J. Vet. Malaysia (2014) 26 (1):8-16

Journal Articles

EFFECTS OF LOCALLY PRODUCED BACTERIAL PHYTASE ON HUMORAL IMMUNITY, LIVE BODY WEIGHT AND BLOOD CHARACTERISTICS IN BROILERS VACCINATED AGAINST NEWCASTLE DISEASE

R. Islam[1*], A. Ideris[1], A. Kasim[2], A.R. Omar[1], A.S. Meor Hussin[3], F. Yasmin[1] and Y. Akter[2]

[1]Faculty of Veterinary Medicine, Universiti Putra Malaysia, UPM Serdang, Selangor, Malaysia
[2]Faculty of Agriculture, Universiti Putra Malaysia, UPM Serdang, Selangor, Malaysia
[3]Faculty of Food Science and Technology, Universiti Putra Malaysia, UPM Serdang, Selangor, Malaysia

SUMMARY

Immune responses in association with body weight performance and hemato-biochemical constituents might influence the Newcastle disease (ND) vaccination by dietary phytase supplementation (*Enterobacter sakazakii* ASUA273). The objective of the study was to determine the effects of bacterial phytase supplementation on humoral immunity in association with live body weight and blood characteristics of broilers. Male-day-old Cobb broilers (n=180) were assigned into four phytase treatments (0, 500, 1000 and 1500 FTU/kg^{-1} of diet) with 12 cages comprised of 3 replicates and each cage contained 15 birds. Birds were maintained on formulated basal diet based with available phosphorus (0.19%) that lasted up to six weeks in feed. Birds were vaccinated with a live ND vaccine at day-old and 21 day-old. Two birds were randomly selected from each treatment weekly. Specific antibody to ND, non-specific immunoglobins (IgM, IgG, and IgA) and live body weight were measured weekly. At the end of experiment, the complete haemato-biochemical constituents were determined. Data of humoral immunity with live body weight and haemato-biochemical values were analysed based on factorial arrangement (treatments×weeks) of completely randomized design (CRD), respectively. Response of the humoral immunity shown that although serum-antibody of ND, IgM, and IgG levels were not improved, the mucosal IgA contents were increased with the increasing phytase doses. The live body weights of broilers were significantly increased (P<0.05) to the graded levels of phytase supplementation throughout the experimental period. Cumulative effects of mucosal IgA contents and live body weights of broilers showed significant (P<0.05) interaction between the effects of phytase levels and effects of weeks. Overall, phytase dose at 1500 FTU/kg^{-1} of diet over the age of 6 weeks-old showed the best performance. Conversely, no significant, sequential, and consistent treatment effects were observed on hemato-biochemical constituents in broiler chickens. It is therefore, concluded that the efficacy of this local phytase was flourished in respective to mucosal IgA contents and live body weights of broilers.

Key Words: Broiler chickens, bacterial phytase, immune responses, body weight, hemato-biochemical constituents

INTRODUCTION

Phytase addition in diets has proven to be an effective and realistic method for ameliorating the phytin (phytic acid and/or phytate) phosphorus (P) digestibility in monogastric animals (poultry, swine, fish and human). In particular, it reduces the phytate content in plant materials and at the same time lowers the phytin P disposal in nature. About 60-80% of the total P contained in feed ingredient of plant origin occurs as phytates and the availability of phytate P is low in poultry due to inadequate phytase activity in the intestine (Sharple *et al.*, 1993). Phytic acid is often considered an anti-nutrient because of its highly reactivity and readily forms of less soluble complexes with cations, carbohydrates and proteins in the small intestine and therefore, less likely to interact with phytase (Angel *et al.,* 2002). Phytase hydrolyzes phosphate group from the phytin yields inorganic P (IUB, 1979). Thus, it diminishes phosphate ester of myo-inositol and directly inhibits chelating potential of minerals, protein or starch with phytin cations (Ravindran *et al.*, 2008; Selle *et al.*, 2007). Consequently, phytase improves the bioavailability of P, di- and trivalent (calcium, cobalt, copper, zinc, magnesium, manganese, iron, potassium and nickel), and ileal digestibility of proteins in animal diet (Ravindran *et al.*, 2006; Selle *et al.*, 2000). Phytase indirectly, plays a major rolein maintain gastro-intestinal tract (GIT) secretion by reducing the phytate activity leadings to less secretion of siliac acid (Cowieson *et al.,* 2004). The industrial production of microbial phytase started in the early 1990s (Wodzinski and Ullah, 1996) and attracted attention from both researchers and entrepreneurs in the areas of nutrition, environmental protection, and biotechnological application. A good source of exogenous phytase was easily produced with biotechnological application (Wodzinski and Ullah, 1996). A variety of microorganisms including bacteria, yeast and fungi had been screened (Yanke *et al.*, 1998; Yoon *et al.*, 1996; Greiner *et al.*, 1993). Current focus on the soil fungus *Aspergillus niger* has allowed commercial production of phytase. Due to some of its biological properties (i.e., substrate specificity, resistance to proteolysis and catalytic efficiency), bacterial phytases have a considerable potential in commercial application (Konietzny *et al.*, 2004). In addition, some bacterial phytases especially those of the genera *Bacillus* and *Enterobacter*, exhibit a pH optimum in the range from 6-8 close to the physiological pH of the chicken gut. In Malaysia, about 30 strains of potential phytase producing bacteria (i.e. *Enterobacter* spp, *Bacillus* spp) were successfully harvested from maize plantation (Anis Shobirin *et al.*, 2009). These strains exhibit a significant amount of phytase activities, but the possibility of using of these phytases has not been investigated and none to

**Corresponding author: Rakibul Islam (R. Islam); Email:rakib_dgvc@yahoo.com*

our knowledge investigated phytase as a superior enzyme to improve chicken performances.

In animals, P is crucial for bone mineralization and cell membrane building. It indirectly, plays a key role in biological function of many metabolic processes. It is essential to supply adequate amounts of P and other nutrients in animal diet to ensure a good health and performances. Phytase supplementation in low P diet may influence chicken health in terms of immune response, body weight, and haemato-biochemical constituents.

