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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Effect of Dietary 1, 25-Dihydroxycholecalciferol Concentration on Growth Performance and Bone Characteristics of Japanese Quail Fed Diet Deficient in Calcium and Phosphorus

A. Sheikhlar and S. Navid
Department of Animal Science, Faculty of Agriculture,
University of Putra Malaysia, 43400, Selangor, Malaysia

Abstract: An investigation was carried out to assay the concentration of dietary 1, 25-dihydroxycholecalciferol [1, 25$(OH)_2D_3$] on growth parameters and bone characteristics includes bone ash, Tibial Dyschondroplasia (TD) incidence and plasma minerals in broiler quails. Diets comprised control diet (diet 1) with sub optimal levels of calcium (0.71%) and total phosphorus (0.37%) and diets 2, 3, 4 and 5 that contained control diet supplemented with 2.5, 4.5, 6.5 or 8.5 µg/Kg 1,25$(OH)_2D_3$. Dietary treatments had no effect ($p>0.05$) on the live weight gain and feed conversion ratio. Bone ash was increased significantly ($p<0.05$) by 6.5 µg/Kg 1, 25$(OH)_2D_3$ at 3 wk of age. Treatments effects were shown ($p<0.05$) on calcium of bone but there was no influence on bone's phosphorus. The incidence and severity of TD were reduced pronouncedly ($p<0.05$) by 6.5 µg/Kg 1, 25$(OH)_2D_3$ at 3 wk of age. Plasma calcium improved significantly ($p<0.05$) however; plasma phosphorus did not show any differences ($p>0.05$) among experimental treatments. In conclusion, dietary level of 6.5 µg/Kg 1, 25$(OH)_2D_3$ showed positive effect on the reduction of TD in broiler quails at 3 wk of age.

Key words: 1, 25-dihydroxycholecalciferol, tibial dyschondroplasia, tibia ash, broiler quail

INTRODUCTION

Vitamin D metabolism in the bird's body is a complex process involving many metabolites. Dietary Vitamins D_2 and D_3 are absorbed through the small intestine and are transported in the blood to the liver where they are converted into 25-hydroxycholecalciferol (25-OHD), the major circulating form of Vitamin D_3. 25-OHD is then transported to the kidneys where it is converted into 1,25-dihydroxycholecalciferol (calcitriol) (1,25-OHD), that is the most biologically active, hormonal metabolite of the vitamin (McDonald and Edwards, 1995). Vitamins D_3 also interact with calcium level in the feed. At low calcium levels, Vitamins D_3 seems to be more effective in reducing TD incidence, than at sufficient calcium levels (Ledwaba and Roberson, 2003).

Several research have also been conducted to show that addition of dietary 1, 25-dihydroxycholecalciferol in feed improves phytate P bioavailability and thereby decreases the incidence of rickets and Tibial Dyschondroplasia (TD) (Edwards, 1993; Roberson and Edwards, 1994). Also addition of the metabolite 1,25-OHD within the range 5-10 µg/kg feed has in many research been reported to alleviate the incidence and severity of TD and rickets and increase bone mineralization, both in diets adequate in calcium and cholecalciferol and at suboptimal levels of calcium and phosphorus in chickens (Roberson and Edwards, 1994; Rennie *et al.*, 1995 and Elliot and Edwards, 1997).

Tibial Dyschondroplasia (TD) in birds are defined as a mass of opaque cartilage lesion occurring in tibiotarsus and tarsometatarsus region. The cartilage lesion originates below the growth plate (physis), remains unvascularized and is the consequence of a failure of transitional (prehypertrophic) chondrocytes to hypertrophy fully (Hargest *et al.*, 1985). The expression and severity of this abnormality are influenced by many factors such as environment (Veltmann and Jensen, 1980), genetics (Leach and Nesheim, 1965; Sheridan *et al.*, 1978) and mycotoxins (Walser *et al.*, 1980), but the etiology of the disease remains unclear. As mentioned above, studies on the evaluation of the effect of 1, 25$(OH)_2D_3$ on TD incidence have much known in chicken, however study in broiler quail is scanty. Therefore this research has conducted to evaluate the influence of 1,25$(OH)_2D_3$ on growth and TD prevalence of quails.

