementation

3.3.1. Growth Performance

The growth performance of broilers fed with either PCTL or P25OHD3 did not differ during all periods ($p \geq 0.103$; Table 7). These results contrast broiler dose-response experiments, in which replacing lower doses of D3 ($\leq$ 2760 IU per kg of feed) with 25OHD3 resulted in higher broiler BW and BWG [11,18]. However, our results are comparable to those observed in a dose–response experiment conducted by Fritts and Waldroup [19], in which replacing 2000 or 4000 IU of D3 with 25OHD3 in broiler diets seems to be ineffective for improving growth performance of broilers. Together with previous experiments, it is possible that broiler dietary 25OHD3 can improve growth performance, but only when replacing lower doses of D3.

Table 7. Effect of post-hatch dietary 25-hydroxycholecalciferol inclusion on broiler growth performance.

Variable [1]	Post-Hatch Diet [2]		SEM	*p*-Value
	PCTL	P25OHD3		
Day 0 BW, g	42	41	0.2	0.103
Day 14 BW, g	429	424	4	0.306
Day 28 BW, g	1559	1563	13	0.814
Day 40 ELBW, g	2854	2891	26	0.321
Starter (day 0 to 14)				
MCFI, g	531	530	6	0.815
MCBWG, g	383	377	4	0.311
MCFCR	1.3886	1.4050	0.01	0.323

Table 7. *Cont.*

Variable [1]	Post-Hatch Diet [2]		SEM	*p*-Value
	PCTL	P25OHD3		
Grower (day 15 to 28)				
MCFI, g	1599	1585	14	0.479
MCBWG, g	1118	1117	13	0.966
MCFCR	1.4282	1.4212	0.01	0.466
Finisher (day 29 to 40)				
MCFI, g	2230	2240	20	0.735
MCBWG, g	1279	1306	18	0.307
MCFCR	1.7318	1.7185	0.01	0.405
Overall (day 0 to 40)				
MCFI, g	4261	4223	51	0.604
MCBWG, g	2721	2726	36	0.926
MCFCR	1.5638	1.5507	0.01	0.128

[1] BW = average live body weight; MCFI = mortality corrected feed intake; MCBWG = mortality corrected body weight gain; MCFCR = mortality corrected feed conversion ratio (MCFI/MCBWG); [2] PCTL = post-hatch control (5000 IU of D3 per kg of broiler feed); P25OHD3 = post-hatch 25OHD3 (2760 IU of 25OHD3 + 2240 of D3 per kg of broiler feed).

3.3.2. Carcass Characteristics

Feeding broilers 25OHD3 (P25OHD3; Table 8) metabolite tended to increase their breast weights ($p = 0.050$) and yield as a proportion of CWOG ($p = 0.053$), bone-in wings ($p = 0.070$) and boneless thigh weights ($p = 0.078$; Table 5). These results are consistent with those of Yarger et al. [11] in which an increase of 0.53 to 0.83% of breast yield (≥48 day of age) in broilers fed 25OHD3 instead of D3 ($p < 0.05$) was observed. Our observations are also congruent with those of Vignale et al. [8], in which feeding 5520 IU of 25OHD3 per kg of feed to broilers for 42 days increased the breast meat yield compared with broilers fed 5520 IU of D3 per kg of feed ($p < 0.05$). Maternal or post-hatch dietary 25OHD3 supplementation did not affect Wooden Breast and White Striping average scores and severity (%) of broiler breasts ($p \geq 0.106$; Table 5; Figure 2C,D). These results suggest that broiler dietary 25OHD3 supplementation increases breast weight and yield as previously demonstrated, but also weights of other carcass components such as wings and thighs.

Table 8. Effect of post-hatch dietary 25-hydroxycholecalciferol inclusion on broiler carcass characteristics at day 40 post-hatch.

