ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 9 (7): 613-622, 2010
ISSN 1682-8356

Effect of Source and Level of Maternal Vitamin D on Carryover to Newly Hatched Chicks[1]

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Abstract: A study was conducted to evaluate the carryover effect of maternal vitamin D level and source on performance and bone development of the progeny. Breeder hens were fed a vitamin D deficient diet for two months to deplete stores. After this period, experimental diets in a factorial arrangement were fed to the hens with five levels of cholecalciferol (0, 300, 600, 1200 and 2400 IU/kg) and two levels of $25OHD_3$ (HyD) (0 and 68 μg/kg) for a total of 10 treatments. At the end of two months on the experimental diets two sets of eggs were hatched. The progeny obtained were placed in battery brooders to 21 days by maternal diet and received a common diet. The first hatch received a diet with no vitamin D supplement whereas the second hatch received a diet with the same nutrient composition but containing 5500 IU/kg of cholecalciferol. The first set of birds responded to the maternal diet supplementation of vitamin D mostly during the first week post hatch with no clear pattern in later stages. The progeny receiving 5500 IU/kg of vitamin D in the diet responded to the maternal vitamin D supplementation even at 21 days and in a clearer trend. Feed conversion and body weight improved as the cholecalciferol level increased and with the inclusion of HyD in the maternal diet. The response when HyD was added was more noticeable at low levels of cholecalciferol supplementation with no difference at higher levels in the hen's diet. Bone development of the progeny was improved with the addition of HyD in the maternal diet; this response was not influenced by increasing levels of cholecalciferol in the breeder diet. This study confirms the importance of the maternal vitamin D carryover for an adequate development of the progeny. Certainly, the vitamin D carryover effect did not overcome the effect of supplementing vitamin D directly in the progeny's diet but it was capable of improving the performance of the progeny even three weeks post-hatch when a high level of cholecalciferol (5500 IU/kg) was present in the diet of the progeny. A carryover effect of HyD when added to the maternal diet was observed in this study, thus the feasibility of using the metabolite to supply vitamin D to the developing embryo was confirmed.

Key words: Vitamin D, cholecalciferol, HyD, carryover, leg problems

INTRODUCTION

Impressive advances in the growth rate of broiler chickens have been observed during the last decades resulting in heavier birds at a lower market age; however, the tradeoff is an increased incidence of skeletal abnormalities (Orth, 1999). Leg abnormalities represent a major concern to the poultry industry due to economic and welfare implications. It is estimated that 2-6% of all broilers display some observable signs of leg disorders (Day, 1990). Leg problems are blamed for causing losses of birds due to mortality, culling and condemnation with an economic impact of $120 million dollars per year to the American broiler industry (Waldenstedt, 2006; Cook, 2000). Benett and IJpelaar (2005) identified skeletal problems as the poultry disease with the highest incidence in the United Kingdom and also as the main cause of loss of animal welfare associated within the poultry industry. Tibial Dyschodroplasia (TD), once considered an insignificant illness, is currently found in 50% of broiler chickens (Leach, 1996). Despite the abundant research conducted and advances in knowledge of requirements for vitamin D, calcium and phosphorus, the unexplained incidence of leg abnormalities remains persistent in the poultry industry (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).

Nutrients supplied in the diet are transferred into the egg yolk by the hen with the embryo recovering them for its development. Nutritional deficiencies and even marginal inadequacies transferred from the breeder during egg formation have a negative impact on the performance of the progeny (Moran, 2007). On the other hand, high levels of certain nutrients deposited by the hen can cause problems to the embryo as serious as those reported for deficiencies (Wilson, 1997). The incubation and neonatal stages now represent over 50% of the productive life of the broiler chicken compared to about 25% decades ago when the selection based on productive parameters started (Ferket, 2009); as a

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consequence, early nutrition has gained special interest as a means of supplying and keeping up with the increasing nutritional needs required by the fast growing broiler strains (Leeson, 2008; Kidd, 2009; Uni and Ferket, 2004; Noy and Sklan, 1998; Lilburn, 1998). 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; Mattila *et al*., 1999; Leeson and Caston, 2003).

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 chicks or poults. 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. 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 may be a selective mechanism giving the embryo the opportunity to control its own supply of the vitamin. From this research, it is suggested that 25-OH in the maternal diet may not be an effective means of increasing the vitamin D supply for the developing embryo and the newly hatched chick. Because maternal stores may play a significant role in alleviating problems associated with early-onset rickets in the young chick a study was conducted evaluating different sources and levels of vitamin D on carryover to the newly hatched chick.

