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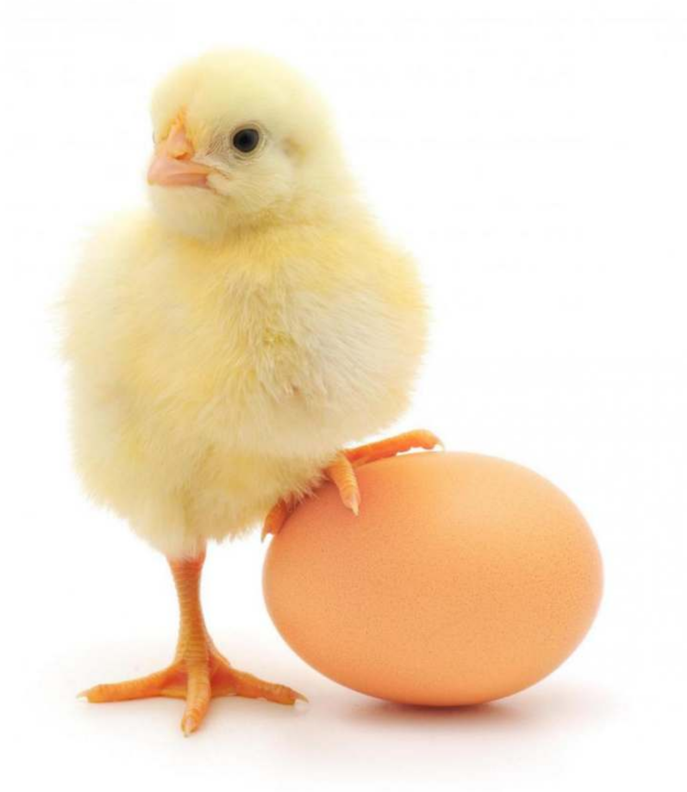

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ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Growth Performance, Bone Mineralization, and Nutrient Retention Responses of Chicks to Dietary Crude Protein and Non-phytate Phosphorus Concentrations

Olayiwola Adeola and Jason Shervago Sands
Department of Animal Science, Purdue University, West Lafayette, IN 47907-2054, USA
E-mail: ladeola@purdue.edu

Abstract: The objective of this study was to investigate possible interactions between dietary crude protein (CP) and phosphorus (P) in broiler chicks. Two CP levels including an adequate NRC-recommended 230 g/kg or an amino acid-supplemented reduced CP 180 g/kg and 4 level - 4.0, 4.5, 5.0, or 5.5 g of non-phytate P (NPP)/kg in a 2 X 4 factorial arrangement were fed to 6 replicate cages of 6 chicks per cage from 10 to 22 d of age. There were main effects of CP ($P < 0.001$) and NPP ($P < 0.05$) concentration on final body weight (FBW), body weight gain (BWG), and gain-to-feed ratio. There was a linear reduction ($P < 0.05$) in feed intake as dietary NPP level increased. Generally, FBW, BWG and FI were linearly reduced ($P < 0.05$) with increasing NPP. Gain-to-feed ratio was lower ($P < 0.05$) for chicks fed the AA-supplemented reduced CP diet than those fed the adequate protein diet. Tibia ash was increased linearly ($P < 0.05$) with increased dietary NPP, but was not affected by CP. There were both linear and quadratic effects ($P < 0.05$) of NPP on retention of dry matter and energy. At either CP level, retention of nitrogen and phosphorus was reduced ($P < 0.05$) with increase in dietary NPP. Retention of P was linearly reduced with increasing NPP concentration and highest in chicks fed the adequate CP diet. These results provide support for an integrated strategy for reducing manure N and P excretion by broiler chickens.

