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Effect of biotin and Ca/P ratio on leg bone deformities and mineralization in broilers fed rations based on palm kernel products

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ABSTRACT

A 2 × 6 factorial experiment was conducted to study the effect of 6 dietary levels of biotin (40, 80, 120, 160, 200 and 240 µg/kg) and 2 Ca/P ratios (1.5:1 and 2:1) on leg deformities and bone mineralization in broilers from day-old to 28 days of age. A Ca/P ratio of 1.5:1 required 160 µg biotin/kg while a ratio of 2:1 reduced the biotin requirement to 120 µg/kg for production of normal bone mineralization and prevention of leg deformities. Furthermore, wider Ca/P ratio (2:1) caused a reduction in the incidence of leg deformities from 2.5 to 1.7%.

Leg disorders in broiler chicken caused retarded growth, reduced feed efficiency, increased mortality and processing losses (Vahl 1985). This study is concerned with the effect of dietary supplemental biotin and Ca/P ratio on leg bone deformities and mineralization in broilers fed practical rations based on palm kernel products.

MATERIALS AND METHODS

In a 2 × 6 factorial format, 12 duplicate groups of day-old commercial broiler chicks with 20 chicks/group were randomly assigned to the 12 dietary treatments, viz. 2 Ca/P ratios (1.5:1 and 2:1) each at 6 biotin levels (40, 80, 120, 160, 200 and 240 µg/kg feed). The test diets were formulated from 2 biotin-deficient basal diets with Ca/P ratios of 1.5:1 and 2:1 (Table 1) supplemented with feed-grade biotin (Rovimix H-2) such that 6 graded levels of the vitamin were obtained at each Ca/P ratio. The chicks were housed in 24 deep litter pens (4.1 m^2, floor area), 20 birds/pen, and offered feed and water *ad lib.* Routine vaccinations were administered. The experiment was terminated when the birds were 28-day-old.

Weekly record of incidence of leg deformities as per cent of birds in a group showing (i) twisted legs, (ii) legs with crooked toes, (iii) difficulty in standing or walking was kept. Birds were also examined for foot pad lesions.

On the 28 day, 4 chicks were randomly selected from each treatment for measurement of leg bone characteristics. Enlargement of hock joint was assessed by measuring the diameter of the joint with vernier calipers and the circumference was estimated. Tibia were removed, weighed,

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the length measured with vernier calipers, and kept for determination of bone ash, Ca and P.

Bone ash was determined on the fat-free dry basis (AOAC 1980). Ca was determined by the atomic absorption spectrophotometric method and P was determined colorimetrically (AOAC 1980). Ca and P contents of the basal diets were similarly analyzed.

Results obtained were subjected to statistical analysis (Steel and Torrie 1960). Significant treatment means were compared by the multiple range test of Duncan (1955).

Table 1. Composition of the biotin-deficient basal diets

Constituent (%)	Ca/P ratio	
	1.5:1	2:1
Yellow maize	54.0	49.5
Palm kernel-meal	18.0	18.0
Blood-meal	10.0	10.5
Fish-meal	2.5	2.5
Brewer's grain	12.2	12.2
Oyster shell	1.0	2.0
Bone-meal	2.0	3.0
Vitamin/mineral premix*	0.1	0.1
Salt (NaCl)	0.2	0.2
Palm kernel oil	0.0	2.0
Chemical analysis		
Ca (%)	1.07	1.72
P (%)	0.73	0.81
Composition (calculated)		
CP (%)	21.26	21.25
ME (Kcal/kg)	2742.00	2752.00
ME/CP	128.73	129.48
Biotin (µg/kg)	38.90	36.20
Linoleic acid (%)	1.40	1.33

*Premix supplied/kg of feed : Vitamin A, 8 000 IU; vitamin D_3, 1 500 IU; vitamin E, 3 IU; menadione sodium bisulphite (vitamin K), 1.5 mg; vitamin B_2, 2.5 mg; calcium d-panthothenate, 3 mg; nicotinic acid, 8 mg; vitamin B_6, 0.3 mg; vitamin B_{12}, 0.008 mg; iron, 15 mg; manganese, 25 mg; copper, 2.5 mg; zinc, 10 mg; iodine, 0.3 mg.

