

Influence of calcium and non-phytate phosphorus deficient diets with phytase on the performance of broilers, serum concentrations of minerals and the activity of alanin transaminase, aspartate transaminase and lactate dehydrogenase

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Abstract: An experiment was conducted to study the effect of microbial phytase (phyzyme XP5000G) supplementation in broiler chicks' diets on the performance, serum total protein (TP), minerals (Ca, Mg and P) and the serum enzyme activities (AST, ALT, LDH). A reference diet adequate in calcium and non-phytate phosphorus (10.0 gkg^{-1} Ca and 5 gkg^{-1} nPP) and two deficient diets in Ca and nPP (8.5 gkg^{-1} Ca and 3.5 gkg^{-1} nPP, and 7.5 gkg^{-1} Ca and 2.5 gkg^{-1} nPP) with or without phytase (0 and 100 mgkg^{-1}) were offered to broiler chicks from 1 to 21 days of age. Although the low-nPP diets had no significant effect on body weight gain (BWG) of chicks ($p>0.05$) They increased ($p<0.01$) feed intake (FI) and feed conversion ratio (FCR) when compared to the low-nPP diet supplemented with enzyme. Phytase had a favorable effect, although non-significantly, on BWG of chicks fed very low level of nPP. Enzyme reduced the feed intake ($p<0.05$) and improved the FCR of Ca-nPP deficient chicks ($p<0.01$). The decrease in Ca-nPP content in the diet caused a significant increase in serum concentration of Ca ($p<0.05$) and decrease in P concentration ($p<0.05$). Low Ca-nPP diets had no influence on serum Mg concentration. Dietary phytase reduced the Ca level and increased the P level ($p<0.05$) of serum in chicks fed with Ca-nPP deficient diets. The activity of LDH increased ($p<0.01$) in response to low dietary Ca and nPP, deficient but there was no influence on serum ALT and AST activity and TP content ($p>0.05$). Phytase supplementation reduced serum ALT ($p<0.05$) and had no effect on AST ($p>0.05$). Serum LDH activity further increased ($p<0.01$) by phytase supplementation. These results demonstrated that the performance of the chicks received low levels of Ca and nPP with phytase was improved and the serum parameters were comparable to those chicks fed with normal Ca-nPP diet.

Key words: phytate phosphorus, phyzyme, broiler performance.

Introduction

Most cereal grains and oilseeds contain phytin between 0.7% and 2%, which serves as the storage form of phosphorus, representing 50% to 85% of the

total P (Cheryan 1980).

Dietary phytin P is poorly digested by monogastric animals, as seen in the excretion of considerable quantities of P in manure from intensive operations. The poor digestive utilization of phytin-bound P by poultry and its consequences on diet cost,

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environment, and digestibility of minerals and proteins have led to extensive research efforts directed toward understanding the process of phytic acid digestion.

There are several reviews on nutritional (Cheryan, 1980, Ravindran *et al.*, 1995, Selle *et al.*, 2000, Adeola and Sands 2003) and environmental (Jongbloed and Lenis, 1998) consequences of phytin, as well as the application, structure, and kinetic properties of phytase (Dvorakova, 1998; Liu *et al.*, 1998; Keshavarz, 2000; Yu *et al.*, 2004). Phytase is an enzyme that hydrolyzes phytate to inositol and inorganic phosphate. The enzyme is at low level in the chicken gastro-intestinal tract but is present in some cereals such as wheat and triticale and at high concentration in microbial sources. Supplementation of diets with microbial phytase has proven to be an effective and realistic method for enhancing the digestibility of phytic acid (Nys *et al.*, 1999). Phytase reduces the need for supplemental inorganic P to the diet and hence, the amount of P excreted into the environment (Coelho, 1999, Kies, 1999, Selle *et al.*, 2000, Kies *et al.*, 2001, Adeola and Sands 2003). However, little is known about the effect of diets deficient in Ca and nPP, and with phytase on serum enzyme activity of broiler chicks. Therefore, the objective of this experiment was to evaluate the effect of phytase on body weight gain, feed intake, feed conversion ratio, and serum concentrations of Ca, P, Mg and total proteins as well as the activity of enzymes AST, ALT and LDH, in corn-soy bean meal based diets deficient in Ca and nPP compared to a nutritionally adequate diet for broiler chicks.

Materials and Methods

Six hundred 1-day old Ross male, broiler chicks (43 ± 1.5 g BW) were allotted to five treatments in a completely randomized design. Each treatment was replicated four times with thirty chicks in each replicate. The chicks were provided the experimental diets and the experiment lasted for 21 days. The birds were housed in an environmentally controlled room with raised wire floors. They were exposed to continuous light for the first 2 days, then to 23 h light and 1h darkness until 21 days of age. Chicks, feed, and

water were checked twice daily, and feed in the mash form and water was provided on an ad libitum basis throughout the experiment.