Key words: Bone ash, broiler chicks, nitrogen, growth response, phosphorus

Introduction

There is a scarcity of information on the relationship between dietary crude protein (CP) and non-phytate phosphorus (NPP) concentrations in poultry and its subsequent influence on the metabolic utilization of these minerals. Phosphorus is intricately involved in numerous metabolic processes requiring energy in the form of high-energy phosphates, including the activation of numerous enzymes and signal proteins, and in protein synthesis and degradation. Several studies in pigs have shown that P requirement is linked to the concentration of protein in the diet (Reinhart *et al*., 1976; Mahan *et al*., 1980) and that higher concentrations of P are required to maximize N deposition in pigs (Carter and Cromwell, 1998). Higher N retention, higher urea concentration in plasma, liver and kidney tissues and a significant reduction in renal glutamate dehydrogenase activity (a central enzyme of amino acid degradation in kidney mitochondria), in P-depleted rats have been reported (Huber and Breeves, 1999). In chickens, reducing dietary crude protein from 206 to 166 g/kg was shown to reduce growth, but improved feed efficiency and tibia P content (Shafey and Mcdonald, 1991). Lim *et al*. (2001) reported that reductions in dietary NPP led to a decrease in N retention in broiler chickens. Ferguson *et al*. (1998) fed adequate or reduced CP and P concentrations to broiler chicks and observed significant CP by P interactions for growth performance. The reductions in performance were partially ameliorated when chicks were fed diets containing low P and low CP. Ferguson *et al*. (1998) also reported significant interactions between CP and NPP when litter P was measured as an indicator of P excretion. Litter P concentration was significantly lower when a low P with the NRC-recommended CP was fed compared to litter from cages of chicks provided low P, low CP diets.

Due to their potential detrimental impact, there is increased interest in reducing the excretion of N and P in poultry manure (Sharpley, 1999). Strategies employed to reduce N excretion have not considered the possible influence of P and vice versa. This study examines the relationship between dietary N and P concentrations on their utilization by broiler chicks. The objective of this study was to examine the interactive effects of dietary N and NPP levels on growth performance, bone mineralization, and nutrient utilization in broiler chicks.

Materials and Methods

Experimental Procedures

Dietary Treatments: Diets were arranged in a 2 X 4 factorial consisting of 2 CP levels including an adequate NRC-recommended CP (230 g/kg) or an amino acid-supplemented reduced CP (180 g/kg) and 4 NPP levels including 4.0, 4.5, 5..0 or 5.5 g/kg. The CP level of the diet was manipulated by varying the concentration corn and soybean meal (Table 1). Non-phytate P level was increased by the addition of monosodium phosphate to the basal diets. Diets were randomly assigned to cages within a block. A source of acid insoluble ash (AIA) was added to the diet at a rate of 10 g/kg as an indigestible marker.

Table 1: Composition of basal diets, (As fed)

Crude protein, g/kg	230	180
Non-phytate phosphorus, g/kg	4.0	4.0
Ingredient		
Corn	500	640
Soybean Meal	330	200
Corn Oil	60	50
Fish Meal	50	50
Limestone	25	25
Monosodium Phosphate	6.75	7.50
Celite	10	10
Salt	4	4
Vitamin-Mineral Premix[a]	3	3
Lysine-HCl	0	1
DL-Methionine	1	1.8
Threonine	0	0.7
Isoleucine	0	1
Corn Starch	10.3	6
Calculated Nutrients and Energy		
Protein, g/kg	232.6	181.4
ME, kcal/kg	3,251	3,287
Ca, g/kg	10.5	10.2
P, g/kg	6.67	6.35
NPP, g/kg	4.0	4.0
Ca: P	1.57	1.61

[a]Supplied the following per kg diet: Vitamin A, 5,484 IU; Vitamin D_3, 2,643 ICU; Vitamin E,11 IU; Menadione sodium bisulfite, 4.38 mg; Riboflavin, 5.49 mg; d-pantothenic acid, 11 mg; Niacin, 44.1 mg; Choline chloride, 771 mg; Vit B_{12}, 13.2 ug; Biotin, 55.2 ug; Thiamine mononitrate, 2.2 mg; Folic acid, 990 ug; Pyridoxine hydrochloride, 3.3 mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 ug.

