UNIVERSITI PUTRA MALAYSIA

EFFECTS OF LOCALLY PRODUCED MICROBIAL PHYTASE ON HUMORAL IMMUNITY AND BLOOD CHARACTERISTICS IN BROILERS VACCINATED AGAINST NEWCASTLE AND INFECTIOUS BURSAL DISEASES

RAKIBUL ISLAM

FPV 2014 15

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By

RAKIBUL ISLAM

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfilment of the requirement for the degree of Doctor of Philosophy

April 2014

DEDICATION

This thesis is dedicated to my beloved father (Md Afsar Ali Molla) and mother (Sajeda Begum).

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

EFFECTS OF LOCALLY PRODUCED MICROBIAL PHYTASE ON HUMORAL IMMUNITY AND BLOOD CHARACTERISTICS IN BROILERS VACCINATED AGAINST NEWCASTLE AND INFECTIOUS BURSAL DISEASES

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April 2014

Supervisor: Professor Datin Paduka Aini Ideris, PhD
Faculty: Veterinary Medicine

In a phosphorus (P) deficient animal diet, phytase supplementation improves the bioavailabilities of P as well as other nutrients by phytate hydrolysis and indirectly, plays a role in biological function of many metabolic processes. In consequence, hypothetically, chicken health in terms of immune responses associated with hematological parameters, blood biochemical constituents and live body weight might be influenced. Phytase production commercially, focuses only on the soil fungus *Aspergillus*, but many possible sources of microbial novel phytases remain unexplored. In Malaysia, around 30 strains of potential phytase producing soil bacteria were successfully harvested and they show good enzymatic activities and characteristics favouring physiology of chicken gut. In addition, some bacterial phytases, especially those of the genera *Bacillus* and *Enterobacter*, exhibits a pH optimum ranging from 6.0 to 8.0, close to the physiological pH of the stomach of chicken. Newcastle disease (ND) and infectious bursal disease (IBD) are the most important diseases for poultry worldwide, which can cause huge economic losses in the poultry industry. In Malaysia, therefore, the possibility of using the locally produced bacterial phytase from *Enterobacter sakazakii* ASUA273 in broilers fed low P diet (0.19%) and vaccinated against ND and IBD could be justified. The objective of the study was to determine the effects of microbial phytases on humoral immunity and blood characteristics in association with the live body weights of broilers vaccinated with ND and IBD vaccines. Five experiments (Experiment I, II, III, IV and V) were carried out. The first three trials (Experiment I, II and III) were carried out in broilers fed low P diet with four doses (0 FTU/kg^{-1}, 500 FTU/kg^{-1}, 1000 FTU/kg^{-1}, and 1500 FTU/kg^{-1} of diet) of local bacterial phytase grouped as T0 (control), T1, T2, and T3, accordingly on ND, IBD, and both of ND and IBD vaccinations, respectively. In each trial, 180 day-old-male broilers were allocated to four treatment groups with 12 cages comprising three replicates, each cage containing 15 birds. The last two trials (Experiment IV and V) were conducted with broiler chickens fed the same diet, with two doses (0 FTU/kg^{-1} and 1500 FTU/kg^{-1} of diet) of Natuphos® grouped as T0 (control) and T1, accordingly on ND and IBD vaccinations, respectively. Ninety (92) day-old-male broilers per trial were randomly assigned into two treatments, each contained three replicates

