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ANIMAL HUSBANDRY & VETERINARY SCIENCE | RESEARCH ARTICLE

Evaluation of humoral immune response, body weight and blood constituents of broilers supplemented with phytase on infectious bursal disease vaccination

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Abstract: Phytase inclusion in phosphorus (P) deficient diet increases the bioavailability of nutrients and plays, indirectly, a role in biological function of many metabolic processes. The possibility of using phytase in diet might influence immune, growth and blood performances of animals. The objective of the study was to assess the effect of local bacterial phytase on humoral immunity in association with weight and blood characteristics of infectious bursal disease (IBD) vaccinated broilers. Male-day-old Cobb-broilers were assigned into four groups based on phytase treatments (0, 500, 1,000 and 1,500 fitase units per kg of diet) with 12 cages comprising three replicates per treatment, each treatment containing 15 birds. They were vaccinated with an IBD vaccine (IBD UPM93) and were fed formulating P (0.19%) deficient diet from 1 to 42 day of age. Results indicated that although serum IBD antibody, IgM, and IgG were not increased, mucosal IgA contents were increased with increasing phytase doses. Data on bird's growth performance revealed that

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Rakibul Islam is an Associate Professor in the Department of Physiology and Pharmacology at Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. He has completed DVM and MS in Physiology from the Bangladesh Agricultural University, Mymensingh, Bangladesh. He has also held a PhD degree in the field of Immunology from the Universiti Putra Malaysia (UPM), Malaysia. He carries on research on nutritional effects of organic and inorganic substances (enzyme, hormone, microorganism, medicinal herb and plant) onto animal health. He has published several papers in national and international journals. This paper was culled from the PhD thesis supervised by Aini Ideris, Azhar Kasim, Abdul Rahman Omar and Anis Shobirin Meor Hussin. Farhana Yasmin was his lab-mate who helped to collect the data and write the manuscript. The collective interest of the research group was therefore on production of potential local bacterial phytase from *Enterobacter sakazakii* ASUA273 in maize plantation at Malaysia for sustainable and beneficial poultry feed additive.

PUBLIC INTEREST STATEMENT

Phytase addition in diets has proven to be an effective and realistic method for improving phytate phosphorus bio-availability in simple digestion, alleviating phytate anti-nutritional effect, and reducing environmental phosphorus pollution. So, it has been broadly used in animal diets as a feed additive and has been attracted in the areas of nutrition, environmental protection, and biotechnological application. In Malaysia, about 30 strains of phytase producing bacteria were successfully harvested from maize plantation. However, the possibility of using these local phytases in animal diets have not been investigated. Phosphorus is crucial for animal growth and indirectly, plays a key role in many metabolic processes. In low phosphorus diet, phytase application may eventually influences chicken health in terms of immune, blood and weight characteristics. Therefore, the study was being conducted to explore the potentiality and consequence of phytase in infectious bursal disease vaccinated boilers for better performances.

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cumulative live weights were increased significantly ($p < 0.05$) to graded levels of phytase and the highest enzyme level commenced best performance. Results suggest that the local phytase supplementation in low phosphorus diet will improve mucosal IgA contents and body weights of birds.

Subjects: Environment & Agriculture; Bioscience; Food Science & Technology

Keywords: phosphorus deficient diet; local bacterial phytase; infectious bursal disease vaccinated broilers; humoral immunity; weight and blood characteristics

1. Introduction

The enzyme phytase is a special class of phosphomonoesterase (*myo*-inositol-hexakisphosphate phosphohydrolase) that is capable of catalyzing the sequential release of phosphate group from phytin (phytic acid and phytate) in plant materials to yield inorganic phosphorus (P) and lower phosphorylated *myo*-inositol derivatives and thus, decreases the phytin P disposal in nature (International Union of Biochemistry, 1979; Wyss et al., 1999). Phytin is ubiquitous among plant components, comprising 1–5% (w/w) of cereal grains, oilseeds, legumes, pollen, and nuts (Gibson & Ullah, 1990). It is often considered an anti-nutrient because of its highly reactivity and readily forms less soluble complexes with Ca, Fe, Mg, Cu, Zn, proteins and/or amino acids in the small intestine and, therefore, less likely to interact with phytase (Ravindran, Cowison, & Selle, 2008; Selle & Ravindran, 2007). Phytase increases the bioavailability of phytate phosphorus (P) with other nutrients in plant originating feed and/or food of monogastric animals (swine, poultry, pre-ruminant calves, and fish) including human (Ravindran, Morel, Partridge, Hruby, & Sands, 2006; Selle, Ravindran, Caldwell, & Bryden, 2000). By reducing the phytate activity on gastro-intestinal (GIT), phytase also leads to less secretion of sialic acid from GIT and maintains GIT health. In animals, P is a crucial for bone mineralization and cell membrane building, and plays a key role in biological function of many metabolic processes. To ensure a good health status and performance, it is therefore, essential to supply adequate amounts of P and other nutrients in poultry diet. In consequence, phytase addition in low P diet may theoretically, influences chicken health in terms of immune responses in association with blood and growth characteristics. Hence, it has attracted great attention from both researchers and entrepreneurs in the areas of nutrition, environmental protection, and biotechnological application.

