EFFECT OF HIGH LEVELS OF CHOLECALCIFEROL ON PERFORMANCE, SOME BLOOD CONISTITENTS, BONE QUALITY AND MINERAL RETENTION IN BROILER CHICKS FED LOW LEVELS OF CALCIUM AND PHOSPHORUS

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ABSTRACT

A 6-wk feeding trail with 216 unsexed day-old Hubbard chicks was conducted to study the effects of supplementing extra levels (3000 vs. 7000 and 8000 I.U) of cholecalciferol on performance, bone quality and mineral retention in broiler chicks fed suboptimal levels of calcium and non-phytate phosphorus (0.5% Ca and 0.25% NPP). A control diet containing recommended levels of Ca, NPP and cholecalciferol (1%, 0.5% and 3000 I.U, respectively). Each diet was fed ad *libitum* to 6 replicates containing 9 chicks in each.

The results indicated that:

- 1- Productive performance (body weight, body weight gain, feed consumption and feed conversion ratio) didn't affected significantly (P≤0.05) by inclusion deficient Ca and NPP diets with high levels of cholecalciferol.
- 2-Plasma Ca and P weren't influenced significantly (P≤0.05) by the reduction of Ca and P with extra levels of cholecalciferol.
- 3- Dry tibia weight, tibia ash%, tibia Ca and P weren't significantly affected by reducing dietary levels of Ca and NPP with high levels of cholecalciferol.
- 4- Birds fed extra levels of cholecalciferol (7000 or 8000 I.U) retain more Ca and P as percentage of intake compared with control birds with significant differences (P≤0.05)

In conclusion, we recommended that higher levels of cholecalciferol supplementation to low Ca and NPP diet improved the performance, bone quality and minerals retention in order to reduce phosphorus pollution as well as feed cost.

Keywords: Bone quality, low calcium, low phosphors, cholecalciferol, growth, broiler chicks.

INTRODUCTION

Phosphorus (P) from plant feed ingredients is not completely available for chicken due to its complex bond with inositol and divalent cations like Ca, Mg, Cu, Zn, Fe, Mn, etc. The complex is called as phytate. Therefore, inverse relation exists between dietary phytate and the solubility/ availability of these minerals to poultry (Nwokola and Bragg, 1977; Eardman, 1979; Kornegay et al., 1996). Use of plant feed ingredients in chicken diet, result in excretion of P and other minerals bound with phytate in considerable quantities and cause environmental pollution. Dietary calcium (Ca) and P at their recommended concentrations is known to reduce the utilization of PP (Ballam et al., 1985; Schoner et al., 1993; Qian et al., 1994). The evidence of increased PP availability from plant feed ingredients at their sub-optimal concentrations of P (Davies et al., 1970; Onyango et al., 2006), existence of gut mucosal phytase activity (Bitar and Reinhold, 1972; Onyango et al., 2001) and its enhancement with D3 supplementation (Onyango et al., 2006) reducing cost

of supplemented P (inorganic phosphorus) and minimizing phosphorus excretion (Rama Rao et al., 2006b; 2007). Dietary cholecalciferol has long been known to increased phytate phosphors digestibility and reduce the rachitogenic nature of low-ca, high phytate diets (Mellanly, 1950; Steenlock and Heirting, 1955). The purpose of this study was to examine whether increased dietary cholecalciferol in deficient Ca and P broiler diet would equivalent to recommended Ca an NPP diet to recover performance and bone quality demands.

MATERIALS AND METHODS

The present study was carried out at the poultry nutrition farm, Poultry Production Department, Faculty of Agriculture, Ain Shams University.

Two hundred and sixteen unsexed one day — old of Hubbard broiler chicks were randomly distributed into 4 treatments. Each treatment comprised of 54 chicks which divided into 6 replicates of 9 chicks each. The chicks were reared p to 6 weeks of age in wire-floored batteries. The control diet (T1) was formulated according to the manual guide of Hubbard broiler chicks which containing calcium 1%, available phosphorus 0.5% and 3000 I.U cholecalciferol at the starter period and for other period grower (ca 1%, A.P0.5%) and finisher (ca 0.9%, A.P0.45%) with the same level of cholecalciferol. Then tested diets were containing the half of calcium and available phosphorus requirements with graded levels of cholecalciferol 3000 (T2), 7000 (T3) and 8000 (T4) International unite (IU). All diets were iso calorie and iso nitrogen, experimental diets were listed in (Table 1).