of 15 chicks each. They were maintained on formulated experimental basal diet based on available phosphorus (0.19%), lasted up to six weeks of age with feed, and water made attainable for *ad libitum* consumption. Birds received two doses of ND vaccines (ND 'V4 HR') at day-old and 21 day-old, respectively and one dose of IBD vaccine (IBD UPM93) at 10 day-old. Two birds were randomly selected weekly, from each treated group (8 and 4 birds per replicate were selected from experiments using local phytase and Natuphos® supplementation, respectively) and live body weights were measured. These birds were then slaughtered for blood collection to prepare serum for quantification of antibody (Ab) titers, IgM, and IgG and jejunal fluid were collected to quantify IgA throughout the experiment. At the end of experiment, blood was furthermore collected for determining the complete hemogram and blood biochemistry. Antibody titers (ND and IBD), IgM, IgG, and IgA were detected by ELISA using commercial kits. Although a hematology analyzer using commercial reagents measured the complete hemogram, other parameters (differential leukocyte count (DLC), packed cell volume (PCV), icterus index, and total plasma protein) were determined manually. All blood biochemical constituents were determined with the help of a chemistry analyzer. Data of humoral immunity with live body weights, and blood characteristics were analyzed based on factorial arrangement (treatments × weeks) of completely randomized design (CRD) and CRD, respectively. Results of humoral immunity of vaccinated broilers showed that serum Ab titers (ND and IBD), IgM, and IgG contents did not increase by phytase supplementation in low P diet. However, mucosal secretory IgA concentrations of vaccinated birds increased consistently and significantly ($P<0.05$) with increasing phytase doses throughout the experiments. Results of live body weights of broilers showed that body weights were linearly and significantly ($P<0.05$) increased to graded levels of phytase supplementation at weekly intervals. Cumulative effects of mucosal IgA contents and live body weights of broilers also showed the significant interaction between effects of phytase levels and effects of weeks. Overall, phytase dose at 1500 FTU/kg^{-1} of diet and over the age of 6 weeks showed the best performance. On the overall, findings on complete hemogram and blood biochemical constituents did not show any consistent and significant ($P<0.05$) difference that would suggest that phytase supplementation in corn-soybean based P deficient diet affected the health of broilers. It was therefore, concluded that the locally produced bacterial phytase obtained from *Enterobacter sakazakii*273 could be as effective as the commercially produced fungal phytase (Natuphos®). Further researches are recommended to determine the optimum level of available P in order to produce maximum performances (body weight gain, feed intake, feed conversion ratio, bone mineralization, mineral retention, and P excretion in the environment) using larger number of chickens. The cell-mediated immunity in broilers vaccinated against ND and IBD vaccines should also be measured to assess the real effect on immune response by local phytase supplementation in low P diet. In addition, phytase from ASUIA273 can be used to a diet to determine the impacts on vitamin-D, parathormone, glucocorticoids, and thyroids in animal body.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN FITASE MIKROB HASILAN TEMPATAN KE ATAS IMMUNITI HUMORAL DAN CIRI-CIRI DARAH PADA AYAM PEDAGING YANG DIVAKSINASI TERHADAP PENYAKIT NEWCASTLE DAN PENYAKIT BERJANGKIT BURSA

