Iran Agricultural Research 22:120-137 (2003)

EFFECTS OF CITRIC AND ASCORBIC ACIDS, VITAMIN D_3 AND CALCIUM ON EFFICACY OF MICROBIAL PHYTASE IN A CORN-SOYBEAN MEAL-BASED DIET: BROILER PERFORMANCE AND NUTRIENT DIGESTIBILITY FROM 1 TO 21 D OF AGE

M. AFSHARMANESH, A.H. SAMIE AND J. POURREZA[1]

Department of Animal Science, College of Agriculture, Isfahan University of Technology, Isfahan, I.R. Iran.

(Received: February 22, 2002)

ABSTRACT

This study was conducted to determine the additive effects of microbial phytase, organic acids (citric, ascorbic), vitamin D_3 and Ca on broiler performance and nutrient digestibility in a corn-soybean meal-based diet from 1 to 21 d of age. Broilers were fed the following basal diets at either 0.79 or 0.9 % of dietary Ca: 1) a negative control corn-based diet (NC), 0.315% available P; 2) NC + 500 phytase units (FYT) kg^{-1} diet; 3) phytase + 2%citric acid; 4) phytase + citric acid + 200 mg kg^{-1} diet ascorbic acid; 5) phytase + citric acid + ascorbic acid + 200 µg kg^{-1} diet vitamin D_3; 6) NC plus 0.135% available P. The 12 experimental diets were fed to four pen replicates of 20 birds each. Increasing dietary Ca from 0.79 to 0.9%, negatively influenced feed conversion ratio but improved feed intake and protein digestibility. Added phytase improved body weight, feed conversion ratio, tibia ash and P and protein digestibility. Subsequent addition of citric acid and ascorbic acid to feed along with phytase increased 10, 8 and 57% body weight, feed efficiency and P retention, respectively, above levels attained with negative control diet. The body weight of chicks fed the positive control diet was similar to those that received the phytase and organic acids supplemented low-P diet. The tibia ash content and P digestibility of broilers fed the diet containing phytase, organic acids and vitamin D_3 was 28.9 and 57% more than the negative control diet, respectively. Data presented clearly indicated that

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1. Former Ph.D. Student, Assistant Professor and Professor, respectively.

supplementation of corn-soybean low-P meal-based diet with appropriate concentrations of phytase, citric acid, ascorbic acid and vitamin D_3, leads to substantial increases in broiler performance and nutrient digestibility and would have an environmental benefit of reducing P and N concentrations of broiler manure.

Key words: Ascorbic acid, Broilers, Citric acid, Phytase, Vitamin D_3.

تحقیقات کشاورزی ایران

۲۲: ۱۲۰-۱۳۷(۱۳۸۲)

تأثیر سیتریک و اسکوربیک اسید، ویتامین D_3 و کلسیم بر کارآیی فیتاز میکروبی در جیره های بر پایه ذرت و کنجاله سویا: عملکرد پرنده و قابلیت هضم مواد مغذی از سن ۱ تا ۲۱ روزگی

محسن افشارمنش، عبدالحسین سمیع و جواد پوررضا

به ترتیب، دانشجوی دکتری، استادیار و استاد بخش علوم دامی دانشکده کشاورزی دانشگاه صنعتی اصفهان، اصفهان، جمهوری اسلامی ایران.

چکیده

این مطالعه به منظور تعیین اثر آنزیم فیتاز و اسیدهای آلی (سیتریک و اسکوربیک)، ویتامین D_3 و سطوح مختلف کلسیم بر عملکرد جوجه های گوشتی و قابلیت هضم مواد مغذی (سن ۱ تا ۲۱ روزگی) در جیره های بر پایه ذرت و کنجاله سویا انجام شد. جوجه های گوشتی با جیره های زیر در دو سطح ۰/۷۹ و ۰/۹ ٪ کلسیم تغذیه شدند. جیره ها شامل: ۱) جیره شاهد منفی حاوی ۰/۳۱۵

