

EFFECT OF PHYTASE SUPPLEMENTATION ON THE PERFORMANCE OF BROILERS GROWN TO MARKET WEIGHTS

H.A. ABDELLATIF* and AZZA M. KAMAL**

*Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Cairo University, Giza,

**Department of Biochemistry, Animal Health Research Institute, Dokki, Giza, Egypt.

Received: 1. 3. 2003.

Accepted: 12.4.2003.

SUMMARY

An experiment was conducted using day-old Hubbard® broilers in order to study the effect of microbial phytase on the performance of broilers in a commercial farm for 39 days. The control diet adequate in non-phytate phosphorus (0.5%) was fed to one house with 14751 birds. The experimental diet (low in non-phytate phosphorus 0.4%) was fed to another house of 15345 birds after being modified using the matrix values for Ronozyme® P CT (phytase). Parameters measured included body weight gain, feed conversion, mortality, profit per bird, tibia ash (total ash, calcium, phosphorus, magnesium and zinc percentages), serum calcium, phosphorus, alkaline phosphatase as well as fecal calcium and phosphorus output. Phytase supplementation (750 U/kg) to the low non-phytate phosphorus diet significantly improved body weight gain. Phytase-supplemented birds consumed more feed than the non-supplemented ones but feed conversion was

not different between the two groups as well as mortality %. However, phytase supplementation significantly ($P < 0.01$) increased serum calcium and phosphorus levels and reduced alkaline phosphatase. Furthermore, phytase supplementation significantly increased tibia weight, length and zinc content but had no effect on tibia total ash, calcium, phosphorus or magnesium content. Fecal calcium and phosphorus output was significantly ($P < 0.01$) reduced by 32.9% and 27.9% respectively in the phytase-supplemented group. Results suggest that dietary phosphorus can be reduced without seriously affecting the skeleton of broilers and that phytase would have other benefits by reducing the cost of the diet as well as improving birds liveweight gain, in addition to reducing environmental pollution as manifested by reduced fecal phosphorus output.

Key words: (Phytase, broilers, performance, phosphorus, calcium, tibia)

INTRODUCTION

Phosphorus is one of the most essential macro-mineral required by animals as it is involved in many important metabolic processes in the body.

Therefore, it is important that animals receive adequate supply of phosphorus in their diet. Phosphorus sources are the third most expensive ingredients in poultry diet after protein and energy sources. About 50-80% of phosphorus present in cereal grains and soybeans is in the form of phytate phosphorus (Jongbloed et al., 1991; Ravindran 1995 and Broz, 1997). The ability of poultry to utilize phosphorus bound to phytic acid (phytate phosphorus) is generally assumed to be poor (Ravindran et al., 1995; Kornegay, 1996). Phytic acid is believed to have anti-nutritive effects due to its ability to bind other nutrients as calcium, copper, magnesium, zinc (Vohra et al., 1965; Oberleas, 1973; Morris, 1986) proteins and lipids (Cosgrove, 1966). The bioavailability of phosphorus in corn and soybean meal for pigs and poultry ranges from 10-30% (Nelson, 1967; Calvert et al., 1978; Jongbloed and Kemme, 1990).

This low bioavailability of phytate phosphorus creates two problems, first the need to include inorganic phosphorus supplements to poultry diets second, the excretion of large amounts of phosphorus in manure which in turn constitutes an environmental hazard. The addition of exogenous phytase has been used to reduce the pollutant resi-

dues, through improving the utilization of phytate bound minerals in pig and poultry diets and decreasing the use of inorganic sources. The amount of phytate degraded by dietary phytase inclusion reduces the need for inorganic phosphorus addition by 1 to 1.2 g/kg in practical diets for pigs and poultry (Yi et al., 1994).

