INTERNATIONAL JOURNAL OF HEALTH & ANIMAL SCIENCE & FOOD SAFETY

UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE VETERINARIE PER LA SALUTE, LA PRODUZIONE ANIMALE E LA SICUREZZA ALIMENTARE

Effect of phytase supplementation on apparent phosphorus digestibility and phosphorus output in broiler chicks fed low-phosphorus diets.

Xian R. Jiang[1, 2], Fa H. Luo[1], Shu G. Wu[1*], Hai J. Zhang[1], Valentino Bontempo[2], Ming R. Qu[3], Hong Y. Yue[1], Guang H. Qi[1]

[1] *Key Laboratory of Feed Biotechnology of the Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences, China.*

[2] *Dipartimento di Scienze Veterinarie per la Salute, la Produzione Animale e la Sicurezza Alimentare, Università di Milano, Italy.*

[3] *Animal Science and Technology College, Jiangxi Agricultural University, China.*

ABSTRACT.

This study was conducted to evaluate the effect of supplemental phytase in broiler chicks fed different low levels of total phosphorus (P) on the apparent phosphorus digestibility (APD) and phosphorus output (PO) in the faeces and ileal digesta. After fed a standard broiler starter diet from day 0 to 14 post-hatch, a total of 144 male broiler chicks were allocated to 6 groups for a 7-d experiment with a 2 × 3 factorial design comparing phytase (supplemented without (CTR) or with 400 FTU/kg phytase (PHY)) and total P levels (2.0, 2.5 and 3.0 g/kg). The faecal samples were collected from day 17 to 21 post-hatch. At 22 days of age, all the chicks were slaughtered and collected the ileal digesta. Phytase supplementation significantly ($P < 0.01$) increased APD and decreased PO in the faeces and ileal digesta in comparison with the CTR group. In addition, PO in the faeces expressed as g/kg DM diets and faeces (Diet × P level, $P = 0.047$ and < 0.01, respectively) as well as PO in the ileal digesta expressed as g/kg DM digesta (Diet × P level, $P = 0.04$) were affected by diet and P level, which were due to the significant reduction ($P < 0.01$) by PHY supplementation to the diets with 3.0 g/kg total P. The results evidenced that supplemental phytase improved the APD and PO when chicks was fed 3.0 g/kg total P diet, while lower total P levels may limit exogenous phytase efficacy.

KEYWORDS

Apparent phosphorus digestibility; chicks; low-phosphorus; phosphorus output.

PAGES

9 – 17

REFERENCES

Vol. 2 No. 1 (2015)

ARTICLE HISTORY

Submitted: January 29, 2015
Revised: March 29, 2015
Accepted: March 31 2015
Published: April 26 2015

CORRESPONDING AUTHOR

Shu Geng Wu,
Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China

Zhongguancun Nandajie 12, 100081, Beijing, China

e-mail: wushugeng@caas.cn
phone: +86 1082106097
fax: +86 1082106054

JOURNAL HOME PAGE

riviste.unimi.it/index.php/haf

1 Introduction

Monogastric animals, such as poultry and pig, have virtually no phytase activity of their own. Thus, the availability of phosphorus (P) in feedstuffs of plant origin is generally very low, ranging from 30 to 40% (Nelson *et al.*, 1968). To increase P bioavailability, the most commonly used method is supplementing high dosage of inorganic P in feed, which leads the excretion of large amounts of P in animal manure. Consequently, the cost of feed and the environmental adverse impact are increased. Moreover, phytate limits the availability of several other essential nutrients, such as minerals, protein and amino acids (Biehl and Baker, 1996). Many studies showed that microbial phytase can be used to increase the availability of P and reduce its excretion (Waldroup *et al.*, 2000; Paik, 2003). Previous studies have mainly focused on the utilization of 3-phytase (EC 3.1.3.8) derived from the Aspergillus niger (Panda *et al.*, 2007) as feed additives for broilers. Shieh and Ware (1968) reported that 3-phytase can catalyze the conversion of myo-inositol hexakisphosphate and water to 1L-myo-inositol 1, 2, 4, 5, 6-pentakisphosphate and orthophosphate.

