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

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

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

By

RAKIBUL ISLAM

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

RAKIBUL ISLAM

April 2014

Pengerusi: Professor Datin Paduka Aini Ideris, PhD
Fakulti: Perubatan Veterinar

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.

ACKNOWLEDGEMENTS

All credit goes to Almighty Allah with Darood and Salam to Rasulullah Sallallaho Alaihe Wasalam, who has enabled me to carry out the study and to build up this thesis. Henceforth, I wish to acknowledge my parents, parents in-law, uncles, aunts, brothers, sisters, teachers, as well as another kith and kin for their best wishes and assistance throughout the time on earth. My special fascination is accorded to my wife and son for their great sacrifices, understanding, and love in every part of domestic life.

I am immensely indebted to my supervisor Professor Datin Paduka Dr. Aini Ideris for her illustrious patience, keen interest, scholastic guidance, continuous inspiration, upscale advice, affectionate feeling, radical investigation, constructive criticism, sympathetic supervision, as well as financial sponsorship in all phases of this study and preparing the manuscript. Definitely, her ethical support and generosity were of great help to me. My cordial appreciations are forwarded to my supervisory committee members, Professor Dr. Abd. Rahman Omar, Assoc. Prof. Dr. Azhar Kasim and Dr. Anis Shobirin Meor Hussin for their valuable instruction, ever and endless encouragement, and precious idea throughout the research work. I would like to express my cardinal respect with profound gratitude to Prof. Dr. Hair Bejo and Associate Prof. Dr. Jalila Abu for their sincere vision and suggestions during the study.

I would like to express my special gratitude to Mr. Syed Osthman bin Syed Zain, Principal Researcher at Standards and Industrial Research Institute of Malaysia (SIRIM) for providing phytase enzyme throughout this project. I am also very grateful to Dr. Kartini Ahmad, Dr. Khandakar Nurul Islam, Rafidah Mohd Ariff, Saniza Sakinah Yusof, Khin San Mu, Nur Liyana binti Jufika Ahmad, Haryati Shila Mohamad Wali and Mohamed Ibrahim Saeed for their enthusiastic encouragement, moral support, meaningful suggestion, kind help, and spent time with me whenever I faced difficulties in the work.

I am very much thankful to all the labmates and staff in Virology, Hematology and Biochemistry, Serology laboratories and others in the Faculty of Veterinary Medicine in sharing their scientific knowledge and help, towards the completion of the study, as well as their patience and tolerance. Many thanks are also due to the staff of a poultry unit under the Department of Animal Science, Faculty of Agriculture for their technical co-operation. I as well would like to take this opportunity to acknowledge my enormous debt to my colleagues and employer at Hajee Mohammad Danesh Science and Technology University, Bangladesh for their magnificent devotion and granting my leave to pursue my study respectively. Finally yet importantly, I would like to thank all individuals who were directly or indirectly involved in this work for making everything worthwhile during the study.

This work was conducted under the financial support of Universiti Putra Malaysia and Ministry of Science, Technology and Innovation (MOSTI), Malaysia, project number BT0106-01 (07-01-08 – EIB011)

This thesis was submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the supervisory committee were as follows:

Aini Ideris, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Abdul Rahman Bin Omar, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Azhar Bin Kasim, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Anis Shobirin Binti Meor Hussin, PhD
Senior Lecturer
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

_____
BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

Declaration by the student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice –Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature:…… Date: 30th Dece

Name and Matric No: RAKIBUL ISLAM, (GS23840)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

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□FR□HQ□WJ□DH□JQL□FR□H 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.

REFERENCES

Adeola, O., and Sands, J. S. (2003). Does supplemental dietary microbial phytase improve amino acid utilization? A perspective that it does not. *Journal of Animal Science.* 81(E. Suppl. 2):E78–E85.

Adeola, O., Olukosi, O. A., Jendza, J. A., Dilger, R. N. and Bedford, M. R. (2006). Response of growing pigs to *Peniophora lycii-* and *Escherichia coli*-derived phytases or varying ratios of calcium to total phosphorus. *Animal Science*. 82: 637-644.

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 (4): 266-271.

Akyurek, H., Senkoylu, N. and Ozduven, M. L. (2005). Effect of microbial phytase on growth performance and nutrients digestibility in broilers. *Pakistan Journal of Nutrition.* 4: 22-26.

Alam, M. J., Howlider, M. A. R., Pramanik, M. A. H. and Haque, M. A. (2003). Effect of exogenous enzyme in diet on broiler performance. *International Journal of Poultry Science*. 2: 168-173.

Al-Harthi, M. A. (2006). Impact of supplemental feed enzymes, condiments mixture or their combination on broiler performance, nutrients digestibility and plasma constituents. *International Journal of Poultry Science* 5 (8): 764-771.

Ali, M. S. (1995). Effect of replacement of fishmeal by Soybean meal on the performance of broilers. M. S. thesis. Cited by Ahmed, F., Rahman, M. S., Ahmed, S. U. and Miah, Y. U. 2004. Performance of broiler on phytase supplemented soybean meal based diet. *International Journal of Poultry Science*. 3 (4): 266-271.

Anderson, P. A. (1985). Interactions between proteins and constituents that affect protein quality. In: digestibility and amino acid availability in cereals and oilseeds (Finley, G. W. and Hopkins, D. T., Eds), *American Association of Cereal Chemists*, St. Paul, Minnesota, pp 31–36.

Anderson, R. J. (1914). A contribution to the chemistry of phytin. *Journal of Biological Chemistry.* 17: 171-190.

Andersson, H., NaÈvert, B., Bingham, S. R., Englyst, H. N. and Cummings, J. H. (1983). The effects of breads containing similar amounts of phytate but different amounts of wheat bran on calcium, zinc and iron balance in

man. *British Journal of Nutrition.* 50: 503-510.

Angel, R., Applegate, T. J. and Christman, M. (2000a). Effects of dietary non-phytate phosphorus (nPP) on performance and bone measurements in broilers fed on a four-phase feeding system. *Poultry Science.* 79(Suppl. 1):21-22.

Angel, R., Applegate, T. J., Christman, M and Mitchell, A. D. (2000b). Effect of dietary non-phytate phosphorus (nPP) level on broiler performance and bone measurements in the starter and grower phase. *Poultry Science*. 79(Suppl. 1):22.

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.

Anis Shobirin, M. H., Farouk, A., Hamzah, M. S., and Greiner, R. (2007). Phytate-degrading enzyme production by bacteria isolated from Malaysian soil. *World Journal of Microbiology and Biotechnology.* 23: 1653-1660.

Anna Czech and Eugeniusz R. Grela. (2004). Biochemical and hematological 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.

Applegate, T. J., Joern, B. C., Nussbaum-Wagler, D. L., and Angel, R. (2003). Water soluble phosphorus in fresh broiler litter is dependent upon phosphorus concentration fed but not on fungal phytase supplementation. *Poultry Science.* 82: 1024-1029.

Assuena, V., Junqueira, O. M., Duarte, K. F., Laurentiz, A. C., Filardi, R. S. and Sgavioli, S. (2009). Effect of dietary phytase suplementation on the performance, bone densitometry, and phosphorus and nitrogen excretion of broilers. *Revista Brasileira de Ciência Avícola*. 11(1): 25-30.

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.

Augspurger, N. R. and Baker. D. H. (2004). High dietary phytase levels maximize phytate-phosphorus utilization but do not affect protein utilization in chicks fed phosphorus- or amino acid-deficient diets. *Journal of Animal Science.* 82: 1100□1107.

Augspurger, N. R., Webel, D. M., Lei, X. G. and Baker. D. H. (2003). Efficacy of an *E. coli* phytase expressed in yeast for releasing phytate-bound phosphorus in young chicks and pigs. *Journal of Animal Science.* 81: 474-483.

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 (2): 160-168.

Austin, S., Bingham, E. T., Koegel, R. G., Mathews, D. E., Shahan, M. N. and Straub, R. J. (1994). An overview of a feasibility study for the production of industrial enzymes in transgenic alfalfa. In: R. K. Bajpai and A. Prokop. *Re combinant DNATechnology II* (pp. 234□244). New York: Academy of Sciences.

Baker, D. H. (1998). Beyond phosphorus: phytase effects on protein, energy and trace mineral utilization of swine and poultry. In: BASF Technical Symposium. BASF Corporation, Mount Olive, N. J., pp:48-62.

