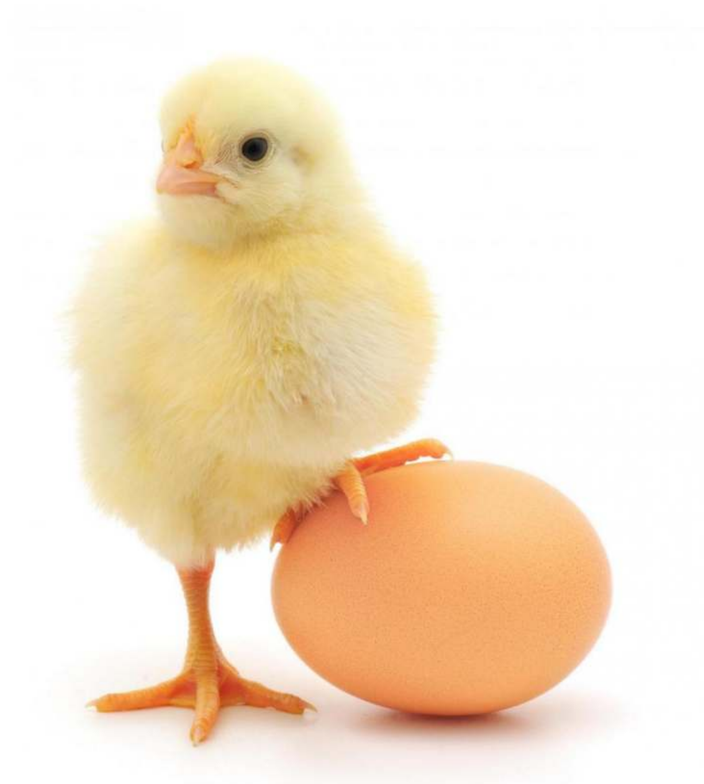
ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 5 (2): 162-168, 2006
ISSN 1682-8356

The Effect of Phytase and *Saccharomyces cervisiae* (Sc47) Supplementation on Performance, Serum Parameters, Phosphorous and Calcium Retention of Broiler Chickens

H.A. Ghasemi[1], A.M. Tahmasbi[1], Gh. Moghaddam[1], M. Mehri[2], S. Alijani[1], E. Kashefi[1] and A. Fasihi[1]
[1]Department of Animal Science, University of Tabriz, Iran
[2]Department of Animal Science, University of Tehran, Iran

Abstract: An experiment was performed to assess the potential of microbial phytase and *Saccharomyces cervisiae* (Sc47) on improving the utilization of phytate P and subsequently on broiler performance fed wheat-corn-soybean meal basal diet from 7 – 49 day of age. A 3×2×2 factorial arrangements of treatments were used: 3 levels of Sc47 (0.0, 0.1 and 0.2 % of diet); two levels of phytase (0 and 800 U / kg of diet) and two levels of nonphytate P (50% and 100% of NRC (1994) recommended). Each treatment contained 3 pens with 15 birds/pen. Measurements included body weight gain (BWG), feed conversion ratio (FCR), serum parameters, Tibia ash, P and Ca retention. During the experimental periods effects of Non phytate phosphorous (NPP) levels on all measured parameters was significant (P<0.01). Phytase inclusion at 800 U/kg of diet induced improvement (P<0.05) in BWG, FCR, tibia ash, P and Ca retention and also increased concentration of serum P and protein (p<0.05). Inclusion phytase on the diet containing the 50% NRC (1994) NPP resulted improvement on the utilization of phytate P and Ca by increasing P and Ca retention which led to an increase in toe ash content. The experiment demonstrated dietary supplementation of *Saccharomyces cervisiae* had a positive influence on the bird's performance. However, significant improvement (P<0.05) was observed in BWG and FCR during starter phase in chickens fed the diets containing *Saccharomyces cervisiae*. Maximum responses to live yeast achieved when broiler chicks were fed diet with 100% NRC (1994) recommended available phosphorous. In conclusion, the finding of this study suggested combination of supplemental phytase and live yeast (Sc47) had some beneficial effects on improving the nutritive value of broilers diets in particular in the low level of NPP.