A variety of microorganisms including bacteria, yeast, and fungi (Greiner, Konietzny, & Jany, 1993; Yanke, Bae, Selinger, & Cheng, 1998; Yoon et al., 1996) has been screened for obtaining a potential phytase. Due to some biological properties, bacterial phytase especially those of genera *Bacillus* and *Enterobacter*, exhibits a pH optimum in the range from 6–8, close to the physiological pH of chicken gut (Konietzny & Greiner, 2004). In Malaysia, about 30 strains of potential phytase producing bacteria (*Enterobacter* sp, *Bacillus* spp, *Pantoea* sp.) were successfully harvested from maize roots (Anis Shobirin, Farouk, & Greiner, 2009). Although the phytase from *Enterobacter sakazakii* ASUA279 has exhibited a significant enzymatic activity on growth and mineral performances of broilers (Khin, 2011), the possibility of using other phytases has not been investigated. There are, however, conflicting reports concerning the ability of phytase for growth with hematological performances (Czech & Grela, 2004; El-Badry, Mahrousa, Fatouh, & Abd El-Hakim, 2008; Islam et al., 2014) and blood biochemistry (Al-Harthi, 2006; Eisa Adel, El-Hamied, & Sahar, 2003; El-Badry et al., 2008; Ghasemi et al., 2006; Islam et al., 2014; Shehab, Kamelia, Khedr, Tahia, & Esmaeil, 2012), and very limited information on immune response in animal body (Islam et al., 2014; Liu, Ru, Li, Cowieson, & Cheng, 2008). Additionally, infectious bursal disease (IBD) is the most important disease for poultry in worldwide, which can cause significant economic losses of poultry industry. For better animal performance, more studies that are comprehensive need to elucidate the efficacy of bacterial phytase on immunity in association with growth, hematology and blood biochemistry. In chicken, therefore, the local phytase supplemented low P diet of IBD vaccinated broilers could be justified for getting a potentially superior enzyme. The objective of this study was to determine the effect of locally produced bacterial phytase from *Enterobacter sakazakii* ASUA273 on humoral immune response against IBD vaccine in association with cumulative live weight and blood constituents of broiler chickens.

2. Materials and methods

2.1. Bacterial phytase
The locally produced, rice bran fermented, crude and liquid, bacterial phytase synthesized from *Enterobacter sakazaki* ASUA273 was obtained from Standards and Industrial Research Institute of Malaysia (SIRIM) and the experiment was conducted at Faculty of Veterinary Medicine of Universiti Putra Malaysia. The activity of this crude enzyme was varied from 3–7 fitase units (FTU) per ml and was stored at 4°C in chiller before being used.

2.2. Chickens and their management
A number of 180 male-day-old broilers (Cobb) of nearly similar live body weight were obtained from a commercial hatchery. They were allocated to 12 cages corresponding to three replications of four phytase doses, each cage containing 15 birds. These birds were reared in steel netted cages with newspaper litter until 2 weeks of age, and then the paper was removed, and birds were kept in same cages up to 6 weeks of age in an automatic environmentally controlled room with continuous lighting and ventilation. Feed in dry mash form and fresh water were offered *ad libitum* basis consumption throughout the experiment. At the 10-day-old, birds were received a dose of live IBD vaccine (IBD UPM93, Malaysian Vaccines and Pharmaceuticals Sdn. Bhd) by the eye drop method, followed by manufacturer's directions.

2.3. Experimental diet
The experimental negative control diet was corn-soybean meal based low P (0.19%), and formulated to meet or exceed NRC recommendations (NRC, 1994) for the 0-42-d-old broiler. Composition and calculated analysis of this diet are presented in Table 1. The diets supplemented with 0 FTU/kg, 500 FTU/kg, 1,000 FTU/kg and 1,500 FTU/kg were named as T0, T1, T2, and T3, respectively. The enzyme was added just prior to give feed to chicks at a day.