The interest in the supplementation of phytase in low P diets for monogastric animals has got great attention due to environmental concerns and high cost of inorganic P. Many previous studies demonstrated that phytase supplementation to low-P diets improved performance and P utilization of broiler chicks (Viveros *et al.*, 2002; Rutherfurd *et al.*, 2004; 2012; Jiang *et al.*, 2013). However, it might be also interesting to investigate whether phytase supplementation could consistently improve P use of broiler chicks fed the P deficient diets even far from the requirement for normal growth. Therefore, we conducted this study to evaluate the effect of supplemental phytase on the apparent P digestibility and P output in the faeces and ileal digesta of broiler chicks fed different low-P levels of diets.

2 Materials and Methods

2.1 Bird husbandry and dietary treatments

All the experimental procedures were approved by the Animal Care and Use Committee of the Feed Research Institute of the Chinese Academy of Agricultural Sciences.

The feeding trial of this study was carried out in Nan Kou pilot base of the Chinese Academy of Agricultural Sciences. Two hundred Arbor Acres male broiler chicks were obtained from a local hatchery, wing-banded, and reared in electrically heated battery brooders maintained at temperatures of 33°C from day 1 to day 7 post-hatch and 30° C from day 8 to day 14 post-hatch. During this time, chicks were provided free access to water and a standard maize soybean meal starter diet containing 230 g of CP/kg, 13.39 MJ of MEn/kg, 10.0 g of Ca/kg, and 6.8 g of total P/kg. At day 15 post-hatch, after overnight feed withdrawal, a total of 144 chicks were weighed and divided into 6 homogeneous groups. Six replicate cages (60×50×50 cm, length×width×height) were used per treatment with 4 chicks per cage. Chicks had free access to feed and water from d 15 to 21 post-hatch, and battery temperature was maintained at 27°C during this period. Lights were continuously on the first day post-hatch, after which a

ISSN: 2283-3927

23L:1D lighting schedule was maintained all through the duration of the feeding trial. Feed and water were provided *ad libitum* throughout the trial.

The experiment was a 2×3 factorial arrangement of the treatments with diet (unsupplemented control [CTR] or supplemented with 400 FTU/kg phytase [PHY]) and 3 total phosphorus levels (2.0 g/kg [2.0P], 2.5 g/kg [2.5P] and 3.0 g/kg [3.0P]). 1 FTU is the amount of enzyme which liberates 1 µmol of inorganic phosphate per minute from sodium phytate at pH 5.5 and 37°C. The phytase, whose type and source of extraction were 3-phytase (EC 3.1.3.8) derived from *Aspergillus Neiger*, was purchased from BASF Vitamins Co., Ltd., China with activity of 5,000 FTU/g. Soybean meal was the only phosphorus source. Diets (Table 1) were semipurified, consisting primarily of cornstarch, dextrose, and soybean meal, and were formulated deceed the NRC (1994) recommendations to ensure maximum responses with phytase. Chromium oxide (Cr_2O_3) was incorporated into diets (3.5 g/kg, as-fed) to calculate nutrient utilization via the index method. Diets in pellet form and water were provided *ad libitum*. Mortalities were recorded daily.

2.2 Sample Collection and Chemical Analyses

After two adaptation days at 15 and 16 days post-hatch, faecal samples were collected twice per day at 6 AM and 6 PM from pans beneath each cage from day 17 to day 21 post-hatch for 5 days. At end of each collection period, cage samples were pooled. All the faecal samples were frozen and stored at -20°C before sending to laboratory for digestibility assays. At 22 day post-hatch, chicks were euthanized via carbon dioxide asphyxiation, and ileal digesta samples were collected by dissecting a segment of the ileum defined as extending from Meckel's diverticulum to the ileocecal junction. Contents of this segment were squeezed into a plastic container, pooled per cage of 4 chicks, and subsequently lyophilized. Freeze-dried digesta and faecal samples and, along with air-dried diet samples, were ground through a 0.75-mm sieve using a grinding mill to facilitate chemical analyses. Determination of dry matter (DM) was performed using the Association of Analytical Communities (AOAC, 2005) official method AOAC 930.15. The contents of phosphorus and chromium were analyzed as described by Dilger and Adeola (2006).

