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Performance, Bone Parameters and Phosphorus Excretion of Broilers Fed Low Phosphorus Diets Supplemented with Phytase from 23 to 40 Days of Age

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Abstract: An experiment was conducted using 300 growing Ross 308 broilers from 23 to 40 days of age. Six dietary treatments were formulated. A basal diet contained 1.48% Dicalcium Phosphate (DCP) was used as a control diet (diet 1). Diets 2 and 3 contained 50% and 25% of the DCP of diet 1 (0.74 and 0.37%, respectively), while diet 4 was formulated without DCP. Diets 3 and 4 were fed without or with supplemented phytase enzyme (500 U/kg). Every dietary treatment was fed to 5 replicates (10 chicks each). The results showed no significant differences between birds fed diets containing 1.48 % DCP or 0.74% DCP on BWG, FI and FCR. Reducing dietary DCP level to 0.37% slightly decreased BWG compared with birds fed 0.74% DCP with inferior value of FCR. When DCP was removed from the diet BWG significantly ($p<0.01$) decreased and FCR recorded worth value. Addition of 500 U phytase/kg to diet 3 of 0.37% DCP significantly enhanced BWG ($p<0.01$), feed intake and FCR ($p<0.05$). Addition of phytase to the diet of no DCP did improve neither BWG nor FCR. Decreasing dietary DCP did not significantly affect length, weights and width of tibia either with or without phytase supplementation. However, birds fed the highest level of dietary DCP showed the highest values of tibia weight and length among the different groups. Tibia breaking strength (kgf) significantly ($p<0.001$) decreased as dietary DCP level decreased. Addition of phytase significantly ($p<0.001$) improved tibia breaking strength and tibia ash %. Addition of phytase to diet of low DCP did increase tibia Ca and P to reach values comparable with those of the control diet. Decreasing dietary DCP showed significant ($p<0.001$) decrease in the excreted Ca and P. Addition of phytase to diets of low or no DCP also decreased ($p<0.001$) the excreted Ca and P. This means that phytase increased the utilization of dietary Ca and P. The excreted Ca and P decreased by 41.22% and 55.26%, respectively, when birds were fed diet of no DCP compared to those fed the control diet. Also, addition of phytase enzyme to diets of low or no DCP decreased the excreted percentage of Ca and P. It could be concluded that reducing dietary P level and using phytase enzyme could be used to limit quantity of P excreted from broilers. This reduce such impact in environmental pollution.

Key words: Growing broilers, low phosphorus, phytase, bone, phosphorus excretion

INTRODUCTION

Broiler diets consist of plant products often contains large amounts of unavailable phosphorus know as phytates which accounts for 60-80% of the total phosphorus present in plant feeds. To meet the dietary Phosphorus (P) requirements of poultry, diets are often supplemented with inorganic P. The unused portions of the supplemental phosphorus as well as the indigestible phytate are excreted, resulting in high concentrations of P in the manure. Cowieson *et al*. (2004) reported that eighty-two percent of the phytate consumed in poultry diets is recovered in the excreta. This increases the cost of the diets and contributes to environmental pollution (Pallauf *et al*., 1994; Musapuor *et al*., 2006). Recently one of the most effective factors in environment contamination is especially contamination by phosphorus.

To reduce the concentration of P in excreta, broilers can be fed to requirement, essentially by reducing overfeeding of P via decreasing the amount of P from inorganic sources. In conjunction with feeding closer to requirements, the addition of enzymes enhances P availability and thus P use by the broiler from diets can substantially decrease P in litter. Angel *et al*. (2005) reported that under commercial conditions broiler litter P was reduced by 30% when diet P was reduced by 10%.

Nahm (2002) reported that using phytase enzymes, combined with reductions in the amount of P in diets, have been shown to effectively reduce excreted P concentrations. Angel *et al*. (2005) recommended using moderately high concentrations of available P in the pre-starter and starter phase combined with no added inorganic P in the finisher and withdrawal phases.

Adding phytase enzyme to broiler diets to improve utilization of phosphorus and other minerals could help lower the need to add expensive commercial inorganic phosphate (dicalcium phosphate) to poultry rations, thus

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reducing the cost of poultry feed. Using phosphorus from the organic phytate compound could also help reduce phosphorus contamination in ground water, a benefit for improving the environment (Huff *et al*., 1998; Scott *et al*., 1999).