Breeder hens were fed different sources of vitamin D (Vit. D_3 or 25-OH) with different levels of vitamin D_3. Chicks were hatched from eggs from the treated hens and the vitamin D levels in the liver as well as mineralization of newly hatched chicks were determined. Feeding trials were carried out to evaluate the carryover effects on the growth and bone formation of the young chick. Results of this study should provide information regarding best source and level of vitamin D to use in breeder diets 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 a breeder company[3]. For a two month period birds were fed a vitamin D deficient diet to deplete liver stores. At the end of two months, hens received the experimental diets. 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 to provide 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. Each of the resulting 10 diets was fed to two replicate pens of ten hens. At the end of two months on test diets eggs were set for hatching. The response of the breeders in terms of performance, mineralization and vitamin D status parameters is described in a separate report (Coto *et al*., in press).

At time of hatch, samples of chicks were sacrificed to determine tibia ash as a parameter of mineralization. Progeny were placed in battery brooders and grown to 21 days by maternal diet. Six birds were placed per pen with the number of replicates varying due to differences on egg production and hatchability among maternal treatments. The number of pen replicates obtained is detailed in Table 1.

Two hatches of birds were studied. All birds were fed a common diet that differed only in the vitamin D_3 content between batches (Table 2). The first batch was fed a vitamin D_3 deficient diet in order to increase the sensibility to the maternal diet; whereas, the second batch was fed a diet containing 5500 IU/kg of vitamin D_3 to simulate commercial conditions. The common diets contained reduced levels of Ca and P to increase the sensitivity to the variables of study. At hatch time, blood samples from the progeny by maternal diet were collected via heart puncture. Each sample was centrifuged to separate plasma from red blood cells. The vitamin D content of the samples was determined by a commercial laboratory to evaluate the vitamin D status[4]. At days 7, 14 and 21 the birds were weighed and feed consumption was determined. At day 21 the birds were killed by CO_2 inhalation. Toes were removed from all birds and ashed by pen to determine bone mineralization (Yan *et al*., 2005). Tibiae from both legs were removed and scored for tibial dyschondroplasia using the scoring system of Edwards and Veltmann (1983). Any bird that died during the course of the study was weighed to adjust feed conversion.

Pens means served as the experimental unit for statistical analysis. Data were subject to analysis of variance using the general linear model procedure of SAS (1991). The model included main effects of maternal vitamin D_3 level and source and the two way interaction. Percentage data were transformed to $\sqrt{n+1}$. Data are presented as natural numbers. 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 $p<0.05$ unless otherwise stated. All procedures used during this study were approved by the University of Arkansas Animal Care committee.

Table 1: Number of pen replicates by maternal diet and by hatch

Treatment	Vitamin D (IU/kg)	HyD (µg/kg)	Hatch	
			First	Second
1	0	0	1	2
2	300	0	8	8
3	600	0	12	16
4	1200	0	6	7
5	2400	0	12	11
6	0	68	8	9
7	300	68	8	12
8	600	68	10	16
9	1200	68	14	19
10	2400	68	10	14

Table 2: Composition (%) and calculated nutrient content of diets for hatched chicks

Ingredient	Chick
Yellow corn	61.908
Soybean meal	35.371
Ground limestone	0.518
Defluorinated phosphate	1.165
Sodium chloride	0.419
L-Lysine	0.069
MHA 84[1]	0.200
Vitamin premix[2]	0.250
Mintrex P_Se mineral mix[3]	0.100
Total	100.000
ME (kcal/lb)	1399.73
Crude protein (%)	23.42
Calcium (%)	0.70
Available P (%)	0.35
Lysine (%)	1.33
Tryptophan (%)	0.28
Threonine (%)	0.90
TSAA (%)	0.98

[1]Novus International, St. Louis Mo. [2]Provides per kg of diet: vitamin A (from vitamin A acetate) 7714 IU; vitamin E (from dl-alpha-tocopheryl acetate) 16.53 IU; vitamin B12 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione (from menadione dimethylpyrimidinol) 1.5 mg; folic acid 0.9 mg; choline 1040 mg; thiamin (from thiamin mononitrate) 1.54 mg; pyridoxine (from pyridoxine HCl) 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg; Se 0.1 mg. [3]Provides as mg/kg: 40 Mn, 20 Cu, 40 Zn, 0.30 Se complexed with methionine hydroxy analogue (Novus International, St. Louis Mo)

RESULTS AND DISCUSSION

Vitamin D status: 25-hydroxy-cholecalciferol is utilized as the indicator of preference to measure the vitamin D status since it represents the major circulating form of this vitamin (DeLuca, 2004). As much as 80% of the activity of the circulating vitamin D is attributed to 25OHD$_3$ (Ovesen *et al*., 2003).

