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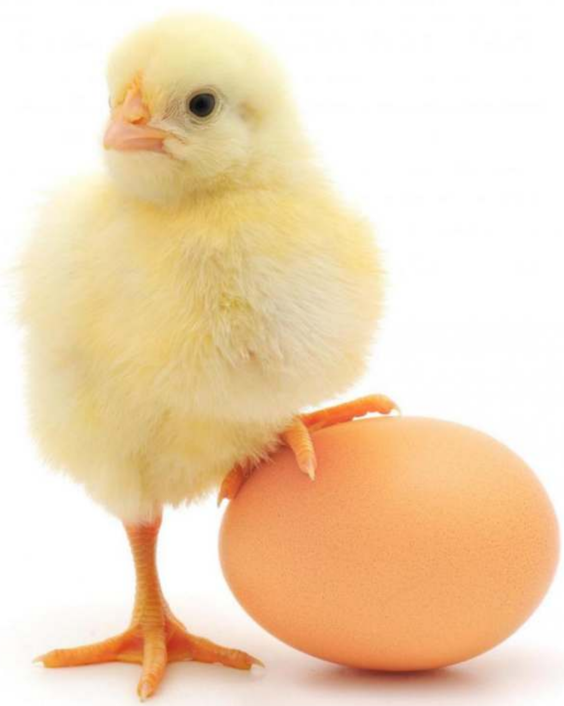

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Effect of Source and Level of Vitamin D on the Performance of Breeder Hens and the Carryover to the Progeny[1]

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Abstract: A study was conducted to evaluate the effect of the vitamin D level and source on the performance of broiler breeders and the deposition of this vitamin in egg yolk. Pullets reaching sexual maturity were depleted of vitamin D stores by feeding a vitamin D deficient diet during an eight week period. Following depletion, an experimental design was utilized consisting of a 5 x 2 factorial arrangement with four levels of dietary cholecalciferol (0, 300, 600, 1200 and 2400 IU/kg) and two levels of 25-hydroxycholecalciferol (25-OH, supplied as HyD) (0 and 68 µg/kg) for a total of 10 treatments. Each experimental diet was fed to two pens with 10 hens and 2 roosters that each received the experimental diets. Levels of 25-OH in plasma and in egg yolk were measured right after the depletion period and during the experimental phase. Performance parameters such as body weight, hen-day production, egg-shell thickness and egg mass were measured weekly. After the depletion period the level of 25-OH in plasma and egg yolk was below the detection limit confirming the depletion status. During the experimental phase the amount of 25-OH in plasma and egg yolk was higher as the cholecalciferol increased. When HyD was fed the level of 25-OH in plasma and egg yolk was higher than obtained when cholecalciferol was fed. Increasing levels of cholecalciferol improved egg shell thickness, hen-day production and egg mass. The addition of HyD improved egg-shell thickness, hen-day production and egg mass. The effect of HyD on performance was more noticeable at low levels of cholecalciferol with no difference at higher levels of cholecalciferol in the diet.

Key words: Breeders, cholecalciferol, 25-hydroxycholecalciferol, vitamin D, carryover

INTRODUCTION

Unexplained incidences of rickets ("field rickets") in young chicks and poults has plagued the poultry industry for decades, despite advances in our knowledge of requirements for vitamin D, calcium and phosphorus (Hurwitz *et al*., 1973; Walser *et al*., 1980; Olson *et al*., 1981; Troup, 1982; Bar *et al*., 1987; Kradel and Keene, 1988; Huff *et al*., 1999). The skeletal development of the embryo starts as early as a few days after incubation with calcium, phosphorus and vitamin D deposited in the egg playing a crucial role (Araujo-Torres *et al*., 2009). Skeletal abnormalities can be observed even few days after hatch, suggesting the supply of adequate amounts of nutrients during incubation as a strategy to explore in order to reduce the incidence of leg abnormalities (Waldenstedt, 2006). Carryover of vitamin D from the hen to her progeny may be a factor to consider when examining the problem of rickets in the young chick or poult. The vitamin D content of eggs can be increased by increasing the amount of vitamin D in the diet of hens (Romanoff and Romanoff, 1949).

Little is known about vitamin D requirements for broiler breeders. Kramer and Waibel (1978) fed turkey hens vitamin D from either cholecalciferol (Vit. D_3) or 25-hydroxycholecalciferol (25-OH, Hy-D) from 0-2400 IU/kg of feed and showed differences in occurrence of rickets in the progeny grown to 2 wks on diets with no vitamin D. Poults from hens fed 0-300 IU/kg of vitamin D, regardless of source, had 40 to 60% higher incidence of leg problems. None of the poults from hens fed 2400 IU/kg from Vit. D_3 were affected, but 28% of those from hens fed equivalent amounts of Hy-D had leg problems. Driver *et al*. (2006) reported a reduction of leg abnormalities in the progeny of hens fed 2000 IU/kg of cholecalciferol compared to the progeny of hens fed 250 IU/kg of cholecalciferol.

According to Fraser and Emtage (1976) 90% of the vitamin D deposited in the egg occurs as Vit. D_3, with only 5% occurring as 25-OH. This mechanism incorporating vitamin D into the yolk may be a selective mechanism giving the embryo the opportunity to control its own supply. In contrast, the vitamin D supply to the mammalian fetus across the placental wall is mainly 25-OH (Weisman *et al*., 1976).

In recent years, the poultry industry has responded to the problem of field rickets by increasing the amount of vitamin D in the maternal diet, often using the more expensive 25-OH form. From the research of Fraser and Emtage (1976) it appears that this may not be an effective means of increasing the vitamin D needs for the developing embryo and for improving liver stores in the newly hatched chick.

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Because maternal stores may be a significant factor in alleviating problems associated with early-onset rickets in the young chick, the present study was conducted to evaluate different sources and levels of vitamin D on breeder performance and egg deposition to aid in overcoming early-onset rickets in chicks.

MATERIALS AND METHODS

Broiler breeder pullets (Cobb 500) nearing sexual maturity (24 weeks of age) were placed in litter floor pens with 10 hens placed in each of 20 pens. These birds had been grown on a restricted feeding program to maintain body weight similar to recommendations by the breeder. For a two month period, hens were control-fed a nutritionally complete diet (Table 1)[3] using a vitamin premix with no supplemental vitamin D to deplete liver stores (Table 2). Atencio *et al.* (2005a) found that a depletion period of 6-7 weeks was necessary to deplete vitamin D stores in breeder hens. At the end of two months two egg yolks samples per pen were collected. Also, blood samples from the brachial vein were collected from two hens per pen. Plasma was separated from the red blood cells by centrifugation. The vitamin D content of the samples was determined by a commercial laboratory to evaluate the vitamin D status[4]. Once the depletion period was concluded, birds were assigned to experimental treatments.