Using phytase enzyme allowed the reduction of inorganic phosphorus levels of the diets, decreasing expenses without negative effect on broiler performance (Plumstead *et al*., 2008).

During the finishing period (23-40 days of age) broilers consumed more than 60% of there total feed consumption. Reducing dietary P levels and/or using phytase enzyme on such phase may help on reducing the excreted P. Therefore, the objective of this study was to evaluate the influence of reducing dietary phosphate with or without phytase supplementation on performance, bone measurements Ca and P excretion of broiler chicks from 23 to 40 days of age.

MATERIALS AND METHODS

A number of 500 one-day old Ross 308 broiler chicks has been grown on floor ben in warmed fumigated brooder house to 23 days of age. Chicks were fed a starter-grower diet till 22 days of age. The starter-grower diet (Table 1) was formulated to cover the recommended requirements of all the nutrients. The dietary levels of Ca and NPP were 1 and 0.5%, respectively, for the starter-grower phases. After fasting overnight chicks were individually weighed and 300 chicks of uniform weights were used in this experiment.

Four finisher diets were formulated; diet 1 a control diet contained 1.48% DCP, diet 2 contained 0.74% DCP (50% DCP of the control), diet 3 contained 0.37% DCP (25% DCP of the control) and diet 4 with no added DCP. 500 U phytase enzyme/kg were added to diets 3 and 4 to perform diets 5 and 6. Thus a number of 6 treatments were examined. These diets were formulated to be isonitrogenous and isocaloric and contained nutrients adequate to cover the requirements of Ross broilers.

Every dietary treatment was fed to 5 replicates of 10 chicks each. The average initial live body weights of all replicates were nearly similar. Replicates were randomly allocated in batteries of three-tier system divided into 30 compartments (5 replicates X 6 dietary treatments). Feed and water were allowed for *ad libitum* consumption from 23 to 40 days of age. At the end of the experiment birds were fasted overnight, individually weighed and feed consumption was recorded per replicate. Gain in body weight and feed conversion ratio were calculated. Samples of excreta were collected from all treatments to determine Ca and P content.

Six birds per treatment were randomly taken to study bone measurments. Birds were individually weighed, slaughtered, feathered and eviscerated. The right tibia was removed and cleaned of all adhering flesh, extracted with ethanol and then with diethyl ether. After recording the overall length and width of tibia, bones were oven dried at 105^{o}C to the constant weight. The dried fat-free bones were ashed in a muffle furnace at 600^{o}C for 6 h. Tibia bone strength (breaking strength) was measured on apparatus IPNIS and expressed in kilograms necessary for bone to be broken (Masic *et al*., 1985).

Calcium and phosphorus were determined in excreta and dried fat-free bones ash based on the Official Methods of Analysis of AOAC (1990).

Throughout the experimental period, birds were vaccinated against Avian Flue, ND, IB and IBD. After such medical treatments, a dose of vitamins (AD_3E) was offered in the drinking water for the successive 3 days.

Data were statistically analyzed for analysis of variance using the General Liner Model of SAS (1990) as one way analysis of variance. Significant differences among treatment means were separated by Duncan's new multiple rang test (Duncan, 1955) with a 5% level of probability.

RESULTS

Body Weight Gain (BWG), Feed Intake (FI) and Feed Conversion Ratio, feed/gain (FCR) of broiler chicks fed the different dietary treatments is summarized in Table 2.

The results showed that birds fed diet 1 containing 1.48% DCP exhibited the highest level of body weight gain (1128 g) and feed intake (1987 g) and the best value of FCR (1.76) among treatments. However, no significant differences were detected between such birds and those feed diet 2 containing 0.74% DCP on BWG, FI and FCR. Reducing dietary DCP level to 0.37% slightly decreased BWG compared with birds fed 0.74% DCP and showed inferior value of FCR (1.83). When DCP was removed from the diet (diet 4) BWG significantly ($p<0.01$) decreased (948 g) and FCR recorded worth value being 1.86.

Feed intake was not significantly affected by reducing DCP from 1.48 to 0.74 or 0.37%, while removal of DCP (0.0%, diet 4) significantly ($p<0.001$) decreased FI compared to the control diet. Phytase supplementation to diet 4 of no DCP content did numerically ($p>0.05$) enhance feed intake.