2.4. Sampling and measurement
At ages of 1–6 weeks, before giving feed, two birds from each treatment (eight birds per replication) were randomly selected and live body weights were recorded by digital balance. The selecting two were then slaughtered, and bloods were collected into vacutainer tubes without anticoagulant to get serum for measuring of antibody (Ab) to infectious bursal disease virus (IBDV), IgM and IgG (Bush, 1975). To quantify the secretory mucosal IgA, the jejunal fluid was then collected from the slaughtering birds immediately, according to the method described by Liu et al. (2008). The Specific Ab to IBDV was detected by using Infectious Bursal Disease Virus Antibody Test kit (FlockChek®, IDEXX Laboratories, USA). Chicken IgM, Chicken IgA and Chicken IgG ELISA Quantitation Set (Bethyl Laboratories, Inc., USA) were also used to determine non-specific of IgM, IgA and IgG, respectively. On the age of 6 week, blood samples were also collected from the slaughtering chickens into vacutainer tubes with anticoagulant lithium heparin to obtain whole blood for measurement of hemato-biochemical constituents. The complete hemogram such as total erythrocyte count (TEC), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), total leukocyte count (TLC), thrombocyte, icterus index (II), and plasma protein (PP) were measured by hematology analyzer (Abbott CELL-DYN 3700 Hematology Analyzer, GMI), using the available commercial kits. Whereas the parameters of differential leukocyte count (DLC) including, heterophil, eosinophil, basophil, lymphocyte, and monocyte, and packed cell volume (PCV) were determined manually. Using the available commercial kits, the biochemical constituents (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 analyzed by chemistry analyzer (HITACHI 902, Japan).

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Table 1. Ingredient compositions (g/kg) and calculated nutritive values of the negative control (low in P) experimental basal diets

Ingredient	P deficient (0.19%) negative basal diet			
	T0 (0 FTU/kg)	T2 (500 FTU/kg)	T2 (1,000 FTU/kg)	T3 (1,500 FTU/kg)
Corn grain	522.5	522.5	522.5	522.5
Rice bran	150	150	150	150
Soybean meal	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	Volume depended on enzyme activity			
Calculated nutritive values				
Metabolisable energy (kcal/kg)	3000.6			
Crude protein (%)	19.98			
Ca (%)	1.189			
Total P (%)	0.609			
nPP (%)	0.193			
K (%)	0.973			
Cl (%)	0.237			
Mg (%)	0.292			
Na (%)	0.152			
Gly & Ser (%)	0.91			
Leu (%)	1.7			
Met & Cys (%)	0.34			
Thr (%)	0.75			

Note: FTU: fitase units.

*In the Lutamix Gladron 528 (Gladron®), the followings were provided per kilogram of diet: vitamin A, 50.000 MIU; vitamin D3, 10.000 MIU; vitamin E, 75.000 g; vitamin K, 20.000 g; vitamin B1, 10.000 g; vitamin B2, 30.000 g; vitamin B6, 20.000 g; vitamin B12, 0.100 g; calcium D-pantothenate, 60.000 g; nicotinic acid, 200.000 g; folic acid, 5.000 g; biotin, 235.00 mg; and antioxidant, anticaking and carrier were added to 1 kg.

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

2.5. Statistical analysis

All the experiments were conducted using completely randomized design 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 $p \leq 0.05$.

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3. Results and discussion
The chickens were almost healthy throughout the experimental period with the exception of lameness of some birds at their advanced ages to higher doses (1,000 FTU/kg and 1,500 FTU/kg of diet) of phytase supplementation. Rice bran fermented crude phytase contains more rice bran with high level of P that might be lead to rickets (Islam et al., 2014).

3.1. Effects on humoral immunity
At weekly, the effects of locally produced bacterial phytase on Ab of IBDV, IgM, IgA and IgG in broiler chickens are shown in Tables 2–5, respectively. The results indicated that the Ab of IBD was almost same to all treatments at each week. However, at the ages of 1–5 weeks, the Ab titers were significantly ($p < 0.05$) different between treated groups, but the values were very inconsistent to their respective doses. Data of both serum IgM and IgG contents in broilers chickens demonstrated that the concentrations were also almost same to all treatments during the experiment. However, although the levels of IgM at 2–4 weeks and of IgG at 4 and 6 weeks were showed significantly ($p < 0.05$) different, the levels were not consistent to their corresponding doses. The effect of phytase

Table 2. Specific Ab titers of infectious bursal disease virus in broiler chickens (weekly intervals) fed on a low P basal diet supplemented with locally produced bacterial phytase and vaccinated with an infectious bursal disease vaccine

Treatments	Antibody titer (mean)					
	1st week	**2nd week**	**3rd week**	**4th week**	**5th week**	**6th week**
T0 (0 FTU/kg)	3,560[c]	1,239.3[ab]	360.3[a]	835.3[b]	1,920.7[b]	1,535.0[a]
T1 (500 FTU/kg)	2,560.7[b]	828.0[bc]	571.0[a]	633.3[a]	1,701.3[a]	1,335.3[a]
T2 (1,000 FTU kg)	2,563.3[b]	1,347.0[a]	242.7 [a]	996.0[a]	1,189.0[a]	1,795.3[a]
T3 (1,500 FTU kg)	3,963.7[a]	673.3[c]	810.3[a]	653.7[a]	1,640.0[a]	1,568.0[a]
LSD$_{0.05}$	930.39	455.51	631.61	1,363.3	1,163.9	667.73

Notes: Values within a column with no common superscript differ significantly ($p \leq 0.05$).