Newcastle disease (ND) is the most important poultry disease worldwide, even in Malaysia. Outbreak of ND is a serious problem in the poultry industry contributing both high morbidity and mortality rates up to 100%. There were limited information of phytase activity on body immunity and many contradictions of phytase effects on blood characteristics in animal body. Only one study by Liu *et al*. (2008) reported the effect of fungal phytase on immune response in ND vaccinated broilers. Therefore, the possibility of using of the local bacterial phytase obtained from *Enterobacter sakazakii* ASUA273 could be justified in broilers vaccinated with a ND vaccine. The current experiment was to determine the effects of local phytase towards humoral immunity [Ab titers of ND (IgM, IgG, and IgA)] in association with live body weights, complete haemogram and blood biochemistry of broilers on ND vaccination in evaluating chicken health.

MATERIALS AND METHODS

Phytase

The locally producedrice bran fermented, crude and liquidbacterial phytase synthesised from *Enterobacter sakazakii* ASUA273 was obtained from the Standards and Industrial Research Institute of Malaysia (SIRIM). The activity of the enzyme was ranged from 3-7 FTU/ml (FTU is the unit of phytase activity) depended on its crudity (rice bran present in enzyme itself, water volume, contamination of site enzymes). This enzyme was stored at 4^0C in chiller and mixed into feed prior to feeding every day to avoid feed rancidity. The amount of it was depended on supplementation of phytase levels based on its activity.

Management of Birds

One hundread and eighty day-old-male broiler chickens (Cobb) of nearly similar body weight (range, 38-42 g) were obtained from a commercial hatchery. The birds were housed up to 6 weeks of age in 12 steel-netted cages kept in an automatic environmentally controlled room with continuous lighting and ventilation. Each cage contained 15 birds with three replications. The experimental feed was designed with P (0.19%) deficient diet as shown in Table 1.

Table 1. Ingredient compositions (g) and calculated values of the negative control (available P: 0.19%) experimental basal diets

Ingredient Compositions	Diet (Local Phytase) in FTU/kg^{-1} of diet			
	T0 (0)	**T1 (500)**	**T2 (1000)**	**T3 (1500)**
Corn grain	522.5	522.5	522.5	522.5
Rice bran	150	150	150	150
SBM	280	280	280	280
Corn oil	11	11	11	11
Methionine	0.3	0.3	0.3	0.3
NaCl	3.2	3.2	3.2	3.2
Ca carbonate	27	27	27	27
Ca phosphate	3	3	3	3
Vitamins†	2	2	2	2
Trace mineral‡	1	1	1	1
Local Phytase	0	Required volume	Required volume	Required volume
Natuphos®5000				
Total (g)	**1000**	**1000**	**1000**	**1000**
Calculated Nutritive Values				
ME (Kcal/kg)	3000.6	3000.6	3000.6	3000.6
CPr (%)	19.98	19.98	19.98	19.98
Ca (%)	1.189	1.189	1.189	1.189
Total P (%)	0.609	0.609	0.609	0.609
aP (%)	0.193	0.193	0.193	0.193
K (%)	0.973	0.973	0.973	0.973
Cl (%)	0.237	0.237	0.237	0.237
Mg (%)	0.292	0.292	0.292	0.292
Na (%)	0.152	0.152	0.152	0.152
Gly&Ser (%)	0.91	0.91	0.91	0.91
Leu (%)	1.7	1.7	1.7	1.7
Met &Cys (%)	0.34	0.34	0.34	0.34
Thr (%)	0.75	0.75	0.75	0.75

†In the Lutamix Gladron 528 (Gladron®), the followings were provided per kilogram of diet: vitamin A, 50.00 MIU; vitamin D3, 10.00 MIU; vitamin E, 75.00 g; vitamin K, 20.00 g; vitamin B1, 10.00 g; vitamin B2, 30.00 g; vitamin B6, 20.00 g; vitamin B12, 0.10 gm; calcium D-pantothenate, 60.00 g; nicotinic acid, 200.00 g; folic acid, 5.00 g; biotin, 235.00 mg; and antioxidant, anti-caking and carrier were added to 1 kg.

‡ In the Gladron Poultry Mineral (Gladron®), the following were supplied per kilogram of diet: selenium, 0.20 gm; iron, 80.00 g; manganese, 100.00 g; zinc, 80.00 g; copper, 1.50 g; potassium chloride, 4.00 g; mangnesium oxide, 0.60 g, sodium bicarbonate, 1.50 g, iodine, 1.00 g; cobalt, 0.25 g; and calcium carbonate was added to 1kg.

Abbreviation Key: ME (Metabolic energy), CPr (Crude protein), aP (available P), g (Gram), mg (Milligram), kg (Kilogram), IU (International unit), Gly (Glycine), Ser (Serine), Leu (Leucine), Met (Methionine), Cys (Cysteine), Thr (Threonine)

It was calculated by using Microsoft Excel 2010 software to ensure adequate nutrient requirements of broilers recommended by NRC (1994). The diet supplemented with phytase at the levels of 0, 500, 1000 and 1500 FTU/kg^{-1}of diet were grouped as T0, T1, T2 and T3, respectively. Feed in dry mash form and fresh water were offered *ad libitum*. Birds received two doses of live ND vaccine (ND "V4HR", Malaysian Vaccines and Pharamaceuticals Sdn. Bhd.) at day-old and 10-day-old respectively, as instructed by the manufacturer.

Sampling and Measurement

Two birds at the age of 1, 2, 3, 4, 5 and 6 weeks, were randomly selected and picked up from each treatment (8 birds per replicate). Live body weights were recorded. The selected birds were slaughtered and bloods were collected into vacutainer tubes without anticoagulant to obtain serum for measurement of Ab titers of ND, IgM and IgG (Bush, 1975). For measurement of IgA, the jejunal fluid was collected from these birds according to the method described by Liu *et al.* (2008). Antibody of NDV was detected using the Newcastle disease virus antibody test kit (FlockChek, IDEXX Laboratories, USA). Chicken IgM, Chicken IgAand Chicken IgG ELISA quantitation sets (Bethyl Laboratories, Inc., USA) were used to determine IgM, IgA and IgG, respectively. Blood samples were collected from the 6-weeks-old birds into vacutainer tubes with lithium heparin to obtain whole blood for measurement of haemato-biochemical constituents. The complete haemogram parameters such as total erythrocyte count (TEC), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), total leukocyte count (TLC), thrombocyte, icterusindex, and plasmaprotein were measured by haematology analyser (Abbott CELL-DYN 3700, GMI) using the available commercial kits. The packed cell volume (PCV), differential leukocyte count (DLC) (heterophil, eosinophil, basophil, lymphocyte and monocyte), icterus index and total plasma protein were measured manually according to the methods described by Othman *et al*. (1994). The biochemical constituents including albumin, total protein, alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), cholesterol, triglyceride, glucose, calcium, phosphorus, sodium, potassium, chloride, urea, creatinine and uric acid were determined using the chemistry analyser (HITACHI 902, Japan). All laboratory analysis was conducted at clinical haematology and biochemistry laboratory of Faculty of Veterinary Medicine, Universiti Putra Malaysia.