Ballam, G. C., Nelson, T. S. and Kirby, L. K. (1984). Effect of fiber and phytate source and of calcium and phosphorus level on phytate hydrolysis in the chick. *Poultry Science.* 63: 333-338.

Banerjee, G. C. (1998). A text book of animal husbandry. In 8th edition. *Poultry Nutrition* (pp. 895-897). New Delhi: Oxford & IBH Publishing Co. Pvt. Ltd.

Banks, K. M., Thompson, K. L., Jaynes, P. and Applegate, T. J. (2004). The effects of copper on the efficacy of phytase, growth and phosphorus retention in broiler chicks. *Poultry Science.* 83: 1335-1341.

Barrier-Guillot, B., Casado, P., Maupetit, P., Jondreville, C. and Gatel, F. (1996a). Wheat phosphorus availability: 1-In vitro study; Factors affecting endogenous phytasic activity and phytic phosphorus content. *Journal of the Science of Food and Agriculture.* 70: 62-68.

Barrier-Guillot, B., Casado, P., Maupetit, P., Jondreville, C. and Gatel, F. (1996b). Wheat phosphorus availability: 2-In vivo study in broilers and pigs; relationship with endogenous phytasic activity and phytic phosphorus content in wheat. *Journal of the Science of Food and Agriculture.* 70:69-74.

BASF Corporation. (2001). Natuphos Update: Phosphorus release comparison between phytase enzymes. BASF Brochure.

Bayry, J., Lacroix-Desmazes, S., Kazatchkine, M. D., Hermine, O., Tough, D. F., and Kaveri, S. V. (2005). Modulation of dendritic cell maturation and function by B lymphocytes. *Journal of Immunology*. 175: 15-20.

Bedford, M. R. (2000). Exogenous enzymes in monogastric nutrition-their current value and future benefits. *Animal Feed Science and Technology*. 86: 1-13.

Bedford, M. R. and Schulze, H. (1998). Exogenous enzymes for pigs and poultry nutrition. *Research Review*. 11: 91-114.

Bedford, M. R., Murphy, C., Persia, M. E. (2007). A holo-analysia of trials investigating the gain and feed conversion benefits of Quantum™ phosphate supplementation to broilers under a variety of managerial environmental and dietary conditions, *Poultry Science*, 86 (Suppl. 1): 673 (Abstract).

Biehl, R. R. and Baker, D. H. (1997). 1*α*-Hydroxycholecalciferol does not increase the specific activity of intestinal phytase but does improve phosphorus utilization in both cecectomized and sham-operated chicks fed cholecalciferol-adequate diets. *Journal of Nutrition.* 127: 2054-2059.

Biehl, R. R., Baker, D. H., and DeLuca. H. F. (1998). Activity of various hydroxylated vitamin D3 analogs for improving phosphorus utilisation in chicks receiving diets adequate in vitamin D3. *British Poultry Science*. 39: 408-412.

Bjorck, I. M., and Nyman, M. E. (1987). *In vitro* effect of phytic acid and polyphenol on starch digestion and fibre degradation. *Journal of Food Science.* 52: 1588-1594.

Bogin, E. and Israeli, B. (1976). Enzyme profile of heart and skeletal muscles, liver and lung of roosters and geese, *Zentralbl Veterinarmd* A. 23: 152-7.

Boling, S. D., Douglas, M. W., Snow, J. L., Parsons, C. M. and Baker, D. H. (2000). Citric acid does not improve phosphorus utilization in laying hens fed a corn-soybean meal diet. *Poultry Science.* 79: 1335-1337.

Boling-Frankenbach, S. D., Snow, J. L., Parsons, C. M. and Baker, D. H. (2001). The effect of citric acid on the calcium and phosphorus requirements of chicks fed corn-soybean meal diets. *Poultry Science.* 80:783-788.

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 and Proteomics* 4: 43-51.

Bramaud C., Aimar P., and Daufin G. (1995). Thermal isoelectric precipitation

of alpha-lactalbumin from a whey protein concentrate - influence of protein□calcium complexation. *Biotechnology and Bioengineering.* 47: 121-130.

Brenes, A., Viveros, A., Arija, I., Centeno, C., Pizarro, M. and Bravo, C. (2003). The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. *Animal Feed Science and Technology*. 110: 201-219.

Brinch-Pedersen H., Sorensen, L. D., and Holm, P. B. (2002). Engineering crop plants: getting a handle on phosphate. *Trends in Plant Science.* 7:118-125.

Broz, J., Oldale, P., Perrin-voltz, A. H., Tychen, G., Schulze, J., and Simoes Nunes, C. (1994). Effects of supplemental phytase on performance and phosphorus utilization in broiler chickens fed a low phosphorus diet without addition of inorganic phosphates. *British Poultry Science*. 35: 273-280.

Brune, M., Rossander, L., Hallberg, L., Gleerup, A. and Sandberg, A. S. (1992). Iron absorption from bread in humans: inhibiting effects of cereal ®ber, phytate and inositol phosphates with different numbers of phosphate groups. *Journal of Nutrition.* 122: 442-449.

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 i Great Britain by Unwin Brothers Limited, The Gresham Press, old woking, Surrey.

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.

Caldwell, R. A. (1992). Effect of calcium and phytic acid on the activation of trypsinogen and the stability of trypsin. *Journal of Agricultural Food Chemistry.* 40: 43-46.

Camden, B. J., Morel, P. C. H., Thomas, D. V., Ravindran, V. and Bedford, M. R. (2001). Effectiveness of exogenous microbial phytase in improving the bioavailabilities of phosphorus and other nutrients in maize-soya-bean meal diets for broilers. *Animal Science.* 73: 289-297.

Camovale, E., Lugaro, E. and Lombardi-Boccia, G. (1988). Phytic acid in faba bean and pea: Effect of protein availability. *Cereal Chemistry.* 65: 114-117.

Campbell, G. L., and Bedford, M. R. (1992). Enzyme applications for monogastric feeds: a review. *Canadian Journal of Animal Science.* 72: 449-466.

Cao, L., Wang, W., Yang, C., Yang, Y., Diana, J., Yakupitiyage, A., Luo, Z., and Li, D. (2007). Application of Microbial Phytase in Fish Feed. *Enzyme and Microbial Technology.* 40: 497-507.

Carlos, A. B. and Edwards, Jr. H. M. (1998). The effects of 1,25-dihydrocholecalciferol and phytase on the natural phytate phosphorus utilization by laying hens. *Poultry Science*. 77: 850-858.

Carlos, A. B., and Edwards Jr., H. M. (1997). Infuence of soybean particle size and chlortetracycline on the utilization of phytate phosphorus by broilers. *Poultry Science.* 76 (Suppl.): 234.

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

Cawly, R. W. and Mitchell, T. A. (1968). Inhibition of Wheat Alpha-amylase by Bran Phytic Acid. *Journal of the Science of Food and Agriculture*. 19: 106

Chen, C. C., Hunag, C. T., and Cheng, K. J. (2001). Improvement of phytase thermostability by using sorghum liquor wastes supplement with starch. *Biotechnology Letters.* 23: 331-333.

Cheryan, M. (1980). Phytic acid interactions in food systems. *CRC Critical Review Food Science Nutrition*. 13: 297-335.

Cheryan, M., Anderson, F. W., and Grynspan, F. (1983). Magnesium-phytate complexes: effect of pH and molar ratio on solubility characteristics. *Cereal Chemistry*. 60: 235-237.

Choi, Y. M., Suh, H. J. and Kim, J .M. (2001). Purification and properties of extracellular phytase from *Bacillus* sp. KHU-10. *Journal of Protein Chemistry.* 20: 287-292.

Correll, D. L. (1998). The role of phosphorus in the eutrophication of receiving waters: a review. *Journal of Environmental Quality*. 27: 261-266.

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

Cowieson, A. J. (2005). Factors that influence the nutritional value of maize for broilers. *Animal Feed Science and Technology*. 119: 293-305.

Cowieson, A. J., Acamovic, T., and Bedford, M. R. (2006a). Phytic acid and phytase: Implications for protein utilization by poultry. *Poultry Science.* 85: 878-885.

Cowieson, A. J., Acamovic, T., and Bedford, M. R. (2006b). 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 metabolisability of minerals and energy. *Poultry Science.* 85: 1389-1397.