All birds were reared under similar environmental, managerial and hygienic conditions. Feed and water were provided ad libitum. Vaccination programs were applied according to the scheme of vaccination, used in the laboratory.

Body weight and feed consumption were recorded weekly. Weight gain, feed conversion ratio (gain/feed) were calculated weekly.

The total excreta were excreta were collected at 28 and 42 days, feed consumption was recorded during the same period. The excreta sample were dried in a 70°C oven, grounded and stored until analysis.

At 28 and 42 days of age, six birds from each treatment (one from each replicate) having average body weight around the treatment were selected and sacrificed by cervical dislocation. Blood sample were taken.

Tibia of both legs were removed, cleaned of flesh and all soft tissue, oven-dried and dry tibia weight, length, breaking strength were determined. The tibias were ground for procedure of the chemical analysis.

The excreta and tibia samples were dried at 105°C until constant weight, ashed at 600°C for 4 hour, dissolved by using 3 N HCi, and then filtered. Calcium and phosphorus content were then assayed in the filtrate by a colorimetric method pursuant to AOAC (1990). Apparent retention (percentage of intake) of Ca and P were calculated for each treatment.

Blood sample were immediately centrifuged at 3000 rpm for 10 minutes to separate plasma. Plasma calcium, phosphorus and alkaline phosphatase were assayed by colorimetric method using commercial kits of *Spinreact* Company, Spain.

Table (1): Composition and calculated analysis of Centrol and tested diets of first and second experiment

	Sta	rter	Gro)Well	Fini	sher
	One day	y to 14 d.	15 to	28 d.	29 to	42d.
Ingredients (%)	Control	Tested	Centrol	Tested	Control	Tested
Yellow com	56.10	56.92	59.51	60.24	62.16	62.91
Soy bean meal (44%)	28.80	33.31	26.30	31.10	23.10	27.4
Corn gluten meal (62%)	9.00	5.8	7.10	3.64	7.40	4.3
Soy oil	1.46	1.46	2.6	2.6	3.32	3.32
Limestone	1.60	0:77	1.72	0.82	1.58	0.63
Monocalcium phosphate	1.85	0.62	1.65	0.55	1.4	0.45
Vit. and min premix	0.30	0.30	0.30	0.30	0.30	0.30
Salt (NaCl)	0.25	0.25	0.25	0.25	0.25	0.25
DL - Methionine	0.25	0.27	0.25	0.28	0.19	0.22
HCI lysine	0.39	0.30	0.32	0. 22	0.30	0.22
Calculated analysis:						
ME kcal/kg	3000	3003	3103	3103	3200	3206
Cp (%)	23.02	23.00	21:03	21.06	20.03	20.06
Ca (%)	1.00	0.50	1.00	0.50	0.90	0.45
Av. ph (%)	0.50	0.25	0.45	0.23	0.40	0.20
Methionine	0.66	0.66	0.62	0.62	0.55	0.56
Methiasine + cystine (%)	1.05	1.05	840	0.98	0.90	0.90
Lysine (%)	1.40	1.40	1.25	1.25	1.15	1.15

*Each 3 kg of premix containing: 15000000 I.U VIT. A, 50 g. VIT. E, 3000 mg. VIT. K3, 3000 mg. VIT. B1, 8000 mg. VIT. B2, 4000 mg. VIT. B6, 20 mg. VIT. B12, 15000 mg. Pantothenic acid, 60000 mg. Nixcka, 1500 mg. Folic acid, 200 mg. Biotin, 200000 mg vitC, 700 gm. Cheline chioride, 80 gm. Mg. B0 gm. Zn, 80 gm. tron, 10 gm. Cu, 1 gm. lodine, and 0.2 gm. Seleniam, where CaCo₃ was taken as a carrier up to 3kg, the inclusion rate was 3kg premix / Ton feed.