درصد فسفر قابل دسترس، ۲) جیره شاهد منفی به علاوه ۵۰۰ واحد فیتاز در هر کیلوگرم جیره، ۳) جیره شاهد منفی به علاوه فیتاز و ۲ درصد سیتریک اسید، ۴) جیره شاهد منفی به علاوه فیتاز، سیتریک اسید و ۲۰۰ میلی گرم در کیلوگرم اسکوربیک اسید، ۵) جیره شاهد منفی به علاوه فیتاز، سیتریک اسید، اسکوربیک اسید و ۲۰۰ میکرو گرم در کیلو گرم ویتامین D_3، ۶) جیره شاهد حاوی ۰/۴۵ درصد فسفر قابل دسترس (توصیه شورای پژوهش های ملی آمریکا). تیمارهای آزمایشی هر کدام در ۴ تکرار و ۲۰ قطعه جوجه گوشتی در هر تکرار اجرا شدند. افزایش کلسیم جیره از ۰/۷۹ به ۰/۹٪ تاثیر منفی بر ضریب تبدیل خوراک داشت اما مصرف خوراک و قابلیت هضم پروتئین را بهبود بخشید. افزودن فیتاز به جیره موجب بهبود وزن بدن، ضریب تبدیل خوراک، خاکستر درشت نی و قابلیت هضم فسفر و پروتئین شد. افزودن سیتریک اسید و اسکوربیک اسید به همراه فیتاز، وزن بدن، بازده غذایی و ابقای فسفر را در مقایسه با جیره شاهد به ترتیب ۱۰، ۸ و۵۷ ٪ افزایش داد. وزن بدن جوجه های تغذیه شده با جیره شاهد مشابه جوجه هایی بود که جیره شاهد منفی تکمیل شده با فیتاز و اسیدهای آلی را دریافت کردند. خاکستر درشت نی و قابلیت هضم فسفر در جوجه های تغذیه شده با جیره دارای فیتاز، اسیدهای آلی و ویتامین D_3 به ترتیب ۱۸ و ۵۷ درصد بالاتر از جوجه های تغذیه شده با جیره شاهد منفی بود. نتایج این مطالعه نشان داد که تکمیل نمودن جیره های کم فسفر بر پایه ذرت و کنجاله سویا با مقادیر مناسب فیتاز، سیتریک اسید، اسکوربیک اسید و ویتامین D_3 موجب افزایش قابل ملاحظه ای در عملکرد جوجه های گوشتی و قابلیت هضم مواد مغذی می شود و با کاهش مقدار نیتروژن و فسفر مدفوع، اثرهای محیطی سودمندی را به همراه دارد.

INTRODUCTION

The effectiveness of phytase enzyme in poultry feeding is limited. This phenomenon is caused mainly by inhibition of the enzyme by inorganic phosphate and by a partial rather than complete dephosphorylation of feed phytates. In many studies (13, 24) the effectiveness of phytase was negatively related to the amount of inorganic P in the diet. The level of

dietary Ca may also affect the utilization of phytic acid through the formation of insoluble Ca phytate and/or reduction of phytase activity (4). Cholecalciferol (vitamin D_3) plays a role in Ca and P absorption and therefore influences their utilization. Mohammed *et al.* (16) reported that cholecalciferol supplemention of poultry diets increased phytic acid-P utilization.

Several studies have shown that the optimal pH of microbial phytase occurs at two peaks, the highest activity was observed at a pH of 5.0 to 5.5 and the second highest activity was at a pH of 2.5 (22). A typical corn-soybean meal based diet for poultry using dicalcium phosphate as the inorganic P source has a pH of approximately 6.0. The addition of organic acids such as citric and ascorbic acids is known to lower diet pH. Because the site of phytase activity is primarily the stomach (24), lowering the dietary pH might reduce the pH of the stomach digesta and thereby increase the effectiveness of microbial phytase. It is widely held that high levels of inorganic Ca have a negative influence on the efficacy of phytase. It has been proposed that the poor solubility of Ca-phytate (23) and other mineral-phytate complexes (15) renders phytate resistant to phytase activity. These mineral-phytate complexes are usually formed at a pH that is above, or at the upper end of the activity spectrum of microbial phytase. In the upper regions of the small intestine the pH of the digesta will increase and thus favor the reformation of mineral-bound phytase-resistant forms of phytate. Thus, the prevailing pH in the gut may have an important influence on the efficacy of phytase. The bi-phasic pH profile of microbial phytase activity (21) indicates that subtle changes in pH of the upper digestive tract could influence the activity of the feed enzyme. *In vitro*, 0.5 M Ca was ineffective as an inhibitor of microbial phytase at pH 5, while 0.005 M Ca caused complete inhibition when the pH of medium was increased to 7.5 (15). Recent work has indicated that supplementing a P-deficient corn-soybean meal diet with citric acid resulted in an increase in tibia ash and weight gain as compared to those consuming diets with no added citric acid (3). Also, citric acid has been known to be a chelator for Ca (10). Improving the efficiency of phytase could lead to reduction of feed costs and to a greater use of phytase which would be of environmental importance.