Addition of 500 U phytase/kg to diet 3 of 0.37% DCP (diet 5) slightly improve BWG and FCR. Feed conversion ration value of such diet (0.37% DCP + phytase) being 1.77 was comparable to that of the control diet (1.48% DCP) being 1.76. Addition of phytase to the diet of no DCP (diet 6) did improve neither BWG nor FCR.

The effects of reducing DCP and phytase addition on bone measurements of broiler chicks fed the different dietary treatments are shown in Table 3.

Level of dietary DCP did not significantly affect weight, length or width of tibia. Decreasing dietary DCP did not significant effect on length, weights and width of tibia

Table 1: Formulation and nutrient composition of starter-grower period (0-22 days of age) and experimental diets for finisher period (23-40 days of age)

		Experimental diets			
Ingredients (%)	Starter-grower diet	Diet 1	Diet 2	Diet 3	Diet 4
Yellow corn	50.37	60.00	61.04	61.61	62.18
Soybean meal (44%)	36.30	26.00	26.00	26.00	26.00
Corn gluten meal (60%)	5.00	6.00	6.00	6.00	6.00
Soy oil	4.00	4.00	3.70	3.50	3.30
Dicalcium phosphate	1.90	1.48	0.74	0.37	0.00
Limestone	1.20	1.42	1.42	1.42	1.42
Vitamin and Mineral mix(1)	0.30	0.30	0.30	0.30	0.30
Salt	0.30	0.30	0.30	0.30	0.30
L-Lysine HCl	0.33	0.30	0.30	0.30	0.30
DL-Methionine	0.30	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00	100.00
Calculated composition(2)					
Crude protein	23.05	20.00	20.00	20.00	20.00
ME (Kcal/kg)	3052.00	3200.00	3200.00	3200.00	3200.00
Lysine	1.42	1.15	1.15	1.15	1.15
Methionine + Cystine	1.07	0.90	0.90	0.90	0.90
Thrionine	0.94	0.74	0.74	0.74	0.74
Calcium	1.00	0.95	0.79	0.71	0.63
Total P	0.76	0.64	0.51	0.44	0.37
Nonphytate P	0.50	0.40	0.27	0.20	0.13

(1) Vitamin-mineral mixture supplied per Kg of diet: Vit A, 12000 I.U; Vit D_3, 2200 I.U; Vit E, 10 mg; Vit K_3, 2 mg; Vit B_1, 1 mg; Vit B_2, 4 mg; Vit B_6, 1.5 mg; Vit B_{12}, 10 µg; Niacin, 20 mg; Pantothenic acid, 10 mg; Folic acid, 1 mg; Biotin, 50 µg; Choline chloride, 500 mg; Copper, 10 mg; Iodine, 1 mg; Iron, 30 mg; Manganese, 55 mg; Zinc, 50 Mg and Selenium, 0.1 mg. (2)According to NRC, 1994

Table 2: Growth performance of broiler chicks fed the dietary treatments from 23 to 40 days of age

Dietary treatments			Body weight gain (g)	Feed intake (g)	Feed conversion ratio
Diet No.	**DCP (%)**	**Phytase (U/kg)**			
1	1.48	0	1128^{a}	1987^{a}	1.76^{b}
2	0.74	0	1052^{ab}	1875^{ab}	1.78^{b}
3	0.37	0	1024^{bc}	1876^{ab}	1.83^{ab}
4	0.00	0	948^{c}	1765^{b}	1.86^{a}
5	0.37	500	1046^{abc}	1851^{ab}	1.77^{b}
6	0.00	500	977^{bc}	1824^{ab}	1.87^{a}
SE of means			±15.49	±21.81	±0.01
Significances			**	*	*

a-bMeans within each column for each effect with no common superscript are significantly different (p<0.05). *p<0.05; **p<0.01

either with or without phytase supplementation. However, birds fed the highest level of dietary DCP (1.48%) showed the highest values of tibia weight and length among the different groups. Tibia breaking strength (kgf) significantly (p<0.001) decreased as dietary DCP level decreased. Birds fed the control diet of 1.48% DCP recorded the highest values of tibia breaking strength being 35.27 kgf. The corresponding values for birds fed 0.74, 0.37% or no dietary DCP were 29.22, 30.42 and 21.19 kgf, respectively.