Key words: Phytase, *Saccharomyces cervisiae*, non phytate phosphorous, broiler

Introduction

Dietary requirements for P and its availability in feedstuffs of plant origin are important issues in poultry nutrition. About of 60 to 70% of P in plant based ingredient commonly used for poultry diets occur as phytate P (myo-inositol hexaphosphate) which were poorly used by poultry (Perney *et al.*, 1993) due to lack of adequate amounts of endogenous phytase in their gastro-intestinal tracts to fully hydrolyze the phytate molecule (Ravindran *et al.*, 1995). In this condition, three main problems may poses for poultry: 1) additional requirement for inorganic phosphorous to diets 2) the excretion of large amounts of no utilized phosphorous in manure, and 3) reduction of availability of several other nutrients, such as Protein, Ca, Cu, and Zn by phytate (Yi *et al.*, 1996; Ceylan *et al.*, 2003). Consequently, phytase supplementation to poultry diets, to release the bound phosphorous and other nutrients, has been very beneficial in solving the aforementioned problems as shown in recent intensive research (Zyla *et al.*, 2000; Snow *et al.*, 2003). In other hand, the increase in P utilization and retention in poultry has great benefits associated with the environment. Various microorganisms such as fungi, yeasts and bacteria have to capacity produce phytase. The use of live yeast culture has been reported to release 75 to 80% of the phytate phosphorous within 4 hours *in vitro*, and to utilize P efficiently in P deficient diets fed to growing chickens (Thayer and Jackson, 1975). Kumprechtova *et al.* (2000) found that supplementation of *Saccharomyces cervisiae* into poultry diets have positive effect on nutrients utilization. The effects of *Saccharomyces cervisiae*, microbial phytase and their combination on bird's performance in the adequate or deficient P in the poultry diets has not fully investigated. Therefore, the purpose of the present research was to investigate the influence the NPP levels, supplemental phytase and live yeast (Sc47) on utilization of phytate P and broiler performance. Furthermore, the interaction of these three factors in broilers was examined.

Materials and Methods

Five hundred and forty-day old Ross broiler chicks were used from 7-49 days old to investigate the utilization of phytate P and broiler performance as influences by microbial phytase, live yeast and their combinations. Diets were formulated diet based on corn-wheat-soybean meal to meet either 50 or 100% of available

Table 1: Composition of the basal diets

	Experimental diets1(%)					
	Starter diet		Grower diet		Finisher diet	
Item	50% NRC	100% NRC	50% NRC	100% NRC	50% NRC	100% NRC
Ingredient Corn	42.32	41.32	47.64	47.64	52.29	52.29
Wheat	20.00	20.00	20.00	20.00	20.00	20.00
Soybean meal	28.97	28.97	23.03	23.03	18.80	18.80
Fish meal	2.00	2.00	2.00	2.00	2.00	2.00
Alfalfa meal	2.00	2.00	2.00	2.00	2.00	2.00
Fat meal (8000kcal/kg)	2.80	2.80	2.55	2.55	2.45	2.45
Dicalcium Phosphate[1]	0.27	1.28	0.21	1.14	0.08	93.0
Oyster shell	1.76	1.18	1.63	1.10	1.53	1.05
DL-Methionine	0.13	0.13	0.11	0.11	0.03	0.03
Lysine	0.00	0.00	0.09	0.09	0.07	0.07
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[3]	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Enzyme[4]						
Calculated composition						
ME, kcal/kg	3023.33	3023.33	3083.24	3083.24	3118.27	3118.27
CP (%)	21.65	21.65	20.28	20.28	18.54	18.54
Ca (%)	0.94	0.94	0.87	0.87	0.78	0.78
Total P (%)	0.50	0.68	0.47	0.63	0.43	0.58
NPP[5] (%)	0.21	0.42	0.19	0.38	0.17	0.34
Lysine (%)	1.31	1.31	1.11	1.11	0.95	0.95
Met + Cys (%)	0.88	0.88	0.69	0.69	0.58	0.58
Methionine (%)	0.56	0.56	0.41	0.41	0.31	0.311

