

The Use of Phytase and Low Phosphorus Levels in Broiler Diets with Different Metabolizable Energy Levels

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Abstract

The objective of this study was to determine the effects of dietary available phosphorus (AP), phytase supplementation and metabolizable energy (ME) levels on performance and bone and blood characteristics of broiler chickens. An experiment in completely randomized design with $2 \times 2 \times 2$ factorial arrangement were conducted, in which two phytase levels (0 and 500 mg/kg), two AP levels (NRC and 15% lower) and two ME levels were used. The ME ratios to CP and other nutrients (except phosphorus) were equal in all treatments. Average weight gain, feed intake, and feed conversion ratio (FCR) of starter, grower and total rearing period were analyzed. Bone calcium and phosphorus, blood calcium, phosphorus and alkaline phosphatase at 6 weeks of age were analyzed. Based on growth parameters, low AP level had equal or better effects than high AP level. Chickens fed diets with high ME levels had better performance. Phytase had no significant effects on weight gain, whereas it enhanced FCR. Adding phytase in low energy diets improved body weight gain and FCR. Phytase in diets with low AP levels significantly enhanced FCR. Using low AP in high and low energy diets, caused better FCR in starter and whole rearing phase consequently. Available phosphorus and phytase supplementations had no significant effects on tibia ash; however, it was inversely affected by low AP level. The results of this experiment indicated that it is possible to decrease dietary AP level up to 15 percent less than NRC recommends. Adding AP and phytase did not significantly affect growth parameters of the chicks fed low energy diets.

Key Words: Available Phosphorus, Metabolizable energy, Phytase, Broiler

Introduction

Phosphorus (P) is an essential mineral for growing birds and there are growing concerns regarding the effects of excreta P on eutrophication of surface waters (Waldroup, 1999). Phytate reduces phosphorus and mineral availability, lessens nitrogen utilization and amino acid, lipid and starch digestibility and inhibits the activity of digestive enzymes (Ravindran et al., 1995; Rutherford, et al., 2004). Microbial phytase overcome these effects and increases ileal digestible and apparent metabolizable energy (Camden et al., 2001; Ravindran et al., 2000) and improves ileal amino acid digestibility in broilers (Camden et al., 2001; Rutherford, et al., 2004; Ravindran et al., 2000).

According to Yan et al. (2003), non-phytate phosphorus (NPP) level for maximizing tibia ash is higher than its recommended levels for optimizing weight gain, feed conversion ratio (FCR) and mortality rates. It is also less than NRC recommendations (1994). Phosphorus requirement for weight gain, feed intake and bone weight decreases with high environmental temperatures, however, in sudden high environmental temperatures, phosphorus deficiency can cause heat stress and mortality (Persia et al., 2003). Environmental temperature interacts with dietary P level in parameters such as bone strength, bone ash and mortality. The response of the chicks to dietary P level is influenced by chick strain and environmental temperature (Orban and Roland, 1990). In 4 to 8 wk old broilers, metabolizable energy (ME) and NPP levels had no interactions (Waldroup et al., 1974). The birds are weak in utilizing phytate P, so their excreta contain higher P, resulting in more environmental P pollution (Ravindran et al., 1995). Faster growing birds utilize less phytate P because of lesser feed retention time. So, slower growing birds that utilize phytate P better, release more P for energy utilization and therefore, they are better energy-utilizers (Ankra-Badu et al., 2004).

There are little studies on the relations of dietary ME and P levels in broilers nutrition. Phosphorus contributes in different cellular enzymatic reactions, especially in energy exchange reactions in the body. In sever P deficiencies, like rickets blood P level is so high. It is reported that bone catabolism provides organic P required for the synthesis of high energy compounds and P containing enzymes (Scott et al., 1982).

Phosphorus containing compounds, such as adenosine triphosphate plays a critical role in energy metabolism. Furthermore, using phytase is a way to overcome non-available P problem in broiler nutrition. However, the relationship between phytase, phytate P and dietary ME requires more clarification. The objective of the present study was to investigate the effects of low P and phytase levels with different ME on broiler performance and some bone and blood characteristics.