The objective of this study was to determine the effectiveness of dietary Ca, reducing diet acidity and cholecalciferol supplementation as a means to improve phytase efficacy in corn-soybean meal diets fed to broiler chickens.

MATERIALS AND METHODS

Housing and Diets

A total of 960 day-old (Ross×Ross) broiler chicks were used in this experiment. All chicks were randomly distributed into 48 floor pens (20 chicks per pen), each floor pen contained one bell-shaped waterer, one hand-filled hanging feeder, one brooding light and approximately 10 cm deep dry-wood shaving litter. The temperature was maintained at 32±1°C in the first week and was reduced by 3°C wk^{-1} to 21°C. Lighting was continuous, and water and feed were provided *ad-libitum*.

The experimental design consisted of a 6×2 factorial arrangement of dietary treatments with four pen replicates. The experimental diets consisted of 6 corn-soybean meal-based diets. The basal diet was formulated to contain lower available P (0.315%) than NRC (17) recommendation (Table 1). Diets were formulated at the same protein and energy concentration. Therefore, broilers were fed the following basal diets at either 0.79 or 0.90% of dietary Ca: 1) a negative control corn-soybean meal-based diet, 0.315% available P (NC); 2) NC + 500 phytase units (FYT) kg^{-1} diet; 3) NC + 500 FYT kg^{-1} diet and 2% citric acid; 4) NC + 500 FYT kg^{-1} diet, 2% citric acid and 200 mg kg^{-1} ascorbic acid; 5) NC + 500 FYT kg^{-1} diet, 2% citric acid, 200 ppm ascorbic acid, and 200 μg kg^{-1} diet vitamin D_3; 6) and a positive control diet, NC + 0.135% available P (from dicalcium phosphate). One phytase unit is the activity of phytase that generates 1 micromole of inorganic phosphorus per minute from an excess of sodium phytate at pH 5.5 and at 37°C. The concentration of dietary NPP (diet 6) in basal diet was calculated to meet total P content of the test diets. Thus, the experimental diets would have the same nutrient content and the same Ca: NPP ratios as the positive control diet only if total dephosphorylation of feed phytates took place in gastrointestinal tract. To avoid the confounding effect caused by the presence of non-starch polysaccharides in corn-soybean, all diets were supplemented with a xylanase (Ronozyme Wx is granulated and heat

stable) at levels recommended by the manufacturer (50 mg kg^{-1}). This product contained 1000 endoxylanase Exu g^{-1} xylanase activity as a main activity (Hoffmann-La Roche Inc. Nutley, NJ 07110-1199).

Table 1. Composition and nutrient content of the low-P negative control basal diet†.

Ingredient	% of diet
Corn	55.35
Soybean meal	38.04
Sunflower oil	2.5
Alfalfa meal	1.00
Oyster shell	1.2
DL-methionine	0.20
Salt	0.32
DCP	0.91
Vitamins and minerals §	0.50
Composition	
Metabolizable energy (kcal kg^{-1})	2971
Crude protein	22.50
Available P	0.315
Ca	0.79
Methionine	0.48
Lysine	1.14

† Additionally, the low-P negative control diets that contained 0.9% of Ca were formulated from the above diet by increasing oyster shell concentration at the expense of corn. At each level of Ca, the following experimental diets were formulated: 1) a negative control (NC) diet, 0.315% available P; 2) NC+500 phytase units kg^{-1} diet; 3) NC plus 500 phytase units ha^{-1} diet and 2% citric acid 4) NC plus 500 phytase units kg^{-1} diet, 2% citric acid kg^{-1} diet and 200 mg ascorbic acid kg^{-1} diet; 5) NC plus 500 phytase units kg^{-1} diet, 2% citric acid, 200 mg ascorbic acid kg^{-1} diet and 200 µg vitamin D_3 kg^{-1} diet and 6) NC plus 0.125% available P (from dicalcium phosphate). Dietary additions were added at the expense of corn and metabolizable energy was adjusted by increasing the amounts of vegetable oil.