RESULTS AND DISCUSSION

Incidence of leg bone abnormalities (Table 2) was significantly affected both by biotin and Ca/P ratio. Abnormalities such as crooked toes, bowed or twisted legs were pronounced with majority of affected birds having difficulty in standing or walking. In addition, foot dermatitis characteristic of biotin deficiency

Table 2. Incidence of leg deformities (%) in broiler chicks as affected by supplemental biotin at different Ca/P ratios*

Ca/P ratio	Biotin (mcg/kg)	Week* 3	4
1.5:1	40	5.0a	7.5a
	80	2.5b	5.0b
	120	0.0c	2.5c
	160	0.0c	0.0d
	200	0.0c	0.0d
	240	0.0c	0.0d
	Mean		2.5
2:1	40	2.5b	5.0b
	80	2.5b	5.0b
	120	0.0c	0.0d
	160	0.0c	0.0d
	200	0.0c	0.0d
	240	0.0c	0.0d
	Mean		1.7

*Mean values in a column bearing different subscripts differ significantly at $P<0.05$; weeks 1, 2 showed 0.

(Whitehead and Bannister 1978) developed crooked toes in birds. The foot pads were swollen and contained haemorrhagic fissures. Cracked and swollen areas occurred on the bottom of the feet. These results also indicated a higher biotin requirement (160 µg/kg) for prevention of leg deformities at lower Ca/P ratio (1.5:1) than at the higher Ca/P ratio (120 µg/kg at a Ca/P ratio of 2:1). Furthermore, higher Ca/P ratio in the diet of broilers caused a marked reduction in the mean incidence of leg deformities from 2.5 to 1.7% among experimental birds. These findings underline the significance of both Ca and P in preventing the incidence of leg disorders in broiler chicken (Edwards 1984 and Kling 1985).

The size of hock joint and length of tibia were not significantly affected by the dietary treatment (Table 3). Both parameters were positively but not significantly correlated with dietary biotin level (Table 4). Results on leg bone biometric measurements obtained in this study tended to suggest that leg disorders developed in broiler chicks were not likely to be perosis as the classical characteristic of perotic joint are not discovered in this case. Indeed, the nonsignificant positive correlation between biotin and size of hock joint (Table 4) indicated that higher levels of the vitamin rather than deficient levels caused an enlargement of the joint though not significantly. This observation together with a small increase in the length of the tibia at higher dietary biotin levels may be attributed to the growth promoting effect of higher level of the vitamin (Oloyo and Ogunmodede 1992).

Table 3. Size of hock joint and tibia's weight, length, ash, calcium and phosphorus of birds fed graded levels of biotin at different Ca/P ratios*

Ca/P ratio	biotin (µg/kg)	Size of hock joint (cm)	Tibia				
			Weight (g)	Length (cm)	Ash (%)	Calcium (%)	Phosphorus (%)
1.5:1	40	4.5	3.1b	6.1	39.7b	28.3b	8.8
	80	4.4	3.5ab	6.1	39.5bc	28.8b	8.9
	120	4.5	3.9a	6.3	38.7c	28.4b	8.6
	160	4.5	3.7a	6.3	38.9c	28.8b	9.4
	200	4.6	3.8a	5.9	39.5bc	29.5b	9.1
	240	4.4	3.6a	6.1	40.1b	29.6b	9.1
2:1	40	4.2	2.5c	5.7	41.6a	30.5a	8.5
	80	4.4	2.5c	5.8	41.3a	30.4a	8.5
	120	4.4	2.6c	6.1	41.8a	30.6a	8.6
	160	4.2	2.3c	5.9	41.7a	31.0a	8.9
	200	4.3	2.4c	5.7	42.0a	31.2a	9.2
	240	4.2	2.5c	6.1	42.1a	31.0a	9.2
	± SEM**	0.07	0.17	0.06	00.39	0.30	0.09

*Mean values in a column bearing different subscripts differ significantly at P<0.05; **SEM, standard error of the mean.

Table 4. Estimated regression line showing the relationship between dietary biotin and the parameter

Parameter	r-value*	t-value*	Regression line+
Size of hock joint (cm^3)	0.297	0.623	Y = 4.7085 + 4.61607145E-04X
Tibia weight (g)	0.204	0.416	Y = 3.0092261 + 1.71957E-04X
Tibia length (cm)	0.697	1.943	Y = 6.65325 + 7.55803574E-04X
Tibia ash (%)	0.238	0.490	Y = 40.512963 + 4.43121E-04X
Calcium (%)	0.396	0.862	Y = 29.488889 + 1.50793E-03X
Phosphorus (%)	0.367	0.789	Y = 8.8185186 + 5.8201E-04X

*, Non-significant at $P<0.05$; + Y, parameter; X, biotin (μg/kg feed).