There has been a recent interest among poultry nutritionists in studying the effects of phytase on broiler performance. It was found that supplementing phytase to grower diets containing reduced levels of non-phytate phosphorus and calcium significantly improved performance and bone strength of broilers (Sohail and Roland, 1999). Namkung and Leeson (1999) showed that growth rate and feed conversion of birds fed the low P diets containing microbial phytase were comparable with or even better than those for birds fed the control diets. They also found that phytase improved the digestibilities of essential amino acids, which was also confirmed by Ravindran et al. (2001). Additional studies showed the beneficial effects of phytase in terms of improving P utilization via increased plasma phosphate and tibia ash content (Klünter and Steimle, 2001).

Most published studies evaluating the usefulness of microbial phytase in poultry feeding have been conducted using corn and soybean meal based diets. Corresponding information on diets with poultry byproducts in addition to corn and soybean meal is lacking. Therefore this trial was de-

signed in order to study the effect of dietary phytase supplementation on performance of broilers fed corn-soybean meal diet besides poultry by-products as an animal protein from day old until market weight.

MATERIALS AND METHODS

Birds, diets and experimental design:

In a commercial farm located in Alexandria province, 14751 day old broiler chicks (Hubbard®) served as control were fed a diet adequate in non-phytate phosphorus (0.51%) during the starter period and then 0.45% and 0.42% during the grower and finisher periods respectively. In another house the experimental group (15345 Hubbard® broiler chicks) were fed a diet lower in non-phytate phosphorus with 0.42% during the starter period and then 0.37% and 0.33% during the grower and finisher periods respectively. The experimental diet was supplemented with 750 phytase units /kg (Ronozyme® P CT) throughout the trial and modified on a least-cost basis by reducing the non-phytate phosphorus taking in consideration the matrix value for phytase. According to the international system for defining enzymes activity, one phytase unit is defined as the quantity of enzyme that liberates 1 μ mol of inorganic P/minute from 1.5 mM of sodium phytate at pH 5.5 and 37°C. The used diets are shown in Tables 1-3. Birds of both treatments were housed in controlled temperature houses with 24 hour illumina-

tion and presented with food and water ad libitum throughout the 39-d trial. Birds that died from both treatments during the study were weighed and production parameters were adjusted for mortality.

The variables measured were liveweight gain, feed intake, feed conversion ratio, fecal calcium and phosphorus output, serum calcium, phosphorus and alkaline phosphatase, tibia weight, length and ash (total ash, calcium, phosphorus, magnesium and zinc).

Serum calcium, phosphorus and alkaline phosphatase:

Serum was separated from blood samples collected from 50 birds per treatment on 21 d and 39 d of the experiment. Serum calcium levels were determined according to the method of Gindler and King (1972); serum phosphorus levels were determined according to the method of Goodwin (1970) while alkaline phosphatase activity was measured according to the method of Kind and King (1954).

Tibia bone analyses:

At 39 d of age, 20 birds per treatment were selected at random from each house and slaughtered. The right tibia was dissected by removing any adhering tissue then measured, weighed and dried at 105°C until a constant weight and then ashed in a

muffle furnace at 550°C. Tibia total ash, calcium, phosphorus, zinc and manganese were analyzed using atomic absorption spectrophotometer according to the method of (AOAC 1990).

Fecal calcium and phosphorus:

At 21 d and 39 d of age, fecal samples were collected from each house at different locations, dried and analyzed for calcium and phosphorus content.

Statistical Analysis:

Data were analyzed statistically by running the student-t test according to Snedcor and Cochran (1974) using Statview 512+ (1986) software program. Statements of probability are based upon ($P < 0.05$).

RESULTS AND DISCUSSION

Overall performance:

Liveweight gain was increased ($P < 0.01$) by dietary phytase addition (1.767 kg vs. 1.683 kg) as shown in Table 2. Such results could be explained in part by the increased phosphorus availability resulting from phytase supplementation. In addition, another reason for the improved weight gain was the possibility that phytase improved dietary quality and release of other trace minerals and amino acids that were bound to the phytate molecule. These results are supported by the results of Beers and Jongbloed (1992), Simon

et al. (1992), Qian et al. (1996), Sohail and Roland (1999), Namkung and Leeson (1999) and Zhang et al. (2000). Selle et al. (1999) reported an increase in weight gain in broilers fed meat and bone meal in broiler standard diet supplemented with 600 phytase U/kg over the non-supplemented ones, but this increase was insignificant. Contrary to our results, Zanini and Sazzad (1999) found no positive effect of phytase supplementation on body weight of broilers and such response was attributed to the large variation in mean values because of the small number of birds they used in their trial (96 day-old chicks).