The experimental treatments consisted of a 2 x 5 factorial arrangement with two levels of supplemental Hy-D (0 or 68 µg/kg) and five levels of supplemental Vit D_3 (0, 300, 600, 1200, or 2400 IU/kg) for a total of 10 experimental diets. A large lot of the basal diet was mixed and divided into two aliquots. One received no 25-OH while the other was supplemented with 68 µg/kg. These two aliquots were each then divided into two aliquots with one receiving no cholecalciferol (low D3) while the other was supplemented to provide 2400 IU/kg of cholecalciferol (high D3). The low D3 and high D3 diets were then blended as needed to provide the intermediate levels of cholecalciferol. Diets were analyzed for content of cholecalciferol and 25-hydroxy-cholecalciferol by a laboratory specializing in these analyses (DSM Nutritional Products. Basel, Switzerland). Each of the resulting 10 diets was fed to two replicate pens of ten hens and two roosters. Diets were control fed on a daily basis as needed to maintain body weight of the hens as recommended by the breeder. Male broiler breeders were fed for the two month depletion period on a vitamin D- adequate diet and during the test period were given an oral dosage of 20,000 IU vitamin D per month by gavage to ensure adequate levels for fertility.

The birds were kept in litter floor pens in a blackout house of commercial design with softwood shavings

Table 1: Composition (%) and calculated nutrient content of diets for broiler breeders

Ingredient	%
Yellow corn	68.115
Poultry oil	0.500
Soybean meal	22.120
Ground limestone	6.980
Defluorinated phosphate	1.510
Sodium chloride	0.350
L-Lysine HCl	0.000
MHA 84[1]	0.075
Vitamin premix[2]	0.250
Mintrex P_Se mineral mix[3]	0.100
Total	100.000
ME (kcal/lb)	1325.00
Crude protein (%)	15.91
Calcium (%)	3.21
Available P (%)	0.38
Lysine (%)	0.85
Tryptophan (%)	0.19
Threonine (%)	0.64
TSAA (%)	0.66

[1]Novus International, St. Louis Mo. [2]See Table 2 for fortification levels of supplemental vitamins. [3]Provides as mg/kg: 40 Mn, 20 Cu, 40 Zn, 0.30 Se complexed with methionine hydroxy analogue (Novus International, St. Louis Mo)

Table 2: Vitamin supplementation levels in test diets compared to average vitamin fortification levels for commercial broiler breeders

			Industry fortification levels (BASF 1997)		
Vitamin	Units/kg	Test premix A[1]	Average	High 25%	Low 25%
Vitamin A	IU	16,500	11,410	16,321	8,215
Vitamin D	IU	0	3,370	4,845	2,455
Vitamin E	IU	44	30.70	45.85	17.29
Niacin	mg	66	40.74	62.57	27.37
Pantothenic acid	mg	22	15.52	22.73	10.75
Riboflavin	mg	13.2	8.94	12.87	6.40
Menadione	mg	3.3	1.91	3.31	0.91
Thiamin	mg	4.4	2.20	3.83	1.11
Pyridoxine	mg	6.6	3.49	5.96	1.67
Folic acid	mg	2.2	1.23	1.96	0.78
Biotin	mg	0.28	0.18	0.24	0.11
Vitamin B_{12}	mg	0.03	0.016	0.024	0.010
Choline	mg	966	----	-----	-----

[1]When added to diet at 0.25%. Test Premix B is same with 5500 IU/kg supplemental vitamin D

over concrete floors. Each pen was equipped with two tube feeders and an automatic water source. Incandescent lights with a 100 watt bulb per pen suspended approximately 8 ft above the litter provided 14 h of light daily. Temperature and airflow were adjusted through the use of thermostatically controlled gas brooders and ventilation fans.

Records were maintained for rate of egg production, egg weight and egg shell thickness. Egg weight and shell thickness were determined on a one-day sample weekly during the study. Eggs were weighed, broken and the contents removed, allowed to dry overnight and shell thickness determined with a ratchet-stop micrometer[5]. At the end of two months on test diets, samples of eggs were collected and the vitamin D content of the yolks determined by a commercial laboratory[6]. Additional samples of eggs were collected for hatching.

Pens means served as the experimental unit for statistical analysis. Data were subjected to analysis of variance using the general linear model procedure of SAS (1991). The model included vitamin D source and level as the main effects and two-way interactions between the main effects. Significant differences among or between means were separated by repeated *t*-tests using the least square means option of SAS software. All statements of significance are based on $p \le 0.05$.

RESULTS AND DISCUSSION

The calculated level of 25-hydroxy-cholecalciferol was 68 µg per kg while the mean analyzed value was 68.2±4.8 (mean±SD). For cholecalciferol, analyzed values for the lower levels of supplementation appeared higher than anticipated while at the higher levels of supplementation there was good agreement with calculated values (Table 3). Because 25-OH is the major circulating form of the vitamin D, it is utilized as the indicator of preference to measure the status of this vitamin. Ovesen *et al.* (2003) reported that as much as 80% of the circulating vitamin D activity is due to 25-OH. The downside is the high variability associated with the analysis of this metabolite (DeLuca, 2004). An advantage of 25-OH is its longer half-life of approximately 21 days compared to several days for vitamin D_3 (Yarger *et al.*, 1995). On the other hand, cholecalciferol is not found in circulation in the blood stream for a long time, instead; it is taken up rapidly by the adipose tissue for storage or by the liver for metabolism (Jones *et al.*, 1998).

Levels of 25-OH obtained in plasma and egg yolk after the 8 week depletion period were below the detection limit. This in agreement with Atencio *et al.* (2005a) who reported that between 6-7 weeks is required for broiler breeders to use the vitamin D reserves and become depleted. This result was also confirmed by the pattern observed for eggshell thickness (Fig. 1), as it was reduced progressively throughout the depletion period.

