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Short Communication

Evaluation of one-alpha-hydroxy-cholecalciferol alone or in combination with cholecalciferol in Ca–P deficiency diets on development of tibial dyschondroplasia in broiler chickens

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ABSTRACT

This experiment was conducted to determine whether dietary cholecalciferol will alleviate a calcium and phosphorous (Ca–P) deficiency when one-alpha-hydroxy-cholecalciferol, 1α(OH)D_3, is supplemented, and to determine the effects of adequate and inadequate Ca–P when 1α(OH)D_3 is supplemented and vitamin D_3 is adequate. A total of 144 one-d-old broiler chicks (Ross 308) were allocated to 3 treatments. The dietary treatments were as follows: treatment A, adequate Ca–P + cholecalciferol + 5 μg/kg 1α(OH)D_3; treatment B, inadequate Ca–P + cholecalciferol + 5 μg/kg 1α(OH)D_3; treatment C, inadequate Ca–P + 5 μg/kg 1α(OH)D_3. All diets were mixed with 500 FTU/kg of phytase, and cholecalciferol was provided in 5,000 IU/kg except for treatment C that fed diets without vitamin D_3. The Ca–P levels in the adequate diets were 0.90% Ca, 0.66% total phosphorus (tP); 0.75% Ca, 0.59% tP; 0.69% Ca, 0.54% tP for the starter, grower and finisher periods. At d 42 of age, broilers were inspected for incidence and severity of tibial dyschondroplasia (TD). The results showed that inadequate Ca–P supplementation with cholecalciferol significantly decreased the incidence of TD, score and tibia ash compared with broilers fed the same diet in the absence of cholecalciferol ($P < 0.05$). The broilers fed inadequate Ca–P diets with cholecalciferol were unable to achieve the same tibia ash and incidence of TD as those fed Ca–P adequate diets ($P < 0.05$). In conclusion, this trial suggests that broilers fed an inadequate Ca–P diet with 1α(OH)D_3 and adequate level of cholecalciferol are unable to sufficient bone formation. There was no indication that 1α(OH)D_3 in the absence of cholecalciferol was effective in reducing TD whereas it could improve tibia ash.

1. Introduction

Avian tibial dyschondroplasia (TD), a disease commonly found in modern meat type birds such as broilers, is very sensitive to the vitamin D metabolism. The major perturbation leading to the TD is that the chondrocytes do not fully hypertrophy but rather accumulate along the growth plate of long bones such as the tibia (Farquharson and Jefferies, 2000). These abnormal accumulations may extend to large opaque plugs that eventually contribute to bone abnormalities. In addition to the vitamin D status of the bird, the dietary Ca–P level influences this insufficiency of chondrocyte maturation. Whitehead et al. (2004) reported that high dietary concentrations of vitamin D_3 could prevent TD. It has been previously shown that addition of vitamin D metabolites to the diet of broiler chickens reduced the incidence of TD (Roberson and Edwards, 1996; Elliot and Edwards, 1997; Mitchell et al., 1997a,b; Xu et al., 1997; McCormack et al., 2004; Nääs et al., 2012; Atencio et al., 2005a).

As an analog of vitamin D, one-alpha-hydroxy-cholecalciferol, 1α(OH)D_3, has been demonstrated to improve growth performance, tibia quality and P utilization in broilers (Snow et al., 2004). Edwards (2002) indicated that addition of 1α(OH)D_3 enhanced

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phytate phosphorus utilization in broilers, although the intestinal phytase activity of broilers was not influenced via 1α(OH)D_3. Landy et al. (2015) reported that in Ca–P deficiency diets and without vitamin D_3, addition of 1α(OH)D_3 improved tibia ash, Ca and P of broilers. However, when vitamin D_3 was enough, tibia qualities of broilers was not improved by 1α(OH)D_3 addition.

This experiment was conducted to determine whether dietary cholecalciferol will alleviate a Ca–P deficiency when 1α(OH)D_3 is supplemented, and to determine the effects of adequate and inadequate Ca–P when 1α(OH)D_3 is supplemented and vitamin D_3 is adequate.

2. Materials and methods

2.1. Ethical matters

The broilers were purchased from a local hatchery and reared in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals. Furthermore, the handling of birds complied with the ethical guidelines of the Isfahan University's Ethical Committee (approval ref No. 2015-234).

