EFFICACY OF A LOCALLY PRODUCED MICROBIAL PHYTASE FROM *ENTEROBACTER SAKAZAKII* ASUIA273 ON BODY WEIGHT AND HEMATO-BIOCHEMICAL CONSTITUENTS IN BROILER CHICKENS

R. Islam[1], I. Aini[1], K. Azhar[2], A. R. Omar[1], Anis Shobirin Meor Hussin[3] and F. Yasmin[1]

[1]Faculty of Veterinary Medicine, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[2]Department of Animal Science, Faculty of Agriculture, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[3]Department of food Technology, Faculty of food Science & Technology, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

SUMMARY

An experiment was carried out in broiler chicks fed different doses of locally produced microbial phytase supplementation to observe their growth performance as health status, and to investigate the changes of hematological and biochemical values. A total of 144 chicks (Cobb) at one-day old were allocated to 4 treatment (T) groups with 12 cages comprising 3 replicates, each cage containing to 12 birds. Experimental formulating diets arranged with 4 levels of 0, 500, 1000 and 1500 phytase enzyme unit (FTU/kg^{-1}) as considered as T_1, T_2, T_3, T_4 respectively. They were maintained formulating diet on these dietary treatments from 1 to 42 d of age with feed and water made available for ad libitum consumption. At 1 week interval 2 birds from each treatment were weight through out experimental period for assessing the growth performance. For determining the changes of hematological (RBC, Hb, PCV, MCV, MCHC, WBC, Heterophil, Eosinophil, Basophil, Lymphocyte, Monocyte, Thrombocyte and Icterus Index) and biochemical (Albumin, Total Protein, ALT, ALP, AST, GGT, LDH, Cholesterol, Triglyceride, Glucose, Ca, P, Na, K, Cl, Urea, Creatine and Uric acid) values at the age of 6 weeks randomly selected 2 birds were slaughtered and blood were collected. Data were subjected to analysis of variance (ANOVA) using the least significant difference (LSD) by PC-SAS software (SAS Institute, 2009). Data showed that body weight was not affected at periods of 1st and 2nd weeks of age among different treatment groups. But, at ages from 3rd to 6th weeks, weight gains at four treatment groups were increased almost sequentially and consistantly, and had been showed more different and significant ($p \leq 0.05$) increased at 4th and 5th weeks of age from the control. No significant and constant treatments effects were observed on blood and biochemical parameters except eosinophil. Accordingly, it can be recommended to use an uncentrifuged microbial phytase in broiler diet during the period from 4th – 5th weeks of age, to achieve increased weight gain without changing hemato-biochemical parameters.

INTRODUCTION

Phytase (myo-inositol-hexakisphosphate phosphohydrolase) is the only recognized enzyme that is capable of catalyzing the phytin (phytic acid and phytate) in feeds to release inorganic phosphorus (IP) as well as inositol (Nelson, 1967), and also to loosen trace minerals, protein, amino acid, and starch that bound with phytin. As more P is removed from phytate leading to more breakdown of intact IP-6, the less able it is to bind or chelate minerals, starch or proteins either directly or via ionic bridges (Selle and Ravindran, 2007). Decreasing the binding of these compounds through the use of phytase may directly improve the bioavailability and digestibility not only of phosphorus and divalent or trivalent cations (Ca, P, Mg, Zn, Cu, Co, Mn and Fe), but also indirectly increase energy and nitrogen utilization (Selle et al., 2000; Kornegay et. at, 1999; Han et al., 1998; Pallauf and Rimbach, 1997; Harland and Morris, 1995; Graf, 1986; Thompson, 1986; Wise, 1983; Reddy *et al.*, 1982). Eventually body weight gain of an animal is enhanced by applying this enzyme in diet. Recently, it has been suggested that phytate alone is more of a potent antinutrient than previously thought, and as such its presence results in a significant loss of endogenous nutrients and energy in the form of mucins, intestinal cells and perhaps pancreatic enzymes (Cowieson et. al., 2004).