Animals and management: Day-old broiler chicks were wing-banded and maintained on a standard 230 g CP/kg starter diet for 10 d. Two-hundred and eighty-eight chicks were selected, blocked according to body weight such that the average body weight (BW) within a block was similar for all 8 diets. Dietary treatments were fed to 6 replicate cages of 6 chicks. Feed was weighed into buckets for each cage and is added to the feeding troughs as needed. Birds were provided ad libitum access to water and dietary treatments from d 11 to 22, and battery temperatures were maintained at 32 and 27°C from d 8 to 14 and d 15 to 22, respectively. Individual BW of birds and feed consumption per cage were recorded at 8 and 22 d of age. Bird management and handling procedures used in this trial were as described by Onyango *et al.* (2004) and approved by the Purdue Animal Care and Use Committee. Excreta samples were collected from beneath each cage between d 17 and 21 and dried in a forced-air oven at 55°C for 5 d as described by Dilger et al. (2004). Birds were euthanized by carbon dioxide asphyxiation on d 22 and left tibia and left toe from each bird were excised and cleaned as described by Dilger *et al.* (2004), sealed in plastic bags, and stored at -4°C pending further analysis for ash.

Chemical analysis: Excreta collected were pooled and stored at -20°C in sealed plastic bags until subsequent analysis. After thawing, excreta were oven dried at 55°C and dried samples were ground to pass through a 1-mm screen. Ground samples were oven dried at 100°C for 2 h for dry matter determination according to AOAC (1995). Feed (0.9) and excreta samples (0.4g) were weighed into porcelain crucibles and allowed to ash overnight in a muffle furnace at 600°C. Samples were digested using nitric sulfuric and hydrochloric acids. A 15-ml aliquot of a nitric-hydrochloric acid solution was added to the previously ashed sample and approximately 0.5 ml of concentrated sulfuric was added to each sample (AOAC, 1995). Samples were placed on a preheated hot plate at 300°C and allowed to boil gently for approximately 15 min. The samples were allowed to cool before being transferred to a 250-ml volumetric flask and brought to volume with de-ionized, distilled water. Total inorganic P concentration in feed and excreta digests was determined by colorimetric analysis using a commercially available diagnostic kit, procedure # 670 (Sigma Chemical Co., St. Louis, MO). Feed and excreta digests were reacted with ammonium molybdate in an acid solution to form phosphomolybdate. A mixture of sodium bisulfite, sodium sulfite and 1-amino-2-naphthol-4-sulfonic acid reduced the phosphomolybdate to form a phosphomolybdenum blue complex. The phosphate concentration was measured at a

Table 2: Growth response of broiler chicks (expressed on a per bird basis) fed 230 or 180 g crude protein (CP)/kg and 4.0, 4.5, 5.0, or 5.5 g non-phytate phosphorus (NPP)/kg[a]

										P-value[b]		
	230				180						NPP	
Item	4.0	4.5	5.0	5.5	4.0	4.5	5.0	5.5	SEM	CP	L[c]	Q[d]
Body weight, g	701	729	700	665	683	646	641	608	19.4	***	*	
Weight gain, g	480	506	477	443	461	424	419	386	19.4	***	*	
Feed intake, g	641	676	627	573	686	637	599	574	23.5		*	
Gain/feed, g:g	0.75	0.75	0.76	0.77	0.67	0.67	0.70	0.67	0.02	***		

[a]Values are least square means; six cages each with six chicks per cage; non-phytate phosphorus supplied by monosodium phosphate. [b]For P-value, ***, **, and * represent effects at P < 0.001, P < 0.01, and P < 0.05, respectively. [c]Linear effect. [d]Quadratic effect.

wavelength of 660 nm on a micro-plate reader. Acid insoluble ash was determined using the method described by Vogtmann *et al.* (1975). A 5 g (feed) or 4 g sample (excreta) was placed in a previously weighed glass beaker to which 50 ml of 4-N HCl was added. The beaker was covered with a watch glass and boiled gently for 45 min. The slurry was then filtered through ashless filter paper and washed twice with double distilled water. The filter paper containing the washed residue was placed in a dried, pre-weighed crucible and dried for 24 h at 70°C. The dried residue was then ashed at 600°C for at least 4 h, allowed to cool in a desiccator, and weighed to determine the weight of the cooled ash (acid insoluble ash).

