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INFLUENCED BY DIETARY CALCIUM-PHOSPHORUS RATIOS

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ABSTRACT
The study tried to establish the optimum dietary calcium-phosphorus ratio (Ca:P) for normal limb development in broiler chickens. To achieve this, a total of 150 four weeks old Hybro strain of broiler chickens were randomly assigned to five treatment groups of 30 birds made up of three replicates of ten birds each. The groups named: T_1, T_2, T_3, T_4 and T_5 were finished on five dietary Ca:P ratios of 2:1, 2.5:1, 3:1, 3.5:1 and 4:1 respectively for a period 35 days. Leg deformity, tibial length, width, weight, ash, Ca and P concentrations constituted the major response criteria. The Ca:P ratio of 2:1 and 2.5:1 had 6.67% leg deformity each. The longest tibiae of 8.92 cm and heaviest tibiae of 12.30g were recorded for 2.5:1 and 4:1 groups respectively. Tibial ash and Ca concentration increased with increasing dietary Ca:P ratio. At higher Ca:P ratio than 3:1, tibiae tended to be heavier such that final body weight may become a function of the bone not necessarily flesh. The study recommended a Ca:P ratio of 3:1 as the optimum on account of normal tibial development in a hot dry environment.

KEYWORDS: Broiler chickens, Optimum Ca:P ratio, Tibial morphology.

INTRODUCTION

Skeletal abnormality represents a big concern within the poultry industry. It has been observed that the higher nutrient demand in a shorter period of time by the fast growing broiler chickens has led to an increased incidence of leg disorders (Cesar-Coto *et al.,* 2008). Although it has been proposed that modern broiler has a high capacity to adapt to calcium (Ca) or phosphorus (P) deficiency (Bar, et *al.,* 2003; Yan *et al.,* 2005) it was earlier reported that numerous skeletal disorders were associated with dietary imbalances and deficiencies with Ca and P being the most implicated (Long, *et al.,* 1984; Woolford, 1985; Shafey , 1993 ; Cook , 2000 and Whitehead *et al.,* 2004).

According to the earlier report of Van Eekeren et *al.* (1995) the ratio of Ca to P for growing chickens should be within the range of 1:1 to 2:1.To this end, Jacob *et al.* (1998) cautioned that young birds should not be fed high Ca diet because the Ca:P ratio will be unbalanced , resulting in increased mortality and morbidity. On the contrary, Cesar-Coto et al. (2008) indicated that birds fed diet with 0.2% or 0.4% Ca : NPP (Non-phytate phosphorus) ratio had significantly lower incidence of Tibial dyschondroplasia (TD) than birds fed diets with 0.2% less Ca or those fed the 2:1 Ca:NPP ratio. This observation was in line with the earlier report of Whitehead *et al.* (2004) who observed higher incidence of TD in diets with low Ca levels. It was clear that ratio of $\geq$ 2:1 Ca:NPP tended to give better tibial development. Therefore, the objective of this study was to determine the optimum dietary Ca:P ratio, for normal limb development in broiler finisher chickens, under a hot-dry environmental condition.

MATERIALS AND METHODS

The study was conducted at the Teaching and Research Farm of the Department of Animal Science, University of Maiduguri, North-eastern Nigeria. Located on latitude *11^0 15'N* , longitude *30^0 05'E* and an altitude of 345m above sea level , the city of Maiduguri is a typical Semi arid area with temperature of $\geq 40^0$ C , RH of 5% - 44% between the months of March and July (Alaku, 1983; Pillar, 1986; Fada and Rayer, 1988).

The study utilized 150 broiler chickens aged 28 days. The birds were randomly assigned to five treatment groups of 30 birds with replicates of 10 birds each in a completely randomized design. The five groups named T_1,T_2,T_3,T_4 and T_5 were finished on five isocaloric-isonitrogeneous diets of varying Ca –P ratios of: 2:1, 2.5:1, 3:1,3.5:1 and 4:1 respectively (Table 1). Feeding and watering were *ad libitum* during the five weeks study period.

At the end of the 9^{th} week of age, total number of leg deformity was recorded for the various treatment groups. Five birds from each of the replicates were slaughtered and drumsticks removed to obtain the tibiae. The latter were first defleshed in strong, then weak, potassium hydroxide (KOH) solution at 105^{0c} for two hours. The bones were then dried in a porcelain crucible and vacuum oven. They were cooled and weighed afterward. Length and diameter of each bone was measured using venire calipers. The distance from cranial to distal extremities of a tibia was the length while diameter was taken at the nutrient foramen. The bones *were* then kept at room temperature for 48 hrs.