Cowieson, A. J., Acamovic, T., Bedford, M. R. (2004). The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. *British Poultry 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. *British Journal 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.

Dale, N. (1996). Variation in feed ingredient quality. *Animal Feed Science and Technology.* 59: 129-135.

Danek, N., Kaplan, O., Avci, M. and Can, A. (2007). Effects of microbial phytase on growth performance, carcass yield, biochemical parameters, oxidative stress and faecal phosphorus content of Japanese quails. *Journal of Animal and Veterinary Advances.* 6(8): 1031-1035

Dannis John Cosgrove, and Irving, G. C. J. (1980). Inositol Phosphates: *Their Chemistry, Biochemistry and Physiology* (pp. 191). Illustrated. Elsevier Scientific Pub Co.

de Boland, A. R., Garner, G. B., and O'Dell, B. L. (1975). Identification and properties of phytate in cereal grains and oilseed products. *Journal of Agricultural and Food Chemistry.* 23: 1186.

De Rham, O., and Jost, T. (1979). Phytate-protein interactions in soybean extracts and low-phytate soy protein products. *Journal of Food Science.* 44: 596-601.

Deepa, C., Jeyanthi, G. P., and Chandrasekaran, D. (2011). Effect of phytase and citric acid supplementation on the growth performance, phosphorus, calcium and nitrogen retention on broiler chicks fed with low level of available phosphorus. *Asian Journal of Poultry Science.* 5: 28-34

Denbow, D. M., Ravindran, V., Kornegay, E. T., Yi, Z., and Hulet, R. M. (1995). Improving phosphorus availability in soybean meal for broilers by supplemental phytase. *Poultry Science.* 74: 7831-1842

Deshpande, S. S., and Cheryan, M. (1984). Effects of phytic acid, divalent cations, and their interactions on alpha-amylase activity. *Journal of Food*

Science. 49: 516-519.

Dilger, R. N., Onyango, E. M., Sands, J. S., and Adeola, O. (2004). Evaluation of microbial phytase in broiler diets. *Poultry Science*. 83. 962-970.

Drezner, M. K. (2002). Phosphorus homeostasis and related disorders. In John P. Bilezikian, Lawrence G. Raisze, and Gideon A. Roden. *Principles of Bone Biology*. 1 (pp: 321□338). Acadimic Press, California, USA.

Driver, J. P., Pesti, G. M., Bakalli, R. I. and Edwards, H. M. (2005). Effects of calcium and nonphytate phosphorus concentrations on phytase efficacy in broiler chicks. *Poultry Science.* 84:1406-1417.

Edwards Jr., H. M. (1983). Phosphorus. I. Effect of breed and strain on utilization of suboptimal levels of phosphorus in the ration. *Poultry Science*. 62: 77-84.

Edwards Jr., H. M. (2002). Studies on the efficacy of cholecalciferol and derivatives for stimulating phytate utilization in broilers. *Poultry Science*. 81: 1026-1031.

Edwards Jr., H. M. and Veltman Jr. J. R. (1983). The role of calcium and phosphorus in the etiology of tibial dyschondroplasia in young chicks. *Journal of Nutrition*. 113: 1568-1575.

Edwards, H. M. Jr. (1991). Nutritional strategies to reduce phosphorus excretion by broilers, In effects of phytate utilization by monogastric animals. *Proceedings of the Georgia Nutrition Conference for Feed Manufacturers*. Atlanta. (pp. 1-6). Flengan Yan. 2001. University of Arkansas, USA

Edwards, H. M. Jr., Elliot, M. A., and Sooncharernying, S. (1992). Quantitative substitution of 1,25-dihydroxycholecalciferol and 1-hydroxy-cholecalciferol for cholecalciferol in broiler diets. (pp 567-571) *in*: □3□U□R□F□H□H□G□L□Q□J□V□□ □R□I□□ □W□K□H□□ □;□,□;□□ Netherlands.

Edwards, H. M. Jr., Palo, P., Soonchaerenying, S. and Elliot, M. A. (1989). Factors influencing the bioavailability of phytate phosphorus to chickens. In: *Nutrient Availability: Chemical and Biological Aspects* (Southgate, D., Johnson, I. and Fenwick, G. R., Eds). (pp: 271-276.) The Royal Society of Chemistry, Cambridge.

Edwards, Jr. H. M. (1993). Dietary 1, 25-dihydroxycholecalciferol supplementation increases natural phytate phosphorus utilization in chicks. *Journal of Nutrition.* 123: 567-577.

Eeckhout, W. and de Paepe, M. (1994). Total phosphorus, phytate-

phosphorus and phytate activity in plant feedstuffs. *Animal Feed Science and Technology*. 47: 19-29.

Eisa, M. A. Adel, and El-Hamied, S. Sahar. (2003). Clicicopathological studies on bio-stimulant agent in broiler chickens. *Kafr El-Sheikh Veterinary Medicine Journal.* 1 (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 (IV): 1059-1081.

Elkin, R. G. (2002). Nutritional components of feedstuff: a qualitative chemical appraisal of protein. In J. M. McNab and K. N. Boorman (eds). *Poultry Feedstuffs: Supply, Composition and Nutritive value.* (pp: 57-81) CABI Publishing.

El-Sherbiny, A. E, Hassan, H. M. A., Abd-Elsamee, M. O., Samy, A and Mohamed, M. A. (2010). Performance, bone parameters and phosphorus excretion of broilers fed low phosphorus diets supplemented with phytase from 23 to 40 days of age, *International Journal of Poultry Science.* 9 (10): 972-977.

Erdman, J. W. Jr. (1979). Oilseed phytates: nutritional implications. *Journal of the American Oil Chemists Society.* 56: 736-741.

European Commission. (2000). Health and consumer protection Directorate General. Directorate B - Scientific health Opinions. Unit B3- Unit B3- Management of scientifia commttees ll.

Farrell, D. J., Martin, E., Dupreez, J. J., Bongarts, M., Betts, M., Sudaman, A., and Thomson, E., (1993). The beneficial effects of a microbial feed phytase in diets of broiler chickens and ducklings. *Journal of Animal Physiology and Animal Nntrition.* 69: 278-283.

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.

Furrer, O. J., Stauffer, W. (1987). P-Verlagerung im Boden und Auswaschung. In: FAC Oktobertagung 1987: Phosphat in Landwirtschaft und Umwelt, Eidgenössische Forschungsanstalt für Agrikulturchemie und Umwelthygiene. FAC, Liebefeld-Bern: 83-90.

Gatlin, D. M. lll, Barrows, F. T., Braown, P., Dabrowski, K., Gaylord, T. G., Hardy, R. W., Herman, E., Hu, G., Krogdahl, A., Nelson, R., Overturf, K., Rust, M., Sealy, W., Skonberg, D., Souza, E. J., Stone, D., Wilson, R. and Wurtele, E. (2007). Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquaculture Research*. 38: 551-579.

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 (2): 162-168.

Gibson, D. M. and Ullah, A. H. J. (1990). Phytase and their actions on phytic acid. In D. J. Morre, W. F. Boss, and F. A. Loewus. *Inositol Metabolism in Plants* (pp. 77). New York: Wiley-Liss, Inc.

Godoy, S., Chicco, C., Meschy, F. and Requena, F. (2005). Phytic phosphorus and phytase activity of animal feed ingredients. *Interciencia*. 30: 24-28.

Golian, A. and Maurice, D. V. (1992). Dietary poultry fat and gastrointestinal transit time of feed and fat utilization in broiler chickens. *Poultry Science.* 71: 1357-1363.

Graf, E., (1986). Chemistry and applications of phytic acid: An overview. In E. Graf. *Phytic Acid: chemistry and applications* (pp. 173-194). Minneapolis: Pilatus Press.

Greiner, R., and Konietzny, U. (2006). Phytase for food Aplication. *Food Technology and Biotechnology.* 44: 125-140.

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

Greiner, R., Larsson Alminger, M., Carlsson, N. G., Muzquiz, M., Burbano, C., Cuadrado, C., Pedrosa, M.M. and Goyoaga, C. (2002). Pathway of dephosphorylation of *myo*-inositol hexakisphosphate by phytases of legume seeds. *Journal of Agricultural and Food Chemistry.* 50: 6865-6870.

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.