The level of 25OHD$_3$ in plasma of the progeny at hatch as influenced by the maternal diet is shown in Fig. 1. The 25OHD$_3$ level in plasma of the progeny was higher when the breeder hens were fed with HyD. A response for 25OHD$_3$ in plasma was observed at high levels of cholecalciferol in the maternal diet, no response was obtained at low levels of 25OHD$_3$ in the maternal diet. The plasma 25OHD$_3$ response obtained when cholecalciferol was fed as the only source of vitamin D in the maternal diet was not as pronounced as that observed when HyD was included in the breeder diet and was only observed at high levels of cholecalciferol in the breeder diet. A higher level of 25OHD$_3$ in plasma has been also reported when feeding HyD directly in the diet of broiler chickens (Ferket and Gernat, 2002). This response can be explained by the longer half life of 25-OHD$_3$ of around 21 days compared to several days for cholecalciferol (Yarger *et al*., 1995). Once cholecalciferol is absorbed; it is taken up rapidly by the adipose tissue for storage or by the liver for metabolism (Jones *et al*., 1998).

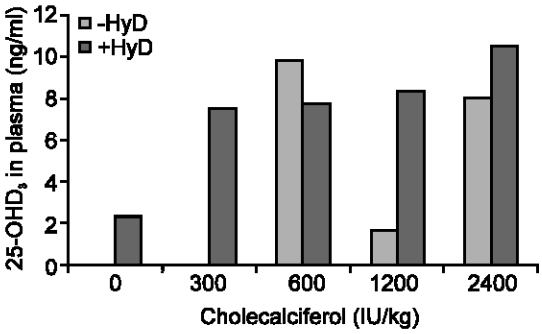

Fig. 1: Effect of the maternal vitamin D level and source on 1 day progeny plasma levels of 25-OHD$_3$[5]

The incubation and first days post hatch are acquiring especial attention since they can play a critical role to reach the genetic potential of the fast growing broilers strains utilized today (Kidd, 2009). The higher load of 25OHD$_3$ in one day old chicks when including HyD in the maternal diet can represent a means to supply the vitamin D demands of the embryo for adequate development and to overcome the delayed activation of the vitamin D absorption machinery during the first days post hatch when the nutrient supply is shifted from egg components to feed (Uni and Ferket, 2004). This higher supply of vitamin D to the progeny can entail an earlier and thus improved activation of metabolic functions such as bone mineralization and immune response which are associated with vitamin D (Holick, 2003).

Progeny fed no vitamin D in the diet

Performance: The table containing the ANOVA with the probability and details of the effects of the dietary factors on mortality, feed:gain ratio and body weight is shown in Table 3. At 7 days, mortality was influenced by the maternal vitamin D source with a numerical response ($p = 0.06$) observed between maternal vitamin D$_3$ level and source. Chicks from breeder hens receiving HyD in the diet had a significantly reduced mortality. Also, a numerical reduction in 7d mortality was observed as HyD was added at different levels of vitamin D in the maternal diet with the exception of the highest vitamin D$_3$ level in the maternal diet where a higher mortality was

Table 3: Effect of source and level of vitamin D in broiler breeder diet on performance of offspring fed diet with no supplemental Vitamin D

