A FURTHER LOOK AT PHOSPHORUS, PHYTATE, AND PHYTASE IN MONOGASTRIC NUTRITION

by

Olufemi Babatunde

A Dissertation

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Doctor of Philosophy

Department of Animal Sciences

West Lafayette, Indiana

May 2022

THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Layi Adeola, Chair

Department of Animal Sciences

Dr. Kolapo Ajuwon

Department of Animal Sciences

Dr. Darrin Karcher

Department of Animal Sciences

Dr. Darryl Ragland

Department of Veterinary Medicine

Approved by:

Dr. Zoltan Machaty

Dedicated to God, my wife, and my family

ACKNOWLEDGMENTS

I would like to appreciate God almighty for his grace and mercies in completing my doctoral program. I would also like to express my sincere appreciation to my advisor, Dr. Layi Adeola, who has been more of a father and mentor to me, and for the opportunity he granted to me in continuing to work with him for my Ph.D. I appreciate the many opportunities he gave me to grow, put my skills to use, and even travel internationally for conferences. I am truly indebted to him. His mentorship, guidance, and leadership has greatly influenced the scientist I have become today. His work ethics and his passion for quality research has continued to inspire me and I am driven towards achieving even greater things. I also use this opportunity to appreciate my super committee members: Drs. Kolapo Ajuwon, Darryl Ragland, and Darrin Karcher for their encouragement throughout my program, suggestions, comments, and push towards a successful dissertation work. They have been the very best mentors I could have asked for and I am ever grateful for having them in my life.

I also use this opportunity to appreciate all members of the Adeola Lab both past and present, without whom I would not have been able to complete my research. I appreciate Pat Jaynes for her immense support both at the farm and at the lab with analyzing samples. She has been a life saver most times. I also appreciate my current lab mates; Chan Sol Park, Ayodeji Aderibigbe, Abidemi Adekoya, Jing Yuan, Sakkariya Nalukudy Paramba (Zak), Opeadura Osunbami, Tanner Wise, Jung Sung, Emmanuel Alagbe, Kayode Balogun, and Vitor Haetinger and past labmates; Drs. Tingting Wang, Hang Lu, Ruqing Zhong, Qifan Yu, Guillermo Fondevila, Saheed Osho, Matthew Aardasma, Fengrui Zhang, Pengcheng Xue and Mr. Yu Shi, for their help during my research and for being such amazing friends that made this journey memorable. I appreciate all Animal Sciences staff both past and present, including the administrative staff, farm crew, animal house staff and feed mill staff for being a good support system to me throughout my program. Special thanks to Marlene Miller, Pat Jonas, Barry Delks, Mike Zeltwanger, JoAnn Gaylon, Blaine Brown and several others who have become great friends over time.

I would like to appreciate all my friends from Upper Room Christian Fellowship, African Christian Fellowship, Matthew and Abby Jordan, and the Upper Room Worship Team for being a wonderful spiritual family here at Purdue. I would especially thank the families of Fola and Bunmi Bolaji, Kenny and Fola Ayano, the Ajuwons, the Bugusus, the Nderitus, Ikenna Okekeogbu, the Okezues, Pastor Bayo and Jummy, and countless others who were my support system during my

program. I especially thank my friends Paul Oladele, Tobi Ogunribido, and Emmanuel Alagbe who later became my roommates when I couldn't get an apartment during my last semester at Purdue. I would also like to appreciate my brother Oluwadamilola Babatunde and my aunt Mrs. Toyin Adeniran for being the first people to push me towards studying for an advance degree in the US and Dr Tayo Faniran for helping with my GRE and suggesting Purdue University to me on the day of the exam. Special thanks to my friend, brother, and mentor; Dr. Saheed Osho who truly put me on his shoulders and made my sojourn at Purdue seem easy. His advice, mentorship, and encouragement both professionally and personally has been very cherished and valuable. His love for my family has been incomparable and I am truly grateful for his gift of friendship. As I tell him often, 'I cant pay his money'.

Finally, I would like to appreciate the love of my life, my darling wife Motunrayo. Even if it pains me that we had to live separately throughout my masters and doctoral programs, you still supported my dreams and stood by me. You have been my rock when I wanted to give up and the pain and loneliness became overwhelming. Our home was practically sustained through countless video calls and messages at every time of the day. Your sacrifice to keep us going was selfless particularly with the unfavorable time difference. I am happy I could go through this and get this degree for both of us. You have mattered the most and I love you the most. This acknowledgment is not complete without my heartfelt appreciation to my parents; Dr. and Mrs. Moses Babatunde for their love and support throughout my program and for taking good care of my wife in my absence. You have been amazing, and your prayers and words of wisdom have kept me going. I also say a big thank you to my siblings and their spouses; Dami and Sarah; Ted and Temi; and Praise, for their love, support, and encouragement during my program. I do hope I have made you all proud of this great achievement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

NOMENCLATURE

SYMBOL	DESCRIPTION
AA	Amino acid
ADG	Average daily gain
ADFI	Average daily feed intake
$ADFI_{time}$	Average daily feed intake at each time point
AID	Apparent ileal digestibility
AME	Apparent metabolizable energy
AMEn	Nitrogen-corrected apparent metabolizable energy
ANF	Anti-nutritional factor
ATP	Adenosine triphosphate
ATTD	Apparent total tract digestibility
BEL	Basal endogenous loss
BW	Body weight
Ca	Calcium
CM	Canola meal
CCM	Corn and canola meal
CR	Chromium
D_{time}	Number of days between each time point
$Diff_{BW}$	Differences in body weight at each time point
DM	Dry matter
DNA	Deoxyribonucleic acid
Exp	Experiment
FGF23	Fibroblast growth factor 23
FI	Feed intake
FYT	Phytase units
G:F	Gain to feed ratio
$G:F_{time}$	Gain to feed ratio at each time point
IP	Inorganic phosphorus

LI	Large intestine
mRNA	Messenger ribonucleic acid
NC	Negative control
nPP	Non-phytate phosphorus
P	Phosphorus
PC	Positive control
PI	Analyzed phosphorus of intake
PFD	Phosphorus-free diet
PhyG	Novel consensus bacterial 6-phytase variant
PP	Phytate phosphorus
$PRet_{time}$	Phosphorus retention at each time point
PTH	Parathyroid hormone
RNA	Ribonucleic acid
SBM	Soybean meal
SI	Small intestine
SID	Standardized ileal digestibility
STTD	Standardized total tract digestibility
TTR	Total tract retention
WSP	Water-soluble phosphorus

ABSTRACT

The objective of this dissertation was to investigate areas that needed further research with regards to phosphorus, phytate, and phytase in monogastric nutrition. To fulfill this objective, a total of 6 studies were carried out.

Study 1 was designed to evaluate the additivity of apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of phosphorus (P) in mixed dies containing corn and soybean meal (SBM) and fed to broiler chickens. There were 7 dietary treatments in a randomized complete block design with body weight (BW) serving as the blocking factor. Treatments consisted of 4 semi-purified diets prepared to contain either corn or SBM as the sole source of P with or without phytase supplementation at 1,000 FYT/kg; 2 mixed diets containing corn and SBM with or without phytase addition; and a P-free diet (PFD) formulated to determine the basal endogenous loss (BEL) of P. A total of 512 day-old broiler chicks were fed a commercial starter diet for 21 days while experimental diets were fed for 3 days (day 22-24 post hatching). The BEL of P in broiler chickens as determined by the PFD was 166 mg/kg dry matter (DM). The SID of P in corn and SBM was 52.2 and 65.4 %, respectively. Phytase addition improved ($P < 0.05$) both the AID and SID of P in the ingredient and mixed diets. There was no difference between the predicted and determined AID and SID of P in the mixed diets and were thus additive. It was concluded that the AID and SID of P in corn and SBM were additive with or without phytase addition. However, we could not be certain if the assumption of additivity will hold true in mixed diets containing ingredients with a higher phytate content and if age of birds affected the BEL of P.

In the 2nd study, the additivity of AID and SID of P in mixed diets containing corn and canola meal (CCM) was investigated in broiler chickens at 2 ages. A total of 588 broiler chickens was used in this study. Dietary treatments were arranged as a 2 × 3 × 2 factorial with 2 ages (day

13 and 21), 3 diets (corn, canola meal, and CCM), and 2 levels of phytase (0 and 1,000 FYT/kg) in a randomized complete block design. A PFD was fed to birds at both ages to determine the effects of age on the BEL of P. A commercial starter diet were fed from day 1 to 10 or 18 post hatching and then experimental diets was fed for 3 days until day 13 or 21, respectively. The AID and SID of P was higher ($P < 0.05$) in birds at day 13 as compared with birds at day 21 regardless of phytase addition. Similarly, the BEL of P in younger birds was higher ($P < 0.01$) than in older birds at d 21. Regardless of age or phytase supplementation, the predicted and determined AID and SID of P in the CCM diets were similar and thus additive. In conclusion, age influenced the BEL of P and the utilization of minerals in broiler chickens while the assumption of additivity held true when mixed diets containing CCM was fed to birds at both ages with or without phytase addition.

In order to evaluate the efficacy of a new consensus phytase variant, the 3rd and 4th studies investigated the responses of broiler chickens to varying concentrations of phytate phosphorus (PP) and the new consensus bacterial 6-phytase variant (PhyG) in the starter and grower phases, respectively. Responses evaluated included growth performance, tibia ash, AID, and total tract retention (TTR) of nutrients. A total of 1,152- and 768-day-old male broiler chickens were used in the starter and grower phases, respectively. Dietary treatments were arranged as a 3 × 5 + 1 factorial with a nutrient-adequate positive control (PC) (2.8 g PP/kg) and 15 nutrient-reduced negative control (NC) diets with 3 levels of phytate (NC1, NC2, and NC3 with 2.3, 2.8, and 3.3 g PP/kg respectively), and 5 levels of PhyG (0, 500, 1,000, 2,000, and 4,000 FYT/kg). Rice bran served as the main source of PP in the experimental diets. All treatments had 6 replicates with 12 birds/cage in the starter phase (day 1 to 11 post hatching) and 8 birds/cage in the grower phase (day 12 to 23 post hatching). Birds fed the PC had greater responses ($P < 0.05$) as compared with

birds fed the NC2 diets without phytase at the starter and grower phases. There was no interaction between PP and PhyG on responses of birds in the starter phase but there was an interaction effect ($P < 0.05$) on the AID of some amino acids (AA) in birds at the grower phase. Increasing levels of PP reduced ($P < 0.05$) the growth performance, Ca and P utilization of birds but had no effect on the tibia ash in the starter phase and grower phases. Phytase supplementation improved ($P < 0.05$) the growth performance, AID and TTR of nutrients, and tibia ash of birds in the starter and grower phases. In conclusion, increasing PP levels reduced some responses of birds in the starter and grower phase while PhyG inclusion improved all responses of broiler chickens at both phases.

The time effects of phytase on the P utilization of growing and finishing pigs was investigated in the 5^{th} and 6^{th} studies, respectively using growth performance, apparent total tract digestibility (ATTD) of nutrients, P excretion, and plasma mineral concentrations as evaluation parameters. In both studies (Exp 1 and 2), treatments were arranged as a 3 × 4 factorial in a randomized complete block design with 3 corn-SBM based diets including a P-adequate PC, a low-P NC, and NC + 1,000 FYT/kg; and 4 sampling time points at days 7, 14, 21, and 28 in Exp 1 and days 14, 26, 42, and 55 in Exp 2. Ninety-six growing pigs were used in both trials with an average BW of 19.8 ± 1.16 kg in Exp 1 and 49.8 ± 3.21 kg in Exp 2. Each treatment had 8 replicates evenly divided between barrows and gilts, and 4 pigs per pen. There was an interaction ($P < 0.01$) between time and diet on some growth performance parameters in pigs in Exp 1 but none in Exp 2. Pigs fed the PC had greater ($P < 0.05$) growth performance and ATTD of nutrients as compared to pigs fed the NC in both trials. Phytase supplementation improved ($P < 0.05$) the ATTD of P and Ca in pigs as compared with pigs fed the NC. There was an interaction ($P < 0.05$) between time and diet on the total and water-soluble P lost from pigs in Exp 1. Supplementing the NC with phytase reduced the water-soluble P by 45, 32, and 35 % over the growing, finishing, and the entire

grow-finish period, respectively. In Exp 2, plasma concentrations of P were increased ($P < 0.05$) over time. In conclusion, phytase improved the responses of growing pigs however, some of these responses were influenced over time.

In summary, the AID and SID of P in corn, SBM, and canola meal are additive in complete diets with or without the inclusion of phytase. The BEL of P could be affected by the age of birds. A new consensus phytase variant seems to be efficient in improving the productivity of broiler chickens regardless of the presence of varying concentrations of PP and the growth phase of the birds. Phytase supplementation improves the P utilization and reduces the P loss of growing and finishing pigs however, this may be influenced by time within each growth phase.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

Monogastrics are single stomached animals that do not chew the cud. Although there are several animals referred to as monogastrics, swine and poultry are of agricultural importance because of their role in supplying some of the major animal proteins (meat and eggs) consumed by the human population. Poultry can be categorized into egg and meat-producing birds with broiler chickens serving as the major meat producers. In 2020, over 100 million metric tons of broiler meat was produced worldwide (Shahbandeh, 2021), with the United States and Brazil leading the global production of broiler chickens. Meanwhile, the production of pork was estimated at 110 million metric tons in 2020 with China leading the production of hogs and being the largest importer of pork products worldwide (FAO, 2021; Shahbandeh, 2021). It is projected that the production of both swine and broiler chickens will almost double by 2040 thus, research that improves the productivity and global output of these animals are always ongoing. Research on monogastric nutrition is of great importance being that the major cost of producing these animals is attributed to feed. The 3 most expensive constituents of animal feed include energy, protein, and phosphorus (P) hence, a big portion of monogastric nutrition research focuses on the improved utilization of these components by broiler chickens and pigs.

Phosphorus is one of the most abundant minerals in the world and plays an important role in the sustenance of all life. Phosphorus is a major constituent of the skeletal system and is required in maintaining the stability and shape of most cells. Phosphorus is involved in several biochemical reactions including the formation of adenosine triphosphate (ATP) which is the energy currency of the cell (Boling et al., 2000a). Phosphorus can be found in the earth crust and in plants as a phosphate compound with other minerals but never as a free element. Howbeit, its form in most plants is not

readily available to broiler chickens and swine due to the inadequacy of endogenous enzymes required to hydrolyze the phosphate compound. The use of P by broiler chickens and pigs have been extensively evaluated and information on the sources, functions, toxicity, deficiency, digestion, absorption, regulation, excretion, and factors affecting its utilization are available in the literature.

Phytate is the main form of P found in cereal grains and oilseeds which comprise the main feed ingredients used in the formulation of diets for broiler chickens and pigs. Phytate is often regarded as an anti-nutritional factor (ANF) in monogastric nutrition because of its ability to bind P, other nutrients such as calcium (Ca), proteins and amino acids (AA), starch, and even enzymes such as salivary amylase hereby inhibiting their function and utilization in broiler chickens and swine. Several studies have evaluated the negative effects of phytate on the utilization of P and on the productivity of both pigs and broiler chickens resulting in measures that have been adapted by commercial farmers to counteract this effect (Cabahug et al., 1999; Blavi et al., 2019). Some of these measures include the addition of more readily available inorganic P (IP) which are mined from the earth crust, or the use exogenous enzymes such as phytase into the diets of broiler chickens and pigs.

Phytase is an enzyme capable of hydrolyzing phytate in a stepwise manner and releasing bound P and other nutrients for use by animals (Selle et al., 2009). They are known to occur naturally in some plants or could be synthesized by microbes such as fungi and bacteria. Phytase exists minimally in the gut of broiler chickens and pigs and so have to be included from exogenous sources into their diets. Because phytase has been proven to be effective in breaking down the phytate complex, extensive research has been carried out to study its composition, mode of action, effects and efficacy, synthesis and production, toxicity, etc. In monogastric nutrition research, previous work has investigated the effects of phytase on several parameters in broiler chickens and pigs including growth performance, nutrient utilization, skeletal development, and nutrient loss (Selle et al., 2000; Blavi et al., 2019). Similarly, the impacts of environmental, nutritional, or physiological

(animal-based) factors on phytase effects in broiler chickens and pigs have been previously examined (Plumstead et al., 2008; Zaefarian et al., 2013; Babatunde et al., 2019a, b).

The available information on P, phytate, and phytase has helped pushed the boundaries on what we currently know about P in monogastric nutrition and has contributed immensely to the outstanding production of broiler chickens and pigs occurring presently when compared with production over the last 5 to 6 decades. It has also contributed to the exceptional progress in enzyme technology seen within the last 3 decades. However, to meet up with the projected increase in both demand and supply of animal proteins, there is the need to continuously explore and investigate this area of research. The derivation of new information on P, phytate, and phytase in monogastric nutrition will support efforts to formulate diets that meet the exact requirements of animals, develop new and more efficient phytase products, increase the productivity of broiler chickens and pigs, and reduce the environmental impact and carbon footprint of animal production.

The objective of this literature review is to describe P, its properties, functions, and attributes. This literature review will also describe phytate and phytase in monogastric nutrition and present current information on their effects on the phenotypic characteristics of broiler chickens and swine. Lastly this review will explore some areas that require further research when P, phytate, and phytase utilization are considered.

1.2 Monogastrics

Monogastrics are animals with a simple single-chambered stomach and could be herbivores, carnivores, or omnivores. They cannot readily digest fibrous materials or extract energy from cellulose digestion as compared with ruminants, however, their ability to digest cellulose may vary amongst species. Monogastrics of agricultural importance include poultry which could be further subdivided into meat-type birds such as broiler chickens, turkeys, and ducks or egg-type birds such

as layers or quail. Swine is another example of monogastrics with a high contribution to the supply of meat proteins to the human population.

1.2.1 Broiler Chickens

Broiler chickens are birds that have been bred specifically for meat purposes and are one of the most consumed meat products around the world. Between 1961 and 2019, the world's broiler meat production increased from 9 to 132 million tons due to growing demand for chicken (FAO, 2020). The United States is the world's leading producer of broiler meat with approximately 17 % of the global output (FAO, 2020). Currently, most commercial broilers reach market weight between 4 to 6 weeks although there are slower growing breeds that reach market weight at about 14 weeks. Advances in selection, breeding, and genetics have improved both the size of birds, growth rate, and feed efficiency as compared with birds from the 90's. Howbeit, the huge sizes, rapid growth and development of broilers combined with current management practices have raised various challenges with modern broiler chicken strains. Some of these challenges include cardiovascular dysfunctions such as sudden death syndrome and ascites; skeletal dysfunctions such as dyschondroplasia and osteodystrophy; integument lesions such as hock burns and breast blisters; and even ocular dysfunction due to lightning conditions (Lauber and Kinnear, 1979; Bessei, 2006). Some of these challenges may be mitigated by nutritional interventions or changes to current management practices.

1.2.2 Swine

The domestic pig is an omnivorous even-toed ungulate considered a subspecies of the Eurasian boar. Over the last 8,000 years, they have been raised primarily for their meat called pork. Currently, over one billion pigs are butchered worldwide each year. In 2021, the global pork

production was led by China with over 40 million tons of pork products followed closely by the European Union and the United States (Shahbandeh, 2021). It is estimated that the global consumption of pork would increase by up to 120 million metric tons by 2027 (Shahbandeh, 2021). Current breeds of pigs have been genetically selected for low back fat and high fecundity with the average sow producing up to 25-30 piglets per year. However, with an average mortality rate of 5 to 10 %, only about 20 pigs per sow may be marketed per year. Although this has raised the bar towards high productivity for commercial farmers, it has also increased the occurrence of health issues in pigs such as the prolapse epidemic. The average pig reaches market weight of 125 kg by 18 to 20 weeks of age and will gain about 0.8 kg per day during the grow finish stage with a feed conversion ratio of 2.75 (Lineen et al., 2005). Due to the high amount of feed consumed by pigs, they also produce a huge amount of waste that is a source of environmental concern today (Angel et al., 2005). To fully investigate the environmental impact of commercial production of broiler chickens and swine, we need to examine how they digest and absorb nutrients.

1.2.3 Digestion and Absorption of Nutrients in Monogastrics

In monogastrics such as pigs, the process of digestion starts from the mouth with the teeth helping to grind and break down food particles into smaller sizes. While grinding occurs, there is a mixing of the food particles with saliva which helps to lubricate the process of swallowing and initiate the digestion of carbohydrates through the release of the enzyme amylase (Baker, 2017). After chewing and swallowing, the food passes through the esophagus into the stomach with the aid of peristaltic movements in the gut. The stomach is a muscular organ used for storage and also for the breakdown of nutrients in food using a mixture of acids such as hydrochloric acid and digestive enzymes such as pepsinogen before it is passed on to the small intestine (SI). The presence of acids in the stomach reduces the pH of the region to a range of 1.5 to 2.5 which helps to kill any microbes

that was ingested with the feed (Baker, 2017). The passage of the digesta or chyme into the SI is regulated by the pyloric sphincter to prevent overwhelming the system and to ensure efficient digestion and absorption of nutrients. The SI which is divided into 3 segments (duodenum, jejunum, and ileum) is the major site of nutrient digestion and absorption. The pancreas and biliary systems are responsible for the secretions of digestive enzymes, sodium bicarbonate, and other endocrine excretions such as glucagon and insulin. In the duodenum, the digestive enzymes released help to break down proteins, fats, and carbohydrates while the sodium bicarbonate increases the pH of the digesta to prevent the destruction of the cells in the region (DeRouchey et al., 2009).

As the digesta flows down into the jejunum and ileum, further breakdown of nutrients as well as absorption occurs in the brush border membrane or intestinal mucosa through the help of finger-like projections called villi. Released nutrients such as AA, simple sugars, minerals, etc., are absorbed through the villi and into the bloodstream where they are transported into the liver through the portal vein. However, fat is absorbed through the villi into the lymphatic system and then to general circulation through the thoracic duct. Undigested feed materials from the SI are passed on to the large intestine (LI) which is divided into the cecum and colon. Microbial fermentation and the absorption of water occurs in the LI. Volatile fatty acids and some vitamins produced by the action of microbes in the LI are reabsorbed by the animal. At this point, any leftover digesta is regarded as waste and passed out through the rectum and anus. A pictorial representation of the digestive tracts of pigs is presented in Figure 1-1.

The digestion of nutrients in broiler chickens is similar to what occurs in swine however, the digestive system of broiler chickens (Figure 1-2) is slightly different from pigs. Broiler chickens have beaks instead of mouths and are without teeth. Food flows through the esophagus into a crop where it is stored for a short period. Afterwards the digesta flows into a proventriculus, which is like the stomach in pigs, and then into the gizzard where grinding occurs. Thereafter, the digesta flows

into the SI where digestion and absorption take place and then into the LI. At the ileocolonic junction, two ceca connect to the gut through duct openings and contain microbes which carry out fermentation of substrates. Undigested materials move from the LI to the cloaca and exit the body through the vent. Due to the inefficiencies of the digestive system, not all ingested nutrients are digested and absorbed by the animal. This inefficiency could be dependent on various factors including the wear and tear of the digestive tract, the physiological state of the animal, environmental conditions, dietary factors, and even the health status of the animal. Although all living organisms produce waste, the nutrient density of the waste from commercial production of broiler chickens and swine is of imminent concern to researchers due to its negative impact on the environment.

1.2.4 Environmental Concerns with Monogastric Production

The high rate of producing broiler chickens and swine under current commercial management practices has contributed to the ongoing environmental issues around the world. The huge amount of waste from commercial farms and improper waste disposal mechanisms has led to environmental challenges such as eutrophication, acidification, and climate change (Andersen, 2015). On average, it is estimated that hog farms produce about 1.5 gallons of manure per pig per day including any wastewater used on site and this quantity could increase with the weight of the pig (Figure 1-3) (Andersen, 2015). Similarly, there is staggering amount of waste being produced from commercial broiler chickens' farms that include manure, sick and dead animals, feed additives, and pathogens (Blue, 2021). Waste from monogastric production contain high amount of nutrients such as P, nitrogen, potassium, and heavy metals that contaminate the soils, and pollute the air and water systems (Petersen et al., 2007). In particular, P and nitrogen from manure have been identified as major polluters of surface waters, groundwaters, and marine waters through the runoff from agricultural soils. This runoff damages the ecosystem through eutrophication of lakes and streams,

deterioration of water quality, destruction of aquatic life, and the reduction of the recreational values of water bodies (Zhang et al., 2017). Managing the loss of nutrients such as P and nitrogen from the commercial production of broiler chickens and swine is of paramount importance to monogastric nutritionists. In particular, P management has received widespread interests from scientists worldwide and remains an area of ongoing research if the negative impact of livestock production on the environment is to be reduced.

1.3 Phosphorus

Phosphorus, which is the 13th element to be discovered, is a highly reactive chemical element with an atomic mass of 30.97 and is one of the most abundant minerals on earth. Existing naturally in either the red or white form, P is never found as a free element on earth but in combination with other minerals as a phosphate. Phosphorus is essential to the sustainability of life and must be replaced into the soil after it has been drawn up by plants hence its application in commercial agricultural fertilizers. Phosphorus also makes up about 1 % of an animal's body weight and plays a role in several biochemical reactions necessary to support growth and development (Boling et al., 2000a).

1.3.1 Sources of Phosphorus

The largest source of P on earth comes from sedimentary rocks such as phosphate rocks (calcium phosphate) and its concentration in the earth's crust is estimated at 4×10^{15} metric tons. Examples include monocalcium phosphates, dicalcium phosphates, monosodium phosphates, etc. Despite the abundance of P on earth, only a small percentage can be mined due to physical, energy, economical, or legal constraints. Other sources of P include ocean sediments, soils, plants, and animals. Due to high demand of P for agricultural purposes including fertilizer manufacturing

(which accounts for about 80 % of demand), animal feed, and food additives, it is estimated that global shortages in the supply of P may occur in the nearest future (Cho, 2013). Phosphorus is present in most plants as phytate P (PP) and serves as a source of P for animals, however, its bioavailability to animals varies depending on the species. Meanwhile, P is also present in the flesh and skeletal system of most vertebrates, eggshells of avian and reptilian species, and shells of some aquatic life such as oysters and mollusks. Of major importance to monogastric nutrition are IP sources and PP from plants.