Table 3: Vitamin D analysis of breeder hen diets[1]

Diet	Cholecalciferol IU/kg Claim	Cholecalciferol IU/kg Result	Hy-D ug/kg Claim	Hy-D ug/kg Result
1	0	50	0	0
2	300	680	0	0
3	600	900	0	0
4	1200	1410	0	0
5	2400	2530	0	0
6	0	0	68	64.2
7	300	400	68	37.6
8	600	715	68	65.8
9	1200	1225	68	73.0
10	2400	2815	68	73.8

[1]Analysis conducted by DSM Nutritional Products Ltd., Basel, Switzerland

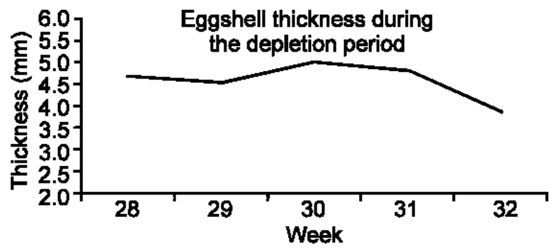

Fig. 1: Eggshell thickness during the depletion period

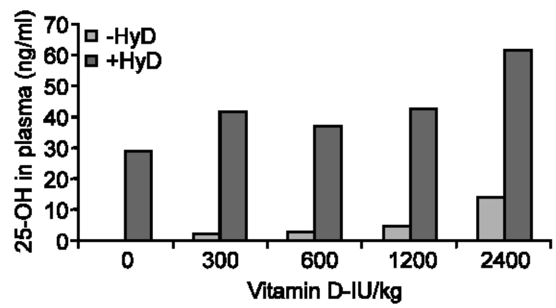

Fig. 2: Effect of Vitamin D level and source on plasma levels of 25-OH in the breeder

The level of 25-OH in plasma by vitamin D level and source is depicted in Fig. 2. Breeder hens receiving HyD in the diet had higher levels of 25-OH in plasma. This response can be explained by the higher potency of HyD which is considered to be 1.5 to 2.2 times higher than for cholecalciferol (McNaughton *et al.*, 1977). Another approach to explain this result is based on the higher absorption rate of 25-OH with respect to cholecalciferol (Atencio *et al.*, 2005b). On this regard, Bar *et al.* (1980) reported that overall absorption of 25-OH with respect to cholecalciferol was significantly higher with rates of 83.6% and 66.5%, respectively. An increasing trend for the metabolite in plasma was also observed as the cholecalciferol level increased, however, values obtained were not as high as the obtained for HyD.

The effect of vitamin D source and level in the egg yolk concentration of 25-OH is observed in Fig. 3. It is known

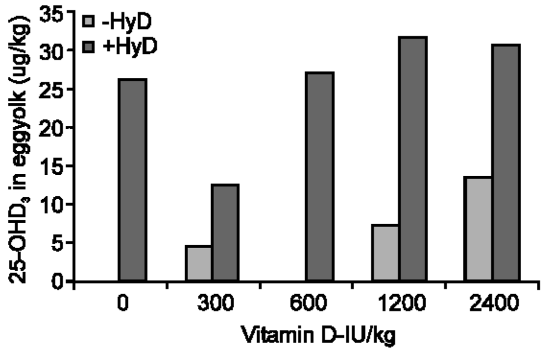

Fig. 3: Effect of Vitamin D level and source in egg yolk levels of 25-OH in the breeder

that egg levels of vitamin D can be modified by varying the levels of this vitamin in the diet (Naber, 1993). The concentration of 25-OH in the egg yolk was higher as the cholecalciferol level increased. This result in agreement with Mattila *et al*. (1999) who observed increased levels of cholecalciferol and 25-OH in egg yolk as increasing levels of dietary cholecalciferol were fed. Mattila *et al*. (1999) fed cholecalciferol levels from 1000 IU/kg to 8600 IU/kg of feed obtaining concentrations of 25-OH in egg yolks ranging from 5-11 μg/kg.

Moreover, the response obtained for 25-OH in egg yolk was greater when HyD was fed compared to the concentration obtained when cholecalciferol was present in the diet. The higher absorption rate of 25-OH and its higher half-life with respect to cholecalciferol can explain this result (Atencio *et al*., 2005b). Cholecalciferol must undergo a metabolic hydroxylation in liver to be converted into 25-OH whereas HyD supplies 25-OH directly. This metabolite is also known to have a higher affinity toward the transporter in the bloodstream making the deposition of 25-OH in egg yolk from HyD more efficient (Jones *et al*., 1998; Kawazoe *et al*., 1996). Contrary to the report by Fraser and Emtage (1976), the presence of 25-OH in the egg yolk when HyD was fed and its higher response compared to cholecalciferol suggest that the metabolite can effectively supply the vitamin to the developing embryo.

Eggshell thickness, hen-day production, egg mass and body weight was also recorded on a weekly basis during the depletion period and were reported according to the future treatment assigned. Table 4 shows the egg shell thickness recorded during the depletion period. Overall, eggshell thickness was reduced as the depletion period progresssed. Table 5 reports the hen-day production during the depletion period; during the first weeks of the depletion period hen-day production was not affected; however, during the last weeks of the depletion period a steep reduction on the hen-day production was observed. Figure 4 also confirms the effect of the depletion period on the hen-day production. Table 6 shows the egg mass response during the depletion period, no clear cut pattern was observed for egg mass with respect to the week during the depletion phase. The effect of the depletion period on body weight is shown in Table 7, there was no clear effect observed for body weight when analyzing within future treatments. Body weight week by week throughout the depletion phase followed an increasing trend; this was expected since increasing levels of feed were fed weekly. The ANOVA table detailing the probability of the experimental diets on weekly egg shell thickness, hen-day egg production, egg mass and body weight is shown in Table 8. The influence of the vitamin D level and source on egg shell thickness is shown in Table 9. A significant weekly effect of the vitamin D level and vitamin D source was observed on egg shell thickness. In general, at increasing levels of vitamin D the egg shell thickness increased with two weeks where no effect was obtained. The HyD supplementation resulted on significantly higher egg shell thickness in most of the weeks comprising the experimental period. There was also a

Table 4: Effect of vitamin D source and level on egg shell thickness (mm) of broiler breeders the depletion period. All hens were fed a common diet

Future treatment		Week of age 28	29	30	31	32
Vit D_3 IU/kg						
0		4.61	4.61	5.00	4.93	3.74
300		4.81	4.69	5.06	4.68	3.79
600		4.67	4.31	4.72	4.59	3.78
1200		4.57	4.49	5.05	4.82	3.88
2400		4.66	4.49	5.10	5.01	4.09
HyD						
No		4.62	4.49	5.00	4.83	3.70
Yes		4.71	4.54	4.97	4.78	4.00
Vit D_3	**HyD**					
0	No	4.66	4.72	4.89	4.84	3.26
0	Yes	4.57	4.50	5.11	5.02	4.22
300	No	4.93	4.53	5.08	4.71	3.50
300	Yes	4.68	4.86	5.05	4.65	4.07
600	No	4.45	4.21	4.90	4.85	3.49
600	Yes	4.90	4.40	4.55	4.32	4.08
1200	No	4.58	4.48	5.05	4.73	4.19
1200	Yes	4.57	4.50	5.04	4.90	3.57
2400	No	4.49	4.53	5.10	5.00	4.07
2400	Yes	4.83	4.45	5.10	5.02	4.10
Mean		4.67	4.53	4.99	4.81	3.87