Phytic acid (myo-inositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate), an organic phosphate, is a phosphorylated cyclic sugar alcohol. The anion form of phytic acid, phytate is the form present in all plants. In mature seeds, phytic acid is present as a complex salt of calcium (Ca), magnesium (Mg), potassium and possibly protein and starch and this complex or chelated molecule is known as phytin (Lott, 1984). As a matter of fact the primary constituents of diets for animals are plant-based ingredients containing anti-nutritive components of phytic acid, and about two-thirds of the P of plant origin is present as phytic acid in the form of myo-inositol hexakis dihydrogen phosphate (NRC, 1994). In addition to phytic acid has strong chelating

potential and forms a wide variety of insoluble salts with di as well as trivalent cations at intestinal p^H and potentially render the mineral unavailable to the animal (Pallauf and Rimbach, 1997). Indeed, monogastric animals (poultry, swine, pre-ruminant calves) including human cannot synthesize phytin degrading enzyme "phytase". To meet dietary P requirement of these animals, inorganic P (dicalcium phosphate) or exogenous phytase are commonly added to commercial diet. However, di-calcium phosphate supplementation is not only expensive but also leads to environmental problems by over supplementation. Excess P from the feces is easy to access ground water, rivers, lakes and oceans, and can lead to mortality of aquatic animals by stimulating algae growth (Musapuor *et al.*, 2006; Sharple *et al.*, 1993; Liu *et al.*, 1998). So, addition of phytase as a feed additive to the diet largely for poultry and swine, and to some extent for fish has been shown to enhance the bioavailability of phytate P as well as to improve the utilization of other nutrients that are bound to plant phytate, and offers interesting opportunities for farmers to reduce feed costs and lessen environmental pollution of minerals.

For these mentioned reasons at present phytase is being used randomly in poultry industry to get more production and reduce P pollution. Although nutrients digestibility are important measures of any dietary changes in animal body; growth performance, hematologic values, biochemical constitutes are generally more sensitive than nutrients bioavailability for evaluating animal's health status. But, research in these contests at Malaysia is the very limited, and also having some contradictions among several researchers. So, more studies that are comprehensive are needed to elucidate the consequence of phytase in animal body. Therefore, the current experiment was planned with the objective of to assess the effect of locally produced microbial phytase supplementation on the body weight, investigation the changes of hematologic values, biochemical constituents.

MATERIALS AND METHODS

Phytin Degrading Enzyme "Phytase"

The current experiment was conducted at faculty of veterinary medicine of University Putra Malaysia. Tested material (rice bran fermented uncentrifuged phytase synthesized from *Enterobacter sakazakii* ASUA 273) was obtained from Standards and Industrial Research Institute of Malaysia (SIRIM).

Birds, Feeding and Management

In this research, a total number of 144, one-day-old male broiler chicks (Cobb strain) of nearly similar live body weight were obtained from a commercial hatchery. The birds were housed in an environmentally controlled automatic climatic chamber for 6 weeks with continuous lighting and controlled ventilation. Temperature was maintained at 30-32^{0}C for the first week and then gradually reduced according to normal management practices until a temperature of at around 25^{0}C which was maintained during remaining period. The chicks were randomly assigned to 1 control and 3 experimental groups comprising 3 replicates of 12 birds each, and were placed in separate cages. There were fed a basal diet grouped T_1 (control) or the basal diet supplemented with 500 FTU kg^{-1}, 1000 FTU kg^{-1} and 1500 FTU kg^{-1} grouped as T_2, T_3 and T_4 respectively. The enzyme "phytase" was added just prior to give feed to chicks at a day. The basal diet was formulated to cover nutrient requirements of broiler chicks as recommended by NRC (1994). The ingredients and calculated analysis of the experimental basal diets are shown in Table 1. Feed in a dry mash form and fresh water were offered ad libitum basis consumption throughout the experimental period.