1.3.2 Functions of Phosphorus

Phosphorus is one of the most abundant minerals in the body and functions in several biochemical reactions required to sustain life (Razzaque, 2011). Phosphorus is present in every cell of the body where it is a component of the cell structure in the form of phospholipids. Phosphorus also serves in the formation of ATP which is required as an energy source for all cells, and as an initiation factor for protein synthesis. Phosphorus is a component of DNA and RNA which carry the genetic information of every organism. Several enzymatic or hormonal reactions require P for activation or phosphorylation (Crenshaw, 2001) or as a part of their components e.g., hemoglobin. Intracellular P ions are important for oxidative phosphorylation and approximately 20 % of it is found in the mitochondria, 30 % in the endoplasmic reticulum, and the rest in the lysosomes, nucleus, and golgi complex (Razzaque, 2011). Phosphorus will also act as a buffer to neutralize acids, maintain normal pH in the blood, and function to support healthy brain and nervous system activity. About 85 % of P in the body is found in the skeletal system and in the teeth where it works with Ca to maintain integrity, strength, and growth (Oster et al., 2016). The remaining P is found in the viscera and skeletal muscle with a minute amount found in extracellular fluids (Gaasbeek and Meinders, 2005)

1.3.3 Phosphorus Digestion and Absorption in Monogastrics

The form of P in diets could determine the rate at which it is digested in the intestine. When P is in the phytin form, as found in plants, it is rarely digested by broiler chickens or pigs except in the presence of phytase enzyme. However, when P is in its inorganic form it is readily digested by monogastrics. Regardless of the form of P in diets, P becomes soluble in its ionic form and is absorbed along the entire length of the gastrointestinal tract. The SI is the main site of P absorption as compared to other sections of the gut while absorption of P in the colon is often regarded as being physiologically irrelevant (Sabbagh et al., 2011). Usually, P is absorbed and transported through the apical membrane by one of two mechanisms namely the passive and sodium (Na)-dependent transport pathways. The passive or paracellular transport depends on the electrochemical gradient across the epithelial layer with movement occurring through the tight junction complexes formed by the interaction of adhesive proteins of adjacent epithelial cells (Sabbagh et al., 2011). There are two families of Na-dependent P transporters responsible for the inward transport of extracellular P and are called type II and III transporters (Virkki et al., 2007). Type III transporters include Pit1 and Pit2 and are found across a wide variety of cell types where they are involved in supplying cells with IP to meet individual cell functions (Miyamoto et al., 2007).

The type II P transporters are more common and are responsible for the majority of P movement in the kidneys (NaPIIa and NaPIIc) and the gut (NaPIIb). Type II P transporters are electrogenic and will transport P with a stoichiometry of 3:1 Na^{+}:HPO^{2-}_{4} in the intestines or kidneys. There is a high affinity for P by NaPIIb in the brush border membrane of the gut resulting in its saturation under most dietary conditions. Moreover, under low dietary P conditions, and independent of Ca levels, NaPIIb plays a role in upregulating the supply of P to the body (Saddoris et al, 2010) and may account for up to 78 % of the total P transport (Eto et al., 2006). Previous work has reported that feeding P deficient diets will upregulate NaPIIb transport in the gut by increasing its maximal

capacity for absorption by almost 50 % (Hattenhaur et al., 1999). This increase in the maximum capacity of the transporter during P deficiency is usually accompanied by an increase in the expression of membrane-bound NaPIIb cotransporter proteins in the SI. However, there has not been consistency with the NaPIIb mRNA expression under similar conditions and this has been reported to be dependent on the severity and length of the exposure to P deficiency (Segawa et al., 2004; Saddoris et al., 2010). Other factors that can influence the expression of NaPIIb in the gut include vitamin D, thyroid hormone, glucocorticoids, estrogens, epidermal growth factor, fibroblast growth factor 23 (FGF23), and metabolic acidosis (Marks et al., 2010).

1.3.4 Phosphorus Regulation in Monogastrics

Phosphorus balance is tightly controlled by a complex inter-organ communication between the kidneys, intestine, parathyroid glands, and bones (Figure 1-4; Drezner, 2002). The regulation of P occurs through the control of intestinal absorption and renal excretion. Dietary P levels and 1,25-dihydroxyvitamin D_3 (calcitriol) directly influence intestinal absorption of P through the regulation of NaPIIb activities. For example, low dietary P induces the release of calcitriol from the kidneys, which in turn upregulates intestinal expression of NaPIIb and an increase intestinal P absorption (Komaba and Fukagawa, 2016). Calcitriol will also increase the mobilization of P from the bones and soft tissues to meet the body's demand for P (Drezner, 2002). Urinary excretion of excess P helps to maintain serum P levels. After glomerular filtration in the kidneys, most of the P is absorbed in the proximal tubule and is mediated by NaPIIa and NaPIIc transporters (Segawa et al., 2009). The amount of P transporters expressed in the kidneys is regulated by the parathyroid hormone (PTH) and FGF23. The PTH hormone reduces the expression of P transporters in the proximal tubule by reducing their gene transcription and driving internalization from the brush border membrane (Komaba and Fukagawa, 2016) thus increasing the excretion of P from the kidneys. Meanwhile,

FGF23 functions by binding with its receptor in the presence of the obligate cofactor Klotho (Urakawa et al., 2006), and increasing P excretion by reducing the expression of NaPIIa. The concerted action of the increased or decreased circulating levels of calcitriol and PTH on the intestines, bones, and kidneys, helps to maintain the circulating levels of P in the blood (Schroder et al., 1996).

1.3.5 Phosphorus and Calcium

Together, P and Ca are the most abundant minerals in the body, and they interact on several levels and in various biochemical reactions in the body. Phosphorus and Ca are required for bone mineralization and are deposited as hydroxyapatite on bones and teeth where they help to maintain strength and integrity (Oster et al., 2016). Calcium is also found in the blood, muscle, tissues, cells, and extracellular fluids. Calcium plays a role in intracellular signaling, nervous system functions, enzyme secretions, muscle contractions, and blood clotting (Lamberg-Allardt and Kemi, 2017). Intracellular and extracellular Ca is more tightly regulated than P and excess is usually excreted through the urine, feces, or sweat (Votterl et al., 2021). Regulation of Ca is controlled by PTH and calcitriol in the kidneys, bones, and intestines. Phosphorus has a high affinity for Ca in the lumen and would usually form strong bonds in the gut. Since both minerals have similar absorption pathways, maintaining an appropriate Ca to P ratio in diets is important in ensuring adequate metabolism of both minerals in the gut and preventing antagonistic effects (Letourneau-Montminy et al., 2012). A Ca :P ratio of 1:1 to 2:1 is usually desirable when feeding corn-soybean based diets to broiler chickens or swine (NRC 1994, 2012). However, several factors could impact the Ca :P ratio in animals including diet formulations, deficiency of vitamin D, or even the source and bioavailability of either mineral in diet. A deviation on either side of Ca to P ratio could hinder the utilization of the other mineral in the gut (Stein, 2016). Increased levels of Ca in the gut decreases

the bioavailability of P by affecting the pH and reducing the efficiency of enzymes actions. When plant materials are fed to monogastric animals, Ca would also bind with PP, forming insoluble complexes, and preventing its utilization by the animals (Selle et al, 2009).

1.3.6 Phosphorus Deficiency

A deficiency of P in the body is known as hypophosphatemia and is caused by the inadequate consumption of P over an extended period of time. The form of P in diets may also cause deficiency in animals particularly if P is present as phytate which is the most common storage form in plants. Slow growth rate is a common symptom of P deficiency in young animals, other symptoms include developments of rickets and rough hair coats. In older animals, P deficiency will cause loss of weight, anorexia, and lethargy at an early stage and may later develop into osteomalacia, pica, abnormal gait, and lameness in latter stages. In broiler chickens and pigs, feeding of low-P diets over extended periods of time results in reduced body weight (BW), feed intake (FI), and feed efficiency (Brana et al., 2006; Merriman et al., 2017; Babatunde et al., 2019a). Previous studies have also reported a decline in energy utilization, protein and amino acids utilization, mineral utilization, and poor bone development when low-P diets were fed to pigs or broiler chickens (Blavi et al., 2019; Babatunde et al., 2020).

1.3.7 Phosphorus Toxicity

Phosphorus toxicity due to excessive retention of P can cause a wide range of damage to cells and tissues. Although not very common in animals, hyperphosphatemia can occur due to decreased urinary excretion of P in association with chronic renal failure. This leads to elevated serum and plasma P levels especially when P uptake is not reduced dramatically. Other causes of hyperphosphatemia could be decreased intracellular P uptake, cellular release of P after lysis,

vitamin D toxicity, excess bone resorption, hypoparathyroidism, acidosis, rhabdomyolysis, or hemoconcentration (Komaba and Fukugawa, 2016). Toxicity of P in animals can result in impaired fertility, increased cell death, reduced bone mineralization, vascular calcification, endothelial dysfunction, renal dysfunction, bone disease, and increased tumorigenesis (Marraffa et al., 2004; Razzaque, 2011).

1.3.8 Phosphorus Bioavailability

Although there are many sources of P that could be included in diets for broiler chickens and pigs, the availability and utilization of P in these sources differ. It is important to determine the bioavailability of nutrients in feed ingredients so as to adequately determine the proportion of the nutrient that could be utilized by the animal. Information on bioavailability also ensures the adequate supply of nutrients such as P required by animals. As a general rule, P from plants is less available than animal sources, and both are less available than IP (Plants < Animals < IP). Plant P are the least available to broiler chickens and pigs because they are stored as phytate in most oilseed and cereals and cannot be readily hydrolyzed due to the inadequate amounts of endogenous phytase present in the gut of monogastrics (Ravindran et al., 2000). Inorganic P are readily digested and absorbed by pigs or broiler chickens because of their rapid solubility in the upper digestive tract thus, they are often referred to as being highly bioavailable. There are several methods adapted by researchers in determining the bioavailability of P in a feedstuff. The use of the slope ratio method with a reference diet has been utilized in ranking the availability of P in feed ingredients. However, a drawback to this method is its inability to determine digestibility. The use of digestibility trials has become more common in determining the availability of nutrients such as P in ingredients for broiler chickens and pigs (Adeola, 2001). Parameters such as growth performance, P and Ca utilization, ash content in bone, and plasma P concentrations are commonly used in digestibility trials to determine the

bioavailability of P in feedstuffs for monogastrics (Adedokun et al., 2004; Petersen et al., 2011). During the formulation of diets for monogastrics, information on the bioavailability of P in feed ingredients contribute towards meeting the standardized total tract digestible (STTD) P requirement for pigs or the non-phytate P requirement for broiler chickens.

1.4 Phytate

Phytic acid (Myo-inositol-1,2,3,4,5,6-hexakisdihydrogenphosphate) is the main storage form of P in plants including cereals, oilseeds, and legumes (Humer, 2015). However, phytic acid is unstable in its free form and would usually form complexes with metallic ions such as Ca, Fe, Zn, Mn, K, and Mn forming salts known as phytates (Figure 1-5) (Lott et al., 2000). Phytate has a high negative charge in the stomach due to the acidic nature of its 12 replaceable protons. By the time it moves into the SI, the phosphate groups will usually carry 1 or 2 negatively charged oxygen atoms (Humer, 2015). In cereals such as corn, PP is mostly found in the germ of the grains while in oilseeds and legumes, it is found in the aleurone layers and outer bran. Generally, PP forms the bulk of total P found in cereals and their by-products such as corn, wheat, rice bran, and oat bran as compared with legume seeds (Viveros et al., 2000). However, the PP contents of plant materials may be affected by climatic conditions, genetics, type of soil, and fertilizer application (Humer et al., 2015). Similarly, the utilization of PP in plant materials by broiler chickens or pigs may differ due to several factors that could include presence of enzymes, accessibility to the PP in the seeds, stability of the phytate structure, or other intrinsic qualities of the plant material (Leske and Coon, 1999).

1.4.1 Binding Properties of Phytate

Phytate is often regarded as an ANF because of its ability to bind to several nutrients and hinder their utilization in broiler chickens or pigs (Ravindran et al., 2000). The pH of the gut

influences the solubility and electronegativity of phytate; hence in the lower pH of the stomach/gizzard/proventriculus, phytate is more soluble but has a lower negative charge. As digesta moves from the stomach to the SI, the increasing pH causes an increase in the electronegativity and precipitation of phytate. This increases the formation of *de novo* complexes which reduces the absorption of minerals and other nutrients in the gut (Selle et al., 2009). The extreme negative charge on phytate also predisposes it to binding tightly with other positively charged cations in the gut (Cowieson et al., 2016). Of importance to monogastric nutrition is the phytate-Ca bonds formed in the upper gastrointestinal tract due to the high concentration of Ca in diets for monogastrics. Studies have reported that high Ca diets reduce PP absorption in broiler chickens or pigs and an increase in PP concentration in diets also increases the animal's Ca requirement (Nelson et al., 1968; Cowieson et al., 2016). Phytate may also influence the uptake of Ca in the gut by interacting and inhibiting Na secretion in the SI which in turn affects the Na-dependent transporters needed to transport Ca across the brush border membrane (Ravindran et al., 2008). Asides from Ca, phytate also has a high affinity for Zn which impedes its utilization due to formation of strong and insoluble complexes (Oatway et al., 2001). Furthermore, phytate has been known to negatively affect Fe metabolism and the bioavailability of other trace minerals such as lead or cadmium (Lind et al., 1998; Sandberg et al., 1999).

It has also been observed that phytate will form strong complexes with proteins and AA hereby reducing their utilization in broiler chickens and pigs (Urbano et al., 2000; Selle et al., 2012; Adedokun et al., 2015). However, the stability of the phytate-protein bonds may be dependent on its solubility across different feedstuffs (Champagne et al., 1985). In addition, phytate is known to alter and reduce protein solubility, enzymatic activity, and proteolytic digestibility (Selle et al., 2000; Humer at al., 2015). This is because proteolytic enzymes such as trypsin, pepsin, or chymotrypsin are less likely to hydrolyze phytate-protein bonds (Cervantes et al., 2011). Furthermore, phytate may

hinder AA uptake in the SI by hindering the actions of Na-dependent transporters and the Na-K pump (Ravindran et al., 2008). Phytate may also interact and bind with starch directly or indirectly by hindering the activities of endogenous amylases through the formation of complexes, or by interaction with the Ca required for enzyme activity (Rickard and Thompson, 1997; Humer at al., 2015). Some studies have reported reduced utilization of energy and starch in diets containing phytate by broiler chickens and pigs (Olukosi et al., 2007; Woyengo et al., 2010). Kumar et al. (2010) reported the formation of lipophytins, which are complexes between phytate and lipids or its derivatives, and insoluble soaps which hinder the utilization of energy from fat sources. A study by Camden et al. (2001) also confirmed reduced energy utilization from starch, lipids, and proteins by broiler chickens in the presence of phytate.

1.4.2 Other Attributes of Phytate

Aside from its impact in animal nutrition, phytate plays other roles in human nutrition and health and also has environmental applications (Crea et al., 2008). Phytate has been reported to serve as an antioxidant in the body (Graf and Eaton, 1990) by inhibiting the production of hydroxyl radicals and normalizing cell homeostasis (Kumar et al., 2010). Phytate also prevents kidney stone formation (Munoz and Valiente, 2003); possesses anticarcinogenic and antineoplastic properties (Graf and Eaton, 1993; Shamsuddin, 2002); and supports the treatment of diseases such as HIV (Raboy, 2003). Thompson (1993) suggested that dietary phytate may be beneficial to diabetic patients due to its ability to lower blood glucose levels by reducing starch digestion and slowing down the emptying of the stomach. It has also been suggested that phytate may reduce blood clots, triglycerides, and cholesterol thus preventing heart diseases (Onomi et al., 2004). In addition, phytate has industrial and environmental applications such as immobilization and *in situ* treatment of soils contaminated with minerals and heavy metals (Ulusoy and Simsek, 2005; Crea et al., 2008).

1.5 Phytase

Phytase also known as myo-inositol hexakiphosphate phosphohydrolase is an enzyme capable of hydrolyzing phytate in a stepwise manner and releasing P, lower inositol phosphate esters, and even myoinositol (Lei and Porres, 2003). Phytase has become more increasingly included in diets for poultry and swine because of its proven ability to improve P utilization from plant materials containing phytate (Woyengo et al., 2010; Babatunde 2019a,b).

1.5.1 History, Structure, and Types of Phytase

Phytase was first discovered in the early 90s as naturally occurring in plant materials such as wheat and rice, and in plant by-products such as rice bran. Phytase was also observed to occur endogenously in animals and could be produced by microbes such as bacteria, yeast, and fungi (Wodzinski and Ullah, 1996). The earliest phytases were derived from the fungus *Aspergillus niger* and launched commercially in 1991 under the brand name Natuphos®. However, because of the high cost of producing phytase, it was not widely utilized in commercial production until the early 2000s when a more effective phytase from the bacteria *Escherichia coli* was introduced (Rodriguez et al., 1999). Since then, several generations of phytases have been developed and made commercially available. Phytases have generally been divided into 2 groups depending on the initiation site of P hydrolysis on the carbon ring of inositol. Microbial phytases especially from fungi are often regarded as 3-phytases because they initiate the splitting of the phosphate group at carbon 3 on the inositol ring while plant phytases or 6-phytases hydrolyze the P group on carbon 6 (Lei and Porres, 2003). However, exceptions such as bacterial phytases will often act as 6-phytases (Lassen et al., 2001). Microbial phytases have often been reported to be more effective than plant phytases; and bacterial phytases have been reported to be more efficacious than fungal phytases in hydrolyzing phytate when added to broiler chicken and swine diets (Kornegay, 2001; Kerr et al., 2010).

Phytase can also be categorized based on its catalytic mechanism. The most common group of phytases in this category belong to the class of histidine acid phosphatases which are acidic phosphatases and characterized by a conserved active site hepta-peptide motif and a catalytically active dipeptide (Lei and Porres, 2003). Other categories include the alkaline phytase group or β-propeller phosphatase, metalloenzymes group or purple acid phosphatase, and dual-specificity phosphatase or protein tyrosine phosphatase (Lei et al., 2013). Some common trade names of commercial phytases available include Natuphos®, Allzyme®, Quantum Blue®, OptiPhos®, Ronozyme HiPhos®, and Axtra® PHY. Each of these phytases differs in protein of origin, microbial expression, and optimum pH and temperature (Dersjant-Li et al., 2015).

1.5.2 Properties of Phytase

Phytase activity is often measured by the amount of IP released per minute from Na- phytate at a pH of 5.5 and a temperature of 37°C (International Union of Biochemistry, 1979). Phytase activity may be influenced by several characteristics of the enzyme including substrate specificity, pH, optimum temperature, proteolytic resistance, and thermostability (Lei and Porres, 2003). Most microbial phytases have a high affinity for phytic acid while plant phytases and some fungal phytases have a broader substrate specificity with a higher affinity for the lower inositol phosphates (Wyss et al., 1999). Phytase from *Bacillus sp.* have been known to preferentially degrade phytate into inositol tri-phosphates (Kerovuo et al., 2000). Most phytases have an optimal pH of 2.5 to 6 but some prefer neutral or alkaline environments. Plant or microbial phytases seem more active at a temperature between 45 and 60°C and would preclude a full activity of phytases at stomach temperatures in poultry and swine (Lei and Porres, 2003). Often, commercial feeds are pelleted, and this requires phytase enzymes to be able to withstand high temperatures to avoid loss of activities during the pelleting process. The high thermostability of phytase could affect their activity under thermoneutral

conditions resulting in some enzymes being chemically coated. However, coating may compromise the release and activity of the enzyme in the stomach and so spraying of the phytase enzymes on feed after pelleting is often practiced commercially to preserve enzyme activity. A strong resistance to the action of proteolytic enzymes in the gut is often required for effective phytase enzymes. Igbasan et al. (2000) observed that bacterial phytases were more resistant to proteolytic digestion as compared to fungal phytases.

1.5.3 Mode of Action

In general, phytase will hydrolyze phytate in a stepwise manner by initiating the removal of the first phosphate group from a fully phosphorylated phytic acid resulting in a penta-phosphate phytic acid. Subsequently, the esters of inositol are released in a descending order of preference (Wyss et al., 1999). This means that all phytate in the gut are hydrolyzed by phytase into penta-phosphate esters before the hydrolysis into lower esters are initiated in a likewise manner. Under perfect conditions, the complete hydrolysis of phytate results in the release of myo-inositol, six P ions, and any other associated nutrient such as proteins, starch, and minerals that were bound in the phytic structure (Yu et al., 2012). However, in practical conditions, the hydrolysis of phytate is usually incomplete resulting in a mixture of lower inositol esters (Dersjant-Li et al., 2015). The hydrolysis of phytate occurs more effectively under acidic conditions as found in the stomach or crop/proventriculus-gizzard of swine and broiler chickens, respectively. Data from previous studies indicate that an early and quick hydrolysis of the higher esters of inositol including phytate and penta-phosphate inositol in the upper gastrointestinal tract result in increased utilization of P, Ca, and other nutrients hereby reducing the anti-nutritional effects of phytate (Morales et al., 2011; Yu et al., 2012).

1.5.4 Effects of Phytase in Monogastric Nutrition

Over the last 3 decades, several studies have reported the beneficial effects of phytase in monogastric nutrition and has probably been referred to as the most consistent feed enzyme currently on the market. This benefit has been attributed to the ability of phytase to degrade the phytic bonds present in plant materials and releasing the complex-bound nutrients for use by animals. Phytase has been proven to increase the BW, BW gain, feed intake (FI), and feed efficiency of both poultry (broiler chickens, ducks, laying hens) and pigs regardless of the age or stage of production when diets low in available P was fed (Selle et al., 2000; Adeola, 2018, Taylor et al., 2018; Blavi et al., 2019). The digestibility and retention of minerals such as P, Ca, Zn, Mg, and Mn by poultry and pigs have also been improved with phytase supplementation (Waldroup et al., 2000; Zyla et al., 2011; Veum and Liu, 2018; Babatunde et al., 2020). Similarly, the utilization of energy (Selle and Ravindran, 2007; Woyengo and Wilson, 2019), dry matter (Zhang et al., 2000, Babatunde et al., 2019a,b), and nutrients such as crude fiber (Attia et al., 2002), proteins and AA (Rutherfurd et al., 2004; Cowieson and Bedford, 2009; Babatunde et al., 2020) and fats (Ravindran et al., 2000) has been reportedly improved by phytase supplementation.

The positive effects of phytase on the utilization of other nutrients besides P have often been referred to as the extra-phosphoric effects of phytase (Gehring et al., 2013). This is because the primary function of the phytase enzyme is to release P from the phytate complex., The additional benefits on the utilization of other nutrients by poultry and pigs have been observed and reported (Cervantes et al., 2011; Gehring et al., 2013). Some studies have reported that dry matter, energy, and proteins are not consistently improved by phytase inclusion in poultry and swine diets. However, this observation has been attributed to several reasons including the choice of indigestible markers, species (pigs vs. broiler chickens), and nature of phytate in diets (Onyango et al., 2005; Adeola and Cowieson, 2011; Veum and Liu, 2018). Phytase supplementation has also been reported to improve

serum P, Ca, and myo-inositol (Madrid et al., 2013; Babatunde et al., 2019a,b), bone mineral deposition as observed with tibia or toe ash (Musapuor et al., 2005; Olukosi et al., 2013; Veum and Liu, 2018), and egg production and qualities (Ahmadi et al., 2007; Kim et al., 2017). Furthermore, phytase has been reported to reduce P concentration in the manure of poultry and pigs particularly in the water-soluble form which plays a role in eutrophication (Angel et al., 2005; Jendza et al., 2009).

1.5.5 Factors Affecting Phytase Efficacy

Despite the beneficial effects of phytase in monogastric nutrition, some factors may affect its efficacy in improving the productivity of poultry and swine. These factors may include enzyme-related properties as described previously, or dietary and animal-based factors as discussed below.

1.5.5.1 Diet-Related Factors

Several dietary factors may influence the efficacy of phytase, but the magnitude of effect may vary among these factors. The type and amount of phytate in the diet could influence the efficacy of phytase. Some studies have reported differences in the hydrolyzing effect of phytase when exposed to phytate from different plant materials such as canola meal, soybean meal, sunflower meal, or rice bran (Leske and Coon, 1999). These differences have been attributed to the chemical composition, concentration, location of phytate, and contribution of endogenous phytase in grains and oilseeds (Dersjant-Li et al., 2015). The concentration of phytate substrate as compared with dosage of phytase included in diets may also affect the efficacy of phytase as the substrate to enzyme ratio may be too small or wide. Some studies have reported reduced effects of phytase when the phytate concentrations of diets were increased and phytase dose kept constant (Cabahug et al.,

1999; Ravindran et al., 2000) while an increased effect of phytase was observed when phytate was kept constant and phytase dose increased up to extreme levels (Shirley and Edwards, 2003).

The ratio of Ca to P and the level of IP in diets have been known to influence the efficacy of phytase. Since phytate is known to bind Ca and other nutrients in the intestinal tract, an excess of Ca in the diet reduces the solubility of phytate hereby reducing the access of phytase to the complex and decreasing its efficacy (Plumstead et al., 2008; Dersjant-Li et al., 2015). In addition, the particle size of limestone, which is a major source of Ca, has been reported to influence the efficacy of phytase due to the increased solubility of Ca in finely ground limestone (Manangi and Coon, 2007). Similarly, the presence of sufficient levels of IP in diets may reduce the efficacy of phytase in improving P utilization as compared with feeding low-P diets since the P requirement of the animal is sufficiently met by the IP source (Zaefarian et al., 2013). In addition, the length of feeding low-P diets has been reported to influence the efficacy of phytase in broiler chickens. Birds are able to adapt to the P deficiency in diets by manipulating their P homeostasis thus reducing the need for phytase to release P for use (Babatunde et al., 2019a,b). Furthermore, the presence of other nutrients such as excess zinc (Augspurger et al., 2004), organic acids (Boling et al., 2000b), Na levels, and dietary electrolyte balance levels (Ravindran et al., 2008) may impact the efficacy of phytase. Lastly, the combination of phytase with other enzymes such as carbohydrases and proteases has sometimes resulted in synergistic effects on growth performance and nutrient utilization of broiler chickens and pigs (Olukosi et al., 2007; Woyengo and Nyachoti, 2011).