Han, Y. M. and Lei., X. G. (1999). Role of glycosylation in the functional expression of an *Aspergillus niger* phytase (*phyA*) in *Pichia pastoris*. *Archives of Biochemistry and Biophysics*□□□□□□□□□□□□□□□□□□□í□

Harland, F. B., and Morris, E. R. (1995). Phytin: A good or a bad food component. *Nutrition Research.* 15: 733-754.

Huff, W. E., Moore, Jr., P. A., Waldroup, P. W., Waldroup, A. L., Balog, J. M., Huff, G. R., Rath, N. C., Daniel, T. C., and Raboy, V. (1998). Effect of dietary phytase and high available phosphorus corn on broiler chicken

performance. *Poultry Science. 77*: 1899-1904.

Idriss, E. E., Makarewicz, O., Farouk, A., Rosner, K., Greiner, R., Bochow, H., Richter, T., and Borris, R. (2002). Extracellular phytase activity of *Bacillus amyloliquefaciens* FZ45 contributes to its plant growth promoting effect. *Microbiology.* 148: 2097-2109.

Igbasan, F. A., Manner, K., Miksch, G., Borriss, R., Farouk, A. and Simon, O. (2000). Comparative studies on the in vitro properties of phytases from various microbial origins. *Archives of Animal Nutrition-Archiv fur Tierernahrung.* 53: 353-373.

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

Jadhav, N. V., Suranagi, M. D., and Anjaneya, S. N. (2009). Bone morphology and mineralization of broiler chicken as influenced by dietary phytase supplementation and partial replacement of SBM and dicalcium phosphate. *Indian Journal of Poultry Science*. 44 (1): 69-71

Jang, D. A., Fadel, J. G., Klasing, K.C., Mireles, A. 1., Ernst, R. A., Young, K. A., Cook, A. and Raboy, V. (2003). Evaluation of low-phytate corn and barley on broiler chick performance. *Poultry Science*. 82: 1914-1924.

Jariwalla, R. J., Sabin, R., Lawson, S., and Herman, Z. S. (1990). Lowering of serum cholesterol and triglycerides and modulation of divalent cations by dietary phytate. *Journal of Applied Nutrition.* 42: 18-28.

Johnston, S. L., and Southern, L. L. (2000). The effect of varying mix uniformity (simulated) of phytase on growth performance, mineral retention, and bone mineralization in chicks. *Poultry Science*. 79:1485-1490.

Jongbloed, A. W., Kemme, P. A., and Mroz, Z. (1990). The effect of *Aspergillus niger* phytase in diets for pigs on concentration and apparent digestibility of dry matter, total phosphorus, and inositol phosphates in different sections of the alimentary tract (pp: 28). Report IVVO no. 221. Instituut voor Veevoedingsonderzoek.

Juanpere, J., Perz-vendrell, A. M. and Brufau, J. (2004). Effects of microbial phytase on broilers fed barley-based diets in the presence or not of endogenous phytase. *Animal Feed Science and Technology*. 115: 265-279.

Kasim, A. B. and Edwards, Jr. H. M. (2000). Effect of sources of maize and maize particle sizes on the utilization of phytate P in broiler chicks. *Animal Feed Science and Technology.* 86: 15-26.

Kasim, A. B., and Edwards Jr., H. M. (1998). The analysis of inositol

phosphate forms in feed ingredients. *Journal of the Science of Food Agriculture*. 76: 1-9

Kerovou, J., von Weymarn, N., Povelainen, M., Auer, S., and Miasnikov, A. (2000). A new efficient expression system for Bacillus and its application to production of recombinant phytase. *Biotechnology Letters.* 22: 1311-1317.

Kerovuo, J., Lauraeus, M., Nurminen, P., Kalkinnen, N. and Apajalahti, J. (1998). Isolation, characterization, molecular gene cloning and sequencing of a novel phytase from *Bacillus subtilis*. *Applied and Environmental Microbiology.* 64: 2079-2085.

Kerr, M. J., Classen, H. L. and Newkirk, R. W. (2000). The effects of gastrointestinal tract micro-flora and dietary phytase on inositol hexaphosphate hydrolysis in the chicken. *Poultry Science.* 79: Suppl. 1, 11 (Abstr.).

Keshavarz, K. (2000). Nonphytate phosphorus requirement of laying hens with and without phytase on a phase feeding program. *Poultry Science.* 79: 748-763.

Keshavarz, K. (2003). The effect of different levels of nonphytate phosphorus with and without phytase on the performance of four strains of laying hens. *Poultry Science.* 82: 71-91.

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. PhD thesis (pages: 211). Universiti Putra Malaysia, Malaysia.

Kies, A. K., Van Hemert, K. H. F., and Sauer, W. C. (2001). Effect of phytase on protein and amino acid digestibility and energy utilization. *World's Poultry Science Journal.* 57. 110-124.

Kliment, Martin and Aneglovicova, Maria. (2011). The effect of microbial phytase on blood performance of broiler chickens. *Animal Science and Biotechnologies*. 44 (1):58-61.

Knuckles, B. E. and Betschart, A. A. (1987). Effects of phytate and other myo-inositol phosphate esters on alpha-amylase digestion of starch. *Journal of Food Science*. 52: 719-721.

Kornegay E. T., Ravindran V. and Denbow D. M. (1996). Improving phytate phosphorus availability in corn and soyabean meal for broilers using microbial phytase and calculation of phos-phorus equivalency values for phytase. *Poultry Science*. 75: 240-249.

Kornegay, E. T. (1996). Effect of phytase on bioavailability of phosphorus, calcium, amino acids, and trace minerals in broilers and turkeys. BASF Technical Symp., Atlanta, GA. p. 39.

Kornegay, E. T. (2001). Digestion of phosphorus and other nutrients, the role of phytase and factors influencing their activity. In M. R. Bedford and G. C. Partridge (eds). *Enzymes in farm animal nutrition.* CABI Publishing, New York, NY, pp: 237-272.

Kornegay, E. T., Denbow, D. M., Yi, Z., and Ravindran, V. (1996). Response of broilers to graded levels of microbial phytase added to maize-soyabean meal-based diets containing three levels of non-phytate phosphorus. *British Journal of Nutrition.* 75: 839-852.

Kumar, A., and Chauhan, B. M. (1993). Effects of phytic acid on protein digestibility (*in vitro*) and HCl-extractability of minerals in pearl millet sprouts. *Cereal Chemistry.* Khartoum, Sudan, 70: 504-506.

Lamosa, P., Burke, A., Peist, R., Huber. R., Liu, M. Y., Silva, G., Rodrigues-Pousada, C., LeGall, J., Maycock, C. and Santos, H. (2000). Thermostabilization of proteins by diglycerol phosphate, a new compatible solute from the hyperthermophile *Archaeoglobus fulgidus*. *Applied and Environmental Microbiology*. 66: 1974-1979.

Lee, S. Y., Kim, J. S., Kim, J. M., An, B. K. and Kang, C. W. (2010). Effects of multiple enzyme (ROVABIO® Max) containing carbohydrolases and phytase on growth performance and intestinal viscosity in broiler chicks fed corn-wheat-soybeanmeal based diets. *Asian Australian Journal of Animal Sciences.* 23(9): 1198-1204.

Leeson, S. (1993). Recent advances in fat utilization by poultry. In Recent Advances in Animal Nutrition in Australia. The University of New England, Armidale, NSW, pp: 170-198.

Leeson, S. and Summers, J. D. (1997). Commercial Poultry Nutrition (pp. 355). 2nd edition, Guelph, Canada: University Book.

Legin, E., Copinet, A., and Duchiron, F. (1998). Production of thermostable amylolytic enzymes by *Thermococcus hydrothermalis*. *Biotechnology Letters.* 20: 363-367.

Lei, X. G., Ku, P. K., Miller, E. R., Yokoyama, M. T., and Ullrey, D. E. (1994). Calcium level affects the efficacy of supplemental microbial phytase in corn-soybean meal diets of weanling pigs. *Journal of Animal Science*. 72: 139-143

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 (6): 1117-23.

Lenis, N. P., and Jongbloed, A. W. (1999). New technologies in low pollution swine diets: Diet manipulation and use of synthetic amino acids, phytase and phase feeding for reduction of nitrogen and phosphorus excretion and ammonia emission □±review-. *Asian-Australian Journal Animal of Sciences.* 12: 305-327.

Lescoat, P., Travel, A., and Nys, Y. (2005). Lois de réponse des volailles de chair à l'apport de phosphore. *INRA Productions Animales*. 18: 193-201.

