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Effects of Phytase on Broilers Performance and Body Status of Phosphorus

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Abstract:

This experiment was conducted to investigate the effect of microbial phytase supplementation on broilers performance, nutrient digestibility, carcass cuts and body status of Ca and P. A total of 200 day-old Cobb-500 chicks were used in the experiment. Birds were partitioned into five experimental groups of 40 birds each treatment was composed of 4 replicates with 10 birds in each. The control group was fed a commercial starter and finisher diet. The second treatment group was fed a phosphorus deficient diet, while the third, fourth and fifth treatments groups were fed a phosphorus deficient diets plus the microbial phytase. Phytase enzyme was incorporated at levels 1000, 2000 and 3000 PU/kg feed for the last three treatments, respectively. In the last week of experiment, four birds from each replicate were used in metabolic trial. However, at time of termination of the experiment, the same birds were killed for carcass cuts and tibia ash content investigations. Results of the experiment showed that the addition of phytase enzyme to P- deficient diets significantly improved ($P<0.5$) broilers performance. However, feed intake, feed conversion ratio and tibia minerals were significantly increased ($P<0.05$) in birds fed P- deficient diets supplemented with phytase. Phytase supplementation had no significant effect on carcass cuts and dressing percent compared to

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birds fed the low P diets. Also, results of this investigation showed that phytase enzyme increased (P<0.05) the digestibility of dry matter, crude protein and ash.

الملخص:

تم إجراء هذه التجربة للتعرف على اثر استخدام أنزيم الفايتيز على الصفات الإنتاجية لدجاج اللحم من نوع كوب 500 ، و على معدلات استهلاك العلف و التحويل الغذائي و نسب التصافي ، إضافة إلى صفات الذبائح و معاملات الهضم و مستويات الكالسيوم و الفسفور في عظم الدجاج. و قد استخدم في التجربة 200 صوص لحم بعمر يوم واحد في التجربة التي استمرت 42 يوما. و تم توزيع الصيصان على خمس مجموعات احتوت كل منها 40 صوصا، بينما قسمت كل مجموعة إلى أربع مكررات احتوت كل منها 10 صيصان. و قد كانت المجموعات كالتالي: المجموعة الأولى هي مجموعة الشاهد و التي تشابة العلائق التجارية المستخدمة في المزارع ، بينما كانت المجموعة الثانية تحوي نسب اقل من الفسفور المقررة لصيصان اللحم، أما المجموعات الثلاث الأخرى فقد احتوت نسب من الفسفور مشابهة للمجموعة الثانية إلا انه أضيف إليها أنزيم الفايتيز بمعدل 1000، 2000 و 3000 وحدة لكل كغم من العلف، على الترتيب. علما ان ذات المعاملات كانت في كل من العلف البادئ و النهائي. و قد تم عمل تجربة هضم في الأيام الثلاثة الأخيرة من التجربة حيث تم اخذ اربعة صيصان من كل من مكررات المجموعات ، و التي تم ذبحها على عمر 42 يوما من اجل دراسة بعض خصائص الذبائح و مستوى الكالسيوم و الفسفور في العظم. و قد بينت النتائج أن للأنزيم المضاف تأثير معنوي على معدلات الوزن عندما أضيف إلى العلائق الفقيرة بالفسفور ابتداء من الأسبوع الرابع، كما انه ادى الى زيادة معنوية في استهلاك العلف للصيصان المغذاة على العلائق الفقيرة بالفسفور أيضا. و كان للأنزيم نفس الأثر على معدلات التحويل الغذائي . هذا و لم يكن لاضافة الانزيم اثر على قطع الذبائح و مكونات الجهاز الهضمي. و بينت التجربة أن اضافة أنزيم الفايتيز الى العلائق الفقيرة بالفسفور قد أدى إلى زيادة معدلات الهضم لكل من المادة الجافة، البروتين و الرماد الخام. هذا و يمكن الاستنتاج انه من الممكن توفير كمية لا يستهان بها من النفقات على مصادر الفسفور حيث من الممكن إضافة كميات اقل من هذه المصادر لدى استخدام أنزيم الفايتيز.