Sampling and Measurement

For assessment of growth performance, at 1st, 2nd, 3rd, 4th, 5th, 6th, and 7th week of age, before giving feed, two (2) birds from each treatment (8 birds per replicate) were selected and picked up randomly, and average body weights (BW) were recorded by digital balance. At the age of 6 weeks the, bloods were collected from the slaughtering chickens into vacutainer tubes with anticoagulant lithium heparin to obtain whole blood for measurement of hemato-biochemical values. After estimation of hematological parameters, blood samples were centrifuged at 5000rpm for 10 minutes and serum was collected and stored at -20^{0}C. The DLC (Heterophil, Eosinophil, Basophil, Lymphocyte and Monocyte) was enumerated manually and other parameters (TEC, Hb., PCV, MCV, MCHC, TLC, Thrombocyte, Ic Index and TPP) were measured by hematology analyzer (Abbott CELL-DYN 3700 Hematology Analyzer, GMI) using commercial reagents. Although Na, K and Cl were determined using electrodes, other biochemical constituents (Albumin, Total Protein, ALT, ALP, AST, GGT, LDH, Cholesterol, Triglyceride, Glucose, Ca, P, Urea, Creatine and Uric acid) were determined

using available commercial kits with the help of chemistry analyzer (HITACHI 902, Japan).

Table 1: Ingredients and nutrient composition of the experimental basal diets

Ingredients	Amount (gm kg^{-1})
Corn grain (dent yellow)	430
Rice bran (bran with germ)	100
SBM (seeds without hulls)	370
Corn oil (refined)	51
Methionine	2
NaCl	4.5
Ca carbonate	28.5
Ca phosphate	11
Vitamins	2
Trace minerals	1
Total	1000
Nutrient composition (calculated)	Total value
Metabolizable energy (Kcal/Kg)	3200
Crude protein (%)	23
Ca (%)	1.4
Cl (%)	0.3
Total P (%)	0.7
Non phytate P (%)	0.34
Glycine + Serine (%)	2.1
Leucine (%)	1.9
Methionine + Cystine	0.37
Threonine	0.86

Statistical Analysis

All the experiments were conducted using completely randomized design (CRD) with three replications. The data were subjected to analysis of variance (ANOVA) and tested for significance using the least significant difference (LSD) by PC-SAS software (SAS Institute, 2009). Differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Growth performance

The average body weight gain of chicks at 1st, 2nd, 3rd, 4th, 5th and 6th weeks fed on different phytase doses are presented in table 2.

Values having the same latter(s) in a column do not differ significan tly at the 5% level of probability.

The main effects data indicated that body weight gains were not affected at periods of 1st and 2nd weeks of age among different treatment groups. But, at ages from 3rd to 6th weeks, weight gains at four treatment groups were increased gradually, and had been showed more different and significant ($p \leq 0.05$) increased at 4th and 5th weeks of age from the control.

Table 2: Effect of phytase on body weight at weekly interval

Phytase doses	Body weight gain (mean)					
	1st week	2nd week	3rd week	4th week	5th week	6th week
0 FTU/Kg of feed	117.167[a]	315.00 [a]	645.50 [a]	1139.67[b]	1881.67[b]	2680.8 [a]
500 FTU/Kg of feed	116.667 [a]	312.17 [a]	660.67 [a]	1295.33[a]	2073.67[a]	2704.7 [a]
1000 FTU/Kg of feed	124.000 [a]	305.17 [a]	665.00 [a]	1279.50[ab]	2107.17[a]	2718.3 [a]
1500 FTU/Kg of feed	125.667 [a]	295.67 [a]	685.50 [a]	1298.50[a]	2163.83[a]	2754.0 [a]
$LSD_{0.05}$	10.096	81.493	80.05	141.92	176.92	260.67