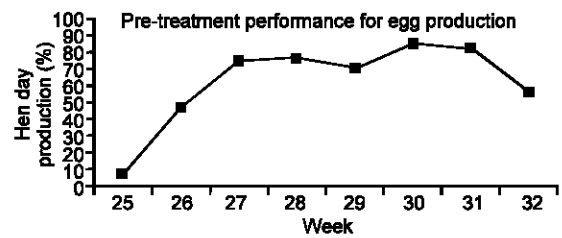

Fig. 4: Effect of the depletion period on hen day production

Table 5: Effect of vitamin D source and level on hen-day egg production during the depletion period. All hens were fed a common diet

Future treatment		Week of age 25	26	27	28	29	30	31	32
Vit D_3 IU/kg									
0		6.75	46.50	66.75	71.00	62.50	84.00	84.50	54.25
300		9.50	45.75	82.75	82.75	73.50	88.00	81.75	47.25
600		10.25	41.50	68.00	72.00	69.25	85.00	81.25	59.75
1200		4.75	50.00	75.50	82.00	77.00	88.25	81.00	53.25
2400		4.50	48.50	78.25	72.00	67.50	76.00	82.75	63.50
HyD									
No		7.30	52.30	80.40	77.40	73.70	85.70	83.70	57.30
Yes		7.00	40.60	68.10	74.50	66.20	82.80	80.80	53.90
Vit D_3	**HyD**								
0	No	9.00	64.50	78.00	84.00	73.50	83.00	84.00	52.00
0	Yes	4.50	28.50	55.50	58.00	51.50	85.00	85.00	56.50
300	No	10.00	51.00	84.00	84.00	73.00	92.00	89.50	52.00
300	Yes	9.00	40.50	81.50	81.50	74.00	84.00	74.00	42.50
600	No	10.50	47.00	73.50	63.50	71.50	84.00	79.50	58.50
600	Yes	10.00	36.00	62.50	80.50	67.00	86.00	83.00	61.00
1200	No	2.50	47.50	76.00	77.00	75.50	83.50	78.00	54.50
1200	Yes	7.00	52.50	75.00	87.00	78.50	93.00	84.00	52.00
2400	No	4.50	51.50	90.50	78.50	75.00	86.00	87.50	69.50
2400	Yes	4.50	45.50	66.00	65.50	60.00	66.00	78.00	57.50
Mean		7.15	46.45	74.25	75.95	69.95	84.25	82.25	55.60

Table 6: Effect of vitamin D source and level on egg mass (g/day) during the depletion period. All hens were fed a common diet

Future treatment		Week of age 28	29	30	31	32
Vit D_3 IU/kg						
0		55.95	56.72	58.08	59.22	56.74
300		56.37	57.76	57.69	59.00	56.51
600		55.68	57.77	58.09	58.24	56.17
1200		55.91	57.43	58.97	59.51	58.51
2400		56.18	57.82	58.31	59.66	57.01
HyD						
No		55.88	57.08	58.29	59.15	56.29
Yes		56.15	57.92	58.16	59.10	57.69
Vit D_3	**HyD**					
0	No	54.84	57.18	58.87	58.74	54.83
0	Yes	57.05	56.27	57.30	59.69	58.65
300	No	55.58	56.38	56.70	58.61	54.87
300	Yes	57.17	59.13	58.67	59.40	58.15
600	No	55.77	56.47	58.14	57.50	54.04
600	Yes	55.59	59.07	58.05	58.98	58.30
1200	No	57.25	58.03	58.52	60.42	60.43
1200	Yes	54.58	56.84	59.42	58.59	56.60
2400	No	55.99	57.36	59.23	60.49	57.29
2400	Yes	56.38	58.27	57.39	58.83	56.73
Mean		55.95	57.51	58.29	59.11	57.02

significant two-way interaction between vitamin D level and source. In those weeks where the interaction was observed birds receiving low levels of vitamin D supplemented with HyD had a thicker egg shell than those birds fed without HyD supplemented. The HyD effect was not obtained at higher levels of vitamin D. This result is in agreement with Fritts and Waldroup (2003) who reported an improvement on performance parameters in broilers when HyD was supplemented at lower levels of vitamin D supplementation. Table 10 shows the effect of the experimental diets on the hen-day egg production. A significant effect of the vitamin D level on hen-day egg production was obtained during a few weeks within the experimental phase, with birds receiving increasing levels of vitamin D having higher hen-day production rates. Atencio *et al*. (2006) reported a reduction in hen-day egg production after 56 weeks of age when feeding low levels of vitamin D and no reduction in those breeder hens fed higher levels of the vitamin. Atencio *et al*. (2006) also reported that the vitamin D requirement for maximum response in breeder hens was higher postpeak. There was significant effect of the vitamin D source at week 44 with no significant effect for the remaining weekson hen-day egg production. At week 44, hens receiving HyD in the maternal diet had a significantly higher hen-day egg production. Driver *et al*. (2006) reported that older birds deposited more vitamin D in eggs than young pullets and hypothesized that either the greater egg production or the higher requirement of the pullet impairs the vitamin D transfer into the egg.

The effect of the vitamin D level and source on egg mass is shown in Table 11. A significant effect of vitamin D level was observed during the last week of the experimental phase where at lower levels of vitamin D in the diet egg mass was reduced. An effect of the vitamin D source was observed only during two weeks of the experimental phase, with birds receiving feed supplemented with HyD having higher egg mass. A two way interaction between vitamin D source and level was observed during four weeks throughout the experimental phase; no clear-cut pattern on this response was observed. Table 12 shows the effect of the vitamin D

Table 7: Effect of vitamin D source and level on weekly body weight (kg) of broiler breeder hens during the depletion period. All hens were fed a common diet