Leske, K. L., and Coon, C. N. (1999). A bioassay to determine the effect of phytase on phytate phosphorus hydrolysis and total phosphorus retention of feed ingredients as determined with broilers and laying hens. *Poultry Science.* 78: 1151-1157.

Lester, J. S. (1989). Nutrition and diet in human health and disease. 3rd edition, Philadaelphia and London: W. B. Saunder Company.

Lewandowski, A. H., Campbell, T. W. and Harrison, G. J. (1986). Clinical Chemistries. In: Clinical Avian Medicine, Harrison, G.J. and L.R. Harrison (Eds.). Philadelphia, W.B. Saunders, pp: 717.

Leytem, A. B., Thacker, P. A., and Turner, B. L. (2007). Phosphorus characterization in feces from broiler chicks fed low-phytate barley diets. *Journal of Science of Food and Agriculture*. 87: 1495-1501.

Li, D., Che, X., Wang, Y., Hong, C., and Thacker, P. A. (1998). Effect of microbial phytase, vitamin D3 and citric acid on growth performance and phosphorus, nitrogen and calcium digestibility in growing swine. *Animal Feed Science and Technology.* 73: 173-186.

Liebert, F., Wecke, C. and Schoner, F. J. (1993). Phytase activity in different gut contents of chickens as dependent on levels of phosphorus and phyatse supplementation. Pages 202□205 in Proc. 1993 Symp. *Enzymes in Animal Nutrition*. Karthause Ittingen, Switzerland

Liener, I. E. (1989). Antinutritional factors in legume seeds: State of art. In: J. Huisman, T. F. B. Van der Poel and I. E. Liener. *Recent Advances of Research in Antinutritional Factors in Legume Seed* (pp. 6- 13). Pudoc, Wageningen, The Netherlands.

Lilburn, M. S., Ngidi, E. M., Ward, N. E. and Llames, C. (1991). The influence of severe drought on selected nutritional characteristics of commercial corn hybrids. *Poultry Science*. 70: 2329-2334.

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.

Liu, Z., H. Wang, E. Xiu Wang, H. Xu, D. Gao, G. Zhang, P. Chen and D. Liu,

(2007). Effect of Wheat Perling on Flour Phytase Activity, Phytic Acid, Iron and Zinc Content. *Food Science and Technology*.

Maenz, D. D. (2001). Enzymatic characteristics of phytases as they relate to their use in animal feeds. *In Enzymes in Farm Animal Nutrition*. M. R. Bedford and G. G. Partridge, (eds). pp: 61-84. CABI Publishing, New York, NY.

Maenz, D. D., and Classen, H. L. (1998). Phytase activity in the small intestinal brush border membrane of the chicken. *Poultry Science.* 77: 557-563.

Maenz, D. D., Engele-Schann, C. M., Newkirk, R. W. and Classen, H. L. (1999). The effects of minerals and mineral chelators on the formation of phytase-resistant and phytase-susceptible forms of phytic acid in solution and in slurry of canola meal. *Animal Feed Science and Technology*. 77: 177-192.

Maga J. A. (1982) Phytate: its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. *Journal of Agriculture and Food Chemistry.* 30: 1-9.

Manangi, M. K., Coon, C. N. (2008). Phytate phosphorus hydrolysis in broilers in response to dietary phytase, calcium, and phosphorus concentrations. *Poultry Science.* 87:1577-1586.

Martin, J. A., Murphy, R. A., and Power, R. F. F. (2005). Purrification and Physico-Chemical Characterisation of Genetically Modifed Phytases Expressed in Aspergillus awamori. *Bioresource Technology*. 97.

McCuaig L. W., Davies M. I., and Motzok I. (1972). Intestinal alkaline phosphatase and phytase of chicks: Effect of dietary magnesium, calcium, phosphorus, and thyroactive casein. *Poultry Science*. 51: 526-530.

McDonald, P. Edwards, R. A., Greenhalgh, J. F. D., Morgan, C. A., Sinclair, L. A. and Wilkinson, R. G. (1995). Animal Nutrition, 7th edition. In John Wiley and Sons (pp. 541 and 554). New York. Pearson.

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

Mitchell, R. D., and Edwards, Jr., H. M. (1996a). Effects of phytase and 1,25-dihydroxycholecalciferol on phytate utilization and the and the quantitative requirement for calcium and phosphorus in young broiler chickens. *Poultry Science.* 75: 95-110.

Mohammed, A., Gibney, M. J., and Taylor, T. G. (1991). The effects of dietary levels of inorganic phosphorus, calcium and cholecalciferol on the

digestibility of phytate-P by the chick. *British Journal of Nutrition.* 66: 251-259.

Moran, E. T. (1985). Digestion and absorption of carbohydrates in fowl and events through perinatal development. *Journal of Nutrition*. 115(5): 665-674

Morris, E. R. (1986). Phytate and mineral bioavailability. In E. Graf. *Phytic acid: Chemistry and application* (pp. 57-76). Minneapolis: Pilatus Press.

Mroz, Z., Jongbloed, A. W. and Kemme, P. A. (1994). Apparent digestibility and retention of nutrients bound to phytate complexes as influenced by microbial phytase and feeding regimen in pigs. *Journal of Animal Science*. 72: 126-132.

Murai, A., Kita, K., Tsuruta, S., and Okumara, J. (2001). Interaction of phytase and glutamic acid on bone ash response in chicken thigh. *Journal of Poultry Science.* 38: 146-151.

Musavi, A., Mirghelenj, S. A. And Rezaei, M. (2009). Effects of microbial phytase on performance and bone strength in broiler chickens. *Journal of Animal and Veterinary Advances* 8(8): 1466-1471.

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 (2): 60-66.

Navert, B., Sandstrom, B. and Cederblad, A. (1985). Reduction of phytate content of bran by leavening in bread and its effect on zinc absorption in man. *British Journal of Nutrition.* 53: 47-53.

Nayini, N. R. and Markakis, P. (1986). In *Phytic acid; chemistry and applications* (Graf, E., ed.), pp. 101□1ŧ07. Pilatus Press, Mineapolis, MN,

Nelson, T. S. (1967) The Utilization of phytate phosphorus by chick - a review. *Poultry Science.* 46: 862-871.

Nelson, T. S. (1976). The hydrolysis of phytate phosphorus by chicks and laying hens. *Poultry Science*. 55: 2262-2264.

Nelson, T. S., and L. K. Kirby (1987). The calcium binding properties of natural phytate in chick diets. *Nutrition Reports International*. 35: 949-956.

Newkirk, R. W., and Classen, H. L. (2001). The non-mineral nutritional impact of phytate in canola meal fed to broiler chicks. *Animal Feed Science and Technology*. 91: 115-128.

NRC. (1994). Nutrient Requirements of Poultry, Ninth revised edition. National research council. National Academy Press, Washinton, DC.

Nunes, S. (1993): Evaluation of phytase resistance in swine diets to different pelleting temperatures. In: Proc. 1st Symp. *Enzymes in Animal Nutrition.* Kartause-Ittingen, Switzerland. Pp: 269-271.

Nys, Y., Frapin, D. and Pointillart, A. (1996). Occurrence of phytase in plants, animals, and microorganisms. In: M. B. Coelho and E. T. Kornegay (Ed.) *Phytase in Animal Nutrition and Waste Management*: *A BASF Reference Manual*, 1996. Pp: 213□236. BASF, Mt. Olive, NJ.

O´Dell, B. L. and de Boland, A. R. (1976). Complexation of phytate with proteins and cations in corn germ and oilseed meals. *Journal of Agricultural Food Chemistry*. 24: 804-808.

Oberleas, D. (1973). Tixicant Occerring Naturally in Foods, 2nd Edition. *In Phytates* (pp. 363-371). Washington, D. C.: National Academy of Sciences.

Oberleas, D. and Harland, B. F. (1996). Impact of phytate on nutrient availability. pp. 211-219 in *Phytase in Animal Nutrition and Waste Management*. M. B. Coelho and E. T. Kornegay Eds. BASF Corporation, Mount Olive, NJ.

Obon, J. M., Manjon, A. and Iborra, J. L. (1996). Comparative thermostability of glucose dehydrogenase from *Haloferax mediterranei*. Effects of salts and polyols. *Enzyme and Microbial Technology*, 19: 352-360.