Addition of phytase enzyme to diet 3 significantly (p<0.001) increased tibia breaking strength and reach a value comparable to that of the control diet being 37.03 kgf. On the other hand, slight improve (p>0.05) in tibia breaking strength was detected when diet 4 of no DCP was supplemented with phytase (diet 6).

Effect of reducing DCP and phytase addition on tibia ash, tibia Ca and P (%) content of chicks fed the different dietary treatments are shown in Table 4. Reducing dietary DCP from 1.48 (diet 1) to 0.74 % (diet 2) did not significantly affect tibia ash %. Reduction of DCP to 0.37 or 0.0% (diet 3 and 4) showed significant (p<0.001) lower tibia ash % compared to the control diet of 1.48% DCP. Addition of phytase to such diets (3 and 4) significantly (p<0.001) improved tibia ash % and reached values being 42.92 and 40.29%, respectively. This means that, phytase addition to diets of less or no DCP made birds able to retain more ash in tibia. Values of tibia Ca and P % showed gradual decrease (p<0.001) with decreasing dietary DCP. Addition of phytase to diet 3 of 0.37% DCP did increase tibia Ca and P to reach values comparable with those of diet 1 of 1.48% DCP (the control). Tibia Ca and P % of the control diet (diet 1) were 52.41 and 20.82%, respectively. The corresponding values recorded for diet 5 (0.37% DCP + phytase) were 51.51 and 20.19%, respectively. Also, addition of phytase to diet 4 of no DCP increased tibia Ca and P %.

Table 3: Tibia bone measurements of broiler chicks fed the dietary treatments from 23 to 40 days of age

Dietary treatments			Tibia weight (g)	Tibia length (cm)	Tibia width (cm)	Tibia strength (kgf)
Diet No.	**DCP (%)**	**Phytase (U/kg)**				
1	1.48	0	5.28	9.13	0.85	35.27[a]
2	0.74	0	4.68	8.28	0.85	29.22[b]
3	0.37	0	4.92	8.44	0.85	30.42[b]
4	0.00	0	4.48	8.43	0.82	21.19[c]
5	0.37	500	4.85	8.97	0.82	37.03[a]
6	0.00	500	4.86	9.04	0.81	23.64[c]
SE of means			±0.106	±0.116	±0.008	±1.48
Significances			NS	NS	NS	***

[a-b]Means within each column for each effect with no common superscript are significantly different (p<0.05). ***p<0.001; NS: Not Significant (p>0.05)

Table 4: Tibia ash, Ca and P% of broiler chicks fed the dietary treatments from 23 to 40 days of age

Dietary treatments			Tibia ash (%)	Tibia minerals Ca (%)	P (%)
Diet No.	**DCP (%)**	**Phytase (U/kg)**			
1	1.48	0	41.82[ab]	52.41[a]	20.82[a]
2	0.74	0	40.27[bc]	48.87[b]	20.10[a]
3	0.37	0	39.61[c]	45.74[c]	19.04[b]
4	0.00	0	36.44[d]	43.17[d]	17.88[c]
5	0.37	500	42.92[a]	51.51[a]	20.19[a]
6	0.00	500	40.29[bc]	46.27[c]	18.44[bc]
SE of means			±0.52	±0.80	±0.23
Significances			***	***	***

[a-b]Means within each column for each effect with no common superscript are significantly different (p<0.05). ***p<0.001

The effects of reducing DCP and phytase addition on calcium and phosphorus excretion of broiler chicks fed the different dietary treatments are shown in Table 5. Decreasing dietary DCP showed significant (p<0.001) decrease in the excreted Ca and P. The excreted Ca decreased from 2.96% for birds fed the control diet (1.48% DCP) to 1.74% for those fed diet 4 of 0% DCP. The corresponding values for the excreted P were 1.52 and 0.68%, respectively. Addition of phytase to diets 3 and 4 of 0.37 or 0% DCP also decreased (p<0.001) the excreted Ca and P %. This means that addition of phytase increased the utilization of dietary Ca and P.

The excreted Ca and P decreased by 41.22% and 55.26%, respectively, when birds were fed diet of no DCP compared to those fed the control diet of 1.48% DCP. Also, addition of phytase enzyme to diets of low or no DCP decreased the excreted percentage of Ca and P.

DISCUSSION

The results of the present study showed that dietary DCP could be reduced by 50% on broiler diets from 23 to 40 days of age with no significant effect upon performance and bone parameters. Pronounced beneficial effect was observed regarding P excreted and its impact on environmental pollution that limits soil and water contamination.