§ Supplied per kilogram of diet: Vitamin A, 9,000 IU; cholecalciferol, 2,000 IU; vitamin E; 18 IU, vitamin K3, 2 mg; vitamin B_{12}, 0.015 mg; thiamine, 1.8 mg; riboflavine, 6.6 mg; folic acid, 1 mg; biotin, 0.10; niacin, 35 mg; pyridoxine, 4 mg; choline chloride, 250 mg; ethoxyquine, 0.125; manganese SO_4, 100 mg; copper SO_4, 10 mg; selenium (sodium selenate), 0.2 mg; iodine (EEI), 1 mg; zinc- SO_4, 100 mg; Fe, 50 mg.

Sample Collection and Assays

Chicks were weighed at 0 and 21 d of age and feed consumption was measured weekly throughout the experiment. Birds that died during the experiment were recorded daily and used to adjust feed consumption data. At 16th day of age, chromic oxide (Cr_2O_3) was also included in all diets (0.3%) and fed for 5 days as an analytical marker for determination of nutrient digestibility. At 21st d of age, six birds from each pen were killed by cervical dislocation and the small intestine was immediately exposed. The contents of the lower ileum were expressed by finger pressure into plastic containers. The ileum was defined as that portion of the small intestine extending from the vitelline diverticulum to a point 40 mm proximal to the ileo-caecal junction. The ileum was divided into 2 halves and the digesta were collected from the lower half towards the ileo-caecal junction. Digesta from birds within a pen were pooled, resulting in 4 samples per dietary treatment. The digesta samples were frozen immediately after collection and subsequently lyophilized. Tibia samples were obtained by severing the left tibia. The tibias of 3 birds within a pen were pooled. The tibial bones were boiled to remove any traces of meat. They were solvent-extracted to remove fat and then dried and ashed.

Excreta were dried and ground to pass through a 1-mm sieve and feed samples were also ground to pass through a 1-mm sieve. Phosphorus concentrations in feed and excreta were determined colorimetrically by the molybdo-vanadate method (1). The protein content (N×6.25) of diet and individual samples of excreta was determined by the Kjeldahl method after acid digestion. Dietary and fecal chromium (Cr) contents were analyzed by the procedure described by Fenton and Fenton (7) using spectrophotometry. Phosphorus and protein retention was calculated using the following formula:

100%-[100% × (Cr concentration in feed ÷Cr concentration in excreta) × (P or protein concentration in excreta ÷ P or protein concentration in feed)].

Statistical Analysis

Two-way analysis of variance was employed to determine the main effects (different basal diets and Ca) and their interaction by using Proc

Anova (14) and separation of means was by Duncan's multiple range test ($P<0.05$).

RESULTS

Body Weight

Significant main effects of dietary additions were observed for 21-d body weight. Generally, phytase addition to basal diet increased body weight of broilers at 21 d of feeding (Table 2). Subsequent addition of citric acid significantly increased body weight and was sufficient to increase body weight to values that did not differ from those found in positive control diet. Subsequent supplementation of ascorbic acid and vitamin D_3 to diets that contained phytase and citric acid did not increase body weight above level attained with phytase and citric acid. The body weight of chicks that received the positive control diet was similar to those that received the phytase and organic acids.

Feed Intake

Significant main effects of dietary additions and Ca level were observed for feed intake (Table 2). In comparison to the positive control, all the diets resulted in lower feed consumption. The greatest increase in feed intake occurred in birds that were fed with the positive control diet and 0.90% dietary Ca.