Nitrogen concentrations in feed and excreta were determined by the combustion method using a LECO model FP-2000 N analyzer (Leco Corp, St. Joseph, MN.) and energy was determined by adiabatic bomb calorimetry (Model 1261, Parr Instrument Co., Moline, IL) as described by Adeola and Bedford (2004). Fat from tibiae and toes was extracted for 16 h with ethanol, followed by a 16-h extraction with ethyl ether in a soxhlet apparatus, oven-dried at 100°C, and ashed in a muffle furnace at 600°C for approximately 18 h to determine fat-free ash as described by Onyango *et al.* (2003).

Statistical analysis: Data were analyzed as a randomized complete block design using the GLM procedure of SAS (SAS, 2001). Cage served as the experimental unit for all nutrient balance and growth performance variables. Orthogonal polynomial contrasts were used to determine significant linear and quadratic responses of nutrient balance and growth performance to dietary NPP level.

Results

Growth performance and bone mineralization: There was no interaction between CP and NPP for any of the performance criteria evaluated (Table 2). Average final body weight (FBW) body weight gain (BWG) and gain-to-feed (GF) ratio were affected by CP (P < 0.001) and NPP (P < 0.05) concentrations, whereas feed intake was only affected (P < 0.001) by NPP concentration. Chicks that received the 230 g CP/kg diet were heavier (P < 0.001) FBW than those that received the 180 g CP/kg diet. Increasing NPP concentration led to linear reductions in FBW, BWG and FI in chicks that received the 180 g CP/kg diet. Feed intake was significantly reduced (P < 0.05) at NPP levels above 4.5 g/kg at both levels of dietary CP. Gain to feed ratio was lower (P < 0.05) for chicks fed the 180 g CP/kg diet than the 230 g CP/kg diet. There was no interaction between CP and NPP for tibia and toe ash (Fig. 1 and 2). Neither the tibia nor toe ash was significantly affected by CP concentration. Tibia and toe ash responded linearly (P < 0.001) to increasing dietary NPP level.

Nutrient retention: Dry matter and energy intake, output, and retention data are shown in Table 3. There was an effect (P < 0.05) of dietary CP on DM and energy retention. The amount and percentage of DM retained responded linearly and quadratically (P < 0.01) to dietary NPP. The amount of energy retained was affected (P < 0.001) by dietary NPP concentration. The response to dietary NPP level in energy retention was both linear and quadratic (P < 0.05) as observed for dry matter retention. The main effects of CP (P < 0.001) and NPP (P < 0.001) were significant for N intake (Table 4). As expected, N intake was higher (P < 0.05) for chicks that received 230 g CP/kg than the 180 g CP/kg diet. There was a linear reduction (P < 0.05) in N intake with increasing dietary NPP.

Nitrogen retained by chicks fed the 230 g CP/kg was significantly higher (P < 0.01) than those that received 180 g CP/kg. There were linear and quadratic reductions (P < 0.01) in N retention with increasing NPP concentrations. Phosphorus intake was linearly increased with increasing dietary NPP level (Table 4). There was an increase (P < 0.01) in P output at the highest concentration of NPP (5.5 g/kg) regardless of the concentration of CP. The amount of retained P was significantly affected by CP concentration (P < 0.05) and NPP (P < 0.05) concentration. Chicks that received the 230 g CP/kg and 4.5 or 5.0 g NPP/kg retained more P than chicks fed 4.0 or 5.5 g NPP/kg at the same CP concentration. When expressed as a percent of intake, P retention was reduced (P < 0.01) in chicks receiving the highest concentration of NPP regardless of the CP concentration.

Table 3: Dry matter and energy retention of broiler chicks (expressed on a per bird basis) fed 230 or 180 g crude protein/kg and 4.0, 4.5, 5.0, or 5.5 g non-phytate phosphorus/kg[a]

	230				180					P-value[b]		
											NPP	
Item	4.0	4.5	5.0	5.5	4.0	4.5	5.0	5.5	SEM	CP	L[c]	Q[d]
Dry Matter												
Intake, g	580	634	567	549	627	593	544	522	21.9			
Output, g	126	134	117	137	140	114	111	115	5.12	*e		**
Retained, g	454	500	450	413	486	480	433	407	19.19		*	**
Retained, %	78.2	78.9	79.2	75.1	77.6	80.9	79.6	77.9	0.60	**e	*	*
Energy												
Intake, kcal	2663	2824	2653	2372	2866	2623	2476	2359	97.7			
Output, kcal	443	462	412	455	499	390	388	408	16.7	*e	*	*
Retained, kcal	2220	2362	2241	1918	2367	2233	2088	1950	86.2		**	
Retained, %	83.3	83.7	84.4	80.7	82.6	85.2	84.3	82.8	0.49	*e	*	*