Data of concerned traits were subjected to one - way analysis of variance with levels of CC used as the main effect using General Linear Model (GLM) procedure of SAS user's Guide (1995) according to the following model:

 $Y_{ik} = \mu + C_i + e_{ik}$ Where; μ = overall mean, C_i = levels of cholecalciferol,

e $_{1\text{ k}}$ = experimental error. Individual effects of cholecalciferol levels were compared using Duncan's multiple range tests (1955) at α level equal to 0.05 or 0.01.

RESULTS AND DISCSSION

Growth performance

Table 2 shows the results of body weight, body weight gain, feed consumption, feed conversion ratio and mortality number during the different periods. The overall results indicated that supplementations cholecalciferol in deficient Ca and P diets have a significant effect on performance traits, while the differences between birds fed 7000 or 8000 I.U and birds fed control diets didn't reach to significant case.

Birds fed control diet recorded the best body weight compared with 3000, 7000 and 8000 I.U (2199, 1886, 2152 and 2180g). These results were agreed with finding of Rio Garcia et al., (2006), (Rama Rao et al., 2008) and

[®] Calculated analysis of the experimental diets were done according to (NRC, 1994).

 ^{*} Calculated on basis average available phosphorus in plant feedstuffs equal 30% of total phosphorus (NRC, 1994)

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Haq Nawaz *et al.* (2008) who found that addition of cholecalciferol in deficient Ca and P diets improve body weight as well as body weight gain.

Dietary cholecalciferol has a significant positive effect on feed consumption and feed conversion ratio, birds fed either control diet or 7000 and 8000 I.U get the best values compared with 3000 I.U. These results were in harmony with those observed by Papeosva et al. (2008) and Haq Nawaz et al. (2008).

Table (2): Effect of low levels of Calcium and phosphorus with different levels of Cholecalciferol on broiler productive performance.

_	ieaela oi cuoied	calciteroi on	prolier produc	cuve perionni	ance.
Items	Control	3000 I.U	7000 I.U	8000 1.U	Sig.
		Body weight	(g)		
Initial	42.82±1.56	42.56±1.86	42.93±1.63	42.57±1.22	NS
2 weeks	398.65±4.36	353.77±4.25	386.29±4.89	394.32±4.32	**
4 weeks	1185.3 ±33.36	1005.4b±55.32	1118.4*±38.57	1165.6 ±40.57	*
0ale=	2199°±15.32	1886 ^b ±17.23	2152 °±22.48	2180. °±25.12	
6 weeks	(100)	(85.7)	(97.8)	(99.1)	_
	` ,	Body weight gai	in (g) ` ´	, ,	
0-2 week	355.83±3.28	293.17±3.51	343.36±3.78	351.75±3.61	•
2-4 week	786.71°±7.31	651.61 ^b ±8.28	732.10°±8.54	771.25°±7.11	*
4-6 week	1013.88°±10.52	880.63 ^b ±10.02	1033.97°±12.57	1014.68°±12.32	*
0-6 week	2156.4°±10.51	1843.4 ^b ±10.54	2109.4°±10.47	2137.7°±10.10	*
	Fe	ed consumption ((g/bird)		
0-2 week	452.32±2.11	467.25±1.51	460.24±2.41	459.94±1.68	NŞ
2-4 week	1294.78±8.85	1356.14±9.68	1321.24±8.86	1310.30 ^b ±8.65	NS
4-6 week	2074.26±11.55	2175.03±11.50	2098.97±11.89	2129.00±11.12	NS
n Carret	3821.4 ^b ±11.55	3998.4°±11.55	3880.4 ^b ±11.55	3899.2b±11.55	**
0-6 week	(100)	(104.6)	(101.5)	(102)	
Feed conversi	lon ratio (g feed / g	gain)	•	, ,	
0-2 week	1.27°±0.02	1.59°±0.05	1.34 ^b ±0.03	1.30 ⁵ ±0.07	**
2-4 week	1.64 ^b ±0.08	2.07°±0.08	1.80 ^b ±0.10	1.69 ^b ±0.10	•
4-6 week	2.04 ^b ±0.08	2.46 ⁴ ±0.08	2.03b±0.10	2.09 ^b ±0.10	•
• • • •	1.77 ^b ±0.10	2.17°±0.10	1.84 ⁵ ±0.10	1.82 ^b ±0.10	*
0-6 week	(100)	(122.6)	(103.9)	(102.8)	
Mortality	1	1	0	1	•
number					

* B.C. * Means within the same row with different superscripts are significantly different at P<0.05, Sig.= Significance, NS= Non Significant, * (P≤0.05), ** (P≤0.01).