Feed Conversion Ratio

For the whole experimental period, feed conversion ratio was influenced by dietary additions and dietary Ca (Table 2). Conversion ratio of feeds that contained 0.79% dietary Ca were superior to those containing 0.90% Ca (1.52 vs 1.58 g g^{-1}). Addition of phytase was not sufficient to decrease feed conversion ratio to values that differ from those found in negative control diets. However, chicks fed diets supplemented with phytase and organic acids were significantly ($P<0.05$) more efficient (1.48 g g^{-1}) than chicks fed no dietary supplements (1.60 g g^{-1}).

Table 2. Performance of broilers fed corn-soybean meal-based diets with different dietary additions (phytase, citric acid, ascorbic acid and vitamin D_3) and different calcium concentrations from 1 to 21 d of age.

	Calcium (%)								
	Body weight (g) 21 d			Feed intake 1-21 d g b^{-1} d^{-1}			Feed conversion ratio 1-21 d		
Supplements to basal diet	Total	0.79	0.90	Total	0.79	0.90	Total	0.79	0.90
(0.315% AP)[1]		468	466		33.7b	34.8a		1.52b	1.58a
None	430c	437	424	32.7b	32.8	32.5	1.60a	1.58	1.62
PHYT	461b	461	460	34.4b	34.7	34.1	1.57ab	1.58	1.56
PHYT+ CA	474ab	476	472	34.3b	33.1	35.4	1.53ab	1.47	1.58
PHYT+ CA+ AA	474ab	474	474	33.3b	33.4	33.3	1.48b	1.48	1.48
PHYT+CA+AA+VIT D_3	458b	453	463	34.1b	32.9	35.2	1.56ab	1.53	1.599
0.135% AP	502a	504	500	36.9a	35.2	38.4	1.55ab	1.47	1.62
Model	P>F			P>F			P>F		
Dietary addition (Da)	**			**			0.17		
Calcium (Ca)	0.81			**			**		
Da x Ca	0.97			0.15			0.42		

*, ** Indicate significance ($P<0.05$ and $P<0.01$, respectively) for main effects and interactions.
abcd Different letters beside mean values indicate significant differences between mean values
[1]Supplements were the following: PHYT = phytase, 500 units (FYT) kg^{-1}; CA = citric acid, 20 g kg^{-1}, AA = ascorbic acid, 200 mg kg^{-1}, VIT D_3 = vitamin D_3, 200 µg kg^{-1}

Table 3. Effect of different dietary addition (phytase, citric acid, ascorbic acid and vitamin D_3) and different calcium concentration on the percentage of tibia ash and P and protein retention of broilers aged 21 d.

	Calcium (%)								
		Tibia ash %			Phosphorus retention%			Protein retention %	
Supplements to basal diet	Total	0.79	0.90	Total	0.79	0.90	Total	0.79	0.90
(0.315% AP)[1]		48.0	48.7		49.3	50.5		65.6b	68.8a
None	42.5c	43.8	41.3	37.7c	34.4de	40.9d	67.2ab	67.1	67.2
PHYT	47.6b	47.7	47.6	52.8b	49.1c	56.9abc	70.1a	67.0	73.1
PHYT+ CA	46.7b	46.2	47.3	54.9ab	51.9bc	57.9ab	68.7a	68.3	69.1
PHYT+ CA+ AA	46.9b	46.1	47.8	59.3a	62.0a	57.0abc	67.9ab	67.4	68.4
PHYT+CA+AA+VIT D_3	54.8a	52.2	57.5	59.1a	58.0ab	60.1ab	66.4ab	64.7	68.1
0.135% AP	51.5a	52.2	50.8	35.4c	40.4d	30.4e	63.1b	59.3	67.0
Model	P>F			P>F			P>F		
Dietary addition (Da)	**			**			0.06		
Calcium (Ca)	0.55			0.42			**		
Da x Ca	0.41			**			0.41		

*, ** Indicate significance ($P<0.05$ and $P<0.01$, respectively) for main effects and interactions.
abcd Different letters beside mean values indicate significant differences between mean values.
[1]Supplements were the following: PHYT = phytase, 500 units (FYT) kg^{-1}; CA = citric acid, 20 g kg^{-1}, AA = ascorbic acid, 200 mg kg^{-1}, VIT D_3 = vitamin D_3, 200 µg kg^{-1}.