Dicalcium phosphate 220 g/kg Ca and 187 g/kg P. [2]Supplied per kilogram of diet: 600 IU Vit A, 800 IU Vit D_3, 8mg Vit E, 2mg Vit B6, 8mg Vit B_{12}, 10mg Nicotin amid, 0.3mg Folic acid, 20 mg D-Biotin and 160 mg Choline Chloride. [3]Supplied per kilogram of diet: 32 mg Mn, 16 mg Fe, 24mg Zn, 2mg Cu, 800µg I, 200µg Co and 60 µg Se. [4]Enzyme sources were either phytase (Natuphos® 5000 which was added to each diet at levels of either 0 or 800 U/kg phyatse respectively) or *Saccharomyces cervisiae* (Sc47 which added to each diet at levels of 0.0, 0.1 and 0.2 % of diet) [5].Non-Phytate Phosphorous

phosphorous allowances recommended by NRC (1994) for different periods of study. Composition of the diets is shown in Table 1. Three replicate pens (fifteen birds per pen) of a completely randomized design were used in a 3×2×2 factorial arrangement of treatments with 3 levels of Sc47 (0, 0.1 and 0.2% of diets), 2 levels of phytase (0, 800 U /kg diet) and 2 levels NPP (50 and 100% NRC). Supplemented microbial phytase in the diet (800 unit of phytase per kg diet) considered to be sufficient for maximum economical release of P as recommended by some investigator (Kornegay *et al.*, 1996;Yi *et al.*, 1996; Yan *et al.*, 2001 and 2003).

The unit of phytase activity was defined as the amount of enzyme that liberates 1µ mol of inorganic phosphate/min from 5.1mM sodium phosphate at pH 5.5 at 37°C. Phytase was Natuphos® 5000 which was determined to contain 3645 U/g phytase activity and based on company information it had no detectable side enzyme activities.

At day 1 of age, chicks were randomly assigned to pens and fed recommended diets (NRC, 1994), without any supplementation. At the start of experiment (7 day-old) diets were formulated for starter (7-21d), grower (21-42) and finisher (42-49) phases, except for phosphorous which content either 100 or 50% NRC (1994) recommended for available phosphorous (Table 1). Dietary supplement were added to ration in the total period of experiment. Chicks were exposed to continuous fluorescent light. All diets were provided for *ad libitum* consumption in mash form and birds had unlimited access to water. Chickens were weighed at 7, 21, 42 and 49d on cage basis to determine weight gain. Feed consumption was measured daily, the refusals feed were discarded and feed efficiencies were calculated (total feed: total gain).

At the end of trial (49d), one bird was randomly selected from each pen and blood samples were taken from wing vein by using vacuum syringe. Blood serum was harvested by centrifugation (10 min at 3000× g) and stored at –20°C for later analysis. Serum samples were

Table 2: Effect of dietary treatments on body weight gain and feed conversion ratio

Diet	NPP[1] Levels	phytase Levels (U/kg diet)	Sc47[2] Levels (g/ kg diet)	Body weight gain (g) 7-21 d	21-42 d	42-49 d	7-49 d	Feed conversion ratio (g:g) 7-21 d	21-42 d	42-49 d	7-49 d
1	50	0	0	485.91	1164.90	428.96	2079.78	1.74	2.35	3.00	2.34
2	50	0	1	468.25	1202.57	435.70	2106.52	1.68	2.20	2.87	2.23
3	50	0	2	473.58	1221.57	432.90	2128.05	1.64	2.18	2.83	2.19
4	50	800	0	489.31	1389.14	476.57	2355.01	1.62	2.06	2.60	2.08
5	50	800	1	483.73	1406.07	485.91	2375.71	1.64	2.05	2.59	2.08
6	50	800	2	503.48	1412.99	485.11	2401.57	1.57	2.01	2.54	2.03
7	100	0	0	497.81	1402.65	488.36	2388.82	1.59	2.03	2.58	2.05
8	100	0	1	521.94	1412.44	492.83	2477.21	1.53	1.98	2.53	1.99
9	100	0	2	509.42	1425.31	476.24	2410.97	1.51	1.97	2.48	1.97
10	100	800	0	515.44	1480.61	607.14	2603.19	1.52	1.92	2.25	1.90
11	100	800	1	508.31	1431.29	508.30	2447.90	1.60	1.97	2.47	2.00
12	100	800	2	536.28	1413.52	498.67	2448.46	1.51	1.99	2.49	1.99
SEM				4.83	7.53	30.84	36.76	0.016	0.014	0.101	0.027
P-value				0.05	0.05	NS	NS	NS	NS	NS	NS
NPP levels											
50% NRC				484.04	1299.54	475.53	2241.11	1.65	2.14	2.27	2.16
100% NRC				514.87	1427.64	511.92	2454.43	1.54	1.98	2.47	1.98
P-value				0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
phytase level (FUT/ kg diet)											
0				492.82	1304.91	459.17	2256.89	1.62	2.12	2.72	2.13
800				506.07	1422.27	510.28	2438.64	1.58	2.00	2.49	2.01
P-value				0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Sc47 level (g/ kg diet)											
0				497.12	1359.33	500.26	2356.70	1.62	2.09	2.61	2.09
1				495.56	1363.09	480.69	2339.34	1.61	2.05	2.62	2.07
2				505.69	1368.35	473.23	2347.26	1.56	2.04	2.59	2.04
P-value				0.05	NS	NS	NS	0.05	0.05	NS	NS
InteractionNPP level × phytase				NS	0.01	NS	0.01	0.01	0.01	NS	0.01
NPP level × Sc47				0.05	0.01	NS	NS	NS	0.01	NS	0.05
Phytase × Sc47				0.01	0.01	NS	NS	0.01	0.01	NS	0.01
NPP level ×phytase × Sc47				0.05	0.05	NS	NS	NS	NS	NS	NS[1]