Introduction:

Phytate is the form in which large portion of phosphorus is present in plant feed ingredients. This makes it difficult for non ruminants to gain their requirements out of being fed with these ingredients (Rezaei, et al., 2007). Phytate can bind minerals and proteins in aqueous medium (Sebastian, et al., 1997). Phytase can help in improving the availability of phytate bound phosphorus and reducing phosphorus levels in excreta from intensive livestock operations. Nelson (1967) and Kornegay (1999) reported that phytase supplementation improved the utilization of phytate P derived from plant feedstuffs, and decreased excretory P by approximately one-third without depressing performance.

Phytic acid was considered as the major storage form of phosphorus. Phosphorus from phytic acid is of great importance as this acid has a high P content (28.2%), and the major portion of poultry and pig diets consists of plant derived ingredients, where high levels of phytic acid is available. The ability of poultry and pigs to use phytate

P is poor (Ravindran, et al., 2006; Wu, et al., 2003; NRC, 1994) due to insufficient quantities or lack of intestinal phytase secretion. As a result of this, large amounts of P are excreted in feces causing an environmental hazards, especially in areas of intensive livestock operations. the focus of recent research was on effects of phytase enzyme during the starter phase of broilers, however, effects on growing and finishing phases are lacking.

The objectives of the present study were to investigate the influence of phytase enzyme supplementation on the performance, digestibility, carcass merits and P status (tibia ash contents) of broiler chickens fed corn-soybean based diets.

Materials and Methods

Ration preparation:

An experimental ration was formulated to meet all nutrient requirements as specified by the last edition of National Research Council (NRC, 1994) for chicken and designated as the control diet. Another ration was also formulated to meet the requirement except for phosphorus and designated as P-low diet (Table 2 and 3).

Rations used in the experiment were:

Diet 1: Control diet contains the recommended levels of Ca and P, with no phytase enzyme.

Diet 2: Diet low phosphorous with no phytase enzyme.

Diet 3: low phosphorous diet which contains 1000 PU/Kg diet enzymes.

Diet 4: low phosphorous diet which contains 2000 PU/Kg diet enzymes.

Diet 5: low phosphorous diet which contains 3000 PU/Kg diet enzymes.

Table 1. Composition and chemical analysis of the 5 starter experimental rations used in the experiment.

Diet	Control	P-low	P-low.+ 1000PU */kg	P-low.+ 2000PU/kg	P-low.+ 3000PU/kg
Diet composition %					
Corn	57	57	57	57	57
Soybean meal	37.5	37.5	37.5	37.5	37.5
Oil	3	3	3	3	3
Limestone	1	1.32	1.32	1.32	1.32
Di-calcium Phosphorous	0.75	0.42	0.42	0.42	0.42
Premix	0.75	0.75	0.75	0.75	0.75
Phytase enzyme	0	0	1000	2000	3000
Chemical analysis%					

Dry matter	90.1	90	90	89.9	89.9
Crude protein	22	22.1	21.9	22	22.2
Crude fiber	4.25	4.25	4.25	4.25	4.25
Crude fat	5.46	5.46	5.46	5.46	5.46
Ash	5.04	5.04	5.04	5.04	5.04
Calcium	0.77	0.77	0.77	0.77	0.77
Phosphorous	0.4	0.34	0.34	0.34	0.34
ME, kcal/kg	2800	2850	2830	2810	2820

Contents Premix Per 1 Ton Feed: Vitamin A 12 IU, Vitamin D3 3 IU, Vitamin E 50 IU, Vitamin K3 2.5g, Vitamin B1 1g, Vitamin B2 7mg, Panototic Acid 14mg, Niacin 37mg, Vitamin B6 3mg, Vitamin B12 10mg, Folic Acid 1mg, Biotin 150 mg, Cholin 200mg, Cobalt 0.20mg, Copper 15mg, Iron 20mg, Manganese 80mg, Iodine 1.20mg, Selenium 0.20mg, Zinc 50mg, Lysine 1500mg.
*Phytase unit.