		Mortality (%)			Feed:Gain Ratio			Body weight (kg)		
Breeder treatment		0-7 d	0-14 d	0-21 d	0-7 d	0-14 d	0-21 d	7 d	14 d	21 d
Vit D_3 IU/kg										
0		11.68	13.68	14.75	1.260^{ab}	1.336	1.274	0.137^{ab}	0.333	0.539
300		9.37	15.36	22.93	1.248^{b}	1.386	1.462	0.123^{b}	0.272	0.431
600		7.66	14.23	22.10	1.304^{a}	1.443	1.372	0.131^{b}	0.275	0.407
1200		7.00	9.42	12.34	1.249^{b}	1.351	1.159	0.145^{a}	0.309	0.478
2400		3.60	11.18	17.03	1.226^{b}	1.378	1.358	0.141^{a}	0.289	0.424
HyD										
No		13.32^{a}	11.62	19.97	1.298^{a}	1.369	1.240	0.128^{b}	0.299	0.477
Yes		2.40^{b}	10.53	15.68	1.217^{b}	1.389	1.411	0.143^{a}	0.292	0.435
Vit D	**HyD**									
0	No	17.00	0.00^{c}	17.00^{abc}	1.292	1.298^{c}	1.086	0.132^{ab}	0.372^{a}	0.632^{a}
0	Yes	6.37	10.37^{bc}	12.50^{bc}	1.228	1.374^{abc}	1.462	0.141^{a}	0.295^{a}	0.446^{ab}
300	No	18.75	11.85^{abc}	22.87^{abc}	1.313	1.346^{abc}	1.280	0.105^{b}	0.271^{a}	0.448^{ab}
300	Yes	0.00	18.87^{ab}	23.00^{abc}	1.183	1.427^{ab}	1.644	0.140^{a}	0.273^{a}	0.414^{ab}
600	No	15.33	26.58^{a}	37.50^{a}	1.364	1.507^{a}	1.526	0.114^{b}	0.223^{b}	0.326^{c}
600	Yes	0.00	1.88^{c}	6.70^{c}	1.245	1.379^{abc}	1.217	0.149^{a}	0.328^{a}	0.488^{ab}
1200	No	14.00	14.00^{abc}	16.83^{bc}	1.274	1.303^{c}	1.041	0.149^{a}	0.337^{a}	0.554^{a}
1200	Yes	0.00	4.85^{bc}	7.84^{c}	1.223	1.400^{abc}	1.278	0.141^{a}	0.282^{a}	0.402^{bc}
2400	No	1.54	5.66^{bc}	5.66^{c}	1.247	1.393^{abc}	1.265	0.141^{a}	0.293^{a}	0.424^{bc}
2400	Yes	5.66	16.70^{ab}	28.40^{ab}	1.205	1.364^{abc}	1.451	0.142^{a}	0.284^{a}	0.424^{bc}
D level	Pdiff	0.65	0.71	0.51	0.01	0.09	0.28	0.0004	0.19	0.15
	SEM	6.879	8.334	10.20	0.038	0.055	0.202	0.008	0.032	0.059
D source	Pdiff	0.003	0.79	0.43	0.0002	0.52	0.12	0.0009	0.69	0.19
	SEM	3.141	3.819	4.64	0.017	0.026	0.093	0.004	0.014	0.027
Level x source	Pdiff	0.06	0.003	0.0005	0.26	0.01	0.08	<0.0001	0.0007	0.0008
	SEM	12.971	15.715	19.236	0.071	0.104	0.378	0.0150	0.060	0.111

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

reported when HyD was added. This result in agreement with (Araujo-Torres *et al*., 2009) who observed a reduction of mortality of the progeny at 7 days when 35 µg/kg of HyD was added to the maternal diet containing 2000 IU/Kg of vit D_3. (Atencio *et al*., 2005) observed a numerical reduction on mortality when HyD was added in the maternal diet at the same inclusion rate as cholecalciferol. This result was explained by the higher potency of HyD compared to cholecalciferol which is estimated to be 1.5 to 2 times higher and the higher affinity of vitamin D binding protein towards HyD (McNaughton *et al*., 1977). At day 14 and 21, there was a significant interaction between maternal vitamin D_3 level and source on mortality. No clear-cut trend was observed for these interactions. Stevens *et al*. (1983) working with 4 week-old turkey embryos found that the progeny of hens fed vitamin D levels as low as 300 IU/kg had a higher mortality rate. Moreover, Atencio *et al*. (2005) reported a numerical reduction in mortality of the progeny at 16 days as the vitamin D level was increased from 78-313 IU/kg. In this regard, Atencio *et al*. (2006) reported that the breeder requirement of vitamin D_3 for late embryo mortality reduction is 1393 IU/kg and 2759 IU/kg for the 26-36 week period and the 37-66 week period, respectively.

At 7 days, the feed:gain ratio was significantly influenced by the vitamin D_3 level and the vitamin D_3 source. The feed:gain ratio of the progeny tended to improve as the maternal level of vitamin D_3 increased. Also, when the maternal diet was supplemented with HyD there was a significant improvement of the feed:gain ratio. At 14 days, there was a significant interaction between the maternal vitamin D_3 level and source where no clear trend was obtained. At 21 days, the feed:gain ratio was not significantly influenced by vitamin D source and level. Lofton and Soares (1986) fed broiler chickens increasing levels of vitamin D from 0-8000 IU/kg observing a trend for improvement in the feed:gain ratio as the vitamin D increased.