The improved liveweight gains in our study were mediated primarily via increased feed intake or increased nitrogen retention (Selle et al. 1999). This may be suggestive of improvements in the utilization of nutrients other than phosphorus. Furthermore, Cabahug et al. (1999) recorded increases in the apparent ileal digestibility of amino acids in response to phytase supplementation. Keis and Selle (1998) reviewed the negative effects of phytic acid on protein utilization by broilers which is the rationale for such responses to added phytase.

Phytase supplementation (750 U/kg) resulted in increased feed intake ($P < 0.05$) (Table 2) compared to the control birds (3.51 vs 3.37 kg/bird). Similar results were obtained by Denbow et al.

1995) who reported that both added inorganic phosphorus and phytase increased feed intake with the greatest response to added phytase at the lowest non-phytate phosphorus level. Food conversion (FCR) was numerically better for the phytase-supplemented birds but such difference was insignificant ($P>0.05$). Similar results were also recorded by Selle et al. (1999) who found a significant increase in feed intake as a result of phytase supplementation without influencing the FCR. In our study, the magnitude of response to phytase could have been higher if we had other diets with much lower non-phytate phosphorus. Such diets with very low non-phytate phosphorus are impractical and would have caused economic losses to the producer.

FCR for the phytase-supplemented group was numerically better than the control group (1.99 vs. 2.0) similar results were also reported by Swiatkiewicz et al. (2001) and Cabahug et al. (1999) who also found that food/gain ratio was linearly decreased with added phytase in both the medium and high phytic acid diets. The marginal improvement in FCR in our study may be also suggestive of improvements in the utilization of nutrients other than phosphorus.

Mortality was not influenced by dietary treatment as should be expected and we did not have such low dietary non-phytate phosphorus to seriously

harm the birds' health. Such results were reported by others (Yan et al., 2001).

The profit per bird in the phytase-supplemented group was better than in the control group (2.34 Egyptian pounds vs. 1.34) as shown in Table 2.

Such improvement in profit could result in higher net returns to the farm when phytase is used in broiler production. Based on the prices of the diet ingredients, modification of the experimental diet resulted in a reduction in ingredients cost of feeding. Such reduction in the cost of feeding coupled with improved liveweight gain in the phytase-supplemented group resulted in increased profit per bird. Similar findings of improved feeding cost were obtained by Selle et al (1999). Therefore, phytase has the potential to reduce the cost liveweight gain depending on relative feed ingredients prices.

Serum calcium, phosphorus and alkaline phosphatase:

Serum calcium, phosphorus and alkaline phosphatase values for the two sampling periods (21 d and 39 d) are presented in Table 3. Dietary supplementation of birds with 750 U/kg phytase resulted in significantly ($P<0.01$) increased serum levels of calcium and phosphorus. In addition phytase supplementation significantly ($P<0.01$) reduced serum alkaline phosphatase levels.

Atia et al. (2000) found that phytase supplementation to growing turkeys significantly improved serum phosphorus level and reduced serum alkaline phosphatase when birds were fed calcium adequate diets and low in non-phytate phosphorus.

This confirms that phytase supplementation was also able to correct blood values for such nutrients when diets were formulated with low non-phytate phosphorus. Kl_{nter} and Steimle (2001) reported that phytase supplementation increased the concentration of inorganic phosphate and reduced the concentration of calcium in the plasma compared to the control birds. The response became greater with increasing dietary inclusion level of the phytase up to 750 U/kg. In the present study, such favorable results in response to phytase supplementation are in agreement with liveweight gains and bone measurements, which confirm that more nutrients were retained and made more available for utilization.