Blood parameter

Table 3 shows values of blood Ca, P and alkaline phosphatase, dietary cholecalciferol has a significant effects on blood parameter (Ca, P and alkaline phosphatase). It was clear that birds fed deficient calcium and phosphorus diets supplemented with high concentration of cholecalciferol (7000 and 8000 I.U) gave values practically like control group, Plasma calcium of birds fed control, 7000 and 8000 I.U were 11.121, 10.923 and 11.042 mg/dl respectively without any significant differences. On the other hand, birds fed deficient calcium and phosphorus with 3000 I.U cholecalciferol get the less value (7.121 mg/dl) with significant difference against all treatments. The same trend was observed in plasma phosphorus. Alkaline phosphatase values in birds fed deficient diet were higher than control, but the differences between 8000 I.U and control group wasn't significant. These results were in agreement with those found by Rama Rao

J. Agric. Sci. Mansoura Univ., 34 (12), December, 2009

et al. (2006), Bolu et al. (2006) and Lofton and Soares (1986) who found that decreasing dietary calcium and phosphorus did not affect plasma calcium, phosphorus and alkaline phosphatase activity in diets containing extra levels of cholecalciferol.

Table (3): Effect of low levels of Calcium and Phosphorus with different

	Jevels of Choleca	on so	we blood co	resucherics.	
Items	Control	3000 I.U	7000 I.U	8000 I.U	Sig.
		Calcium (mg/	an -		
4 week	9.965°±0.41	6.233°±0.41		9.745°±0.45	*
6 week	11.121 ±0.81		40 0225 10 02	11.042 ±0.81	
o waek				11.042 IV.01	
•		^o hosphorus (m		_	
4 week	5.785°±0.12	3.333°±0.12	5.423 ±0.12	5.531°±0.12	*
6 week				5.996° ±0.19	•
OWCON				J.330 IU. 13	
	Alka	fine Phosphata	S8 (U/QI)		
4 week	1.855°±0.08	5.232*±0.09	3.063 ⁸ ±0.08	2.911b±0.08	*
6 week	2.022° ±0.16	7.121 ±0.16	3.211 ^b ±0.21	3.101 ^{bc} ±0.27	*

* h * Means within the same row with different superscripts are significantly different at P<0.05, Sig.= Significance, NS= Not significant, * (PS0.05), ** (PS0.01).

Bone quality

Data in Table 4 showed that increasing dietary cholecalciferol has a significant positive effect on bone quality measurements. Birds fed either 7000 or 8000 I.U gave the best values compared with 3000 I.U. Control birds get the superior values but the differences between control and 7000 or 8000 weren't significant. Tibia length of birds fed control diet was the best during 4 and 6 weeks of age but the differences with either 7000 or 8000 I.U weren't significant. While birds fed deficient diet with 3000 I.U was the worst on among all treatments with significant differences. The same trend was observed in Tibia width and tibia breaking strength. These results were in harmony with those found by Edwards (2002) who found that Supplemental D₃ appeared to prevents incidence of rickets and improves the bone ash contents, and Baker et al. (1998) who found that with a D₃ concentration of 1250 micro g/kg (250 times the recommended by the NRC) bone ash was increased (p<0.05).

Table (4): Effect of low levels of Calcium and Phosphors with different levels of Cholecalciferol on Bone quality parameters at 4 and 6

Mee	ks or age.			•	
items	Control	3000 I.U	7000 I.U	8000 I.U	Sig.
		Tibia Length (C	im)		
4 week	8.96°±0.07	5.14 ^b ±0.07	8.45°±0.07	8.60°±0.07	*
6 week	9.68 ² ±0.06	5.36 ^b ±0.06	9.32°±0.06	9.57°±0.06	*
		Tibia Width (C	m)		
4 week	0.79°±0.05	0.48°±0.05	0.60 ^b ±0.05	0.72°±0.05	*
6 week	0.98°±0.08	0.51°±0.08	0.88 ^b ±0.08	0.92°±0.08	*
	Tibia Br	eaking Strengti	h (Kg/Cm²)		
4 week	17.29°±0.93	9.59 ^b ±0.91	16.95°±0.89	17.09*±0.90	*
6 week	22.62ª ±0.92	9.86 ^b ±0.88	21.86° ±0.86	22.40° ±0.87	*_

4.5. Means within the same row with different superscripts are significantly different at P<0.05, Sig.= Significance, NS= Not significant, * (P≤0.05), ** (P≤0.01).