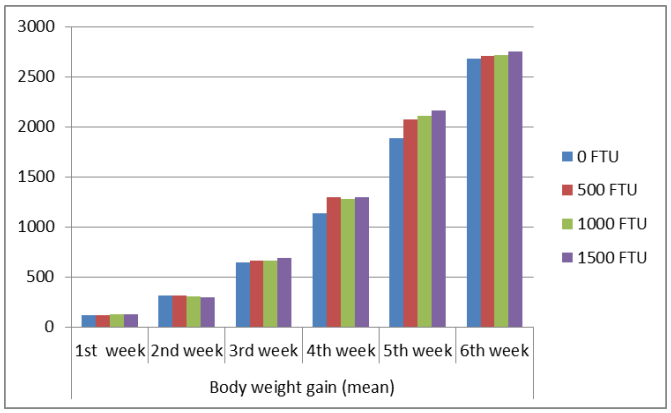

Figure 1: Effect of phytase on body weight at weekly interval

It would be concluded that supplementation of phytase to a diet had a greater effect on growth performance at the age of 4th and 5th weeks than earlier and latter ages of broiler chickens. This was likely because birds were at a more vigorous growth stages at 4th and 5th weeks of ages than at 1st, 2nd, 3rd and 6th weeks of ages. On the other hand these contrasting results may be due to a number of factors including phytase quality (source and storage), ingredients (types, source and phytate content), dietary characteristics (processing, CP, ME, mineral contents). Indeed, these data suggest that performance of broiler chickens may be increased in diets supplemented with phytase, which is generally correlate with previous works (Nelson et al., 1968; 1971; Simons et al., 1990; Zhu et al., 1990; Broz et al., 1994; Kornegay and Denbow, 1996; Kornegay et al., 1996; Mitchell and Edwards, 1996a; 1996b; qian et al., 1996, 1997; Sebastian et al., 1996a, 1996b; Biehl and Baker, 1997; Gordon and Rolan, 1997; Huff et al., 1998; Viveros et al., 2002).

No obvious clinical symptoms were manifested until 5th weeks of age. But, later age at 6th weeks, few birds with phytase supplemented groups were showing slight weight loss and simply mild lameness. This symptom might be due imbalance Ca:P ratio that leads to rickets, suggest that at 6 weeks of age P requirement would be lower than the earlier for bone mineralization. So, to maintain Ca:P ratio at blood level Ca is withdrawn from bone, and bone would be softening. It is evident that uncentrifuged phytase from rice bran fermented organisms contain more P leads to disturbance of Ca:P ratio. In spite of some shortcomings it can be explained that the efficacy of phytase in broiler chicks is more obvious at 4th and 5th weeks of age compare to other ages.

It is often difficult to define the optimal level of uncentrifuged phytase supplementation because this involves not only levels of both dietary phytate P and non-phytate P but also levels other minerals and the specific activity of the enzyme product used. Moreover, the physiological status of the birds affects its response to phytase. In the present study, the results between the 3 levels of phytase supplementation for growth performance indicate that the level of supplementation was more economically beneficial at 500 FTU/kg of feed for this particular phytase source under the conditions specified in this experiment. This is consistent with the report of Zhou et al. (2008).

Hematological parameters:

Effect of phytase enzyme supplementation on the hematological parameters of control and enzyme treatment groups of broiler chicken at the age of 6th weeks are depicted in table 3.