Tibia bone measurements:

Tibia bone measurements are presented in Table 4. Dietary phytase supplementation significantly increased tibia weight ($P < 0.01$) and length ($P < 0.05$) than the control ones. However, there was a trend regarding the total ash % which was numerically higher in the phytase supplemented group ($P = 0.09$), such result probably was due to the variation of results within the treated group.

Tibia calcium, phosphorus and magnesium were not significantly affected as a result of dietary phytase supplementation. On the other hand, tibia zinc was significantly higher in the phytase-supplemented group ($P < 0.05$). Other workers have demonstrated the impact of phytase on tibia parameters. Yan et al. (2001) reported that phytase supplementation improved tibia ash markedly at lower levels of non-phytate phosphorus; at higher levels of non-phytate phosphorus that approached or surpassed the amount needed to maximize tibia ash, the addition of phytase was of little or no benefit, as should be expected. Qian et al. (1996) found that dietary supplementation of broiler diets with 1050 U of phytase significantly increased the tibia length while total ash percentage was not affected as well as tibia magnesium content, but zinc content was significantly increased. Zanini and Sazzad (1999) reported that dietary phytase addition led to an improvement in the DM intake, resulting in higher intakes of nitrogen, phosphorus, calcium and zinc. It is suggested that this effect is related to improved retention of such nutrients and to the increase in the calcium and zinc concentrations in the tibia. The results suggest that stronger bones of broilers fed diets containing high level of non-phytate phosphorus or supplemented with phytase resulted from better mineralization of bone.

Fecal calcium and phosphorus output:

Fecal calcium and phosphorus data are shown in Figure 1. Phosphorus is a critical component in poultry waste that could leave the site of application and can cause environmental pollution (Kornegay, 1996). Supplementing poultry diets with phytase seems to be a practical approach to decrease environmental pollution. In our study as shown in Figure 1, fecal calcium and phosphorus was significantly ($P < 0.01$) reduced by 32.9 % and 27.9% respectively in the phytase-supplemented group. Our findings generally confirmed the earlier results obtained by Yi et al. (1996), which showed that dietary supplementation of phytase improved phosphorus retention by 8.3 to 22.0% when compared with negative control diets.

Much larger reductions in phosphorus excretion were observed when phytase-supplemented groups were compared with positive control diets (Zhang et al. 2000). Kornegay (1999) suggested that when phytase is added at 500 to 750 U/kg diet, phosphorus excretion is reduced to 31.8 to 35.7% compared with phosphorus excretion when recommended phosphorus levels are fed. Our re-

sults confirm the hypothesis that dietary phytase improves calcium and phosphorus retention as manifested by reduced fecal output of these two nutrients.

Conclusion

Phytase supplementation significantly improved the growth performance of broiler chickens over the non-supplemented birds. The results also demonstrated that phosphorus and calcium utilization was improved as manifested by increased serum calcium and phosphorus levels due to phytase supplementation. Furthermore, phytase-supplemented birds excreted less calcium and phosphorus than non-supplemented birds, which indicates better calcium and phosphorus retention and can reduce environmental pollution. Tibia parameters were also improved with phytase supplementation. Therefore, broilers can be fed a diet lower in non-phytate phosphorus after taking in consideration the matrix values for phytase without jeopardizing normal bone development. Finally, phytase supplementation can reduce the cost of feed by reducing the amount of phosphorus supplements to the feed.

Table (1): Composition of diets fed to broiler chickens during starter, grower, and finisher phases

Ingredients	Starter control %	Starter + Phytase %	Grower control %	Grower + Phytase %	Finisher control %	Finisher + Phytase %
Corn, yellow	53.61	54.41	54.81	55.97	60.42	61.11
SBM(44%CP)	35.29	35.15	33.14	32.11	26.75	26.62
Vegetable oil	4.22	3.88	5.46	5.06	4.73	4.51
Poultry byproducts	3	3	2.71	3.30	5.0	5.0
Bone meal	2.69	1.98	2.35	1.61	1.97	1.07
Limestone	0.35	0.71	0.65	1.0	0.51	0.86
Salt	0.3	0.3	0.3	0.3	0.3	0.3
Premix*	0.3	0.3	0.3	0.3	0.3	0.3
DL-Methionine	0.24	0.24	0.28	0.28	0.20	0.20
(75%)Liquid Phytase	0	0.03	0	0.03	0	0.03
Calculated Analysis						
Energy, Kcal / kg	3042	3036	3135	3134	3184	3185
Protein%	21.87	21.87	20.86	20.87	19.90	19.9
Calcium%	1.1	1.0	1.1	1.0	0.95	0.88
Non-phytate P,%	0.51	0.42	0.45	0.37	0.42	0.33