Oleh

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Di dalam diet haiwan yang kurang fosforus (P), tambahan fitase memperbaiki bioketersediaan P dan nutrient-nutrien lain dengan hidrolisis fitat dan secara tidak langsung, memainkan peranan dalam banyak fungsi biologi proses metabolik. Disebabkan itu, secara hipotesisnya, kesihatan ayam dalam terma tindakbalas keimunan yang berkaitan dengan parameter-parameter hematologi, komposisi biokimia darah dan berat badan semasa hayat mungkin turut dipengaruhi. Penghasilan fitase secara komersial tertumpu hanya kepada kulat tanah *Aspergillus,* banyak kemungkinan sumber baru fitase mikrob yangbelum diterokai. Di Malaysia, lebih kurang 30 strain bakteria tanah yang berpotensi menghasilkan fitase telah berjaya diperolehi dan mereka menunjukkan aktiviti enzim yang baik dan ciri cenderung kepada fisiologi usus ayam. Tambahan pula, beberapa fitase bakteria, terutama yang tergolong dalam genus *Bacillus* dan *Enterobacter*, mempamerkan pH optimum dalam julat 6.0 hingga 8.0, hampir kepada pH fisiologi perut ayam. Penyakit Newcastle (ND) dan penyakit berjangkit bursa (IBD) adalah penyakit-penyakit yang paling penting bagi ternakan ayam di seluruh dunia, yang mana boleh menyebabkan kerugian ekonomi yang ketara dalam industri ternakan ayam. Oleh itu, di Malaysia, kebarangkalian menggunakan fitase bakteria yang dihasilkan secara tempatan daripada *Enterobacter sakazakii* ASUA273 pada ayam pedaging yang diberi diet rendah P (0.19%) dan divaksinasi terhadap ND dan IBD boleh dipertimbangkan dengan wajar. Objektif kajian ini adalah untuk mengenal pasti kesan fitase mikrob ke atas imuniti humoral dan ciri darah yang berkaitan dengan berat badan ayam pedaging ke atas vaksinasi ND dan IBD. Lima kajian (Eksperimen I, II, III, IV dan V) telah dijalankan. Tiga ujian pertama (Eksperimen I, II dan III) telah dijalankan pada ayam pedaging yang diberi makan diet rendah P dengan empat dos (0 FTU/kg^{-1}, 500 FTU/kg^{-1}, 1000 FTU/kg^{-1} dan 1500 FTU/kg^{-1} dalam diet) fitase bakteria yang dihasilkan secara tempatan, dengan dibahagikan sebagai T0 (kawalan), T1, T2, dan T3 sewajarnya ke atas vaksinasi ND, IBD dan kedua-dua ND dan IBD masing-masing. Dalam setiap ujian, 180 ekor anak ayam jantan pedaging berusia sehari dibahagikan kepada empat kumpulan rawatan dengan 12 sangkar, terdiri daripada tiga replikat, dengan setiap sangkar mengandungi 15 ekor ayam. Dua ujian terakhir (Eksperimen IV dan V) telah dijalankan ke atas anak-anak ayam pedaging yang diberi makan diet yang sama dengan dua dos (0 FTU/kg^{-1} dan 1500 FTU/kg^{-1} dalam diet) Natuphos® yang digolongkan sebagai T0 (kawalan) dan T1 sewajarnya ke atas vaksinasi ND dan IBD masing-masing. Anak ayam jantan berusia sembilan puluh (90) hari setiap ujian telah dikendalikan secara rawak kepada dua rawatan, setiap satu mengandungi tiga replikat dengan 15 ekor anak ayam. Kesemuanya diberikan diet eksperiman asas yang diformulasi berdasarkan fosforus sedia-ada (0.19%) yang mencukupi hingga enam minggu jangka umur dengan makanan, dan bekalan air sedia ada bagi penggunaan *ad libitum*. Ayam-ayam menerima dua dos vaksin ND (ND 'V4 HR') pada hari pertama dan hari ke-21, dan satu dos vaksin IBD (IBD UPM93) pada hari kesepuluh masing-masing. Setiap minggu, dua ayam dipilih secara rawak daripada setiap rawatan (8 dan 4 ayam setiap replikat dipilih bagi eksperimen fitase tempatan dan tambahan Natuphos ® masing-masing) dan ditimbang untuk menilai berat badan semasa hayat. Ayam-ayam tersebut kemudian disembelih untuk mendapatkan darah bagi persediaan serum untuk mengetahui titer antibodi-antibodi (Ab), IgM, dan IgG dan cecair jejunal bagi mengetahui kuantiti IgA di sepanjang eksperimen. Di akhir tempoh eksperimen, darah dikumpulkan sekali lagi bagi menentukan hemogram lengkap dan komposisi biokimia darah. Titer antibodi (ND dan IBD), IgM, IgG dan IgA telah dikesan menggunakan kit komersial ELISA. Walaupun penganalisa hematologi menggunakan reagen-reagen komersial mengukur hemogram lengkap, parameter-parameter lain (kiraan bezaan leukosit (DLC), isipadu sel termampat (PCV), indeks ikterus dan jumlah protein plasma) telah ditentukan secara manual. Kesemua komposisi biokimia darah telah diketahui dengan bantuan mesin penganalisa kimia. Data berat badan hayat yang berkenaan dengan imuniti humoral dan ciri-ciri darah telah dianalisa berdasarkan susunan berfaktor (rawatan x minggu) reka bentuk rawak (CRD) dan CRD sepenuhnya masing-masing. Keputusan tindak balas keimunan humoral ayam pedaging divaksinasi menunjukkan bahawa kandungan serum titer Ab (ND dan IBD), IgM dan IgG tidak meningkat dengan tambahan fitase dalam diet rendah P. Walau bagaimanapun, kepekatan rembesan IgA mukosa ayam-ayam divaksinasi telah meningkat dengan konsisten dan nyata ($P<0.05$) dengan peningkatan dos fitase di sepanjang eksperimen. Data berat badan hayat ayam-ayam pedaging menunjukkan bahawa berat badan adalah meningkat secara linear dan nyata ($P<0.05$) ke tahap berperingkat oleh penambahan fitase pada selangan mingguan. Kesan-kesan terhimpun kandungan mukosa IgA dan berat badan hayat ayam pedaging juga menunjukkan interaksi yang ketara antara kesan-kesan tahap fitase dan kesan-kesan minggu. Keseluruhannya, dos fitase sebanyak 1500 FTU/kg^{-1} dalam diet dan usia melebihi 6 minggu menampakkan pencapaian terbaik. Secara keseluruhannya, keputusan hemogram lengkap dan komposisi biokimia darah tidak menunjukkan perbezaan yang konsisten dan nyata ($P<0.05$) yang boleh mencadangkan bahawa tambahan fitase dalam diet kurang P berasaskan jagung-kacang soya menjejaskan kesihatan ayam pedaging. Oleh itu, boleh disimpulkan bahawa fitase bakteria hasilan tempatan yang didapati dari *Enterobacter* sakazakii273 mempunyai kesan yang sama dengan fitase kulat yang dihasilkan secara komersial (Natuphos®). Kajian selanjutnya dicadangkan bagi menentukan tahap