2.3 Calculations and Statistical Analyses

Apparent P digestibility (*i.e.* faecal and ileal digestibility) was calculated using the index method according to the following equation:

$$APD\ (\%) = 100 - [(Cr_I/Cr_O) \times (P_O/P_I) \times 100] \quad [1]$$

where APD is apparent P digestibility (calculated for ileal digesta or faecal samples) expressed as a percentage, Cr_I is the chromium concentration of dietary intake, Cr_O is the chromium concentration of output (as analyzed in ileal digesta or faeces), P_O is the P

ISSN: 2283-3927

Table 1. Composition and nutrient content of the experimental diets (as fed basis)[1]

	CTR			PHY		
	2.0P	**2.5P**	**3.0P**	**2.0P**	**2.5P**	**3.0P**
Ingredient, g/kg						
Corn starch	589.4	490.8	389.3	589.4	490.8	389.3
Dextrose	90.0	90.0	90.0	90.0	90.0	90.0
Soybean meal	294.2	379.8	465.5	294.2	379.8	465.5
Soybean oil	12.0	25.0	40.0	12.0	25.0	40.0
Limestone	4.8	6.0	7.0	4.8	6.0	7.0
Salt	2.5	2.5	2.5	2.5	2.5	2.5
Lysine	3.0	2.0	2.0	3.0	2.0	2.0
Methionine	1.0	0.8	0.6	1.0	0.8	0.6
Choline chloride	0.6	0.6	0.6	0.6	0.6	0.6
Mineral premix [2]	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin premix [3]	1.5	1.5	1.5	1.5	1.5	1.5
Phytase, FTU/kg	--	--	--	400	400	400
Calculated and analyzed composition						
Metabolizable energy, MJ/kg	12.27	12.27	12.31	12.27	12.27	12.31
CP, g/kg	132.1	169.6	207.0	132.1	169.6	207.0
Analyzed DM, g/kg	892.1	873.7	875.4	872.6	885.0	870.9
Analyzed Ca, g/kg	2.7	3.4	4.1	2.7	3.4	4.1
Analyzed total P, g/kg	2.1	2.7	3.0	2.1	2.7	3.0

1. CTR = basal diet without phytase supplementation; PHY = CTR + 400 FTU/kg phytase; 2.0 P = 2.0 g/kg total P level; 2.5P = 2.5 g/kg total P level; 3.0 P = 3.0 g/kg total P level.

2. Provided the following per kg of diet: Cu 8mg, Zn 75 mg, Fe 80 mg, Mn 100 mg, Se 0.15 mg, I 0.35 mg.

3. Provided the following per kg of diet: retinyl acetate, 4.3 mg; cholecalcipherol, 0.0625 mg; DL-alpha-tocopherol, 18.75 mg; menadione, 2.65 mg; cyanocobalamin, 0.025 mg; biotin, 0.0325 mg; folic acid, 1.25mg; niacin, 50 mg; D-pantothenic acid, 12 mg; riboflavin, 6 mg; thiamin, 2 mg

concentration of output (as analyzed in ileal digesta or faeces), and P_I is the P concentration of dietary intake. All analyzed values were expressed as grams per kilogram of DM.

ISSN: 2283-3927

Calculation of P output, expressed on a dry matter intake (DMI) basis, utilized the ratio of chromium intake to chromium output:

$$P_{O\text{-}DMI}\ (g/kg) = P_{O\text{-}DMO}\ (g/kg) \times (Cr_I/Cr_O) \quad [2]$$

where $P_{O\text{-}DMI}$ and $P_{O\text{-}DMO}$ represent P output concentrations (as analyzed in ileal digesta or faeces) on DMI and DM output bases, respectively, and Cr_I and Cr_O represent chromium concentrations of intake and output (of either ileal digesta or faeces), respectively.

Data were analyzed as a completely randomized block design by ANOVA, as implemented in the MIXED procedure of SAS v. 9.2 (SAS Institute Inc., Cary, NC). A model for a 2×3 factorial design was applied. The model statement included the effects of diet (CTR and PHY), total P levels (2.0, 2.5 and 3.0 g/kg) and interactions among those factors. Cage served as the experimental unit for all statistical analyses. Treatment differences were assessed by using the least squares means with a Tukey adjustment. Treatment effects were considered significant at $P \leq 0.05$.