* Premix supplied / kg feed , vitamin A 12 MIU , vitamin D3 2.2 MIU, vitamin E 10,000 mg , vitamin k3 2,000 mg , vitamin B1 1,000 mg , vitamin B2 5,000 mg , niacin 30,000 mg , panatothenic acid 10,000 mg , vitamin B6 1,500 mg , vitamin B12 10 mg , folic acid 1,000 mg , biotin 50 mg , choline chloride 500,000 mg , selenium 100 mg , copper 10,000 mg , iron 30,000 mg , manganese 60,000 mg , zinc 50,000 mg , iodine 1,000 mg , cobalt 100 mg.

Table (2): Effect of phytase supplementation on overall performance of broiler chickens

Parameter	Control	Phytase supplemented
Number of birds	14751	15345
Phytase, U/kg diet	0	750
Liveweight gain, kg	1.683	1.767**
Feed consumption (kg/bird)	3.37	3.51*
FCR	2.0	1.99
Mortality, %	5.24	5.28
Profit/bird (EGP)	1.34	2.34

* (P<0.05), ** (P<0.01), EGP = Egyptian Pound

Table (3): Effect of phytase supplementation on serum calcium, phosphorus and alkaline phosphatase levels in broilers

Age (days)	Control			Phytase- Supplemented		
	Ca (mg/dl)	P (mg/dl)	ALP (U/L)	Ca (mg/dl)	P (mg/dl)	ALP (U/L)
21	9.90	5.10	389.0	10.95**	6.34**	371.5**
39	9.92	5.30	357.63	12.00**	6.64**	347.5**

Values are means, ** (P <0.01) compared to the control, ALP= alkaline phosphatase.

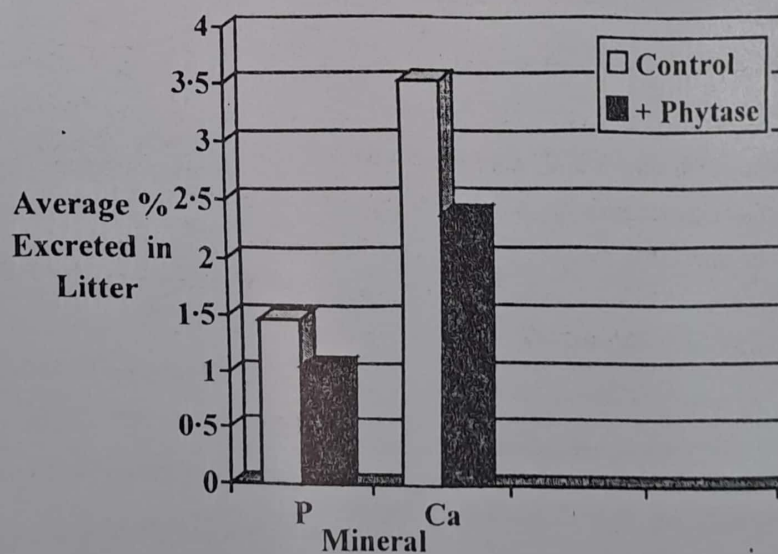


Fig. (1): Average % excreted calcium and phosphorus in the manure of broilers.