1.5.5.2 Animal-Related Factors

Although phytase efficacy is mostly affected by dietary factors, it has been reported that the age of animals or the specie could also affect phytase efficacy. Babatunde et al. (2019a,b) observed that younger broiler chickens within the first 2 weeks were able to utilize phytase more efficiently

in releasing P and other nutrients as compared with older birds. Similar age effects of phytase were also reported between pigs in the nursery and grow-finish phases (Cambria-Lopez et al., 2020). Different species of animals also utilize phytase at varying degrees and this might be attributed to the various intrinsic qualities of the animal. Regardless, phytase increases the utilization of P and other nutrients in most animal species including poultry, swine, fishes, and ruminants (Kincaid et al., 2005; Kumar et al., 2012; Adeola, 2018; Blavi et al., 2019). The location of phytase activity in the gastrointestinal tract has been reported to influence its efficacy (Dersjant-Li et al., 2015). In broiler chickens and pigs, the stomach and upper gastrointestinal tract are the site of highest exogenous phytase activity due to the favorability of the acidic environment which allows the release of nutrients before absorption occurs in the SI (Yi and Kornegay, 1996; Marounek et al., 2010). However, in the absence of exogenous enzymes, endogenous phytases have been reported to be more active in the colon and caecum due to increased microbial activity in the LI (Pagano et al., 2007; Marounek et al., 2010). This causes a release of soluble P in the hindgut that is not well absorbed by animals but lost in the manure (Seynaeve et al., 2000).

1.5.6 Super-Dosing Effects of Phytase

Under ideal conditions, phytase should completely hydrolyze the phytate complex in the gut and release all bound P and myoinositol for use by the animal. However, studies have shown that this does not occur when traditional doses of 500 or 1,000 phytase units/kg diet are included in diets for poultry or swine. At these traditional doses, there is more release of the lower esters of phytate in addition to P in the gut of animals (Bello et al., 2019). When high doses of phytase ($\geq$ 2,000 phytase units/kg) have been included in the diets of poultry or pigs, there has been an increase in the release of P as well as other nutrients resulting in greater performance and productivity (Shirley and Edwards, 2003; Taheri and Taherkhani, 2015). This indicates that there is a dose-response

relationship with phytase supplementation as increased inclusion of phytase in animal diets consistently increases the response of animals in terms of growth performance and nutrient utilization (Ravindran et al., 2000; Babatunde et al 2019a). This observation is sometimes termed the super-dosing effect of phytase and seems to work in tandem with the extra-phosphoric effects of phytase. Previous studies report an increase in the utilization of AA and other nutrients with increasing phytase doses (Cowieson et al., 2011; Walk et al., 2013). Possible mechanisms for the super-dosing effects of phytase include an increased destruction of the phytate complex which releases more P for use by the animal. The increased release of P could restore the Ca to P ratio and resulting in the increased absorbance of both nutrients in the gut. Furthermore, the total degradation of the phytate complex releases myoinositol which has been reported to have insulin mimetic properties resulting in the modulation of glucose homeostasis (Cowieson et al., 2013). Myoinositol is also involved in cellular and lipid signaling; osmolarity in the tissues of the brain and kidney medulla; as well as supporting the health of animals (Gonzalez-Uarquin et al., 2020). Some studies have reported increased performance and bone stability when myoinositol was supplemented in animal diets (Zyla et al., 2012; Lee et al., 2017). Therefore, if phytase can completely degrade phytate into P and myoinositol then the combined benefits of the metabolism of both nutrients may explain some of the super-dosing effects of phytase observed in monogastric nutrition. However, super-dosing phytase in laying hens has been reported to negatively affect growth performance and egg production probably because layer diets contain high levels of Ca. The further release of Ca by high doses of phytase could tip the Ca: P ratio and hinder the utilization of both nutrients by birds (Skrivan et al., 2018).

1.6 Future Considerations on Phosphorus, Phytate, and Phytase Utilization

Although the area of P, phytate and phytase utilization has been well explored in monogastric nutrition, there are still several areas that require considerations and further research as the demand for sustainable practices in animal husbandry keep increasing. To reduce the loss of P to the environment, research that explores the formulation of diets that meet the exact requirements of animals as well as interventions that increase digestive efficiency need to be carried out.

1.6.1 Basal Endogenous Loss of Phosphorus

The accurate determination of P digestibility in feed ingredients for animals is the cornerstone for estimating P requirements and formulating diets to meet requirement in animals. One method for determining P digestibility in animals is by collecting digesta from the ileum after euthanizing poultry or through a surgically fitted T-canula in pigs. The use of the index method is a common way to determine apparent ileal digestibility (AID) and it requires the use of index markers. The equation for calculating the AID of a nutrient such as P and as described by Adeola (2001) is as follows

$$\text{AID of P, \%} = 100 - [(CR_I/CR_O) \times (P_O/P_I) \times 100]$$

where CR_i and CR_o are the concentration of Cr (g/kg DM) in diet and ileal digesta, respectively; P_i and P_o are the concentration of P (g/kg DM) in diet and ileal digesta respectively. However, the standardized ileal digestibility (SID) of nutrients have been considered more accurate than the AID in determining digestibility of nutrients because it puts into consideration the basal endogenous loss (BEL) of nutrients (Adeola et al., 2016). The BEL of nutrients is that amount of nutrient that is synthesized and secreted endogenously by the animal but not reabsorbed, thus, it is lost through the gastrointestinal tract. The BEL of nutrients in animals can be determined using several methods

including feeding a nutrient-free diet, regression method or isotope marked techniques (Adeola et al., 2016). When the BEL of P is determined, it becomes easier to calculate the SID of P using the following equations

$$BEL = (CR_I/CR_O) \times Po;$$

$$\text{SID of P, \%} = \text{AID of P} + (100 \times BEL/Pi),$$

Where the parameters CR_i, CR_o, P_i, and P_o are as described above. Considerable research has been carried out on the BEL and SID of AA in both poultry and swine with several reviews in the literature on the subject (Nyachoti et al., 1997; Adeola et al., 2016; Ravindran, 2021). However, the same cannot be said for the determination of BEL or SID of P in pigs and poultry. Some work in this regard has been done with swine nutrition as the use of STTD of P in feed ingredients has become common practice during the formulation of diets (Petersen and Stein, 2006; NRC, 2012). However, this is not the case in poultry nutrition where the non-phytate P in feed ingredients is more commonly used for formulating diets. Similarly, the methodology for determining SID of P in poultry has been based mostly on the regression method (Dilger and Adeola, 2006). However, this could be prone to errors due to the dependence on the extrapolation to zero on the regression curve or the specificity to a feed ingredient. The utilization of other methods such as feeding a P-free diets to poultry requires further investigation to provide values that will more accurately represent the BEL of P in birds as have been done with the BEL of AA (Osho et al., 2019). It may also be important to investigate factors, such as age, that may influence the BEL of P as have been done previously with the BEL of AA in broiler chickens (Adedokun et al., 2011). Obtaining accurate values for the BEL of P in broiler chickens would be important in determining the SID of P in feed ingredients which would eventually provide values that accurately predict the utilization of P in broiler chickens. This information would

support formulation of appropriate diets for birds and prevent wastage and loss of P into the environment.

1.6.2 Additivity of Digestible Phosphorus

In ensuring that best feeding practices are used for monogastric nutrition, another area of research to explore is the additivity of digestible P in broiler chickens. When formulating diets for poultry or swine, the assumption of additivity is always considered, and it states that the amount of available nutrients in a mixed diet is equal to the sum of available nutrients originating from each individual feed ingredient (Fang et al., 2007). Several studies have investigated and verified the assumption of additivity of AA digestibility in feed ingredients and mixed diets for broiler chickens and pigs (Xue et al., 2014; Osho et al., 2019) but relatively few studies have done the same for P in both species, but particularly in broiler chickens. In swine, the additivity of the STTD of P in mixed diets has been verified in studies carried out by Fang et al. (2007) and She et al. (2018) but there is little or no information on the additivity of SID of P in mixed diets for poultry. It is also of importance to verify the additivity of AID and SID of P in ingredients with different PP levels. Because poultry and swine cannot utilize the PP efficiently in feed ingredients without the aid of exogenous enzymes, the utilization of P in low-PP ingredients such as corn and soybean meal would differ from high-PP ingredients such as canola meal or rice bran. Therefore, it is imperative to verify the assumption of additivity from mixed diets containing both low and high-PP feed ingredients as these are ingredients that comprise practical and commercial poultry or swine diets. Lastly, there is little or no information on the additivity of P digestibility in feed ingredients or mixed diets fed to either broiler chickens or pigs and containing phytase. Considering that phytase is increasingly being included in commercial diets for poultry and swine, it becomes necessary to confirm the assumption of additivity of P digestibility in mixed diets as phytase would influence the utilization of P in both species.

1.6.3 New Generation of Phytases

Since the inception of the first commercially available fungal-derived phytase in 1991, several generations of phytase have been developed over the last 3 decades. Newer generations of phytase are derived from bacteria and have been proven to be more effective than the fungal phytases as a feed additive (Lei et al., 2013). Currently, phytase inclusion in poultry and swine diets is approximately 70 % and the worth of the global phytase industry is projected to rise above $1 billion by 2025 (Acumen Research and Consulting, 2021). There are projections of astronomical increases in the human population and the demand for food over the next 20 years. Therefore, new phytase products that are more efficient than the current products in the market have to be continuously developed to support the increased production of livestock. This raises the need to evaluate the efficacy of these new phytase products and ensuring that efficient and rigorous trials are carried out with poultry and swine. The evaluation process must include factors that will challenge the phytase products and should be carried out over different growth phases. Including varying levels of PP as a challenge factor for new phytase products, particularly from feed ingredients with relatively inaccessible PP such as rice bran (Leske and Coon, 1999), should help evaluate the efficacy of the products. Similarly, information from such studies should produce nutrient matrix values that will support the appropriate formulation of diets for monogastrics while ensuring minimal wastage of nutrients.

1.6.4 Development of Assays for Phytase Activity

Current assays (Engelen et al., 1994) used in the measurement of phytase activity are carried out at a pH of 5.5 which is not reflective of the pH in the upper gastrointestinal tract where optimal phytase activity takes place. New assays that analyze phytase activity at acidic pH (2 to 4) should be developed to aid the appropriate comparison of activity between several available phytase

products. Similarly, current assays measure phytase activity using Na-phytate as the substrate but in practicality, phytase substrates are usually from plant materials fed to either poultry or swine. Therefore, new assays should utilize phytate substrates from plant materials such as soybean meal in determining the activity of phytase (Dersjant-Li et al., 2015).

1.6.5 Time Effects on Phytase Efficacy

Several studies have investigated the efficacy of phytase in improving P utilization in both poultry and swine (Kerr et al., 2010; Blavi et al., 2019; Babatunde et al., 2020). Some studies have also investigated factors that may influence the efficacy of phytase enzymes including age, feeding length, Ca solubility and concentration in poultry and swine (Augspurger et al., 2004; Manangi and Coon, 2007; Babatunde et al., 2019a,b). However, there seems to be a knowledge gap on the time effects of phytase in improving P utilization particularly in swine. Although phytase improves P utilization in pigs during the nursery, growing, and finishing phases (Jendza et al., 2005), it is not clear if the effect of phytase is constant during the period within each phase. Unlike poultry with short growth phases, swine may be fed diets for as long as 6 months before reaching market weight. This indicates that changes in phytase effects may occur over that relatively long period of time. Similarly, the waste from swine production during that period are enormous and if not properly managed could result in environmental damages due to the presence of excess P in the manure. Therefore, it is imperative that within each growth phase, the effects of time on phytase efficacy and phosphorus utilization should be investigated. This information may prove useful if time-dependent initiatives are being considered in reducing the loss of P from commercial swine production while ensuring sustainable agriculture.

1.7 Summary

In summary, this literature review describes the nutrition and utilization of nutrients in monogastrics such as poultry and swine. In addition, information on P utilization in monogastrics are reviewed. The subject of phytate and its attributes are discussed while the history, characteristics, and actions of phytase are outlined using reports from previous studies. Factors that may affect the utilization of phytase are briefly elucidated. Lastly, areas of future considerations in the utilization of P, phytate, and phytase by monogastrics are detailed in this literature.

1.8 Objectives

The objective of this dissertation was to further evaluate the subject of P, phytate, and phytase in the nutrition of broiler chickens and swine. To achieve this aim, 2 studies were carried out to determine the additivity of AID and SID of P in mixed diets containing either corn, soybean meal, or canola meal with or without phytase and fed to broiler chickens. One of these studies also investigated the effects of age on the BEL of P in broiler chickens fed a P-free diet. Furthermore, 2 studies were carried out to investigate the responses of broiler chickens in the starter and grower phases to varying concentrations of PP and a novel consensus bacterial phytase. Lastly, 2 studies were carried out to investigate the time effects of phytase on P utilization in pigs fed low-P diets during the grower and finisher phases.

1.9 References

Acumen Research and Consulting. 2021. Phytase Market Global Size Worth Over $1 Billion By 2025. https://www.acumenresearchandconsulting.com/phytase-market.

Adedokun, S.A., O. Adeola, C. M. Parsons, M. S. Lilburn, and T. J. Applegate. 2011. Factors affecting endogenous amino acid flow in chickens and the need for consistency in methodology. Poult. Sci. 90:1737–1748.

Adedokun, S., A. Owusu-Asiedu, D. Ragland, P. Plumstead, and O. Adeola. 2015. The efficacy of a new 6-phytase obtained from *Buttiauxella spp*. expressed in *Trichoderma reesei* on digestibility of amino acids, energy, and nutrients in pigs fed a diet based on corn, soybean meal, wheat middlings, and corn distillers' dried grains with solubles. J. Anim. Sci. 93:168–175.

Adedokun, S. A., J. S. Sands, and O. Adeola. 2004. Determining the equivalent phosphorus released by an Escherichia coli-derived phytase in broiler chicks 1. Can. J. Anim. Sci. 84:437–444.

Adeola, O. 2001. Digestion and balance techniques in pigs. In: A. J. Lewis, and L. L. Southern, editors, Swine nutrition. 2nd ed. CRC Press, Washington, DC, USA. pp. 903–916.

Adeola, O. 2018. Phytase in starter and grower diets of White Pekin ducks. Poult. Sci. 97 592–598.

Adeola, O., and A. J. Cowieson. 2011. Board-invited Review: Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. J. Anim. Sci. 89:3189–3218.

Adeola, O., P. C. Xue, A. J. Cowieson, and K. M. Ajuwon. 2016. Basal endogenous losses of amino acids in protein nutrition research for swine and poultry. Anim. Feed Sci. Tech. 221:274–283.

Ahmadi, A., A. Saki, M. M. Tabatabaie, S. A. H. Siyar, H. Aliarabi, K. H. Zaboli, and S. Mirzaei. 2007. Effect of different sources of phytase supplementation on the performance and egg quality of laying hens. Proceedings of the 19th Australian Poultry Science Symposium, Sydney, New South Wales, Australia. February 12-14, 2007; pp 76–78.

Andersen, D. 2015. Does hog size impact manure production? The Manure Scoop blog https://www.nationalhogfarmer.com/environment/does-hog-size-impact-manure-production.

Angel, C. R., W. J. Powers, T. J. Applegate, N. M. Tamim, and M. C. Christman. 2005. Influence of phytase on water-soluble phosphorus in poultry and swine manure. J. Environ. Qual. 34:563–571.

Attia, Y. A., S. A. Abdel-Rahman, and E. M. A. Qota. 2002. Effects of microbial phytase with or without cell-wall splitting enzymes on the performance of broilers fed marginal levels of dietary protein and metabolizable energy. Egypt. Poult. Sci. 21:521–547.

Augspurger, N. R., J. D. Spencer, D. M. Webel, and D. H. Baker. 2004. Pharmacological zinc levels reduce the phosphorus-releasing efficacy of phytase in young pigs and chickens. J. Anim. Sci. 82:1732–1739.

Babatunde, O. O., A. J. Cowieson, J. W. Wilson, and O. Adeola. 2019a. Influence of age and duration of feeding low-phosphorus diet on phytase efficacy in broiler chickens during the starter phase. Poult. Sci. 98:2588–2597.

Babatunde, O. O., A. J. Cowieson, J. W. Wilson, and O. Adeola. 2019b. The impact of age and feeding length on phytase efficacy during the starter phase of broiler chickens. Poult. Sci. 98:6742–6750.

Babatunde, O. O., J. A. Jendza, P. Ader, P. Xue, S. A. Adedokun, and O. Adeola. 2020. Response of broiler chickens in the starter and finisher phases to three sources of microbial phytase. Poult. Sci. 99:3997–4008.

Baker, S. D. 2017. Digestive system 4-H animal science lesson plan nutrition level 2, 3. University of Idaho Extension. www.uidaho.edu/extension/4h.

Bello, A., Y. Dersjant-Li, and D. R. Korver. 2019. The efficacy of 2 phytases on inositol phosphate degradation in different segments of the gastrointestinal tract, calcium and phosphorus digestibility, and bone quality of broilers. Poult. Sci. 98:5789–5800.

Bessei, W. 2006. Welfare of broilers: A review. World's Poult. Sci. J. 62: 455–466.

Blavi, L., C. J. Munoz, J. N. Broomhead, and H. H. Stein. 2019. Effects of a novel corn-expressed E. Coli phytase on digestibility of calcium and phosphorous, growth performance, and bone ash in young growing pigs. J. Anim. Sci. 97:3390–3398.

Blue, M. 2021. Ecological impact of chicken farming. Sciencing. https://sciencing.com/ecological-impact-chicken-farming-5005.html.

Boling, S. D., M. W. Douglas, M. L. Johnson, X. Wang, C. M. Parsons, K. W. Koelkebeck, and R. A. Zimmerman. 2000a. The effects of dietary available phosphorus levels on performance of young and older laying hens. Poult. Sci. 79:224–230.

Boling, S. D., D. M. Webel, I. Mavromichalis, C. M. Parsons, and D. H. Baker. 2000b. The effects of citric acid on phytate-phosphorus utilization in young chicks and pigs. J. Anim. Sci. 78:682–689.

Brana, D. V., M. Ellis, E. O. Castaneda, J. S. Sands, and D. H. Baker, 2006. Effect of a novel phytase on growth performance, bone ash, and mineral digestibility in nursery and grower-finisher pigs. J. Anim. Sci. 84:1839–1849.

Cabahug, S., V. Ravindran, P. H. Selle, and W. L. Bryden. 1999. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus contents. I. Effects on bird performance and toe ash. Br. Poult. Sci. 40:660–666.

Cambra-López, M., A. Cerisuelo, P. Ferrer, L. Ródenas, R. Aligué, V. Moset, J. J. Pascual. 2020. Age influence on effectiveness of a novel 3-phytase in barley-wheat based diets for pigs from 12 to 108 kg under commercial conditions. Anim. Feed Sci. Tech. 267:114549.

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

Cervantes, M., R. Gomez, S. Fierro, M. A. Barrera, A. Morales, B. A. Araiza, R. T. Zijlstra, J. Sanchez, and W. C. Sauer. 2011. Ileal digestibility of amino acids, phosphorus, phytate and energy in pigs fed sorghum-based diets supplemented with phytase and pancreatin. J. Anim. Physiol. Anim. Nutr. 95:179–186.

Champagne, E. T., R. M. Rao, J. A. Liuzzo, J. W. Robinson, R. J. Gale, and F. Miller. 1985. The interactions of minerals, proteins, and phytic acid in rice bran. Cereal Chem. 62:231–238.

Cho, R. 2013. Phosphorus: Essential to life—are we running out? State of the planet; News from the Columbia Climate School. https://news.climate.columbia.edu/2013/04/01/phosphorus-essential-to-life-are-we-running-out/.

Cowieson, A. J., and M. R. Bedford. 2009. The effect of phytase and carbohydrase on ileal amino acid digestibility in monogastric diets: Complementary mode of action? World's Poult. Sci. J. 65:609–624.

Cowieson, A. J., A. Ptak, P. Mackowiak, M. Sassek, E. Pruszynska-Oszmalek, K. Zyla, S. Swiatkiewicz, S. Kaczmarek, and D. Jozefiak. 2013. The effect of microbial phytase and myo-inositol on performance and blood biochemistry of broiler chickens fed wheat/corn-based diets. Poult. Sci. 92:2124–2134.

Cowieson, A. J., J. P. Ruckebusch, I. Knap, P. Guggenbuhl, and F. Fru-Nji. 2016. Phytate-free nutrition: A new paradigm in monogastric animal production. Anim. Feed Sci. and Tech. 222:180–189.

Cowieson, A. J., P. Wilcock, and M. R. Bedford. 2011. Super-dosing effects of phytase in poultry and other monogastrics. Worlds. Poult. Sci. J. 67:225–235.

Crea, F., C. De Stefano, D. Milea, and S. Sammartano. 2008. Formation and stability of phytate complexes in solution. Coord. Chem. Rev. 252:1108–1120.

Crenshaw, T. D. 2001. Calcium, phosphorus, vitamin D, and vitamin K in swine nutrition. Swine Nutrition, A. J. Lewis, and L. L. Southern, ed. CRC Press, Boca Raton, FL, USA. pp 187–212.

DeRouchey, J., B. Goodband, M. Tokach, S. Dritz and J. Nelssen. 2009. Digestive system of the pig: Anatomy and function. Proceedings of the 2009 Swine Profitability Conference, Kansas State University; KS, USA; February 3, 2009. www.thepigsite.com/articles/digestive-system-of-the-pig-anatomy-and-function

Dersjant-Li, Y., A. Awati, H. Schulze, and G. Partridge. 2015. Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. J. Sci. Food Agric. 95:878–896.

Dilger, R. N., and O. Adeola. 2006. Estimation of true phosphorus digestibility and endogenous phosphorus loss in growing chicks fed conventional and low-phytate soybean meals. Poult. Sci. 85:661–668.

Drezner, M. 2002. Phosphorus homeostasis and related disorders. In: Principles in Bone Biology. Editors: Bilezikian J., L. Raisz, and G. Rodan. Academic Press; New York, NY. USA. pp. 321–338.

Engelen, A. J., F. C. van der Heeft, P. H. G. Randsdorf, and E. L. C. Smit. 1994. Simple and rapid determination of phytase activity. J. AOAC Int. 77:760–764.

Eto, N, M. Tomita, and M. Hayashi. 2006. NaPi-mediated transcellular permeation in the dominant route in intestinal inorganic phosphate absorption in rats. Drug Metab. Pharmacokinet. 21:217–221.

Fang, R. J., T. J. Li, F. G. Yin, Y. L. Yin, X. F. Kong, K. N. Wang, Z. Yuan, G. Y. Wu, J. H. He, Z. Y. Deng, and M. Z. Fan. 2007. The additivity of true or apparent phosphorus digestibility values in some feed ingredients for growing pigs. Asian-Aust. J. Anim. Sci. 20:1092–1099.

Food and Agriculture Organization (FAO). 2020. Gateway to poultry production and products. http://www.fao.org/poultry-production-products/production/en/.

Food and Agriculture Organization (FAO). 2021. Overview of global pork market developments in 2020. https://www.pig333.com/latest_swine_news/fao-overview-of-global-pork-market-developments-in-2020_17273/.

Gaasbeek, A., and A. E. Meinders. 2005. Hypophosphatemia: an update on its etiology and treatment. Am. J. Med. 118:1094–1101.

Gehring, C. K., M. R. Bedford, and W. A. Dozier. 2013. Extra-phosphoric effects of phytase with and without xylanase in corn-soybean meal-based diets fed to broilers 1. Poult. Sci. 92:979–991.

Gonzalez-Uarquin, F., M. Rodehutscord, and K. Huber. 2020. Myo-inositol: its metabolism and potential implications for poultry nutrition—a review. Poult. Sci. 99:893–905.

Graf, E., and J. W. Eaton. 1990. Antioxidant functions of phytic acid. Free Radical Biol. Med. 8:61–69.

Graf, E., and J. W. Eaton. 1993. Suppression of colonic cancer by dietary phytic acid. Nutr. Cancer, 19:11–19.

Hattenhaur, O., M. Traebert, H. Murer, and J. Biber. 1999. Regulation of small intestinal Na-Pi type IIb cotransporter by dietary phosphate intake. Am. J. Physiol. Gastrointest. Liver Physiol. 277:756–762.

Humer, E., C. Schwarz, and K. Schedle. 2015. Phytate in pig and poultry nutrition. J. Anim. Physiol. Anim. Nutr. 99:605–625.

Igbasan, F. A., K. Manner, G. Miksch, R. Borriss, A. Farouk, and O. Simon. 2000. Comparative studies on the in vitro properties of phytases from various microbial origins. Arch. Anim. Nutr. 53:353–373.

International Union of Biochemists (IUB). 1979. Enzyme nomenclature: Recommendations of the Nomenclature Committee of the International Union of Biochemistry. Academic Press, New York. NY. USA. pp 247.

Jendza, J. A. and O. Adeola. 2009. Water-soluble phosphorus excretion in pigs fed diets supplemented with microbial phytase. Anim. Sci. J. 80:296–304.

Jendza, J. A., R. N. Dilger, S. A. Adedokun, J. S. Sands, and O. Adeola. 2005. Escherichia coli phytase improves growth performance of starter, grower, and finisher pigs fed phosphorus-deficient diets. J. Anim. Sci., 83:1882-1889.

Kerovuo, J., and S. Tynkkynen. 2000. Expression of *Bacillus subtilis* phytase in *Lactobacillus plantarum* 755. Lett. Appl. Microbiol. 30:325–329.

Kerr, B. J., T. E. Weber, P. S. Miller, and L. L. Southern. 2010. Effect of phytase on apparent total tract digestibility phosphorus in corn-soybean meal diets fed to finishing pigs. J. Anim. Sci. 88:238–247.

Kim, J. H., F. M. Pitargue, H. Jung, G. P. Han, H. S. Choi, and D. Y. Kil. 2017. Effect of super dosing phytase on productive performance and egg quality in laying hens. Asian. Austral. J. Anim. Sci. 30:994–998.

Kincaid, R. L., D. K. Garikipati, T. D. Nennich, and J. H. Harrison. 2005. Effect of grain source and exogenous phytase on phosphorus digestibility in dairy cows. J. Dairy Sci. 88:2893–2902.

Komaba, H. and M. Fukagawa. 2016. Phosphate—a poison for humans? Kidney Int. 90:753–763.

Kornegay, E. T. 2001. Digestion of phosphorus and other nutrients : The role of phytases and factors influencing their activity. Enzym. Farm Anim. Nutr.:237–271.

Kumar, V., A. K. Sinha, H. P. S. Makkar, G. and K. Becker. 2010. Dietary roles of phytate and phytase in human nutrition: A review. Food Chem. 120:945–959.

Kumar, V., A. K. Sinha, H. P. S. Makkar, G. De Boeck, and K. Becker. 2012. Phytate and phytase in fish nutrition. J. Anim. Physiol. Anim. Nutr. 96: 335–364.