[a]Values are least square means; six cages each with six chicks per cage; non-phytate phosphorus supplied by monosodium phosphate. [b]For P-value, ***, **, and * represent effects at $P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively. [c]Linear effect. [d]Quadratic effect.
[e]Interaction of crude protein and non-phytate phosphorus.

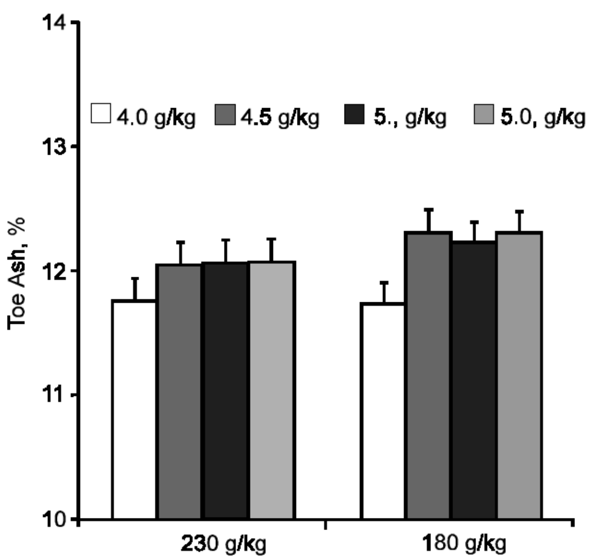

Fig. 1: Toe ash response of broiler chicks fed 230 or 180 g crude protein/kg and 4.0, 4.5, 5.0, or 5.5 g non-phytate phosphorus/kg. Values are least square means; six cages each with six chicks per cage; non-phytate phosphorus supplied by monosodium phosphate. Standard error of the mean (SEM) was 0.18. Linear effect of NPP at ($P < 0.01$)

Discussion

The purpose of this study was to investigate possible interactions between dietary CP and NPP concentrations on growth performance and nutrient utilization in broiler chicks. No significant interactions between CP and NPP were detected for any of the growth performance criteria evaluated, however all performance criteria were affected by the concentration of CP and NPP. Growth performance of birds on the 230 g CP/kg diet are consistent with previous observations (Onyango *et al.* 2003; Dilger *et al.*, 2004). Blair *et al.* (1999) have shown that performance is not compromised in chicks fed AA-supplemented reduced CP concentrations. However, in the study by Blair *et al.* (1999) the CP concentration in the control and low CP diets were 21 and 18%, respectively, with the appropriate supplementation of crystalline amino acids. Comparatively, the CP concentrations in the current study were 23 and 18% in the high and low CP diets, respectively. A possible reason for the observed reduction is the fact that diets were formulated on a total amino acid and not a digestible amino acid basis. Despite the fact that amino acids were added to the reduced protein diet to equal concentrations in the 230 g CP/kg diet, the digestible amino acid concentration in the 180 g CP/kg diet might have been below requirement. The reduction in CP was achieved by reducing the amount of soybean meal with a concomitant increase in corn, in which the inherent digestibility of amino acids is lower than that of soybean meal (NRC, 1994). Another possible reason for the observed reductions in performance by chicks fed the AA-supplemented reduced CP concentration might have been a deficiency of amino nitrogen. Soybean meal supplies over half of the CP in the AA-supplemented reduced CP diet. Fernandez *et al.* (1994) reported that the order of amino acid limitation in soybean meal is (1) sulfur-containing amino acids, (2) threonine, (3) lysine and valine and (4) amino nitrogen. Deschepper and De Groote, (1995) achieved similar performance in chicks fed AA-supplement reduced CP diets compared to chicks fed control diets, when the reduced CP diets were supplemented with crystalline, essential and non-essential amino acids or with amino acids based on body protein profile. This was not achieved when the reduced CP diets were supplemented with crystalline amino acids to NRC-recommendation.