Table (4): Effect of phytase supplementation on tibia measurements of broilers at slaughter (39 days of age)

Parameter	Control	Phytase (750 U/kg)	P Value
Weight, g	16.38 ±0.64	19.87 ±0.74	P<0.01
Length, cm	10.04 ±0.18	10.78 ±0.09	P<0.05
Ash %	37.30 ±0.45	38.26 ±0.61	P=0.09
Calcium, %	42.6 ±0.42	43.0 ±0.32	P=0.82
Phosphorus, %	11.7 ±0.27	12.3 ±0.33	P=0.73
Magnesium, %	3.0 ±0.12	3.4 ±0.3	P=0.84
Zinc, mg %	3.28 ±0.68	5.4 ±0.74	P=0.03

Values are means ± SE.

ACKNOWLEDGMENT

The authors would like to express their appreciation for the full cooperation of Dr. A. Soliman Chairman of British Egyptian Company for General Development (Galina). Special thanks to Engineers Rami Ahmed, Fouad Amer and Hamdi El Said for their help during the experiment.

REFERENCES

- Association of Official Analytical Chemists 1990. Official Methods of Analysis. Washington, DC, AOAC.
- 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. Poultry Sci. 79:231-239.
- Beers, S. B. M., and Jongbloed, A. W. 1992. Effect of supplementary *Aspergillus niger* phytase in diets of piglets on their performance and apparent digestibility of phosphorus. Anim. Prod. 55:425-430.
- Broz, J. 1997. Application of phytase in animal feeds in Proceedings 5th Regional ASA-Feed Technology Workshop, May 25-29, 1997. Pp 1-7, The Westin, Changmai, Thailand.
- Cabahug, S., Ravindran, V., Ravindran, G., 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. Poultry Sci. 40:660-666.
- Calvert, C. C., Besecker, R. J., Plumlee, M. P., Cline, T. R. and Forsyth, D. M. 1978. Apparent digestibility of phosphorus in barley and corn for growing swine. J. Anim. Sci. 47:420-426.

- sgrove, D. J. 1966. The chemistry and biochemistry of inositol polyphosphates. *Rev. Pure Appl. Chem.* 16:209-224.
- Denbow, D. M., Ravindran, V., Kornegay, E. T., Yi, Z., and Hulet, R. M. 1995. Improving phosphorus availability for broilers by supplemental phytase. *Poultry Sci.* 74:1831-1842.
- Hindler, E. M. and King, J. D. 1972. Rapid colorimetric determination of calcium in biological fluids with methyl thymol blue. *Am. J. Clin. Path.* 58:376-382.
- Woodwin, J. E. 1970. Quantification of serum inorganic phosphorus, phosphate and urinary phosphate without preliminary treatment. *Clin. Chem.* 16:776-780.
- Longbloed, A. W., Everts, H. and Kemme, P. A. 1991. Phosphorus availability and requirements in pigs. In *Recent Advances in Animal Nutrition 1991*, Butterworth, England, pp 65-80.
- Longbloed, A. W. and Kemme, P. A. 1990. Apparent digestible phosphorus in the feeding of pigs in relation to availability, requirement and environment. 1. Digestible phosphorus in feedstuffs from plant and animal origin. *Neth. J. Agric. Sci.* 38:567-575.
- Kies, A. K. and Selle, P. H. 1998. A review of the antinutritional effects of phytic acid on protein utilization by broilers. *Proceedings of the Australian Poultry Science Symposium*. Ed. D. Balnave. 10:28-131.
- Kind, P. R. and King, E. G. 1954. Estimation of plasma phosphatase by determination of hydrolyzed phenol with amino-antipyrine. *J. Clin. Path.* 7:322.
- Klinter, A. M and Steimle, C. 2001. Effect of *Peniophora lycii* phytase in broiler chickens fed a low phosphorus diet. 13th Euro. Symp. Poult. Nutr. Oct. 2001, Blankenberge, Belgium. 188-189.
- Kornegay, E. T. 1996. Phytase in poultry and swine phosphorus management, *Proceedings of the Eastern Nutrition Conference*, pp. 71-113 (Nova Scotia, Canada).
- Kornegay, E. T. 1999. A review of phosphorus digestion and excretion as influenced by microbial phytase in poultry. *BASF Technical Symposium, January 16, Atlanta, GA.* BASF Corp., Mount Olive, NJ.
- Morris, E. R. 1986. Phytate and dietary mineral bioavailability. Pages 57-76 in: *Phytic acid: Chemistry and Applications*. E. Graf, ed. Pilatus Press, Minneapolis, MN.
- Namkung, H. and Leeson, S. 1999. Effect of phytase enzyme on dietary nitrogen-corrected apparent metabolizable energy and the ileal digestibility of nitrogen and amino acids in broiler chicks. *Poultry Sci.* 78:1317-1319.
- Nelson, T. S. 1967. The utilization of phytate phosphorus by poultry- A review. *Poultry Sci.* 46:862-871.
- Oberleas, D. 1973. Phytates. Pages 363-371 in: *Toxicants Occurring Naturally in Foods*. National Academy of Sciences, Washington, DC.
- Qian, H., Veit, H. P., Kornegay, E. T., Ravindran, V. and Denbow, D. M. 1996. Effects of supplemental phytase and phosphorus on histological and other tibial bone characteristics and performances of broilers fed semi-purified diets. *Poultry Sci.* 75:618-626.
- Ravindran, V., Kornegay, E. T., Potter, L. M., Ogunabameru, B. O., Welten, M. K., Wilson, J. H. and Potchanakorn, M. 1995. An evaluation of various response criteria in assessing biological availability of phosphorus for broilers. *Poultry Sci.* 74:1820-1830.
- Ravindran, V., Selle, P. H., Ravindran, G., Morel, P. C. H., Kies, A. K. and Bryden, W. L. 2001. Microbial phytase improves performance, apparent metabolizable energy, and ileal amino acid digestibility of broilers fed a lysine-deficient diet. *Poultry Sci.* 80:338-344.