Lamberg-Allardt, C., and V. Kemi. 2017. Interaction between calcium and phosphorus and the relationship to bone health. In: Clinical aspects of natural and added phosphorus in foods. nutrition and health. Editors; Gutiérrez O., K. Kalantar-Zadeh, and R. Mehrotra. Springer, New York, NY, USA.

Lassen, S. F., J. Breinholt, P. R. Ostergaard, R. Brugger, A. Bischoff, M. Wyss, and C. C. Fuglsang. 2001. Expression, gene cloning, and characterization of five novel phytases from four basidiomycete fungi: *Peniophora lycii*, *Agrocybe pediades*, *Ceriporia sp*., and *Trametes pubescens*. Appl. Environ. Microbiol. 67:4701–4707.

Lauber, J. K., and A. Kinnear. 1979. Eye enlargement in birds induced by dim light. Can. J. Ophthal. 14: 265–269.

Lee, S. A., D. Nagalakshmi, M. V. L. N. Raju, S. V. Rama Rao, and M. R. Bedford. 2017. Effect of phytase superdosing, myo-inositol and available phosphorus concentrations on performance and bone mineralization in broilers. Anim. Nutr. 3:247–251.

Lei, X. G., and J. M. Porres. 2003. Phytase enzymology, applications, and biotechnology. Biotech. Lett. 25:1787–1794.

Lei, X. G., J. D. Weaver, E. Mullaney, A. H. Ullah, and M. J. Azain. 2013. Phytase, a new life for an 'old' enzyme. Annu. Rev. Anim. Biosci. 1:283–309.

Lesjak, M., S. K. S. Srai. 2019. Role of dietary flavonoids in iron homeostasis. Pharmaceuticals (Basel) 12:119.

Leske, K. L., and C. N. Coon. 1999. A bioassay to determine the effect of phytate phosphorus hydrolysis and total phosphorus retention of feed ingredients as determined with broilers and laying hens. Poult. Sci. 78:1151–1157.

Létourneau-Montminy, M. P., C. Jondreville, D. Sauvant, and A. Narcy. 2012. Meta-analysis of phosphorus utilization by growing pigs: effect of dietary phosphorus, calcium and exogenous phytase. Animal 6:1590–1600.

Lind, Y., J. Engman, L. Jorhem, and A. W. Glynn. 1998: Accumulation of cadmium from wheat bran, sugar-beet fibre, carrots and cadmium chloride in the liver and kidneys of mice. Br. J. Nutr. 80:205–211.

Linneen, S. K., R. Mikesell, L. F. Kime, and J. K. Harper. 2005. Swine Production. Agric. Altern. Penn State Extension, PA, USA. https://extension.psu.edu/swine-production.

Lott, J., I. Ockenden, V. Raboy, and G. Batten. 2000. Phytic acid and phosphorus in crop seeds and fruits: A global estimate. Seed Sci. Res. 10:11–33.

Madrid, J., S. Martinez, C. Lopez, and F. Hernandez. 2013. Effect of phytase on nutrient digestibility, mineral utilization and performance in growing pigs. Livest. Sci. 154:144–151.

Manangi, M. K., and C. N. Coon. 2007. The effect of calcium carbonate particle size and solubility on the utilization of phosphorus from phytase for broilers. Int. J. Poult. Sci. 6:85–90.

Marks, J., E. S. Debnam, and R. J. Unwin. 2010. Phosphate homeostasis and the renal-gastrointestinal axis. Am. J. Physiol. Renal Physiol. 299:285–296.

Marounek, M., M. Skrivan, O. Rosero, and O. Rop. 2010. Intestinal and total tract phytate digestibility and phytase activity in the digestive tract of hens fed a wheat–maize–soyabean diet. J. Anim. Feed Sci. 19:430–439.

Marraffa, J. M., A. Hui, and C. M. Stork. 2004. Severe hyperphosphatemia and hypocalcemia following the rectal administration of a phosphate-containing Fleet pediatric enema. Pediatr. Emerg. Care. 20:453–456.

Merriman, L. A., C. L. Walk, M. R. Murphy, C. M. Parsons, and H. H. Stein. 2017. Inclusion of excess dietary calcium in diets for 100- to 130-kg growing pigs reduces feed intake and daily gain if dietary phosphorus is at or below the requirement. J. Anim. Sci. 95:5439–5446.

Miyamoto, K., M. Ito, S. Tatsumi, M. Kuwahata, and H. Segawa. 2007. New aspect of renal phosphate reabsorption: the type IIc sodium-dependent phosphate transporter. Am. J. Nephrol. 27:503–515.

Morales, G. A., F. J. Moyano, and L. Marquez. 2011. In vitro assessment of the effects of phytate and phytase on nitrogen and phosphorus bio accessibility within fish digestive tract. Anim. Feed Sci. Technol. 170:209–221.

Muñoz, J. A., and M. Valiente. 2003. Determination of phytic acid in urine by inductively coupled plasma mass spectrometry. Anal. Chem. 75:6374–6378.

Musapuor, A., J. Pourrez, A. Samie, and H. M. Shahrbabake. 2005. The effect of phytase and different level of dietary calcium and phosphorous on phytate P utilization in laying hens. Int. J. Poult. Sci. 8:560–562.

National Research Council (NRC). 1994. Nutrient requirements of poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC, USA.

National Research Council (NRC). 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC, USA.

Nelson, T. S., J. J. McGillivray, T. R. Shieh, R. J. Wodzinski, and J. H. Ware. 1968. Effect of phytate on the calcium requirement of chicks. Poult. Sci. 47:1985–1989.

Nyachoti, C., C. F. M. de Lange, B. W. McBride, and H. Schulze. 1997. Significance of endogenous gut nitrogen losses in the nutrition of growing pigs: a review. Can. J. Anim. Sci. 77:149–163.

Oatway, L., T. Vasanthan, and J. H. Helm. 2001. Phytic acid. Food Rev. Int. 17:419–431.

Olukosi, O. A., C. Kong, F. Fru-Nji, K. M. Ajuwon, and O. Adeola. 2013. Assessment of a bacterial 6-phytase in the diets of broiler chickens. Poult. Sci. 92:2101–2108.

Olukosi, O. A., J. S. Sands and O. Adeola. 2007. Supplementation of carbohydrases or phytase individually or in combination to diets for weanling and growing-finishing swine. J. Anim. Sci. 85:1702–1711.

Onomi, S., Y. Okazaki, and T. Katayama. 2004. Effect of dietary level of phytic acid on hepatic and serum lipid status in rats fed a high-sucrose diet, Biosci. Biotech. Biochem. 68:1379–1381.

Onyango, E. M., M. R. Bedford, and O. Adeola. 2005. Efficacy of an evolved *Escherichia coli* phytase in diets of broiler chicks. Poult. Sci. 84:248–255.

Osho, S. O., O. O. Babatunde, and O. Adeola. 2019. Additivity of apparent and standardized ileal digestibility of amino acids in wheat, canola meal, and sorghum distillers dried grains with solubles in mixed diets fed to broiler chickens. Poult. Sci. 98:1333–1340.

Oster, M., F. Just, K. Busing, P. Wolf, C. Polley, B. Vollmar, E. Murani, S. Ponsuksili, and K. Wimmers. 2016. Toward improved phosphorus efficiency in monogastrics-interplay of serum, minerals, bone, and immune system after divergent dietary phosphorus supply in swine. Am. J. Physiol. Regul. Integr. Comp. Physiol. 310:917–925.

Pagano, A. R., K. R. Roneker, and X. G. Lei. 2007. Distribution of supplemental *Escherichia coli* AppA2 phytase activity in digesta of various gastrointestinal segments of young pigs. J. Anim. Sci. 85:1444–1452.

Petersen, G. I., C. Pedersen, M. D. Lindemann, and H. H. Stein. 2011. Relative bioavailability of phosphorus in inorganic phosphorus sources fed to growing pigs. J. Anim. Sci. 89:460–466.

Petersen, S.O., S. G. Sommer, F. Beline, C. Burton, J. Dach, J. Y. Dourmad, A. Leip, T. Misselbrook, F. Nicholson, H. D. Poulsen, G. Provolo, P. Sorensen, B. Vinneras, A. Weiske, M. P. Bernal, R. Bohm, C. Juhasz, and R. Mihelic. 2007. Recycling of livestock manure in a whole-farm perspective. Livest. Sci. 3:180–191.

Petersen, G. I., and H. H. Stein. 2006. Novel procedure for estimating endogenous losses and measurement of apparent and true digestibility of phosphorus by growing pigs. J. Anim. Sci. 84:2126–2132.

Plumstead, P. W., A. B. Leytem, R. O. Maguire, J. W. Spears, P. Kwanyuen, and J. Brake. 2008. Interaction of calcium and phytate in broiler diets. 1. Effects on apparent prececal digestibility and retention of phosphorus. Poult. Sci. 87:449–458.

Raboy, V. 2003. Myo-Inositol-1,2,3,4,5,6-hexakisphosphate. Phytochem. 64:1033–1043.

Ravindran, V. 2021. Progress in ileal endogenous amino acid flow research in poultry. J. Anim. Sci. Biotechnol. 12:1-12. https://doi.org/10.1186/s40104-020-00526-2.

Ravindran, V., S. Cabahug, G. Ravindran, P. H. Selle, and W. L. Bryden. 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorous levels: Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. Br. Poult. Sci. 41:193–200.

Ravindran, V., A. J. Cowieson, and P. H. Selle. 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.

Razzaque, M. S. 2011. Phosphate toxicity: new insights into an old problem. Clin. Sci. (Lond.) 120:91–97.

Rickard, S. E., and L. U. Thompson. 1997. Interactions and biological effects of phytic acid. In: Antinutrients and phytochemicals in food. Editors; F. Shahidi. Am. Chem. Soc. Washington, DC, USA. pp. 294–312.

Rodriguez, E., J. M. Porres, Y. Han, and X. G. Lei. 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. Arch. Biochem. Biophys. 365:262–267.

Rutherfurd, S. M., T. K. Chung, P. C. H. Morel, and P. J. Moughan. 2004. Effect of microbial phytase on ileal digestibility of phytate P, total P, and amino acids in a low-P diet for broilers. Poult. Sci. 83:61–68.

Sabbagh, Y., H. Giral, Y. Caldas, M. Levi, S. C. Schiavi. 2011. Intestinal phosphate transport. Adv. Chronic Kidney Dis. 18:85–90.

Saddoris, K. L., J. C, Fleet, and J. S. Radcliffe. 2010. Sodium-dependent phosphate uptake in the jejunum is post-transcriptionally regulated in pigs fed a low-phosphorus diet and is independent of dietary calcium concentration. J. Nutr. 140:731–736.

Sandberg, A. S., M. Brune, N. G. Carlsson, L. Hallberg, E. Skoglund, and L. Rossan-der-Hulthen. 1999. Inositol phosphates with different numbers of phosphate groups influence iron absorption in humans. Am. J. Clin. Nutr. 70:240–246.

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

Segawa, H., I. Kaneko, S. Tamanaka, M. Ito, M. Kuwahata, Y. Inoue, S. Kato, and K. Miyamoto. 2004. Intestinal Na-Pi cotransporter adaptation to dietary Pi content in vitamin D receptor null mice. Am. J. Physiol. Renal Physiol. 287:39–47.

Segawa, H., A. Onitsuka, J. Furutani, I. Kaneko, F. Aranami, N. Matsumoto, Y. Tomoe, M. Kuwahata, M. Ito, M. Matsumoto, M. Li, N. Amizuka, and K. Miyamoto. 2009. Npt2a and Npt2c in mice play distinct and synergistic roles in inorganic phosphate metabolism and skeletal development. Am. J. Physiol. Renal Physiol. 297:671–678.

Selle, P., A. J. Cowieson, N. Cowieson, and V. Ravindran. 2012. Protein–phytate interactions in pig and poultry nutrition: A reappraisal. Nutr. Res. Rev. 25:1–17.

Selle, P. H., A. J. Cowieson, and V. Ravindran. 2009. Consequences of calcium interactions with phytate and phytase for poultry and pigs. Livest. Sci. 124:126–141.

Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Tech. 135:1–41.

Selle, P. H., V. Ravindran, A. Caldwell, and W. L. Bryden. 2000. Phytate and phytase: consequences for protein utilisation. Nutr. Res. Rev. 13:255–278.

Selle, P. H., V. Ravindran, and G. G. Partridge. 2009. Beneficial effects of xylanase and/or phytase inclusions on ileal amino acid digestibility, energy utilisation, mineral retention, and growth performance in wheat-based broiler diets. Anim. Feed Sci. Tech. 153:303–313.

Seynaeve, M., G. Janssens, M. Hesta, C. van Nevel, and R. O. De Wilde. 2000. Effects of dietary Ca/P ratio, P level and microbial phytase supplementation on nutrient digestibilities in growing pigs: precaecal, post-ileal and total tract disappearances of OM, P and Ca. J. Anim. Physiol. Anim. Nutr. 83:36–48

Shabandeh, M. 2021. Global pork production in 2020 and 2021, by country. Statista. https://www.statista.com/statistics/273232/net-pork-production-worldwide-by-country/.

Shamsuddin, A. M. 2002. Anti-cancer function of phytic acid. Int. J. Food Sci. Tech. 37:769–782.

She, Y., Q. Wang, H. H. Stein, L. Liu, D. Li, and S. Zhang. 2018. Additivity of values for phosphorus digestibility in corn, soybean meal, and canola meal in diets fed to growing pigs. Asian-Aust. J. Anim. Sci. 31:1301–1307.

Shirley, R. B., and H. M. Edwards Jr. 2003. Graded levels of phytase past industry standards improves broiler performance. Poult. Sci. 82:671–680.

Skrivan M., M. Englmaierova, and V. Skrivanova. 2018. Negative effect of phytase superdosing in laying hens. Czech J. Anim. Sci. 63:182–187.

Stein, H. H. 2016. Calcium digestibility and requirements for digestible calcium by growing pigs. Proceedings of the 16th Annual Midwest Swine Nutrition Conference. Indianapolis, IN, USA. pp. 57–61.

Taheri, H. R., and S. Taherkhani. 2015. Effect of phytase superdoses and citric acid on growth performance, plasma phosphorus and tibia ash in broilers fed canola meal-based diets severely limited in available phosphorus. Poult. Sci. J. 3:27–36.

Taylor, A. E., M. R. Bedford, S. C. Pace, and H. M. Miller. 2018. The effects of phytase and xylanase supplementation on performance and egg quality in laying hens. Br. Poult. Sci. 59:554–561.

Thompson, L. U. 1993. Potential health benefits and problems associated with antinutrients in foods. Food Res. Int. 26:131–149.

Ulusoy, U., and S. Simsek. 2005. Lead removal by polyacrylamide-bentonite and zeolite composites: Effect of phytic acid immobilization. J. Hazard. Mat. 127:163–171.

Urakawa, I., Y. Yamazaki, T. Shimada, K. Lijima, H. Hasegawa, K. Okawa, T. Fujita, S. Fukumoto, and T. Yamashita. 2006. Klotho converts canonical FGF receptor into a specific receptor for FGF23. Nature. 444:770–774.

Urbano, G., M. Lopez-Jurado, P. Aranda, C. Vidal-Valverde, E. Tenorio, and J. Porres. 2000: The role of phytic acid in legumes: antinutrient or beneficial function? J. Physiol. Biochem. 56:283–294.

Veum, T. L., and J. Liu. 2018. The effect of microbial phytase supplementation of sorghum-canola meal diets with no added inorganic phosphorus on growth performance, apparent total-tract phosphorus, calcium, nitrogen and energy utilization, bone measurements, and serum variables of growing and finishing swine. Livest. Sci. 214:180–188.

Virkki, L. V., J. Biber, H. Murer, and I. C. Forster. 2007. Phosphate transporters: a tale of two solute carrier families. Am. J. Physiol. Renal Physiol. 293:643–654.

Viveros, A., C. Centeno, A. Brenes, R. Canales, and A. Lozano. 2000. Phytase and acid phosphatase activities in plant feedstuffs. J. Agric. Food Chem. 48:4009–4013.

Votterl, J. C., J. Klinsoda, I. Hennig-Pauka, D. Verhovsek, and B. U. Metzler-Zebeli. 2021. Evaluation of serum parameters to predict the dietary intake of calcium and available phosphorus in growing pigs. Transl. Anim. Sci. 5:1-13. doi:10.1093/tas/txab059.

Waldroup, P. W., J. H. Kersey, E. A. Saleh, A. Fritts, F. Yan, H. L. Stillborn, R. C. Crum Jr., and V. Raboy. 2000. Non phytate P requirement and P excretion of broilers chicks fed diets composed of normal or high available phosphate corn with and without microbial phytase. Poult. Sci. 79:1451–1459.

Walk, C. L., M. R. Bedford, T. S. Santos, D. Paiva, J. R. Bradley, H. Wladecki, C. Honaker, A. P. McElroy. 2013. Extra-phosphoric effects of superdoses of a novel microbial phytase. Poult. Sci. 92:719–725.

Wodzinski, R. J., and A. H. J. Ullah. 1996. Phytase. Adv. Appl. Microbiol. 42:263–303.

Woyengo, T. A., and C. M. Nyachoti. 2011. Review: Supplementation of phytase and carbohydrases to diets for poultry. Can. J. Anim. Sci. 91:177–192.

Woyengo, T. A., B. A. Slominski, and R. O. Jones. 2010. Growth performance and nutrient utilization of broiler chickens fed diets supplemented with phytase alone or in combination with citric acid and multi carbohydrase. Poult. Sci. 89:2221–2229.

Woyengo, T. A., and J. Wilson. 2019. Enhancing nutrient utilization in maize for broiler chickens by superdosing phytase. Anim. Feed Sci. Tech. 252:34–40.

Wyss, M., R. Brugger, A. Kronenberger, R. Romy, R. Fimbel, G. Oesterhelt, M. Lehmann, A. P. G. M. Van Loon. 1999. Biochemical characterization of fungal phytases (myo-inositol hexakisphosphate phosphohydrolases): catalytic properties. Appl. Environ. Microbiol. 65: 367–373.

Xue, P. C., D. Ragland, and O. Adeola. 2014. Determination of additivity of apparent and standardized ileal digestibility of amino acids in diets containing multiple protein sources fed to growing pigs. J. Anim. Sci. 92:3937–3944.

Yi, Z., and E. T. Kornegay. 1996. Sites of phytase activity in the gastrointestinal tract of young pigs. Anim. Feed Sci. Tech. 61:361–368.

Yu, S., A. J. Cowieson, C. Gilbert, P. Plumstead, and S. Dalsgaard. 2012. Interactions of phytate and myo-inositol phosphate esters (IP1-5) including IP5isomers with dietary protein and iron and inhibition of pepsin. J. Anim. Sci. 90:1824–1832.

Zaefarian, F., J. F. Romero, and V. Ravindran. 2013. Influence of a microbial phytase on the performance and the utilisation of energy, crude protein and fatty acids of young broilers fed on phosphorus-adequate maize- and wheat-based diets. Br. Poult. Sci. 54:653–660.

Zhang, Z. B., R. R. Marquardt, W. Guenter, J. Cheng, and Z. Han. 2000. Predication of the effect of enzymes on chick performance when added to cereal-based diets: Use of a modified log-linear model. Poult. Sci. 79:1757–1766.

Zhang, D., X. Wang, and Z. Zhou. 2017. Impacts of small-scale industrialized swine farming on local soil, water, and crop qualities in a hilly red soil region of subtropical China. Int. J. Environ. Res. Public Health 14:1524.

Zyla, K., M. Mika, R. Dulinski, S. Swiatkiewicz, J. Koreleski, H. Pustkowiak, and J. Piironen. 2012. Effects of inositol, inositol-generating phytase B applied alone, and in combination with 6-phytase A to phosphorus-deficient diets on laying performance, eggshell quality, yolk cholesterol, and fatty acid deposition in laying hens. Poult. Sci. 91:1915–1927.

Zyla, K., M. Mika, S. Swiatkiewicz, J. Koreleski, and J. Piironen. 2011. Effects of phytase B on laying performance, eggshell quality and on P and Ca balance in laying hens fed P-deficient maize-soybean meal diets. Czech J. Anim. Sci. 56:406–413.

1.10 Figures

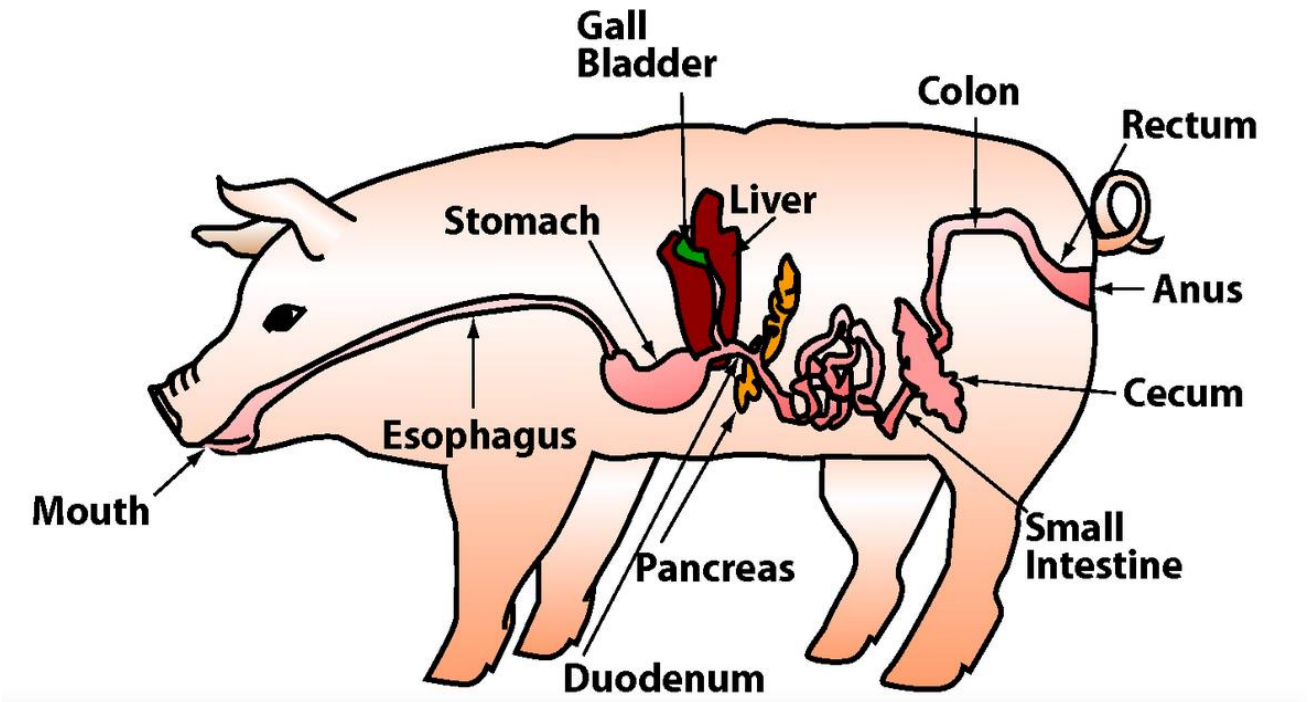


Figure 1-1. The digestive tract of a pig.
Source: Agadventures (https://agadventures.weebly.com/uploads/9/3/6/9/9369630/srlesson1.pdf).

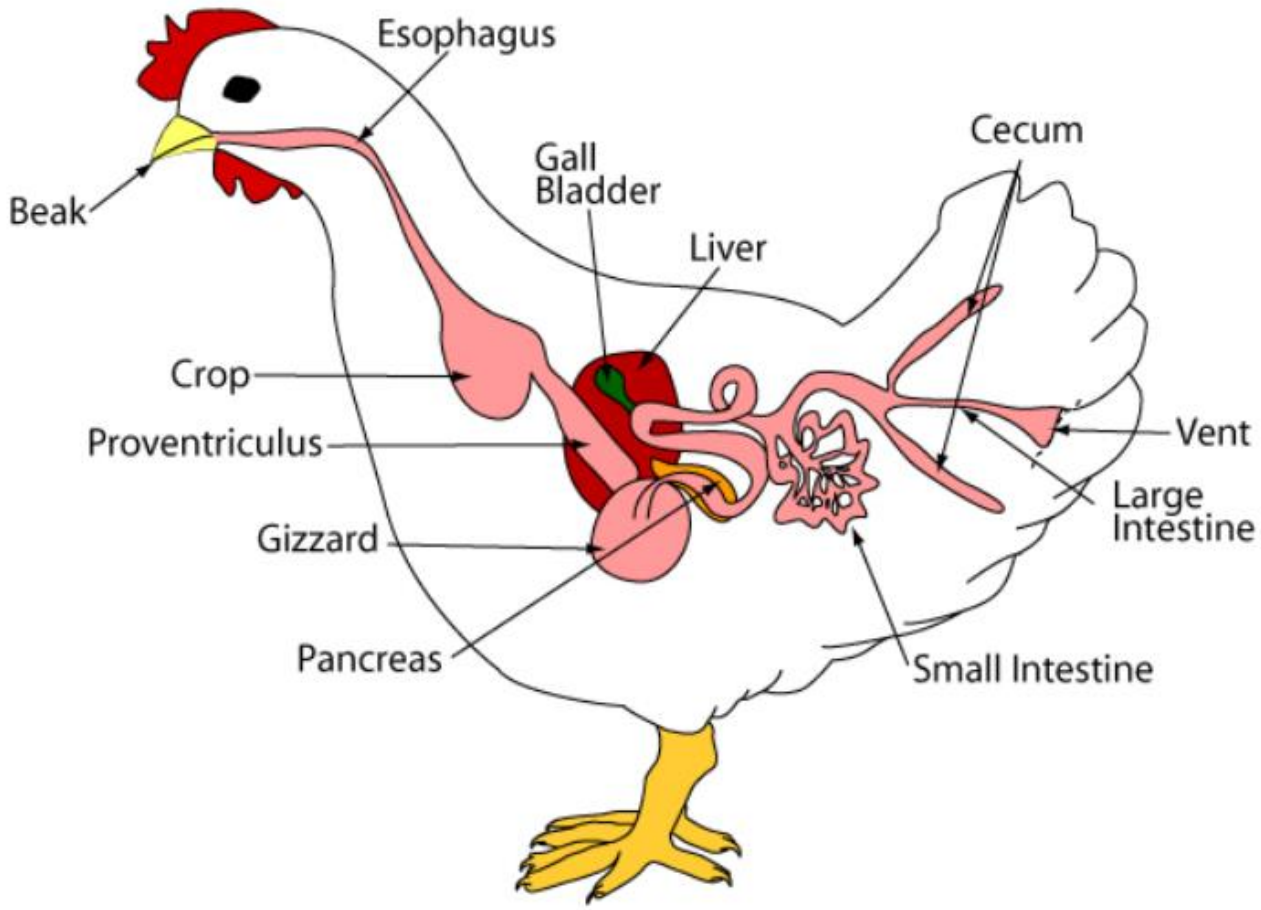


Figure 1-2. The digestive tract of a broiler chicken.
Source: Agadventures (https://agadventures.weebly.com/uploads/9/3/6/9/9369630/srlesson1.pdf).