Statistical Analysis

Data of ND Ab titers (IgM, IgG, and IgA contents) and live body weights were analysed using a completely randomised design (CRD) based on 4 × 6 factorial arrangement, with dietary levels of phytase and intervals of week (6 weeks) being the main factors. The complete haemogram and blood biochemical constituents were analysed using CRD, with levels of phytase being the main factor. The data were subjected to analysis of variance (ANOVA) and tested for least significant difference (LSD) to assess the effects of phytase doses and their interaction with weeks. All analysis was conducted with SAS software (SAS Institute, 2009).

RESULTS

The chickens were healthy throughout the experiment with a mortality of less than 2%. Effects of local bacterial phytase on humoral immunity and blood characteristics in association with live body weights of broilers fed with low P (0.19%) diet and vaccinated against ND were described as below.

Humoral Immunity

The graded levels (0, 500, 1000, and 1500 FTU/kg^{-1} of diet) of local phytase supplementation weekly on humoral immunity [specific Ab titre of ND and non-specific Igs (IgM, IgG and IgA)] of broilers fed with P deficient diet on ND vaccination were summarised in Table 2 and 3. Effects data of graded levels of local phytase supplementation on serum Ab titres of ND in broilers indicated that the increasing or decreasing titres were not consistent with the increasing week (Wk) intervals (Table 2). Results of cumulative values of ND titres were significantly different ($P<0.05$), but the values of titres at T0 (1498.1 ng/ml), T1 (1869.5 ng/ml), T2 (1715.2 ng/ml) and T3 (1869.0 ng/ml) were inconsistent to the graded levels of phytase supplementation throughout the experiment (Table 3). Cumulative values at weekly were 6874.3, 1771.3, 449.8, 654.5, 557.0 and 346.8 over the age of Wk1, Wk2, Wk3, Wk4, Wk5 and Wk6, respectively had shown inconsistent and significant differences ($P<0.05$). The ND titre at the age of Wk1 was higher compared to the other weeks. No interaction was detected between the effects of phytase doses and the effects of week intervals on Ab production of ND.

The serum IgM concentrations of broilers at weekly showed that although serum IgM contents at group T2 were not different ($P>0.05$) compared to other treated groups (T0, T1 and T3), it was significantly different ($P<0.05$) to week intervals (Table 2). However, the inconsistent patterns of both insignificant and significant values of serum IgM contents weekly were almost the same to the graded levels of phytase supplementation. Results of cumulative values of serum IgM levels on T0 (12290 ng/ml), T1 (12801 ng/ml), T2 (10923 ng/ml), and T3 (11553 ng/ml) also demonstrated that serum IgM contents at different levels of phytase supplementation were not significantly different ($P>0.05$) (Table 3). The cumulative values of serum IgM contents at Wk1, Wk2, Wk3, Wk4, Wk5 and Wk6 were 10456, 11003, 10283, 14041, 11505 and 14062 ng/ml, respectively which had significant differences ($P<0.05$) but the values were inconsistent to week intervals. Overall, the interaction between the effects of local phytase doses and the effects of weeks on serum IgM contents was not observed.

Table 2. Effects of dietary local phytase supplementation at weekly on humoral immunity and live body weights of broilers fed low P diet and vaccinated with a ND vaccine

Parameters	T0 (Control: 0 FTU/kg^{-1} of diet)	T1 (500 FTU/kg^{-1} of diet)	T2 (1000 FTU/kg^{-1} of diet)	T3 (1500 FTU/kg^{-1} of diet)
Serum Ab titer of ND				
Week 1	5607.3^{a}	7173.7^{a}	6610.3^{a}	8106.0^{a}
Week 2	1477.7^{b}	2121.7^{b}	2037.3^{b}	1448.7^{b}
Week 3	525.0^{b}	374.3^{d}	312.7^{c}	587.3^{c}
Week 4	895.3^{b}	658.3^{d}	466.0^{c}	598.3^{bc}
Week 5	226.3^{b}	1165.0^{c}	593.7^{c}	243.0^{c}
Week 6	257.0^{b}	625.3^{d}	271.0^{a}	233.7^{c}
$LSD_{0.05}$	1508.6	477.77	1198.3	854.71
Serum IgM Level (ng/ml)				
Week 1	11285^{b}	10072^{b}	9296^{a}	11173^{ab}
Week 2	10017^{b}	10033^{b}	11630^{a}	12333^{ab}
Week 3	11329^{b}	10788^{b}	9445^{a}	9570^{b}
Week 4	15507^{a}	16059^{a}	10463^{a}	14136^{a}
Week 5	9641^{b}	14028^{ab}	11302^{a}	11049^{ab}
Week 6	15963^{a}	15825^{a}	13404^{a}	11054^{ab}
$LSD_{0.05}$	3246.8	4407	5060	4124.5
Serum IgG Level (ng/ml)				
Week 1	14931^{ab}	17822^{a}	9337.3^{ab}	18563^{a}
Week 2	12109^{b}	15498^{ab}	8229.7^{b}	13069^{b}
Week 3	15585^{a}	15438^{ab}	9052.3^{ab}	16357^{ab}
Week 4	13516.7^{ab}	11479^{bc}	11028.3^{a}	14700^{ab}
Week 5	11708^{b}	9370^{c}	9579.0^{ab}	12531^{b}
Week 6	13972.0^{ab}	10421.7^{c}	10285.7^{a}	15584^{ab}
$LSD_{0.05}$	3448.4	4678.5	2041.8	5127
Mucosal IgA level (ng/ml)				
Week 1	742.3^{c}	856^{d}	991^{c}	1111^{d}
Week 2	4063^{c}	3638^{d}	5813^{c}	6243^{d}
Week 3	15659^{b}	20002^{c}	20724^{b}	23495^{c}
Week 4	55062^{a}	63864^{b}	60441^{a}	69454^{b}
Week 5	65639^{a}	77250^{a}	68365^{a}	83669^{a}
Week 6	60918^{a}	67081^{b}	65933^{a}	72895^{ab}
$LSD_{0.05}$	11037	7449.8	10981	11789
Live Body Weight (g)				
Week 1	117.17^{f}	133.50^{f}	147.17^{f}	151.17^{f}
Week 2	288.00^{e}	313.83^{e}	323.17^{e}	338.83^{e}
Week 3	612.17^{d}	645.67^{d}	688.33^{d}	708.83^{d}
Week 4	1042.33^{c}	1178.67^{c}	1257.50^{c}	1283.50^{c}
Week 5	1494.67^{b}	1597.83^{b}	1679.00^{b}	1690.17^{b}
Week 6	1866.83^{a}	2043.50^{a}	2115.33^{a}	2159.00^{a}
$LSD_{0.05}$	70.067	43.229	115.33	87.556

Means with the same letter (s) in a column are not significantly different at the 5% level of probability.