A corn- soybean meal diet (C-SBM), adequate in all nutrients (except Ca and nPP) for experimental purposes was used. The diet (Table 1) provided 220gkg^{-1} crude protein and 2900 kcal ME kg^{-1} , and met all other nutrient requirements (NRC, 1994). The dietary treatments were: 1) C-SBM, 10gkg^{-1} Ca and 5gkg^{-1} nPP (Diet 1); 2) C-SBM, 8.5gkg^{-1} Ca and 3.75gkg^{-1} nPP (Diet 2); 3) C-SBM, 7.5gkg^{-1} Ca and 2.5gkg^{-1} nPP (Diet 3); 4) Diet 2 + 100mgkg^{-1} phytase (Phyzyme XP5000G, Danisco Animal Nutrition, Marlborough, Wiltshire, SN8 1XN, UK); 5) Diet 3 + 100mgkg^{-1} phytase. The amount of enzyme added to the diet was based on the manufacturer's recommendations.

Growth Performance

At the end of experimental period, the birds were weighed and feed consumption was recorded for feed efficiency computation. Feed conversion ratio (FCR) was calculated from the ratio of feed intake (FI) to body weight gain (BWG) for the 21 days of experimental treatment.

Serum Ca, P, Mg, ALT, AST, LDH and TOP

At 21 days of age, two birds were randomly selected from each pen and blood samples (10ml) were obtained by bleeding. Blood tubes were centrifuged at $1200 \times g$ and supernatants were collected in 1.5 ml Eppendorf tubes and deep frozen (-20°C) until measurement of minerals (Ca, P, Mg), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and total protein (TOP) in serum using commercial diagnostic kits and according to the standard procedures (Eppendorf Blood Analyzer, Model EPOS 5060, Netheler-Hinz GmbH, Hamburg, Germany) (AOAC, 1990).

Statistical Analysis

The experiment had a complete random design (ANOVA) (Minitab 13.2 statistical package, Minitab Inc. State College) with a 2×2 factorial arrangement



(two diets deficient in Ca and nPP and two levels of enzyme) with a reference diet adequate in Ca and nPP with no enzyme. Fisher's LSD method was used to find confidence intervals for all pair wise differences between means. The general linear model was used to determine the main effects of factors and any possible interaction between factors.

Results

The influence of phytase enzyme on body weight gain, feed intake, feed conversion ratio of the chicks and the concentration of Ca, Mg, P and TP in serum and the activity of enzymes ALT, AST and LDH, is shown in Table 2.

There was no significant difference ($p>0.05$) in BWG of chicks among treatments. However, those chicks offered with Ca-nPP deficient diets had lower BWG values than the Ca-nPP adequate diet. Adding phytase tended to increase the BWG of Ca-nPP deficient groups to the level of Ca-nPP adequate diet.

There was a numeric increase in feed intake of the birds who received the lowest level of Ca-nPP diet compared to Ca-nPP adequate birds (30g), but phytase reduced significantly ($p<0.05$) the amount of feed consumed by those chicks fed the diet with the lowest level of Ca-nPP by almost 100g.

Those birds offered with the lowest level of Ca-nPP diet had the highest FCR and the birds received the same diet with enzyme had the lowest FCR among all treatments ($p<0.01$).

The serum concentration of Ca decreased and concentration of P increased when the level of Ca and P in the experimental diet decreased ($p<0.05$). However, the serum concentrations of Ca and P in birds provided with the same diets containing enzyme, were comparable to Ca-nPP adequate diet.

Different dietary treatments had no effect on serum concentrations of Mg and TOP, and serum activity of AST.

The serum activity of ALT decreased and the activity of LDH increased ($p<0.05$) in response to the diets deficient in Ca-nPP. Phytase had no influence on ALT activity in Ca-nPP deficient birds ($p>0.05$). However the enzyme further increased the activity of LDH in Ca-nPP deficient groups ($p<0.01$).

Discussion

Almost 70-80% of the phosphorus in feedstuffs of plant origin and 61-70% phosphorus found in corn-soybean meal diets is in the form of phytate phosphorus (Kies *et al.*, 2001, Naher 2002). The inability of poultry to utilize phytate phosphorus, due to lack of endogenous phytase, results in the addition of inorganic feed phosphates to poultry diets in order to meet the phosphorus requirements of poultry (Maenz and Classen 1998, Yu, *et al.*, 2004).

The adverse effects of phytate phosphorus could possibly be overcome by dietary supplementation of exogenous phytases. Addition of exogenous phytases to the diet has been reported to improve growth performance of broilers (Ahmad *et al.*, 2000, Naher 2002) and also improve phosphorus digestibility for certain plant based feedstuffs and overall utilization of the broiler diets (Rutherford *et al.*, 2002). An addition of phytase to diets reduces the excretion of P in manure and helps to correct the possible environmental pollution (Simons *et al.*, 1990, Roberson and Edwards 1994, Kornegay and Harper 1997, Coelho 1999, Kies 1999, Selle *et al.*, 2000, Kies *et al.*, 2001, Adeola and Sands 2003).