3 Results

Graded levels of dietary P intake and the supplementation of phytase, did not affect normal digestive functions as was reflected by changes in apparent DM digestibility values (Table 2). Phytase supplementation significantly ($P < 0.01$) increased APD and decreased PO in the faeces and ileal digesta in comparison with the CTR group, when these were expressed as g/kg DM digesta, faeces or diets. Compared with the CTR chicks fed 3.0P, dietary PHY increased APD in faeces (linear contrast, $P = 0.02$) and ileal digesta (linear contrast, $P = 0.02$) of 3.0P chicks. PHY supplementation decreased PO in the ileal digesta of birds fed 2.5 g/kg (linear contrast, $P < 0.01$) and 3.0 g/kg (linear contrast, $P = 0.010$) total P diets compared to the CTR chicks fed 2.5P and3.0P, respectively, when these were expressed as g/kg DM digesta. Birds fed 2.0P had less PO in the faeces than fed 3.0P (linear contrast, $P < 0.01$), when these were expressed as g/kg DM faeces. However, compared to the birds fed 2.0P, chicks fed 2.5P had an increased APD in the ileal digesta (linear contrast, $P = 0.04$), and chicks fed 3.0P had an increased APD in the faeces (linear contrast, $P = 0.02$). Moreover, APD in the faeces of 2.5P chicks and in the ileal digesta of 3.0P chicks had tendencies to be higher than in the corresponding parts of 2.0P birds (linear contrast, $P = 0.051$ and 0.056, respectively). In addition, PO in the faeces expressed as g/kg DM diets and faeces (Diet × P level, $P = 0.047$ and < 0.01, respectively) as well as PO in the ileal digesta expressed as g/kg DM diets and digesta (Diet × P level, $P = 0.04$) were affected by diet and P level, and PO in the ileal digesta expressed as g/kg DM diets had a tendency to be affected by diet and P level (Diet × P level, $P = 0.059$). The interactions can be attributed to the significant reduction (linear contrast, $P < 0.01$) by PHY supplementation to the diets with 3.0 g/kg total P, but no differences were found in others total P levels diets.

ISSN: 2283-3927

Table 2. Effect of phytase on total P output, and apparent P digestibility in the faeces and ileal digesta of chicks fed different levels of P

	CTR			PHY				*P*-value		
	2.0P	**2.5P**	**3.0P**	**2.0P**	**2.5P**	**3.0P**	**SEM**	**Phytase**	**P level**	**Interaction**
No of pen	6	6	6	6	6	6				
P intake, g/kg of DMI	2.3	3.1	3.4	2.4	3.1	3.5	-	-	-	-
Faeces										
ADD, %	81.32	80.23	81.21	80.78	81.54	80.83	1.00	0.87	0.98	0.60
APD, %	57.33	63.52	60.22	60.89	69.17	74.77	2.94	<0.01	0.02	0.16
PO g/kg of DMI	0.99^{b}	1.15^{ab}	1.34^{a}	0.94^{b}	0.96^{b}	0.88^{b}	0.08	<0.01	0.19	0.047
PO g/kg of DMF	5.24^{bc}	5.80^{b}	7.12^{a}	4.88^{c}	5.16^{bc}	4.60^{c}	0.19	<0.01	<0.01	<0.01
Ileal digesta										
ADD, %	80.07	78.84	77.95	78.89	82.55	78.66	1.20	0.28	0.15	0.14
APD, %	42.62	49.16	44.86	46.70	65.97	68.39	4.91	<0.01	0.03	0.16
PO, g/kg of DMI	1.33	1.60	1.85	1.28	1.06	1.11	0.14	<0.01	0.41	0.059
PO g/kg of DMD	6.79^{ab}	7.53^{ab}	8.45^{a}	6.05^{bc}	6.03^{bc}	5.15^{c}	0.50	<0.01	0.69	0.04

a-c . Data in the same row with different superscripts differ significantly ($P<0.05$).

CTR = basal diet without phytase supplementation; PHY = CTR + 400 FTU/kg phytase; 2.0 P = 2.0 g/kg total P level; 2.5P = 2.5 g/kg total P level; 3.0 P = 3.0 g/kg total P level; PO = P output; ADD = apparent dry matter digestibility; APD = apparent P digestibility; DMD = dry matter digesta; DMF = dry matter faeces; DMI = dry matter intake.

