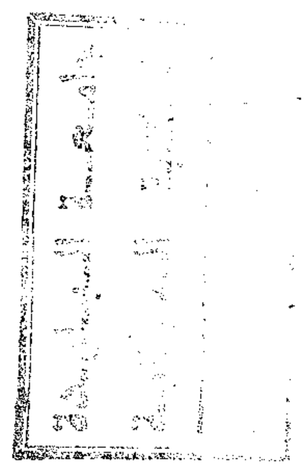

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Enhancement of phytate phosphorus availability in the diets of commercial broilers and layers

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Abstract

A reference diet (10.0 g Ca and 4.5 g nonphytate phosphorus, NPP kg^{-1}) and two basal diets (basal diet A, 10.0 g Ca and 3.0 g NPP and basal diet B, 7.5 g Ca and 3.0 g NPP kg^{-1}) with or without additives were fed to broilers from 3 to 30 days of age. Phytase (500 and 500 phytase units, kg^{-1}), cholecalciferol (100 or 200 μg kg^{-1}), yeast culture (13.5×10^9 *Saccharomyces cerevisiae* cells kg^{-1}) were supplemented to basal diet A and phytase (250 phytase units kg^{-1}) or cholecalciferol (100 or 200 μg kg^{-1}) were added to basal diet B. Phytase supplementation to basal diet A significantly ($P \leq 0.05$) improved weight gain compared to unsupplemented basal diet A. Supplementation of either phytase or cholecalciferol to basal diet B resulted in comparable weight gain and feed efficiency as those fed the reference diet. Tibia ash content was not improved when the above supplements were added to basal diets compared to reference diet fed birds. However, supplementation of cholecalciferol at 200 μg kg^{-1} basal diet A significantly improved the tibia ash content. Highest P retention and serum inorganic P were observed, when both the basal diets were supplemented with phytase. A reference diet (36.0 g Ca and 2.0 g NPP kg^{-1}) and two basal diets (basal diet A: 36.0 g Ca and 1.0 g NPP kg^{-1} and basal diet B: 36.0 g Ca and 1.5 g NPP kg^{-1}) were prepared. No inorganic P supplement was present in basal diet A. Both the basal diets were supplemented with phytase (250 PU), cholecalciferol (200 mcg kg^{-1}) or yeast culture (13.5×10^9 *Saccharomyces cerevisiae* cells kg^{-1}). All the diets were fed to layers for 57 days from 338 to 394 days of age. Feeding of basal diet A resulted in significantly ($P \leq 0.05$) lower egg production (62.8 eggs per 100 birds per day) compared to those fed the reference diet (90.8 eggs per 100 birds per day), however, phytase supplementation to this diet improved ($P \leq 0.05$) egg production (91.0 eggs per 100 birds per day), feed per dozen eggs, egg weight, shell quality and final body weight ($P \leq 0.05$) to the level of birds fed reference diet. Cholecalciferol or yeast culture supplementation failed to improve the layer performance. Feeding of basal diet B with or without supplementation resulted in layer performance that was similar to the reference diet fed layers, suggesting that under

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this experimental conditions 1.5 g NPP kg^{-1} diet is sufficient for WL layer. Results of this study indicate that dicalcium phosphate can completely be replaced with phytase (250 phytase units kg^{-1} diet) in WL layer diet.

Keywords: Chicken; Phytate; Phytase; Cholecalciferol; Supplements

1. Introduction

Phosphorus (P) is a critical and expensive mineral in poultry nutrition. Animal protein supplements are rich in P whose availability is generally considered as 1.0 while the availability of P from vegetable protein supplements is only about 0.30 (NRC, 1994). Vegetable protein supplements are being currently used in increased quantity in place of animal protein supplements due to high cost, nonavailability and presence of *E. coli* and *Salmonella* in animal protein supplements.

The major portion of P present in cereals, cereal byproducts and vegetable protein supplements is in the form of phytic acid and its salts, Phytate. Phosphorus from phytate is poorly available to the chicken due to lack of phytase in the digestive system. Due to this, a considerable amount of P from poultry diets is being excreted into the surrounding environment. Information on the effect of supplemental phytase (Broz et al., 1994; Kornegay et al., 1994), cholecalciferol or its metabolites (Mohammed et al., 1991; Edwards, 1993) in diets is limited and variable. Two experiments were conducted to improve the bioavailability of phytate phosphorus (PP) in commercial broilers and WL layers by fortifying their diets with phytase, cholecalciferol or yeast (*Saccharomyces cerevisiae*) culture.