Non- phytate phosphorous, [2]*Saccharomyces cervisiae* NS indicated insignificant difference; P < 0.05 indicated significant difference

thawed at room temperature and its total protein, P and Ca were measured using a biochemical analyzer Kits. Also, one bird from each replicate was killed by cervical dislocation, and its left tibia bone was removed and kept frozen at -20°C until require for analysis. For determination tibia ash, the tibiae bones were boiled to remove any traces of flesh. They were solvent-extracted to remove fat, dried and ashed to measure bone ash percentage.

At 45d, one bird from each dietary treatment was selected and transformed to metabolic cages to determine P and Ca apparent digestibility. During days 46 to 48 the excreta samples were freeze-dried and sieved through a 1-mm screen to remove feathers and then were ground and packed in plastic bags for storage before analysis. Dry matter was determined according to AOAC (1984) procedures. Following a nitric-percholoric acid wet digestion, P concentrations were determined by Spectrophotometer (AOAC, 1984). Concentration of Ca was determined by using atomic absorption spectrophotometer and finally apparent retention of P and Ca were calculated.

The data for all response variables were analyzed as a completely randomized design based on 3×2×2 factorial arrangement using the General Linear Model (GLM) of SAS (1996). Differences between treatments were compared by the Duncan's multiple range test following ANOVA, and values were considered statistically differences at P<0.05. Results are reported as least square means with standard error.

Results

The effects of NPP level, phytase and Sc47 on body weight gain, feed conversion ratio are presented in Table 2. Overall diet effect along with the main and interaction effect are presented. Main effects of NPP levels and phytase supplementation on the BWG and FCR were significant (P<0.01) during experimental period. During starter period, BWG was significantly (P<0.01) affected by Sc47.No significantly response, however, was observed in BWG by addition of live yeast from 21-49 days of age.

Interaction between phytase, level of NPP and Sc47 on BWG was significant only during phytase was greater in the diets containing recommended level of P. Addition of both phytase and Sc47 in low P diets, significantly

Table 3: Effect of dietary treatments on tibia ash, P and Ca digestibility

Diet	NPP[1] Levels	phytase Levels (U/kg diet)	Sc47[2] Levels (g/ kg diet)	Tibia ash (%)	P retention (%)	Ca retention (%)
1	50	0	0	41.75	49.66	66.02
2	50	0	1	43.08	48.77	65.93
3	50	0	2	43.58	49.54	67.39
4	50	800	0	46.65	63.02	68.50
5	50	800	1	45.43	62.39	69.55
6	50	800	2	46.22	64.03	69.74
7	100	0	0	45.93	49.71	68.17
8	100	0	1	45.48	50.94	68.43
9	100	0	2	45.25	51.90	68.81
10	100	800	0	46.15	56.42	69.91
11	100	800	1	45.44	54.78	71.71
12	100	800	2	45.21	55.83	71.44
SEM				0.43	0.73	0.74
P-value				NS	NS	NS
NPP levels						
50% NRC				44.45	56.24	67.85
100% NRC				45.58	53.26	69.74
P-value				0.01	0.01	0.01
phytase level (FUT/ kg diet)						
0				44.18	50.09	67.49
800				45.85	59.41	70.14
P-value				0.01	0.01	0.01
Sc47 level (g/ kg diet)0				45.12	54.70	68.15
1				44.86	54.22	68.91
2				45.06	55.33	69.34
P-value				NS	NS	0.05
InteractionNPP level × phytase				0.01	0.01	NS
NPP level × Sc47				NS	NS	NS
Phytase × Sc47				NS	NS	NS
NPP level ×phytase × Sc47				NS	NS	NS