Bone measurements

Table 5 shows bone measurements, which didn't influenced by decreasing dietary calcium and phosphorus with extra levels of cholecalciferol. Birds fed deficient calcium and phosphorus diet with 3000 I.U gave the worst values compared with other treatments. At 6 weeks of age DTW% of birds fed either control or 7000 and 8000 were 0.501, 0.462 and 0.490% respectively without any significant differences, while DTW% of birds fed deficient diet with 3000 I.U was 0.312% which was significant low among all experimental treatments. Values of tibia ash, tibia Ca and tibia p% were in the same trend of DTW%, birds fed deficient diet with 3000 I.U gave the inferior values of ash, tibia Ca and P% among all treatments with significant differences. These results were in harmony with those observed by Santos, Y et al. (2005) who indicated that according tibia breaking strength, bones were stronger in high vitamin D₃ diets than control group for treated birds.

Table (5): Effect of low levels of Calcium and Phosphors with different levels of Cholecalciferol on Bone measurements at 4 and 6

241	ooks of age.				
Items	Control	3000 I.U	7000 I.U	8000 i.U	Sig.
		DTW (%)			
4 week	0.436 ^a ±0.002	0.257 ^b ±0.004	0.401°±0.002	0.420°±0.002	• .
6 week	0.501°±0.006	0.312b±0.004	0.462 ±0.006	0.490°±0.003	*
		Tibia Ash (%)		
4 week	42.85°±0.33	35.21°±0.33	41.89°±0.33	42.12°±0.33	*
6 week	43,12°±0.22	36.41 ^b ±0.48	42.80°±0.40	43.03°±0.53	•
		Tibia Ca (%)			
4 week	13.95°±0.77	9.12 ^b ±0.47	13.52°±0.22	13.60°±0.52	•
6 week	14,41°±0.63	9.58 ^b ±0.63	14.02°±0.54	14.18°±0.32	*
		Tibia P (%)			
4 week	7,50°±0.21	4.21 ^b ±0.21	6.72°±0.71	6.99°±0.11	*
6 week	7.80°±0.31	4.52 ^b ±0.11	7.01°±0.11	7.11 ^a ±0.12	*

**5. ** Means within the same row with different superscripts are significantly different at P<0.05, Sig.= Significance, NS= Not significant, * (P≤0.05), ** (P≤0.01).

DTW (%) = Relative dry tibla weight to body weight

Mineral retention

Table 6 shows mineral retention values of experimental treatments. Birds fed either 7000 or 8000 I.U retain more Ca and P compared with control group with significant differences. Values of Ca retention% of birds fed control diet, deficient diets with 7000 and 8000 I.U were 44.94%, 57.57% and 59.80% respectively with significant differences, it was observed that these decrease were dramatically. While birds fed deficient Ca and P with 3000 I.U was the worst in Ca retention% (37.75%). The same trend was observed in P retention% but it wasn't dramatically like Ca retention%. These results were in harmony with those found by (Mellanly, 1950; Steenlock and Heirting, 1955) who found that dietary cholecalciferol has long been known to increased phytate phosphors digestibility and reduce the rachitogenic nature of low-ca, high phytate diets. Generally, improvement of PP utilization upon vitamin D₃ supplementations might be accredited to increased biosynthesis

and/or activity of intestinal phytase (Shafey et al., 1991), increased phytate hydrolysis by stimulation Ca absorption making phytate more sensitive to hydrolysis and utilization (Mohammed et al., 1991) or increased P absorption (Wasserman and Taylor 1973; Tanka and DeLuca 1974).