2. Materials and methods

Maize and soyabean meal were analyzed for calcium (Ca), total phosphorus (TP) and PP (Haugh and Lantzsch, 1983) and proximate constituents (AOAC, 1990). The nonphytate phosphorus (NPP) content of the feed ingredients was calculated by subtracting PP fraction from the TP content.

Two experiments were conducted to enhance the bioavailability of PP in commercial broilers and WL layers by supplementing the diets with phytase, cholecalciferol or yeast culture.

2.1. Experiment 1

This experiment was designed to increase the availability of PP in commercial broiler diets. A reference diet (10.0 g Ca and 4.5 g NPP kg^{-1}) and two basal diets: basal diet A, normal level of Ca and low in NPP (10.0 g Ca and 3.0 g NPP kg^{-1}) and basal diet B, low in Ca and NPP (7.5 g Ca and 3.0 g NPP kg^{-1}) were formulated (Table 1). Basal diet A was supplemented with Alltech's phytase (250 or 500 phytase units (PU) kg^{-1}), cholecalciferol (100 or 200 μg kg^{-1}) or yeast (13.5×10^9 *Saccharomyces cerevisiae* cells

Table 1
Composition of diets (g kg^{-1}), and their determined and calculated analysis, fed to commercial broiler males (3–30 d of age).

Ingredient	Reference	Basal A	Basal B
Yellow maize	609.3	611.7	619.3
Soybean meal	348.5	348.5	348.5
Common salt	4.0	4.0	4.0
Dicalcium phosphate	17.1	9.1	9.1
Oyster shell grit	15.2	20.8	13.2
DL-Methionine	2.5	2.5	2.5
Vitamin premix[a]	1.7	1.7	1.7
Trace mineral premix[b]	1.2	1.2	1.2
Coccidiostat[c]	0.5	0.5	0.5
Nutrient Composition			
Determined analysis (g kg^{-1})			
Crude protein	233.0	233.3	233.9
Total phosphorus	5.04	5.09	5.11
Nonphytate phosphorus[d]	4.52	3.02	3.03
Calcium	10.12	10.02	7.53
Calculated analysis (g kg^{-1})			
Metabolizable energy (MJ kg^{-1})	11.81	11.84	11.95
Lysine	12.53	12.54	12.56
Methionine	6.22	6.22	6.24
Methionine+Cystine	9.67	9.68	9.70
Threonine	8.29	8.29	8.31

[a] Vitamin premix provided (mg kg^{-1} diet): thiamine, 1; pyridoxine, 2; cyanocobalamine, 0.01; niacin, 1.50; pantothenic acid, 10; tocopherol, 10; riboflavin, 5; menadione, 1; retinol acetate, 8250 IU; cholecalciferol, 1200 ICU; choline, 650.
[b] Trace mineral premix provided (mg kg^{-1} diet): zinc, 80; manganese, 90; iron, 60; copper, 5.
[c] Coban TM (monensin sodium 0.10 w/w).
[d] Based on analysed values of feed ingredients.

kg^{-1}). Whereas, basal diet B was supplemented with phytase (500 phytase units kg^{-1}) or cholecalciferol (100 or 200 μg kg^{-1}). Levels of dicalcium phosphate (DCP), oyster shell grit and maize were adjusted to obtain the desired levels of NPP and Ca. Metabolizable energy, crude protein and essential amino acid contents were kept constant (NRC, 1994) in all the experimental diets.

Three hundred fifty two Hubbard day old male broiler chicks were distributed equally at random into 44 electrically heated wire floor galvanized iron battery brooder cells. During the initial 2 days, chicks were fed on ground yellow maize only.

Each diet was fed ad libitum to four replicate groups of eight chicks each, from 3 to 30 days of age. Uniform and standard managemental practices were followed during the course of experiment. Individual body weight, feed intake of each replicate, leg abnormality score (Watson et al., 1970) of individual birds were recorded at weekly interval through 30 days of age. Feed conversion ratio (FCR) was calculated at weekly intervals. Blood samples were collected from three birds per replicate on day 31 of age. Sera was pooled for each replicate and analyzed for Ca (AOAC, 1990) and inorganic P (Fiske and Subba Row, 1925) content.