optimum P sedia ada bagi menghasilkan pencapaian maksimum (pertambahan berat badan, pengambilan makanan, nisbah penukaran makanan, mineralisasi tulang, pengekalan mineral dan pengumuhan P ke persekitaran) menggunakan bilangan ayam yang lebih besar. Keimunan berperantara sel pada ayam pedaging yang divaksinasi terhadap ND dan IBD juga seharusnya ditentukan bagi menilai kesan sebenar ke atas tindak balas keimunan oleh penambahan fitase tempatan ini dalam diet rendah P. Sebagai tambahan, fitase dari ASUIA273 boleh digunakan dalam diet bagi menentukan kesan-kesan ke atas vitamin-D, parathormon, glukokortikoid, dan tiroid dalam badan haiwan.

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I certify that a Thesis Examination Committee has met on 21 April 2014 to conduct the final examination of Rakibul Islam on his thesis entitled "Effects of Locally Produced Microbial Phytase on Humoral Immunity and Blood Characteristics in Broilers Vaccinated Against Newcastle and Infectious Bursal Diseases" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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A. niger	*Aspergillus niger*
aa	Amino acid
Ab	Antibody
Ag	Antigen
Alb	Albumin
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AME	Apparent metabolizable energy
aP	Available phosphorus
AST	Aspartate aminotransferase
BWG	Body weight gain
Ca	Calcium
CAID	Coefficient of apparent ileal digestibility
CF	Crude fiber
Chol	Cholesterol
Cl	Chloride
CLBW	Cumulative live body weight
Co	Cobalt
CPr	Crude protein
Creat	Creatinine
Cu	Copper
Cys	Cysteine
DCP	Dicalcium phosphate
dl	Deciliter
DLA	Differential leukocyte count
DM	Dry matter
E. coli	*Escherichia coli*
E. sakazakii	*Enterobacter sakazakii*
EDTA	Ethylenediaminetetraacitic acid
ELISA	Enzyme-linked immunosorbent assay
FCR	Feed conversion ratio
Fe	Iron
FI	Feed intake
g	Gram
GGT	Gamma glutamyl transpeptidase
GI	Gastro-intestinal
GIT	Gastro-intestinal tract
Glu	Glucose
Gly	Glycerine
Hb	Hemoglobin
hr	Hour
HRP	Horseradish peroxidase

I	Iodine
IBD	Infectious bursal disease
IBDV	IBD virus
IFCC	International federation of clinical chemistry
Ig	Immunoglobin
II	Icterus index
iP	Inorganic phosphorus
IUB	International union of biochemistry
K	Potassium
kcal	Kilocalorie
kg	Kilogram
LDH	Lactate dehydrogenase
Leu	Leucine
M	Molarity
MCHC	Mean corpuscular Hb concentration
MCV	Mean corpuscular volume
ME	Metabolizable energy
Met	Methionine
Mg	Magnesium
mg	Milligram
ml	Milliliter
mmol	Milimole
Mn	Manganese
Mo	Molybdenum
MSIgA	Mucosal secretory IgA
Na	Sodium
ND	Newcastle disease
NDV	ND virus
Ng	Nanogram
Ni	Nickel
nm	Nanometer
NRC	National research council
OD	Optical density
P	Phosphorus
P. lycii	*Peniophora lycii*
PAGE	Plolyacrylamide gel electrophoresis
PCV	Packed cell volume
PLT	Platelet
PP	Phytin phosphorus
RBC	Red blood cell
SAS	Statistical analysis system
SBM	Soy-bean meal
Se	Selenium
SIRIM	Standards and industrial research institute of Malaysia

TEC	Total erythrocyte count
Temp	Temperature
TLC	Total leukocyte count
TMB	Tetramethylbenzidine
tP	Total phosphorus
TPr	Total protein
Trig	Triglyceride
UA	Uric acid
uL	Microliter
Umol	Micromole
UPM	Universiti Putra Malaysia
USA	United State of America
WBC	White blood cell
WIC	WBC impedance count
Wk	Week
WOC	WBC optical count
Zn	Zinc