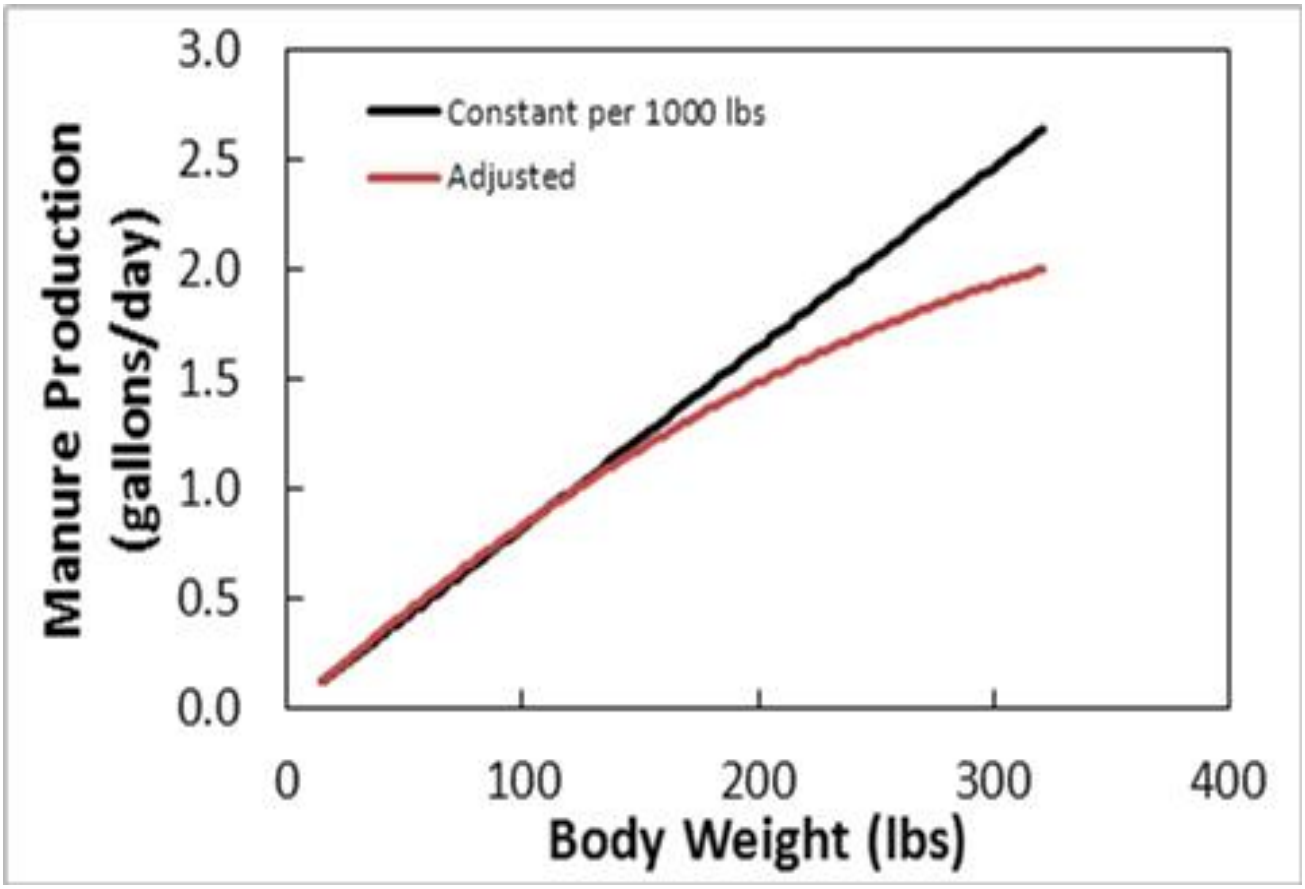


Figure 1-3. Swine manure production as related to body weight of swine.
Source: Anderson (2015).

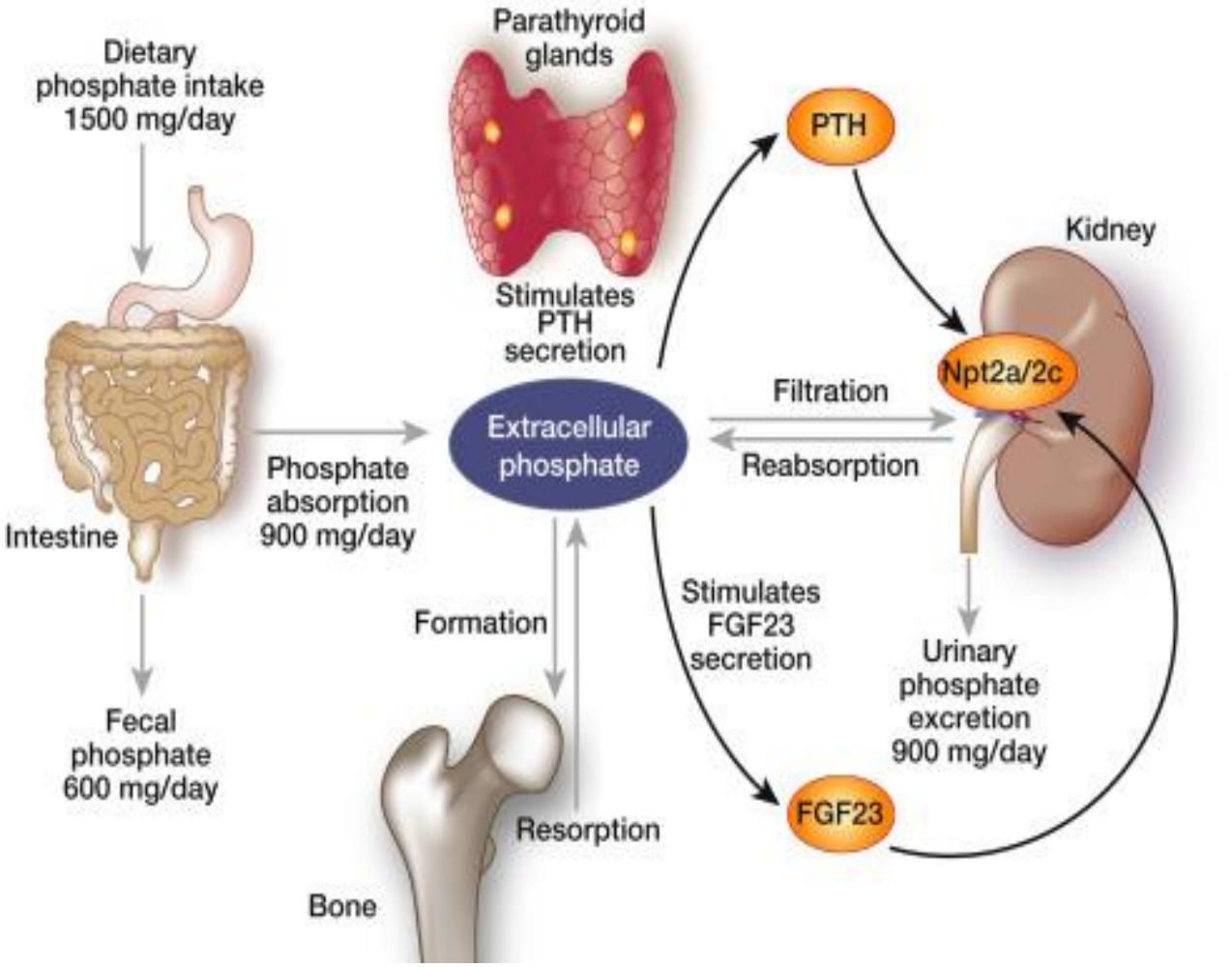


Figure 1-4. Regulation of phosphorus through inter-organ communication in the body. Source: Komaba and Fukagawa (2016).

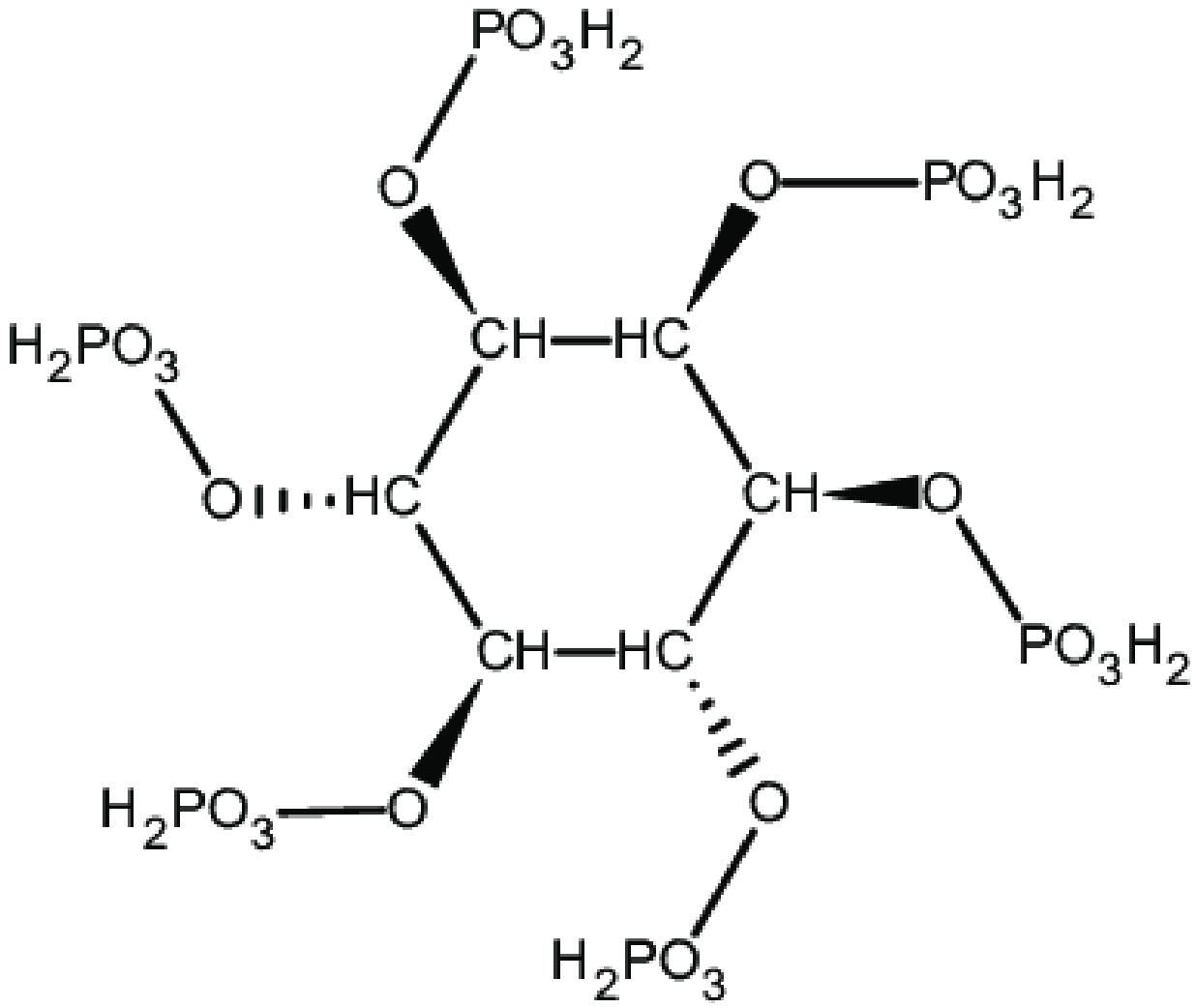


Figure 1-5. Structure of phytate.
Source: Lesjak and Srai (2019).

CHAPTER 2. ADDITIVITY OF APPARENT AND STANDARDIZED ILEAL DIGESTIBILITY OF PHOSPHORUS IN MIXED DIETS CONTAINING CORN AND SOYBEAN MEAL FED TO BROILER CHICKENS

2.1 Abstract

Phosphorus is an integral part of diet formulation for broiler chickens as P is required for various biochemical processes essential to life. A study was designed to examine the additivity of apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of P in mixed diets containing corn and soybean meal (SBM) with or without phytase supplementation. Birds were fed a commercial starter diet from d 0 to 21 post hatching and then allotted to 7 dietary treatments in a randomized complete block design with BW as a blocking factor. Four semi purified diets were prepared to contain corn or SBM as the sole source of P with or without the addition of phytase at 1,000 phytase units/kg of diet. Two mixed diets were also prepared to contain corn and SBM with or without the addition of phytase at 1,000 phytase units/kg diet. A P-free diet (PFD) was formulated to determine the basal ileal endogenous loss of P. There were 16 replicate cages of the PFD and 8 replicate cages of the 6 experimental diets with 8 birds per replicate cage for a total of 512 birds. Diets were fed for 3 d. The ileal digesta of birds was collected from the distal two-thirds of the ileum on d 24 post hatching. The SID of P in corn and SBM were 52.2 and 65.4% respectively (SEM = 1.37). The addition of phytase improved ($P < 0.05$) both the AID and SID of P in the corn, SBM, and mixed diets. The determined AID or SID in corn and SBM with or without phytase were used to predict the AID or SID in the mixed diets. There were no differences between the predicted and determined digestibility values in the mixed diets for either AID or SID of P and thus additive. Phytase supplementation of the mixed diet did not influence the additivity of AID or SID. In

conclusion, the apparent or standardized ileal digestibility of P in corn and soybean meal were additive in the mixed diets containing corn and soybean meal with or without the addition of phytase.

Key words: additivity, corn, digestibility, phosphorus, soybean meal

2.2 Introduction

Phosphorus is one of the important nutrients, which should be considered when formulating diets for broiler chickens. The concentration of non-phytate P has been used to describe the availability of P in feed ingredients as well as the P requirements of broiler chickens (NRC, 1994). However, a portion of the non-phytate P may be unavailable to birds due to the intrinsic characteristics of various feed ingredients such as their phytate content or other anti-nutritional components (Liu et al., 2012). Similarly, a portion of P in phytate may become partially available to broiler chickens due to the limited action of endogenous phytase enzymes. Thus, to prevent the consequent loss of P into the environment due to the overfeeding of P to broiler chickens, it is necessary to accurately determine the digestible P in feed ingredients for broiler chickens.

The basal endogenous loss (BEL) of P is important in measuring the actual digestibility of P in diets either by using the regression method to determine the true ileal digestibility of P as described by Dilger and Adeola (2006), or by feeding a P-free diet (PFD) to determine the standardized P digestibility. Although some studies have reported the BEL of P using the regression method (Dilger and Adeola, 2006; Mutucumarana et al., 2014), there are limited studies estimating the BEL of P in broiler chickens using a PFD. The use of the regression method in determining endogenous losses may be prone to errors due to the specificity to a feed ingredient and the dependence on the extrapolation to zero on the regression curve. Several points on the regression line may be required thus increasing the treatments required and the cost of running experimental trials. Thus, nitrogen free diets are more common in determining the BEL of amino acids in broiler

chickens (Kong and Adeola, 2013; Osho et al., 2019) as compared to the regression method. The ileal BEL of P is important in calculating the standardized ileal digestibility (SID) of P. Ileal digestibility of nutrients may show a more accurate picture of nutrient utilization from broiler diets as compared with total tract utilization due to the action of microbes in the large intestine and the presence of urine in the excreta (Ravindran et al., 1999b). Thus, estimating the SID of P instead of standardized total tract retention may be more useful in formulating diets for broiler chickens.

It is also of importance to verify the assumption of additivity of the apparent ileal digestibility (AID) and SID of P in mixed diets for broiler chickens. In the formulation of diets, it is assumed that the amount of available nutrients in a mixed diet is equal to the sum of available nutrients originating from each of the individual feed ingredients (Fang et al., 2007). However, there is a lack of information that the AID and SID of P measured in feed ingredients are additive in mixed diets for broiler chickens, although previous studies have been reported on the additivity of P digestibility in pigs (Fang et al, 2007; She et al, 2018).

The supplementation of phytase in diets has been known to improve the digestibility of P in broiler chickens (Babatunde et al., 2019a,b), and is now commonly included in diets for broiler chickens. For the appropriate use of phytase in mixed diets, matrix values of phytase have been routinely used when formulating diets (Adeola and Cowieson, 2011), and therefore, a certain value of the available P concentration has been given to phytase even though phytase per se does not contain P at all. Moreover, efficacy of phytase may vary among feed ingredients depending on the concentration of phytate, leading to the changes in matrix values of phytase added into diets consisting of various feed ingredients. Therefore, using digestibility of P elevated by phytase in each feed ingredient when formulating diets may provide the accurate concentration of available P in mixed diets. However, information for the additivity of P digestibility elevated by phytase in mixed diets is scarce. Therefore, the objective of this study was to examine the additivity of AID and SID

of P in mixed diets containing corn and soybean meal (SBM) with or without phytase supplementation. The study was designed to test the null hypothesis that the determined AID or SID of P in mixed diets containing corn and SBM was not different from the predicted values calculated from the AID or SID of P in each feed ingredient regardless of the addition of phytase.

2.3 Materials and Methods

All protocols of animal experiments were reviewed and approved by the Purdue University Animal Care and Use Committee.

2.3.1 Birds, Management, and Experimental Design

Male broiler chicks (Cobb 500, Siloam Springs, AR) were donated by a commercial hatchery at hatch. Birds were tagged with identification numbers and housed inside battery cages equipped with thermostatically controlled heaters (model SB 4T, Alternative Design Manufacturing, Siloam Springs, AR) and located in an environmentally controlled room. Birds had free access to water via water nipples and were fed a commercial starter diet prepared to meet or exceed the nutrient requirements of broiler chicks from d 0 to 21 post hatching (NRC, 1994). On d 21 post hatching, each bird was weighed individually and allotted to experimental diets. The experiment was conducted as a randomized complete block design with birds assigned into 6 experimental diets with 8 replicate cages and a PFD with 16 replicate cages. Each replicate cage contained 8 birds, and BW was used as a blocking factor. Birds were fed experimental diets for 3 days.

2.3.2 Experimental Diets

A PFD was prepared with dextrose and gelatin to estimate the basal ileal endogenous loss (BEL) of P in broiler chickens. Four diets were formulated to contain corn or SBM as the sole source of P with or without the inclusion of phytase at 1,000 phytase units (FYT)/kg diet (Table 1). Two

mixed diets containing corn and SBM with or without the inclusion of phytase at 1,000 FYT/kg were formulated. Phytase (RONOZYME® HiPhos, DSM Nutritional Products, Kaiseragust, Switzerland) premix was prepared with dextrose to contain 50 FYT/g and added at 20 g/kg diets in mash form. The calculated P levels were 0, 26, 30 and 39 g/kg for the PFD, corn, SBM and corn-SBM mixed diets, respectively. The ratio of total Ca to non-phytate P (nPP) was maintained at 2:1. All other mineral contents met or exceeded the requirement estimates of broiler chickens (NRC, 1994). Chromic oxide was incorporated into the diets at 5g/kg as an indigestible marker.

2.3.3 Sampling Procedures and Chemical Analysis

On d 24 post hatching, all birds were euthanized by CO_2 asphyxiation. Ileal digesta was flushed from the distal two thirds of the ileum with distilled water into plastic containers, pooled by cage, and stored in -20^{o}C. Frozen ileal digesta was dried in a forced-air drying oven at 56˚C until constant weight.

Diets and dried ileal digesta were finely ground using an electric coffee grinder and analyzed for DM, CP, GE, Cr, P and Ca concentrations. Dry matter was determined by placing samples in a drying oven for 24 h at 105˚C (Precision Scientific Co., Chicago, IL; method 934.01; AOAC, 2000). Nitrogen contents in ingredients, diets, and ileal digesta samples were determined by the combustion method (TruMac N; LECO Corp., St. Joseph, MI; method 990.03; AOAC, 2000), and the CP content was calculated by multiplying the content of nitrogen by 6.25. Gross energy was determined in diet samples using the isoperibol bomb calorimeter (model 1261; Parr Instrument Co., Moline, IL). Chromium concentrations in the diets and ileal digesta were determined following a wet-ash digestion of samples as previously described by Fenton and Fenton (1979). Phosphorus concentration was determined from digested samples by spectrophotometry (Spark 10 M; Tecan Group Ltd., Männedorf, Switzerland), with absorbance read at 630 nm, while Ca concentrations in

samples were determined by flame atomic absorption spectrometry using a Varian Spectr.AA 220FS (Varian Australia Pty Ltd., Victoria, Australia; Babatunde et al., 2019a).

2.3.4 Calculation and Statistical Analysis

The AID (%), BEL (g/kg DM intake), and SID (%) were calculated using the equations described by Adeola (2001):

$$\text{AID, } = 100 - [(CR_I/CR_O) \times (P_O/P_I) \times 100]$$

$$\text{BEL} = (CR_I/CR_O) \times \text{Po}$$

$$\text{SID} = \text{AID} + (100 \times \text{BEL/Pi}),$$

where CR_i and CR_o are the concentration of Cr (g/kg DM) in diets and ileal digesta, respectively; P_i and P_o are the concentration of P (g/kg DM) in diets and ileal digesta respectively; and the BEL of P was calculated with birds fed the PFD. Data for AID and SID in ingredients and mixed diets were analyzed using the mixed procedure of SAS (SAS Inst. Inc., Cary, NC) with diet, phytase, and their interaction as fixed variables, and block as a random variables. Statistical significance was set at a $P < 0.05$. Cage served as the experimental unit.

The AID or SID of P in corn and SBM determined in semi-purified diets were used for calculating the predicted AID (%) or SID (%) of P in the mixed diets according to the following equation (Xue et al., 2014);

$$AID_P = [(AID_{Corn} \times P_{Corn}) + (AID_{SBM} \times P_{SBM})]/ (P_{corn} + P_{SBM})$$

in which AID_P (%) is the predicted AID of P in the mixed diet; P_{Corn} and P_{SBM} are the concentrations (g/kg) of P contributed by corn and SBM calculated by multiplying the concentration of P (%) in the ingredient by the proportion of the ingredient in the mixed diet; AID_{Corn} and AID_{SBM} are the

determined AID of P (%) in the ingredient. The predicted SID of P (%) in mixed diet was calculated using the same equation except that SID replaced AID.

Differences between determined and predicted AID or SID in the mixed diets were analyzed using a one-sample two-tailed t-test by TTEST procedure of SAS to test the difference from zero as follows:

Hypothesis: H_0: Determined – Predicted = 0

H_1: Determined – Predicted ≠ 0

$P > 0.05$ = Additive

With the predicted being one mean value for each of AID or SID, the difference between determined and predicted AID or SID for each of 8 replicates was calculated as:

Difference = Determined – Predicted

The Difference for each replicate was output into SAS and analyzed using the following Proc TTEST code: Proc ttest h0=0 plots(showh0) sides=u alpha=0.05; var Difference;.

2.4 Results and Discussion

All birds were healthy during the experimental period and readily consumed all the experimental diets except the PFD. No mortality or leg problems were recorded during this period. The average initial BW of birds at d 21 was 789 g. However, after consuming the experimental diets for 3 d, the average BW of birds fed the corn, SBM and mixed diets were 951, 961, and 998 g (with phytase), and 944, 951, and 973 g (without phytase), respectively, while birds fed the PFD lost weight and had an average BW of 740 g. Similarly, the feed intake of birds fed the corn, SBM and mixed diets for 3 d were 331, 271, and 302 g/bird (with phytase) and 318, 281, and 289 g/bird

(without phytase), respectively while the birds fed the PFD had a feed intake of 76 g/bird. This indicated the significant role of P in the metabolism of broiler chickens and negative impacts of a deficiency of P on the growth and development of broiler chickens (Adeola and Walk, 2013). Analyzed composition of DM, CP, P and Ca in the feed ingredients are similar to concentrations reported by the NRC (1994) and Mutucamarana et al., (2015). The concentration of P in the PFD was −0.13 g/kg while the concentration of P and other nutrients in the ingredients and mixed diets were similar to calculated values (Table 2).

There are limited studies that use PFD to determine the BEL of P in broiler chickens. However, the composition of the PFD used in this trial was similar to that fed to broiler chickens (Liu et al., 2012, 2013) and pigs (Petersen and Stein, 2006) with the exception that crystalline amino acids were not included in the PFD used in the current study. The source of CP (i.e., gelatin) in the PFD used in this trial was also different from that used in the study by Mutucumarana and Ravindran (2016) where dried egg albumen served as the main source of CP. The estimated BEL of P in broiler chickens after feeding the PFD for 72 h was 0.166 g/kg DM intake. From previous work in our lab, it was reported that feeding P-deficient diets to broiler chickens over a long period of time could influence the P digestibility as birds are able to adapt to the deficiency by secreting P-containing compounds into the gut (Babatunde et al., 2019a, b). Thus, ileal digesta was collected after 72 h of feeding to estimate the endogenous flow of P in the gut. Although ileal P endogenous loss values are scarce in literature, the value estimated in this trial was lower than that reported by Rutherfurd et al. (2002, 2004) where a range of 260 to 450 mg/kg DM intake was estimated. The greater BEL of P reported in these trials may be a result of the high levels of synthetic amino acids and the absence of Ca in the PFD. The total absence of Ca in their PFD may result in the increased resorption of minerals from the skeleton, thus affecting the metabolism of the birds (Mahan, 1982; Plumstead et al., 2008). Another method that has been used in previous studies to estimate the endogenous loss

of P in broilers is the regression method. However, values from this method are highly variable and could be prone to errors because values are ingredient-specific and involve the extrapolation to zero P intake. Nonetheless, the BEL of P from this trial was similar to values from Dilger and Adeola (2006) where the prececal endogenous P loss was estimated at 176.5 mg/kg DM intake by regression analysis. The BEL of P from the current trial is also slightly greater than the 123 and 109 mg/kg DM intake reported in Liu et al. (2012, 2013). An important difference is that BEL of P were estimated in the excreta after feeding the PFD to broiler chickens for 4 h and collecting the excreta for 52 h. Estimating the BEL of P from the excreta may not give a true picture of the endogenous flow of P due to the activities of phosphorus utilizing bacteria that may be present in the ceca and the presence of urine in the excreta. A study by Borda-Molina et al. (2016) reported the presence of microbes (*Clostridium spp*) in the cecum of broilers with the capacity to produce phytases that could further increase the hydrolysis of phytate for P release. Since there could be reverse peristalsis in the gut of broiler chickens, released P in the ceca may be absorbed by epithelial cells in the small intestine thus, misrepresenting the actual P utilization of the birds. Therefore, it may be pertinent to estimate the endogenous flow of P from the ileum as have been established with amino acids in several studies to prevent the influence of microbes on BEL values (Ravindran et al., 1999; Osho et al., 2019).