Okubu, K., Meyers, D. V. and Iacobucci, G. A. (1976). Binding of phytic acid to glycinin. *Cereal Chemistry*. 53: 513-524.

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.

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

Orban, J. I., Adeola, O. and Stroshine, R. (1999). Microbial phytase in finisher diets of white pekin ducks: effect on growth performance, plasma phosphorus concentration, and leg bone characteristics. *Poultry Science* 78: 366-377.

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

Pallauf, J. and Rimbach, G. (1996). Nutritional significance of phytic acid and phytase. *Archives of Animal Nutrition*. 50: 301-319.

Pallauf, J. and Rimbach, G. (1997). Effect of supplemental phytase on mineral and trace element bioavailability and heavy metal accumulation in pigs with different type diets. In M.B. Coehlbo and E.T. Kornegay *Phytase in Animal Nutrition and Waste management*. Ed. BASF publication DC9601.

Pallauf, J., Hohler, D. and Rimbach, G. (1992). Effekt einer zulage an mikrobieller phytase zu einer mais- soja-diat auf die scheinbare absorption von Mg, Fe, Cu, Mn und Zn sowie auf parametter des zinkstatus beim ferkel. *Journal of Animal Physiology and Animal Nutrition.* 68: 1-8.

Panda, A. K., Rama Rao, S. V., Raju, M. V. L. N., and Bhanja, S. K. (2005) Effect of microbial phytase on production performance of white leghorn layers fed on a diet low in non-phytate phosphorus. *British Poultry Science*. 46: 464-469.

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.

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

Phillippy, B. (1999). Susceptibility of wheat and Aspergillus niger phytases to inactivation by gastrointestinal enzymes. *Journal of Agricultural and Food Chemistry*. 47: 1385-1388.

Phillippy, B. Q., Johnston, M. R., Tao, S. H. and Fox, M. R. S. (1988). Inositol phosphates in processed foods. *Journal of Food Science*. 53: 496-499.

Pirgozliew, V., Oduguwa, O., Acamovic, T. And Bedfordt, M. R. (2007). Diets containing Escherrichia coli-□G□H□U□L□Y□H□G□□ □S□K□\□W□D□ turkeys: effect on performance, metabolizable energy, endogenous secretions, and intestinal morphology. *Poultry Science.* 86: 705-713.

Plumstead, P. W., Romero-Sanchez, H., Maguire, R. O. Gernat, A. G., and Brake, J. (2007). Effects of phosphorus level and phytase in broiler breeder rearing and laying diets on live performance and phosphorus excretion. *Poultry Science.* 86: 225-231.

Pomeranz, Y. (1973). Structure and mineral composition of cereal aleurone cells as shown by scanning electron microscopy. *Cereal Chemistry*. 50: 504-511.

Pond, W. G., Church, D. C. and Pond, K. R. (1995). Basic Animal Nutrition and Feeding. (4th edition). Pp: 615. John Wiley and Sons, New York.

Powar, V. K. and Jagannathan, V. (1982). Purfication and Properties of Phytate-Specific Phosphatase from *Bacillus subtilis*. *Journal of Bacteriology.* 151: 1102-1108.

Powell, S., Bidner, T. D. and Southern, L. L. (2011). Phytase supplementation improved growth performance and bone characteristics in broilers fed varying levels of dietary calcium. *Poultry Science.* 90: 604□±
608.

Prattley, C. A. and Stanley, D. W. (1982). Protein-phytate interactions in soybeans. I. Localization of phytate in protein bodies and globoids. *Journal of Food Biochemistry.* 6: 243-253.

Qian, H., Kornegay, E. T. and Denbow, D. M. (1997). Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol, an the calcium:Total phosphorus ratio in broilers diets. *Poultry Science.* 76: 37-46.

Qian, H., Kornegay, E. T. and Denbow, D. W. (1996b). Phosphorus equivalence of microbial phytase in turkeys diets as influenced by calcium to phosphorus ratios and phosphorus levels. *Poultry Science*. 75: 69-81.

Qian, H., Veit, H. P., Kornegay, E. T., Ravindran, V. and Denbow, D. M. (1996a). Effects of supplemental phytase and phosphorus on histological and other tibial bone characteristics and performances of broilers fed semi-purified diets. *Poultry Science.* 75: 618-626.

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

Raboy, V. and Gerbasi, P. (1996). Genetics of myoinositol phosphate synthesis and accumulation. In B. B. Biswas and S. Biswas. *Subcellular Biochemistry.* Vol. 26 (pp. 257-285). Myoinositol phosphates, phosphoinositides, and signal transduction. New York, NY: Plenum Press.

Raghavan, Dr. V. (2011). □$□6□(□$□1□¶□6□□ □S□WORLD POULTRY-
Else□Y□L□H□U□□□9□R□O□X□P□H□□□□□□□□□□□□1□R□□□□□□□

Rama Rao, S. V., Panda, A. K., Raju, M. V. L. N., Shyam, Sunder, G. and Praharja, N. K. (2003). Requirements of calcium for commercial broilers and white leghorn layers at low dietary phosphorus levels. *Animal Feed Science and Technology*. 106: 199-208.

Rama Rao, S. V., Raju, M. V. L. N., Reddy, M. R. and Pavani, P. (2006). Interaction between dietary calcium and nonphytate phosphorus levels

on growth, bone mineralization and mineral excretion in commercial broilers. *Animal Feed Science and Technology*. 132: 135-150.

Rama-Rao S. V., Ravindra Reddy V., Ramasubba Reddy V. (1999). Enhancement of phytate phosphorus availability in the diets of commercial broilers and layers. *Animal Feed Science Technology*. 79: 211-222.

Ravindran, V. (1995). Phytases in poultry nutrition. An overview. Proceeding, *Australian Poultry Science Symposium*. 7: 135-139.

Ravindran, V. and Blair, R. (1999). Feed resources of poultry production in Asia and the Pacific Region. L. Energy resources. *World's Poultry Science Journal.* 47: 213-231.

Ravindran, V. Kornegay, E. T., Denbow, D. M., Yi, Z. and Hulet, R. M (1995b). Response of turkey poults to three levels of Natuphos® phytase added to soybean meal based semi-purified diets containing three levels of non-phytate phosphorus. *Poultry Science*. 74: 1843-1854.

Ravindran, V., Bryden, W. L. and Kornegay, E. T. (1995a) Phytates: occurrence, bioavailability, and implications in poultry nutrition. *Poultry and Avain Biology Review.* 6(2):125□143

Ravindran, V., Cabahug, S., Ravindran, G. and Bryden, W. L. (1999). Influence of microbial phytase on apparent ileal amino acid digestibility of feedstuffs for broilers. *Poult. Sci.* 78: 699-706.

Ravindran, V., Cabahug, S., Ravindran, G., Selle, P. H. and Bryden, W. L. (2000). Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate □S□K□R□V□S□K□R□U□X□V□□□O□H□Y□H□O□V□□□□□ÿ□ÿ□□□□ digestibility and nutrient retention. *British Poultry Science*. 41: 193-200.

Ravindran, V., Cowieson, A. J. and Selle, P. H. (2008). Influence of dietary electrolyte balance and microbial phytase on growth performance, nutrient utilization, and excreta quality of broiler chickens. *Poult. Sci.*, 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 utilization in broiler starters fed diet containing varying concentrations of phytic acid. *Poultry Science*. 85: 82-89.

Ravindran, V., Selle, P. H., Ravindran, G., Morel, P. C. H. Kies, A. K. and Bryden, W. L. (2001). Microbial phytase improves performance, apparent metabolizable energy, and ileal amino acid digestibility of broilers fed a lysine-deficient diet. *Poultry Science*. 80: 338-344.

Reddy, N. R. (2002). Occurrence, distribution, content, and dietary intake of

phytate. In: Reddy, N. R., Sathe, S. K. (eds). *Food phytates*. CRC Press, Boca Raton, pp 25–51

Reddy, N. R., Sathe, S. K. and Salunkhe, D. K. (1982). Phytates in legumes and cereals. *Advance in Food Research*. 28: 1-92.

Reddy, N. R., Sathe, S. K., and Pierson, M. D. (1988). Removal of phytate from great northern beans (Phaseolus Vulagaris) and its combined density effect. *Journal of Food Science*. 53: 107.