[1]Non- phytate phosphorous. [2]*Saccharomyces cervisiae*. NS indicated insignificant difference; P < 0.05 indicated significant difference

improved BWG (308.57 g) compare with unsupplemented diet in this ration.

However, at higher levels of P at which the dietary NPP level was sufficient, addition of phytase and Sc47 had no significant effect on BWG, accounting for the significant phytase × P level × Sc47 interaction.

Difference in FCR was noted (P<0.05) between chicks fed the three level of Sc47 only during starter and grower phases. Interaction between NPP level, phytase and Sc47 was not significant during period of study, but all binary interaction effects were significant (P<0.05) in the total of experiment. In contrast to the phytase, which had a higher effect on low NPP diets, a greater improvement in FCR was observed when Sc47 supplementation was added to the diets with high NPP level compared with diets containing low NPP level.

Effects NPP levels and phytase were significant (P<0.01) on tibia ash, P and Ca apparent retention (Table 3). Additionally, the 100% of NRC (1994) NPP level increased tibia ash and Ca retention but, decreased P retention compared the 50 % of NRC (1994) NPP level. Tibia ash and P retention did not significantly affect by Sc47 supplementation. However, chicks fed diet supplemented with Sc47 marginally had a higher tibiae ash compared with chicks fed diet without Sc47 supplementation. Increasing Sc47 in diets linearly improved Ca retention (P<0.05). Significant interaction (P<0.01) was found between phytase supplementation and levels of NPP on tibia ash and phosphorous apparent retention. Supplementation of phytase, significantly improved tibia ash and P retention when added to diets with low level of NPP; however this result was not clarified at normal level of NPP.

Serum protein, P and Ca were considerably affected by main effects of NPP level in diet (P<0.01). A significant increase in serum P and total protein was also observed in birds receiving phytase but no differences in concentration of serum Ca were found (Table 4).

Table 4: Effect of dietary treatments on Serum Parameters

NPP[1] Levels	phytase Levels (U/kg diet)	Sc47[2] Levels (g/ kg diet)	Serum Parameters Phosphorus (mg/100ml)	Calcium (mg/100ml)	Total protein (g/100ml)
50	0	0	41.75	49.66	66.02
50	0	1	43.08	48.77	65.93
50	0	2	43.58	49.54	67.39
50	800	0	46.65	63.02	68.50
50	800	1	45.43	62.39	69.55
50	800	2	46.22	64.03	69.74
100	0	0	45.93	49.71	68.17
100	0	1	45.48	50.94	68.43
100	0	2	45.25	51.90	68.81
100	800	0	46.15	56.42	69.91
100	800	1	45.44	54.78	71.71
100	800	2	45.21	55.83	71.44
SEM			0.43	0.73	0.74
P-Value			NS	NS	NS
NPP levels					
50% NRC			44.45	56.24	67.85
100% NRC			45.58	53.26	69.74
P-Value			0.01	0.01	0.01
phytase level (FUT/ kg diet)					
0			44.18	50.09	67.49
800			45.85	59.41	70.14
P-value			0.01	0.01	0.01
Sc47 level (g/ kg diet)					
0			45.12	54.70	68.15
1			44.86	54.22	68.91
2			45.06	55.33	69.34
P-value			NS	NS	0.05
Interaction					
NPP level × phytase			0.01	0.01	0.01
NPP level × Sc47			NS	NS	NS
phytase × Sc47			NS	NS	NS
NPP level ×Phytase × Sc47			NS	NS	NS

[1]Non -phytate phosphorous. [2]*Saccharomyces cervisiae*. NS indicated insignificant difference; P < 0.05 indicated significant difference

Although concentrations of P and Ca in serum were not affected by addition of live yeast in diets, however, Sc47 significantly (P<0.05) increased concentration of serum protein. At 100% of NRC (1994) NPP level, concentration of serum P, Ca and protein were higher than 50% NPP level. There was significant interaction between NPP level and Sc47 supplementation on serum Ca and protein.