Table 3. Interaction of effects of local phytase levels and week intervals (treatments × weeks) on humoral immunity and live body weights of broilers fed low P diet and vaccinated with a ND vaccine

Parameters	Effects of Treatment (T)					Effects of Week (Wk)							Interaction (treatments × weeks
	Phytase level (FTU/kg^{-1} of diet)												
	T0 (Control: 0)	T1 (500)	T2 (1000)	T3 (1500)	$LSD_{0.05}$	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	$LSD_{0.05}$	
Serum Ab titer of ND	1498.1^{b}	1869.5^{a}	1715.2^{ab}	1869.0^{a}	365.2	6874.3^{a}	1771.3^{b}	449.8^{c}	654.5^{c}	557.0^{c}	346.8^{c}	551.4	NS
Serum IgM Level (ng/ml)	12290^{a}	12801^{a}	10923.0^{a}	11553.0^{a}	2585.9	10456.0^{b}	11003.0^{b}	10283.0^{b}	14041.0^{a}	11505.0^{ab}	14062.0^{a}	2773.5	NS
Serum IgG Level (ng/ml)	13637.1^{b}	13338.1^{b}	9585.4^{c}	15134.1^{a}	1354.7	15163.0^{a}	12227.0^{ab}	14208.0^{a}	12681.0^{ab}	10797.0^{b}	12566.0^{ab}	3302.4	NS
Mucosal IgA level (ng/ml)	33681^{c}	38783.0^{b}	37045.0^{bc}	42811.0^{a}	3606.3	925.0^{d}	4939.0^{d}	19970.0^{c}	62205.0^{b}	73731.0^{a}	66709.0^{ab}	7657.8	<0.05
Live body Weight (g)	903.53^{c}	985.5^{b}	1035.0^{a}	1055.3^{a}	36.5	137.0^{f}	315.0^{e}	63.7^{d}	1190.5^{c}	1615.4^{b}	2046.2^{a}	4261.3	<0.05

Means with different superscripts within the row were significant different (P<0.05); Not significant (NS), P≥0.05

The serum IgG concentrationsof broilers weekly revealed the significant differences (P<0.05) but the concentrations were not consistent to the graded levels of phytase supplementation (Table 2). Results of cumulative data of serum IgG contents of broilers showed that the cumulative values of serum IgG contents were inconsistently and significantly (P<0.05) different (Table 3). The cumulative serum IgG contents at T0, T1, T2 and T3 were 13637.1, 13338.1, 9585.4 and 15134.1 ng/ml, respectively and at Wk1, Wk2, Wk3, Wk4, Wk5 and

Wk6 were 15163, 12227, 14208, 12681, 10797 and 12566 ng/ml, respectively. These data indicated that cumulative values were not consistent with the increasing of phytase levels and week intervals. Overall, no interaction between the effects of phytase doses and the effects of weeks was observed.

The mucosal secretory IgA (MSIgA) concentrations of ND vaccinated broilers weekly showed significantly increased (P<0.05) MSIgA contents with the increasing week intervals at each level of phytase supplementation (Table 2). The cumulative MSIgA contents values were significantly increased (P<0.05) with the increased phytase doses and the ages of birds (Table 3). Cumulative effects of enzyme supplementation on MSIgA contents showed that values of phytase treated birds at T1 (38783 ng/ml), T2 (37045 ng/ml), and T3 (42811 ng/ml) were higher than the control birds without phytase T0 (33681 ng/ml). Furthermore, the cumulative values of MSIgA contents on Wk1, Wk2, Wk3, Wk4, Wk5 and Wk6 were 925, 4939, 19970, 62205, 73731 and 66709 ng/ml, respectively had significant differences (P<0.05) with the increased week intervals. The supplementing phytase at T3 (1500 FTU/kg^{-1} of diet) showed the heist MSIgA concentration of 42811 ng/ml, whereas, the heist MSIgA levels were found at Wk5 was 73731 ng/ml. Overall, there was an interaction between the effects of graded levels of phytase supplementation and the effects of week intervals on MSIgA levels of ND vaccinated broilers.

Live Body Weight

Weekly effects data of graded levels of phytase supplementation on live body weights of broilers (Table 2) indicated that body weights at each treatment were consistently and significantly increased (P<0.05) to week intervals throughout the experimental period. Cumulative effects of live body weights (CLBW) of broilers demonstrated that the body weights were linearly and significantly increased (P<0.05) to graded levels of phytase supplementation and week intervals (Table 3). The CLBWs of phytase treated birds on T1 (985.5 g), T2 (1035.0 g) and T3 (1055.3 g) were higher the control birds T0 (903.5 g). The CLBWs over the age of Wk1, Wk2, Wk3, Wk4, Wk5 and Wk6 were 137.0, 315.0, 663.8, 1190.5, 1615.4 and 2046.2 g, respectively. The phytase dose T3 (1500 FTU/kg^{-1} of diet) overthe age of 6-week-old showed the best performance. The strong interaction between the effects of graded levels of phytase supplementation and the effects of week intervals was detected.

Complete Haemogram

Effects of graded levels of locally produced bacterial phytase supplementation at Wk6 on complete haemogram in ND vaccinated broiler chickens were summarised in Table 4.The results shown that only the basophil counts were significantly decreased (P<0.05) with the graded levels of phytase supplementation. Numbers of basophils were 3.67%, 2.50%, 2.33% and 1.50% at T0, T1, T2 and T3, respectively. Other parameters of complete haemogram showed notdifferent (P>0.05) with the increasing levels of phytase supplementation.

Blood Biochemistry

Effects of graded levels of locally produced bacterial phytase supplementation at Wk6 on blood biochemical constituents in ND vaccinated broiler chickens werepresented in Table 5. The biochemical constituents were consistent and with no significant differences (P<0.05) observed at different parameters to the graded levels of local bacterial phytase supplementation.