A metabolism trial of 3-day duration was conducted during 31–33 days of age. Three birds per replicate were selected at random and were housed in raised wire floor battery brooders. Records of feed intake, excreta voided and dry matter content of feed and excreta were recorded. The samples of feed and excreta were analyzed for TP. The P retention was expressed as proportion of P retained per unit weight of P intake.

On day 31 of age, three birds per replicate were selected at random and slaughtered by cervical dislocation.

Both tibia were freed of soft tissue including the diaphysis and defattened by soaking in petroleum ether for 48 h. Dried bone samples were ashed at 600 ± 30°C for 12 h. Bone ash and Ca and P content of the tibia ash were analyzed (AOAC, 1990).

2.2. *Experiment 2*

Utilization of PP from layer diets was studied by employing Alltech's phytase (250 phytase units kg^{-1}), cholecalciferol and yeast culture supplemented diets fed to Single Combed White Leghorn hens. Basal diet A contained no supplemental inorganic P (1.05 g NPP kg^{-1}), while basal diet B contained 1.5 g NPP kg^{-1}, lower than the reference diet (2.0 g NPP kg^{-1}). Calcium level was maintained at 36.0 g kg^{-1} in all the diets (Table 2). Both basal diets were supplemented with phytase (250 phytase units/kg), cholecalciferol (200 μg kg^{-1}) or yeast culture (13.5×10^9 *Saccharomyces cerevisiae* cells kg^{-1} diet). Dicalcium phosphate, oyster shell grit and sawdust were added to a basal mixture of maize and soyabean meal to arrive at the desired levels of Ca and NPP. Sawdust was soaked in water for 2 h to remove any water soluble nutrients and extraneous contaminants and it was sun dried before incorporating in the diets. Each diet was fed ad libitum to five groups of six layers maintained in individual cages from 338 to 394 days of age.

Light was provided for 17 h daily. Hen day egg production, body weight, and FCR were recorded and compiled for every 14 day period. All the eggs that were produced during the last 3 consecutive days at every 14 day period were collected to measure egg weight, shell thickness with Dial Thickness Gage (Mitutoto, No. 7301) and shell content. At the end of experiment, about 5 ml blood was drawn out from the left jugular vein from each bird, three birds per replicate and sera was pooled for each replicate. The Ca (AOAC, 1990) and inorganic P (Fiske and Subba Row, 1925) contents of sera were analyzed.

The experimental results were subjected to statistical analysis (Snedecor and Cochran, 1980) by using randomized block design and the differences between the treatment means were compared with Duncan's multiple range test (Duncan, 1955).

3. Results and discussion

Calcium (Ca), total phosphorus (TP) and phytate phosphorus (PP) content of different feed ingredients and mineral sources are presented in Table 3. The NPP content of maize and soyabean meal were 0.71 and 1.79 g kg^{-1}, respectively.

Table 2
Composition of diets (g kg^{-1}), and their determined and calculated analysis, fed to commercial WL layers (335–377 d of age).

Ingredient	Reference	Basal A	Basal B
Yellow maize	704.75	704.75	704.75
Soybean meal	180.0	180.0	180.0
Common salt	4.0	4.0	4.0
Dicalcium phosphate	4.9	0.0	2.18
Oyster shell grit	104.0	107.5	106
Saw dust[c]	–	1.4	0.72
DL-Methionine	1.0	1.0	1.0
Vitamin premix[a]	0.35	0.35	0.35
Trace mineral premix[b]	1.0	1.0	1.0
Nutrient Composition			
Determined analysis (g kg^{-1})			
Crude protein	159.14	160.08	159.98
Total phosphorus	4.27	3.34	3.75
Nonphytate phosphorus[d]	2.00	1.08	1.56
Calcium	36.21	36.08	36.12
Calculated analysis (g kg^{-1})			
Metabolizable energy (MJ kg^{-1})	11.72	11.72	11.72
Lysine	7.92	7.92	7.92
Methionine	3.52	3.52	3.52
Methionine + Cystine	6.45	6.45	6.45
Threonine	6.08	6.08	6.08

[a] Vitamin premix provided (mg kg^{-1} diet): thiamine, 1; pyridoxine, 2; cyanocobalamine, 0.01; niacin, 1.50; pantothenic acid,10; tocopherol, 10; riboflavin, 5; menadione, 1; retinol acetate, 8250 U; cholecalciferol, 1200 ICU; choline, 650.
[b] Trace mineral premix provided (mg kg^{-1} diet): zinc, 104; manganese, 108; iron, 40; copper, 4; iodine, 4.
[c] Water soaked and sun dried.
[d] Based on analysed values of feed ingredients.