Materials and Methods

Four hundred and eighty broiler chicks (Ross strain) were fed a standard diet for one week. Birds were allocated to 24 litter pens and randomly assigned to eight dietary treatments (each in 3

replicates) with $2 \times 2 \times 2$ factorial arrangements. Treatments were two levels of ME (2850 and 2950 kcal/kg in starter and 2930 and 3030 kcal/kg in grower period), two levels of AP (equal or 15 percent lower than NRC recommendations) and two levels of phytase (Natuphos-1000) supplementation (0 and 500 mg/kg). Diets were formulated according to NRC guidelines (1994; Table 1). Mono-calcium phosphate, CaCO₃ and washed grits were used to formulate diets. Chicks were subjected to a normal cyclic temperature of summer season (averagely 27.2 to 35.7 degree C°) and allowed *ad libitum* access to feed and water. The Birds and feed residues of experimental pens were weighted weekly. Average weight gain, feed intake and feed conversion ratio (FCR) of starter and grower and total period were calculated.

On day 42, four birds from each pen were bled by brachial venipuncture. Blood samples were centrifuged at $3500 \times g$ for 15 minute to separate serums. Serum calcium, P and alkaline phosphatase were determined by using Autoanalyser Technicon RA-1000 (Technicon Instruments Corporation, New York, U.S.A.). At age of 42 days, two birds were randomly selected and euthanized (one male and one female) from each pen and the left tibias of euthanized birds were separated. The soft tissues around bones were removed, dried and analyzed for ash, calcium and phosphorus (AOAC, 1990). Data from both male and female birds averaged and used for statistical analysis. Data in percent scale were transformed by $\arcsin x^{-1}$, and then subjected to statistical analysis, but the original data is presented.

Table 1. Ingredients and composition of the experimental diets of starter and grower periods

Ingredients (%)	ME level: AP level:	starter				grower			
		Low		high		low		high	
		-15%	NRC ²	-15%	NRC	-15%	NRC	-15%	NRC
Ground yellow corn		60.65	60.65	57.82	57.82	67.22	67.22	62.29	62.29
Soybean meal (45% CP)		36.45	36.45	35.30	35.30	30.32	30.32	32.65	32.65
Fish meal (65% CP)		-	-	2.20	2.20	-	-	-	-
Soybean oil		-	-	2.00	2.00	-	-	2.60	2.60
Iodized salt		0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Ca Co ₃		0.54	0.28	0.59	0.33	0.80	0.60	0.76	0.55
Mono-calcium phosphate		1.05	1.41	0.85	1.21	0.69	0.96	0.75	1.04
DL-Methionine		0.14	0.14	0.14	0.14	0.05	0.05	0.06	0.06
Vitamin-mineral premix ¹		0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Washed grit		0.37	0.27	0.30	0.20	0.120	0.05	0.09	0.01
Calculated analysis									
ME	kcal/g	2850	2850	2950	2950	2930	2930	3030	3030
CP	%	20.48	20.48	21.20	21.20	18.31	18.31	18.93	18.93
Methionine	%	0.46	0.46	0.49	0.49	0.35	0.35	0.37	0.37
Methionine + cystine	%	0.80	0.80	0.83	0.83	0.66	0.66	0.68	0.68
Calcium	%	0.89	0.89	0.92	0.92	0.82	0.82	0.85	0.85
Total Phosphorus	%	0.76	0.83	0.75	0.82	0.67	0.72	0.68	0.73
Available Phosphorus	%	0.34	0.40	0.35	0.42	0.27	0.32	0.28	0.33
Ca / AP ratio		2.61	2.22	2.61	2.22	3.02	2.57	3.02	2.57

¹supplied per kilogram of diet: vitamin A, 10,000 IU; Cholecalciferol, 2,000 IU; vitamin E, 10 mg; vitamin K₃, 2 mg; thiamine, 1 mg; riboflavin, 5 mg; pyridoxine, 2 mg; vitamin B₁₂, 0.0154 mg; niacin, 125mg; calcium pantothenate, 10 mg; folic acid, 0.25 mg; biotin, 0.02 mg; selenium, 0.1 mg; iron, 40 mg; copper, 12 mg; zinc, 120 mg; manganese, 100 mg; iodine, 2.5 mg; cobalt, 0.75 mg; BHT, 30 mg.