Future treatment		Week of age 25	26	27	28	29	30	31	32
Vit D_3 IU/kg									
0		3.004	3.063	3.246	3.378	3.378	3.471	3.609	3.545
300		2.985	3.013	3.146	3.256	3.256	3.366	3.480	3.449
600		3.002	3.035	3.224	3.321	3.303	3.430	3.538	3.526
1200		2.952	2.996	3.156	3.253	3.272	3.339	3.470	3.451
2400		2.927	3.010	3.163	3.271	3.271	3.396	3.494	3.501
HyD									
No		3.000	3.040	3.193	3.298	3.298	3.407	3.526	3.486
Yes		2.948	3.006	3.182	3.293	3.293	3.394	3.511	3.504
Vit D_3	**HyD**								
0	No	3.074	3.103	3.248	3.340	3.340	3.445	3.609	3.529
0	Yes	2.935	3.023	3.244	3.415	3.415	3.496	3.609	3.561
300	No	2.956	2.973	3.119	3.245	3.245	3.356	3.458	3.396
300	Yes	3.014	3.054	3.174	3.266	3.266	3.376	3.503	3.503
600	No	2.988	3.034	3.223	3.340	3.303	3.461	3.538	3.536
600	Yes	3.017	3.036	3.225	3.303	3.303	3.399	3.538	3.515
1200	No	3.003	3.054	3.211	3.295	3.333	3.393	3.525	3.491
1200	Yes	2.901	2.938	3.101	3.211	3.211	3.286	3.415	3.411
2400	No	2.981	3.039	3.163	3.270	3.270	3.379	3.499	3.475
2400	Yes	2.873	2.981	3.164	3.271	3.271	3.413	3.489	3.528
Mean		2.974	3.023	3.187	3.296	3.296	3.400	3.518	3.496

Table 8: Part I. Probabilities and mean standard errors of the effects of vitamin D sources and levels on weekly breeder hen performance

Measurement		Week of age 33	34	35	36	37	38	39	40	41	42	43	44
Shell thickness (mm)													
Vit D level	P diff	0.16	0.001	0.05	0.001	<0.0001	0.39	0.41	0.009	0.03	0.0003	<0.0001	0.002
	SEM	0.319	0.098	0.094	0.138	0.119	0.228	0.165	0.113	0.139	0.114	0.084	0.140
Vit D source	P diff	0.79	0.001	<0.0001	0.001	0.0004	0.24	0.89	0.33	0.23	0.006	0.002	0.005
	SEM	0.143	0.064	0.057	0.079	0.071	0.121	0.105	0.067	0.079	0.069	0.053	0.079
Level x Source	P diff	0.30	0.001	0.002	<0.0001	0.0004	0.42	0.64	0.52	0.08	0.0001	0.0004	0.01
	SEM	0.554	0.156	0.155	0.223	0.168	0.386	0.234	0.145	0.228	0.179	0.121	0.209
Coefficient of variation		17.23	11.45	11.44	12.68	10.57	11.92	13.40	10.13	9.87	11.58	8.49	10.05
Egg production (%)													
Vit D level	P diff	0.62	0.71	0.34	0.08	0.03	0.04	0.04	0.06	0.07	0.006	0.07	0.04
	SEM	11.46	6.51	5.55	4.85	3.84	4.61	3.97	4.76	4.23	3.69	4.78	4.47
Vit D source	P diff	0.64	0.18	0.94	0.16	0.10	0.40	0.59	0.18	0.61	0.22	0.07	0.01
	SEM	7.24	4.12	3.51	3.07	2.43	2.92	2.51	3.01	2.67	2.33	3.03	2.83
Level x Source	P diff	0.99	0.59	0.45	0.34	0.07	0.49	0.66	0.52	0.63	0.18	0.07	0.16
	SEM	16.21	9.20	7.85	6.58	5.42	6.52	5.61	6.73	5.98	2.22	6.77	6.32
Coefficient of variation		60.33	22.49	13.92	15.64	10.47	11.30	11.04	13.74	13.68	11.68	13.36	14.52
Egg mass (g/day)													
Vit D level	P diff	0.27	0.25	0.85	0.33	0.45	0.76	0.48	0.21	0.38	0.81	0.12	0.03
	SEM	2.96	1.31	0.95	1.13	1.13	1.86	1.34	1.35	1.20	1.20	1.83	1.25
Vit D source	P diff	0.41	0.002	0.05	0.23	0.42	0.74	0.61	0.29	0.32	0.21	0.75	0.98
	SEM	1.34	0.84	0.57	0.64	0.74	0.99	0.85	0.80	0.83	0.73	1.04	0.74
Level x Source	P diff	0.15	0.002	0.004	0.02	0.15	0.14	0.15	0.45	0.08	0.44	0.16	0.01
	SEM	2.96	2.12	1.55	1.82	2.02	3.16	1.89	2.14	1.69	1.95	3.05	1.83
Coefficient of variation		9.06	9.80	7.53	7.91	7.89	7.08	7.23	8.16	6.50	7.88	8.04	7.94
Body weight (kg)													
Vit D level	P diff	0.82		0.87	0.78	0.84	0.91	0.82	0.84	0.79	0.90		
	SEM	0.068		0.068	0.066	0.072	0.073	0.072	0.077	0.081	0.083		
Vit D source	P diff	0.96		0.54	0.76	0.94	0.98	0.80	0.87	0.75	0.57		
	SEM	0.042		0.042	0.042	0.044	0.045	0.045	0.047	0.049	0.050		
Level x Source	P diff	0.89		0.75	0.96	0.87	0.91	0.99	0.97	0.82	0.89		
	SEM	0.096		0.094	0.101	0.106	0.100	0.107	0.105	0.121	0.124		
Coefficient of variation		10.08		9.88	9.97	10.17	10.14	10.14	10.59	10.92	11.08		

Week effect	Shell thickness (mm)	Egg production (%)	Egg mass (g/day)	Body weight (kg)
P>F	<0.0001	<0.0001	<0.0001	<0.0001
SEM	0.073	0.681	2.791	0.031

Table 9: Effect of vitamin D source and level on egg shell thickness (mm) of broiler breeders