Treatment 0 (T0) = Basal diet (Negative control) without phytase; Treatment 1 (T1) = Basal diet with phytase (500 FTU/kg of diet); Treatment 2 (T2) = Basal diet with phytase (1,000 FTU/kg of diet); Treatment 3 (T3) = Basal diet with phytase (1,500 FTU/kg of diet); FTU: Fitase units; LSD: Least significant difference.

Table 3. Blood IgM content (weekly intervals) of broiler chickens fed on a low P diet supplemented with locally produced bacterial phytase and vaccinated with an infectious bursal disease vaccine

Treatments	IgM concentration (mean)					
	1st week	**2nd week**	**3rd week**	**4th week**	**5th week**	**6th week**
T0 (0 FTU/kg)	18,858.0[a]	19,832.0[a]	19,010.7[a]	14,358.0[a]	12,091.0[a]	14,092.0[a]
T1 (500 FTU/ kg)	15,665.0[a]	18,525.3[b]	17,016.3[b]	14,380.0[a]	13,478.0[a]	14,913.0[a]
T2 (1000 FTU/kg)	17,169.0[a]	18,211.0[b]	18,064.7[ab]	8,177.0[b]	7,982.0[a]	12,675.0[a]
T3 (1500 FTU/kg)	14,197.0[a]	20,512.3[a]	19,499.0[a]	11,950.0[ab]	8,423.0[a]	10,210.0[a]
LSD$_{0.05}$	7,110.6	808.86	1,678.6	5649.1	7,012	4,748.9

Notes: Values within a column with no common superscript differ significantly ($p \leq 0.05$).

Treatment 0 (T0) = Basal diet (Negative control) without phytase; Treatment 1 (T1) = Basal diet with phytase (500 FTU/kg of diet); Treatment 2 (T2) = Basal diet with phytase (1,000 FTU/kg of diet); Treatment 3 (T3) = Basal diet with phytase (1,500 FTU/kg of diet); FTU: Fitase units; LSD: Least significant difference.

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Table 4. Mucosa IgA content (weekly intervals) of broiler chickens fed on a low P diet supplemented with locally produced bacterial phytase and vaccinated with an infectious bursal disease vaccine

Treatments	IgA concentration (mean)					
	1st week	**2nd week**	**3rd week**	**4th week**	**5th week**	**6th week**
T0 (0 FTU/kg)	293.67[a]	3630[b]	13,932[a]	13,735[a]	20,550[a]	13985[b]
T1 (500 FTU/kg)	415.00[a]	6546[ab]	18,924[a]	19,480[a]	29,766[a]	26016[b]
T 2 (1000 FTU/kg)	342.33[a]	13,169[a]	18,841[a]	28,243[a]	41,461[a]	45417[ab]
T3 (500 FTU/kg)	415.67[a]	8,814[ab]	19,296[a]	42,755[a]	32,839[a]	64467[a]
LSD$_{0.05}$	185.76	7,589.4	11,757	35,271	32,558	34591

Notes: Values within a column with no common superscript differ significantly ($p \le 0.05$);

Treatment 0 (T0) = Basal diet (Negative control) without phytase; Treatment 1 (T1) = Basal diet with phytase (500 FTU/kg of diet); Treatment 2 (T2) = Basal diet with phytase (1,000 FTU/kg of diet); Treatment 3 (T3) = Basal diet with phytase (1,500 FTU/kg of diet); FTU: Fitase units; LSD: Least significant difference.

Table 5. Blood IgG content (weekly intervals) of broiler chickens fed on a low P diet supplemented with locally produced bacterial phytase and vaccinated with an infectious bursal disease vaccine

Treatments	IgG concentration (mean)					
	1st week	**2nd week**	**3rd week**	**4th week**	**5th week**	**6th week**
T0 (0 FTU/kg)	1,0759[a]	13,879[a]	10,153[a]	17,009[b]	15,889[a]	17,035[a]
T1 (500 FTU/ kg)	12,276[a]	19,606[a]	11,109[a]	18,004[a]	16,550[a]	17,170[a]
T2 (1,000 FTU/kg)	11,223[a]	24,639[a]	7,997[a]	14,846[c]	13,789[a]	14,607[b]
T3 (1,500 FTU/kg)	9,835[a]	15,398[a]	8,769[a]	17,677[ab]	13,789[a]	17,336[a]
LSD$_{0.05}$	6,884.1	3,448.5	3,973.1	843.23	3,557.1	1,307.4

Notes: Values within a column with no common superscript differ significantly ($p \le 0.05$).