2.2. Animals and dietary treatments

One hundred forty-four 1-d-old broiler chicks (as-hatched) were randomly assigned to 3 dietary treatments for 6 wk. The broilers were fed starter diets from 1 to 10 d of age, grower diets from 11 to 24 d, and finisher diets from 25 to 42 d (Table 1). The dietary were formulated by Amino-feed software to meet nutrient requirements of broilers (Ross 308, 2014) except for Ca, P and cholecalciferol. The dietary treatments were as follows: treatment A, adequate Ca–P + cholecalciferol + 5 μg/kg 1α(OH)D_3; treatment B, inadequate Ca–P + cholecalciferol + 5 μg/kg 1α(OH)D_3; treatment C, inadequate Ca–P + 5 μg/kg 1α(OH)D_3. The Ca–P levels in the adequate diets were 0.90% Ca, 0.66% total phosphorus (tP); 0.75% Ca, 0.59% tP; 0.69% Ca, 0.54% tP for the starter, grower and finisher periods. The Ca and P levels in the inadequate diets were 0.80% Ca, 0.61% tP; 0.65% Ca, 0.54% tP; 0.59% Ca, 0.49% tP for the starter, grower and finisher periods. All diets were mixed with 500 FTU/kg of Phyzyme XP 5000 phytase (Danisco Animal Nutrition) and 5 μg/kg of 1α(OH)D_3 (Vitamin Derivatives Inc., Georgia, USA), and cholecalciferol was provided in 5,000 IU/kg except for treatment C that fed diets without vitamin D_3. Each replicate was assigned to a clean floor pen (120 cm × 120 cm × 80 cm) for 6 wk, and feed and water were provided for *ad libitum* consumption throughout the entire experimental period. To prevent exposure to ultraviolet light, the windows in broiler house were covered with clear plastic, and incandescent bulbs provided lighting. Except for d 1, a period of 23 h light and 1 h of darkness lighting schedule was applied. The experimental house temperature was controlled at 32 °C during the starter phase, 27 °C during the grower phase and 22 °C for the rest of the experiment.

2.3. Chemical analysis

At 42 d of age, 2 male broilers per pen were chosen, based on the average weight of the group, and sacrificed by cervical dislocation, and the right tibia was evaluated for TD as described by Edwards and Veltmann (1983). The left tibia was removed from each bird for bone ash analysis on a dry fat-free basis (method 22.10; AOAC, 1995).

2.4. Statistical analysis

The data were subjected to analysis of variance procedures appropriate for a completely randomized design using the GLM procedures of SAS (SAS Institute Inc., Cary, NC) to estimate the significance of the treatment effects. Means were compared using the LSD method. Statements of probability are based on $P < 0.05$.

3. Results

Data on tibia quality are summarized in Table 2. Treatments failed to induce any effect on tibia diameter, though it tended to

Table 1
The ingredient (as-fed basis) and calculated composition of experimental diets.

Item	Starter		Grower		Finisher	
	Adequate	Deficient	Adequate	Deficient	Adequate	Deficient
Ingredients, g/kg						
Corn	558.56	567.76	572.27	578.26	631.51	639.16
Soybean meal	385	383	360	360	308	307
Soybean oil	15	12.50	32	30	26.80	24.19
Monocalcium phosphate	12.70	10.50	10.19	7.95	8.98	6.74
$CaCO_3$	14.90	13.10	12.12	10.34	11.50	9.71
NaCl	3.50	3.50	3.50	3.50	3.45	3.45
Trace mineral premix[1]	1.25	1.25	1.25	1.25	1.25	1.25
Vitamin premix[2]	1.25	1.25	1.25	1.25	1.25	1.25
DL-methionine	3.17	3.16	3.07	3.07	2.86	2.85
L-lysine	1.70	1.72	1.57	1.60	1.63	1.65
L-threonine	1.05	1.06	0.91	0.92	0.80	0.80
Choline chloride	1.20	1.20	1.10	1.10	1.15	1.15
NH_4Cl	0.72	0.71	0.77	0.76	0.82	0.80
Calculated composition, %						
ME, kcal	2,870	2,870	3,010	3,010	3,040	3,040
CP	22	22	21	21	19.08	19.09
Ca	0.900	0.800	0.750	0.650	0.690	0.590
Total P	0.660	0.611	0.591	0.542	0.546	0.497
Nonphytate P	0.380	0.330	0.320	0.270	0.290	0.240
Na	0.155	0.155	0.155	0.155	0.150	0.150
Cl	0.360	0.360	0.360	0.360	0.360	0.360

[1] Provided the following per kg of diet: Mg, 120 mg; Fe, 20 mg; Cu, 16 mg; Zn, 110 mg; Se, 0.3 mg; I, 1.25 mg.
[2] Provided the following per kg of diet: vitamin A, 12,000 IU; vitamin D_3, 5,000 IU; vitamin E, 80 IU; vitamin K, 3.2 mg; riboflavin, 8.6 mg; vitamin B_{12}, 0.017 mg; pantothenic acid, 20 mg; nicotinic acid, 65 mg; folic acid, 2.2 mg.

Table 2
Effect of dietary Ca–P levels, cholecalciferol, and 1α(OH)D_3 supplementation on tibia parameters of broilers at 42 d.