Treatment		Week of age 33	34	35	36	37	38	39	40	41	42	43	44
Vit D_3 IU/kg													
0		3.06	3.59^{c}	3.83^{b}	3.90^{d}	4.06^{c}	4.42	4.27	4.00^{b}	3.78^{c}	3.61^{b}	3.69^{c}	3.81^{c}
300		3.98	3.56^{c}	4.09^{a}	4.65^{bc}	4.36^{bc}	4.26	4.35	4.25^{ab}	4.07^{abc}	4.24^{a}	3.98^{b}	3.86^{c}
600		3.22	3.70^{bc}	4.15^{a}	4.48^{c}	4.42^{b}	4.60	4.04	4.32^{a}	3.86^{bc}	4.00^{a}	3.98^{b}	4.13^{bc}
1200		3.16	4.42^{a}	4.15^{a}	5.00^{a}	4.92^{a}	4.76	4.25	4.51^{a}	4.18^{ab}	4.25^{a}	4.16^{ab}	4.25^{ab}
2400		3.14	3.88^{b}	3.97^{ab}	4.91^{ab}	4.54^{b}	4.65	4.49	4.42^{a}	4.23^{a}	4.18^{a}	4.30^{a}	4.46^{a}
Hy-D													
No		3.29	3.57^{b}	3.84^{b}	4.33^{b}	4.29^{b}	4.63	4.27	4.26	3.96	3.92^{b}	3.91^{b}	3.94^{b}
Yes		3.34	4.08^{a}	4.24^{a}	4.85^{a}	4.63^{a}	4.44	4.29	4.35	4.09	4.19^{a}	4.14^{a}	4.26^{a}
Vit D_3	**HyD**												
0	No	3.02	2.96^{d}	3.30^{e}	3.09^{d}	3.44^{d}	4.28	4.15	3.84	3.49	3.01^{b}	3.23^{c}	3.40^{c}
0	Yes	3.09	4.22^{b}	4.36^{a}	4.70^{bc}	4.68^{abc}	4.56	4.39	4.17	4.07	4.21^{a}	4.16^{ab}	4.23^{ab}
300	No	3.89	3.21^{d}	4.40^{d}	4.47^{bc}	4.27^{c}	4.59	4.33	4.18	4.11	4.14^{a}	3.93^{b}	3.44^{c}
300	Yes	4.08	3.91^{bc}	4.20^{abcd}	4.83^{abc}	4.44^{c}	4.94	4.37	4.33	4.02	4.34^{a}	4.02^{b}	4.28^{ab}
600	No	2.96	3.30^{d}	3.97^{d}	4.43^{c}	4.35^{c}	4.71	4.03	4.24	3.87	3.98^{a}	3.96^{b}	4.13^{b}
600	Yes	3.47	4.09^{b}	4.33^{ab}	4.54^{bc}	4.50^{bc}	4.48	4.04	4.40	3.84	4.02^{a}	4.00^{b}	4.13^{ab}
1200	No	3.29	4.69^{a}	3.99^{cd}	4.73^{bc}	4.87^{ab}	4.73	4.45	4.58	3.96	4.16^{a}	4.13^{ab}	4.20^{ab}
1200	Yes	3.03	4.14^{b}	4.31^{abc}	5.27^{a}	4.97^{a}	4.80	4.04	4.44	4.40	4.35^{a}	4.19^{ab}	4.31^{ab}
2400	No	3.27	3.71^{c}	3.93^{cd}	4.92^{ab}	4.52^{bc}	4.85	4.38	4.43	4.36	4.33^{a}	4.26^{ab}	4.55^{a}
2400	Yes	3.00	4.04^{bc}	4.02^{bcd}	4.90^{abc}	4.56^{bc}	4.44	4.61	4.40	4.11	4.04^{a}	4.33^{a}	4.37^{ab}
Mean		3.22^{f}	3.86^{e}	4.08^{d}	4.65^{a}	4.51^{b}	4.58^{ab}	4.28^{c}	4.31^{c}	4.00^{de}	4.09^{d}	4.03^{d}	4.17^{cd}

^{abcd}Means in column with no common superscript differ significantly ($p<0.05$)

Table 10: Effect of vitamin D source and level on hen-day egg production

Treatment		Week of age 33	34	35	36	37	38	39	40	41	42	43	44
Vit D_3 IU/kg													
0		32.00	55.75	76.75	53.50	63.00^{b}	73.00^{bc}	65.25^{b}	62.75	51.75	49.75^{b}	60.25	49.50^{b}
300		26.00	51.75	72.25	53.75	68.00^{b}	70.75^{c}	62.50^{b}	57.75	57.25	53.75^{b}	66.00	55.75^{ab}
600		50.25	63.50	86.00	67.00	81.00^{a}	87.25^{ab}	79.75^{a}	76.75	69.75	70.50^{a}	80.00	69.00^{a}
1200		43.50	56.50	77.25	63.50	74.00^{ab}	86.50^{ab}	77.25^{a}	74.00	67.00	70.00^{a}	78.50	66.75^{a}
2400		38.25	62.00	86.75	72.25	80.25^{a}	90.50^{a}	74.25^{ab}	75.50	63.25	66.75^{a}	73.50	67.00^{a}
Hy-D													
No		35.50	53.70	79.60	58.70	70.10	79.80	70.80	66.30	60.80	60.00	67.30	55.70^{b}
Yes		40.50	62.10	80.00	65.30	76.40	83.40	72.80	72.40	62.80	64.30	76.00	67.50^{a}
Vit D_3	**HyD**												
0	No	27.50	44.50	67.50	42.50	48.50	68.00	63.00	55.00	49.00	39.00	42.50	38.00
0	Yes	36.50	67.00	86.00	64.50	77.50	78.00	67.50	70.50	54.50	60.50	78.00	61.00
300	No	22.50	49.00	72.00	46.50	69.00	72.00	63.50	55.00	56.50	53.00	63.00	48.50
300	Yes	29.50	54.50	72.50	61.00	67.00	69.50	61.50	60.50	58.00	54.50	69.00	63.00
600	No	45.50	59.00	90.00	67.00	83.00	90.50	82.00	79.50	73.50	70.50	83.00	69.00
600	Yes	55.00	68.00	82.00	67.00	79.00	84.00	77.50	74.00	66.00	70.50	77.00	69.00
1200	No	43.00	50.00	82.00	65.00	73.50	79.00	71.50	67.00	61.50	68.00	73.00	54.50
1200	Yes	44.00	63.00	72.50	62.00	74.50	94.00	83.00	81.00	72.50	72.00	84.00	79.00
2400	No	39.00	66.00	86.50	72.50	76.50	89.50	74.00	75.00	63.50	69.50	75.00	68.50
2400	Yes	37.50	58.00	87.00	72.00	84.00	91.50	74.50	76.00	63.00	64.00	72.00	65.50
Mean		38.00^{f}	57.90^{e}	79.80^{ab}	62.00^{de}	73.25^{ab}	81.60^{a}	71.80^{c}	69.35^{cd}	61.80^{de}	62.15^{de}	71.65^{c}	61.60^{de}

^{abc}Means in column with no common superscript differ significantly ($p\leq 0.05$)

level and source on the weekly body weight, there was no significant effect of the vitamin D level and source on body weight.