Discussion

In nature, plants P exist mainly in a form of phytate so that availability of P in most feedstuff is less than 50% (NRC, 1994). Therefore, it is necessary to provide an adequate of this nutrient in broiler diets. Fortified diets with phytase enzyme can decrease the level of phytate P excretion and improve phytate utilization by birds (Jalal and Scheideler, 2001). Results of the present study demonstrate that the bird performance was maximally affected by increasing dietary phosphorous concentration. Broiler responded well to the recommended NPP by NRC (1994). When adequate amount of NPP (100% NRC) provided in the diets, bird exhibit an average 9.6 percent higher in body weight gain and FCR was decreased as 0.18 than deficient NPP diets (50% NRC).

Phytase supplementation improved body weight gain, feed conversion ratio, tibia ash, P and Ca retention indicating a better utilization of phytate and other dietary constituents. These results are in agreement with previous reports in broilers (Kornegay *et al.*, 1996; Yi *et al.*, 1996; Qian *et al.*, 1996; Yan *et al.*, 2001 and 2003).

Improvement in P and Ca retention was reported Qian *et* *al.* (1997). Phytase release P and Ca from the insoluble salts of phytic acid, and potentially makes Ca available for absorption in birds. In other hand, the effectiveness of microbial phytase for improves the utilization of phytase P and Ca by broilers is influenced by the Ca: P ratio (Qian *et al.*, 1997). However, the dietary requirement of Ca can be reduced in diets supplemented with phytase, which increase liberation of bounded nutrients in the phytate complex (Sebastian *et al.,* 1996, Qian *et al.*, 1996). In our study, Ca retention increased approximately 4% by fortified diets due to the inclusion phytase enzyme in the diets. This was an interesting finding that ought to be kept in mind in connection with the correct fixing of the Ca content in diet supplemented with phytase.

Thayer and Jackson (1975) reported that live yeast culture had improved the P utilization in growing chicks. The data from our experiment indicated that supplementation of diet with Sc47 trend to have a positive effects (although the differences between means were not significant) on the bird's performance and linearly increased BWG and decreased FCR. The lack of significant effects in bird's performance and blood parameters especially during the finishing periods due to Sc47 supplementation, despite of some improvements at starter periods, has been reported by several studies (Brake, 1991, Bradley and Savage, 1995). In contrast to phytase on improvement of P bioavailability, in our study, Sc47 (as a sources of enzyme for examined diets) did not produce the similar effects in all deliberate parameters. However, maximum responses to supplement of Sc47 achieved when broiler chicks were fed diet with 100% NRC (1994) recommended NPP. Nevertheless, more research is required to investigate higher level of Sc47 on diets containing different level of NPP in the diet.

It is generally accepted that bone mineralization, which measured by ash content of tibia, is a good indicator of P and Ca utilization. The finding from this study indicated that complementation of diets with phytase significantly improved bone mineralization of phytase. This finding is agreement with several investigators (Sebastian *et al.*, 1996 and 1997; Applegate *et al.*, 2003).

The blood measurements presented in Table 4 show clearly that serum P, Ca and protein responded to P adequacy and show that presence phytase and Sc47 supplementations influenced these parameters. Increases in concentrations of P, Ca and total protein in the serum were consisted with growth response.

From our present results, we concluded that supplementation of phytase alone or combination of phytase and Sc47, significantly improve phytate phosphorus utilization in broiler diets. This finding importance to the poultry industry since it may reduce a production cost and provides a strategy to reduction in environmental pollution due to phytate.

References

Applegate, T.J., R. Angel and H.L. Classen, 2003. Effect of dietary calcium, 25 Hydroxycholecalciferol, or bird strain on small intestine Phytase activity in broiler chickens. Poult. Sci., 82: 1140- 1148.

Association of Official Analytical Chemists, 1984. Official Methods of Analysis. 14th ed. Association of Official Analytical Chemists, Washington, DC.