As expected, a decrease in dietary calcium and available phosphorus caused a negative effect on overall performance of chicks. These results were in agreement with those reported by others (Qian *et al.*, 1996, Punna and Roland 1999, Rama Rao *et al.*, 1999, Yu *et al.*, 2004). Similarly, the positive effect of phytase on performance of chicks seen in this experiment, has already been reported by many workers (Broz *et al.*, 1994, Sebastian *et al.*, 1996, Rama Rao *et al.*, 1999, Ahmad *et al.*, 2000, Yu *et al.*, 2004). As shown here and indicated by others, microbial phytase seems to be effective in releasing Ca and P in diets deficient in Ca and P (Qian *et al.*, 1996, Gordon and Roland 1998, Cabahug *et al.*, 1999, Sohail and Roland, 1999). The beneficial effect of phytase on growth performance has been related to (1) the release of minerals and trace elements from complexes with phytic acid, and increase in digestibility and availability of macro- and micro-elements (Knuckles and Betschert 1987, Qian *et al.*,



1996, Sebastian *et al.*, 1996, Selle *et al.*, 2000, Adeola and Sands 2003), (2) the utilization of inositol (Simons *et al.*, 1990), (3) increased starch digestibility (Knuckles and Betschart 1987) and (4) increased utilization of protein (Farrell *et al.*, 1993).

In the present study, reducing the Ca and nPP content of diet from 10 to 7.5 gkg⁻¹ and from 5 to 2.5 gkg⁻¹ depressed numerically BDG of the birds, and dietary phytase showed a numeric improvement on the BWG. The results fully agreed with the reports indicating that the increase in concentration of phytase in SBM based diets might increase the body weight of chicks (Salobir *et al.*, 1998, Rama Rao *et al.*, 1999, Ahmad *et al.*, 2000, Moshad 2001). However, Mohanna and Nys (1999), Wilson *et al.*, (1999) and Pizzolante *et al.*, (2002) did not notice a positive influence of phytase on body weight gain of the experimental birds.

An increase in FI of the chicks offered the lowest level of Ca-nPP in their diet might be in order to compensate factors influencing the need for calcium and phosphorus. This result is in contrast with many workers who demonstrated that a reduction in Ca and nPP of diet reduced the feed consumption of the chicks (Sohail and Roland 1999, Boling *et al.*, 2000, Johnston and Southern 2000, Watson 2002). Juanpere *et al.*, (2004) showed that a reduction in nPP of broiler chick diets decreased daily feed intake and water consumption and a reduction in bird growth.

On the other hand, in the experiment presented here, a decrease in feed intake of the birds offered the Ca-nPP deficient diets with phytase was probably due to the better digestion and utilization of dietary nutrients. However, this result is in contrast with the results of Denbow *et al.*, (1995) and Sebastian *et al.*, (1996, 1997), Sohail and Roland (1999), Boling *et al.*, (2000), Johnston and Southern (2000), Watson (2002), Brenes (2003) and Juanpere *et al.*, (2004). The above authors suggested that the increase in BWG of chicks feed with the dietary phytase was due to an increase in FI.

An improvement in FCR of the birds by the addition of phytase, was similar to those reported by Broz *et al.*, (1994), Kornegay *et al.*, (1996), Gordon and Roland (1998), Rama Rao *et al.*, (1999),

Waldroup *et al.*, (2000) and Yan *et al.*, (2001).

An investigation was carried out by Ahmed *et al.*, (2004) on the performance of broiler chicks who were fed soybean meal (SBM) based diet incorporating phytase with the levels of 0.0, 0.50, 1.00 and 1.50 gkg⁻¹ diet for better utilization of the basal diet. It was noted that the growth rate, feed intake, feed consumption, dressing yield and profitability increased as the level of phytase supplementation increased. It was suggested that 1.50 gkg⁻¹ phytase may be incorporated in SBM based broiler diets in order to overcome effectively overcome the antinutritive effect of phytate phosphorus on broiler performance.

An increase in serum concentration of Ca and a decrease in concentration of P in response to decreasing Ca and nPP levels of the diet, has also been observed in chickens and turkeys (Fernandes *et al.*, 1999, Atia *et al.*, 2000, Brenes *et al.*, 2003). The increase in serum Ca level of experimental chicks in response to decreasing dietary Ca and nPP level, might be related to an increase in Ca retention, which has been also indicated by others (Taylor and Dacke 1984, Sebastian *et al.*, 1996, Fernandes *et al.*, 1999). An increase in P retention in broilers and pullets fed low nPP diets was related to a decrease in plasma P, indicating that birds had a greater ability to retain P from diets with lower nPP content (Ravindran *et al.*, 2000, Keshavarz 2000).