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

Ribeiro A. M. L., Mireles A. J. and Klasing K. C. (2003). Interactions between dietary phosphorus level, phytase supplementation and pelleting on performance and bone parameters of broilers fed high levels of rice bran. *Animal Feed Science and Technology.* 103: 155-161.

Rickard, S. E., and Thompson L. U. (1997). Interactions and biological effects of phytic acid. In: F. Shahidi (ed). *Antinutrients and Phytochemicals in Food*. pp. 294-312. American Chemical Society, Washington DC.

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.

Rodriguez, E., Porres, J. M., Han, Y. and Lei, X. G. (1999). Different sensitivity of recombinant *Aspergillus niger* phytase (r-phyA) and *Escherichia coli* pH 2.5 acid phosphatase (r-AppA) to trypsin and pepsin *in vitro*. *Archives of Biochemistry and Biophysics.* 365: 262-267.

Rosen, G. (2003). Microbial phytase in broiler nutrition. In: P.C. Garnsworthy and J. Wiseman, Editors, *Recent Advances in Animal Nutrition*, Nottingham University Press, Nottingham, UK: 105–117.

Rutherfurd, S. M., Chung, T. K. and Moughan, P. J. (2002). The effect of microbial phytase on ileal phosphorus and amino acid digestibility in the broiler chicken. *British Poultry Science.* 43: 598–606.

Sandberg, A. S. (2002). *In vitro* and *in vivo* degradation of phytate. In R. Reddy and S. K. Sathe. Food Phytase (pp. 134). London: CRC Press LLC.

Sandberg, A. S. and Andersson, H. (1988). Effect of dietary phytase on the digestion of phytate in stomach and small intestine of humans. *Journal of Nutrition.* 118: 469-473.

Sandberg, A. S., and Andlid, T. (2002). Phytogenic and microbial phytases in

human nutrition. *Int. Journal of Food Science and Technology.* 37: 823-834.

□6□$□6□□ □,□Q□V□W□L□W□X□W□H□□□□ □□□□□□□□□□□□□□□□□ □ Institute Inc., Cary, NC, USA.

Scheideler S. E., and Ferket P. R. (2000). Phytase in broiler rations effect on carcass yields and incidence of tibial dyschondroplasia. *Journal of Applied Poultry Research.* 9: 468-475.

Scheideler, S. E., and Sell J. L. (1987). Utilization of phytate phosphorus in laying hens as influenced by dietary phosphorus and calcium. *Nutrition Reports International.* 35: 1073-1081.

Schoner, V.F.J., Hoppe, P. P. and Schwarz, G. (1991). Comparative effects of microbial phytase and inorganic phosphorus on performance and on retentions of phosphorus, calcium and crude ash in broilers. *Journal of Animal Physiology and Animal Nutrition.* 66: 248.

Schroder, B., Breves, G. and Rodehutscord, M. (1996). Mechanisms of intestinal phosphorus absorption and availability of dietary phosphorus in pigs. *Dtsch Tierarztl Wochenschr.* 103: 209□214

Schwarz, G. (1994). Phytase supplementation and waste management. Pages 21-44 in: Proceedings BASF symposium Arkansas Nutrition Conference. BASF Corp., Mount Olive, NJ.

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

Sebastian, S., Touchburn, S. P., Chavez, E. R. and Lague, P. C. (1996b). Efficacy of supplemental microbial phytase at different dietary calcium levels on growth performance and mineral utilization of broiler chicks. *Poultry Science.* 75: 1516-1523.

Selle, P. H, Ravindran, V., Bryden, R. A. and Scott, T. (2006). Influence of dietary phytase and exogenous phytase on amino acid digestibility in poultry. A review. *Journal of Poultry Science*. 43: 83-103.

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

Selle, P. H. and Ravindran, V. (2007). Microbial phytase in poultry nutrition. A review. *Animal Feed Science and Technology.* 35: 1-41.

Selle, P. H., Ravindran, V. Pittolo, P. H. and Bryden, W. L. (1999). An evaluation of microbial phytase in sorghum-based broiler diets.

Proceeding, *Australian Poultry Science Symposium.* 11: 97-100.

Shafe, T. M., McDonald, M. W. and Pym, R. A. E. (1990). Effects of dietary calcium, available phosphorus and vitamin D on growth rate, food utilization, plasma and bone constituents and calcium and phosphorus retention of commercial broiler. *British Poultry Science*. 31: 587-602.

Shafey, T. M., McDonald, M. W. and Dingle, J. G. (1991). Effects of dietary calcium, available phosphorus concentration on digesta pH and on the availability of calcium, iron, magnesium and zinc from the intestinal contents of meat chickens. *British Poultry Science*. 32: 185-194.

Shah, V. and Parekh, L. J. (1990). Phytase from *Klebsiella* sp. No. PG-2: purification and properties. *Indian Journal of Biochemistry and Biophysics.* 27: 98-102.

Sharpley, A. N., Chapra, S. C., Wedepohl, R., Sims, J. T., Daniel, T. C. and K. R. Reddy. (1994). Managing agricultural phosphorus for protection of surface waters: Issues and options. *Journal of Environmental Quality*. 23: 437-451.

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 Hematological Parameters of Japanese Quails. *Journal of Animal Science Advances.* 2(9): 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.

Silversides, F. G., Scott, T. A. and Bedford, M. R. (2004). The effects of phytase enzyme and level on nutrient extraction by broilers. *Poultry Science.* 83: 985-989.

Simons, P. C., Versteegh, H. A. J., Jongbloed, A. W., Kemme, P. A., Stump, P., Bos, K. D., Wolters, M. G. E., Beudeker, R. F. and Verschoor, G. J. (1990). Improvement of Phosphorus Availability by Microbial phytase in Broilers and Pigs. *British journal of Nutrition.* 64: 525-540.

Singh, M. and Krikorian, A. D. (1982). Inhibition of trypson activity in vitro by phytate. *Journal of Agricultural and Food Chemistry.* 30: 799-800.

Singh, P. K., and Khatta, V. K. (2003). Effect of phytase supplementation on the performance of broiler chickens fed wheat based diets. In. *Journal Animal Nutrition*. 20: 57-62.

Snow, J. L., Baker, D. H. and Parsons, C. M. (2004). Phytase, citric acid, □D□Q-hydroxychole- calciferol improve phytate phosphorus utilization in chicks fed a corn-soybean meal diet. *Poultry Science*. 83: 1187-1192.

Stefan, H., Anja, K., Edzard, S., Joerg, B., Markus, L., and Oskar, Z. (2005). Biotechnological production and applications of phytases. *Applied Microbiology and Biotechnology.* 68: 588-597.

Suzuki, U., Yoshimura, K., and Takaishi, M. (1907). Ueber ein Enzym □³□3□K□\□W□D□V□H-oxymethy□D□V□H□Q□³□$□G□LK□S□KG□RU *Bulletin of the College of Agriculture.* Tokyo Imperial University. 7: 503□512.

Takemasa, M., Murakami, H. and Yamazaki, M. (1996). Reduction of phosphorous excretion in chicks by addition of yeast phytase. *Japanese Poultry Science*. 33(2): 104-111.

Tanaka, Y. and DeLuca, H. F. (1974). Stimulation of 24,25-dihydroxyvitamin D3 production by 1,25-dihydroxyvitamin D3. *Science.* 183 (130): 1198-1200.

Taylor, T. C. (1965). The availability of calcium and phosphorus of plant material for animals. *Proceeding of the Nutrition Society.* 24: 105□112

Thiel, U., and Weigand, E. (1992). Influence of dietary zinc and microbial phytase supplementation on Zn retention and Zn excretion in broiler chicks. In: *Proc. 19th World's Poultry Congress,* 20-24 Sept., Amsterdam, Vol. 3, pp. 460.

Thiel, U., Hoppe, P. P., Schoner, F. J., and Yeigan, E. (1993). Influence of microbial phytase supplementation on the retention of Zn, P, and Ca in broiler chicks. *Proceedings of the Society of Nutrition Physiology.* 47: 20.

Thompson, L. U. (1986). Phytic acid: a factor influencing starch digestibility and blood glucose response. In E. Graf. *Phytic Acid: Chemistry and Applications* (pp. 173-194). Minneapolis: Pilatus Press,

Thompson, L. U. and Serraino, M. R. (1986). Effect of Phytic Acid on Rape Seed Protein Digestibility and Amino Acid Absorption. *Journal of Agricutural Chemistry*. 34 (3): 468-469.