Whitehead (1977) neither did observe perosis in birds fed biotin-deficient diet, nor perosis always related to the biotin content of the diet. It was, however, indicated that although perosis may be a consequence of embryonic biotin deficiency, it was not a typical sign of an uncomplicated dietary biotin deficiency in the chick. Jensen (1978) also reported that perosis accounted for little of the considerable leg weakness encountered in the broilers.

The nonsignificant effect of dietary treatment on tibia length tended to suggest that bone length was normal in experimental chicks. However, crooked toes and foot pad lesions that developed in broilers fed 40-80 μg biotin/kg diet containing higher Ca/P ratio, and those given 40-120 μg biotin/kg diet with lower Ca/P ratio made it difficult for affected birds to move and hence resulted in the twisting of the legs. These observations are in agreement with those reported by Stappers and Vahl (1982) on the development of twisted legs in broilers.

Very high demand placed on the bones in the legs, particularly the tibiotarsi, for supporting the entire body in rapidly growing broiler chicken has made important the study of leg bone mineralization and strength. Tibia's weight, ash, Ca except P were significantly affected by the dietary treatments (Table 3). All the parameters were positively though not significantly correlated with dietary biotin level. Birds fed diet with the lower Ca/P ratio had heavier tibia, but contained lower amounts of ash and Ca. Edwards (1984) confirmed involvement of Ca/P ratio in the mineralization of leg bone. Kling (1985) reported a significant correlation between bone mineralization and the Ca/P ratio with the highest rate of mineralization occurring in birds fed diet with the highest Ca/P ratio tested. These results therefore suggested that tibia weight was not due to bone mineralization; rather the heavier tibia noted in birds given lower Ca/P ratio might be because of the heavier body weight recorded in the group as reported by Oloyo and Ogunmodede (1992). The positive correlation between biotin and the mineral elements is suggestive of the involvement of the vitamin in the utilization of Ca and P as well as mineralization of the leg bone.

From the foregoing therefore it may be concluded that to promote optimum utilization of Ca and P, adequate mineralization of the leg bone and prevention of the incidence of leg deformities, 160 μg biotin/kg was required when Ca/P ratio in the diet was 1.5:1, whereas 120 μg biotin/kg was adequate when Ca/P ratio was increased to 2:1.

REFERENCES

AOAC. 1980. *Official Methods of Analysis.* 13th edn. Association of Official Analytical Chemists, Washington DC.

Duncan D B. 1955. Multiple range and multiple F test. *Biometrics* **2**: 1-42.

Edwards (Jr) H M. 1984. Studies on the etiology of tibia dyschondroplasia in chickens. *Journal of Nutrition* **114**: 1001-13.

Jensen L S. 1978. Trace elements in broiler nutrition. Third Annual Carolina Poultry Nutrition Conference 9-13.

Kling L J. 1985. The effect of calcium and phosphorus on the incidence of tibial dychondroplasia in young chicks. *Nutrition Report International* **32**(6): 1473-80.

Oloyo R A and Ogunmodede B K. 1992. Preliminary investigation on the effect of dietary supplemental biotin and palm kernel oil on blood, liver and kidney lipids in chicks. *Archives of Animal Nutrition* **42**:263-72.

Strappers H P and Vahl H A. 1982. Afwisselend verlichten van slachtkuikens. CLO - Institut voor de veevoeding. De Schothorst. Publication No. 158.

Steel R G D and Torrie J H. 1960. *Principle and Procedures of Statistics.* McGraw-Hill Book Co., New York.

Vahl H A. 1985. Leg disorders in broiler chickens. *Poultry - Misset* **1**(5): 12-15.

Whitehead C C. 1977. The use of biotin in poultry nutrition. *World's Poultry Science Journal* **33**(3) 140-54.

Whitehead C C and Bannister D W. 1978. Studies on the biotin requirements of broilers. Proceedings of 16th World's Poultry Congress, Rio De Janeiro, Brazil. Vol. T2: 1927-38.