CHAPTER 1

INTRODUCTION

Phytase is an enzyme of phosphomonoesterase, capable of catalyzing the sequential release of a phosphate group from phytin in plant materials to yield inorganic phosphorus (iP) and lower phosphorylated *myo*-inositol derivatives such as phosphate ester of mono, di, tri, tetra, and penta phosphates (IUB, 1979; Wyse *et al.,* 1999). It was first discovered by Suzuki *et al*., 1907 and the industrial production of this enzyme was started in the early 1990s (Wodzinski and Ullah, 1996). Phytase addition in diets has proven to be an effective and realistic method for ameliorating the phytin digestibility in monogastric animals (swine, poultry, pre-ruminant calves, fish and humans), in particular, for the reduction of phytate content in feed and food and at the same time, lowers the phytin phosphorus (PP) disposal in nature. Since 1990, it has been broadly used in animal diets as a feed additive and has attracted great attention from both researchers and entrepreneurs in the areas of nutrition, environmental protection, and biotechnological application.

Phytase inhibits chelating potential of di- and trivalent cations (Ca, Co, Cu, Zn, Mg, Mn, and Fe), proteins and/or amino acids, or possibly starch with phytin either directly or via ionic bridges (Ravindran *et al*., 2008; Selle *et al*., 2006 and 2007) and consequently, improves not only the P bioavailability but also other nutrients. In addition to it preserves detrimental effects of hepatic antioxidants, and endogenous depletion from the gastrointestinal tract (GIT) of broilers by reducing the phytate activity on tract lead to less secretion of sialic acid (Cowieson *et al*., 2004, 2006, and 2007; Ravidran *et al*., 2000). This acid is an indicator of mucin losses from the GIT and accomplice with health problems such as cellular senescence, bacterial infection, certain pathological conditions, and osmotic fragility. Phytase also prevents the cadmium absorption by liberating zinc from phytate. Indirectly, it reduces the risk of heavy-metal poisoning and microbial pollution in animals caused by P rich compounds (Ca and Na phosphate) and bone meal, respectively (Cowieson *et al.,* 2004). Moreover, certain *myo*-inositol have been proposed to have a novel metabolic, and beneficial health (risk of heart disease, renal stone formation, and certain type of cancers) effects (Zhou *et al*., 1995).

Phytic acid exists in plant materials as phytate, a mixed cation salt of phytic acid, or a complex of phytate and protein, also known as phytin. It is ubiquitous among plant components, comprising 1-5% (w/w) of cereal grains, oilseeds, legumes, pollen, and nuts (Gibson and Ullah, 1990). The primary constituents of poultry and swine diets are plant-based ingredients incorporating phytate phosphorus (PP), which is not available to animals for absorption unless the phosphate group is removed from the inositol molecule by either intrinsic feed phytase, intestinal phytase or exogenous microbial phytase (Sandberg, 2002). However, phytin is considered as an anti-nutritional factor because it chelates various nutrients (multivalent cations, proteins and/or amino acids, and possibly starch) and reduces their bioavailability in animals (Reddy *et al*., 1982; Pallauf and Rimbach, 1997).

Intrinsic plant phytases are partially or totally inactivated by high steam-pelleting temperatures during the production stages (Ravindran *et al*., 1995a) and the simple stomached animals can barely utilize organically bound P, and other nutrients owing to lack or inadequate phytase activity in their GIT. Consequently, when poultry does not metabolize phytate, a substantial quantity of undigested PP is discharged in animal feces that tend to exacerbate the P pollution in environment, particularly in the waste stream at intensive livestock operations (Jang *et al*., 2003). Intestinal hydrolysis of dietary phytate is mainly achieved using exogenous microbial phytases (Ravindran *et al*., 1995a; Schroder *et al.,* 1996; Wodzinski and Ullah, 1996; Maenz *et al*., 1998; Brinch-Pedersen *et al*., 2002; Applegate *et al*., 2003; Vohra and Satyanarayana, 2003). Accordingly, feed distributors in the world have begun to formulate poultry diets with supplemental microbial phytase in order to improve feedlot productivity, effectively obviate P waste in nature, and lessen feed cost by using the limit of iP for their daily requirements.