²according to NRC (1994)

Data were subjected to analysis of variance using the general linear Models procedures of SAS software (SAS Institute, 1985) in a completely randomized design with a $2 \times 2 \times 2$ factorial arrangement. Treatment means were compared using Duncan's new Multiple Range Test (Steel and Torrie, 1980).

Results

As shown in Table 2, high ME rations increased birds weight gain and feed efficiency. In addition, low dietary AP levels decreased FCR, especially in starter period ($P < 0.05$). Adding Phytase in bird's rations decreased FCR and serum calcium, while it increased bone calcium content. Phytase also increased feed efficiency (2.03 vs. 2.21) and weight gain of chicks fed low energy diets, while it decreased serum calcium (8.8 vs. 9.5 mg/dl) ($P < 0.05$). Phytase had no effects on performance and blood calcium of chicks fed high energy rations.

Phytase in both levels of AP caused better FCR (Table 3). It increased tibia ash and bone calcium and decreased serum calcium of birds fed NRC-based AP diets ($P < 0.05$).

Low AP level reduced starter FCR, especially in high ME diets ($P < 0.05$). In both levels of dietary ME, lowered AP level reduced tibia ash (Table 4).

Experimental treatments did not affect breast and thigh muscle percentages. Phytase decreased carcass yield (73.8 vs. 71.64 percent), especially in diets with standard AP levels (Table 3). Abdominal fat significantly ($P < 0.05$) increased due to low AP (2.43 vs. 2.02 percent) and non-significantly in high ME diets (2.33 vs. 2.12 percent). Low AP had a significantly increasing effect on abdominal fat of chicks fed high energy diets ($P < 0.05$) (Table 4).

Table 2. Effects of dietary ME and AP level and Phytase on broiler performance

	Phytase (mg/kg)		ME level		AP level (%)	
	0	500	low	high	NRC	-15 %
Average daily gain (g/day)	49.2	51	48 ^b	52.3 ^a	49.6	50.6
Daily feed intake (g)	101.9	99.7	101.5	100.1	100.7	100.8
Feed conversion ratio (g/g)	2.08 ^a	1.96 ^b	2.12 ^a	1.92 ^b	2.04	2.00
Starter average daily gain (g/day)	42.20	40.50	39.80 ^b	42.80 ^a	40.8	41.80
Starter feed conversion ratio (g/g)	1.21	1.24	1.28 ^a	1.17 ^b	1.27 ^a	1.18 ^b
Grower Average daily gain	61.10	60.50	58.60 ^b	63.00 ^a	60.5	61.10
Grower Feed conversion ratio (g/g)	2.19	2.17	2.27 ^a	2.10 ^b	2.17	2.19
Bone calcium (%)	15.40 ^b	19.30 ^a	17.30	17.50	15.40	19.30
Serum calcium (mg/dl)	9.60 ^a	8.90 ^b	9.20	9.40	9.40	9.20

^{a,b} numbers with different subscripts in each row from each block of data differed significantly ($P < 0.05$)

Discussion

In the recent years, considerable reports about using of P and phytase in broilers nutrition have been published. They mainly studied effects of phytase and relevant dietary P levels on performance, bone quality and mortality rates. In the current experiment, effect of phytase on chick performance was consistent with Camden et al. (2001) and Yan et al. (2003) findings. In the present study, significant effects of phytase on birds FCR were observed. Sebastian et al. (1996) indicated that adding phytase in birds' rations increased tibia ash and bone calcium contents, whereas it decreased blood calcium concentrations. In the present study, reduced level of AP increased feed efficiency and did not have negative effects on birds' performance, especially in the starter period. It seems that dietary AP level can be decreased by 15 percent of NRC values by supplementing phytase in the rations (Table 3). It is noticed that AP level required for optimum tibia ash, weight gain, FCR and mortality rate is orderly increased (Yan et al., 2001 and 2003; Sebastian et al., 1996). In the present study, the results observed by phytase and AP levels were similar to that reported by Yan et al. (2001).