In this study, phytase supplementation to the low Ca-nPP diets reduced Ca and increased P concentrations in serum. Similar results have also been observed by Sebastian *et al.*, (1996), Han *et al.*, (1997), Orban *et al.*, (1999), Rama Rao *et al.*, (1999) and Hassen and Chauhan (2003), Brenes *et al.*, (2003) and Juanpere *et al.*, (2004). Perney *et al.*, (1993) and Ahmad *et al.*, (2000) indicated that the decreased P excretion in birds fed low-P diets with phytase, might be due to the increased bioavailability of both P and Ca, because both are part of the same complex and are released by the phytase enzyme at the same time. When birds were fed diets with phytase, the phosphate group of phytate was degraded and absorbed, and then used for growth. The increased bioavailability of dietary P by adding phytase has also



Table 1: Formulation of diets and chemical composition of control and experimental rations

Ingredients (gkg ⁻¹)	Treatments		
	Diet 1	Diet 2	Diet 3
Corn	533	547	560
Soybean Meal	394	392	390
Vegetable Fatty Acid	30.4	24.6	20.0
Di-Calcium Phosphate	21.4	14.2	7.1
Limestone	10.1	10.5	11.3
DL-Methionine	1.8	1.8	1.8
L-Lysine	0.2	0.2	0.2
Salt	4.0	4.0	4.0
Vitamin + Mineral Premix*	5.0	5.0	5.0
Chemical Analysis (calculated)			
Metabolizable Energy (kcalkg ⁻¹)	2900	2900	2900
Crude Protein	220	220	220
Crude Fiber	41.0	41.1	41.2
Total Fat	49.4	43.1	40.5
Arginine	15.8	15.8	15.8
Lysine	13.0	13.0	13.0
Methionine	5.4	5.4	5.4
Methionine + Cystine	9.2	9.2	9.2
Threonine	9.0	9.0	9.0
Tryptophan	2.7	2.7	2.7
Total Calcium	10	8.5	7.5
Total Phosphorus	8.0	6.75	5.50
Non-Phytate Phosphorus	5.0	3.75	2.5

*, The vitamin and mineral premix supplied per kg of diet; Vit A 9000IU, Vit D3 2000IU, Vit E 18IU,

Vit B1 1.8mg, Vit B2 6.6mg, Vit B3 (Calcium Panthotenate) 10mg, Vit B5 (Niacin) 30mg, Vit B6 3mg, Vit B12 0.015mg, Biotin 0.1mg, Folic Acid 1.0mg, Cholin Chloride 500mg, Vit K 2mg, Manganese 99.2mg, Iron 50mg, Zinc 84.7mg, Copper 10mg, Iodine 1mg, Selenium 0.2mg.

been reported by Nys *et al.*, (1999) and Leske and Coon (1999).

Dietary phytic acid might increase the excretion of endogenous compounds by broilers. However, the supplementation of phytase seems to reduce the excretion of compounds with endogenous origin (Cowieson *et al.*, 2004).

In the present study, the concentration of total protein and magnesium were not affected either by the dietary concentration of Ca and nPP or by the presence of phytase in the diet. However, it has been documented that the serum total protein and magnesium levels significantly increased when the diet of chicks was supplemented with dietary phytase (Hassen and Chauhan 2003, Brenes *et al.*, 2003).

In an experiment by Brenes *et al.*, (2003), it was

demonstrated that by decreasing the nPP levels in the diet, plasma Ca concentration as well as AST, ALP, and LDH activities were increased and plasma P and TOP content were reduced. Phytase supplementation increased linearly plasma Ca, P, Mg, and TOP content, and serum AST, ALT and LDH activities, but reduced linearly serum ALP activity.

A difference in an activity of serum ALT in response to Ca-nPP deficient diets demonstrated in here and reported by Brenes *et al.*, (2003), could be due to the higher activity of enzyme ALT reported here. Adding phytase to the diet could not elevate the activity of the enzyme in this experiment, which is in contrast with their report.

The activity of enzyme AST was not affected either by changes in the dietary concentration of Ca



Table 2: The influence of Phytase on growth performance, serum protein (TOP), minerals (Ca, P, Mg) and enzyme activity (ALT, AST, LDH) of chicks fed Ca-P deficient diets up to 21 days of age.

	Diet 1	Diet 2	Diet 3	Diet 2+ Enz	Diet 3+ Enz	Pooled SEM	Diet	Enzy me	Diet x Enz
BWG ¹ (g)	725	720	713	715	724	18.2	NS	NS	NS
FI ² (g)	1033 ^{ab+}	1021 ^{ab}	1063 ^b	997 ^a	994 ^a	24.2	NS	*	NS
FCR ³	1.346 ^a	1.340 ^a	1.407 ^b	1.318 ^a	1.300 ^a	0.0179	NS	**	NS
Ca (mg/dl)	7.114 ^a	7.617 ^a	8.729 ^b	7.840 ^a	7.486 ^a	0.2569	*	NS	NS
P (mg/dl)	6.971 ^a	6.217 ^{ab}	5.350 ^b	6.660 ^a	6.367 ^{ab}	0.3118	*	NS	NS
Mg (mg/dl)	2.483	2.366	2.554	2.454	2.596	0.1204	NS	NS	NS
TOP ⁴ (g/100ml)	3.029	2.801	2.823	2.798	2.977	0.0993	NS	NS	NS
ALT ⁵ (U/L)	13.75 ^a	13.71 ^a	12.63 ^{ab}	13.43 ^a	11.5 ^b	0.53	*	NS	NS
AST ⁶ (U/L)	284	243	252	254	275	7.4	NS	NS	NS
LDH ⁷ (U/L)	2535 ^a	2831 ^{ab}	3170 ^b	3773 ^c	3843 ^c	181.8	*	**	NS