At 7 days, body weight was significantly influenced by the maternal vitamin D_3 level and source with an interaction between vitamin D_3 level and source. In general, body weight increased as the vitamin D_3 level in the hen diet increased. Also, the progeny of hens receiving HyD in the diet had a significantly improved body weight at 7 days. There was also a trend to increase body weight at 7 days when HyD was supplemented at low levels of vitamin D in the maternal diet with no difference at higher levels of maternal vitamin D_3 in the diet. At days 14 and 21, body weight of the progeny was significantly affected by an interaction between HyD and vitamin D_3 level in the maternal diet; however, no clear trend was observed in both cases.

Table 4: Effect of source and level of vitamin D in broiler breeder diet on leg conditions of progeny fed diet with no supplemental vitamin D

			21 d		
Breeder treatment		1 d Tibia ash (%)	Toe ash[6] (%)	TD Incidence[7] (%)	TD Severity[3] (%)
Vit D_3 IU/kg					
0		25.96[b]	11.440	80.00	43.02
300		25.87[b]	10.65	97.50	80.20
600		28.48[a]	11.52	97.69	86.16
1200		30.20[a]	11.12	94.56	80.64
2400		29.27[a]	10.52	98.05	72.33
HyD					
No		27.64	11.30	94.67[b]	79.34
Yes		28.17	10.59	99.22[a]	80.36
Vit D	**HyD**				
0	No	22.85[c]	13.22	60.00	0.00
0	Yes	28.54[b]	9.65	100.00	86.04
300	No	23.45[c]	11.56	95.00	73.33
300	Yes	28.29[b]	9.73	100.00	87.08
600	No	28.00[b]	11.49	97.04	88.33
600	Yes	28.94[b]	11.54	98.33	84.00
1200	No	32.85[a]	11.62	90.55	75.27
1200	Yes	27.55[b]	10.61	98.57	86.01
2400	No	31.02[ab]	10.55	96.11	80.41
2400	Yes	27.51[b]	10.49	100.00	64.25
Vitamin D level	P diff	0.003	0.22	0.57	0.33
	SEM	0.906	0.418	2.060	6.053
Vitamin D source	P diff	0.52	0.07	0.02	0.85
	SEM	0.572	0.285	1.406	4.131
Source x level	P diff	0.0003	0.29	0.66	0.18
	SEM	1.281	0.682	2.913	8.560

[abc]Means in column with no common superscript differ significantly ($p<0.05$)

Griminger (1966) reported an improvement in body weight of the progeny fed a vitamin D deficient diet as the maternal diet was supplemented with increasing levels of vitamin D. This response was not observed during the first week but in most of the weeks post hatch. The delayed response toward maternal vitamin D supplementation can be explained by the lower demand of nutrients of the slow growth rate birds utilized decades ago.

Bone development: The ANOVA detailing the probability and the effects of the various maternal dietary factors on one day tibia ash, 21 day toe ash, 21 day TD incidence and TD severity is shown in Table 4. One day tibia ash was significantly influenced by the vitamin D level and an interaction between vitamin D_3 level and HyD supplementation in the maternal diet. When the maternal diet contained the two lowest levels of vitamin D_3 (0 and 300 IU/kg) a lower one day tibia ash was obtained compared to the other levels; no significant difference was observed among maternal levels of vitamin D_3 equal or greater than 600 IU/kg. For the two-way interaction, one day tibia ash was higher at lower levels of vitamin D_3 in the maternal diet when HyD was added with no difference at vitamin D_3 levels higher than 600 IU/kg. This result is in agreement with Atencio *et al.* (2006) who observed an increased 1 day body ash of the progeny from hens fed increasing levels of vitamin D. Atencio *et al.* (2006) also estimated that between 2000 and 4000 IU/Kg of vitamin D in the maternal diet is required for maximum body ash of the progeny. This result also confirms the importance of the maternal vitamin D supply for adequate skeletal development (Wilson, 1997). Toe ash was not significantly influenced by the maternal vitamin D_3 level and source. However, a numerical effect ($p = 0.07$) was observed when HyD was present in the maternal diet where the progeny of hens fed HyD reported lower toe ash at day 21. In contrast, Griminger (1966) working with progeny fed a vitamin D deficient diet observed an increased 4 week tibia ash as the vitamin D level in the hen diet increased. In the present study there was a significant effect of the supplementation of HyD in the maternal diet on TD incidence of the progeny at 21 days, an increase in the incidence of TD was observed as HyD was added to the maternal diet. No significant effects were found for TD severity. Noteworthy are the high rates of TD incidence and severity which demonstrates the importance of supplementing vitamin D_3 and adequate amounts of Ca and P in the diet for a proper development of the bone structure of the chick. Toe ash and the TD score were evaluated at day 21; at this point it is possible that any potential effect of the maternal vitamin D carryover had disappeared because of the depletion of stores

supplied by the hen and the absence of vitamin D in the diet of the progeny. The different response observed here compared to that reported by Griminger (1966) can be due to the higher requirement of the broiler strains utilized at the present time compared to those utilized decades ago.