3.1. Experiment 1

The effect of feeding broilers with diets containing varying levels of Ca and NPP with or without supplementation of various levels of phytase, cholecalciferol, or yeast is presented in Table 4.

Table 3
Analyzed chemical composition (g kg^{-1} dry matter) of feed ingredients

Feed ingredient	Calcium	Total phosphorus	Phytate Phosphorus
Yellow maize	2.02	2.67	1.96
Soybean meal	4.41	5.81	4.02
Dicalcium phosphate	210.14	193.10	0.00
Oyster shell	330.00	0.00	0.00

Table 4
Performance of commercial male broilers (3–30 d of age), fed low phosphorus diets supplemented with phytase, cholecalciferol or yeast (*Saccharomyces cerevisiae*) culture.

Treatment	Weight gain (g)	Feed intake (g/b/d)	Feed conversion ratio	Tibia ash (g/kg^{-1})	Ca in tibia ash (g/kg^{-1})	P in tibia ash (g/kg^{-1})	Serum Ca ($mg\ dl^{-1}$)	Serum Pi ($mg\ dl^{-1}$)	P retention (g/g)
Ref. diet (10.12 g Ca and 4.52 g NPP/kg diet)	1105^{f}	63.2^{ef}	1.603^{ab}	467^{c}	365	182.2	11.49	5.64^{d}	0.220^{a}
Basal diet A (10.02 g Ca and 3.02 g NPP/kg diet)	914^{ab}	55.8^{abc}	1.716^{b}	422^{ab}	360	177.1	12.06	3.31^{ab}	0.221^{a}
Basal diet A + phytase[a]	991^{cd}	59.4^{bcde}	1.677^{ab}	434^{b}	362	178.2	12.33	3.27^{ab}	0.495^{ef}
Basal diet A + phytase[b]	1003^{cd}	60.1^{cdef}	1.555^{a}	430^{b}	376	181.6	11.61	4.43^{bcd}	0.537^{g}
Basal diet A + cholecalciferol[c]	901^{a}	53.2^{a}	1.654^{ab}	398^{a}	355	175.5	12.10	3.19^{ab}	0.428^{c}
Basal diet A + cholecalciferol[d]	910^{ab}	54.5^{ab}	1.677^{ab}	445^{bc}	361	177.9	11.75	3.92^{abc}	0.441^{cd}
Basal diet A + yeast[e]	961^{bc}	57.9^{abcd}	1.687^{ab}	422^{ab}	373	175.0	11.72	2.80^{a}	0.451^{d}
Basal diet B (7.53 g Ca and 3.03 g NPP/kg diet)	1030^{de}	58.5^{bcde}	1.589^{ab}	424^{b}	363	177.8	11.78	3.56^{ab}	0.410^{b}
Basal diet B + phytase[b]	1081^{ef}	63.9^{f}	1.655^{ab}	431^{b}	364	183.3	12.27	5.04^{cd}	0.652^{h}
Basal diet B + cholecalciferol[c]	1065^{ef}	61.3^{def}	1.624^{ab}	430^{b}	365	179.7	11.71	4.12^{bc}	0.426^{c}
Basal diet B + cholecalciferol[d]	1042^{de}	60.1^{cdef}	1.616^{ab}	424^{b}	368	178.8	12.02	3.64^{ab}	0.439^{cd}
SEM (±)	20.02	1.59	0.0446	8.6	5.3	4.10	0.523	0.403	0.00502

Means within a column having different superscripts are significantly ($P \leq 0.05$) different.
[a] Phytase contained 250 PU kg^{-1}.
[b] Phytase contained 500 PU kg^{-1}.
[c] Cholecalciferol contained 100 μg kg^{-1}.
[d] Cholecalciferol cotained 200 μg kg^{-1}.
[e] Yeast contained 13.5×10^{9} *Saccharomyces cerevisiae* cells kg^{-1} diet.