Item	Treatments				Tibia parameters					
	Supplementation[1]	Cholecalciferol [2]	Total P	Ca	Weight, g	Length, cm	Diameter, cm	Bone ash, %	TD[3], %	Score[4]
A	Phytase + 1α	+	Adequate	Adequate	5.00[a]	9.22[b]	0.61	43.42[a]	40[c]	0.60[b]
B	Phytase + 1α	+	Deficient	Deficient	4.19[b]	9.58[a]	0.56	39.97[b]	60[b]	0.60[b]
C	Phytase + 1α	−	Deficient	Deficient	4.68[ab]	9.62[a]	0.55	44.63[a]	75[a]	1.53[a]
SEM					0.24	0.09	0.04	0.52	7.23	0.32

TD = tibial dyschondroplasia; SEM = standard error of the mean.
[a,b] within a column, means not sharing a common superscript differ ($P < 0.05$).
[1] Supplementation at 500 units/kg of phytase + 5 μg/kg of 1α(OH)D_3.
[2] +, dietary containing 5,000 IU/kg D_3; −, dietary without vitamin D_3.
[3] Percentage of birds indicated TD.
[4] The mean of 4 pens per treatment.

increase in broilers fed Ca–P adequate diets ($P > 0.05$). Treatment C had higher tibia length compared with treatments A, whereas treatment B was intermediate and not different from treatment C. Treatment A had higher tibia weight that came from higher tibia ash compared with treatment B, but did not differ from treatment C that was intermediate. Addition of vitamin D_3 to Ca–P deficient diets without cholecalciferol and containing 5 μg/kg 1α(OH)D_3 led to the lower tibia ash values ($P < 0.05$). The percentage of tibia ash values were lower for the chicks fed Ca–P deficient diets containing cholecalciferol and 5 μg/kg of 1α(OH)D_3 than for chicks fed a normal commercial level of Ca–P. At 42 d of age, supplementation of cholecalciferol to Ca–P deficient diets decreased the TD score ($P < 0.05$). The sagittal sections of the tibias showed over a 75% incidence of TD in broilers fed Ca–P deficiency diets without vitamin D_3 that was differ from broilers supplemented with vitamin D_3 in treatments A and B.

4. Discussion

In the present study, 1α(OH)D_3 negatively influenced bone mineralization when dietary cholecalciferol was adequate. Edwards (2002) reported that addition of 1α(OH)D_3 in basal diets with tP of 7.0 g/kg and without cholecalciferol could improve performance of broilers. However when dietary cholecalciferol was adequate (Biehl et al., 1997), performance of broilers was not improved by 1α(OH)D_3 supplementation. Considering the possibility of interaction between 1α(OH)D_3 and cholecalciferol, the effect of 1α(OH)D_3 alone or in combination with different levels of cholecalciferol should be investigated in broilers chickens.

In this trial feeding inadequate Ca–P diets containing 1α(OH)D_3 in combination with cholecalciferol adversely affect bone development compared with broilers fed inadequate Ca–P diets containing 1α(OH)D_3 in the absence of cholecalciferol. Landy et al. (2015) reported that Ca–P deficiency diets and without vitamin D_3, 1α(OH)D_3 improved tibia parameters of broilers. However, when vitamin D_3 was adequate, tibia qualities of broilers was not improved by 1α(OH)D_3 addition. Similarly, Atencio et al. (2005b) reported that a complement of 25(OH)D_3 enhanced the hen-day egg production in broiler breeders but only at very low levels of overall cholecalciferol addition. Consistently, Edwards (2002) found that an interaction between vitamin D_3 and 1,25$(OH)_2D_3$ exists in tibia ash.

In this trial, feeding inadequate Ca–P diets containing 1α(OH)D_3 in combination with cholecalciferol decreased incidence and severity of TD compared with broilers fed inadequate Ca–P diets containing 1α(OH)D_3 in the absence of cholecalciferol. Elliot and Edwards (1992) reported high incidence of TD which was associated with elevated plasma l,25$(OH)_2D_3$. In contrast, Newbrey et al. (1988) found no correlation between the incidence of TD and plasma l,25$(OH)_2D_3$. Drewe et al. (1988) reported that both l,25$(OH)_2D_3$ and 25(OH)D_3 were enhanced in plasma when 1,25$(OH)_2D_3$ was supplemented to a diet that was inadequate in cholecalciferol. However, Rennie et al. (1993) reported no changes in plasma l,25$(OH)_2D_3$ when l,25$(OH)_2D_3$ was supplemented to a diet that was adequate in cholecalciferol. It seems that in the present study addition of cholecalciferol to the Ca–P deficiency diet containing 1α(OH)D_3 could decrease l,25$(OH)_2D_3$ plasma level due to interaction between cholecalciferol and 1α(OH)D_3 (Edwards, 2002) and thereby decreased incidence and severity of TD.

5. Conclusion

In conclusion, this trial suggests that broilers fed an inadequate Ca–P diet with 1α(OH)D_3 and adequate level of cholecalciferol are unable to sufficient bone formation. There was no indication that 1α(OH)D_3 in the absence of cholecalciferol was effective in reducing TD whereas it could improve tibia ash. Considering the possibility of interaction between 1α(OH)D_3 and cholecalciferol, the effect of 1α(OH)D_3 alone or in combination with different levels of cholecalciferol should be investigated in broilers chickens.

Conflict of interest

We declare that we do not have any conflict of interest.

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