The phytase increased tibia ash only in standard AP and not in low AP diets, whereas bone calcium resulted from low AP diets with or without phytase was greater than standard AP diets (Table 3). Low AP levels in current study did not pose a serious P deficiency. In addition, phytase could not compensate P deficiency. It is reported that optimum NPP level of broilers is less than that of NRC (1994) and NPP requirements of 3 to 6 weeks old broiler chicks with and without phytase are 0.151 and 0.184 percent, respectively (Yan et al., 2001 and 2003). The limited levels of AP and phytase used in this experiment were not enough to determine the best level of AP in each phytase levels. Phytase caused the most tibia ash in standard AP diets. Phytase supplementation in low AP diets improved bone calcium content compared with no phytase standard AP diets (Table 3). This may be related to different Ca/AP ratios which was greater in high P diets. Serum and alkaline phosphatase did not have significant differences between studied diets plans, but phytase supplementation reduced serum calcium content (8.98 vs. 9.60 mg/dl). Serum calcium of chicks fed high AP and phytase supplemented diets were lower than high AP diets without phytase (Table 3).

Table 3. Interactions of dietary phytase and AP level on broiler performance

Phytase (mg/kg):	AP level: NRC ¹		- 15%		SE
	0	500	0	500	
Average daily gain (g/day)	49.0	50.2	49.4	51.8	1.41
Feed conversion ratio (g/g)	2.09 ^a	1.98 ^b	2.07 ^a	1.93 ^b	0.022
Starter feed conversion ratio (g/g)	1.22 ^{ab}	1.32 ^a	1.2 ^{ab}	1.15 ^b	0.043
Bone calcium (%)	14.2 ^b	16.6 ^{ab}	16.7 ^{ab}	22.0 ^a	-
Serum calcium (mg/dl)	9.9 ^a	8.8 ^b	9.3 ^{ab}	9.1 ^b	-
Tibia ash (%)	40.93 ^b	42.23 ^a	39.99 ^b	40.28 ^b	-
Carcass yield (%)	74.40 ^a	71.40 ^c	73.20 ^{ab}	71.80 ^{bc}	-

^{a-c} numbers with different superscript in each row of data differed significantly ($P < 0.05$)

¹ according to NRC-1994

Table 4. Interaction of dietary ME and AP level on broiler performance

	ME level: low		ME level: high		SE
	Phosphorus level: NRC ¹	- 15%	Standard	- 15%	
Average daily gain (g/day)	47.2 ^b	48.8 ^{ab}	52 ^a	52.5 ^a	1.41
Feed conversion ratio (g/g)	2.16 ^a	2.08 ^b	1.92 ^c	1.92 ^c	0.022
Starter average daily gain (g/day)	39.9 ^b	39.8 ^b	41.7 ^{ab}	43.8 ^a	1.091
Starter feed conversion ratio (g/g)	1.32 ^a	1.25 ^a	1.23 ^a	1.11 ^b	0.043
Grower feed conversion ratio (g/g)	2.26 ^a	2.28 ^a	2.08 ^b	2.11 ^b	0.042
Tibia ash (%)	40.93 ^b	39.26 ^c	42.23 ^a	41.02 ^b	-
Abdominal fat (%)	2.06 ^b	2.18 ^b	1.98 ^b	2.67 ^a	-

^{a-c}numbers with different superscript in each row of data differed significantly ($P < 0.05$)

¹according to NRC (1994)

Regardless of AP level, high dietary ME level improved growth performance and FCR. Reducing AP level in low energy diets enhanced feed efficiency during the course of the study. However, reduced P level in starter period improved feed efficiency in high energy diets, indicating some interactions between rearing phase and P. In the present study, which was conducted in high cyclic environmental temperatures, standard P level did not compensate reduced performance of low energy diets. Moreover, low P level did not decrease high performance attained by high energy diets (Table 4). This is coincided with the classic biochemical findings that enzymatic reactions require low blood P level (Scott et al., 1982) and energy metabolism is not affected by low level of dietary P (Waldroup et al., 1974). Apparently, in low energy diets, in case other nutrients are in higher levels, dietary P may interact with ME via different ways. This is a matter for further investigation. Low P in any ME levels decreased tibia ash percentage. This indicates that low AP level decreased tibia ash in comparison with AP level suggested by NRC (Yan et al., 2003).