Table 3: Effect of phytase on hematological parameters in broiler chickens

Parameters	Units	Levels of Phytase				$LSD_{0.05}$
		0 FTU/Kg of feed	500 FTU/Kg of feed	1000 FTU/Kg of feed	1500 FTU/Kg of feed	
TEC	**$\times 10^{12}$/L**	2.60^{a}	2.50^{a}	2.53^{a}	2.52^{a}	0.15
Hb	**g/L**	135.33^{a}	128.66^{a}	127.66^{a}	128.00^{a}	9.95
PCV	**L/L**	0.31^{a}	0.31^{a}	0.30^{a}	0.30^{a}	0.02
MCV	**fL**	120.8^{a}	123.0^{a}	119.8^{a}	119.2^{a}	5.28
MCHC	**g/L**	432.0^{a}	419.7^{a}	421.0^{a}	426.8^{a}	18.88
TLC	**$\times 10^{9}$/L**	28.43^{a}	25.57^{a}	31.43^{a}	30.42^{a}	10.90
Heterophil	**%**	35.33^{a}	37.00^{a}	35.17^{a}	34.17^{a}	7.78
Eosinophil	**%**	0.103^{a}	0.045^{b}	0.027^{b}	0.045^{b}	0.67
Basophil	**%**	4.74^{a}	4.24^{a}	5.68^{a}	4.44^{a}	2.13
Lymphocyte	**%**	56.50^{a}	56.33^{a}	58.17^{a}	59.17^{a}	7.01
Monocyte	**%**	4.00^{a}	3.33^{a}	3.50^{a}	4.33^{a}	2.85
Thrombocyte	**$\times 10^{9}$/L**	4.42^{a}	3.21^{a}	3.58^{a}	2.20^{a}	4.95
Ic. Index	**unit**	2.00^{a}	3.00^{a}	2.00^{a}	2.00^{a}	1.63
Pl. Protein	**g/L**	36.17^{a}	34.50^{a}	31.67^{a}	32.33^{a}	7.96

Values within a row with no common superscript differ significantly ($p \leq 0.05$)

Table 4: Effect of phytase on biochemical values in broiler chickens

Parameters	Units	Levels of Phytase				$LSD_{0.05}$
		0 FTU/Kg of feed	500 FTU/Kg of feed	1000 FTU/Kg of feed	1500 FTU/Kg of feed	
Albumin	g/L	15.00[a]	15.60[a]	14.28[a]	14.72[a]	1.4703
T. Protein	g/L	37.12[a]	37.68[a]	35.17[a]	36.48[a]	5.5423
ALT	U/L	2.73[a]	1.13[a]	1.18[a]	1.35[a]	2.67
ALP	U/L	2747[a]	2090[a]	1860[a]	1976[a]	1417.6
AST	U/L	363.45[a]	303.25[a]	315.90[a]	346.58[a]	57.407
GGT	U/L	20.83[a]	19.17[a]	19.83[a]	20.00[a]	5.3875
LDH	U/L	2108.7[a]	1904.8[a]	2077.3[a]	2067.7[a]	1405.5
Cholesterol	mmol/L	2.81[a]	2.73[a]	2.66[a]	2.60[a]	0.5418
Triglyceride	mmol/L	0.40[a]	0.53[a]	0.42[a]	0.32[a]	0.2983
Glucose	mmol/L	13.22[a]	14.10[a]	13.97[a]	13.82[a]	1.4301
Ca	mmol/L	1.68[a]	2.01[a]	2.13[a]	2.26[a]	0.6664
P	mmol/L	2.21[a]	1.88[a]	1.80[a]	1.71[a]	0.9863
Na	mmol/L	152.4[a]	151.6[a]	151.2[a]	153.4[a]	3.6872
Ka	mmol/L	6.63[a]	4.03[a]	4.78[a]	3.25[a]	4.3515
Cl	mmol/L	108.7[a]	107.9[a]	107.6[a]	108.5[a]	5.2201
Urea	mmol/L	0.717[a]	0.533[a]	0.567[a]	0.617[a]	0.2952
Creatinine	umol/L	28.33[a]	25.50[a]	25.50[a]	27.33[a]	5.6616
Uric Acid	umol/L	176.0[a]	204.1[a]	158.6[a]	151.4[a]	63.706

Means in a row with no common superscript differ significantly ($p \leq 0.05$)

The data indicated that there were no significant or constant treatment effects on total erythrocyte count (TEC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), total leukocyte count (TLC), heterophil, basophil, lymphocyte, monocyte, thrombocyte, icterus index and plasma protein. Although the lymphocyte was not significant different, it was almost increasing gradually and constantly according to the enzyme doses.

Only the eosinophil was decreased significantly ($p \leq 0.05$) as compared to control group.