Table(2) Composition and chemical analysis of the 5 finisher experimental rations used in the experiment

Diet	Control	P-low	P-low+ 1000PU/ kg	P-low+ 2000PU/kg	P-low.+ 3000PU/kg
Diet composition %					
Corn	58	58	58	58	58
Soybean meal	32	32	32	32	32
Oil	5.4	5.4	5.4	5.4	5.4
Limestone	1.86	2.6	2.6	2.6	2.6
Di-calcium Phosphorous	1.37	0.59	0.59	0.59	0.59
Premix	1.37	1.37	1.37	1.37	1.37
Phytase enzyme	0	0	1000	2000	3000
Chemical analysis%					
Dry matter	90	90.1	89.9	90	90
Crude protein	19.48	19.48	19.48	19.48	19.48
Crude fiber	3.88	3.88	3.88	3.88	3.88
Crude fat	7.93	7.93	7.93	7.93	7.93
Ash	4.87	4.87	4.87	4.87	4.87
Calcium	1.29	1.3	1.3	1.3	1.3
Phosphorous	0.5	0.36	0.36	0.36	0.36

ME, kcal/kg	3000	3050	3030	3010	3020

Contents Premix Per 1 Ton Feed: Vitamin A 8.5 IU, Vitamin D3 2.5 IU, Vitamin E 50 IU, Vitamin K3 2mg, Vitamin B1 0.80 mg, Vitamin B2 6 mg, Panototic Acid 11mg, Niacin 30 mg, Vitamin B6 2.40 mg, Vitamin B12 8 mg, Folic Acid 0.80 mg, Biotin 150 mg, Cholin Chloride 200 mg, Cobalt 0.20 mg, Copper 15 mg, Iron 20 mg, Manganese 80 mg, Iodine 1.20 mg, Selenium 0.20 mg, Zinc 50 mg, Methionin 1100 mg, Lysine 1200 mg.

Performance experiment:

A total 200 of one day-old broiler chicks (Cobb 500) were bought from a local hatchery (Poultry Company of Palestine, Tulkarm, Palestine). Chicks were immediately transferred to the experimental site and divided into five dietary treatment groups of 40 chicks in each. Each group was composed of 4 replicates with 10 chicks in each. Chicks were housed on floor of a suitable size house and managed as any commercial broiler flock. Chicks were weighed at weekly basis till the end of the experiment which lasted for 42 days. Feed intake, body weight and mortality were weekly recorded, and weight gain and feed conversion efficiency were then calculated.

Metabolism trial

A metabolic trial of 6 days duration was conducted during the last three days of feeding trial. This trial included an adaptation period of 3 days prior to collection. During the metabolic trial a total collection of daily feed intake and feces from 4 birds of each treatment was performed. Feed and feces samples were kept for later chemical analysis.

Carcass cuts preparation and sampling:

At termination of the feeding trial, 8 chicks were taken randomly from each feeding group. Chicks were killed according to the routine practices adopted in commercial broiler slaughter house. Total cool carcass weight was recorded from each bird then each carcass was split into its cuts, breast, and thighs where each cut weight was recorded. Weights of wings, neck, head and feet were also recorded. The tibia of each killed bird was removed and frozen for later Ca and P analysis.

Chemical analysis:

Feed and feces were analyzed for dry matter (DM), crude protein (CP), fiber, crude fat (CF), Ca and P according to the A.O.A.C (1995) procedures. Tibia Ca and P contents were determined using the flame photometry procedure.

Statistical analysis:

All data were analyzed by ANOVA using the linear model procedure of SAS (SAS, 1988) to determine the effect of addition of phytase enzyme to broiler rations on body weight development, feed intake, feed conversion, and carcass cuts and toe ash contents.

Results and Discussion

Broiler performance:

The effect of phytase enzyme supplementation on the broilers chicks is shown in Table 3. Reducing P level in the second treatment in both starter and finisher diets depressed body weight starting from week 2, compared to control and diets supplemented with different levels of phytase. This lower body weight was due to the deficiency of P in the broilers fed the lower level of P which is lower than the recommended levels for broilers during starter and finisher periods (NRC, 1994). This effect of P deficiency was also reported in broilers (Sohail and Roland, 1999; Fernandez, et al., 1999; Bozkurt, et al., 2006; Mondal, et al., 2007) and ducks (Orban, et al., 1999).

Phytase supplementation at levels of 1000, 2000 and 3000 PU/kg in both starter and finisher diets solved the problem of P deficiency and resulted in birds average body weights similar to control. Results of this experiment also are in agreement with those of Qian, et al., (1997), Huff, et al. (1998), Namkung and Leeson, (1999), Zyla, et al., (2000) and Bozkurt, et al., (2006) which reported that the growth rate and feed conversion ratio of broilers fed low P diets containing phytase were comparable or even better than those obtained for broilers fed the standard P diets. These results supported the concept that phytase was improving P availability, and P level can be lowered in corn-soybean meal based broiler starter and finisher by the addition of phytase.