Reduction in NPP content of a broiler diet from 4.5 to 3.0 g kg^{-1} depressed ($P < 0.05$) weight gain by 17.3% with Ca level maintained at 10.0 g kg^{-1} (Ca : NPP, 3.33 : 1.0) but only by 6.8% with Ca level at 7.5 g kg^{-1} (Ca : NPP, 2.5 : 1.0). It is evident that maintaining Ca : NPP ratio close to 2 : 1 results in better performance of broilers. Supplementation of basal diet A (Ca 10 and NPP 3.0 g kg^{-1}) with phytase either at 250 or 500 PU kg^{-1} increased weight gains of chicks. Supplementation of basal diet A with yeast or cholecalciferol was found ineffective in influencing the weight gain of broilers. The improvement in growth performance observed in the chicks fed phytase might be due to, (1) the release of P from the phytate- mineral complex (Qian et al., 1996; Sebastian et al., 1996), (2) the utilization of inositol by animals as suggested by Simons et al. (1990), (3) increased starch digestibility (Knuckles and Betschart, 1987) and or (4) increased utilization of protein (Farrell et al., 1993).

Supplementation of basal diet B (Ca 7.5 g and NPP 3.0 g kg^{-1}) with phytase (500 phytase units kg^{-1}) or cholecalciferol (100 μg kg^{-1} diet) improved the growth of broilers (1081 and 1065 g, respectively) to be comparable to that of the reference diet fed group (1105 g). Basal diet B, however, resulted in significantly higher weight gain compared to those fed basal diet A. It implies that phytase supplementation at higher dietary Ca (Basal diet A) was inadequate to greatly improve Ca : P ratio. The effect of cholecalciferol was found only at narrower Ca : NPP (basal diet B). The decreased effect of phytase or cholecalciferol as the Ca : P ratio became wider may be due to that (1) the extra Ca binds with phytate to form an insoluble complex penta–calcium–phytate (Mellanby, 1949) that is less accessible to phytase, (2) the extra calcium probably more importantly, could directly repress phytase activity (Pointillart et al., 1985), (3) it may be possible that the higher content of Ca in the low P diet increased the intestinal pH and reduce the soluble fraction of minerals, consequently reducing their availability for absorption (Shafey, 1993).

Cholecalciferol supplementation (200 μg kg^{-1}) to basal diet A (Ca : NPP, 3.33 : 1) was not effective in improving the weight gain of broilers. However, when it was supplemented to basal diet B (Ca : NPP, 2.5 : 1), weight gain was improved, indicating that cholecalciferol was ineffective in improving the weight gain of broilers fed the diet having a wider Ca : P ratio. Mohammed et al. (1991) observed increased phytate digestibility when cholecalciferol was supplemented to a diet low in Ca and P (5.0 g and 2.6 g NPP kg^{-1}, respectively). This improved phytate digestibility observed may be due to increased intestinal phytase activity with cholecalciferol supplementation to low P (1.6 g NPP kg^{-1}) diet (Waldroup et al., 1964; Davies et al., 1970) and also may be due to increased Ca utilization (Edwards et al., 1992) thereby preventing the formation of insoluble Ca phytate complex which is not accessible for phytase activity.

Feed consumption was significantly ($P \leq 0.05$) reduced when birds were fed basal diet A compared to the reference diet. When basal diet A was supplemented with phytase, there was a tendency for the feed consumption to increase and was similar to the level of the reference diet, possibly due to improved growth. Supplementation of cholecalciferol or yeast to basal diet A did not influence feed consumption. Feed intake was unaltered by feeding basal diet B with or without supplementation of phytase, yeast or cholecalciferol compared to the reference diet. Feed efficiency was significantly improved when basal diet A was supplemented with phytase (500 phytase units kg^{-1}), but it was same in all

other treatments. The increased phytate utilization might have improved overall utilization of the diet (Miles and Nelson, 1974). Similar increase in feed intake, when low P broiler diet was supplemented with phytase was also reported by several investigators (Farrell et al., 1993; Broz et al., 1994; Cantor et al., 1994; Kornegay et al., 1994).

Tibia ash content was significantly ($P \leq 0.05$) lower in chicks fed basal diet A or B (422 and 424 g kg^{-1}, respectively) compared to the reference diet (467 g kg^{-1}). Among various supplements tested only cholecalciferol supplementation at the rate of 200 μg kg^{-1} to basal diet A increased tibia ash content (445 g kg^{-1}) similar to the reference diet fed group. The improved bone mineralization may be attributed to one or more of the following mechanisms (1) increased synthesis or activity of intestinal phytase (Pointillart et al., 1985; Shafey et al., 1991), (2) increased phytate hydrolysis (Mohammed et al., 1991) with enhanced absorption of P (Wasserman and Taylor, 1973; Tanaka and De Luca, 1974). Phytase supplementation to basal diets improved tibia ash content compared to the respective unsupplemented basal diets, which might be due to liberation of inorganic P and Ca from the phytate molecule by supplemental enzyme. Calcium and P content of tibia ash was not significantly influenced by the treatments employed.