Microbial phytase can work more readily at wider temperature ranges from 35-63^{0}C, and broader pH ranges from 2.5-5.5 (Wodzinski and Ullah, 1996). Recent studies show that microbial phytase is most promising for a biotechnological application and for obtaining a good source of microbial phytase, a variety of microorganisms, including bacteria, yeast, and fungi has been screened (Yanke *et al*., 1998; Yoon *et al*., 1996; Greiner *et al*., 1993; Shah and Parekh, 1990; Howson and Davis, 1983; Powar and Jagannathan, 1982). Commercial production currently focuses on only the soil fungus *Aspergillus* (Natuphos®) and many possible sources of microbial novel phytase remain unexplored. Due to some biological properties, such as 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 phytase especially those of the genera *Bacillus* and *Enterobacter*, exhibits a pH optimum in the range from 6-8, close to the physiological pH of the chicken stomach. In poultry ration, therefore, it would be more beneficial as feed additive as well as a real alternative to the fungal phytase. In Malaysia, about 30 strains of potential phytase producing bacteria (*Enterobacter* sp, *Bacillus* spp, *Pantoea* sp.) were successfully harvested from maize plantation (Anis Shobirin *et al*., 2009), and they exhibit a significant amount of phytase activities. However, the possibility of using these phytases in poultry feed has not been investigated.

In Malaysia, there are about 2,500 broiler farms producing over 400 million of birds and poultry meat production is projected to reach 1340.5 thousand metric tonnes in 2010 with an increased rate of 14.95% compared to 1166.1 thousand metric tonnes in 2005 (Raghavan, 2011). Since, productivity measures are very close to global standards, to improve PP utilization, alleviate the anti-nutritional effects of phytate in plant ingredients, and reduce P pollution, the microbial phytase is a novel and cost effective tool in poultry industry. Recently, phytases have been produced locally, and researches are being conducted to explore their potential under a project number: BT0106-01 supported by Ministry of science, technology and innovation (MOSTI). It is of practical interest to investigate the abilities of each type phytase produced by the respective bacteria and elucidate the consequence of phytase in poultry. Additionally, more studies that are comprehensive are needed to obtain a superior enzyme for better chicken performances.

Newcastle disease (ND) and infectious bursal disease (IBD) are the most important poultry diseases in worldwide, even in Malaysia and constitute a serious problem in the poultry industry. The virulent Newcastle disease virus (NDV) is highly contagious and outbreaks of it have a tremendous impact on backyard chickens in developing countries and may experience morbidity, and mortality up to 100% (Center for food security and public health, 2008). The IBD virus (IBDV) □L□V□□□H□[□W□U□H□P□H□O□\□-□□HF FRQWDJLRXV□□ the IBDV in the form of antigenic variants, and hyper-virulent strains has caused the significant economic losses. In infected flocks, morbidity is high, with up to 100% after infection, whilst mortality is variable. In addition to the indirect impact of the IBD is considerable, due to virus-induced immunosuppression and/or potential interactions between IBDV and other viruses, bacteria or parasites.

In animals, P is crucial for bone mineralization, and cell membrane building and indirectly, 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 animal diet. As a feed additive, in a P deficient animal diet, phytase improves the bioavailabilities of P and other nutrients by phytate hydrolysis. In consequence, phytase addition in low P diet may theoretically, influences chicken health in terms of immune responses in association with hematological parameters, blood biochemical constituents and body weight. However, there were many contradictions regarding growth performance, hematological parameters (El-Badry *et al.,* 2008; Anna Czech and Eugeniusz R. Grela, 2004), and blood biochemistry (Shehab *et al.,* 2012; El-Badry *et al.,* 2008; Ghasemi *et al.,* 2006; Al-Harthi, 2006 and Eisa *et al.,* 2003) and very limited information of phytase activity on body immunity. The study by Liu *et al*. (2008) reported the effect of phytase on immune response in ND vaccinated broilers. In Malaysia, therefore, the possibility of using the locally produced bacterial phytase from *E. sakazakii* ASUA273 and commercially produced fungal phytase (Natuphos®) from *A. niger* in broilers fed low P diet (0.19%) on ND and IBD vaccinations could be justified. Therefore, the current experiments were carried out to determine the effects of phytase on humoral immunity and blood characteristics associated with the live body weights of broiler chickens and vaccinated against ND and IBD for evaluating chicken health, with the following specifics objectives:

1) To determine the effects of locally produced bacterial phytase obtained from *E. sakzakii* ASUA273 on humoral immunity, and hemato-biochemical constituents associated with the live body weights of broiler chickens fed on a low P diet and vaccinated against ND, IBD, and both of ND and IBD.
2) To determine the effects of commercially produced fungal phytase (Natuphos®) obtained from *Aspergillus niger* on humoral immunity, and hemato-biochemical constituents associated with the live body weights of broiler chickens fed on a low P diet and vaccinated against ND, and IBD.

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