Treatment 0 (T0) = Basal diet (Negative control) without phytase; Treatment 1 (T1) = Basal diet with phytase (500 FTU/kg of diet); Treatment 2 (T2) = Basal diet with phytase (1000 FTU/kg of diet); Treatment 3 (T3) = Basal diet with phytase (1500 FTU/kg of diet). FTU: Fitase units; LSD: Least significant difference.

on mucosal IgA concentrations in IBD vaccinated broiler chickens indicated that the increasing of mucosal IgA concentrations were continued from the ages of 1 to 6 week with increasing phytase doses and showed significantly ($p < 0.05$) different at the ages of 2 and 6 week.

Antibody plays a key role for the maintenance of protected homeostasis by exposure to environmental stimulation (Coutinho, Kazatchkine, & Avrameas, 1995) and it has been reported that low levels of Ab might be associated with disease susceptibility (Parmentier et al., 2004). In the current experiment, the main affects data of serum specific Ab to IBDV demonstrated that the titers could not be enhanced by dietary phytase addition in a P deficient diet. Over the ages of 1, 4, and 5 week, the Ab levels showed significantly ($p < 0.05$) different, but the titers between treated groups were incongruous increasing or decreasing regardless to their phytase treatments at all ages. However, Liu et al. (2008) reported that phytase (Phyzyme®, *Escherichia coli*-derived phytase, Danisco Animal Nutrition, UK) addition in high phytate (0.44%) diet improved significantly ($p < 0.05$) the anti-Newcastle disease virus (anti-NDV) antibodies at the ages of 3 and 4 weeks, and low phytate (0.22%) diet did not affect the serum Ab production at the ages of 2–4 week birds on ND vaccination. Data also

showed that both of serum IgM and IgG contents in birds were regular and almost same throughout the experiment. However, although IgM levels for the ages of 2–4 weeks and IgG levels for the ages of 4 and 6 weeks demonstrated significantly ($p < 0.05$) different, the values were not consistent with their corresponding levels of phytase. Clearly, in the current study, the elevated or decreased IgM values for unlike ages were inconsistent regardless of phytase treatments. Therefore, the overall results indicated that levels of phytase did not affect serum IgM and IgG concentrations of broiler chickens at IBD vaccination. To our knowledge, there are no reports in these regards due to dietary administration of phytase for broiler chickens.

The main effects data revealed that supplementation of locally produced bacterial phytase improved mucosal secretory IgA (MSIgA) concentrations of broiler chickens throughout the experiment, and prompt with significant ($p < 0.05$) secretions were usually observed from the ages of 4 to 6 week. In the current study, research has shown that the MSIgA contents of vaccinated birds were increased consistently to their relative doses of phytase and IgA levels of phytase treated chickens were always higher than the birds without phytase. Phytase addition, after all, increased the mucosal IgA concentration of broiler chickens, and this agrees results previously reported by Islam et al. (2014) and Liu et al. (2008). They showed that phytase supplementation increased significantly ($p < 0.05$) the MSIgA content of broiler chickens at week of 2–4 for both the low- and high-phytate diets. The mucosal secretory epithelium is responsible for a potential effector tissue of integrated host responses, synthesizing MSIgA to protect gastrointestinal associated port of entry into the body. It is speculated that the degradation products of phytate by phytase could maintain immune activity of mucosal cells (Bozsik, Kokeny, & Olah, 2007; Vucenik & Shamsuddin, 2006). In other study, Kettunen and Rautonen (2005) shown 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 contemplated that birds fed on the phytate diet could influences mucin integrity was thought to be related to the highly (pH dependent) reactive nature of dietetic phytate. When feed was exposed to the low pH conditions, and/or phytase in the proximal gut, phytate was solubilized and could react electrostatically with basic amino acid residues in dietetic protein. According to the study by Vaintraub and Bulmaga (1991), these protein-phytate complexes were variably refractory to digestion by pepsin and solubilization with HCl, leading to a downstream increase in the secretion of mucin by GI epithelium. It was, therefore, the fact that phytase addition could partially ameliorate the adverse effects of phytate in the GIT of broilers by excess secretion of mucin (Cowieson, Acamovic, & Bedford, 2004, 2006). It could contribute to regulate a normal gut ecology that influences 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 & Ravindran, 2007; Cowieson, Ravindran, & Selle, 2008).