With the exception of eosinophil the hematological values are similar and consistent to the findings of Huff et al., (1998). They showed that phytase supplementation at a level of 500 FTU/kg^{-1} had no significant effect on RBC, PCV, Hb, MCV, MCHC, WBC and DLC in broiler chicks. A low eosinophil level (eosinopenia) might be due to increased circulating steroids because inositol may have effect on hormone regulation (ACTH, epinephrine, thyroxine, prostaglandins). Eosinopenia, does not, in and of itself, represent a cause for alarm.

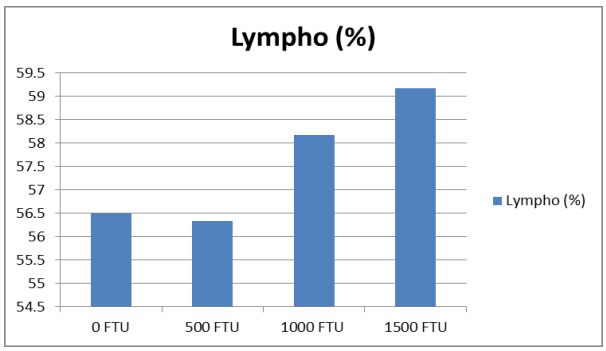

Figure 2: Increased lymphocytic count at various phytase levels

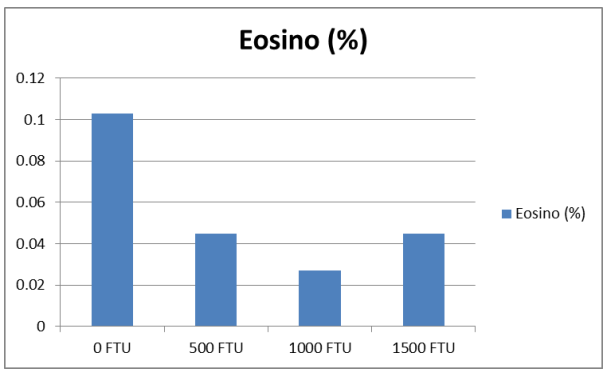

Figure 3: Decreased eosinophilic count at Various phytase levels

A low eosinophil level is usually not a cause for concern and is actually quite common. Some animals with extremely low eosinophil levels lead quite normal lives. So, in this study it can be explained that adding uncentrifuged phytase in broiler diet did not have objectionable changes among the parameters rather than increased lymphocytes may indicate the good sign for health.

Biochemical values:

The effect of phytase doses on biochemical constituents in broiler chickens at 6 weeks of age are summarized in Table 4.

Biochemical constituents of blood plasma such as albumin, total protein (TP), alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST), gamma-glutamyl transferase (GGT), Lactate dehydrogenase (LDH), cholesterol, triglyceride, glucose, Ca, P, Na, K, Cl, Urea, creatine, and uric acid were not affected by the dietary phytase supplementation. But some parameters (ALT, ALP, Cholesterol, P and K) had decreased and some (Glucose and Ca) had increased trend. The decreased trend of serum alkaline phosphatase associated with the diets supplemented with phytase might reflect the down regulation of this enzyme resulting from the increased availability of phosphorus. Besides that perhaps more likely, the inositol may affect cholesterol and glucose metabolism. On the other hand, the findings in this area among the researchers are inconsistent.