Thereafter, bones were set in giant crucibles and furnace at 600^0c for 6.5hrs to get the ash. The latter was weighed and percent ash calculated according to Chung and Baker (1990). The Ca and Mg analyses were done using Atomic Absorption Spectrophotometer (AAS), "Model Analyst 400" with software "Winlab 32" while PO_4 was achieved by the use of Ultraviolet/Visible (UV/V) spectrophotometer "Model Lambda 35" Perkin Elman Machine.

All data collected were subjected to analysis of variance of completely randomized design according to **SAS (1991).**Where necessary**;** means were separated using Least Significant Difference (LSD). Statements of significance were based on P<0.05.

TABLE 1. Composition and Calculated analysis of the experimental broiler finisher diets (%).

	Various Ca/P ratios in the diets				
Ingredients	T_1	T_2	T_3	T_4	T_5
Maize	58.00	58.00	58.00	58.00	58.00
Wheat offal	10.00	10.00	10.00	10.00	10.00
Full-fat Soya	12.00	12.00	12.00	12.00	12.00
Groundnut cake	10.00	10.00	10.00	10.00	10.00
Fish meal	05.00	05.00	05.00	05.00	05.00
Blood meal	01.00	01.00	01.00	01.00	01.00
Bone meal	02.75	01.74	01.06	00.60	00.22
Limestone	00.25	01.26	01.95	02.40	02.78
Min-Vit premix*	00.50	00.50	00.50	00.50	00.50
Salt	00.30	00.30	00.30	00.30	00.30
Methionine	00.20	00.20	00.20	00.20	00.20
Total	100	100	100	100	100
Calculated analysis					
Crude prot (CP)	20.35	20.35	20.35	20.35	20.35
Crude fibre (CF)	3.23	3.23	3.23	3.23	3.23
Ether extract (EE)	5.67	5.67	5.67	5.67	5.67
ME (Kcal/Kg)	2977.36	2977.36	2977.36	2977.36	2977.36
Calcium (Ca)	1.4726	1.4524	1.4386	1.42.96	1.4220
Phosphorus (P)	0.7275	0.5760	0.4725	0.4050	0.3480
Ca: P ratio	2:1	2.5:1	3:1	3.5:1	4:1

***** *Composition of the broiler finisher premix used.*

The Mineral-Vitamin Premix supplied the following nutrients per kg of feed: Vitamin A = 12,000.00 IU, Vitamin E =1000mg, Folic acid = 1000mg, Panthotenic acid = 15000mg, Vitamin B_{12} *= 15000mg,* B_6 *= 2500mg,* B_1 *= 2000mg,* B_6 *= 6000mg, Vitamin K = 2000mg, Chorine = 50,000mg, Manganese = 10,000mg, Vitamin* D_3 *= 25,000IU, Nicotinic acid = 40,000mg, Biotin = 6000mg , Vitamin C = 3000mg, Copper = 15000mg, Cobalt = 250mg and Selenium = 1000mg.*

TABLE 2: Mean tibial osteology parameters of *Hybro* strain of broiler finisher chickens finished on varying Ca:P ratio.

	Various Ca/P ratios in the diets					
Parameters	$T_{1\,(2:1)}$	$T_{2\,(2.5:1)}$	$T_{3\,(3:1)}$	$T_{4\,(3.5:1)}$	$T_{5\,(4:1)}$	SEM
Leg deformity(%)	6.67	6.67	00	00	00	-
Tibial length (cm)	7.56b	8.92^{a}	8.31^{b}	8.36^{b}	8.61^{b}	0.51
Tibial diameter (cm)	1.76^{b}	1.82^{ab}	1.82^{ab}	1.87^{a}	1.79^{b}	0.04
Tibial weight (g)	8.16^{b}	10.87^{ab}	11.24^{ab}	11.33^{ab}	12.30^{a}	1.44
Ashed weight (g)	5.98^{ab}	6.18^{ab}	4.52^{b}	6.14^{ab}	6.93^{a}	0.77
Percent ash	53.34	55.81	56.53	55.33	56.20	2.90^{NS}
Percent calcium	26.77^{b}	24.08^{b}	35.17^{b}	38.94^{b}	50.09^{a}	13.63
Percent phosphate	8.30	3.70	6.00	9.00	3.00	0.37^{NS}
Percent Magnesium	0.93	0.82	0.80	0.80	0.81	0.01^{NS}

… abc Means within the same row bearing different superscripts differ significantly (P<0.05)
NS = Not significant (P>0.05)
Where SEM is standard error of means

RESULTS AND DISCUSSIONS

The T_1 (control) group recorded 6.67% (two birds) leg deformity in the form of "K" shape while same number came down with bulging distal tibia in T_2 during the 6^{th} week of age. This implied that the two ratios were not adequate to meaningfully support proper bone development in broilers. This finding agreed with the report of Cesar-Coto *et al.* (2008) which observed significantly lower incidence of Tibial Dyschondroplasia among birds fed higher Ca:P ratios than 2:1. The result was also supportive of the earlier report of Whitehead *et al.* (2004). No paralysis was observed among all the treatment groups. The observed bulging distal tibia in T_2

might have been predisposed by excessive body weight, postural abnormalities or some ligament rupture as observed by Jordan, (1990).