MATERIALS AND METHODS

Present study was conducted in Poultry Unit of Negine Sabz Company, in Karaj, Iran. A total of 500 day-old unsexed broiler quail were kept in stainless steel starter battery brooders and randomly placed to 20 pens of 25 birds each. Four of the pens were assigned to each of five dietary treatments. The diets were based on corn and soybean meal. The dietary treatments included diet 1 with sub optimal levels of calcium (0.71%) and total phosphorus (0.37%) and diets 2, 3, 4 and 5 contained diet 1 supplemented with 2.5, 4.5, 6.5 or 8.5 µg/Kg 1,25$(OH)_2D_3$. All diets and water were provided for *ad libitum* consumption. The environmental temperature was initially 37°C and gradually reduced by 3°C per week to 28°C in week 3. The experiment was 21 days. The bird

Table 1: Composition of experimental diet

Ingredients (g/kg)	Amount
Corn grain	486
Soybean meal	440.3
Palm oil	40
Limestone	13.5
Dicalcium phosphate (DCP)	3.2
Salt	4
Mineral premix[1]	5
Vitamin premix[2]	5
Lys	1
DL Met	1
Chemical composition	
ME (Kcal/kg)	2960
Crude protein%	24
Ca %	0.71
Available P%	0.21
Total P%	0.37

[1]Mineral premix supplied/kg diet: Cu, < 15 mg; Fe, 70 mg; Zn, < 100 mg; Mn, 80 mg; Se, 0.15 mg ; Co, 0.50 mg; Pb, 50 ppm; Cd ,10 ppm; Hg,0.5 ppm; I < 20 mg.
[2]Vitamin premix supplied /kg diet : vitamin A, 15.00 MIU; vitamin D3 (cholecalciferol), 2.50 MIU; vitamin E, 40 mg; vitamin B_1 (thiamine), 3 mg , vitamin B_2 (riboflavin), 6 mg ; vitamin B_6 (pyridoxine), 4 mg; vitamin B_{12}, 0.04 mg; vitamin PP (niacin), 30 mg; pantothenic acid, 16 mg; vitamin H (biotin), 0.12 mg; vitamin M (folic acid), 1 mg

management was based on the guidelines of the Consortium Guide (1988). Daily feed intake and weekly body weights were recorded throughout the experiment. Feed efficiency was determined from the body weight gain and feed intake data. At the end of experiment (21 d of age), blood samples were taken from 10 birds randomly selected from each replicate using heart puncture technique to measure Ca and P according to AOAC (1990); The plasma from heparinized blood samples was separated by centrifugation at 3,000 x g for 15 min at 4°C and digested with HNO_3. Thereafter, Ca was measured at 422 nm by an atomic absorption spectrophotometer (Z-5000 polarized Zeeman, Hitachi Instruments, Inc., USA) and P at 400 nm by a spectrophotometer (U-2001, Hitachi instruments, Inc., USA). At 21 d of age, 10 birds from each replicate were slaughtered to determine the incidence and severity of TD (Edwards and Veltmann, 1983) and tibia ash content (AOAC, 1990). Right tibia of those birds were freed of soft tissue and weighted. Thereafter bone samples dried at 100 C/4h and were defattened by soaking in petroleum ether for 48h. Then tibia ash was measured.

Statistical analysis: The experimental data were subjected to analysis of variance by the GLM procedure of SAS (SAS Institute, 1991) in a completely randomized design. Pen means were used as the experimental unit. Duncan's multiple range test (Duncan, 1995) was used to determine significance of differences among means at p<0.05.

RESULTS AND DISCUSSION

Live weight gain and feed conversion ratio of broiler quails at 1, 2 or 3 wk of age are presented in Table 2. In the study, it has not seen any dietary treatment effects on live body weight and feed conversion ratio of quails over 3 wk of age. Our findings agree with the findings of some workers that reported supplement of 1, $25(OH)_2D_3$ with 3 - 6µg/kg feed showed no negative effect on growth of chickens when diets contained low dietary calcium concentrations (Elliot *et al*., 1995; Rennie *et al*., 1995 and Roberson and Edwards, 1996) that mentioned 1, $25(OH)_2D_3$ can reduce TD disease without any influence on performance parameters.