Mean values for the ileal DM digestibility in corn and SBM diets were 70.3 and 73.2 % respectively (Table 3). Birds fed the mixed diets had 62.0 and 63.0 % ileal DM digestibility with and without the inclusion of phytase respectively. The AID of P in corn, SBM, and mixed diets were 46.7, 60.9, and 55.8 %, respectively when phytase was not added to the diets. Corn and SBM have low concentrations of P, most of which are bound as phytate complexes (NRC, 2012). Thus, it is not surprising that birds were not able to fully utilize the P present in these feed ingredients. The AID of P in corn and SBM in this trial were lower than values reported by Wu et al. (2004) and Dilger and Adeola (2006), which may be due to cultivar differences in the phytate concentrations. However,

the AID values in the current study were close to values reported by Mutucumurana et al. (2014, 2015). Phytase increases the utilization of P by the hydrolysis of phytate-P complexes present in cereals and oil grains (Dilger et al., 2004; Olukosi et al., 2013). As expected, the addition of phytase in the corn, SBM, and mixed diets improved ($P < 0.01$) the AID of P in birds as compared with the birds fed diets without phytase. A similar trend was observed with Ca digestibility. Birds fed diets without phytase had lower ($P < 0.01$) apparent digestibility of Ca in corn, SBM, and mixed diets as compared with birds fed diets containing phytase.

Standardized ileal digestibility of P has not been widely used in the formulation of diets for broiler chickens as compared with the standardized ileal digestibility of amino acids due to challenges in estimating the BEL of P. More commonly found in literature are values for the true ileal digestibility of P in feed ingredients from regression analysis. However, with increasing concerns for the environmental impact of P excretion and the efforts going towards reducing the environmental footprint of animal production husbandry, it may be relevant to use SID of P values for ingredients when formulating diets for broiler chickens. Similarly, using SID of P values instead of the nPP concentrations in ingredients may also be advantageous, because some portion of the nPP may be unavailable to broiler chickens leading to wastage of dietary P. In pigs, the standardized total tract digestibility of P in feed ingredients has been commonly used for diet formulation (Almeida and Stein, 2010). For an accurate use of SID of P in diet formulation for broiler chickens, further research may be needed to investigate the standardized ileal digestible P requirement of broiler chickens as well as the appropriate methodology to determine the BEL of P in broiler chickens. In the current study, the SID of P in corn, SBM, and mixed diets were determined at 52.2, 65.4 and 59.6 % respectively, without the addition of phytase. Because of the dearth of SID of P values in literature, values from the current studies could not be compared with values from other studies. However, the SID of P values were similar to the standardized P retention in corn, SBM and corn-

SBM-based mixed diets (45, 70, and 53% respectively) reported by Liu et al. (2012), and greater than the true ileal digestibility of P in corn and SBM (34 and 42%, respectively) reported by Trairatapiwan et al. (2018). The differences in the method of estimating the BEL of P and the collection site may explain the disparities in P digestibility values between the current study and other studies. The inclusion of phytase to the corn, SBM, and mixed diets improved ($P < 0.01$) the SID of P by 26, 20 and 23% respectively.

In the current study, there was no difference between the predicted and determined AID or SID of P in the corn-SBM-based mixed diet (Figure 1), which indicated that digestibility values in the mixed diets for either AID or SID are additive. To the best of our knowledge, this is the first to examine the additivity of AID or SID of P in diets for broiler chickens. However, the additivity of P digestibility in feed ingredients has been examined with pigs. In contrast with our study, Fang et al. (2007) observed that AID of P was not additive but the true ileal P digestibility values in feed ingredients containing low concentrations of phytate-P and anti-nutritional factors such as corn and SBM were additive in mixed diets. Differences in the physiology and metabolism of P between pigs and chickens may explain why the differences in findings were observed. In agreement with the current study, She et al. (2018) reported that standardized total tract digestibility of P were additive in mixed diets containing corn, SBM, and canola meal fed to pigs. Zhai and Adeola (2013) also reported additivity for true total tract digestibility of P in mixed diets containing corn and SBM fed to pigs. From these studies, there were indications that P digestibility values that accounted for endogenous losses of P in the gastrointestinal tract were additive in pigs. This may therefore explain why the SID values determined in the current study was additive.

The AID and SID values in the mixed diets containing corn, SBM, and phytase were additive. This study is the first to determine additivity of AID or SID in mixed diets supplemented with phytase and fed to broiler chickens or pigs. Phytase is known to improve the digestibility and

utilization of P in most feed ingredients fed to broiler chickens. However, because of differences in composition and structures of phytate between corn and SBM (Selle and Ravindran, 2007), it was not known if the AID or SID of P in mixed diets containing these ingredients and phytase would be additive. Results from this study revealed that the inclusion of phytase did not affect the additivity of AID or SID of P in mixed diets containing corn and SBM. Whether this holds for mixed diets containing phytate-rich ingredients such as canola meal or rice bran, even in the presence of phytase requires further investigation. Because phytase is more commonly included in commercial broiler diets, care should be taken to not use the AID or SID values in feed ingredients determined without phytase for formulating diets containing phytase as the AID or SID would be underestimated. A more appropriate method is to determine the SID in commonly used feed ingredients with phytase included and use these determined values in the formulation of mixed diets containing phytase for broilers chickens. The knowledge of the P utilization of feed ingredients in the presence of phytase is fundamental and will succor in formulating diets with adequate P levels for broiler chickens while reducing wastage and loss of P to the environment. In conclusion, the apparent or standardized ileal digestibility of P in corn and soybean meal were improved in the presence of phytase. The expected P digestibility in their mixture was not different from the determined digestibility with or without the presence of phytase, indicating additivity of apparent or standardized ileal digestibility of P in mixed diets containing corn and soybean meal fed to broiler chickens.

2.5 References

Adeola, O. 2001. Digestion and balance techniques in pigs. In: A. J. Lewis, and L. L. Southern, editors, Swine nutrition. 2nd ed. CRC Press, Washington, DC. p. 903–916

Adeola, O., and A. J. Cowieson. 2011. Board-invited review: Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. J. Anim. Sci. 89:3189-3218.

Adeola, O., and C. L. Walk. 2013. Linking ileal digestible phosphorus and bone mineralization in broiler chickens fed diets supplemented with phytase and highly soluble calcium. Poult. Sci. 92:2109–2117.

Almeida F. N., and H. H. Stein. 2010. Performance and phosphorus balance of pigs fed diets formulated on the basis of values for standardized total tract digestibility of phosphorus. J. Anim. Sci. 88:2968–2977.

Association of Official Analytical Chemists International. 2000. Official Methods of Analysis. 17th ed. AOAC Int., Gaithersburg, MD.

Babatunde, O. O., A. J. Cowieson, J. W. Wilson, and O. Adeola. 2019a. Influence of age and duration of feeding low-phosphorus diet on phytase efficacy in broiler chickens during the starter phase. Poult. Sci. 98:2588–2597

Babatunde, O. O., A. J. Cowieson, J. W. Wilson, and O. Adeola. 2019b. The impact of age and feeding length on phytase efficacy during the starter phase of broiler chickens. Poult. Sci. 98:6742–6750

Borda-Molina, D., M. Vital, V. Sommerfeld, M. Rodehutscord, and A. Camarinha-Silva. 2016. Insights into broilers' gut microbiota fed with phosphorus, calcium, and phytase supplemented diets. Front Microbiol. 7:2033

Dilger, R. N., and O. Adeola. 2006. Estimation of true phosphorus digestibility and endogenous phosphorus loss in growing chicks fed conventional and low-phytate soybean meals. Poult. Sci. 85:661–668.

Dilger, R. N., E. M. Onyango, J. S. Sands, and O. Adeola. 2004. Evaluation of microbial phytase in broiler diets. Poult. Sci. 83:962–970.

Fang, R. J., T. J. Li1, F. G. Yin, Y. L. Yin, X. F. Kong, K. N. Wang, Z. Yuan, G. Y. Wu, J. H. He, Z. Y. Deng, and M. Z. Fan. 2007. The additivity of true or apparent phosphorus digestibility values in some feed ingredients for growing pigs. Asian-Aust. J. Anim. Sci. 20:1092 - 1099

Fenton, T. W., and M. Fenton. 1979. An improved procedure for the determination of dietary chromic oxide in feed and feces. Can. J. Anim. Sci. 59:631–634.

Kong, C., and O. Adeola. 2013. Additivity of amino acid digestibility in corn and soybean meal for broiler chickens and White Pekin ducks. Poult. Sci. 92:2381–2388.

Liu, S. B., S. F. Li, L. Lu, J. J. Xie, L. Y. Zhang, and X. G. Luo. 2012. Estimation of standardized phosphorus retention for corn, soybean meal, and corn-soybean meal diet in broilers. Poult. Sci. 91:1879–1885.

Liu, S. B., J. J. Xie, L. Lu, S. F. Li, L. Y. Zhang, Y. Jiang, and X. G. Luo. 2013. Estimation of standardized phosphorus retention for inorganic phosphate sources in broilers. J. Anim. Sci. 91:3766–3771

Mahan, D. C. 1982. Dietary calcium and phosphorus levels for weanling swine. J. Anim. Sci. 54:559–564

Mutucumarana, R. K., and V. Ravindran. 2016. Measurement of true ileal phosphorus digestibility in meat and bone meal for broiler chickens using the direct method. Anim. Feed Sci. Tech. 219:249–256

Mutucumarana, R. K., V. Ravindran, G. Ravindran, and A. J. Cowieson. 2014. Measurement of true ileal digestibility of phosphorus in some feed ingredients for broiler chickens. J. Anim. Sci. 92:5520–5529

Mutucumarana, R. K., V. Ravindran, G. Ravindran, and A. J. Cowieson. 2015. Measurement of true ileal phosphorus digestibility in maize and soybean meal for broiler chickens: Comparison of two methodologies. Anim. Feed Sci. Tech. 206:76–86

National Research Council (NRC). 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

National Research Council (NRC). 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Olukosi, O. A., C. Kong, F. Fru-Nji, K. M. Ajuwon, and O. Adeola. 2013. Assessment of a bacterial 6-phytase in the diets of broiler chickens. Poult. Sci. 92:2101–2108.

Osho, S. O., O. O. Babatunde, and O. Adeola. 2019. Additivity of apparent and standardized ileal digestibility of amino acids in wheat, canola meal, and sorghum distillers dried grains with solubles in mixed diets fed to broiler chickens. Poult. Sci. 98:1333–1340

Petersen, G. I., and H. H. Stein. 2006. Novel procedure for estimating endogenous losses and measurement of apparent and true digestibility of phosphorus by growing pigs. J. Anim. Sci. 84:2126–2132.

Plumstead, P., A. Leytem, R. Maguire, J. Spears, P. Kwanyuen, and J. Brake. 2008. Interaction of calcium and phytate in broiler diets. 1. Effects on apparent prececal digestibility and retention of phosphorus. Poult. Sci. 87:449–458

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

Rutherfurd, S. M., T. K. Chung, P. C. H. Morel, and P. J. Moughan. 2004. Effect of microbial phytase on ileal digestibility of phytate phosphorus, total phosphorus, and amino acids in a low-phosphorus diet for broilers. Poult. Sci. 83:61–68.

Rutherfurd, S. M., T. K. Chung, and P. J. Moughan. 2002. The effect of microbial phytase on ileal phosphorus and amino acid digestibility in the broiler chicken. Br. Poult. Sci. 43:598–606.

Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 135:1–41.

She, Y., Q. Wang, H. H. Stein, L. Liu, D. Li, and S. Zhang. 2018. Additivity of values for phosphorus digestibility in corn, soybean meal, and canola meal in diets fed to growing pigs. Asian-Aust. J. Anim. Sci. 31:1301–1307

Trairatapiwan, T., Y. Ruangpanit, O. Songserm, and S. Attamangkune. 2018. Determination of true ileal phosphorus digestibility of maize and soybean meal and true ileal calcium digestibility of soybean meal for broiler chickens. Anim. Prod. Sci. 59:681-687.

Wu, Y. B., V. Ravindran, and W. H. Hendriks. 2004. Influence of exogenous enzyme supplementation on energy utilization and nutrient digestibility of cereal for broilers. J. Sci. Food Agric. 84:1817–1822

Xue, P. C., D. Ragland, and O. Adeola. 2014. Determination of additivity of apparent and standardized ileal digestibility of amino acids in diets containing multiple protein sources fed to growing pigs. J. Anim. Sci. 92:3937–3944

Zhai, H., and O. Adeola. 2013. True total-tract digestibility of phosphorus in corn and soybean meal for fifteen-kilogram pigs are additive in corn–soybean meal diet, J Anim. Sci. 91:219–224

2.6 Tables

Table 2-1. Ingredient composition of experimental diets, g/kg as-fed basis

	Diet[1]						
	0 FYT/kg phytase			1,000 FYT/kg phytase			
Ingredients	Corn	SBM	C+SBM	Corn	SBM	C+SBM	PFD
Ground corn	912.4	-	526.1	912.4	-	526.1	-
Soybean meal	0.0	482.0	386.0	0.0	482.0	386.0	-
Dextrose	20.0	451.7	20.0	-	431.7	-	460.0
Cornstarch	-	-	-	-	-	-	145.0
Gelatin[2]	-	-	-	-	-	-	262.0
Soybean oil	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Cellulose[3]	-	-	-	-	-	-	50.0
Ground limestone	3.6	2.3	3.9	3.6	2.3	3.9	5.5
Salt	4.0	4.0	4.0	4.0	4.0	4.0	-
Potassium carbonate	-	-	-	-	-	-	2.6
Magnesium oxide	-	-	-	-	-	-	2.0
Sodium bicarbonate	-	-	-	-	-	-	7.5
Choline chloride	-	-	-	-	-	-	2.5
Potassium chloride	-	-	-	-	-	-	2.9
Vitamin-mineral premix[4]	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Phytase premix[5]	-	-	-	20.0	20.0	20.0	-
Chromic oxide premix[6]	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Total	1,000	1,000	1,000	1,000	1,000	1,000	1,000

[1]FYT = phytase units; SBM = soybean meal; C+SBM = corn + soybean meal; PFD = phosphorus-free diet.

[2]PB Leiner, Vilvoorde, Belgium.

[3]Solka-floc 40 FCC, International Fiber Corporation, North Tonawanda, NY.

[4]Supplied the following quantities per kg of diet: vitamin A, 5,484 IU; vitamin D_3, 2,643 ICU; vitamin E, 11 IU; menadione sodium bisulfite,4.38 mg; riboflavin, 5.49 mg; $_D$-pantothenic acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B_{12}, 13.2 µg; biotin, 55.2 µg; thiamine mononitrate, 2.2 mg; folic acid, 990 µg; pyridoxine hydrochloride, 3.3mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 µg.

[5]Phytase product contains 2500 phytase unit (RONOZYME® HiPhos, DSM Nutritional Products, Switzerland). Phytase premix was prepared with dextrose to provide 1,000 FYT/kg of diet.

[6]Prepared as 1 g chromic oxide added to 4 g dextrose.

Table 2-2. Analyzed concentration of DM, Gross energy (GE), CP, P, and Ca in experimental diets, g/kg as-fed basis

Item	Diet[1]						
	0 FYT/kg phytase			1,000 FYT/kg phytase			
	Corn	SBM	C+SBM	Corn	SBM	C+SBM	PFD
DM	877	799	869	876	787	865	834
CP	75	228	230	76	229	228	229
GE, kcal/kg	3,946	3,759	4,047	3,924	3,783	4,011	3,811
P	2.67	2.93	3.85	2.62	3.01	3.83	-0.13
Ca	1.96	2.75	3.25	1.98	2.71	3.28	2.68

[1]FYT = phytase units; SBM = soybean meal; C+SBM = corn + soybean meal; PFD = P-free diet.

Table 2-3. Digestibility (%) of nutrients in ingredients and mixed diets[1,2]

	0 FYT/kg phytase			1,000 FYT/kg phytase				*P* value		
Item[3]	Corn	SBM	C+SBM	Corn	SBM	C+SBM	SEM	Diet	Phytase	Diet × Phytase
AID DM	68.9	71.2	63.0	71.7	75.1	62.0	1.46	< 0.01	0.12	0.22
AID Ca	41.0	30.3	42.6	52.8	56.8	58.7	3.15	0.10	< 0.01	0.07
AID P	46.7	60.9	55.8	64.3	78.4	73.5	1.38	< 0.01	< 0.01	1.00
SID P[4]	52.2	65.4	59.6	69.9	82.8	77.3	1.38	< 0.01	< 0.01	1.00

[1]Data are least squares mean of 8 observations.

[2]FYT = phytase units; SBM = soybean meal; C+SBM = corn + soybean meal.

[3]AID = apparent ileal digestibility; SID = standardized ileal digestibility.

[4]For the calculation of SID, values of AID were corrected for the basal endogenous loss of P at 0.166 g/kg

CHAPTER 3. ADDITIVITY OF APPARENT AND STANDARDIZED ILEAL DIGESTIBILITY OF PHOSPHORUS IN MIXED DIETS CONTAINING CORN AND CANOLA MEAL AND BASAL ENDOGENOUS LOSS OF PHOSPHORUS RESPONSES TO PHYTASE AND AGE IN BROILER CHICKENS

3.1 Abstract

The additivity of apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of P in mixed diets containing corn and canola meal (CCM) with or without phytase supplementation and the impact of age on the basal ileal endogenous loss (BEL) of P were investigated in broiler chickens. Treatments were arranged as a 2 × 3 × 2 factorial with 2 ages (d 13 or 21 post hatching), 3 diets (corn, canola meal, or CCM), and 2 phytase levels (0 or 1,000 FYT/kg diet) in a randomized complete block design. There were 8 and 6 birds per cage at d 13 and 21, respectively, and 6 replicate cages per treatment, for a total of 588 birds. A P-free diet (PFD) treatment was included at each age to determine the BEL of P. Birds were fed a commercial starter diet from d 1 to d 10 or 18 and then fed the experimental diets for 3 d until d 13 or 21, respectively. Predicted digestibility values calculated from the individual feed ingredients and the determined values in the mixed diets were used to test additivity in the mixed diets. Chromic oxide was included in diets as an indigestible marker. The ileal digesta, collected from birds at d 13 or 21, was used to determine nutrient digestibility. The AID and SID of P at d 13 was higher ($P < 0.01$) when compared with older birds at d 21 regardless of dietary phytase supplementation. Regardless of age or dietary phytase supplementation, AID and SID of P were additive as there were no differences between predicted and determined values in the mixed diets. The BEL of P (g/kg DM intake) in birds at d 13 was higher ($P < 0.05$) than birds at d 21 (0.197 versus 0.159). In conclusion, age had an impact on the BEL of P and the utilization of minerals in the diets. The

apparent and standardized ileal digestibility of P in the mixed diet containing corn and canola meal were additive regardless of age or phytase supplementation.

Key words: age, additivity, digestibility, mixed diets, phosphorus

3.2 Introduction

Nutritionists consider energy, protein, and P as major factors of economic importance when formulating diets for broiler chickens. Phosphorus plays a major role in several biochemical processes essential to life. Thus, research is always ongoing to better improve its utilization in broiler chickens. One area of concern is the loss of P to the environment from poultry waste, due to the inability of broiler chickens to adequately utilize P present in grains and oilseeds (Adeola and Cowieson 2011). It is therefore of importance to properly understand the dynamics of P utilization in broiler chickens in order to improve efficiency and reduce the loss of P to our soils and aquatic bodies.

The use of non-phytate P (nPP) concentrations of ingredients in the formulation of diets for broiler chickens is common (NRC 1994). However, the use of nPP in diet formulation may be flawed. This is because some of this P may be unavailable for use by broiler chickens due to the intrinsic characteristics of some feed ingredients, and the interactions of P with phytate and other minerals in the gut of broiler chickens (Liu et al. 2012). Therefore, it may be necessary to shift attention to the use of standardized ileal digestibility (SID) as a better tool for estimating P utilization and determining the available P in feed ingredients for broiler chickens.

In order to calculate the SID of P in broiler chickens, there is a need to estimate the basal endogenous loss (BEL) of P in the gastrointestinal tract. Previous work from our lab (Babatunde et al, 2020) estimated the BEL of P in broiler chickens fed corn and soybean meal (SBM) based diets at d 24 post hatching using a P-free diet (PFD). However, Adedokun et al. (2011) has reported

that several factors may influence the estimation of endogenous flow of nutrients in broiler chickens including age of birds and method of estimation. Similarly, in a report by Batal and Parsons (2002), they observed that age had an influence on the utilization of nutrients in broiler chickens, with birds at 2 wk. having an increased utilization of nutrients. Babatunde et al. (2019a) also reported a greater efficiency in the utilization of P and Ca in birds at d 14 as compared to other ages. Nevertheless, due to scarcity of information in literature, we do not know if there is an effect of age on the BEL of P estimated in birds fed a PFD, and if it consequently influences the SID of P in broiler chickens.

Additivity of digestible P is also of importance when formulating mixed diets for broiler chickens as it helps prevent the loss of nutrients. Previous work from our lab (Babatunde et al., 2020) have indicated the additivity of apparent ileal digestibility (AID) and SID of P in broiler chickens fed mixed diets containing corn and SBM with or without phytase supplementation. However, corn and SBM are relatively low in phytate-P (PP). Phytate, a form of P storage in grains and oilseeds, is known to tightly bind P and other nutrients within its structure thus, making them unavailable for use by broiler chickens (Pallauf and Rimbach 1997; Selle et al. 2009). Previous work has established the ability of phytase to hydrolyze phytate and improve the utilization of P and other nutrients in broiler chickens (Dilger et al. 2004; Babatunde et al. 2019a,b). However, it is unclear if the assumption of additivity of P digestibility will be validated in mixed diets containing ingredients such as canola meal (CM) which is high in PP.

Thus, the objective of this study was to investigate the additivity of AID and SID of P in mixed diets containing corn and CM (CCM) with or without the supplementation of phytase at 1,000 FYT/kg diet and to examine the effects of age on the BEL of P in broiler chickens. The study tested the null hypothesis that the predicted AID and SID of P, calculated from the feed ingredients,

were not different from the determined values in the mixed diets with or without the supplementation of phytase, and that there was no impact of age on BEL of P in broiler chickens.

3.3 Materials and Methods

All protocols of animal experiments were reviewed and approved by the Purdue University Animal Care and Use Committee.

3.3.1 Birds and Management

A total of 588 male broiler chicks (Cobb 500, Siloam Springs, AR) was donated from a commercial hatchery at hatch. Birds were tagged with identification numbers and placed in heated battery cages (model SB 4T, Alternative Design Manufacturing, Siloam Springs, AR). Birds were provided water *ad libitum* via water nipples and were fed a commercial starter diet in mash form formulated to contain 13.43 MJ/kg metabolizable energy, 225 g/kg crude protein, 10 g/kg Ca, and 4.6 g/kg nPP. Phytase was not included in the starter diet because all nutrients met or exceeded the nutrient requirements of growing broiler chicks (NRC, 1994) for either 10 or 18 d post hatching. The general health of birds and mortality were monitored daily.

3.3.2 Experimental Design and Diets

This study was a randomized complete block design with 6 dietary treatments and a PFD treatment at each age group. Treatments were arranged as a 2 × 3 × 2 factorial with 2 age groups (d 13 and 21 post hatching), 3 diet types (corn, CM, and CCM), and 2 phytase levels (0 and 1,000 FYT/kg diet). All treatments had 6 replicates cages with either 8 or 6 birds per cage at d 13 or 21 post hatching, respectively. Body weight served as the blocking factor. On d 10 and 18 post hatching, birds were weighed individually, sorted and allotted to dietary treatments, and fed the experimental diets for 3 days until d 13 and 21 post hatching, respectively.

A PFD, prepared with dextrose and gelatin, was used to estimate the BEL of P in broiler chickens as previously described by (Babatunde et al., 2020). Four diets, formulated to contain corn and CM as the sole source of P with or without the inclusion of phytase at 1,000 FYT/kg diet, was used to measure the AID and SID of P in ingredients (Table 1). Digestibility values from these ingredient diets were used to calculate the predicted AID and SID of P in the mixed diets. Two mixed diets containing corn and CM with or without the inclusion of phytase was formulated, and the determined AID and SID of P was estimated. A premix was formulated with phytase (RONOZYME® HiPhos, DSM Nutritional Products, Kaiseragust, Switzerland) and dextrose to contain 50 FYT/g. The premix was included at 20 g/kg to supply 1,000 FYT/kg diet. One FYT is defined as the quantity of enzyme required to liberate 1 µmol of inorganic phosphate/min from 5.0m*M* sodium phytate at pH 5.5 and 37˚C (Engelen at al. 1994). All diets were in mash form. Calcium to nPP ratio was maintained at 2:1, while other mineral content met or exceeded the requirements of broiler chickens (NRC 1994). Chromic oxide was incorporated into the diets at 5g/kg as an indigestible marker.

3.3.3 Sampling Procedures and Chemical Analysis

On d 13 and 21 post hatching, BW of birds and feed intake per cage were measured. All birds were euthanized by CO_2 asphyxiation. Ileal digesta was gently flushed from the distal two thirds of the ileum (section of the intestine between the Merkel's diverticulum and the ileocecal junction) into plastic containers, pooled by cage and stored at −20ºC. Frozen ileal digesta was dried in a forced-air oven at 56˚C. Diets and ileal digesta were finely ground using an electric coffee grinder and analyzed for DM, Cr, P, and Ca concentrations. Dry matter in ingredient, diets and digesta samples were determined using methods described by the AOAC (2000). Chromium concentrations in the diet and digesta samples were determined by spectrophotometry (450 nm;

Spark 10 M; Tecan Group Ltd., Männedorf, Switzerland), following a wet-ash digestion with perchloric and nitric acid (Fenton and Fenton, 1979). Gross energy in the ingredient and diet samples was determined using the isoperibol bomb calorimeter, with benzoic acid as the calibration standard (model 1261; Parr Instrument Co., Moline, IL). Nitrogen concentration in the ingredient and diet samples was determined by the combustion method (TruMac N; LECO Corp., St. Joseph, MI; method 990.03; AOAC, 2000). Multiplying the nitrogen concentration by a factor of 6.25 gave the estimated CP content. Phosphorus and Ca concentrations in the ingredients, diets and ileal digesta samples were determined in methods as previously described by Babatunde et al. (2019a).