4 Discussion

The objectives of the study were to determine whether dietary phytase added to the low total phosphorus (P) diets of broiler chicks would consistently improve apparent P digestibility (APD) and P output (PO) in faeces and ileal digesta, and whether there would be a significant interaction between phytase supplementation and levels of low total P in the diets. Our previous work confirmed that dietary supplementation of phytase can improve growth performance with a higher ash content in bone of chicks fed with low-P diet (4.8 g/kg total P diets) during 8-21 days post-hatch period (Jiang *et al.*, 2013), which may be due to the improvement the availability and absorption of nutrients through increasing the digestibility of the ingested diets (Abudabos, 2012; Attia *et al.*, 2012). Dilger and Adeola (2006) also observed

ISSN: 2283-3927

that low-phytate SBM decreased P output and increased P retention as compared to the conventional SEM. In the present study, positive results were obtained using 3-phytase supplemented to diets with low P (2.0, 2.5 or 3.0 g/kg total P) on apparent P digestibility and P output in both faeces and ileal digesta from day 14 to day 21 post-hatch. Numerous studies have been conducted that confirm the finding of enhanced P uptake in and poultry after treatment with phytase (Nelson *et al.*, 1968; Waldroup *et al.*, 2000; Paik, 2003; Rutherfurd *et al.*, 2004). The improvement in P utilization by supplementing phytase may be related to the increased dephosphorylation of phytate in the small intestine. Rutherfurd *et al.* (2004) reported that microbial phytase supplemented to the diets of chicks improved total P digestibility and increased phytate P disappearance in the ileum.

In this study, higher PO was observed in the faeces of birds fed with 3.0 g/kg total P diets compared to 2.0 g/kg total P diets, which may be due to the increased P intake. However, the interaction between diet and P level evidenced that the high PO in the maximum total P level group was attributed by the unsupplemented group. Moreover, the increased ADP in the faeces and ileal digesta of chicks fed with the diets containing 3.0 g/kg total P were due to the 3-phytase supplementation. In the present study, APD in the faeces were 57-63% and in the ileal digesta were 42-49% in the unsupplemented chicks fed with low-P diets, which were similar as the finding by Sebastian *et al.* (1996) and Rutherfurd *et al.* (2004). Whereas the corresponding value in the low-P diet supplemented with 400 FTU/kg of 3-phytase were 61-75% and 47-68%, respectively. In the present study, phytase improved the ADP and PO in the faeces of broilers from day 14 to day 21 of age mainly reflected in the birds fed with 3.0 g/kg total P diets, interestingly, the observations in the ileal digesta were in accordance with the findings in faeces. Our observation is in agreement with the finding by Dilger and Adeola (2006) that low-phytate SBM improved P output (1.122 vs. 1.808 g/kg of DMI) and retention (65.5 vs. 54.1 %) compared to the conventional SEM mainly in the groups contained 528 g/kg SEM (total P; 3.01 vs. 3.58 g/kg).

The limited improvements of ADP and PO in the diets with 2.0 and 2.5 g/kg total P might be due to the levels of the dietary P concentrations. It is possible that chicks rapidly acclimated to these low P intakes in an attempt to maintain P homeostasis or even "buffer" body P homeostasis for long-term P deficiency. Moreover, the low total P intake may provide limited phytate P for dietary phytase to "recycle", and consequently, rare improvements in P digestibility and excrete were observed in the 2.0 and 2.5 g/kg total P diets even supplemented with 400 FTU/kg 3-phytase. However, it seems likely that the diets containing 3.0 g/kg total P may possess enough phytate P for dietary phytase to hydrolyze and eventually result in the significant improvements in the P utilization. Although Dilger and Adeola (2006) concluded that the P use and endogenous P loss were influenced by dietary phytate content when broiler chicks were fed P-deficient diets and the levels of total P used were in the ranges of 0.83-3.58 g/kg, it is well known that severe P reductions in starter chicks might have jeopardized their skeletal integrity, general health status and survival (Scott *et al.*, 1982). The levels of low total P in diets of the studies which evidenced the improvement in growth performance and P utilization of broiler chicks by that phytase supplementation were in the ranges of 4.8-6.4 g/kg (Viveros *et al.*, 2002; Rutherfurd *et al.*, 2004; 2012; Jiang *et al.*, 2013), which are much higher than the levels (2.0, 2.5 and 3.0 g/kg) used in this study. Thus, phytase supplementation may not consistently improve P utilization during the early life stage of a commercial chicken strain when they are fed severe P-deficient diets.