Table 4. Haematological parameters of broiler chickens fed locally produced bacterial phytase in a P deficient diet and vaccinated with ND vaccine

Parameters	Units	Treatments				$LSD_{0.05}$
		T0 (0 FTU/kg^{-1} of diet)	T1 (500 FTU/kg^{-1} of diet)	T2 (1000 FTU/kg^{-1} of diet)	T3 (1500 FTU/kg^{-1} of diet)	
TEC	**×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	**×10^{9}/L**	28.43^{a}	25.57^{a}	31.43^{a}	30.42^{a}	1 0.90
Heterophil	**%**	35.33^{a}	37.00^{a}	35.17^{a}	34.17^{a}	7.78
Eosinophil	**%**	0.50^{a}	0.83^{a}	0.67^{a}	0.83^{a}	0.67
Basophil	**%**	3.67^{a}	2.50^{ab}	2.33^{ab}	1.50^{b}	2.14
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	**×10^{9}/L**	4.42^{a}	3.21^{a}	3.58^{a}	2.20^{a}	4.95
Icterus. Index	**Unit**	2.00^{a}	3.00^{a}	2.00^{a}	2.00^{a}	1.63
Plasma 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 5. Blood biochemical constituents of broiler chickens fed on a P deficient diet with addition of locally produced bacterial phytase and vaccinated with a ND vaccine

Parameters	Units	Treatments				$LSD_{0.05}$
		T0 (0 FTU/kg^{-1} of diet)	T1 (500 FTU/kg^{-1} of diet)	T2 (1000 FTU/kg^{-1} of diet)	T3 (1500 FTU/kg^{-1} of diet)	
Albumin	**g/L**	15.00^{a}	15.60^{a}	14.28^{a}	14.72^{a}	1.47
T. Protein	**g/L**	37.12^{a}	37.68^{a}	35.17^{a}	36.48^{a}	5.54
ALT	**U/L**	2.73^{a}	1.13^{a}	1.18^{a}	1.35^{a}	2.67
ALP	**U/L**	2747.00^{a}	2090.00^{a}	1860.00^{a}	1976.00^{a}	1417.60
AST	**U/L**	363.45^{a}	303.25^{a}	315.90^{a}	346.58^{a}	57.41
GGT	**U/L**	20.83^{a}	19.17^{a}	19.83^{a}	20.00^{a}	5.39
LDH	**U/L**	2108.70^{a}	1904.80^{a}	2077.30^{a}	2067.70^{a}	1405.50
Cholesterol	**mmol/L**	2.81^{a}	2.73^{a}	2.66^{a}	2.60^{a}	0.54
Triglyceride	**mmol/L**	0.40^{a}	0.53^{a}	0.42^{a}	0.32^{a}	0.30
Glucose	**mmol/L**	13.22^{a}	14.10^{a}	13.97^{a}	13.82^{a}	1.43
Ca	**mmol/L**	1.68^{a}	2.01^{a}	2.13^{a}	2.26^{a}	0.67
P	**mmol/L**	2.21^{a}	1.88^{a}	1.80^{a}	1.71^{a}	0.99
Na	**mmol/L**	152.40^{a}	151.60^{a}	151.20^{a}	153.40^{a}	3.69
K	**mmol/L**	6.63^{a}	4.03^{a}	4.78^{a}	3.25^{a}	4.35
Cl	**mmol/L**	108.70^{a}	107.90^{a}	107.60^{a}	108.5^{a}	5.22
Urea	**mmol/L**	0.72^{a}	0.53^{a}	0.57^{a}	0.62^{a}	0.30
Creatinine	**umol/L**	28.33^{a}	25.50^{a}	25.50^{a}	27.33^{a}	5.67
Uric Acid	**umol/L**	176.00^{a}	204.10^{a}	158.60^{a}	151.40^{a}	63.71

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

DISCUSSION

Antibody (Ab) is an essential biological element prevalent in the healthy resistant repertoire. It plays a key role for the maintenance of protected homeostasis by exposure to environmental stimulation (Coutinho *et al.,* 1995; Bayry *et al*., 2005). Low levels of Ab may be accomplice with disease susceptibility (Parmentier *et al*., 2004). The serum ND Ab titres measured weekly were not increased by dietary phytase supplementation, or even by the challenged of ND vaccine. This indicated that dietary supplement phytase in vaccinated broilers had no impact on ND Ab production. In spite of giving ND vaccine (ND "V4 HR") to broilers at day-old and 21-day-old, the ND titres were significantly decreased over time, which were unexpected finding in this study. High ND titres at first week compared to the other weeks might be due to the availability of maternal Ab. It was therefore, concluded that ND Ab titres of vaccinated broilers were not influenced by phytase supplemented low P diet and/or ND vaccination. It was speculated that the heat resistant highly antigenic "V4" strain of NDV could not be effective for Cobb broiler chickens. The current finding was similar with Liu *et al.* (2008), reported that although phytase (Phyzyme®, *Escherichia coli*-derived phytase) addition in high phytate (0.44%) diet improved the anti-NDV antibodies, the low phytate (0.22%) diet did not affect the serum Ab production at the age of 2, 3 and 4 weeks birds vaccinated against ND. Both the serum IgM and IgG contents of vaccinated broilers were not affected by supplementation of locally produced bacterial phytase in low P diet. To our knowledge, there are limited reports of the phytase supplementation effects on serum IgM and IgG contents in vaccinated broiler birds.