Table 3. Average weekly body weights (g) development of broilers on different treatments.

Age, weeks	Control	P-low	P-low+1000PU/kg	P-low+ 2000PU/kg	P-low+ 3000PU/kg
0	46.5	46.5	46.5	46.5	46.5
1	114.6a	106b	108.9ab	108.9ab	106.9b
2	221.1a	197.5b	217.1a	216.8a	217.6a
3	372.1a	328.6a	357.9a	347.1a	352.6a
4	849a	738b	837.5a	849.8a	862.8a
5	1399.2a	1184.1b	1399.2a	1442.4a	1415a
6	2012a	1568.7b	2011.2a	1991.9a	2016.4a

Rows of different letters means significantly different (P<0.05)

Feed intake:

Feed intake of broilers fed P-deficient diets supplemented with phytase at different levels was similar to those fed control diet. Phytase enzyme supplementation improved (P<0.05) feed intake in broilers fed a P-deficient diets. The results indicated that phytase at levels of 1000 PU/kg and higher released phytate P that was utilized for growth in a similar manner as would P supplied by dicalcium phosphate (Table 4). Similar findings were reported by Mondal, et al., (2007) when broilers were fed with P-deficient diets supplemented with phytase at levels higher than 500 PU/kg. However, phytase levels lower than 500 PU/kg had no impact feed intake and feed conversion efficiency.

Table 4. Average daily feed intake (g) of broilers under different treatments

Age/ weeks	Control	P-low	P-low+ 1000PU/kg	P-low+ 2000PU/kg	P-low+ 3000PU/kg
1	19.21a	17.4b	18.28a	19.06a	18.65a
2	33.19a	28.71b	30.45a	35.21a	33.17a
3	41.1a	40.98b	42.2a	42.15a	43.23a
4	78.6a	76.15b	78.32a	79.96a	80.07a
5	121.46a	113.8b	117.44a	116.46a	121.86a
6	151.18a	114.55b	146.14a	141.77a	149.19a

Rows of different letters means significantly different (P<0.05)

Similar intake trends were observed for duck, turkey and layer diets as reported by (Orban, et al., 1999; Atia, et al., 2000; Ciftci, et al., 2005; Mondal, et al., 2007), respectively.

Feed conversion ratio:

Phytase supplementation at different levels improved (P<0.05) feed conversion ratio of broilers at weight of marketing compared to with low P diets (Table 5). Similar findings were reported by previous research (Huff, et al., (1998), Sohail and Roland, (1999), Ravindan, et al., (2006), Bozkurt, et al., (2006) and Mondal et al. (2007) who reported that phytase supplementation to broiler diets caused numerical improvement in feed efficiency of broilers fed a P-deficient diets fed without phytase.

Table 5. Feed conversion ratio of broilers under different treatments

Age/weeks	Control	P-deficient	P-def. +1000PU/kg	P-def. +2000PU/kg	P-def. +3000PU/kg
Feed Conversion ratio	1.583a	1.833b	1.520a	1.589a	1.599a

Rows of different letters means significantly different (P<0.05)

Dressing and carcass cuts percentages

The parts investigated were thighs, neck, head, back, legs, wings, and breast. Phytase supplementation had no effect on percentages of all cuts (Table 6). These results agrees with previous findings of Angel, et al., (2007) but opposite to those of Pillai, et al., (2006) who showed that phytase supplementation significantly increased percentages of most of carcass merits compared to P-deficient diets.

Table 6. Carcass cuts of broilers under different treatments(% of live weight).

Organs	Control	P-low	P-low +1000PU/kg	P-low +2000PU/kg	P-low +3000PU/kg
Neck	4.6	4.7	4.1	4.7	4.3
Thighs	20.8	22.1	20.3	20.6	20.2
Head	2.1	2	1.99	2.1	1.9
Back	13.9	12.3	12.8	13	13.6
Legs	4.8	5	4.4	4.2	4.1
Wings	7.2	8	7.8	7.6	7.9
Breast	26.6	24.5	24.7	24.3	25.7

The dressing percentage

As shown in table (7) the dressing percent of the birds was not affected by phytase supplementation. However, dressing percent values reported in this experiment were higher to values reported in previous research in broilers fed commercial rations (Khawaja, 2003; Rabayaa, 2003).