ISSN: 2283-3927

5 Conclusions

The observations in this study demonstrated that supplementation of phytase increased apparent P digestibility and deceased P output in both faeces and ileal digesta when broiler chicks were fed the diets with 3.0 g/kg total P. However, the lower total P levels (2.0 and 2.5 g/kg) may limit the effect of exogenous phytase on P utilization and could not be recommended to use.

6 Conflicts of interest

The authors declare that there are no conflicts of interest.

7 Acknowledgements

This work was granted by China Agriculture Research System Poultry-related Science and Technology Innovation Team of Peking, and the National Key Technology Research and Development Program (2011BAD26B04).

References

Abudabos M.A. (2012). Phytate phosphorus utilization and intestinal phytase activity in laying hens. Ital. J. Anim. Sci., 11: 41-46.

AOAC (2005). Official Methods of Analysis, 18th ed., Association Official Analytical Chemists, Arlington, VA, USA.

Attia Y.A., El-Tahawy W.S., Abd El-Hamid A.E., Hassan S.S., Nizza A., El-Kelaway M.I. (2012). Effect of phytase with or without multienzyme supplementation on performance and nutrient digestibility of young broiler chicks fed mash or crumble diets. Ital. J. Anim. Sci., 11: 303-308.

Biehl R.R., Baker D.H. (1996). Efficacy of supplemental 1 alpha-hydroxycholecalciferol and microbial phytase for young pigs fed phosphorus- or amino acid-deficient corn-soybean meal diets. J. Anim. Sci. 74: 2960-2966.

Dilger R.N., Adeola O. (2006). Estimation of true phosphorus digestibility and endogenous phosphorus loss in growing chicks fed conventional and low-phytate soybean meals. Poult. Sci. 85: 661-668.

Jiang X.R., Luo F.H., Qu M.R., Bontempo V., Wu S.G., Zhang H.J., Yue H.Y., Qi G.H. (2013). Effect of non-phytate phosphorus levels and phytase sources on the growth performance, serum biochemical and tibial parameters of broiler chickens. Ital. J. Anim. Sci. 12: 375-380.

ISSN: 2283-3927

National Research Council (1994). Nutrient Requirements of Poultry, 9th rev. ed. Natl. Acad. Press, Washington, DC, USA.

Nelson T.S., Sheih T.R., Wodzinski R.J., Ware J.H. (1968). The availability of phytate phosphorus in soybean meal before and after treatment with a mold phytase. Poult. Sci. 47: 1842-1848.

Paik I.K. (2003). Application of phytase, microbial or plant origin, to reduce phosphorus excretion in poultry production. Asian-Aust. J. Anim. Sci. 16: 124-135.

Panda A.K., Rama Rao S.V., Raju M.V.L.N., Gajula S.S., Bhanja S.K. (2007). Performance of broiler chickens fed low non phytate phosphorus diets supplemented with microbial phytase. J. Poult. Sci. 44: 258-264.

Rutherfurd S.M., Chung T.K., Morel P.C., Moughan P.J. (2004). Effect of microbial phytase on ileal digestibility of phytate phosphorus, total phosphorus, and amino acids in a low-phosphorus diet for broilers. Poult. Sci. 83: 61-68.

Rutherfurd S.M., Chung T.K., Thomas D.V., Zou M.L., Moughan P.J. (2012). Effect of a novel phytase on growth performance, apparent metabolizable energy, and the availability of minerals and amino acids in a low-phosphorus corn-soybean meal diet for broilers. Poult. Sci., 91: 1118-1127.

SAS Institute (2008). SAS/STAT User's Guide, Version 9.2. SAS Institute, Inc., Cary, NC, USA.

Scott M.L., Nesheim M.C., Young R.J. (1982). Nutrition of the chicken. 3rd ed. Scott M.L. & Associates Publ., Ithaca, NY, USA.

Sebastian S., Touchburn S.P., Chavez E.R., 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.

Shieh T.R., Ware J.H. (1968). Survey of microorganisms for the production of extracellular phytase. J. Appl. Microbiol. 16: 1348-1351.

Viveros A., Brenes A., Arija I., 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.

Waldroup P.W., Kersey J.H., Saleh E.A., Fritts C.A., Yan F., Stilborn H.L., Crum Jr R.C., Raboy V. (2000). Nonphytate 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.

ISSN: 2283-3927