In general, when feeding a vitamin D deficient diet to the progeny the carryover effect of the vitamin D was observed during the first week after hatch with no significant difference on later stages. Our results detailed above confirm the presence of a carryover effect of the vitamin D and its importance for the development of the newborn as proposed by Edwards (2000). The presence of more lasting effects of the vitamin D carryover was explored in the following trial including supplemented vitamin D in the diet of the progeny.

Progeny fed 5500 IU/kg of cholecalciferol in the diet

Performance: The table containing the ANOVA with the probability and details on the effects of the dietary factors on mortality, feed:gain ratio and body weight is shown in Table 5. Mortality at days 7, 14 and 21 was significantly influenced by the level of vitamin D in the maternal diet. Here the trend was to increase mortality as the vitamin D level in the maternal diet was increased. This result requires extra consideration since there was a reduced number of pen replicates for those treatments where the maternal diet had low levels of vitamin D (Table 1) and mortality analysis usually requires an increased sample size. Since each pen was allocated six birds the loss of one bird would be very influential on the mortality results. There was no significant effect of the variables studied on the feed:gain ratio at 7 days. The feed:gain ratio at days 14 and 21 was significantly influenced by the maternal vitamin D level and source and by an interaction between vitamin D level and vitamin D source. Increasing levels of vitamin D in the maternal diet improved the feed:gain ratio of the progeny. This result highlights the importance of the maternal supplementation of vitamin D since it was observed even at a high level of vitamin D in the diet of the progeny (5500 IU/kg) and 3 weeks after hatch. This result also supports the idea of maternal vitamin D supplementation as an strategy to deal with new regulations for vitamin D levels in broiler diets like that adopted by European nations where no more than 125 µm/kg (5000 IU/kg) of vitamin D is allowed to fulfill the broiler needs (Waldenstedt, 2006).

The addition of HyD in the maternal diet significantly improved the feed:gain ratio of the progeny at 14 and 21 days. This result can be explained by the higher polarity of 25-OHD allowing a higher intestinal absorption rate (Araujo-Torres *et al*., 2009).

Table 5: Effect of source and level of vitamin D in broiler breeder diet on performance of offspring fed diet with 5500 IU/kg supplemental vitamin D from cholecalciferol

		Mortality (%)			Feed:Gain Ratio			Body weight (kg)		
Breeder treatment		0-7 d	0-14 d	0-21 d	0-7 d	0-14 d	0-21 d	0-7 d	0-14 d	0-21 d
Vit D_3 IU/kg										
0		0.00^{b}	0.00^{b}	0.00^{b}	1.437	1.606^{a}	1.668^{a}	0.133^{c}	0.319^{c}	0.528^{c}
300		2.83^{ab}	3.89^{b}	3.89^{b}	1.341	1.486^{b}	1.562^{b}	0.144^{bc}	0.380^{b}	0.626^{b}
600		1.56^{b}	2.06^{b}	2.06^{b}	1.312	1.423^{b}	1.471^{c}	0.160^{a}	0.432^{a}	0.695^{a}
1200		5.43^{a}	5.43^{ab}	5.43^{ab}	1.328	1.465^{b}	1.490^{bc}	0.157^{ab}	0.418^{a}	0.679^{ab}
2400		5.96^{a}	8.80^{a}	8.80^{a}	1.229	1.375^{c}	1.438^{c}	0.160^{a}	0.439^{a}	0.726^{a}
HyD										
No		3.43	4.96	4.96	1.368	1.521^{a}	1.576^{a}	0.146	0.370^{b}	0.609^{b}
Yes		2.88	3.11	3.11	1.291	1.421^{b}	1.475^{b}	0.155	0.426^{a}	0.693^{a}
Vit D	**HyD**									
0	No	0.00	0.00	0.00	1.610	1.833^{a}	1.930^{a}	0.104^{d}	0.214^{d}	0.339^{d}
0	Yes	0.00	0.00	0.00	1.264	1.380^{c}	1.406^{d}	0.162^{ab}	0.425^{ab}	0.717^{ab}
300	No	4.25	6.37	6.37	1.452	1.533^{b}	1.610^{b}	0.140^{c}	0.354^{c}	0.586^{c}
300	Yes	1.41	1.41	1.41	1.229	1.438^{bc}	1.514^{bc}	0.149^{bc}	0.407^{b}	0.667^{b}
600	No	1.00	2.00	2.00	1.257	1.414^{c}	1.426^{d}	0.164^{ab}	0.423^{ab}	0.702^{ab}
600	Yes	2.12	2.12	2.12	1.368	1.432^{bc}	1.516^{bc}	0.156^{abc}	0.442^{ab}	0.688^{ab}
1200	No	7.28	7.28	7.28	1.316	1.471^{bc}	1.485^{cd}	0.154^{abc}	0.398^{bc}	0.658^{bc}
1200	Yes	3.57	3.57	3.57	1.340	1.459^{bc}	1.494^{cd}	0.160^{ab}	0.439^{ab}	0.700^{ab}
2400	No	4.63	9.18	9.18	1.205	1.355^{c}	1.430^{cd}	0.171^{a}	0.461^{a}	0.759^{a}
2400	Yes	7.28	8.42	8.42	1.252	1.395^{c}	1.446^{cd}	0.150^{bc}	0.417^{b}	0.692^{ab}
Vit D level	P diff	0.04	0.01	0.01	0.31	0.003	<0.0001	0.007	<0.0001	<0.0001
	SEM	2.560	2.976	2.976	0.094	0.053	0.044	0.008	0.020	0.036
Vit D source	P diff	0.71	0.29	0.29	0.16	0.002	0.0003	0.07	<0.0001	0.0001
	SEM	1.254	1.459	1.459	0.046	0.026	0.023	0.004	0.010	0.018
Level x source	P diff	0.45	0.76	0.76	0.07	0.001	<0.0001	0.001	<0.0001	<0.0001
	SEM	4.631	5.384	5.384	0.166	0.096	0.080	0.015	0.037	0.065