+, Values within rows with different superscripts are significantly different ($p < 0.05$)

NS, Not Significant; *, $p < 0.05$; **, $p < 0.01$

1, Body Weight Gain; 2, Feed Intake; 3, Feed Conversion Ratio; 4, Total Protein; 5- Alanine Aminotransferase; 6, Aspartate Aminotransferase; 7, Lactate Dehydrogenase

and nPP or by supplementation of dietary phytase, which is in agreement with the results of Kundu *et al.*, (2004).

Viveros *et al.*, (2002) informed that by decreasing nPP levels of the diet, plasma Ca and Mg concentrations increased, and plasma P and TOP content as well as AST activity were reduced. Phytase supplementation increased plasma P level and serum AST activity, reduced plasma Ca and Mg contents and reduced serum ALT and LDH activities.

An elevation in activity of enzyme LDH in response to the low levels of Ca and nPP in the diet and further increase in LDH activity when the dietary phytase was supplemented has also been shown by Brenes *et al.*, (2003). The activity of LDH is nonspecific for hepatocellular disease in birds and the enzyme has five isoenzymes, which occur in a wide variety of tissues (Zantop 1997, Thrall 2004). However, the activity of the enzyme in normal bird is reported to be less than 1000 U/L. The authors could not find any clear explanation for the increased activity of the LDH enzyme and further works are needed to elucidate such an effect.

In conclusion, the current study confirms that the addition of phytase to the calcium and non phytate phosphorus deficient diets could improve the overall

performance of the broiler chicks, enhance the retention of phosphorus in the body and hence reduce the environmental pollution of phosphorus. However, further studies are needed in order to clarify the influence of phytase on the function of the hepatic enzymes.

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References

1. Adeola, O., Sands, J. S., (2003) Does supplemental dietary microbial phytase improve amino acid utilization? A perspective that it does not. *J. Anim. Sci.* 81:78-85.
2. Ahmad, T., Rasool, S., Sarwar, M., Haq, A., Zia-ul, H. (2000) Effect of microbial phytase produced from a fungus *Aspergillus niger* on bioavailability of phosphorus and calcium in broiler chickens. *Anim. Feed. Sci. Technol.* 83: 103-114.
3. Ahmed, F., Rahman, M. S., Ahmed, S. U. and Miah,



- M. Y. (2004) Performance of Broiler on Phytase Supplemented Soybean Meal Based Diet. *Poult. Sci.* 3:266-271.
4. Atia, F. A., Waibel, P. E., Hermes, I., Carlson, C. W. and Walser, M. M. (2000) Effect of dietary phosphorus, calcium, and phytase on performance of growing turkeys. *Poult. Sci.* 79: 231-239.
 5. Boling, S. D., Douglas, M. W., Shirley, R. B., Parsons, C. M. and Koelkebeck, K. W. (2000) The effects of various dietary levels of phytase and available phosphorus on performance of laying hens. *Poult. Sci.* 79:535-538.
 6. Brenes, A., Viveros, A., Arija, I., Centeno, C., Pizarro, M. and Bravo, C. (2003) The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. *Anim. Feed Sci. Technol.* 110: 201-219.
 7. Broz, J., Oldale, P., Perrin-Voltz, A. H., Rychen, G., Schulze, J. and Nunes, S. C. (1994) Effects of supplemental phytase on performance and phosphorus utilization in broiler chickens fed a low phosphorus diet without addition of inorganic phosphates. *Br. Poult. Sci.* 35: 273-280.
 8. Cabahug, S., Ravindran, V., Selle, P. H. and Bryden, W. L. (1999) Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus contents. I. Effects on bird performance and toe ash. *Br. Poult. Sci.* 40: 660-666.
 9. Campbell, T. W. (2004) Clinical chemistry of birds, In *Veterinary Hematology and Clinical Chemistry* by M. A. Thrall, published by Lippincott Williams and Wilkins Co. ISBN 0-683-30415-1, 479-492.
 10. Cheryan, M. (1980) Phytic acid interactions in food systems. *CRC Critical Reviews in Food Science and Nutrition.* 13: 297-335.
 11. Cowieson, A. J., Acamovic, T. and Bedford, M. R. (2004) The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. *Br. Poult. Sci.* 45: 101 - 108.
 12. Coelho, M. B. (1999) Phosphorus balance of poultry operations with Natuphos[®] phytase. In *Phytase in Animal Nutrition and Waste Management: A BASF Reference*. BASF Corp, Mount Olive. NJ. 735-742.
 14. Denbow, D. M., Ravindran, V., Kornega, E. T., Yi, Z. and Hulet, R. M. (1995) Improving phosphorus availability in soybean meal for broilers by supplemental phytase. *Poult. Sci.* 74:1831-1842.
 15. Dvorakova, J. (1998) Phytase: Sources, preparation and exploitation. *Folia Microbiol.* 43:323-338.
 16. Farrell, D. J., Martin, E., Pareez, J. J., Bongarts, M., Betts, M., Sudaman, A. and Thomson, E. (1993) The beneficial effects of a microbial phytase in diets of broiler chicks and ducklings. *J. Anim. Physiol. Anim. Nutr.* 69: 278-283.
 17. Fernandes, J. I. M., Lima, F. R., Mendonca, C. X., Mabe, I., Albuquerque, R. and Leal, P. M. (1999) Relative bioavailability of phosphorus in feed and agricultural phosphates for poultry. *Poult. Sci.* 78:1729-1736.
 18. Gordon, R. W., Roland, D. A. (1998) Influence of supplemental phytase on calcium and phosphorus utilization in laying hens. *Poult. Sci.* 77: 290-294.
 19. Han, Y. M., Yang, F., Zhou, A. G., Miller, E. R., Ku, P. K., Hogberg, M. G. and Lei, X. G. (1997) Supplemental phytases of microbial and cereal sources improve dietary phytate phosphorus utilization by pigs from weanling through finishing. *J. Anim. Sci.* 75: 1017-1025.
 20. Hassen, H., Chauhan, S. S. (2003) Phytase supplementation on biochemical blood parameters of maize-based-diets-fed broilers. *AgriTopia* 18: 2-3.
 21. Johnston, S. L., Southern, L. L. (2000) The effect of varying mix uniformity (simulated) of phytase on growth performance, mineral retention, and bone mineralization in chicks. *Poult. Sci.* 79: 1485-1490.
 22. Jongbloed, A. W., Lenis, N. P. (1998) Environmental concerns about animal manure. *J. Anim. Sci.* 76: 2641-2648.
 23. Juanpere, J., Pérez-Vendrell, A. M. and Brufau, J. (2004) Effect of microbial phytase on broilers fed barley-based diets in the presence or not of endogenous phytase. *Ani. Feed Sci. Technol.* 115: 265-279.
 24. Keshavarz, K. (2000) Reevaluation of nonphytate phosphorus requirement of growing pullets with and without phytase. *Poult. Sci.* 70: 1143-1153.
 25. Kies, A. K. (1999) Phytase - mode of action. In