The ANOVA table reporting the probability as well as the effects of the experimental diets on overall egg shell thickness, hen-day egg production, egg mass and body weight during the experimental phase is shown in Table 13. A significant effect of the vitamin D level on body weight was observed with no clear trend. Egg shell thickness was significantly affected by the vitamin D level and source. An increased thickening of the egg shell was observed as the dietary level of the vitamin D increased. Hens fed diets containing HyD produced eggs with significantly higher shell thickness. This was in agreement with Charles *et al*. (1978) who observed a significant improvement on shell quality in laying hens by the addition of 25-hydroxycholecalciferol. A significant two-way interaction was observed between vitamin D

Table 11: Effect of vitamin D source and level on egg mass (g/day)

Treatment		Week of age 33	34	35	36	37	38	39	40	41	42	43	44
Vit D$_3$ IU/kg													
0		54.54	58.08	60.77	60.29	62.35	63.14	65.95	63.57	64.87	64.26	62.85	61.81^{b}
300		61.22	55.28	62.05	59.23	60.79	61.55	64.08	62.47	62.44	65.90	64.58	64.96^{ab}
600		56.68	57.02	61.62	61.08	62.81	63.97	63.92	65.98	65.35	65.52	67.34	66.71^{a}
1200		58.53	59.14	61.97	61.53	63.02	64.19	64.75	65.50	63.80	66.15	67.74	65.61^{a}
2400		55.76	56.56	61.98	61.94	63.73	62.51	62.51	63.28	62.92	65.47	64.20	66.27^{a}
Hy-D													
No		56.64	55.44^{b}	60.92^{b}	60.27	62.15	63.29	63.93	63.57	63.31	64.85	65.57	65.06
Yes		58.05	58.99^{a}	62.43^{a}	61.35	62.94	62.85	64.55	64.75	64.45	66.07	65.11	65.08
Vit D$_3$	**HyD**												
0	No	54.70	55.50^{cde}	57.42^{c}	57.63^{b}	60.62	63.09	64.27	61.19	61.38	61.87	59.58	58.57^{c}
0	Yes	54.38	60.66^{abc}	64.12^{a}	62.94^{a}	64.08	63.18	67.64	65.95	68.36	66.64	66.13	65.05^{ab}
300	No	60.72	52.02^{e}	61.48^{ab}	58.14^{b}	58.69	60.74	62.54	61.63	61.53	65.73	65.34	65.07^{ab}
300	Yes	61.73	58.54^{abc}	62.62^{ab}	60.32^{ab}	62.90	62.35	65.62	63.31	63.35	66.07	63.82	64.85^{ab}
600	No	52.89	52.41^{de}	60.33^{bc}	59.77^{ab}	63.04	62.89	62.34	65.88	66.26	64.77	69.02	66.18^{ab}
600	Yes	60.48	61.63^{a}	62.91^{ab}	62.40^{a}	62.59	65.05	65.50	66.09	64.43	66.27	65.66	67.23^{a}
1200	No	59.36	61.48^{ab}	63.69^{a}	63.46^{a}	64.35	63.85	65.97	65.17	64.92	66.74	69.88	68.00^{a}
1200	Yes	57.70	56.80^{bcd}	60.26^{bc}	59.60^{ab}	61.70	64.54	63.54	65.84	62.69	65.55	65.60	63.22^{b}
2400	No	55.55	55.80^{cde}	61.68^{ab}	62.36^{a}	64.05	65.89	64.54	64.00	62.45	65.15	64.05	67.49^{a}
2400	Yes	55.98	57.33^{abc}	62.27^{ab}	61.52^{ab}	63.41	59.13	60.48	62.56	63.40	65.80	64.36	65.05^{ab}
Mean		56.63^{g}	57.34^{g}	61.90^{ef}	60.98^{f}	62.83^{de}	63.09^{cde}	64.25^{abcd}	64.26^{abc}	63.94^{bcd}	65.62^{a}	65.82^{a}	65.33^{ab}

^{abcde}Means in column with no common superscript differ significantly ($p \leq 0.05$)

Table 12: Effect of vitamin D source and level on weekly body weight (kg) of broiler breeder hens

Treatment		Week of age 33	35	36	37	38	39	40	41	42
Vit D$_3$ IU/kg										
0		3.745	3.737	3.709	3.837	3.882	3.884	3.912	3.919	3.942
300		3.670	3.707	3.706	3.810	3.868	3.862	3.853	3.904	3.911
600		3.659	3.698	3.666	3.781	3.815	3.841	3.848	3.832	3.882
1200		3.634	3.635	3.599	3.741	3.797	3.769	3.792	3.807	3.834
2400		3.685	3.695	3.678	3.743	3.834	3.819	3.827	3.824	3.898
HyD										
No		3.677	3.712	3.680	3.785	3.840	3.843	3.852	3.868	3.914
Yes		3.680	3.676	3.662	3.780	3.838	3.827	3.841	3.846	3.873
Vit D$_3$	**HyD**									
0	No	3.729	3.801	3.740	3.873	3.916	3.916	3.933	3.920	3.940
0	Yes	3.761	3.673	3.678	3.800	3.849	3.851	3.892	3.918	3.944
300	No	3.677	3.732	3.708	3.841	3.877	3.858	3.863	3.982	3.985
300	Yes	3.663	3.683	3.704	3.778	3.860	3.865	3.844	3.825	3.837
600	No	3.673	3.736	3.698	3.794	3.844	3.835	3.846	3.825	3.885
600	Yes	3.644	3.660	3.634	3.768	3.786	3.846	3.850	3.839	3.879
1200	No	3.674	3.655	3.608	3.729	3.783	3.791	3.826	3.841	3.888
1200	Yes	3.594	3.614	3.591	3.753	3.811	3.747	3.759	3.773	3.780
2400	No	3.633	3.638	3.649	3.685	3.783	3.813	3.791	3.773	3.870
2400	Yes	3.738	3.752	3.707	3.801	3.885	3.825	3.863	3.876	3.927
Mean		3.679^{c}	3.694^{c}	3.671^{c}	3.781^{b}	3.838^{ab}	3.835^{ab}	3.847^{ab}	3.855^{ab}	3.892^{a}

level and vitamin D source for egg shell thickness which was increased by the supplementation of HyD at low dietary levels of cholecalciferol with no difference at higher levels of cholecalciferol. This result in agreement with Fritts and Waldroup (2003) who observed an improvement in bird response when adding HyD into diets containing low levels of vitamin D. Hen-day production was significantly affected by the vitamin D level and source. Hen-day production tended to be higher at increasing levels of vitamin D. A similar response was observed by Atencio *et al.* (2006) when feeding increasing levels of cholecalciferol in breeder hens. Addition of HyD into the hens diet resulted on a significantly improved hen-day egg production. A two-way interaction between vitamin D level and source was observed with no clear cut response. Vitamin D level and

Table 13: Effect of source and level of vitamin D on average performance of vitamin D-depleted breeder hens over a 12-week period