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

A two-way interaction between the vitamin D level and source was observed where HyD improved the feed:gain ratio with low levels of vitamin D in the maternal diet and with no difference at vitamin D levels higher than 600 IU/kg. This result is in agreement with Roberson *et al.* (2005). Body weight was significantly influenced at days 7, 14 and 21 by increasing levels of vitamin D in the maternal diet. There was a consistent trend of improvement in body weight of the progeny at increasing levels of vitamin D in the maternal diet. This is in agreement with Stevens *et al.* (1984). There was a significant response in body weight at days 14 and 21 and a numerical response at day 7 ($p = 0.07$) when HyD was added in the maternal diet. The progeny of hens receiving HyD showed higher body weight than the progeny of hens without HyD supplemented in the diet. There was a two-way interaction between maternal source and level of vitamin D on body weight; there was a trend to improve body weight of the progeny when HyD was added to those maternal diets containing low levels of vitamin D with no effect of HyD when added to maternal diets with higher levels of vitamin D. Fritts and Waldroup (2003) compared 25OHD$_3$ and cholecalciferol at different inclusion levels in broiler diets observing the former as more metabolically potent than the latter to sustain body weight. This effect was observed at low levels of vitamin D supplementation with no difference at higher levels.

Bone development: The ANOVA detailing the probability and the effects of the various maternal dietary factors on 21 day toe ash, TD incidence and TD severity is shown in Table 6. Toe ash was significantly influenced by the vitamin D source in the maternal diet; the progeny of hens fed HyD had a significantly higher mineralization rate. Stevens *et al.* (1984) working with turkeys observed an increased 18 day tibia ash of the progeny when adding increasing levels of vitamin D$_3$ to the maternal diet. Moreover, Atencio *et al.* (2005) reported an increased 1 day body ash in the progeny of hens fed with 25-OHD$_3$. The response observed when HyD was added to the maternal diet compared to increasing levels of vitamin D3 can be due to the longer half life of HyD. Vitamin D level and source in the maternal diet had no significant effect on the TD incidence. TD severity reported a numerical reduction ($p = 0.08$) when HyD was supplemented in the maternal diet, in agreement with Fritts and Waldroup (2003) when feeding different levels and sources of vitamin D directly in the diet of broiler

Table 6: Effect of source and level of vitamin D in broiler breeder diet on leg conditions of progeny fed common diet with 5500 IU/kg of cholecalciferol