- Phytase in Animal Nutrition and Waste Management: A BASF Reference. BASF Corp, Mount Olive, NJ. 205-212.
26. Kies, A. K., Van Hemert, K. H. F. and Sauer, W. C. (2001) Effect of phytase on protein and amino acid digestibility and energy utilization. *World Poult. Sci. J.* 57:109-125.
 27. Knuckles, B. E., Betschert, A. A. (1987) Effect of phytate and other myo-inositol phosphate esters on alpha-amylase digestion of starch. *J. Food Sci.* 52: 719-721.
 28. Kornegay, E. T., Denbow, D. M., Yi, Z. and Ravindran, V. (1996) Response of broilers to graded levels of microbial phytase added to maize-soyabean-meal-based diets containing three levels of non-phytate phosphorus. *Br. J. Nutr.* 75: 839-852.
 29. Kornegay, E. T., Harper, A. F. (1997) Environmental nutrition: nutritional management strategies to reduce nutrient excretion of swine. *Prof. Anim. Sci.* 13: 99-111.
 30. Kundu, B., Biswas, P., Rajendran, D. (2004) Effect of inclusion of microbial phytase in feed by gradual replacement of dicalcium phosphate on performance of commercial broiler chicken. *Anim. Feed Technol.* 4: 33-41.
 31. Leske, K. L., Coon, C. N. (1999) A bioassay to determine the effects of phytase on phytate phosphorus hydrolysis and total phosphorus retention of feed ingredients as determined with broilers and laying hens. *Poult. Sci.* 78:1151-1157.
 32. Liu, B. L., Rafiq, A., Tzeng, Y. M. and Rob, A. (1998) The induction and characterization of phytase and beyond. *Enzyme Microb. Technol.* 22: 415-424.
 33. Maenz, D. D., Classen, H. L. (1998) Phytase activity in the small intestinal brush border membrane of the chicken. *Poult. Sci.* 77: 557-563.
 34. Maenz, D. D., Engele-Schaan, C. M., Newkirk, R. W. and Classen, H. L. (1999) The effect of minerals and mineral chelators on the formation of phytase-resistant and phytase-susceptible forms of phytic acid in solution and in slurry of canola meal. *Anim. Feed Sci. Technol.* 81:177-192.
 35. Mohanna, C., Nys, Y. (1999) Changes in zinc and manganese availability in broiler chicks induced by vegetal and microbial phytases. *Anim. Feed Sci. Technol.* 77: 241-253.
 36. Moshad, M. A. (2001) An enzyme for better utilization of parboiled rice polish based diet in broilers. M. S. Thesis, Bangladesh Agricultural University, Mymensingh.
 37. Naher, B. (2002) Utilization of parboiled rice polish based diet with supplementation of carbohydrase and phytase in growing ducklings. M. S. Thesis, Bangladesh Agricultural University, Mymensingh.
 38. National Research Council. (1994) Nutrient Requirements of Poultry. 9th rev. Ed. National Academy Press, Washington, DC.
 39. Nys, Y., FrnPPin, D. and Pointillart, A. (1999) Occurrence of phytase in plants, animals and microorganisms. in *Phytase in Animal Nutrition and Waste Management: A BASF Reference*. BASF Corp, Mount Olive, NJ. 213-236.
 40. Orban, J. I., Adeola, O. and Strohshine, R. (1999) Microbial phytase in finisher diets of white pekin ducks: effect on growth performance, plasma phosphorus concentration, and leg bone characteristics. *Poult. Sci.* 78: 366-377.
 41. Perney, K. M., Cantor, A. H., Straw, M. L. and Herkelman, K. L. (1993) The effect of dietary phytase on growth performance and phosphorus utilization of broiler chickens. *Poult. Sci.* 72: 2106-2114.
 42. Pizzolante, C. C., Teixeira, A. S., Santos, C. D., Bertechini, A. G. and Garci, E. A. (2002) Phytase and calcium levels on performance of broiler chickens. *Ciencia-e-Agrotecnol.* 26: 418-425.
 43. Punna, S., Roland, D. A. (1999) Variation in phytate phosphorus utilization within the same broiler strain. *J. NPPpl. Poult. Res.* 8: 10-15.
 44. Qian, H., Kornegay, E. T. and Conner, D. E. (1996) Adverse effects of wide calcium: phosphorus and calcium as influenced by microbial phytase, cholecalciferol and the calcium:total phosphorus ratio in broiler diets. *Poult. Sci.* 76: 37-46.
 45. Rama Rao, S. V., Ravindra Reddy, V. and Ramasubba Reddy, V. (1999) Enhancement of phytate phosphorus availability in the diets of commercial broilers and layers. *Ani. Feed Sci.*