Table 7. Dressing percentage of broilers under different treatments

Parameter	Control	P-deficient	P-def.+ 1000PU/kg	P-def+ 2000PU/kg	P-def+ 3000PU/kg
Live weight (g)	2012	1568	2011	1991	2016
Carcass weight (g)	1489	1145	1468	1453	1472
Dressing percent (%)	74	73	73	73	73

Mineral content of tibia:

The effect of microbial phytase supplementation to low P diets on mineral content is shown in table (8). The percentage of broilers tibia crude ash was significantly increased by the addition of dietary phytase. This agrees with the previous studies dealing with broilers (Sabestian, et al., 1997; Zyla, et al., 2000; Mondal, et al., 2007), pekin ducks (Orban, et al., 1999) and turkeys (Atia, et al., 2000). However, it disagrees with others (Fernandez, et al., 1999; Bozkurt, et al., 2006).

Phytase supplementation to diets increased the content of Ca and P in the tibia compared to diets containing low P. This is a good indication of increased availability of P from phytase mineral complex by the action of phytase (Sabestian, et al., 1996; Mondal, et al., 2007). Our findings are similar to previous work with broilers and ducks, in which dietary phytase increased tibia ash and P percentages.

Bone Zn level was increased ($P<0.05$) when phytase was added in the diet. This result is in agreement with those reported by Zanini and Sazzad, (1999) and Mondal, et al., (2007), where they reported an increase in the concentration of Ca and Zn in the tibia by phytase supplementation. Phytase can decrease Zn availability by chelating divalent Zn which increases its concentration in bone tissues.

Table 8. Tibia minerals in broilers under different treatments, %

Control	P-low	P-low	P-low+ 1000PU/kg	P-low+ 2000PU/kg	P-low+ 3000PU/kg
Ash	9.0a	6.0b	9.2a	10.2a	10.7a
Ca	0.92a	0.81b	1.1a	1.1a	1.0a
P	1.1a	0.9b	1.4a	1.2a	1.2a
Zn	0.22a	0.12b	0.22a	0.27a	0.25a

Rows of different letters means significantly different (P<0.05)

Effect of phytase supplementation on digestibility:

Phytase tended to increase (P<0.05) apparent digestibility of dry matter, crude protein and crude ash (Table 9). These results can be explained by the fact that phytase enzyme had a positive influence on gastrointestinal tract digestive enzymes that leads to the increase in digestibility observed in birds fed with P-deficient diets. These results are in agreement with previous findings on broiler (Ravindran, et al., 1999; Rutherfurd, et al., 2004; Onyange, 2005; Mondal, et al., 2007).

Table 9. Digestibility of nutrients in broilers under different treatments, %.

Trait	Control	P-low	P-low+ 1000PU/kg	P-low+ 1000PU/kg	P-low+ 1000PU/kg
DM	83.0a	80.1b	83.2a	82.9a	83.2a
Crude protein	81.0a	78.1b	82.1a	82.4a	81.8a
Ash	82.6a	79.4b	82.0a	82.4a	82.6a

Rows of different letters means significantly different (P<0.05)

Conclusion and Recommendations:

The study showed that addition of phytase enzyme had a positive effect on birds performance and feed conversion ratio. Costs of broilers rations might be reduced as commercial sources of P in rations are reduced. However, more research is needed to support these findings.

References:

1. **AOAC.** (1995) Official Methods of Analysis Association of Officials Analysis Chemist Vol, -1,16 Ed., AOAC international, Arington, USA.

2. **Angel, R., Saylor, W. W., Mitchell, A. D., Powers, W., and Applegate, T. J.,** (2007). Effect of dietary phosphorous, phytase, and 25-hydroxycholecalciferol on broiler chicken bone mineral-

ization, nutrient utilization, and excreta quality of broiler chickens, Poult. Sci. 87: 1200-1211.

3. **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. Science., 79:231–239.

4. **Bozkurt, M., Cabuk, M. and Alcicek, A.** (2006) The effect of microbial phytase in broiler grower diets containing low phosphorous, energy and protein. J. Poult. Sci., 43: 29-34.

5. **Ciftci, M., Bestami D. and M. Ali Azman, M.** (2005) Effects of microbial phytase supplementation on feed consumption and egg production of laying hens. International Journal of Poult. Science. 4 (10): 758-760.