		21 day		
Breeder treatment		Toe ash[a] (%)	TD incidence (%)	TD severity (%)
Vit D$_3$ IU/kg				
0		12.39	31.48	8.84
300		11.54	28.16	5.55
600		11.72	16.54	2.18
1200		11.59	20.95	2.08
2400		11.65	25.13	2.27
HyD				
No		10.98[b]	21.83	4.09
Yes		12.27[a]	23.57	1.96
Vit D	**HyD**			
0	No	12.90	25.00	12.50
0	Yes	11.88	37.96	5.18
300	No	10.78	25.20	6.66
300	Yes	12.30	31.11	4.44
600	No	11.33	14.34	2.29
600	Yes	12.10	18.75	2.08
1200	No	10.89	17.61	2.85
1200	Yes	12.30	24.29	1.31
2400	No	10.91	30.15	4.54
2400	Yes	12.40	20.11	0.00
Vitamin D level	P diff	0.99	0.19	0.16
	SEM	0.451	7.845	3.050
Vitamin D source	P diff	0.003	0.37	0.08
	SEM	0.329	3.853	1.498
Source x level	P diff	0.87	0.52	0.78
	SEM	0.773	7.586	2.768

[ab]Means in column with no common superscript differ significantly ($p<0.05$)

chickens. Moreover, a numerical trend ($p = 0.16$) was observed; as the vitamin D level was increased in the diet a reduction on TD severity was seen. This result is in agreement with Edwards (2000).

The progeny fed 5500 IU/kg of vitamin D showed a more clear response for TD incidence and TD severity and overall lower rate of TD with respect to the progeny fed without vitamin D in the diet. This demonstrates the role of the maternal vitamin D on reducing the early onset of leg problems and the role of adequate levels of vitamin D in the diet of the progeny on preventing the later onset of leg problems, as reported by Stevens *et al.* (1984). Cholecalciferol absorption pathway requires micelle formation mediated by the action of bile acid and lipase (Ward, 2004). Vieth (1999) reported that the liver and pancreas of the newborn chick reach their maximum development between 3 and 7 days posthatch with full development of digestible and absorptive activities 2 weeks after hatching. Before hatch, lipase from the yolk sac membrane supports absorption for embryo development even a few days before hatch. $25OHD_3$ has a higher absorption rate due to its polarity and a higher activity once within the organism due to its longer half life compare to cholecalciferol (Yarger *et al.*, 1995; Bar *et al.*, 1980). The higher polarity given by the OH group of $25OHD_3$ allows bypassing the micelle formation step required by cholecalciferol for absorption. $25OHD_3$ also offers a higher affinity towards the vitamin D Binding Protein (DBP) transporter in the bloodstream resulting in a longer half-life (21 d) compared to several days half life for vitamin D_3 (Yarger *et al.*, 1995).

Once in the hen's body, the deposition of vitamin D into the egg yolk occurs through the Vitamin D-DBP complex traveling in the bloodstream and further binding the phosvitin protein in yolk by electrostatic bonds (Kawazoe *et al.*, 1996; Mecham and Olcott, 1949). The longer half life of $25OHD_3$ may be a reflection of better timing between the egg yolk formation and the vitamin D deposition into the egg yolk.

The results described in the present study confirm the presence of carryover from both sources of vitamin D (cholecalciferol and HyD) from the hen to the progeny. As expected, this carryover effect was not sufficient to sustain adequate performance of the progeny when no vitamin D was added in the progeny's diet. However, the carryover effect was strong enough to remain active even three weeks after hatching when most of the bone development activity occurs. The carryover effect on performance by HyD was more noticeable at low levels of cholecalciferol in the maternal diet with little or no difference at higher levels of cholecalciferol. The carryover effect on bone development was more related to the presence of HyD in the maternal diet with a better response of the progeny when HyD was in the maternal diet. Our results also confirm that supplementation of HyD in the maternal diet is a feasible option to supply vitamin D to the developing embryo.

ACKNOWLEDGEMENTS

This study was supported by a grant from Cobb-Vantress Inc., Siloam Springs AR. We express our thanks to DSM, Basel Switzerland, for conducting the many assays for this experiment.

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[3]Cobb-Vantress, Inc., Siloam Springs AR, USA.

[4]DSM Nutritional Products Ltd. Basel, Switzerland.

[5]Values depicted represent the average of two samples.

[6]Pens from treaments1 and 6 were not considered in the statistical analysis due to the presence of just one replicate and to balance the analysis respectively.

[7]Pens from treatments 1 and 6 were excluded from the statistical analysis for TD incidence and TD severity due to the presence of only one replicate and to balance the analysis respectively.

[8]Pens from treaments1 and 6 were not considered in the statistical analysis due to the presence of just one replicate and to balance the analysis respectively.