Dietary Ca and NPP content had no effect on serum Ca levels. Serum inorganic P content, however, was significantly ($P \leq 0.05$) reduced in groups fed the basal diets compared to those fed the reference diet. The low serum P levels resulting from low dietary P intake were increased ($P \leq 0.05$) similar to the level of the reference diet fed group when phytase was supplemented (500 PU kg^{-1}) to both basal diets. Yeast or cholecalciferol supplementation did not elicit any effect on serum P content.

Phosphorus retention was not influenced by lowering dietary P from 4.5 to 3.0 g kg^{-1} (basal diet A), whereas, reducing both dietary Ca and P level from 10.0 to 7.5 and 4.5 to 3.0 g kg^{-1} diet, respectively, enhanced P retention from 0.221 to 0.410. This agrees with the findings of Ballam et al. (1985) and Mohammed et al. (1991) who observed higher phytate hydrolysis in the broiler diets containing lower levels of Ca.

Phosphorus retention was increased from 0.221 to 0.537 and 0.410 to 0.652, respectively, when phytase (500 PU kg^{-1}) was supplemented to basal diet A and B, respectively. The increase in P availability and feed intake might be responsible for higher body weight gain with phytase supplementation. These findings are in agreement with those of other investigators (Schoner et al., 1993; Broz et al., 1994; Kornegay et al., 1994; Yi et al., 1994) who reported decreased PP or P excretion when diets were supplemented with phytase.

The results of this experiment indicated that the phytase supplementation to a practical broiler diet containing low Ca and NPP (7.5 and 3.0 g kg^{-1}, respectively) would allow the reduction of the usual addition of inorganic phosphate in the diet and also reduce the amount of P excreted in to the environment. Cholecalciferol (100 μg kg^{-1}) can also be used as an alternative to phytase to improve PP availability in low Ca and NPP commercial broiler diet.

3.2. Experiment 2

Effect of phytase, cholecalciferol or yeast on the performance of WL layers is summarized in Table 5. Feeding basal diet A resulted in a significant ($P \leq 0.05$)

Table 5
Performance of WL layers (335–384 d of age), fed low P diets supplemented with phytase, cholecalciferol or yeast (*Saccharomyces cerevisiae*).

Treatment	Hen day egg production (eggs/100 birds per day)	Feed intake (g/b/d)	Feed (kg)/ 12 eggs	Egg weight (g)	Shell weight (g kg^{-1} egg)	Shell thickness (mm)	Final body weight (g)
Ref. Diet (36.21 g Ca and 2.00 g NPP/kg)	90.8^{cd}	115.7^{e}	1.535^{a}	60.7^{fg}	93.6^{bcd}	0.372^{de}	1559^{c}
Basal diet A (36.08 g Ca and 1.08 g NPP/kg)	62.8^{a}	99.6^{ab}	1.933^{b}	56.8^{bc}	88.1^{a}	0.342^{b}	1361^{ab}
Basal diet A + phytase[a]	91.0^{cd}	111.8^{d}	1.476^{a}	59.8^{efg}	96.6^{d}	0.389^{f}	1557^{c}
Basal diet A + cholecalciferol	64.6^{ab}	101.4^{b}	1.930^{b}	51.1^{a}	87.5^{a}	0.316^{a}	1276^{a}
Basal diet A + yeast	71.4^{b}	97.4^{a}	1.634^{ab}	56.5^{b}	89.9^{ab}	0.361^{cd}	1369^{ab}
Basal diet B (36.12 g Ca and 1.56 g NPP/kg)	87.3^{cd}	113.4^{de}	1.561^{a}	58.5^{cde}	91.2^{abc}	0.364^{cd}	1464^{bc}
Basal diet B + phytase[a]	94.4^{d}	114.2^{de}	1.469^{a}	61.3^{g}	94.4^{cd}	0.383^{ef}	1521^{c}
Basal diet B + cholecalciferol[b]	84.2^{c}	101.9^{b}	1.467^{a}	59.1^{def}	88.8^{a}	0.362^{cd}	1351^{ab}
Basal diet B + yeast[c]	85.9^{c}	105.8^{c}	1.483^{a}	57.5^{bcd}	93.7^{cd}	0.378^{ef}	1434^{bc}
SEM (+)	0.81	0.95	0.1335	0.91	6.01	0.027	11.1