Table (6): Effect of low levels of Calcium and Phosphorus with different levels of Cholecalciferol on Calcium and Phosphors retention.

items	Control	3000 I.U	7000 I.U	8000 I.Ū	Sig.
		Cal	cium	<u> </u>	
Intake	1.98°±0.02	0.98 ^b ±0.02	0.99 ^b ±0.02	1.02 ^b ±0.02	*
Excretion	1.09°±0.04	0.61 ^b ±0.04	0.42°±0.02	0.41°±0.03	*
Retention (g)	0.89°±0.02	0.37°±0.02	0.57°±0.04	0.61 ^b ±0.03	*.
Retention	44.94 ^b ±0.32	37.75°±0.35	57.57*±0.29	59.80°±0.23	
(%)	(100)	(84)	(128.1)	(133)	
		Phos	phorus		
Intake	1.37°±0.003	0.70 ^b ±0.003	0.74 ^b ±0.003	0.73 ^b ±0.003	*
Excretion	0.49 ^a ±0.002	0.32 ^b ±0.002	0.25 ^b ±0.002	0.23 ^b ±0.002	*
Retention (g)	0.88°±0.001	0.38°±0.001	0.49 ^b ±0.001	0.50 ^b ±0.001	*
Retention	63.23 ^b ±0.25	54.28°±0. 23	66.21°±0.28	68.49 ^a ±0.31	*
(%)	(100)	(85.8)	(10 <u>4.</u> 7)	(108.3)_	

^{a, b, c} Means within the same row with different superscripts are significantly different at P<0.05, Sig.= Significance, NS= Not significant,* (P≤0.05), ** (P≤0.01).

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تأثير اضافة مستويات مرتفعة من فيتامين دم على الاداء الانتاجي وبعض مكونات الدم وجودة العظام والمحتجز من العناصر لبداري التسمين المغذاة علسى علائسق منخفضة في محتواها من الكالسيوم والفوسفور

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تم استخدام ٢١٦ كتكوت تسمين غير مجنس عمر يوم لمدة ٦ أسابيع لدراسة تأثير اضافة مستويات مرتفعة (٧٠٠٠ أو ٨٠٠٠ بدلا من ٢٠٠٠ وحدة دولية) من فيتامين دم على الاداء الانتاجي وصفات العظام والمحتجز من العناصر لبداري التسمين المغذاة على مستويات منخفضة من الكالسيوم والفوسفور (٥,٠% كالسسيوم و ٥,٢٠% فوسسفور متاح).

كانت عليقة المقارنة تحتوي على المستويات الموصى بها من الكالسيوم والفوسفور المتاح وفيتامين در (١% و ٥٠٠% و ٢٠٠٠وحدة دولية على الترتيب). كل العلائق تمت تغذيتها على ٦ مكررات يحتوي الواحد منها على ٩ كتاكيت. ويمكن تلخيص النتائج كالتالى:

الداء الانتاجي (وزن الجسم- وزن الجسم المكتسب - الاستهلاك الغذائي - معامل التحويل الغذائي) لم يتأثر معنويا بالتغذية على علائق منخفضة في الكالسيوم والفوسفور مدعمة بمستويات مرتفعة من فيتامين دم

٢- لم يتأثر مستوى كلا من الكالسيوم والفوسفور في بلازما الدم معنويا بخفض الكالسيوم
 والفوسفور وزيادة فيتامين دم

۳- وزن عظمة الساق ونسبة الرماد بها وكذلك نسبة الكالسيوم والفوسفور بها لم يتأثروا
 معنويا بخفض الكالسيوم والفوسفور وزيادة مستوى فيتامين د٣

كانت النسبة المئوية للمحتجز من العناصر الطيور المغذاة على مستويات مرتفعة من فيتامين دم أعلى معنويا من مثيلتها المغذاة على عليقة المقارنة.

ويستنتج من هذه الدراسة أن استخدام مستويات مرتفعة من فيتامين دم لعلائق منخفضة في كلا من الكالسيوم والفوسفور يمكن أن يحسن من الاداء الانتاجي وصفات جودة العظام والمحتجز من العناصر بغرض تقليل التلوث من عنصر الفوسفور بالاضافة لتقليل تكلفة التغذية.

قام بتحكيم البحث

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