In the current study, the phytase doses and week intervals administration has an effect on the mucosal secretory IgA (MSIgA) where the IgA concentrations were observed increased. The increased mucosal secretory IgA (MSIgA) concentrations of vaccinated broilers indicated that phytase supplementation in low P diet affected mucosal IgA contents throughout the experiment. Liu *et al*. (2008) showed similar observation that phytase supplementation increased MSIgA production over the day of 14, 21 and 28 for both the low (0.22%) and high (0.44%) phytate diets. The mucosal secretory epithelium is responsible for a potential effector tissue of integrated host responses where, MSIgA is synthesised to protect gastrointestinal-associated part of entry into the body. The degradation products of phytate by phytase could maintain immune activity of mucosal cells (Vucenik and Shamsuddin, 2006; Bozsik *et al*., 2007). Kettunen and Rautonen (2005) showed that the application of exogenous enzymes (xylanase, amylase, and protease or a combination of enzymes and betaine) in a diet enhanced nutrient uptake by intestinal cells, and increased the IgA levels in the digesta contributed to improvements of immune competence. In the current study, it was speculated that birds fed on the phytate diet could return a lower concentration of IgA in the jejunal mucosa, as a result from dilution with hypersecreted mucin, as stimulated by phytate. Phytate influenced mucin integrity was speculated to be related to the highly (pH dependent) reactive nature of dietetic phytate. When feed was exposed to the low pH conditions in the proximal gut, phytate solubilised and react electrostatically with basic amino acid residues in dietetically protein. Vaintrub and Bulmaga (1991) reported that protein-phytate complexes were variably refractory to digestion by pepsin and solubilisation with HCl, leading to a downstream secretion of mucin by GI epithelium. Therefore, phytase addition could partially ameliorate the adverse effect of phytate in the GIT of broilers by excess secretion of mucin (Cowieson *et al*., 2004; 2006a) with the contributing factor to regulate normal gut ecology. Thus, it influenced host immunological defense mechanisms by enhancing nutrient uptake for the intestinal immune cells and improving mucin integrity, perhaps by reducing the

concentration of saprogenic compounds (Cowieson and Ravindran, 2007; 2008).

The live body weights of broiler chickens were significantly increased with the increasing phytase doses and during the weekly interval of administration. Observations were similar with other studies (Khin, 2011; Nasrollah, 2010; Saima *et al.,* 2009; Ahmed *et al.*, 2004; Shirley and Edwards,2003; Ahmad *et al.*, 2000; Viveros *et al.,* 2002; Cabahug *et al.,* 1999; Qian *et al.,* 1997; Mitchell and Edwards, 1996; Sebastian *et al.*, 1996 and Perney *et al.,* 1993) where supplementing phytase in low P diet improved body weight of broilers.

Supplementation of enzymes in low P diet did not affect the haematological values of broilers except for the basophil count. Basopenia was observed with the increased levels of local bacterial phytase supplementation. This could be due to enzyme crudity (contaminated site enzymes) that led to viral infections, or hormonal impacts (elevated glucocorticoids and/or hyperthyroidism) of birds. A few studies have investigated the influence of dietary microbial phytase on haematological parameters in animals with variable observation (El-Badry *et al.,* 2008; Anna Czech and Eugeniusz, 2004). El-Badry *et al.* (2008) reported that total leucocyte count, total erythrocyte count and packed cell volume were not affected, but blood haemoglobin concentration was increased by dietary available P and/or phytase treatments. The exogenous microbial phytase inclusion to a low P swine diets did not influence packed cell volume and total erythrocyte count but, diminished total leucocyte count and increased haemoglobin content in blood (Anna Czech and Eugeniusz, 2004). In this current study, the overall findings of blood biochemical constituents did not indicate any change that would suggest that locally produced bacterial phytase from *Enterobacter sakazakii* ASUA273 in P deficient diet affected the health of broiler chickens (Shehab *et al.,* 2012; Al-Harthi, 2006; Eisa *et al.,* 2003; Attia, 2003; Qota *et al.*, 2002; Huff *et al.*, 1998). However, others has observed elevated level of ALT, AST, cholesterol, increased of plasma electrolytes (specifically Na and K) (El-Badry *et al.*, 2008), increased level of triglyceride (Danek *et al.*, 2007) and increased level of serum total protein (Ghasemi *et al*., 2006).

In contrast, there is ample evidence to report that supplemental microbial phytase caused significant effect on some blood biochemical parameters. El-Badry *et al.* (2008) showed that ducks fed diet contained lower available P without phytase recorded highest levels of AST and ALT, cholesterol compared to phytase treatments. They also reported that phytase addition was responsible for the slight increase of plasma Na and K of ducks under summer condition. A study with Japanese quails (Danek *et al.,* 2007) showed that phytase supplemented diet significantly affected serum. Ghasemi *et al.* (2006) reported a significant increase of serum total protein in broiler chickens receiving phytase whereas Viveros *et al.* (2002) reported that decreasing dietary levels caused a decrease in serum AST activity due to dietary phytase addition.

Several reports showed that decreased ALP level in chickens (Viveros *et al.,* 2002; Fernandes *et al.,* 1999; Huff *et al.*, 1998) and in turkeys (Attia *et al.,* 2000). A higher phytate might be responsible for the higher ALP activity possibly related to intestinal lesions, skeletal disorders, or liver dysfunctions. Besides that, decreased ALP activity was associated with the increase zinc retention in the chicken intestine (Lei *et al.*, 1993). In contrast, Roberson and Edwards (1994) reported that plasma ALP level was not affected by phytase addition in broiler diets. The enzyme also led to inconsistent decrease of blood urea and creatinine content in birds, but they are generally poor indicators of renal disease and/or muscle injury in birds (Campbell, 2004).

Phytase inclusion in a low P diet did not have an influence on blood Ca and P concentrations (Aureli *et al.,* 2011; Kliment *et al.,* 2011; Zhou *et al.,* 2008; Danek *et al.*, 2007; Rezaei *et al.*, 2007; Catala-Gregori *et al.,* 2006). In contrast, other has reported either elevated blood calcium level (Jadhav *et al.,* 2009) or increased blood P concentration (Aureli *et al.,* 2011; Han *et al.,* 2009; El-Badry *et al.*, 2008; Ghasemi *et al.*, 2006; Onyango *et al.,* 2004) in phytase supplemented diet. These controversy results are probably due to diet factors as well as phytase source, crudity and activity.

CONCLUSION

It was concluded that the supplementation of *Enterobacter sakazakii* ASUA273 phytase to P deficient corn-soybean based diet was an effective means to improve live body weight performance and mucosal IgA contents. The phytase dose of 1500 FTU/kg^{-1} of diet produced better performances than other treatments. The overall findings of blood haemato-biochemical constituents did not affect the health status of broiler chickens. Further researches are recommended to determine the optimum level of available P in order to produce maximum performances. The cell-mediated immunity in broilers vaccinated against ND vaccines should be measured to assess the real effect on immune response by local phytase supplementation in low P diet. It would also be beneficial to investigate the effect of phytase from ASUIA273 to determine enzyme impacts on vitamin-D, parathormone, glucocorticoids, and thyroids in animal body.

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: 764-771.

Ahmad, T., Rasool, S., Sarwar, M., Ahsan-ul-Haq and Zia-ul-Hasan. (2000). Effect of microbial phytase produced from a fungus *Aspergillus niger* on bioavailability of phosphorus and calcium in broiler chickens. Animal Feed Science and Technology. 83: 103-114.