Tibia Ash

The influence of dietary additions on tibia ash percentage was significant (Table 3). Over the whole experimental period, phytase addition increased tibia ash of experimental birds (47.6 vs. 42.5%). Subsequent organic acid supplementation to diet containing phytase did not further improve tibia ash over those observed in chicks fed diets supplemented with phytase alone. The greatest increases in tibia ash were observed in broilers consuming feeds that contained vitamin D_3 and all supplements. The percentage of ash in tibia of birds fed the diet of all dietary additions did not differ significantly from those found in birds fed the positive control diet.

Phosphorus and Protein Retention

There were significant ($P<0.05$) influences of dietary treatment main effects and interaction effects on P retention (Table 3). The lowest P retention was found in birds fed either the positive (35.4%) or the negative (37.7%) control diets. Phytase proved to be the most efficient in increasing P retention (40%) over negative control diet, whereas simultaneous addition of phytase and citric acid and ascorbic acid increased P retention of chicks 12% above levels attained with phytase fed as the sole supplement. Subsequent vitamin D_3 supplementation in diets containing phytase and organic acids did not further improve P retention over those observed in chicks fed diets supplemented with phytase and organic acids.

Each of the dietary supplements significantly increased the mean P retention percentage in both levels of Ca. At 0.79% dietary Ca, the percentage of P retention of birds consuming organic acids and all dietary additions were significantly superior to values found in birds fed diets supplemented with phytase and citric acid, with citric acid or with phytase alone. Increases in the level of dietary Ca, however, reduced the gap between P retention of birds fed diets with all dietary additions and those fed diets supplemented with phytase alone. Increases in the level of dietary Ca reduced the P retention in those fed positive control diet containing 0.44% available P.

There were significant dietary additions and dietary Ca main effects on the amount of protein retention (Table 3). Addition of phytase was sufficient to increase protein retention to values that significantly differ from those found in positive control diets (70.1 vs. 63.1%). Subsequent addition of organic acids and vitamin D_3 to diet containing phytase did not further improve protein retention over those observed in chicks fed diet supplemented with phytase alone. However, chicks fed diet supplemented only with phytase were more efficient in protein retention (70.1%) than chicks fed no dietary supplements (67.2%).

Protein retention was significantly ($P<0.05$) altered by dietary Ca level. The influence of Ca level on protein retention tended to parallel that of feed intake. The overall mean of values of protein retention were higher in birds consuming 0.9% dietary Ca than in those fed 0.79% dietary Ca (Table 3).

DISCUSSION

Over 65% of the total P in corn and soybean meal is bound to phytic acid, the utilization of which is influenced by a variety of factors (19). In animal experiments, dietary phytase activity levels suggested for maximum phytate-P utilization vary widely. These discrepancies may partially be due to variations in dietary factors that influence the efficacy of phytase. The level of dietary Ca is a key factor influencing the performance of broiler chicks fed diets supplemented with phytase. Ballam *et al.* (2) showed that phytate hydrolysis is influenced by Ca and non-phytate P levels and also that 15% inclusion of wheat bran to corn-based diets does not influence the process. The present study showed that, depending on the level of Ca used in the basal diet there would be a varied increase in feed intake, but a "constant" increase in body weight, resulting in varied feed conversion. As there was a proportionally higher feed intake than growth rate of 0.90% as compared to 0.79% dietary Ca that resulted in significantly higher feed conversion for 0.90% dietary Ca. The differences in feed conversion ratio determined for the respective dietary treatments suggest that increasing dietary Ca from 0.79 to 0.90% reduces limitations on feed intake, reducing

the broilers need to be efficient in extracting nutrients to achieve an equal growth rate. We cannot as yet determine how this increase in palatability is achieved or why it was so different between the two levels of Ca tested here. Also, increasing dietary Ca from 0.79 to 0.90%, positively influenced protein retention. This type of increase could be due to the increase in P availability as corn feeds enriched with phytase. It seems, however, that phytase by promoting dephosphorylation, supplied additional available P that was balanced by a relatively high Ca concentration and thereby decreased the pool of minerals forming insoluble protein-mineral-phytate complexes. It has been suggested that phytate complexes with protein because of the presence of divalent cations. These cations, usually Ca, Mg or Zn, act as a bridge between negatively charged protein carboxyl groups and the phytate (18). Although, there were no significant differences in 21 d body weight of broilers fed two levels of Ca, this resulted in a significantly higher feed conversion for birds fed 0.90% Ca compared to those fed 0.79% Ca level.