Table 4: Nitrogen and phosphorus retention of broiler chicks (expressed on a per bird basis) fed 230 or 180 g crude protein/kg and 4.0, 4.5, 5.0, or 5.5 g non-phytate phosphorus/kg[a]

	230				180					P-value[b]		
											NPP	
Item	4.0	4.5	5.0	5.5	4.0	4.5	5.0	5.5	SEM	CP	L[c]	Q[d]
Nitrogen												
Intake, g	24.1	25.2	23.7	21.5	20.4	18.9	17.8	16.5	0.80	***	**	
Output, g	6.83	6.87	6.31	7.47	5.86	4.79	4.62	4.88	0.27	*e		**
Retained, g	17.3	18.3	17.4	14.1	14.6	14.1	13.2	11.6	0.70	***	**	**
Retained, %	71.5	72.8	73.2	65.2	71.3	74.6	73.9	70.5	1.25	*	**	**
Phosphorus												
Intake, kcal	4.53	5.41	5.38	5.60	5.21	5.20	5.12	5.50	0.20		**e	
Output, kcal	1.87	2.04	2.03	2.60	2.39	2.21	2.33	2.70	0.12	**	**	**
Retained, kcal	2.66	3.37	3.34	3.00	2.83	3.00	2.79	2.79	0.16	*		**
Retained, %	58.4	62.4	62.1	53.3	54.3	57.6	54.4	51.1	1.84	***	*	*

[a]Values are least square means; six cages each with six chicks per cage; non-phytate phosphorus supplied by monosodium phosphate. [b]For P-value, ***, **, and * represent effects at P < 0.001, P < 0.01, and P < 0.05, respectively. [c]Linear effect. [d]Quadratic effect. [e]Interaction of crude protein and non-phytate phosphorus.

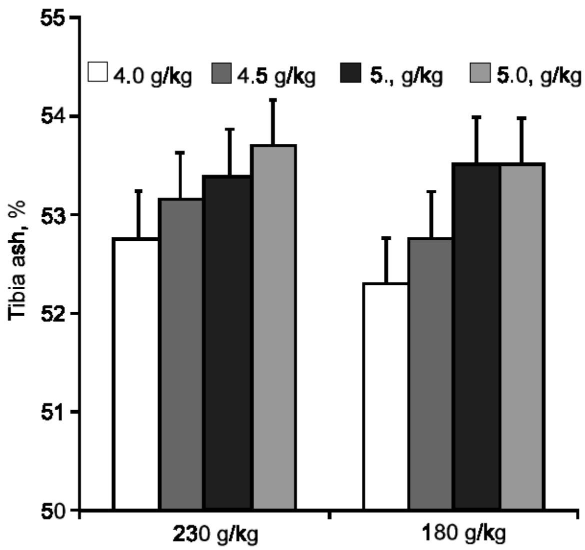

Fig. 2: Tibia ash response of broiler chicks fed 230 or 180 g crude protein/kg and 4.0, 4.5, 5.0, or 5.5 g non-phytate phosphorus/kg. Values are least square means; six cages each with six chicks per cage; non-phytate phosphorus supplied by monosodium phosphate. Standard error of the mean (SEM) was 0.49. Linear effect of NPP at (P < 0.05).

The fact that FI was not affected by CP is evidence that the difference inFI was not due to the inevitable reduction in CP: E ratio when the contribution of soybean is reduced and corn increased, as in the 180 g CP/kg diet. The linear reductions in FBW, BWG and FI above 4.5 and 4.0 g NPP/kg suggests that NPP requirement is higher at the higher CP concentration. The observation that performance is maximized at 4.5 g NPP/kg in the 230 g CP/kg diet is consistent with the NRC-recommended NPP requirement for 0-3 week-old chicks (NRC, 1994).