Ahmed, F., Rahman, M. S., Ahmed, S. U. and Miah, Y. U. (2004). Performance of broiler on phytase supplemented soya meal bean based diet. International Journal of Poultry Science. 3: 266-271.

Angel, R., Dhandu, A. S. and Applegate, T. J. (2002). Phosphorus requirements of broilers and the impact of exogenous phytases. Arkansas Nutrition Conference. University of Arkansas, Fayetteville, AR.

Anis Shobirin, M. H., Farouk, A.and Greiner, R. (2009). Potential phytate-degrading enzyme producing bacteria isolated from

Malaysian maize plantation. African Journal of Biotechnology. 15: 3540-3546

Anna Czech and Eugeniusz R. Grela. (2004). Biochemical and haematological blood parameters of sows during pregnancy and lactation fed the diet with different source and activity of phytase. Animal Feed Science and Technology. 116: 211-223.

Attia, Y.A. (2003). Performance, carcass characteristics, meat quality and plasma constituents of meat type drakes fed diets containing different levels of lysine with or without a microbial phytase. Archives of Animal Nutrition. 66: 39-48.

Aureli, R., Umar Faruk, M., Cechova, Pederson, P. B., Elvig-Joergensen, S. G., Fru, F. and Broz, J. (2011). The efficacy of a novel microbial 6-phytase expressed in *Aspergillus oryzae* on the performance and phosphorus utilization in broiler chickens. International Journal of Poultry Science. 10: 160-168.

Boyd, R. D., Hall, D. and Wu, J. F. (1983). Plasma alkaline phosphatase as a criterion for determining biological availability of phosphorus for swine. Journal of Animal Science. 57: 396.

Bozsik, A., Kokeny, S. and Olah, E. (2007). Molecular mechanisms for the antitumor activity of inositol hexakisphosphate (IP_6). Cancer Genomics Proteomics. 4: 43-51.

Bush, B. M. (1975). Collection of Blood, Plasma, and Serum. In Veterinary Laboratory Manual. 1st ed. Pp. 109-111. Willizm Heinemann Medical Books Ltd, London. Printed in Great Britain by Unwin Brothers Limited, The Gresham Press.

Cabahug, S., Ravindran, V., Selle, P. H. and Bryden, W. L. (1999). Response of broiler chickens to microbial phytase as influenced by dietary phytic acid and non-phytate phosphorus levels I.Effects on bird performance and toe ash content. British Poultry Science. 40: 660-666.

Campbell, T. W. (2004). Clinical chemistry of birds, In Veterinary Haematology and Clinical Chemistry by M. A. Thrall, published by Lippincott Williams and Wilkins Co., 479-492

Catala-Gregori, P., Garcia, V., Hernandez, F., Madrid, J. and Ceron, J. J. (2006). Response of broilers to feeding low-calcium and phosphorus diets plus phytase under different environmental conditions: body weight and tibio tarsus mineralization. Poultry Science. 85: 1923-1931.

Cowieson, A. J., Acamovic, T. and Bedford, M. R. (2006). Supplementation of corn-soy based diets with ab *Eschericia coli*-derived phytase: effects on broiler chick performance and the digestibility of amino acids and the metabolis ability of minerals and energy. Poultry Science. 85: 1389-1397.

Coutinho, A., Kazatchkine, M. D. and Avrameas, S. (1995). Natural autoantibodies. Current Opinion of Immunology. 7: 812-818.

Cowieson, A. J., Acamovic, T. and Bedford, M. R. (2004). The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. BritishPoultry Science. 45: 101-108.

Cowieson, A. J. and Ravindran, V. (2007). Effect of phytic acid and phytase on the flow and amino acid composition of endogenous protein at the terminal ileum of growing broiler chickens. BritishJournal of Nutrition. 98: 745-752.

Cowieson, A. J., Ravindran, V. and Selle, P. H. (2008). Influence of dietary phytic acid and source of microbial phytase on ileal endogenous amino acid flows in broiler chickens. Poultry Science. 87: 2287-2299.

Eisa, M. A. Adel, El-Hamied andSahar, S. (2003).Clinicopathological studies on bio-stimulant agent in broiler chickens. Kafr El-SheikhVeterinary Medicine Journal. 1: 631-644

El-Badry, A. S. O., Mahrousa, M. H., Fatouh, M. H. and El-Hakim, Abd. A. S. (2008). Role of phytase supplementation into Muscovy ducks diet in thermo- and osmoregulation during summer season. Egyptian Poultry Science. 28: 1059-1081.

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

Ghasemi, H. A., Tahmasbi, A. M., Moghaddam, Gh.,Mehri, M., Alijani, S., Kashefi, E. and Fasihi, A. (2006).The effect of phytase and *Saccharomyces cervisiae* (Sc47) supplementation on performance, serum parameters, phosphorus and calcium retention of broiler chickens. International Journal of Poultry Science. 5: 162-168.

Greiner, R., Konietzny, U. andJany, K. D. (1993).Purification and characterisation of two phytases from *Escherichia coli*. Archives Biochemistry and Biophysics. 303: 107-113.

Han, J. C., Yang, X. D., Qu, H. X., Xu, M., Zhang, T., Li, W. L., Yao, J. H., Liu, Y. R., Shi, B. J., Zhou, Z. F. and Feng, X. Y. (2009). Evaluation of equivalency values of microbial phytase to inorganic phosphorus in 22- to 42-day-old broilers. Journal of Applied Poultry Research. 18: 707-715.

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.andRaboy, V. (1998). Effect Of Dietary Phytase And High Available Phosphorus Corn On Broiler Chicken Performance.Poultry Science. 77: 1899-1904.

International Union of Biochemistry (1979). In: Enzyme Nomencluture: Recommendations of the Nomenclature Committee of the International Union of Biochemistry, Academic Press, New York, Pp: 242-247

Kettunen, H. and Rautonen, N. (2005). With betaine and exogenous enzymes towards improved intestinal health and immunity, and better performances of broiler chicks. Poultry Science. 84 (Suppl.1): 47 (abstr.).

Khin, San Mu. (2011). Evaluation of locally produced microbial phytases and their by-products as additives in broiler nutrition. Universiti Putra Malaysia, Malaysia.

Kliment, Martin and Aneglovicova, M. (2011).The effect of microbial phytase on blood performance of broiler chickens. Animal Science and Biotechnologies. 44: 58-61.