On studying reducing dietary P and/or using phytase, different results have been reported. Waldroup *et al*. (2000) reported that using supplemental phytase in conjunction with reduced dietary P levels has been shown to be an effective method of improving P utilization and decreasing P excretion in the manure. They added that NPP could be reduced, than the NRC (1994) requirement, within an age period by 0.075% from 0-21, 21-42 and 42-56 d of age without adverse effects on broiler performance. Also, Applegate *et al*. (2003) found that the NRC (1994) NPP requirement is substantially higher than that required for overall performance in 3 wk phases. Therefore, they concluded that the dietary nonphytate phosphorus levels of broiler diets could be reduced substantially from NRC (1994) recommendations without affecting broiler performance. Fritts and Waldroup (2003) suggested that available P levels can be reduced by up to 30% with no negative impact on broiler performance resulting in a significant reduction of P excretion. Yan *et al*. (2000, 2004) and Angel *et al*. (2005) found no differences in BW when comparing broilers fed the NRC (1994) recommended P level and broilers fed less P but with added phytase.

Earlier, Skinner *et al*. (1992) examined the influence of complete removal of inorganic P from broiler finisher diets on growth, bone strength and the incidence of skeletal abnormalities. There were no negative effects of P removal on growth rate, feed conversion, tibia length or width, or the incidence of leg abnormalities. Furthermore, Kornegay *et al*. (1997) showed that performance measurements increased when P and phytase were added to low P diets. The largest response to phytase was usually obtained at the lower levels of P.

Table 5: Calcium and phosphorus excreted from broiler chicks fed the dietary treatments from 23 to 40 days of age

---------------------- Dietary treatments -----------------------			Excreta Ca %	Decrease Ca % in excreta	Excreta P %	Decrease P % in excreta
Diet No.	**DCP (%)**	**Phytase (U/kg)**				
1	1.48	0	2.96[a]		1.52[a]	
2	0.74	0	2.27[b]	23.31	1.03[b]	32.24
3	0.37	0	2.09[c]	29.39	0.79[c]	48.03
4	0.00	0	1.74[de]	41.22	0.68[d]	55.26
5	0.37	500	1.88[d]	36.49	0.69[d]	54.61
6	0.00	500	1.62[e]	45.27	0.60[e]	60.53
SE of means			±0.09		±0.08	
Significances			***		***	

[a-b]Means within each column for each effect with no common superscript are significantly different ($p<0.05$). ***$p<0.001$

On the other hand, Salem *et al*. (2003) found that decreasing dietary P levels reduced weight gain and feed intake of broiler chicks fed corn soybean meal diet. Phytase supplementation overcame ($p<0.05$) the depression of weight gain and feed intake observed on the low P diet to comparable values of the normal P diet. Studies performed by Plumstead *et al*. (2008) showed that using phytase supplementation allowing the reduction of inorganic phosphorus levels of the diets, decreasing expenses without negative affect on broiler performance.

Regarding feed manipulation to reducing the excreted P, Campestrini *et al*. (2005) reported that birds excrete more than half of phosphorus and nitrogen they consume. Using enzymes in poultry diets improves digestibility and availability of certain nutrients, mainly phosphorus, nitrogen, calcium copper and zinc, diminishing its presence on excreta and hence, its deposition to the environment. Environmental pollution, through fecal excretion of nitrogen and phosphorus, can occur in higher or lower level, depending on the utilization capacity of these nutrients by animals, which is improved with exogenous enzymes addition.

In agreement with different studies (Angel *et al*., 2005; Angel *et al*., 2006; Leytem *et al*., 2007; Plumstead *et al*., 2007) total phosphorus in the excreta increased as the dietary phosphorus level increased. This confirms the importance of feeding close to the P requirement. Also, microbial phytase supplementation with low-Ca, low-P diet can decrease P excretion in the manure and limit soil and water contamination (Musapuor *et al*., 2006).

From the obtained results and forgoing discussion, it could be concluded that reducing dietary P level and using phytase enzyme could be used to limit quantity of P excreted from broilers. This reduces such impact in environmental pollution. Further studies is recommended on removal of DCP from broiler finisher diets since finisher diets are generally only fed in the last week before market and represents approximately 40% of the total feed consumed by a commercial broiler.

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