3.2. Effects on growth performance

To evaluate the growth performance, the effect of locally produced bacterial phytase on body weight during the experimental period (Table 6) revealed that the cumulative body weights of broilers were consistently and significantly ($p < 0.05$) increased with increasing phytase levels and showed more vigorous from the ages of 3 to 5 weeks. Phytase supplemental effect on growth performance revealed that the significant ($p < 0.05$) increasing of body weight gains of vaccinated broiler chickens were recorded from the ages of 1 to 6 weeks. Phytase inclusion at the doses of 500, 1,000, and 1,500 FTU/kg of diet to a low P basal diet produced higher body weight gains of broiler chickens compared to the diet without phytase (0 FTU/kg of diet) and 1,500 FTU/kg of diet commenced better performance than birds of other treated groups. These results were similar to those obtained by Islam et al. (2014), Saima, Khan, Jabbar, Ijaz, and Qadeer (2009), Shirley and Edwards (2003), Cabahug, Ravindran, Selle, and Bryden (1999), Qian, Kornegay, and Denbow (1997), Mitchell and Edwards (1996) and Perney, Cantor, Straw, and Herkelman (1993). They reported that supplementing phytase to the low P diet improved growth performance of broilers throughout the experimental period. In addition, regardless of some conflicting data these results were also similar to those reported by Khin (2011), Nasrollah (2010), Ahmed, Rahman, Ahmed, and Miah (2004), Ahmad, Rasool, Sarwar,

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Table 6. Effects of locally produced bacterial phytase on average body weight gains (weekly intervals) of broiler chickens fed on a low P basal diet and vaccinated with an infectious bursal disease vaccine

Treatments	Body weight gain (g; mean)					
	1st week	**2nd week**	**3rd week**	**4th week**	**5th week**	**6th week**
T0 (0 FTU/kg)	112.0[b]	265.8[b]	550.5[c]	1032.3[c]	1,494.7[c]	1,975.7[b]
T1 (500 FTU/kg)	128.5[ab]	306.3[a]	634.3[b]	1124.5[bc]	1,580.5[b]	2,147.3[a]
T2 (1000 FTU/kg)	148.3[a]	322.0[a]	697.3[a]	1169.5[ab]	1,627.3[ab]	2,213.0[a]
T3 (1500 FTU/kg)	148.7[a]	336.7[a]	713.8[a]	1247.3[a]	1,666.8[a]	2,275.8[a]
LSD$_{0.05}$	21.34	38.67	42.47	100.27	81.13	1,65.83

Notes: Values within a column with no common superscript differ significantly ($p \leq 0.05$).

Treatment 0 (T0) = Basal diet (Negative control) without phytase; Treatment 1 (T1) = Basal diet with phytase (500 FTU/kg of diet); Treatment 2 (T2) = Basal diet with phytase (1,000 FTU/kg of diet); Treatment 3 (T3) = Basal diet with phytase (1,500 FTU/kg of diet); FTU: Fitase units; LSD: Least significant difference.

Haq, and Hasan (2000), Viveros, Brenes, and Centeno (2002) and Sebastian, Touchburn, Chavez, and Lague (1996). The improvement of weight gains depended on phytase source and dose (Khin, 2011; Parr & Wyatt, 2006; Selle & Ravindran, 2007). In contrast, results were contrary with the study of Assuena et al. (2009) who reported that phytase addition linearly decreased weight gains as phytase levels were increased and some other studies (Danek, Kaplan, Avci, & Can, 2007; Onyango, Bedford, & Adeola, 2004) have also suggested that phytase inclusion in a diet did not affect growth performances of birds.

3.3. Effects on hematological parameters

At the age of 6 week, the results of complete hemogram (Table 7) showed that basophil and thrombocyte counts were significantly ($p < 0.05$) decreased to the increasing levels of phytase. Whether, the numbers of basophil were almost consistent to their corresponding treatments. The highest and lowest basophil counts were 4.17 and 1.00% at 0 and 1,500 FTU/kg of diet, respectively. However, the values of thrombocyte at different treatments were verily inconsistent to their corresponding phytase levels. The numbers of thrombocytes (×10^9/L) were 5.95, 1.41, 0.54, and 3.85% to the levels of 0, 500, 1,000 and 1,500 FTU/kg of diet, respectively. To our knowledge, there were no reports on basophil and thrombocyte counts due to dietary administration of phytase for broiler chickens. It was, therefore, a fact that the basopenia with increasing locally produced phytase addition might be due to stress, viral infections, or hormonal imbalances (elevated glucocorticoids and/or hyperthyroidism) of birds. Only a few studies have investigated the influence of dietary microbial phytase on hematological parameters (Czech & Grela, 2004; El-Badry et al., 2008) in animals. El-Badry et al. (2008) reported that white blood cell (WBC), red blood cell (RBC) and packed cell volume (PCV) were not affected, but blood Hb concentration was increased by dietary non phytate P and/or phytase treatments. The exogenous microbial phytase inclusion to a low P sow diet did not influence PCV, RBC, but diminished WBC number and increased Hb content in blood (Czech & Grela, 2004).