Experimental treatments did not affect breast and thigh percentages. Phytase decreased carcass yield (73.8 vs. 71.64 percent). Phytase in low P diets significantly decreased carcass yield (Table 3). Abdominal fat was increased non-significantly due to reduced dietary P (2.43 vs. 2.02 percent) and high energy (2.33 vs. 2.12 percent). Birds fed low AP with high energy diets had greatest abdominal fat (Table 4). This is probably a reason to show that reducing P shifted the energy pathways to accumulate more fat in abdominal cavity. This experiment was carried out in high environmental temperatures, but mortality rate was low. Reducing P level had no impact on mortality rate.

Conclusion

The results of this experiment showed that it is possible to decrease dietary AP by 15 percent lower than NRC recommendations. Altering dietary phytase and AP level could not compensate growth performance reduction due to low dietary ME. Available P level showed some relations with ME levels and growth periods.

References

- Ankra-Badu, G. A., S. E. Aggrey, G. M. Pesti, R. I. Bakalli, and H. M. Edwards. 2004. Modeling of parameters affecting phytate phosphorus bioavailability in growing birds. *Poultry Science*, 83:1083-1088.
- Association of Official Analytical Chemists. 1990. *Official Methods of Analysis*. 15th ed. Association of Official Analytical Chemists, Washington, DC.
- Camden, B. J., C. H. Morel, D. V. Thomas, V. Ravindran, and M. R. Bedford. 2001. Effectiveness of exogenous microbial phytase in improving the bioavailabilities of phosphorus and other nutrients in maize-soybean meal diets for broilers. *Journal of Animal Science*, 73:289-297.
- NRC. 1994. *Nutrient requirements of poultry*. 9th rev. ed. National Academy Press, Washington D.C.
- Orban, J. I., and D. A. Roland. 1990. Response of four broiler strains to dietary phosphorus above and below the requirement when brooded at two temperatures. *Poultry Science*, 69:440-445.
- Persia, M. E., C. M. Parsons, and K. W. Koelkebeck. 2003. Interrelationship between environmental temperature and dietary nonphytate phosphorus in chicks. *Poultry Science*, 82:1616-1623.
- Ravindran, V., W. L. Bryden, and E. T. Cornegay. 1995. Phytates: occurrence, bioavailability and implications in poultry nutrition. *Poultry and Avian Biology Review*, 6:125-143.
- Ravindran, V., S. Cabahug, G. Ravindran, P. H. Selle, and W. L. Bryden. 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus level. I. Effects on apparent metabolizable energy, nutrient digestibility and nutrient retention. *British Poultry Science*, 41:193-200.
- Rutherford, S. M., T. K. Chung, P. C. Morel, and P. J. Moughan. 2004. Effect of microbial phytase on ileal digestibility of phytate phosphorus, total phosphorus, and amino acids in low-phosphorus diet for broilers. *Poultry Science*, 83:61-68.
- SAS. 1985. *SAS procedure guide for personal computers*. Version 6 edition. SAS Institute Inc., Cary, NY.
- Scott, M. L., M. C. Nesheim, and R. J. Young. 1982. *Nutrition of the chicken*. 3rd ed. Ithaca NY, M. L. Scott and Associates.
- Sebastian S., Touchburn S.P., Chavez E.R., Lague P.C. 1996. Efficacy of supplemental microbial phytase at different dietary calcium levels on growth performance and mineral utilization of broiler chickens. *Poultry Science*, 75:516-523.
- Steel, R. G. D., and J. H. Torrie. 1980. *Principles and procedures of statistics*. McGraw-Hill Book Co., NY.
- Waldroup, P. W., R. J. Mitchell, and K. R. Hazen. 1974. The phosphorus needs of finishing broilers in relationship to dietary nutrient density levels. *Poultry Science*, 53:1655-1663.
- Waldroup, P. W. 1999. Nutritional approaches to reducing phosphorus excretion by poultry. *Poultry Science*, 78:683-691.
- Yan, F., J. H. Kersey, and P. W. Waldroup. 2001. Phosphorus requirements of broiler chicks three to six weeks of age as influenced by phytase supplementation. *Poultry Science*, 80:455-459.
- Yan, F., C. A. Fritts, and P. W. Waldroup. 2003. Evaluation of modified dietary phosphorus levels with and without phytase supplementation on live performance and fecal phosphorus levels in broiler diets. 1. Full-term feeding recommendations. *Journal of Applied Poultry Research*, 12:174-182.