Treatment		Body weight (kg)	Shell thickness (mm)	Hen-day production (%)	Egg Mass (g/day)
Vit D_3 IU/kg					
0		3.809^{a}	3.84^{d}	56.69^{b}	61.43^{c}
300		3.768^{a}	4.14^{bc}	56.55^{b}	61.35^{c}
600		3.754^{ab}	4.06^{c}	71.91^{a}	62.53^{ab}
1200		3.704^{b}	4.35^{a}	67.83^{a}	63.15^{a}
2400		3.749^{ab}	4.25^{ab}	69.87^{a}	62.06^{bc}
HyD					
No		3.763	3.99^{b}	62.10^{b}	61.51^{b}
Yes		3.751	4.27^{a}	67.04^{a}	62.69^{a}
Vit D_3	**HyD**				
0	No	3.826	3.41^{d}	47.60^{d}	59.27^{e}
0	Yes	3.793	4.28^{ab}	65.78^{ab}	63.59^{ab}
300	No	3.782	4.01^{c}	54.96^{cd}	60.10^{de}
300	Yes	3.755	4.27^{ab}	58.14^{bc}	62.30^{bc}
600	No	3.766	3.95^{c}	72.75^{a}	61.30^{cd}
600	Yes	3.742	4.18^{b}	71.07^{a}	63.77^{ab}
1200	No	3.729	4.32^{ab}	64.28^{b}	64.51^{a}
1200	Yes	3.680	4.38^{a}	71.39^{a}	61.79^{c}
2400	No	3.711	4.25^{ab}	70.92^{a}	62.40^{bc}
2400	Yes	3.788	4.24^{ab}	68.82^{a}	61.71^{c}
Vit D Level	P diff	0.02	<0.0001	<0.0001	0.005
	SEM	0.233	0.045	1.94	0.42
Vit D Source	P diff	0.57	<0.0001	0.005	0.0006
	SEM	0.014	0.027	1.23	0.25
Level x Source	P diff	0.28	<0.0001	0.001	<0.0001
	SEM	0.032	0.067	2.74	0.63
Coefficient of variation		10.47	15.12	22.46	9.36

^{abcde}Means in column with no common superscript differ significantly ($p \leq 0.05$)

source had a significant effect on egg mass. Egg mass was higher at higher levels of vitamin D in the diet. Also, the addition of HyD to the diet significantly increased egg mass. There was a two-way interaction between vitamin D level and source on egg mass; in general, HyD was effective in increasing egg mass at lower levels of dietary vitamin D with no difference at higher cholecalciferol levels. This result is supported by Fritts and Waldroup (2003). The contribution of parameters such as body weight, egg mass and egg production to evaluate the vitamin D status is minimal since as hens age there is a normal increase in size which allows for the production of larger but fewer eggs. Parameters of mineralization and leg abnormalities and monitoring of vitamin D plasma levels are better indicators of vitamin D status (Driver *et al.*, 2006).

Table 14 shows the effects of maternal vitamin D level and source on fertility and hatchability. The presence of only two repetitions per treatment with two roosters each requires special attention when obtaining conclusions on fertility and hatchability; that is, any fertility problem related to a specific rooster will have a significant impact on these two parameters. The hatchability of fertile eggs parameter represents a more accurate measurement of hatchability since it lessens any potential effect from the roosters. No significant effect of vitamin level and source was obtained. A numerical trend was observed for the vitamin D level ($p = 0.11$) where at higher levels of vitamin D in the hens diet the hatchability of fertility eggs was improved. Atencio *et al.* (2006) reported a significant improvement in hatchability as hen breeders received increasing levels of vitamin D up to 4000 IU/kg. There was also a numerical effect of the vitamin D source on hatchability/fertility ($p = 0.07$). When HyD was supplemented in the breeder diet an improvement on hatchability/fertility was observed. Figure 5 describes the same effect considering the response by pen replicate. It has been reported that Vitamin D deficiency in breeders results in late embryonic mortality and excess dietary levels of vitamin D reduce hatchability (Wilson, 1997). A numerical reduction in hatchability ($p = 0.11$) was observed as the cholecalciferol level was reduced in the breeder diet. Atencio *et al.* (2005a) and Atencio *et al.* (2005b) confirmed this observation when reporting low hatchability at a low levels of vitamin D supplemented in the diet.

Our study confirms that HyD is an effective means to deposit vitamin D into the egg yolk so it can be supplied in the breeder diet with the prospect of promoting an adequate skeletal development of the embryo. The plasma and egg yolk levels of 25-OH were higher when HyD was supplemented in the diet with no similar response obtained when cholecalciferol was supplemented even at high levels.

Table 14: Effect of vitamin D level and source on fertility, hatchability and hatchability/fertility by pen replicate

		Fertility (%)		Hatchability (%)		Hatchability/fertility (%)	
Vit D level (IU/kg)							
0		73.59		30.67		40.43	
300		88.33		59.36		66.42	
600		85.28		58.60		68.25	
1200		80.37		57.33		70.30	
2400		91.46		62.15		65.97	
Vitamin source							
No HyD		80.11		45.97		55.13	
Yes HyD		87.51		61.27		69.41	
Vitamin level * source							
0	No	65.18		15.66		24.97	
0	Yes	82.01		45.68		55.89	
300	No	93.53		58.70		62.78	
300	Yes	83.13		60.03		70.06	
600	No	86.48		60.55		68.56	
600	Yes	84.08		56.66		67.94	
1200	No	68.53		38.24		57.85	
1200	Yes	92.22		76.43		82.76	
2400	No	86.83		56.73		61.50	
2400	Yes	96.09		67.57		70.44	
		P diff	SEM	P diff	SEM	P diff	SEM
Vitamin D Level		0.52	7.57	0.25	10.32	0.11	7.79
Vitamin D source		0.30	4.78	0.13	6.53	0.07	4.93
Level x source		0.53	10.70	0.57	14.60	0.60	11.02
CV		18.06		38.49		25.03	

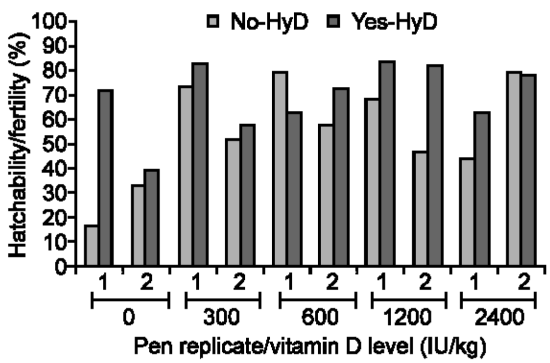

Fig. 5: Effect of vitamin D level and source on hatchability/fertility by pen replicate

HyD supplementation was effective in improving performance and mineralization of the breeder hen only at low levels of vitamin D in the diet with no difference at high levels of vitamin D inclusion.

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