Variable [1]	Post-Hatch Diet [2]		SEM	*p*-Value
	PCTL	P25OHD3		
HWOG, g	2145	2180	22	0.252
CWOG, g	2146	2185	21	0.194
Abdominal fat pads, g	33	33	1	0.680
Breast (boneless, skinless), g	615 ^{y}	637 ^{x}	8	0.050
Tenders, g	119	121	1	0.312
Wings (bone-in), g	210 ^{y}	215 ^{x}	2	0.070
Thighs (boneless), g	270 ^{y}	279 ^{x}	3	0.078
Drums (bone-in), g	245	250	3	0.214
CWOG/HWOG, %	99.6	99.9	0.1	0.290
HWOG/ELBW, %	75.2	75.5	0.3	0.447
CWOG/ELBW, %	75.2	75.7	0.3	0.268

Table 8. *Cont.*

Variable [1]	Post-Hatch Diet [2]		SEM	p-Value
	PCTL	P25OHD3		
Abdominal fat pads/CWOG, %	1.6	1.5	0.03	0.276
Breasts/CWOG, %	28.7 ^{y}	29.1 ^{x}	0.2	0.053
Tenders/CWOG, %	5.5	5.5	0.0	0.885
Wings/CWOG, %	9.8	9.9	0.1	0.285
Thighs/CWOG, %	12.6	12.7	0.1	0.213
Drums/CWOG, %	11.4	11.4	0.1	0.970
Average Wooden Breast score [3]	2.1	2.1	0.1	0.357
Average White Striping score [3]	1.1	1.1	0.05	0.341

[1] HWOG = hot carcass weight without giblets, CWOG = chilled carcass weight without giblets; [2] PCTL = post-hatch control (5000 IU of D3 per kg of broiler chicken feed); P25OHD3 = post-hatch 25OHD3 (2760 IU of 25OHD3 + 2240 of D3 per kg of broiler chicken feed); [3] Wooden Breast and White Striping scores determined with a palpation and visual scoring method, respectively: Score 0 = normal; Score 1 = mild; Score 2 = moderate; Score 3 = severe; ^{x,y} Means within row with unlike letters tend to be different ($0.0501 \leq p \leq 0.10$).

Though no additive or synergistic MDIET × PDIET effects were observed for carcass characteristics, the separate inclusion of 25OHD3 in either the maternal or broiler diet increased tender and breast, wing, and thigh weights, respectively. Together, the changes observed here in carcass parts represent potential for substantial increases in revenue to the poultry industry, as the breasts, tenders, and wings are considered some of the most valuable parts of broiler chicken carcass, particularly in the US marketplace [20,21]. For example, feeding breeder hens 25OHD3 resulted in 6 g of extra tender per broiler compared with broilers from the MCTL-fed breeders (123 vs. 117 g). If tenders are assumed to be market-valued at US$ 3.43 per kg (USDA, 2020), replacing roughly half of the D3 in the broiler breeder diets in a typical complex that processes 1 million birds per week would result in US$ 20,580 of additional revenue. In addition, the inclusion of 25OHD3 in broiler diets resulted in an increase of 21 g of breast meat, which, if breast meat is valued at US$ 1.65 per kg, equates to US$ 34,650 additional revenue for that single complex. A more thorough economic analysis of the cost to benefit ratio of 25OHD3 inclusion in commercial broiler integrator diets is beyond the scope of this work. However, these two simple examples highlight the potential positive economic impact of partially replacing D3 with 25OHD3 in breeder hen and broiler diets.

Overall, no additive or synergistic effects of combining maternal and post-hatch dietary 25OHD3 supplementation on growth performance or carcass characteristics of broilers from young broiler breeder hens were observed in this study. Perhaps the lack of an interactive effect is because this study only focused on the offspring of relatively young (36 to 38-week-old) broiler breeder hens fed for 10 weeks prior to egg collection. It is possible that there may be interactive effects in a follow-up study with offspring from hens fed through the typical end of a commercial laying period. The literature would suggest that the inclusion of 25OHD3 in breeder diets is more beneficial to the offspring of either very young or older broiler breeder hens [12,17]. Regardless, tender weight and yield in broilers from 25OHD3-fed hens, as well as greater breast, wing, and thigh weights in broilers fed a combination of 25OHD3 and D3 post-hatch were observed in this study. The results of this study are overall consistent with the current literature, in terms of the impact of feeding 25OHD3 to broilers post-hatch. However, further research will be necessary to determine the optimal strategy for vitamin D supplementation to maximize both commercial broiler breeder reproductive performance and health, as well as broiler offspring growth performance and carcass yields.

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