Bradley, G.L. and T.F. Savage, 1995. The effect of autoclaving a yeast culture of *Saccharomyces cervisiae* on turkey pullet performance and retention of gross energy, and selected minerals. Anim. Feed. Sci. Tec., 55: 1-7.

Brake, J., 1991. Lack of effect of a live yeast culture on broiler breeder and progeny performance. Poult. Sci., 70: 1037-1039.

Ceylan, N., S.E. Scheideler and H.L. Stilborn, 2003. High available phosphorous corn and phytase in layer diets. Poult. Sci., 82: 789-795.

Jalal, M.A. and S.E. Scheideler, 2001. Effect of supplementation of tow different sources of phytase on egg production parameters in laying hens and nutrient digestibility. Poult. Sci., 80: 1463- 1471.

Kornegay, E.T., D.M. Denbow, Z. Yi and Ravindran, 1996. Response of broilers to graded levels of microbial phytase added to corn-soybean meal based diets containing three levels of non-phytate phosphorous. Br. J. Nutr., 75: 839-852.

Kumprechtova, D.K., P. Zobac and I. Kumprecht, 2000. The effect of *Saccharomyces cervisiae* SC47 on chicken broiler performance and nitrogen output. Czech. J. Anim. Sci., 45: 169- 177.

National Research Council, 1994. Nutrient Requirements of Poultry. 9 th Rev. Ed. National Academy Press, Washington, DC.

Perney, K.M., A.H. Cantor, M.L. Straw and K.L. Herkelman, 1993. The effect of dietary phytase on growth performance and phosphorous utilization of broiler chicks. Poult. Sci., 75: 69-81.

Qian, H., E.T. Kornegay and D.M. Denbow, 1996. Phosphorus equivalence of microbial Phytase in turkey diets as influenced by calcium to phosphorus levels. poult. Sci., 76: 37- 46.

Qian, H., E.T. Hornegay and D.M. Denbow, 1997. Utilization of Phytate phosphorus and calcium as influenced by microbial Phytase, Cholecalciferol, and the calcium: total phosphorus ratio in broiler diets. Poult. Sci., 76: 37- 46.

Ravindran, V., W.L. Bryden and E.T. Kornegay, 1995. Phytates occurrence, bioavailability and implications in poultry nutrition. Poult. Arain Bio. Rev., 6: 125-143.

SAS Institute, 1996. SAS/STAT User's Guide: Statistics, 1996 ed. SAS Institute, Inc., Cary, NC.

Sebastian, S., S.P. Touchburn, E.R. Chavez and P.C. Lague, 1996. Efficacy of supplemental microbial Phytase at different dietary calcium levels on growth performance and mineral utilization of broiler chickens. Poult. Sci., 75: 1516-1523.

Sebastian, S., S.P. Touchburn, E.R. Chavez and P.C. Lague, 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.

Snow, J.L., M.W. Douglas and C.M. Parsons, 2003. Phytase effect on amino acid digestibility in molted laying hens. Poult. Sci., 82: 474- 477.

Thayer, R.H. and C.D. Jackson, 1975. Improving Phytate phosphorus utilization by poultry with live yeast culture. Animal science Research Report MP- 94. Oklahoma State Univ., Still Water. OK.

Yan, F., J.H. Kersey and P.W. Worldroup, 2001. Phosphorus requirements of broiler chicken three to six week of age as influenced by Phytase supplementation. Poult. Sci., 80: 455- 459.

Yan, F., J.H. Kersey, C.A. Fritts and P.W. Waldroup, 2003. Phosphorus requirements of broilers chicks six to nine weeks of age as influenced by Phytase supplementation. Poult. Sci., 82: 294-300.

Yi, Z., E.T. Kornegay, V. Ravindran and D.M. Denbow, 1996. Improving phytate phosphorous availability in corn and soybean meal for broilers using microbial phytase and calculation of phosphorous equivalency value for phytase. Poult. Sci., 75: 240-249.

Zyla, K., A. Wikiera, J. Koreleski, S. Swiathiewicz, J. Pironen and D.R. Ledoux, 2000. Comparison of the efficacies of a Novel *Aspergillus niger* Mycelium with separate and combined effectiveness of phytase, Acid phosphates, and pectinase in dephosphorylation of wheat-based feeds fed to growing broilers. Poult. Sci., 79: 1434-1443.