- Technol. 79: 211-222.
46. Ravindran, V., Bryden, W. L. and Kornegay, E. T. (1995) Phytates: Occurrence, bioavailability and implications in poultry nutrition. *Poult. Avian Biol. Rev.* 6:125-143.
47. Ravindran, V., Cabahug, S., Ravindran, G., Selle, P. H. and Bryden, W. L. (2000) Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorous levels. II. Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. *Br. Poult. Sci.* 41: 193-200.
48. Roberson, K. D., Edwards, H. M. (1994) Effects of 1, 25-dihydroxycholecalciferol and phytase on zinc utilization in broiler chicks, *Poult. Sci.* 73: 1312-1326.
49. Rutherford, S. M., Chung, T. K., Moughan, P. J. (2002) The effect of microbial phytase on ileal phosphorus and amino acid digestibility in the broiler chicken. *Br. Poult. Sci.* 44: 598-606.
50. Salobir, J. (1998) Effect of Xylanase alone and in combination with beta-glucanase on energy diets based on two wheat samples. *Archiv-fur Geflugelkunde.* 62: 209-213.
51. Sebastian, S., Touchburn, S. P., Chavez, E. R. and Lague, P. C. (1996) The effects of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper, and zinc in broiler chickens fed corn-soybean diets. *Poult. Sci.* 75: 729-736.
52. Sebastian, S., Touchburn, S. P., Chavez, E. R. and Lague, P. C. (1997) Apparent digestibility of protein and amino acids in broiler chickens fed a corn-soybean diet supplemented with microbial phytase. *Poult. Sci.* 76:1760-1769.
53. Selle, P. H., Ravindran, V., Caldwell, R. V. and Bryden, W. L. (2000) Phytate and phytase: Consequences for protein utilization. *Nutr. Res. Rev.* 13: 255-278.
54. Simons, P. C. M., Versteegh, H. A. J., Jongbloed, A. W., Kemme, P. A., Stump, P., Bos, K. D., Wolters, M. G. E., Beudeker, R. F. and Verscoor, G. J. (1990) Improvement of phosphorus availability by microbial phytase in broilers and pigs. *Br. J. Nutri.* 64: 525-540.
55. Sohail, S. S., Roland, D. A. (1999) Influence of supplemental phytase on performance of broilers four to six weeks of age. *Poult. Sci.* 78: 550-555.
56. Taylor, T. G., Dacke, C. G. (1984) Calcium metabolism and its regulation. In *Physiology and Biochemistry of the Domestic Fowl*, Edited by Freeman, B. M. Academic Press, London, 126-170.
57. Viveros, A., Brenes, A., Arija, A., Centeno, C. (2002) Effects of Microbial Phytase Supplementation on Mineral Utilization and Serum Enzyme Activities in Broiler Chicks Fed Different Levels of Phosphorus. *Poult. Sci.* 81:1172-1183.
58. Waldroup, P. W., Kersey, J. H., Saleh, E. A., Fritts, C. A., Yan, F., Stilborn, H. L., Crum, R. C. Jr. and Raboy, V. (2000) Non-phytate phosphorus requirement and phosphorus excretion of broiler chicks fed diets composed of normal or high available phosphate corn with and without microbial phytase. *Poult. Sci.* 79:1451-1459.
59. Watson, B. C. (2002) The effects of phytase in nutritionally adequate diets, diets deficient in calcium and phosphorus, and the interactive effects of phytase and *Eimeria acervulina* infection in broiler chicks. M. S. Thesis. Louisiana State University.
60. Wilson, J. H., Kornegay, E. T., Frazer, B. L., Barrios, L., Miller, A. N. and Pettit, S. (1999) The influence of supplemental phytase on broiler bone strength. ASAE-CSAE-SCGR Annual International Meeting, Toronto, Ontario, Canada, ASAE Paper No. 996072, 8.
61. Yan, F., Kersey, J. H. and Waldroup, P. W. (2001) Phosphorus requirements of broiler chicks three to six weeks of age as influenced by phytase supplementation. *Poult. Sci.* 80: 455-459.
62. Yu, B., Jan, Y. C., Chung, T. K., Lee, T. T. and Chiou, P. W. S. (2004) Exogenous phytase activity in the gastrointestinal tract of broiler chickens. *Anim. Feed. Sci. Technol.* 117: 295-303.
63. Zantop, D. W. (1997) *Avian Medicine: Principles and applications*, Wingers Publishing, Inc., Florida, 117.