3.4. Effects on blood biochemical constituents

Over the age of 6 week, the effects of locally produced bacterial phytase on blood biochemical constituents in IBD vaccinated broiler chickens demonstrated that no significant and consistent treatment effects were found in treated birds (Table 8). However, some parameters (ALT, AST, triglyceride, urea, and creatinine) were in increased trend to the phytase treated groups. The main effects data indicated that the locally produced bacterial phytase did not show any significant effects on blood biochemistry during the feeding of low P diet. The findings of unaffected parameters were accorded with those reported by Shehab et al. (2012), Al-Harthi (2006), Eisa Adel et al. (2003), Attia (2003),

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Table 7. Hematological parameters of broiler chickens fed locally produced bacterial phytase in a P deficient diet and vaccinated with an infectious bursal disease vaccine

Parameters	Units	Treatments				LSD$_{0.05}$
		T0 (0 FTU/kg)	T1 (500 FTU/kg)	T2 (1,000 FTU/kg)	T3 (1,500 FTU/kg)	
Total erythrocyte count	×10^{12}/L	2.30[a]	2.47[a]	2.28[a]	2.36[a]	0.32
Haemoglobin	g/L	114.77[a]	118.50[a]	115.67[a]	115.17[a]	16.42
Packed cell volume	L/L	0.27[a]	0.28[a]	0.28[a]	0.27[a]	0.04
Mean corpuscular volume	fL	116.0[a]	134.2[a]	120.0[a]	116.0[a]	35.59
Mean corpuscular haemoglobin concentration	g/L	429.67[a]	428.43[a]	424.00[a]	421.17[a]	24.53
Total leukocyte count	×10^{9}/L	27.90[a]	25.87[a]	29.12[a]	21.05[a]	18.05
Heterophil	%	33.50[a]	38.50[a]	34.33[a]	30.83[a]	15.60
Eosinophil	%	0.17[a]	0.50[a]	0.33[a]	1.00[a]	0.90
Basophil	%	4.17[a]	2.17[ab]	2.33[ab]	1.00[b]	2.45
Lymphocyte	%	56.50[a]	55.33[a]	59.83[a]	61.83[a]	16.05
Monocyte	%	4.50[a]	3.50[a]	3.17[a]	4.17[a]	3.18
Thrombocyte	×10^{9}/L	5.95[a]	1.41[b]	0.54[b]	3.58[ab]	3.31
Icterus index	Unit	2.00[a]	2.00[a]	2.00[a]	2.00[a]	0.00
Plasma protein	g/L	34.67[a]	33.00[a]	34.00[a]	33.83[a]	6.66

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

Treatment 0 (T0) = Basal diet (Negative control) without phytase; Treatment 1 (T1) = Basal diet with phytase (500 FTU/kg of diet); Treatment 2 (T2) = Basal diet with phytase (1,000 FTU/kg of diet); Treatment 3 (T3) = Basal diet with phytase (1,500 FTU/kg of diet); FTU: Fitase units; LSD: Least significant difference.

Qota, El-Ghamry, and El-Mallah (2002) and Huff et al. (1998). In contrast, there is ample evidence reporting that supplemental microbial phytase causes significant effect on some blood biochemical parameters. El-Badry et al. (2008) showed that ducks fed diet contained lower non-phytate 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. In a study with Japanese quails, Danek et al. (2007) showed that phytase supplemented diet significantly affected serum K, and triglyceride. Ghasemi et al. (2006) reported a significant increase of serum TP in broiler chickens receiving phytase. Viveros et al. (2002) reported that decreasing dietary non phytate phosphorus (nPP) levels caused a decrease in serum AST activity, and it was counteracted by dietary phytase addition. On the other hand, some researchers (Attia, 2003; Fernandes et al., 1999; Huff et al., 1998; Viveros et al., 2002) reported that using of phytase in diet increased ALP activity. The higher phytate might be responsible for the higher ALP activity that could be related to intestinal lesions, skeletal disorders, or liver dysfunctions. However, the decrease ALP activity could be associated with the increase in Zn retention in the chicken intestine (Lei, Ku, Miller, Ullrey, & Yokoyama, 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 a generally poor indicators of renal disease and/or muscle injury in birds (Campbell, 2004). In the current study, the blood Ca levels in the birds of phytase treated groups were insignificantly ($p > 0.05$) higher than the