Konietzny, U. and Greiner, R. (2004).Bacterial phytase: potential application, in vivo function and regulation of its synthesis. Brazillian Journal of Microbiology. 35: 11-18.

Lei, X., Ku, P. K., Miller, E. R., Ullrey, D. E. and Yokoyama, M. T. (1993). Supplemental microbial phytase improves bioavailability of dietary zinc to weanling pig. The Journal of Nutrition. 123: 1117-1123.

Liu, N., Ru, Y. J., Cowieson, F.D. Li. and Cheng, X. CH. (2008). Effects of phytate and phytase on the performance and immune function of broilers fed nutritionally marginal diets. Poultry Science. 87: 1105-1111.

Mitchell, R. D., and H. M. Edwards, Jr. (1996). Additive effects of 1,25-dihydroxycholecalciferol and phytase on phytate phosphorus utilization and related parameters in broiler chickens. Poultry Science. 75:111–119.

Nasrollah Vali (2010). Comparison difference levels of phytase enzyme in diet of Japanese Quail (*Coturnix japonica*) and some blood parameters. Asian Journal of Poultry Science. 4: 60-66.

National Research Council (1994). Nutrient requirements of poultry. 9th Rev. Ed. National Academy Press, Washington, DC.

Onyango, E. M., Bedford, M. R. and Adeola, O. (2004). The yeast production system in which *Escherichia coli* phytase is expressed may affect growth performance, bone ash, and nutrient use in broiler chicks. Poultry science. 83: 421-427.

Othman, M. H.,and Ragavan, K. (1994). Laboratory Manual in Haematology. Faculty of veterinary medicine. Universiti Putra Malaysia. Malaysia. Pp: 19-33.

Parmentier, H. K., Baelmans, R., Savelkoul, H. F. J., Dorny, P., Demey, F., and Berkvens, D. (2004). Serum haemolytic complement activities in 11different MHC (B) typed chicken lines. Veterinary Immunology and Immunopathology. 100: 25-32.

Parr, T. and Wyatt, C. (2006).Effect of different phytase sources on broiler performance and yield when added to the mixer and pelleted at two temperatures. Poultry Science. 85[Suppl. 1]: 90.

Perney, K. M., Cantor, A. H., Straw, M. L. and Herkelman, K. L. (1993). The effect of dietary phytase on growth performance and phosphorus utilisation of broiler chickens. Poultry Science. 72: 2106-2114.

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. Poultry Science. 76: 37–46.

Qota, E. M. A., El-Ghamry, A. A. and El-Mallah, G. M. (2002). Nutritional value of soaked linseed cake in broiler diets without or with pro-nutrients or formulating diets based on available amino acids value on performance of broilers. Egyptian Poultry Science. 22: 461- 475.

Raghavan, Dr. V. (2011). ASEAN'S poultry industry. WORLD POULTRY-Elsevier Vol 17, No. 1.

Ravindran, V., Cowison, A. J. and Selle, P. H. (2008). Influence of dietary electrolyte balance and microbial phytase on growth performance, nutrient utilisation, and excreta quality of broiler chickens, Poultry Science. 87: 677-688.

Ravindran, V., Morel, P. C. H, Partridge, G. G., Hruby, M. and Sands, J. S. (2006). Influence of *Escherichia coli*-derived phytase on nutrient utilisation in broiler starters fed diet containing varying concentrations of phytic acid. Poultry Science. 85: 82-89.

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: 55-58.

Roberson, K. D. and Edwards Jr. H. M. (1994). Effects of ascorbic acid and 1,25-dihydroxycholecalciferol on alkaline phosphatase and tibial dyschondroplasia in broiler chickens. British Poultry Science. 35: 763-773.

Saima, M. Z., Khan, U., Jabbar, M. A., Ijaz, M. and Qadeer, M. A. (2009). Efficacy of microbial phytase at different levels on growth performance and mineral availability in broiler chickens. Journal of Animal and Plant Sciences. 19: 58-62.

Sebastian, S., Touchburn, S. P., Chavez, E. R. and Lague, P. C. (1996).The effects of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper, and zinc in broiler chickens fed Cornsoy bean diets. Poultry Science. 75: 729–736.

Selle, P. H.and Ravindran, V. (2007). Microbial phytase in poultry nutrition. Animal Feed Science and Technology. 135:1-2, 1-41.

Selle, P.H, Ravindran, R. A, Caldwell and Bryden, W. L. (2000). Phytate and phytase: consequences for protein utilisation, Nutrition Research Reviews.13: 255-278.

Sharpley, A. N., Smith, Si. and Bain. R. (1993). Nitrogen and phosphorus fate from long-term poultry litter applications to Oklahomasoils. Soil Science Society of America Journal 57: 1131-1137.

Shehab A. E., Kamelia M. Z., Khedr N. E., Tahia E. A. and Esmaeil F. A. (2012). Effect of dietary enzyme supplementation on some biochemical and haematological parameters of Japanese Quails. Journal of Animal Science Advances. 2: 734-739.

Shirley, R. B. and Edwards, Jr. H. M. *(*2003). Graded levels of phytase past industry standards improve broiler performance. Poultry Science. 82*:* 671–680.

Vaintraub, I. and Bulmaga, V. (1991). Effect of phytate in the in vitro activity of disgestive proteinases. Journal of Agricultural Food Chemistry. 39: 859-861.

Vucenik, I. and Shamsuddin, A. M. (2006). Protection against cancer by dietary IP6 and inositol. Nutrition and Cancer. 55: 109-125

Viveros, A., Brenes, A.and Centeno, C. (2002). Effects of microbial phytase supplementation on mineral utilisation and serum enzyme activities in broiler chicks fed different levels of phosphorus. Poultry Science. 81:1172-1183.

Yanke, L. J., Bae, H. D., Selinger, L. B. and Cheng, K. J. (1998). Phytase activity of anaerobic ruminal bacteria. Microbiology. 144: 1565-1573.

Yoon, S. J., Choi, Y. J., Min, H. K., Cho, K. K., Kim, J. W., Lee, S. C. and Jung, Y. H. (1996). Isolation and identification of phytase-producing bacterium, *Enterobacter sp*. 4, and enzymatic properties of phytase enzyme. Enzyme and Microbial Technology. 18: 449-454.

Wodzinski, R.J. and Ullah, A. H. J. (1996). Phytase. Advances in Applied Microbiology. 42: 263-302.