Huff et al., (1998) recorded that diet supplemented with 500 FTU/kg^{-1} of phytase in broiler chicks did not have significant treatment effects on serum levels of Ca, P, uric acid, TP, triglycerides, creatinine, glutamyltranferase and cholinesterase. Still alkaline phosphate and cholesterol levels decreased significantly in these birds. Al-Harthi, (2006) reported that adding of phytase at a level of 1000 FTU/kg^{-1} did not affect plasma TP with their fraction, total lipids, cholesterol, AST and ALT in broiler chicks. On the other hand Danek et al., (2007) found that biochemical parameters (Ca, P, Cu, Zn, Fe, Glu, TP and cholesterol) of Japanese quails were not affected by the dietary phytase supplementation at doses of 500 FTU/kg^{-1}, 750 FTU/kg^{-1} and 1000 FTU/kg^{-1}. But serum K, triglyceride and VLDL in these birds were significantly affected. Contrariwise, Viveros et al., 2002 demonstrated that adding of microbial phytase to low-P diets affected plasma P, Ca, Mg, Zn and TP concentrations, and ALP, AST, ALT and LDH activities. Nasrollah Vila, 2010 reported that phytase content in the diet of Quails were not significantly different for Ca retention, but for P retention, Mg and ALT were significantly different ($p > 0.05$). Zhou et al., (2008) also found that mineral utilization was improved for broiler fed the low-AP diet supplemented with phytase. The results obtained in this study suggest that phytase would modify some serum enzyme activities and increases the availability and use of minerals for growth.

CONCLUSION

The clinical chemistry and hematology data did not indicate any changes at certain age that would suggest that diet supplementation with uncentrifuged phytase affected the health of broiler chickens. Furthermore, the results obtained in this study decided that phytase supplementation had no side effect on hematological parameters and biochemical constituents at serum level. Rather it enhances the growth performance without modifying or changing hemato-biochemical values. It would be necessary for further research to evaluate interaction dietary mineral content, biochemical constituents including inositol and steroid hormones, and the rate of phytase supplementation so that the optimal dose could be used in gaining the most benefit from phytase supplementation.

REFERENCES

Al-Harthi, M. A., 2006. Impact of Supplemental Feed Enzymes, Condiments Mixture or Their Combination on Broiler Performance, Nutrients Digestibility and Plasma Constituents. International Journal of Poultry Science 5(8): 764-771.

Biehl, R. R., And D. H. Baker, 1997. Microbial Phytase Improves Amino Acid Utilization In Young Chicks Fed Diets Based On Soybean Meal But Not Diets Based On Peanut Meal. Poultry Sci. 76:355–360.

Broz, J., P. Oldale, A. H. Perrin-Voltz, G. Tychen, J. Schulze, And C. Simoes Nunes, 1994. Effects Of Supplemental Phytase On Performance And Phosphorus Utilization In Broiler Chickens Fed A Low Phosphorus Diet Without Addition Of Inorganic Phosphates. Br. Poult. Sci. 35:273–280.

Danek, N., Kaplan, O., Avci, M. and Can, A., 2007. Effects of Microbial Phytase on Growth Performance, Carcass Yield, Biochemical Parameters, Oxidative Stress and Faecal Phosphorus Content of Japanese Quails. Journal of Animal and Veterinary Advances 6(8): 1031-1035

Gordon, R. W., And D. A. Roland, Sr., 1997. Performance Of Commercial Laying Hens Fed Various Phosphorus Levels, With And Without Supplemental Phytase. Poultry Sci. 76:1172–1177.

Graf, E., 1986. Chemistry And Applications Of Phytic Acid: An Overview. In: Phytic Acid: Chemistry And Applications. E. Graf, Editor. Pilatus Press, Minneapolis, P. 173-194

Han, Y. M., Roneker, K. R., Pond, W. G. And Lei, X. G. 1998. Adding Wheat Middlings, Microbial Phytase, And Citric Acid To Corn-Soybean Meal Diets For Growing Pigs May Replace Inorganic Phosphorus upplementation. J. Anim. Sci. 76: 2649-2656.

.Harland, F. B., And E. R. Morris, 1995. Phytin: A Good Or A Bad Food Component. Nutr. Res. 15:733-754.

Huff, W. E., Moore Jr, P. A., 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. Poultry Science 77:1899-1904

.

Kornegay, E. T., And D. M. Denbow, 1996. Supplemental Microbial Phytase Improves Zinc Utilization In Broilers. Poultry Sci. 75:540–546.

Kornegay, E. T., V. Ravindran, And D. M. Denbow, 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