In agreement with the current findings, Yoshida and Hoshii (1982) also reported a decrease in FI intake, which led to decreased growth rate in broiler chicks fed high concentrations of available P. Ferguson *et al.* (1998) fed adequate or reduced CP and P concentrations to broiler chicks to evaluate ammonia concentration and litter composition. Feed intake, BWG and GF were reduced when low P and 21% CP diet. The reductions in performance were partially restored when chicks were fed diets containing low P and low CP (Ferguson *et al.*, 1998). Thus, the interaction between CP and P are likely due to difference in dietary CP and available P concentrations. These findings are in agreement with the current study, where in the growth performance on the 180 g CP/kg diet was linearly (P < 0.001) reduced with increasing NPP concentration.

Tibia and toe ash of 22-d old broilers in the current study are similar to those reported by Onyango *et al.* (2003, 2004) and DIlger *et al.* (2004). There were no detectable interactions between dietary CP and NPP for toe or tibia ash response. Tibia and toe ash responded linearly (P < 0.05) to dietary NPP. Lim *et al.* (2001) reported a linear increase in tibia ash as dietary NPP increased, which is in agreement with the current findings. Furthermore, Edwards Jr. (2002) also reported an increase in tibia ash with higher dietary NPP, an observation that is consistent with current study. Dietary CP has been reported to affect bone mineralization in chickens (Skinner *et al.*, 1991). However, CP did not significantly affect percentage bone ash in the current study.

The observed reduction in growth performance is consistent with reduced nutrient intake and retention (Table 3 and 4). Linear reductions in DM intake with increasing NPP concentration led to similar reductions in E, and nitrogen intake. Therefore, the effect of NPP on N and E intake are mediated by reductions in FI. The reductions in intake however, does not explain the numerical increases in output of DM, E, N and P at 5.5 g

NPP/kg. Increases in nutrient output at the highest concentration of NPP suggests that either absorption is reduced, the efficiency of metabolic utilization is lower or that both mechanisms may be responsible. Significant interactions between CP and NPP concentrations were observed for DM and E retention. Based on significant linear and quadratic trends, DM and E retention as a percent of intake appears to be optimized at 5.0 g NPP/kg at the 230 g CP/kg and at 4.5 g NPP/kg for the 180 g CP/kg diet.

Published reports providing insight into the interaction of dietary CP and P, and their subsequent metabolic utilization in the chick are lacking. However, higher N retention, higher urea concentration in plasma, liver and kidney tissues and a significant reduction in renal glutamate dehydrogenase activity (a central enzyme of amino acid degradation in kidney mitochondria), in P-depleted rats have been reported (Huber and Breeves, 1999). The reduction in N intake and retained N by chicks receiving an AA-supplemented reduced concentration is expected and are consistent with previous reports (Deschepper and De Groote, 1995; Ferguson *et al*., 1998; Blair *et al*., 1999). However, lower intake is partially compensated by higher N retention as a percent of intake in chicks fed AA-supplemented reduced CP diets. Nitrogen retention was also affected by NPP concentration with linear increases up a point followed by a decrease. These finding are in agreement with Lim *et al*. (2001) who reported that reductions in dietary NPP led to a decrease in N retention in broiler chickens. Nitrogen retention observed in the current study are similar some of those in the data summarized by Adeola and Sands (2003).

With a quadratic response in P retention to dietary NPP, phosphorus retention appeared to be maximal at 4.5g NPP/kg for the 230 g CP/kg and at 4.0 g NPP/kg for the 180 g CP/kg diet. Ferguson *et al*. (1998) also reported significant interactions between CP and NPP when litter P was measured as an indicator of P excretion. Litter P concentration was significantly lower when low P and control (NRC-recommended CP) was fed compared with litter from cages of chicks provided low P, low CP diets. This implies higher retention of P at the higher CP concentration.

The results of this study suggest that interactions between dietary CP and NPP may influence the utilization of N and P in broiler chicks. At higher dietary CP, higher concentrations of NPP may be required to maximize nutrient retention. On the other hand, reductions in dietary CP may require a lower dietary NPP concentration. The use AA-supplemented reduced CP concentrations is being proposed as a strategy to reduce manure nutrient excretion. Further, strategies employed to reduce P excretion include reductions in dietary P concentration and the addition of supplemental phytase. Interactions between CP and P as observed in the present study require further investigation to determine the optimal dietary NPP concentration in AA-supplemented reduced CP diets. These results provide support for the need to develop integrated strategies for reducing manure N and P excretion by broiler chickens.

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