- Selle, P. H., Ravindran, V., Pittolo, P. H. and Bryden, W. L. 1999. An evaluation of microbial phytase in sorghum-based broiler diets. *Proc. Aust. Poult. Sci. Sym.* 11, 97-100
- Simon, P. C. M., Jongbloed, A. W., Versteegh, H. A. J., and Kemme, P. A. 1992: Improvement of phosphorus availability by microbial phytase in poultry and pigs. Pages 18-37 in: *Proceedings of the Nutrition for the Nineties Conference*, Pitman-Moore, Bloomington, MN.
- Snedcor, G. W. and Cochran, W. C. 1974. *Statistical Methods* 6th ed. Iowa State Univ. Press., Ames IA.
- Sohail, S. S. and Roland, D. A. 1999. Influence of Supplemental phytase on performance of broilers four to six weeks of age. *Poultry Sci.* 78:550-555.
- Swiatkiewicz, S., Koreleki, J. and Koslacz, J. 2001. Effect of phytase on the bioavailability of zinc from inorganic and organic sources in broiler chickens. 13th Eur. Symp. Poult. Nutr. Blankenberge, Belgium 175-176.
- Vohra, P. Gray, G. A. and Kratzer, F. H. 1965. Phytic acid-metal complexes. *Proc. Soc. Exp. Biol. Med.* 120:447-449.
- 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. *Poultry Sci.* 80:455-459.
- Yi, Z., Kornegay, E. T. and McGuirk, A. 1994. Replacement values of inorganic phosphorus by microbial phytase for pigs and poultry. *J. Anim. Sci.* 72:330 (Abstract).
- Yi, Z., Kornegay, E. T., Ravindran, V., and Denbow, D. M. 1996. Improving phytate phosphorus availability in corn and soybean meal for broilers using microbial phytase and calculation of phosphorus equivalency values for phytase. *Poultry Sci.* 75:240-249.
- Zanini, S. F. and Sazzad, M. H. 1999. Effects of microbial phytase on growth and mineral utilization in broilers fed on maize soybean-based diets. *Br. Poult. Sci.* 40:348-352.
- Zhang, Z. B., Kornegay, E. T., Radcliffe, J. S., Denbow, D. M., Veit, H. P. and Larsen, C. T. 2000. Comparison of genetically engineered microbial and plant phytase for young broilers. *Poultry Sci.* 79:709-717