3.3.4 Calculation and Statistical Analysis

The AID of P (%), BEL (g/kg DM intake), and SID of P (%) were calculated using the equations described by Adeola (2001):

$$\text{AID of P, \%} = 100 - [(CR_I/CR_O) \times (P_O/P_I) \times 100]$$

$$\text{BEL} = (CR_I/CR_O) \times Po;$$

$$\text{SID of P, \%} = \text{AID of P} + (100 \times \text{BEL}/Pi),$$

where CR_i and CR_o are the concentration of Cr (g/kg DM) in diet and ileal digesta, respectively; P_i and P_o are the concentration of P (g/kg DM) in diet and ileal digesta respectively; and the BEL of P was calculated from birds fed the PFD.

The AID or SID of P in corn and CM determined in the ingredient diets were used for calculating the predicted AID or SID of P (%) in the mixed diets according to the following equation:

$$AID_P\ (\%) = [(AID_{Corn} \times P_{Corn}) + (AID_{CM} \times P_{CM})]/\ (P_{corn} + P_{CM})$$

in which AID_P (%) is the predicted AID of P in the mixed diet; P_{Corn} and P_{CM} are the concentrations (g/kg) of P contributed by corn and CM and were calculated by multiplying the concentration of P (%) in the ingredient by the proportion of the ingredient in the mixed diet; AID_{Corn} and AID_{CM} are the AID (%) of the P-determined ingredient. The predicted SID of P (%) in mixed diet was calculated using the same equation as with AID except that SID replaced AID of P.

Data for AID and SID of P in ingredients and mixed diets were analyzed using the mixed procedure of SAS (SAS Inst. Inc., Cary, NC) with age, diet, and phytase as fixed variables, and block as a random variable. Differences between means of BEL of P at both ages were analyzed using a two sample, two-tailed t-test by TTEST procedure of SAS. Predicted values were subtracted from determined values for each of AID and SID of P in the mixed diets. Differences were analyzed using the TTEST procedure of SAS to test the difference from zero. Statistical significance was set at $P < 0.05$ and cage served as the experimental unit.

3.4 Results and Discussion

Analyzed DM, CP, GE, P, and Ca in the feed ingredients are similar to values reported by NRC (1994). Analyzed nutrients in experimental diets (Table 2) are similar to values reported by NRC (1994) and Akinmusire and Adeola (2009). Birds at both ages were healthy and consumed the experimental diets readily except for the PFD (Table 3). No mortality was recorded in the study.

There was no interaction between age, diet, and phytase on the AID of DM or Ca in broiler chickens (Table 4). Similarly, there was no interaction between age and diet, age and phytase, and diet and phytase on the AID of DM and Ca in broiler chickens. However, there was an effect of diet on the AID of DM in birds regardless of age or inclusion of phytase. Birds fed the corn diet had the highest ($P < 0.01$) AID of DM when compared to birds fed the CM or CCM diets. Canola meal is known to have a higher concentration of fiber when compared with corn or other feed

ingredients (Bell 1993) and may have resulted in the differences observed with DM digestibility. Birds fed the experimental diets at d 13 post hatching had increased AID of Ca as compared to birds fed the diets at d 21 post hatching. Similarly, birds fed the corn diet had a higher ($P < 0.01$) AID of Ca as compared with birds fed diets containing CM. Canola meal contains relatively high concentrations of phytate (Zhou et al. 1990; NRC 1994) when compared with corn. Phytate is known to hinder the solubility of minerals such as P, Ca, Mg, and other divalent ions in the gut thus, affecting their utilization negatively (Cabahug et al. 1999). It was therefore plausible that the digestibility of Ca in the CM and CCM diets would be lower than in the corn diet. Phytase has the ability to hydrolyze phytate bonds and increase the bioavailability of minerals thus, the increased ($P < 0.01$) digestibility of Ca in diets containing phytase was expected and similar to reports from (Olukosi et al. 2013; Babatunde et al. 2019a).

There was no interaction between age, diet, and phytase on the AID and SID of P in broiler chickens. Similarly, there was no interaction between age and diet on the AID and SID of P in broiler chickens. However, there was an interaction ($P < 0.01$) between age and phytase, and between diet and phytase on the AID and SID of P in broiler chickens. Birds at d 13 post hatching had higher ($P < 0.01$) AID and SID of P as compared with birds at d 21 post hatching when fed diets containing phytase but the reverse was the case when fed diets without phytase. This indicated that older birds were able to utilize phytase more efficiently than younger birds when the AID and SID of P in diets with or without phytase supplementation were compared (23.3 versus 20.0 percentage points improvements). Although this observation was in contrast to reports from Babatunde et al. (2019a,b) where the efficacy of phytase was higher in younger birds, the older birds in this case were more sensitive to the low availability of P in the experimental diets without phytase. Birds fed the corn diet had greater ($P < 0.01$) P digestibility than birds fed the CM or CCM diets. Although the P concentration of corn and CM is relatively low, the PP content of corn

(71%) is lower than that of CM (75%). This indicates that broiler chickens may be able to utilize more of P in corn as compared with CM without the intervention of phytase supplementation. Regardless of age, phytase improved the AID and SID of P in the corn, CM and CCM diets by 19.5, 23.2, and 22.1%, respectively. The increased efficacy of phytase in diets containing CM may have been responsible for the interaction observed with diet and phytase on the AID and SID of P. Because the relative amount of PP was higher in CM, a larger substrate pool for phytase in the CM and CCM diets may have been available. It is also possible that the PP in corn, located in the germ, was not as accessible to phytase enzyme as compared with CM which is obtained from canola seeds that has undergone further processing and oil extraction. Thus, phytase may have had easier access to the PP in CM as compared with corn and explaining its improved efficacy. However, it should be noted that differences in the digestibility of P in corn and CM were relatively minor even though they were statistically different in the absence of phytase.

In the current trial, there were no differences between the determined and predicted AID and SID of P in the CCM mixed diets with or without phytase supplementation and at both ages (Table 5). This indicated that regardless of age or phytase inclusion, the AID and SID of P in mixed diets containing corn and CM were additive. In a previous work from our lab (Babatunde et al., 2020), the assumption of additivity of P digestibility was validated in mixed diets containing corn and SBM with or without phytase supplementation. However, it was unknown if the digestible P in mixed diets containing increased levels of PP would be additive. Similarly, considering the negative effects of inorganic P supplementation on PP utilization in broiler chickens, it may have become necessary to test the additivity of P digestibility in mixed diets containing feed ingredients with high PP concentrations and low inorganic mineral supplements. This information would be important when formulating diets, as CM is increasingly being used as an alternative source of CP instead of SBM for broiler chickens (Khajali and Slominski 2012), and industry-type diets contain

a combination of PP and high inorganic mineral concentrations. Thus, understanding the dynamics of P utilization from CM in broiler chickens would help reduce the amount of P wastage to the environment. Similarly, phytase is increasingly being included in diets for broiler chickens and pigs. With the high levels of PP in CM, phytase inclusion in the diet would be key to improving the utilization of nutrients in CM. Information on the additivity of P digestibility in mixed diets containing phytase and fed to broiler chickens is scarce. Thus, it is important to examine the additivity of AID and SID OF P in mixed diets containing CM and phytase, as the expected elevated levels of P with phytase supplementation would influence diet formulation for broiler chickens.

Osho et al. (2019) reported additivity of AA digestibility in mixed diets containing CM, wheat, and sorghum distillers dried grains with solubles fed to broiler chickens, while Hong et al. (2001) reported additivity for metabolizable energy values in mixed diets containing barley and CM fed to ducks. She et al. (2018) reported additivity of standardized total tract digestibility of P in mixed diets containing CCM and CCM + SBM and fed to pigs. However, this study would be the first to examine additivity of AID and SID of P in CCM mixed diets fed to broiler chickens. In agreement with our previous study (Babatunde et al., 2020), the SID of P was additive in the CCM mixed diets with or without phytase supplementation and fed to broiler chickens. Although, She et al. (2018) reported non-additivity with the apparent total tract digestibility of P in the CCM mixed diet fed in pigs, the AID of P in the current trial was additive. They reasoned that the presence of BEL of P or the intrinsic qualities of the feed ingredient may have impacted the additivity of the apparent digestibility of P. Nonetheless, the difference in their observations and the current trial may be attributed to inherent differences between pigs and chickens or may be due to low concentration of endogenous P in comparison to the P content of the test ingredients in the current trial. It was somewhat interesting to observe that there was more precision between the

predicted and determined AID of P values as compared with SID of P. This is because standardized values are known to be of more accuracy than apparent values due to the removal of the influence of BEL on values (Stein et al., 2005). However, we cannot explain why this occurred in the current trial.

As previously noted, there were no studies that tested the additivity of P digestibility in mixed diets containing phytase and fed to either chickens or pigs except from one recent report (Babatunde et al., 2020). In the current trial, the AID and SID of P were additive in the presence of phytase. Although CM contains more P than SBM, most of it is bound as phytate thus, the additional phosphoric advantage it has over SBM becomes useless in the absence of phytase supplementation. However, with the inclusion of phytase, its potential as a P source increases considerably. Thus, SID values from this trial may be used in diet formulation to estimate P utilization when mixed diets containing CM with or without phytase supplementation are fed to broiler chickens. It is also noted that regardless of the age of the birds, the assumption of additivity for AID and SID of P in broiler chickens was valid. However, both ages used in the current trial were in the starter phase and more research may be needed to validate this claim in birds at the finisher phase.

In the current study, the estimated BEL of P in birds fed the PFD for 3 d until d 13 and 21 post hatching were 0.197 and 0.159 g/kg DM intake, respectively, with birds at d 13 post hatching having a higher ($P < 0.05$) BEL of P compared with birds at d 21 post hatching (Figure 1). There are limited studies in the literature that report values for the BEL of P for broiler chickens fed PFD. More commonly available are values estimated using the regression method. This may be due to the challenges associated with feeding a non-physiological diet to broiler chickens. Similarly, the absence of P in the diet makes it difficult for several biological processes to function properly. A study by Babatunde et al. (2019a) reported that feeding P deficient diet to broiler chickens for a

short period may reduce the ability of birds to adapt to the deficiency either by increasing endogenous intestinal phytase activity or by increasing the expression of Na-dependent P transporters in the epithelium (Fang et al. 2012). The BEL of P estimated for birds at d 21 post hatching was similar to reports from previous work in our lab, where birds at d 24 post hatching had an endogenous P flow of 0.166 g/kg DM intake (Babatunde et al., 2020). The endogenous loss of P in birds at both ages were lower than values reported by Dilger and Adeola (2006) in birds at d 22 post hatching and higher than values reported by Liu et al. (2013). The disparities in values reported by these studies and the current trial may have been as a result of differences in collection site or method of estimating the endogenous flow. Adedokun et al. (2011) reported variations in the estimation of endogenous flow of amino acids (AA) due to the methods used. They postulated that values obtained from feeding nitrogen-free diet were usually lower than values obtained using regression or other methods. However, because the review by Adedokun et al. (2011) focused on the BEL of AA in broiler chickens, further studies may be needed to evaluate the effects of collection site or method of estimation on the BEL of P in broiler chickens.

This study may be the first to evaluate the effect of age on BEL of P in broiler chickens. Adedokun et al. (2007a,b) reported an effect of age on the endogenous flow of AA in broiler chickens and turkey poults fed a nitrogen-free diet. They observed that younger birds at d 5 had an increased endogenous flow of AA when compared with older birds at d 15 and 22. Thus, it is possible that younger birds may be more sensitive to deficiencies in nutrients such as P due to the important role P plays during this phase of growth. There may also be an increase in the activities of endogenous phytases and phosphatases in the intestines of younger birds in order to maintain P homeostasis during deficiency. Since all cells contain P in their cell membrane as phospholipids, cells from the walls of the intestines may be degraded to supply P in a state of acute deficiency.

As birds grow older, the intensity by which palliative actions in the gut occur due to the absence of P may reduce. Additional studies may be required to evaluate these speculations.

In conclusion, results from this trial indicates that age influences the basal endogenous loss of P in broiler chickens and may therefore impact the SID of P values that will be used in the formulating of diets. Birds at d 21 utilized minerals and phytase more efficiently in corn, CM and CCM diets as compared with younger birds. The predicted P digestibility in the corn and canola meal mixed diet was not different from the determined digestibility values with or without phytase supplementation and regardless of age. Thus, indicating additivity of corn and canola meal fed to broiler chickens.

3.5 References

Adedokun S. A., O. Adeola, C. M. Parsons, M. S. Lilburn, and T. J. Applegate. 2011. Factors affecting endogenous amino acid flow in chickens and the need for consistency in methodology. Poult. Sci. 90:1737–1748.

Adedokun S. A., M. S. Lilburn, C. M. Parsons, O. Adeola, and T. J. Applegate. 2007a. Effect of age and method on ileal endogenous amino acid flow in turkey poults. Poult. Sci. 86:1948–1954.

Adedokun S. A., C. M. Parsons, M. S. Lilburn, O. Adeola, and T. J. Applegate. 2007b. Endogenous amino acid flow in broiler chicks is affected by the age of birds and method of estimation. Poult. Sci. 86:2590–2597.

Adeola, O. 2001. Digestion and balance techniques in pigs. In: A. J. Lewis, and L. L. Southern, editors, Swine nutrition. 2nd ed. CRC Press, Washington, DC. p. 903–916.

Adeola, O., and A. J. Cowieson. 2011. Board-invited review: Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. J. Anim. Sci. 89:3189–218.

Akinmusire A. S., and O. Adeola. 2009. True digestibility of phosphorus in canola and soybean meals for growing pigs: Influence of microbial phytase. J. Anim. Sci. 87:977–983.

AOAC International. 2000. Official Methods of Analysis. 17th ed. AOAC Int., Gaithersburg, MD.

Babatunde, O. O., A. J. Cowieson, J. W. Wilson, and O. Adeola. 2019a. Influence of age and duration of feeding low-phosphorus diet on phytase efficacy in broiler chickens during the starter phase. Poult. Sci. 98:2588–2597.

Babatunde, O. O., A. J. Cowieson, J. W. Wilson, and O. Adeola. 2019b. The impact of age and feeding length on phytase efficacy during the starter phase of broiler chickens. Poult. Sci. 98:6742–6750.

Babatunde, O. O., S. O. Osho, C. S. Park, and O. Adeola. 2020. Additivity of apparent and standardized ileal digestibility of phosphorus in mixed diets containing corn and soybean meal fed to broiler chickens. Poult. Sci. 99:6907–6913.

Batal, A. B., and C. M. Parsons. 2002. Effects of age on nutrient digestibility in chicks fed different diets. Poult. Sci. 81:400–407.

Bell, J. M. 1993. Factors affecting the nutritional value of canola meal: A review. Can. J. Anim. Sci. 73: 619–697.

Cabahug, S., V. Ravindran, W. L. Bryden, and P. H. Selle. 1999. Response of broilers to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. 1. Effects on broiler performance and toe ash content. Br. Poult. Sci. 40:660–666.

Dilger, R. N., and O. Adeola. 2006. Estimation of true phosphorus digestibility and endogenous phosphorus loss in growing chicks fed conventional and low-phytate soybean meals. Poult. Sci. 85:661–668.

Dilger, R. N., E. M. Onyango, J. S. Sands, and O. Adeola. 2004. Evaluation of microbial phytase in broiler diets. Poult. Sci. 83:962–970.

Engelen, A. J., F. C. van der Heeft, P. H. G. Randsdorf, and E. L. C. Smit. 1994. Simple and rapid determination of phytase activity. J. AOAC Int. 77:760–764.

Fang, R., Z. Xiang, M. Cao, and J. He. 2012. Different phosphate transport in the duodenum and jejunum of chicken response to dietary phosphate adaptation. Asian-Aust. J. Anim. Sci. 25:1457–1465.

Fenton, T. W., and M. Fenton. 1979. An improved procedure for determination of chromic oxide in feed and feces. Can. J. Anim. Sci. 59:631–634.

Hong D., D. Ragland, and O. Adeola. 2001. Additivity and associative effects of metabolizable energy and amino acid digestibility in barley and canola meal for white pekin ducks. Poult. Sci. 80:1600–1606.

Khajali F., and B. A. Slominski. 2012. Factors that affect the nutritive value of canola meal for poultry. Poult. Sci. 91:2564–2575.

Liu, S. B., S. F. Li, L. Lu, J. J. Xie, L. Y. Zhang, and X. G. Luo. 2012. Estimation of standardized phosphorus retention for corn, soybean meal, and corn-soybean meal diet in broilers. Poult. Sci. 91:1879–1885.

Liu, S. B., J. J. Xie, L. Lu, S. F. Li, L. Y. Zhang, Y. Jiang, and X. G. Luo. 2013. Estimation of standardized phosphorus retention for inorganic phosphate sources in broilers. J. Anim. Sci. 91:3766–3771.

NRC. 1994. Nutrient Requirements of Poultry. 9th rev. e. Natl. Acad. Press, Washington, DC.

Olukosi, O. A., C. Kong, F. Fru-Nji, K. M. Ajuwon, and O. Adeola. 2013. Assessment of a bacterial 6-phytase in the diets of broiler chickens. Poult. Sci. 92:2101–2108.

Osho, S. O., O. O. Babatunde, and O. Adeola. 2019. Additivity of apparent and standardized ileal digestibility of amino acids in wheat, canola meal, and sorghum distillers dried grains with solubles in mixed diets fed to broiler chickens. Poult. Sci. 98:1333–1340.

Pallauf, J., and G. Rimbach. 1997. Effect of supplemental phytase on mineral and trace element bioavailability and heavy metal accumulation in pigs with different type diets. In Phytase in Animal Nutrition and Waste Management. M. B. Coehlbo, and E. T. Kornegay. ed. BASF Publication DC9601. BASF Corporation, Mount Olive, NJ. pp 451-466.

Selle, P. H., A. J. Cowieson, and V. Ravindran. 2009. Consequences of calcium interactions with phytate and phytase for poultry and pigs. Livestock Sci. 124:126–141.

She, Y., Q. Wang, H. H. Stein, L. Liu, D. Li, and S. Zhang. 2018. Additivity of values for phosphorus digestibility in corn, soybean meal, and canola meal in diets fed to growing pigs. Asian-Aust. J. Anim. Sci. 31:1301–1307.

Stein, H. H., C. Pedersen, A. R. Wirt, and R. A. Bohlke. 2005. Additivity of values of apparent and standardized ileal digestibility of amino acids in mixed diets fed to growing pigs. J. Anim. Sci. 83:2387–2395.

Zhou, B., Z. He, H. Yu, and K. D. Mukherjee. 1990. Proteins from double-zero rapeseed. J. Agric. Food Chem. 38:690–694.

3.6 Tables & Figure

Table 3-1. Ingredient composition of experimental diets, g/kg as-fed basis

	Diet[1]						
	0 FYT/kg phytase			1,000 FYT/kg phytase			
Ingredients	Corn	CM	CCM	Corn	CM	CCM	PFD
Ground corn	914.4	-	616.2	914.4	-	616.2	-
Canola meal	-	435.0	300.0	-	435.0	300.0	-
Dextrose	20.0	503.0	20.0	-	483.0	-	460.0
Cornstarch	-	-	-	-	-	-	145.0
Gelatin[2]	-	-	-	-	-	-	262.0
Soybean oil	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Cellulose[3]	-	-	-	-	-	-	50.0
Ground limestone	3.6	-	1.8	3.6	-	1.8	7.5
Salt	4.0	4.0	4.0	4.0	4.0	4.0	-
Potassium carbonate	-	-	-	-	-	-	2.6
Magnesium oxide	-	-	-	-	-	-	2.0
Sodium bicarbonate	-	-	-	-	-	-	7.5
Choline chloride	-	-	-	-	-	-	2.5
Potassium chloride	-	-	-	-	-	-	2.9
Vitamin-mineral premix[4]	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Phytase premix[5]	-	-	-	20.0	20.0	20.0	-
Chromic oxide premix[6]	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Total	1,000	1,000	1,000	1,000	1,000	1,000	1,000

[1]FYT = phytase units; CM = canola meal; CCM = corn + canola meal; PFD = phosphorus-free diet.

[2]PB Leiner, Vilvoorde, Belgium.

[3]Solka-floc 40 FCC, International Fiber Corporation, North Tonawanda, NY

[4]Supplied the following quantities per kg of diet: vitamin A, 5,484 IU; vitamin D_3, 2,643 ICU; vitamin E, 11 IU; menadione sodium bisulfite,4.38 mg; riboflavin, 5.49 mg; $_D$-pantothenic acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B_{12}, 13.2 µg; biotin, 55.2 µg; thiamine mononitrate, 2.2 mg; folic acid, 990 µg; pyridoxine hydrochloride, 3.3mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 µg.

[5]Prepared as 1 g chromic oxide added to 4 g dextrose.

[6]Phytase product contains 2500 phytase unit (RONOZYME® HiPhos, DSM Nutritional Products, Switzerland). Phytase premix was prepared with dextrose to provide 1,000 phytase units/kg of diet.

Table 3-2. Analyzed concentration of DM, Gross energy (GE), crude protein, P and Ca in experimental diets, g/kg as-fed basis

	Diet[1]						
	0 FYT/kg phytase			1,000 FYT/kg phytase			
Item	Corn	CM	CCM	Corn	CM	CCM	PFD
DM	835	856	841	836	865	841	866
CP	74	163	164	75	162	163	228
GE, kcal/kg	3,949	3,825	4,192	3,951	3,873	4,155	3,863
P	2.59	5.09	5.23	2.57	5.10	5.26	0.00
Ca	2.02	3.33	3.03	2.12	3.23	3.10	3.03

[1]FYT = phytase units; CM = canola meal; CCM = corn + canola meal; PFD = phosphorus-free diet.

Table 3-3. Growth performance responses of broiler chickens fed ingredient and mixed diets for 3 d at two ages

Age, d	Diet[1]	Phytase, FYT/kg[2]	Initial BW, g	Final BW, g	Feed Intake, g/bird	No. of replicates
13	Corn	0	278	323	82	6
13	Corn	1,000	277	325	84	6
13	CM	0	278	373	100	6
13	CM	1,000	278	375	99	6
13	CCM	0	278	371	112	6
13	CCM	1,000	277	372	116	6
13	PFD		278	259	24	6
21	Corn	0	702	761	188	6
21	Corn	1,000	702	766	194	6
21	CM	0	702	856	213	6
21	CM	1,000	702	868	220	6
21	CCM	0	703	843	221	6
21	CCM	1,000	702	851	207	6
21	PFD		703	627	35	6
SEM[2]			13.34	15.17	8.12	

[1]CM = canola meal; CCM = corn + canola meal; PFD = phosphorus-free diet.
[2] FYT = phytase units; BW = body weight; SEM = standard error of mean.

Table 3-4. Effect of age, diet and phytase on the digestibility (%) of dry matter, Ca and P in ingredients and mixed diets fed to broiler chickens

Age, d	Diet[1]	Phytase, FYT/kg[2]	AID[3] DM	AID Ca	AID P	SID[3] P	No. of replicates
13	Corn	0	78.4	49.4	51.5	57.9	6
13	Corn	1,000	78.7	62.2	68.9	75.3	6
13	CM	0	75.9	43.2	48.0	51.3	6
13	CM	1,000	77.3	57.1	70.1	73.4	6
13	CCM	0	67.3	46.0	49.3	52.5	6
13	CCM	1,000	72.1	60.4	69.8	72.9	6
21	Corn	0	79.7	45.4	47.1	52.2	6
21	Corn	1,000	80.2	57.1	68.7	73.8	6
21	CM	0	77.7	32.4	43.3	46.0	6
21	CM	1,000	76.0	45.1	67.8	70.5	6
21	CCM	0	71.8	36.4	44.9	47.5	6
21	CCM	1,000	72.3	50.1	68.7	71.2	6
13	Corn		78.6	55.8	60.2	66.6	12
13	CM		76.6	50.1	59.1	62.4	12
13	CCM		69.7	53.2	59.6	62.7	12
21	Corn		79.9	51.3	57.9	63.2	12
21	CM		76.8	38.7	55.6	58.3	12
21	CCM		72.1	43.2	56.8	59.4	12
13		0	73.8	46.2	49.6^{B}	53.9^{C}	18
13		1,000	76.1	59.9	69.6^{A}	73.9^{A}	18
21		0	76.4	38.1	45.1^{C}	48.6^{D}	18
21		1,000	76.2	50.8	68.4^{A}	71.9^{B}	18
	Corn	0	79.0	47.4	49.3^{G}	55.1^{H}	12
	Corn	1,000	79.4	59.6	68.8^{F}	74.6^{F}	12
	CM	0	76.8	37.8	45.7^{H}	48.7^{I}	12
	CM	1,000	76.7	51.1	68.9^{F}	72.0^{G}	12
	CCM	0	69.5	41.2	47.1^{H}	50.0^{I}	12
	CCM	1,000	72.2	55.3	69.2^{F}	72.1^{G}	12
13			75.0	53.0	59.6	63.9	36
21			76.3	44.4	56.7	60.3	36
	Corn		79.2^{P}	53.5^{P}	59.0^{P}	64.9^{P}	24
	CM		76.7^{Q}	44.4^{Q}	57.3^{Q}	60.3^{Q}	24
	CCM		70.9^{R}	48.2^{Q}	58.2^{PQ}	61.1^{Q}	24
		0	75.1	42.1	47.4	51.3	36
		1,000	76.1	55.3	69.0	72.9	36
SEM[4]			1.18	2.31	0.57	0.57	

Table 3-4 continued

***P* values**				
Age x Diet x Phytase	0.60	1.00	0.54	0.54
Age x Diet	0.42	0.09	0.35	0.61
Age x Phytase	0.08	0.70	<0.01	<0.01
Diet x Phytase	0.39	0.86	<0.01	<0.01
Age	0.06	<0.01	<0.01	<0.01
Diet	<0.01	<0.01	<0.01	<0.01
Phytase	0.15	<0.01	<0.01	<0.01

[ABCD]Simple effect means within a column with different superscripts differ ($P < 0.05$)
[FGHI]Simple effect means within a column with different superscripts differ ($P < 0.05$)
[PQR]Main effect means of diet within a column with different superscripts differ ($P < 0.05$)
[1]CM = canola meal; CCM = corn + canola meal.
[2] FYT = phytase units.
[3]AID = apparent ileal digestibility; SID = standardized ileal digestibility.
[4]SEM = standard error of mean (for the 3-way interaction).