تأثیر جیره‌های غذایی دارای کمبود کلسیم و فسفر غیر فیتاته به همراه فیتاز بر روی عملکرد رشدی، غلظت سرمی املاح و فعالیت آنزیم‌های آلانین ترانس آمیناز، آسپارات ترانس آمیناز، لاکتات دهیدروژناز در جوجه‌های گوشتی

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تحت یک آزمایش تأثیر فیتاز میکروبی (فیز آیم ایکس پی ۵۰۰۰) در جیره غذایی جوجه‌های گوشتی بر روی عملکرد پرورنده، پروتئین تام سرمی، املاح و فعالیت آنزیمی سرم (ALT, AST, LDH) مطالعه گردید. یک جیره رفرانس کافی از لحاظ کلسیم و فسفر غیر فیتاته (۱۰ گرم در کیلوگرم کلسیم و ۵ گرم در کیلوگرم فسفر غیر فیتاته) و دو جیره دیگر که دارای کمبود کلسیم و فسفر غیر فیتاته بودند (به ترتیب ۸/۵ گرم در کیلوگرم کلسیم و ۳/۵ گرم در کیلوگرم فسفر و ۷/۵ گرم در کیلوگرم کلسیم و ۲/۵ گرم در کیلوگرم فسفر) بدون و با آنزیم فیتاز تهیه و در اختیار جوجه‌های مورد آزمایش از سن یک روزه تا ۲۱ روزه قرار گرفت. اگرچه جیره‌های دارای کمبود فسفر غیر فیتاته تأثیر معنی داری بر روی افزایش وزن بدن جوجه‌ها نداشتند ($p > 0.05$) این جیره‌ها باعث افزایش میزان مصرف خوراک و افزایش ضریب تبدیل غذا ($p < 0.01$) در مقایسه با گروه‌های مشابه به همراه آنزیم فیتاز گردیدند. فیتاز تأثیر مثبت، اگرچه غیر معنی دار، بر روی افزایش وزن بدن جوجه‌هایی که جیره‌های دارای کمبود شدید فسفر غیر فیتاته بودند، داشت. آنزیم باعث کاهش مصرف خوراک ($p < 0.05$) و بهبودی ضریب تبدیل غذایی جوجه‌های دریافت کننده جیره دارای کمبود فسفر غیر فیتاته گردید ($p < 0.01$) کاهش فسفر غیر فیتاته جیره، باعث افزایش کلسیم سرمی ($p < 0.05$) و کاهش فسفر سرمی ($p < 0.05$) گردید ولی تأثیری بر روی منیزیم سرمی نداشت ($p > 0.05$). فیتاز جیره باعث کاهش سطح کلسیم سرم و افزایش سطح فسفر سرم در جوجه‌هایی که جیره دارای کمبود فسفر غیر فیتاته بود گردید ($p < 0.05$). فعالیت آنزیم LDH تحت تأثیر جیره دارای کمبود کلسیم و فسفر غیر فیتاته افزایش پیدا نمود ($p < 0.01$) در حالی که تأثیری در میزان فعالیت ALT, AST و همچنین مقدار پروتئین تام سرمی مشاهده نگردید ($p < 0.05$). فیتاز باعث کاهش فعالیت ALT سرمی ($p < 0.05$) شد ولی تأثیری بر روی AST نداشت، فعالیت سرمی آنزیم LDH تحت تأثیر فیتاز افزایش بیشتری پیدا نمود ($p < 0.01$).

واژه‌های کلیدی: فیتات فوسفور، فیزیم، برویلر پر فورمانس.