Means within a column having different superscripts are significantly ($P \leq 0.05$) different.
[a] Phytase contained 250 PU kg^{-1}.
[b] Cholecalciferol contained 200 μg kg^{-1}.
[c] Yeast contained 13.5×10^{9} *Saccharomyces cerevisiae* cell kg^{-1} diet.

reduction in egg production compared to the reference diet fed birds, indicating that the plant P from a corn-soya diet is not sufficient for layers. This observation is in corroboration with that of Owings et al. (1977) who observed significant drop in egg production within 4 weeks of commencement of feeding low P diets. Whereas, Davidson and Boyne (1970) reported that layers on all plant P (3.4 g kg^{-1} diet) maintained egg production similar to layers fed supplemental inorganic P. These workers used oats, barley and wheat in the diets as a source of energy which are known to contain higher levels of endogenus phytase (Eeckhout and DePaepe, 1994), which enhances the availability of PP. Due to the presence of inherent phytase, the availability of P from phytate might be more thereby meeting the requirements of NPP without inorganic P supplementation.

Egg production (87.3 eggs per 100 birds per day) of layers on basal diet B (1.5 g NPP kg^{-1}) was similar to those fed the reference diet (90.8 eggs per 100 birds per day), indicating that 1.5 g NPP kg^{-1} diet was sufficient for WL layers. Waldroup et al. (1967) and Vogt (1992) also obtained better egg production at 1.6 g NPP kg^{-1} diet when compared to 2.8 and 3.2 g NPP kg^{-1} diet, respectively. Supplementation of basal diet A with cholecalciferol or yeast culture failed to improve egg production to the level of the reference diet, whereas, supplementation of phytase significantly improved egg production comparable to that of the reference diet. Improved egg production with phytase supplementation to a low P diet (3.7 g kg^{-1}) was also reported by Schoner et al. (1993); Gordon and Roland (1998). Appreciable improvement in egg production was observed with phytase supplementation to basal diet B. Addition of cholecalciferol or yeast to basal diet B did not improve the egg production over basal diet B fed birds. The proportion of defective and/or shelless eggs were more in layers fed basal diet A (0.103) compared to that on basal diet B (0.021) and reference diet (0).

Feed intake followed the trend of egg production. All the treatments that produced higher egg production consumed significantly ($P \leq 0.05$) more feed compared to other groups. Supplementation of basal diet A with phytase resulted in improved feed intake similar to the reference diet fed layers. The improved feed intake by supplementing phytase in layer diet was also observed by Schoner et al. (1991); Huyghebaert et al. (1992); Farrell et al. (1993); Broz et al. (1994); Cantor et al. (1994); Kornegay et al. (1994). Feed efficiency was significantly ($P \leq 0.05$) poorer for groups fed basal diet A and basal diet A plus cholecalciferol, however, supplementation of this diet with phytase or yeast improved feed efficiency, to be comparable to the reference diet fed group. Feed efficiency of layers fed basal diet B was similar to those fed the reference diet. Supplementation of basal diet B with phytase, cholecalciferol or yeast did not influence feed conversion efficiency.

Egg weights were significantly ($P \leq 0.05$) decreased in groups fed either basal diet A or B. However, supplementation of both basal diets with phytase or basal diet A with cholecalciferol improved egg weight similar to those fed the reference diet.

Shell content and shell thickness were significantly ($P \leq 0.05$) inferior in the birds fed basal diet A, but the shell quality of layers fed basal diet B was comparable to that the reference diet. Supplementation of phytase or yeast to basal diet A improved both, the shell content and shell thickness to the level of the reference group, but other supplements were not effective in influencing the shell quality traits.

Body weights were significantly ($P \leq 0.05$) lower with basal diet A and were improved to the level of the reference diet by addition of phytase, but not with cholecalciferol or yeast supplementation. The basal diet B with or without supplementation of phytase or yeast resulted in a comparable body weight with that of reference diet. The improved performance of layers fed phytase supplemented diet may be due to increased P retention as observed in the broiler experiment and also due to increased feed.

Results of the this experiment indicate that phytase (250 phytase units kg^{-1}) supplementation to maize-soya layer diet eliminates inorganic P supplementation without affecting layer performance.

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