The result of the osteology is presented in Table 2 while *Figure 1* shows representative images of the various groups. All the anatomical indices differed significantly (P<0.05) among treatment means. The T_2 group significantly recorded longer tibial length (8.92cm) than the other four groups. This may indicate that ossification was higher in the 2.5:1 ratio group. In other words, such ratio favored more ossification than calcification. Therefore birds in this group were comparatively taller than the others. It was clear that the tibial epiphysial plate will continue to grow, and perhaps, takes longer time than the other groups before all the cartilage changes to bone. It may also imply that other forms of leg deformity may occur but certainly not achondroplastic dwarfism. This observation was inline with the report s of Frandson, (1986) and Classen and Riddel (1989).

On the other hand, the T_4 (3.5:1) group recorded the largest tibial diameter of 1.87cm. This figure was significantly larger than the one recorded for T_1 and T_5. No definite trend was observed in terms of tibial weight. However, the T_5(4:1) group recorded significantly (P<0.05) heavier tibia than T_3 with Ca:P ratio of 3:1. Similarly, T_4(3.5:1) was heavier than those with lower Ca:P ratios(2:1, 2.5:1 and 3:1). This significantly heavier tibia of the T_5 followed by T_4 might have contributed to the heavier body weight recorded by these groups. In other words, the higher ratio of Ca to P translated to heavier body weight by way of heavier bones not necessarily total flesh.

Quantum ash followed similar pattern as the tibial weight. The T_3 and T_5 had the highest concentration of tibial ash of about 56% while T_1 had the least score of 53%. However, no significant (P>0.05) difference was observed among treatment groups in terms of percent ash. This implied that the ratio of Ca to P did not exert significant influence over total bone ash in this study. The observation partly agreed with the report of Yan et al. (2005) who observed significant improvement of toe ash in 2:1 ratio group when supplemented with phytase. This observation in this study also indicated that Ca and P were deposited at varying degrees, for bone development, among the various treatment groups. This may in turn determine the stage of bone development. The observed range of tibial ash in this study (53.34% - 56.57%) was above the 46% and 49% reported by Mc Donald, et al. (1995) and Maynard, et al. (1979) respectively. Percent Magnesium (Mg) was highest in the control while the score 0.8%, being the least was recorded for the T_3 and T_4 groups. The highest score of 0.93% was below the 1.0% reported by Mc Donald et al (1995). Therefore, the tibial Mg was within the normal range

Percent Ca in the tibia appeared to be increasing with increasing ratio of Ca to P after the control. T_5 had significantly (P<0.05) more Ca than the other four groups. T_2 was the least with Ca containing about 24% of the total bone ash while T_5 had about 50% of its ash made up of Ca. The latter score was in conformity with the reports of Frandson (1986); Van Eekeren (1995) and Tekdek (2005) which indicated that Ca constituted the bulk proportion of the total bone ash. Therefore, higher Ca:P ratio favored more calcification of bone. As such stronger bones are associated with higher ratios as evident by heavier bones and higher ash concentration. Percent phosphate did not reveal significant difference, indicating that within the

Ca:P range of 2:1 to 4:1, phosphate showed no significant (P>0.05) variations among the groups. Furthermore, no definite trend was observed for percent phosphate.

CONCLUTION

Calcium and Phosphorus are dietary essential Macro-mineral elements and the ratio between the two is very important in achieving nutritional adequacy in chickens. Limb development, Tibial weight and Tibial dimensions are three physical characteristics of assessing the efficacy of a particular Calcium-Phosphorus ratio.

Tibial strength was higher in diets with Ca:P ratio of >2:1 as evident by higher concentration of tibial ash and calcium (which tended to increase with increasing Ca:P ratio) and absence of any form of leg deformity. At lower ratios, perhaps tibial magnesium tended to increase beyond desirable limit thereby causing weak and possibly deformed tibiae. At higher ratios, bones tended to be heavy, so much so that the bones outrageously contribute to the final body weight of the chicken. Therefore, the study concluded that Ca:P ratio of 3:1 is the optimum for broiler finisher chickens under a hot-dry environment.

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