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Parameters	Units	Treatments				LSD$_{0.05}$
		T0 (0 FTU/kg)	T1 (500 FTU/ kg)	T2 (1,000 FTU/ kg)	T3 (1,500 FTU/kg)	
Albumin	g/L	12.43[a]	11.58[a]	11.10[a]	13.52[a]	2.88
Total protein	g/L	31.92[a]	29.95[a]	32.10[a]	30.11[a]	5.64
Alanine transaminase	U/L	4.39[a]	6.52[a]	13.05[a]	10.38[a]	9.86
Alkaline phosphatase	U/L	1603[a]	1312[a]	1270[a]	1494[a]	678.61
Aspartate transaminase	U/L	366.52[a]	405.17[a]	379.42[a]	420.58[a]	121.85
Gamma-glu-tamyl transferase	U/L	16.00[a]	14.50[a]	15.17[a]	17.50[a]	5.56
Lactate dehydrogenase	U/L	1,855.7[a]	1,998.7[a]	1,818.8[a]	2,107.7[a]	630.14
Cholesterol	mmol/L	2.57[a]	2.36[a]	2.68[a]	2.57[a]	0.66
Triglyceride	mmol/L	0.39[a]	0.41[a]	0.42[a]	0.45[a]	0.22
Glucose	mmol/L	12.60[a]	13.25[a]	13.42[a]	12.88[a]	2.38
Ca	mmol/L	2.76[a]	2.63[a]	2.84[a]	2.75[a]	0.26
P	mmol/L	1.95[a]	1.92[a]	1.95[a]	2.00[a]	0.20
Na	mmol/L	155.1[a]	155.8[a]	158.2[a]	157.2[a]	5.18
K	mmol/L	3.72[a]	3.62[a]	3.15[a]	3.50[a]	0.69
Cl	mmol/L	113.35[a]	112.23[a]	114.37[a]	113.37[a]	5.59
Urea	mmol/L	0.52[a]	0.85[a]	1.00[a]	1.00[a]	0.63
Creatinine	μmol/L	27.83[a]	29.00[a]	29.50[a]	30.83[a]	5.28
Uric acid	μmol/L	272.23[a]	214.43[a]	282.10[a]	228.40[a]	136.12

Table 8. Blood biochemical constitutes of broiler chickens fed on a P deficient diet with addition of locally produced bacterial phytase and vaccinated with an infectious bursal disease vaccine

Values within a row with no common superscript differ significantly (p ≤ 0.05).

Treatment 0 (T0) = Basal diet (Negative control) without phytase; Treatment 1 (T1) = Basal diet with phytase (500 FTU/ kg of diet); Treatment 2 (T2) = Basal diet with phytase (1,000 FTU/kg of diet); Treatment 3 (T3) = Basal diet with phytase (1,500 FTU/kg of diet); FTU: Fitase units; LSD: Least significant difference.

birds of without phytase, and the values of blood P were vice versa. Several studies have reported that phytase inclusion in a low P diet did not have significant (*p* < 0.05) influence on blood Ca and P concentrations (Aureli et al., 2011; Catala-Gregori, Garcia, Hernandez, Madrid, & Ceron, 2006; Danek et al., 2007; Kliment & Aneglovicova, 2011; Rezaei, Borbor, Zaghari, & Teimouri, 2007). In contrast, in a study with broiler, Jadhav, Suranagi, Anjanega, Chandra, and Mallikarjunappa (2009) reported that blood Ca levels were highly significantly (*p* < 0.05) enhanced in the phytase treated group in comparison without phytase. However, several studies have indicated that microbial phytase addition in a low P diet increased plasma P concentration and in contrast decreased plasma Ca content (Aureli et al., 2011; El-Badry et al., 2008; Ghasemi et al., 2006; Han et al., 2009; Onyango et al., 2004). These controversial results are probably due to diet factors as well as phytase source, crudity, purity, and activity.

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4. Conclusion

It can be concluded that the supplementation of ASUIA273 phytase to P deficient (0.19%) broiler diet was an effective mean for improving cumulative body weight, mucosal IgA contents, and the phytase dose of 1,500 FTU/kg of diet produced better performances than other treatments. The overall findings of hematology and blood biochemical constituents did not indicate any change that would suggest that locally produced bacterial phytase obtained from *Enterobacter sakazakii273* in corn-soybean based low P diet affected the health of broilers. It would be necessary for further research to evaluate the interaction of cell-mediated immunity, dietary mineral content, feed intake, feed conversion ratio, bone mineralization, and mineral retention. For yielding the most benefit from this phytase supplementation, research can also be done to optimize P levels in P deficient diet, and measure excreted P levels in feces. By supplementing phytase in animal diet, study can also be carried on to know the impact of inositol, steroid hormone, vitamin-D, parathormone, and thyroid in animal body.

Competing Interests
The authors declare no competing interest.

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