The concentration of plasma calcium, phosphorus and 1, $25(OH)_2D_3$ were not influenced by treatments (Table 3). These results are similar with the reports of Kevin and Edwards (1996) that showed no effect of dietary 1, $25(OH)_2D_3$ on calcium and phosphorus concentration of plasma in broiler chicks at 3 wk of age. Plasma 1, $25(OH)_2D_3$ was unaffected in these studies as well despite the obvious effect dietary 1, $25(OH)_2D_3$ had on the development of TD. The absence of a relationship between TD and plasma 1, $25(OH)_2D_3$ has been shown

Table 2: Effects of dietary treatments on live weight gain and feed conversion ratio of broiler quails over 3 wk of age

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM	P
Live weight gain (g)							
Wk 1	26.5	25.6	25.6	24.9	26.2	1.8	<0.001
Wk 2	45.2	44.9	45.0	45.6	46.1	1.9	<0.001
Wk 3	54.5	55.2	56.0	56.1	57.1	3.0	<0.001
Feed conversion ratio							
Wk 1	1.77	1.82	1.78	1.75	1.81	0.13	<0.001
Wk 2	2.56	2.6	2.49	2.51	2.45	0.17	<0.001
Wk 3	2.58	2.61	2.62	2.55	2.62	0.19	<0.001

[a,b]Means with different superscripts within the same row are different (p<0.05). S.E.M: pooled standard error of mean. P: possibility

Table 3: Effects of dietary treatments on plasma 1, $25(OH)_2D_3$, phosphorus and calcium in broiler quail at 21d

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM	P
1, $25(OH)_2D_3$ (pg/mL)	276	290	294	287	283	36	<0.001
Phosphorus (mmol/L)	2.38	2.40	2.39	2.41	2.41	0.21	<0.001
Calcium (mmol/L)	2.04	2.02	1.94	1.89	1.95	0.18	<0.001

[a,b]Means with different superscripts within the same row are different (p<0.05). S.E.M: pooled standard error of mean. P: possibility

Table 4: Effects of dietary treatments on tibia ash, calcium, phosphorus and incidence and score of TD in broiler quail at 21d

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM	P
Tibia ash (%)	43.00[b]	43.4[b]	45.72[a]	45.60[a]	46.10[a]	2.25	<0.001
Calcium of Tibia (%)	38.38[b]	38.40[b]	39.55[a]	39.74[a]	40.00[a]	1.0	<0.001
Phosphorus of Tibia (%)	18.46	18.53	18.66	18.70	19.10	0.7	<0.001
Incidence of TD (%)	55[a]	54[a]	48[b]	47[b]	45[b]	8.2	<0.001
Score of TD	2.83[a]	2.67[b]	2.66[b]	2.59[b]	2.54[b]	0.14	<0.001

[a,b]Means with different superscripts within the same row are different ($p<0.05$). S.E.M: pooled standard error of mean. P: possibility

before by some researchers (Newbery *et al*., 1988; Elliot and Edwards, 1992).

Additional 1, 25$(OH)_2D_3$ enhanced pronouncedly ($P<0.05$) the bone ash of quails fed diet contained 6.5 µg/Kg 1, 25$(OH)_2D_3$. Calcium content of bone also increased ($P<0.05$) by dietary treatments however, no effect has seen in phosphorus content of bone by supplemental 1, 25$(OH)_2D_3$. 1, 25$(OH)_2D_3$ significantly reduced ($P<0.05$) the incidence of TD and TD severity of birds at 3 wk of age (Table 3). Therefore the effective level of additional 1, 25$(OH)_2D_3$ to increase bone ash and reduce incidence and severity of TD at 3 wk of age was 6.5 µg/Kg in broiler quails. Same results were reported by Kevin and Edwards (1996) in broiler chicken that showed 6 µg/Kg could be an effective level of 1, 25$(OH)_2D_3$ to improve TD incidence and tibia ash percentage. It could be concluded from above that 6.5 µg/Kg of 1, 25$(OH)_2D_3$ in broiler quails reduces the TD prevalence over 3 wk of age.

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