Thomson, L. U. and Yoon, J. H. (1984). Starch digestibility as affected by poly phenols and phytic Acid. *Journal of Food Science*. 49: 1228-1229.

Torre, M. Rodriguez, A. R. and Saura-Calixto, F. (1991). Effects of Dietary Fiber and Phytic Acid on Mineral Availability. *Critical Reviews in Food Science and nutrition*. 1: 1-22.

Traylor, S. L., G. L. Cromwell, M. D. Lindemann, and D. A. Knabe. (2001). Effects of level of supplemental phytase on ileal digestibility of amino acids, calcium, and phosphorus in dehulled soybean meal for growing

pigs. *Journal of Animal Science*. 79: 2634–2642.

Ullah, A. H. J., and Phillippy, B. Q. (1994). Substrate selectivity in *Aspergillus ficuum* phytase and acid phosphatase using *myo*-inositol phosphates. *Journal of Agricultural and Food Chemistry*. 42: 423-425.

Underwood, E. J., and Suttle, N. F. (1999). In: The Mineral Nutrition of Livestock. 3rd Ed. Pp: 1-17. CABI Publishing, CAB International, Wallingford, Oxon, UK.

Urbano, G., Lopez-Jurado, M., Aranda, P., Vidal-Valverde, C., Tenorio, E. and Porres, J. (2000). The role of phytic acid in legumes: antinutrient or beneficial function? *Journal of Physiology and Biochemistry*. 56: 283-294.

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

Vanderhasselt, R. F., Buijs, S., Sprenger, M., Goethals, K., Willemsen, H., Duchateau, L., and Tuyttens, F. (2013). Dehydration indicators for broiler chickens at slaughter. *Poultry Science*. *92*(3): 612-619.

Viveros A., Centeno C., Brenes A., Canales R., Lozano A. (2000): Phytase and acid phosphatase activities in plant feedstuffs. *Journal of Agricultural and Food Chemistry*. 48: 4009-4013.

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

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

Waldroup, P. W. (1996). Calcium and phosphorus for poultry feeds. ASA Technical Bulletin PO27-1996. American Soybean Association, Singapore.

Waldroup, P. W. (1999). Nutritional approaches to reducing phosphorus excretion in poultry. *Poultry Science*. 78: 683-691.

Waldroup, P. W., Kersey, J. H., Saleh, E. A., Fritts, C. A., Yan, F., Stilborn, H. L., Crum Jr., R. C. and Raboy, V. (2000). Nonphytate phosphorus requirement and phosphorus excretion of broilers fed diets composed of normal or high available phosphate corn with and without microbial phytase. *Poultry Science.* 79:1451–1459.

Wang, H. L., Swain, E. W. and Hasseltine, C. W. (1980). Phytase of moulds used in oriental food fermentation. *Journal of Food Science*. 45:1262–1266.

Wasserman, R. H. and Taylor, A. N. (1973). Intestinal absorption of phosphate in the chicks. Effect of vitamin D and other parameters. *Journal of Nutrition.* 103: 586-599.

Weremko, D., Fandrejewski, H. and Zebrowska, T. (1997). Bioavailability of phosphorus in feeds of plant origin for pigs-review. *Asian-Australian Journal of Animal Science*. 10: 551-566.

Wise, A. (1983). Dietary Factors Determining the Biological Activity of Phytates. *Nutrition Abstracts and Reviews.* 53: 791-806.

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

Wu, Y. B., Ravindran, V. and Hendriks, W. H. (2003). Effects of microbial phytase, produced by solid-state fermentation, on the performance and nutrient utilisation of broilers fed maize- and wheat-based diets. *British Poultry Science.* 44: 710-718.

Wu, Y. B., Ravindran, V., Hendriks, W. H., Morel, P. C. H. and Pierce, P. C. H. (2004b). Evaluation of a microbial phytase, produced by solid state fermentation, in broiler diets II. Influence on phytate hydrolysis, apparent metabolizable energy, and nutrient utilization. *Journal of Applied Poultry Research.* 13:561-569.

Wu, Y. B., Ravindran, V., Hendriks, W. H., Morel, P. C. H. and Pierce, J. (2004a). Evaluation of a microbial phytase, produced by solid-state fermentation, in broiler diets, I. Influence of performance, toe ash contents, and phosphorus equivalency estimates. *Journal of Applied Poultry Research.* 13: 373-383.

Wyss M., Pasamontes L., Remy R., Kohler J., Kusznir E., Gadient M., Muller F. and van Loon A.P.G.M. (1998). Comparison of the thermostability properties of three acid phosphatases from moulds: *Aspergillus fumigatus* phytase, *A. niger* phytase, and *A. niger* pH 2.5 acid phosphatase. *Applied and Environmental Microbiology.* 64: 4446-4451.

Wyss, M., Pasamontes, L., Friedlein, A., Remy, R., Tessier, M., Kronenberger, A., Middendorf, A., Lehmann, M., Schnoebelen, L., Röthlisberger, U., Kusznir, E., Wahl, G., Müller, F., Lahm, H.-W., Vogel, K., and van Loon, A. P. (1999). Biophysical characterization of fungal phytases (*myo*-inositol hexakisphosphate phosphohydrolase): molecular size, glycosylation pattern, and engineering of proteolytic resistance. *Applied and Environmental Microbiology.* 65 (2): 359-366.

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

Yi Z., Kornegay E. T. and Meguirk A. (1994a). Replacement value of

inorganic phosphorus by microbial phytase for pigs and poultry. *Journal of Animal Science*. 72: 330-336.

Yi, Z. and Kornegay, E. T. (1996). Sites of phytase activity in the gastrointestinal tract of young pigs. *Animal Feed Science and Technology.* 61: 361-368.

Yi, Z., Komegay, E. T., Lindemann, M. D. and Ravindran, V. (1994b). Effect of Natuphos phytase for improving the bioavailabilities of phosphorus and other nutrients on soybean meal based semi-purified diets for young pigs. *Journal of Animal Science*. 72: 7 (Suppl. 1).

Yi, Z., Kornegay, E. T. and Denbow, D. M. (1996a). Supplemental microbial phytase improves zinc utilization in broilers. *Poultry Science*. 75: 540-546.

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.

Young-Ok Kim, Hyung-Kwoun Kim, Kyung-Sook Bae, Ju-Hyun Yu, and Tae-Kwang Oh. (1998). Purification and properties of a thermostable phytase from *Bacillus* sp. DS11. *Enzyme and Microbial Technology.* 22: 2-7.

Yu, B., Jana, Y. C., Chungb, T. K., Leea, T. T. and Chioua, P. W. S. (2004). Exogenous phytase activity in the gastrointestinal tract of broiler chickens. *Animal Feed Science and Technology.* 117: 295-303.

Zantop D. W. (1997). Biochemistries. In Avian medicine: Principles and Applications. B. W. Ritchie, G. J. Harrison, and L. R. Harrison, ed. Wingers Publ. Inc., pp: 115-129. Lake Worth, FL.

Zhou, J. P., Yang, Z. B., Yang, W. R., Wang, X. Y., Jiang, S. Z. and Zhang, G. G. (2008). Effects of a new recombinant phytase on the performance and mineral utilization of broilers fed phosphorus-deficient diets. *Journal of Applied Poultry Research*. 17: 331-339.

Zhou, J. R.; Erdman Jr., J. W. (1995). Phytic acid in Health and Disease. *Critical Review in Food Science and Nutrition.* 35: 495-508.

Zyla K, Ledoux, D. R. and Veum, T. L. (1995). Complete enzymic dephosphorylation of corn-soybean meal feed under simulated intestinal conditions of the turkey. *Journal of Agricultural and Food Chemistry.* 43: 288-294

□)□\□á□D□□□□□.□□□□□□□.□R□U□H□O□H□V□N□L□□□□-□□□□□□ J., and Ledoux, D. R. (2000). Effects of phosphorolytic and cell wall-degrading enzymes on the performance of growing broilers fed wheat-based diets containing different calcium levels. *Poultry Science.* 79: 66-

76.

Zyla, K., Mika, M., Stodolak, B., Wikiera, A., Koreleski, J. and Swiatkiewicz, S. (2004). Towards complete dephosphorylation and total conversion of phytates in poultry feeds. *Poultry Science*. 83: 1175-1186.