6. **Fernandes, J.I.M., Lima, J., Mendonca, C. X., Mabe, I., Albuquerque, R. and P.M. Leal, P. M.** (1999) Relative bioavailability of phosphorus in feed and agricultural phosphates for poultry. Poult. Sci., 78: 1729-1736.

7. **Huff, W. E., Moore, P. A., JR., Waldroup, P. W., Waldroup, A. L., Balog, J. M., Huff, G. R., Rath, N. C., Daniel, T. C. and Raboy, V.** (1998) Effect of Dietary Phytase and High Available Phosphorus corn on broiler chicken performance. Poult. Science. 77:1899–1904.

8. **Khawaja, Z**. (2003) Utilization of citrus pulp in broiler rations. Master's thesis. Faculty of Graduate Studies, An Najah National University.

9. **Kornegay, E. T**. (1999) Feeding to reduce nutrient excretion: Effects of phytase on phosphorus and other nutrients. Pages 461– 490 in Biotechnology in the Feed Industry. Proc. Alltech's 15th Annu. Symp. T. P. Lyons and K. A. Jacques, ed. Nottingham University Press, Nottingham, UK.

10. **Mondal, M. K., Panda S., and Biswas, P**. (2007) Effect of microbial phytase in soybean meal based broiler diets containing low phosphorous. International Journal of Poultry Science., 6 (3): 201-206, 2007.

11. **Namkung, H., and Leeson. S.**(1999) Effect of phytase enzyme on dietary nitrogen-corrected apparent metabolizable energy and ileal digestibility of nitrogen and amino acids in broiler chicks. Poult. Sci. 78:1317–1319.

12. **National Research Council**. (1994) Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

13. **Nelson, T. S.** (1967) The utilization of phytate phosphorus by poultry-A review. Poult. Sci. 46:862–871.

14. **Onyango, E. M., Bedford M. R., and Adeola, O.** (2005) Efficacy of an evolved Escherichia coli Phytase in diets of broiler chicks. Poult. Science. 84:248–255.

15. **Orban, J.L., Adeola O. and Strashine, S.** (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.

16. **Pillai, P. B., Conner- Dennie, T. O., Owens C. M., and Emmert, J. L.** (2006) Efficacy of an E. Coli Phytase in broiler fed adequate or reduced phosphorus diets and its effects on carcass characteristics. Poultry Sci. 85: 1200-1211.

17. **Qian, H., Kornegay, E. T., and Denbow, D. M**. (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.

18. **Rabayaa, E.** (2003) Utilization of olive cake in broilers ration. Master's thesis. Faculty of Graduate Studies. An Najah National University.

19. **Ravindran, V., Bryden, W. L., and Kornegay, E. T.**(2006) Phytates: Occurrence, bioavailability and implications in poultry nutrition. Poult. Avian Biol. Rev. 6:125–143.

20. **Rezaei, M., Borbor, S., , Zaghari M., and Teimouri, A.** (2007) Effect of Phytase supplementation on nutrients availability and performance of broiler chicks. International Journal of Poultry Science. 6 (1): 55-58

21. **Rutherfurd, S. M., Chung, T. K., Morel P. C. H., and 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. Poultry Science. 83:61–68.

22. **SAS User's Guide: Statistics Version 6.03, 4th Ed.**, (1988) SAS Inst., Cary, NC.

23. **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. Poultry Science. 76:1760–1769.

24. **Sohail, S. S., and Roland, D. A.**(1999) Influence of supplemental phytase on performance of broilers four to six weeks of age. Poult. Sci. 78:550–555.-

25. **Wu, Y. B., Pierce, , J. Hendriks, W.H., and Ravindran, V.** (2003) Comparison of in vitro nutrient release by three enzyme preparations in wheat-and maize-based diets. Proc. Aust. Poult. Sci. Symp. 15:114–118.

26. **Zanini, S. F., and M. H. 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.

27. **Zyla, K., Koreleski, J., Swiatkiewicz Wikiera, A., Kujawski, M., Piironen J., and Ledovx, D. R.** (2000) Effects of phosphorolytic and cell wall-degrading enzymes on the performance of growing broilers fed wheat based diets containing different calcium levels. Poult. Sci., 79: 66-67.