In the current study, the addition of phytase to diet low in P significantly improved body weight, tibia ash and P and protein retentions. This result would be expected, because there was more phytate-bound P present in this diet for responding to the phytase supplementation. Microbial phytase is more efficacious in diets with no or low level of inorganic P supplementation in both swine and poultry (5, 21, 13). Body weight gain and bone ash proportion were found to be sensitive criteria for the assessment of P availability (21). The improvement in the digestibility of protein by addition of phytase was due to the purported ability of phytate P to bind certain gut proteases (20). Decreased feed intake resulting from low levels of available P in diets fed to poultry is a well-known phenomenon (12). In the current study, however, this effect was partly compensated for by enhanced feed efficiency in chicks fed diets supplemented with phytase and organic acids.

Interestingly, certain compounds with chelating capacity have been shown to increase mineral availability when included in plant-based diets fed to animals. Organic acids (citric and ascorbic acids) are often added to diets to improve performance. In the current study, chickens fed diets

enriched with citric acid along with phytase showed 10 and 9% increase in body weight and protein retention above the level attained with the negative and positive control diets, respectively and also was sufficient to increase body weight to values that did not differ from those found in positive control diet. It seems that acidifying the diet by citric acid increased the solubility of phytate, a phenomenon known to occur during seed germination (8) and provided a better environment for the multiple microbial phytase to carry out its function. Boling *et al.* (3) suggested that dietary citric acid improves phytate P utilization in chicks fed phytate containing corn-SBM (soybean meal) diets. Citric acid may have slowed gastric emptying, which would mean an increased transit time through the gastrointestinal tract and thus allow more time for Ca absorption. In addition, Ca has a greater apparent absorption in acidic environments. The lowered pH of the gastrointestinal digesta may also have provided a better environment for endogenous enzymes to work, thus leaving less substrate for Ca to bind with and forming insoluble salts that are not easily absorbed (11).

In the present study, ascorbic acid addition to feeds with 0.79% Ca along with phytase and citric acid increased amount of P retention 26% above level observed in birds fed phytase as the sole supplement. However, the effect was not evident at the highest dietary Ca level. Also addition of ascorbic acid along with phytase and citric acid, markedly improved feed conversion ratio (8%) as compared to negative control. Although, the mode of interaction between phytase and organic acids are unclear, Han *et al.* (9) have suggested two possibilities. First, the organic acids may enhance total P absorption by increasing the solubility of digesta P and prolonging the transit time of digesta in small intestine (11). Second, the organic acids may help provide a better pH environment for phytase to function by acidifying the diets and digesta. Edwards (6) reported that addition of ascorbic acid to the diet resulted in increased duodenal Ca-binding protein and plasma 1, 25 dihydroxycholecalciferol. Therefore, these compounds may improve the apparent efficacy of supplemental dietary phytase by increasing Ca and P uptake from the gut.

Addition of the vitamin D_3 to feeds containing phytase and organic acids, produced an 18% increase in tibia ash above the level observed in

birds fed phytase as the sole supplement. Plausibly, vitamin D_3 may improve the apparent efficacy of supplemental dietary phytase by increasing Ca uptake from the gut, thereby facilitating utilization of digested P by increasing its transport rate and/or by increasing the solubility of phytate in the small intestine and then its accessibility to phytase. Vitamin D has been shown to induce intestinal mucosal phytase activity in chicks (4).

CONCLUSIONS

Data presented clearly indicated that supplementation of corn-soybean low-P meal-based diet with appropriate concentrations of phytase, citric acid, ascorbic acid and vitamin D_3, leads to substantial increases in broiler performance and nutrient digestibility and would have an environmental benefit of reducing phosphorus and nitrogen concentration of broiler manure.

ACKNOWLEDGEMENTS

This study is a part of the senior author's Ph.D. dissertation. The authors would like to thank Isfahan University of Technology for financial support.

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