Table 3-5. Predicted and determined apparent ileal digestibility (AID, %) and standardized ileal digestibility (SID, %) of P in mixed diets containing corn and canola meal with or without phytase supplementation and fed to broiler chickens at two ages

	Phytase,	AID P				SID P			
Age, d	FYT/kg[1]	Predicted[2]	Determined	SEM	P-value	Predicted[2]	Determined	SEM	P-value
13	0	49.2	49.3	0.51	0.40	53.5	52.5	0.51	0.95
	1,000	69.7	69.8	0.25	0.35	74.1	72.9	0.25	0.99
21	0	44.6	44.9	0.38	0.22	48.1	47.5	0.38	0.92
	1,000	68.1	68.7	0.56	0.17	71.6	71.2	0.56	0.74
13		59.6	59.4	0.34	0.37	62.7	63.8	0.34	0.99
21		56.8	56.3	0.40	0.15	59.3	59.8	0.40	0.87
	0	47.1	46.9	0.23	0.18	50.0	50.8	0.23	0.99
	1,000	69.2	68.9	0.26	0.12	72.1	72.8	0.26	0.98

[1]FYT = phytase units; SEM = standard error of mean

[2]Predicted values for the AID and SID of P were calculated by determined values for the AID and SID of P in corn and canola meal respectively.

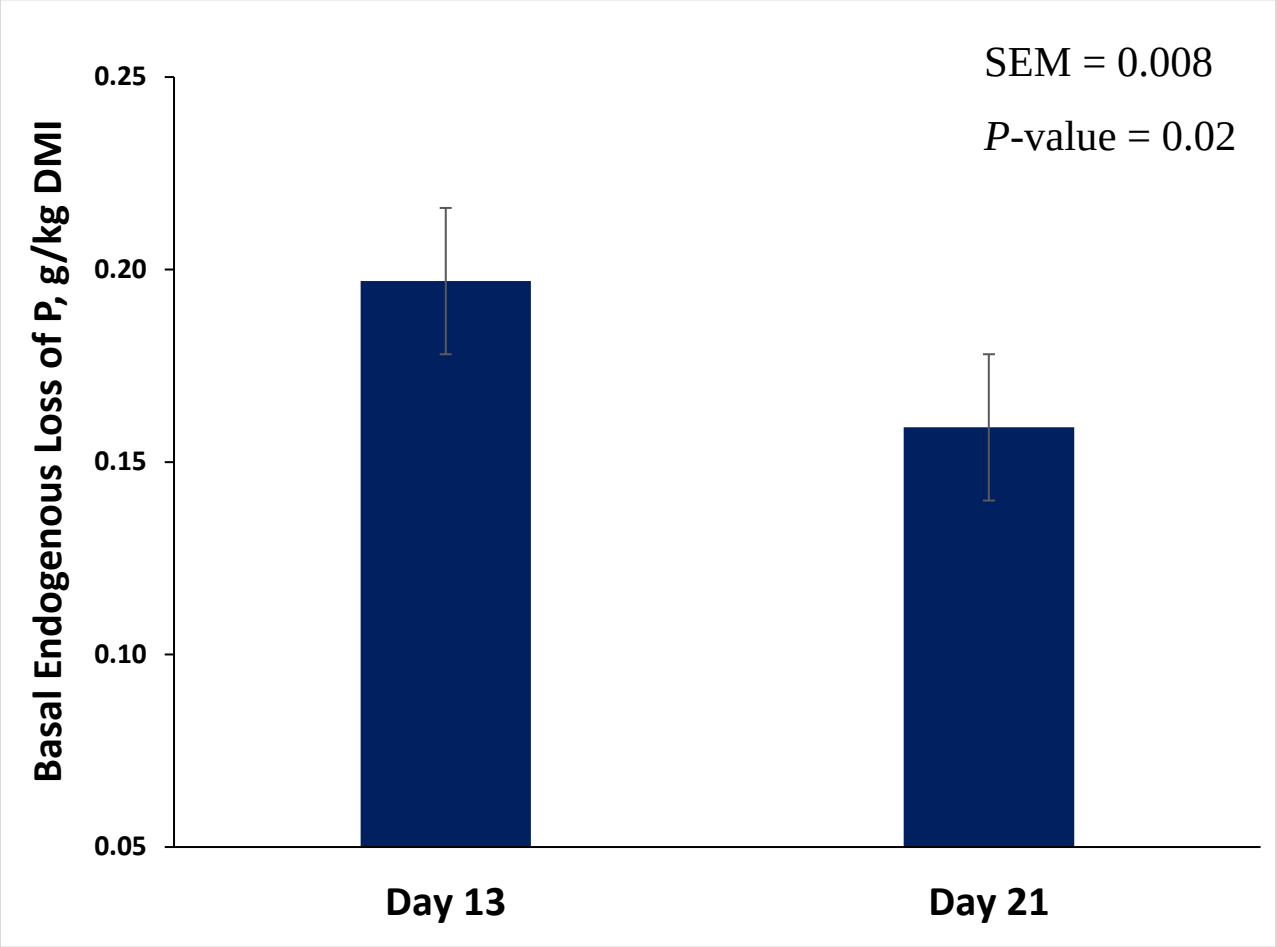


Figure 3-1. Effect of age on the basal ileal endogenous loss of P (g/kg dry matter intake) for broiler chickens fed a P-free diet. Each bar represents the least square means of 6 observations. The basal endogenous loss of P in birds at day 13 and 21 were 0.197 and 0.159 g/kg dry matter intake (DMI), respectively. The standard error of mean (SEM) was 0.008.

CHAPTER 4. EVALUATION OF THE RESPONSES OF BROILER CHICKENS TO VARYING CONCENTRATIONS OF PHYTATE PHOSPHORUS AND PHYTASE. I. STARTER PHASE (DAY 1-11 POST HATCHING)

4.1 Abstract

Growth performance, tibia ash, apparent ileal digestibility (AID), and total tract retention (TTR) of nutrient responses of broiler chickens fed diets containing varying concentrations of phytate P (PP) and a novel consensus bacterial 6-phytase variant (PhyG) from day 1 to 11 post hatching were evaluated with 1,152 broiler chicks. Diets were a nutrient-adequate positive control diet (PC) with 2.8 g PP/kg or one of 15 nutrient-reduced negative control (NC: PC minus 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 g/kg available P, 1.8 g/kg Ca and 0.5 g/kg Na) diets with 3 PP (g/kg) levels, mainly from rice bran, at 2.3 (NC1), 2.8 (NC2), or 3.3 (NC3) and 5 PhyG supplementation at 0, 500, 1,000, 2,000, or 4,000 FYT/kg in a 1 + 3 × 5 factorial. All treatments had 6 replicate cages with 12 birds per cage. Despite comparable PP levels, birds fed the PC diet had greater ($P \leq 0.01$) body weight (BW), feed intake (FI), tibia ash, AID of energy, AA, P, and Ca as compared with birds fed the NC2 without phytase. There was no interaction between PP and phytase for all responses. Increasing PP concentrations linearly decreased ($P < 0.01$) BW, FI, AID and TTR of P and Ca. With phytase supplementation, there was a quadratic response ($P < 0.05$) in BW, FI, tibia ash, and a linear increase ($P < 0.05$) in the AID of energy, nitrogen, and all the measured AA. Increasing phytase dose from 0 to 4,000 FYT/kg increased ($P < 0.01$) AID of P and Ca by 88 and 18%, respectively. There was also a quadratic response ($P \leq 0.05$) on TTR of P and Ca with increasing phytase dose. In conclusion, increasing levels of PP reduced growth performance and most nutrient utilization responses of broiler chickens while phytase

supplementation positively impacted the responses of broiler chickens during day 1 to 11 post hatching.

Key words: broiler chickens, growth performance, nutrient utilization, phytase, phytate

4.2 Introduction

Phosphorus is of utmost importance in broiler chickens due to its role in growth and development. The use of phytase to improve the productivity of broiler chickens through the release of P from feed ingredients has been established by several studies (Coelho and Kornegay, 1999; Adeola and Cowieson, 2011; Babatunde and Adeola, 2021). The efficacy of phytase in broiler chickens can be determined by evaluating the nutrient matrix in birds through responses such as mineral utilization, and bone mineralization. In poultry production, microbial phytases are commonly used instead of fungal phytases due to their proven efficacy as established by several studies (Cabahug et al., 1999; Dilger et al., 2004; Babatunde et al., 2020a). However, the drive for higher production efficiency and environmental sustainability in broiler industry necessitates the need for phytase with improved phosphoric and extra-phosphoric efficacies. Thus, new generation phytases are developed and tested by evaluating their improved efficacy to allow for higher reductions in dietary nutrient.

In most cereals and oilseeds, the majority of P is bound in a complex as phytate P (PP). This inhibits its utilization by broiler chickens, however, phytases hydrolyze the phytate complex and release P and other nutrients for use by the birds (Namkung and Leeson, 1999). By reducing the density of dietary energy, protein and minerals, the efficacy of phytases to degrade phytate and increase nutrient availability to maintain optimum productivity of broiler chickens can be evaluated (Walk and Olukosi, 2019). Phytate is regarded as an antinutritional factor. Thus, several feed ingredients with high PP content such as canola meal or rice bran are not conventionally used

in diets fed to broiler chickens relative to corn and soybean meal in the USA. However, to properly test the efficacy of a new phytase product, it may be important to evaluate it in birds fed diets with varying PP content.

The accessibility of PP to enzymatic hydrolysis is not the same for all feed ingredients. Previous studies have reported differences in the hydrolysis of PP and in the impact of phytase on P utilization from various feed ingredients (Leske and Coon, 1999; Almeida et al., 2017). The PP in rice bran was reported to be the most difficult to hydrolyze even in the presence of phytase when compared with those from other ingredients like canola meal, soybean meal, sunflower meal, or wheat probably due to its high stability (Leske and Coon, 1999; Almeida et al., 2017). Thus, using rice bran as the source of PP should be a more effective test of a new phytase product as compared with other PP sources. In addition, evaluating the phytate-degrading efficiency of phytase in rice bran-containing diets will provide an insight into the phytase`s effectiveness. The high efficacy of phytase to degrade rice bran PP may also encourage the use of ingredients with high PP content in broiler diets, thereby reducing the complete dependence on corn and soybean meal and potentially reducing the variable production cost.

Lastly, it has been observed from previous work in our lab that broiler chickens during the first 2 weeks post hatching were able to utilize P more efficiently than at an older age, and that phytase was more efficacious during this period when mineral digestibility was considered (Babatunde et al., 2019a,b). The starter phase in broiler chickens is characterized by rapid growth and development of tissues, muscles, and bones that form the foundation for the deposition of meat in the latter phases (Batal and Parsons, 2002). Thus, the adequate supply of nutrients including P in this phase is important. Most digestibility studies are carried out with birds at day 21 post hatching with limited studies reporting digestibility in younger broiler chickens. Hence, evaluating

the potency of a new phytase to efficiently degrade rice bran PP during the starter period may give a clear understanding of the efficacy of the phytase in broiler chickens.

Therefore, the objective of this study was to evaluate the effects of PP concentrations from rice bran and varying doses of a novel consensus bacterial phytase variant (PhyG) on broiler chickens in the starter phase (day 1 to 11 post hatching) using growth performance, bone mineralization, apparent ileal digestibility (AID), and total tract retention (TTR) of energy and nutrients as response criteria. This study tested the null hypothesis that there was no effect of PP and phytase concentrations on responses of broiler chickens in the starter phase.

4.3 Materials and Methods

All protocols of animal experiments were reviewed and approved by the Purdue University Animal Care and Use Committee.

4.3.1 Birds and Management

Male Cobb 500 broiler chicks were individually tagged and housed in temperature-controlled battery cages (model SB 4T, Alternative Design Manufacturing, Siloam Springs, AR) located at the poultry unit of the Purdue Animal Science Research and Experimental Station. Birds were given free access to water via water nipples and were fed a commercial starter mash diet formulated to meet or exceed the nutrient requirements of growing broiler chicks (Cobb, 2013) for 1 d as they could not be allocated to dietary treatments at day 0 due to transportation stress. Subsequently, birds were weighed and randomly allotted to treatments and the experimental diets were fed *ad libitum* for 10 d (day 1 to 11 post hatching). Housing temperature and humidity, general well-being of birds, and mortality were observed and recorded daily.

4.3.2 Experimental Design and Diets

This experiment was conducted as a randomized complete block design with dietary treatments arranged as a 1 + 3 × 5 factorial and BW used as the blocking factor. Diets were a nutrient-adequate positive control diet (PC) with 2.8 g PP/kg or one of 15 nutrient-reduced negative control (NC: PC minus 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 g/kg available P, 1.8 g/kg Ca and 0.5 g/kg Na) diets with 3 PP (g/kg) levels, mainly from rice bran, at 2.3 (NC1), 2.8 (NC2), or 3.3 (NC3) and 5 PhyG supplementation at 0, 500, 1,000, 2,000, or 4,000 FYT/kg in a 1 + 3 × 5 factorial. The non-phytate P (nPP) content of the PC and NC diets were 4.3 and 2.3 g/kg, respectively, while the PC contained similar PP content as the NC2 diets. Levels of phytate and phytase used in this trial were those commonly found in commercial or experimental diets. The phytase used in this trial was a novel consensus bacterial 6-phytase variant originating from *Buttiauxella sp.* but expressed in *Trichoderma reesei* (PhyG; Danisco Animal Nutrition, The Netherlands) and screened for a broad pH profile but higher activity in the acidic sections of the gut. Phytase was prepared as a premix with ground corn and included at 50 g/kg of the phytase diets. The PP contents of the NC diets were adjusted through the addition of rice bran and polished rice while soy hulls were used as a filler. Energy and all other nutrients in the PC diet met or exceeded the nutrient requirements of broiler chicks as recommended by Cobb (2013). Titanium dioxide was included into all diets at 5 g/kg as an indigestible marker.

4.3.3 Sample Collection and Chemical Analyses

Excreta was collected twice daily during the last 3 d of the experimental period from pans lined with wax paper and placed under the cages. After collection, excreta was dried in a forced air oven at 56°C for 7 days and stored until analysis. On day 11 post hatching, individual BW of birds and feed intake (FI) per cage were recorded and used to determine the BW gain and gain to

feed ratio (G:F). Furthermore, all birds were euthanized by CO_2 asphyxiation and dissected to collect digesta from the distal two-thirds of the ileum (intestinal section from the Merkel's diverticulum to the ileocecal junction). Ileal digesta was flushed using distilled water into plastic containers, pooled by cage and stored at −20°C until they were freeze dried. The left tibia of 4 median weight birds per cage were collected, defatted using a Soxhlet extractor, weighed and ashed in a muffle furnace at 600°C for 24 h to determine bone ash as described by Ogunwole et al. (2017). Tibia ash was then reweighed to determine ash weight per bone. Dried ileal digesta and excreta samples were ground using a coffee and centrifugal (Retsch ZM 200 GmbH, Haan, Germany) grinder, respectively, and passed through a 0.5-mm screen. Diets, ileal digesta and excreta samples were analyzed for dry matter (DM) by placing in a drying oven for 24 h at 105°C (The Precision Scientific Co., Chicago, IL; method 934.01; AOAC, 2006). Gross energy of diets, ileal digesta and excreta samples was determined by an isoperibol bomb calorimeter using benzoic acid as the calibration standard (Parr 1261; Parr 105 Instrument Co., Moline, IL). Nitrogen content of samples were determined by combustion methods (TruMac N; LECO Corp., St. Joseph, MI, USA; method 990.03; AOAC, 2000) using EDTA as a calibration standard and values were multiplied by a factor of 6.25 to estimate the CP contents. The University of Missouri Experiment Station Chemical Laboratories (Columbia, MO) carried out the AA analyses in the ingredients, diets, and ileal digesta samples. Ground samples were hydrolyzed by 6 M HCl (or BaOH for Trp analysis) at 110 °C for 24 h under nitrogen atmosphere. However, samples used for the analysis of Met and Cys were oxidized by performic acid before acid hydrolysis. The concentrations of AA in samples were analyzed by a high-performance liquid chromatography after post-column derivatization [method 982.30 E (a, b, c); AOAC, 2006]. Titanium concentration in the diets, ileal digesta and excreta samples were determined using methods previously described by Short et al. (1996) at the University of Missouri Experiment Station Chemical Laboratories (Columbia, MO). Following

the wet ash digestion of samples by hydrochloric and nitric acid, P concentration was determined by spectrophotometry with absorbance read at 630 nm (Spark 10 M; Tecan Group Ltd., Männedorf, Switzerland), while Ca and Zn concentrations were determined by flame atomic absorption spectrometry using a Varian Spectr. AA 220FS (Varian Australia Pty Ltd., Victoria, Australia) with absorbance read at 425 and 214 nm, respectively. Phytase activity in diets was analyzed by DuPont Feed Technical Service (Brabrand, Denmark) using methods previously described by Engelen et al. (1994) with modifications from Christensen et al. (2020).

4.3.4 Calculation and Statistical Analysis

The AID and TTR of nutrients in the ileal digesta and excreta were determined using the index method according to the following equation;

$$\text{AID or TTR, \%} = 100 - [(Ti_I/Ti_O) \times (N_O/N_I) \times 100]$$

Where Ti_I is Ti concentration in the diets, Ti_O is the Ti concentration in the ileal digesta or excreta, N_O is the concentration of a nutrient in the ileal digesta or excreta and N_I is the concentration of a nutrient in the diets. The concentration of Ti and nutrients in this equation was expressed as g/kg of DM.

The AID of energy and the apparent metabolizable energy (AME; kcal/kg DM) of the diet was calculated as a product of the coefficient and gross energy concentrations (kcal/kg) in the diet. The nitrogen corrected AME (AMEn) was calculated by correcting for zero N retention using a factor of 8.22 kcal/g N as previously described by Zhang and Adeola (2017).

Data was analyzed using the general linear model procedure of SAS (SAS Inst. Inc., Cary, NC) as 1 + 3 × 5 factorial arrangements of treatments with PC, phytate, phytase, their interactions as fixed effects and replicate blocks as random effects. Using PROC IML to generate appropriate

contrast coefficients, polynomial contrasts were used to compare the PC and NC2 (0 FYT/kg and equivalent phytate-P as the PC), and to determine the linear and quadratic effects of PP and phytase concentrations in the NC diets. Cage served as the experimental unit for all analyses. Statistical significance was set at $P \leq 0.05$ and a trend was set at $0.05 < P \leq 0.1$.

4.4 Results

All birds were healthy throughout the experimental period. Analyzed nutrients and phytase activity in experimental diets were similar with calculated values and within acceptable ranges (Table 2).

Broiler chickens fed the PC diet had a higher ($P < 0.05$) BW, BW gain, FI and gain to feed ratio than birds fed the NC2 without phytase (Table 3). There was no PP level × phytase dose interaction for any of the growth performance and bone ash responses. There was a linear reduction ($P \leq 0.01$) in BW, BW gain, and FI by 4.5, 5.0, and 4.1%, respectively, as PP concentrations increased from 2.3 to 3.3 g/kg in the NC diets. There was no effect of phytate on feed efficiency. With phytase supplementation (from 0 to 4,000 FYT/kg), there was a quadratic response ($P < 0.05$) with BW and FI, and a linear increase ($P < 0.01$) in the BW gain of broiler chickens. Similarly, the gain to feed ratio of broiler chickens was linearly increased ($P < 0.01$) from 703 to 771 g/kg with the addition of phytase. Birds fed the NC2 diet without phytase had lower ($P < 0.01$) tibia ash (%) and tibia ash weight per bone (mg/bone) as compared with birds fed the PC diet (Table 3). Similarly, there was no significant effect of phytate on tibia ash properties. There was a quadratic response ($P < 0.01$) in tibia ash properties, with increases in percentage tibia ash from 37.2 to 47.9% and in tibia ash weight from 289 to 453 mg/bone as phytase concentration increased from 0 to 4,000 FYT/kg.

The AID of DM, energy, P, and Ca was lower ($P < 0.01$) in birds fed the NC2 without phytase as compared with birds fed the PC (Table 4). Similarly, the AID of nitrogen in birds fed the NC2 diet without phytase was 9.3% lower ($P < 0.05$) than that of birds fed the PC diet. There was no interaction between phytate and phytase on the AID of DM, energy, nitrogen, P, Ca, and Zn. Similarly, there was no effect of phytate concentration on the AID of DM, energy, nitrogen, and Zn. However, there was linear decrease ($P < 0.01$) in the AID of P and Ca with increasing PP concentrations. There was a linear increase ($P \leq 0.05$) in the AID of DM, energy, and nitrogen with phytase supplementation. Likewise, the AID of P and Ca was linearly increased ($P < 0.01$) by 88.5 and 18.0%, respectively, with the addition of phytase in the NC diets. Within each NC diet, and in comparison with the NC diets without phytase, the percentage difference in the AID of P was higher with increasing phytase levels (Figure 1). In addition, the increase in the percentage difference of the AID of P was higher in the NC3 diets with 3.3 g PP/kg diet.

There was a significant difference ($P < 0.05$) in the AID of some indispensable AA including His, Met, Thr, Trp, and Val between birds fed the PC diet and the NC2 diet without phytase (Table 5). There was no interaction between PP levels and phytase dose on the AID for any of the indispensable AAs. Although, there was no significant effect of phytate on the AID of most indispensable AA, however, a quadratic trend ($P < 0.1$) was observed with the AID of Arg, His, Leu, Phe, and Val while a quadratic response ($P < 0.05$) was observed with the AID of Met, Thr, and Trp. The lowest AID of all indispensable AA was observed in NC2 diets with 2.8 g PP/kg. When phytase was added to the NC diets, there was a linear increase ($P < 0.01$) in the AID of all indispensable AA, with the highest improvements observed with the AID of Thr, Phe and Trp (7.6, 7.0, and 7.0%, respectively), and the least improvement observed with the AID of Arg (4.8%). For effects on the dispensable AA, birds fed the PC had a higher ($P < 0.05$) AID of Cys, Pro, and Tyr as compared with birds fed the NC2 diet without phytase (Table 6). There was no interaction

between phytate and phytase concentrations on any of the AID of dispensable AA. However, a quadratic trend ($P < 0.1$) and a quadratic response ($P \leq 0.05$) was observed in the AID of all dispensable AA except Glu with increasing PP concentrations. The lowest AID of all dispensable AA was observed in NC2 diets with 2.8 g PP/kg. With the addition of phytase, there was a linear increase ($P < 0.01$) in the AID of all dispensable and total AA.

Birds fed the NC2 diet without phytase had lower ($P < 0.05$) AME, AMEn, and TTR of P and Ca as compared with birds fed the PC diet (Table 7). There was no interaction between phytate and phytase concentrations on the metabolizable energy and the TTR of nutrients. Increasing PP concentrations in NC diets, linearly increased ($P < 0.05$) the TTR of nitrogen while reducing ($P < 0.01$) the TTR of P and Ca. There was no effect of phytate on AME, AMEn, and TTR of DM and Zn. A linear increase ($P < 0.05$) in the TTR of DM, nitrogen and Zn, and a quadratic response ($P \leq 0.05$) with the AMEn and TTR of P and Ca was observed with increasing phytase supplementation.

4.5 Discussion

The use of phytase and its benefits on broiler production have been growing over the years (Sebastian et al., 1996; Selle and Ravindran, 2007; Babatunde et al 2020b). It is probably the enzyme class that has shown the highest consistency in its ability to improve the growth performance, nutrient utilization, or bone mineralization of poultry and swine, while efficiently extracting nutrients from feedstuffs (Adedokun et al., 2015; Babatunde et al., 2019b). Although phytase is an established enzyme, there is always the opportunity to improve the product or the application in poultry and swine. To thoroughly investigate the efficiency of a new phytase product on broiler production, experimental trials that examine their effects on nutrient matrices are important. Growth performance, utilization of nutrients particularly with minerals such as P and

Ca, and bone mineralization of broiler chickens are important parameters necessary to investigate the efficacy of the phytase enzyme. Furthermore, the PP factor is another tool used to investigate the efficacy of phytase. To counter the negative effects of phytate, there arises the need for phytase to hydrolyze phytate quickly and completely in the upper section of the gastrointestinal tract thus, reducing the antinutritive effect of phytate and promoting the extra-phosphoric effects of the phytase (Dersjant-Li et al., 2020). Phytase degrades phytate in a stepwise manner in the gut and releases bound nutrients for use by birds while reducing the amount of minerals such as P wasted through excreta and into the environment. In theory, it is assumed that phytase will hydrolyze most of the phytate content in the diet, however, the accessibility of phytate differs among feed ingredients. Leske and Coon (1999) and Almeida et al. (2017) observed that the accessibility of phytate in rice bran was lower than in canola meal, soybean meal, or sunflower meal. Thus, including rice bran as the source of PP and varying its concentration in the diet, should provide a challenging but adequate substrate from which phytase can hydrolyze while serving as a good tool for evaluating the efficacy of the phytase. Previous studies have reported the efficacy of phytase to improve utilization of nutrients as the PP content of diets was increased (Ravindran et al., 2000, 2006; Liu et al., 2008)

In the current trial and in agreement with several studies (Sebastian et al., 1996; Babatunde et al 2019ab), there was a difference in the growth performance of birds fed the PC diet and the NC2 diet. Although both diets had similar PP content, they differed in their nPP content with the NC2 diet having the lower concentration. The impact of the unavailability of the P in phytate was evident in birds fed the NC2 diet without phytase as they had lower BW, BW gain and feed efficiency as compared with birds fed the PC diet. Broiler chicks have been known to perform poorly when deficient of P and other nutrients as they play a huge role in supporting growth and development particularly at the starter phase (Babatunde et al., 2019a). In the presence of increased

PP concentrations in the diets, a negative impact was observed in the growth performance of broiler chicks. Cabahug et al. (1999) observed a similar decline in growth performance of broiler chickens when dietary PP was increased from 2.9 to 4.4 g/kg using rice pollards. This observation was expected as phytate is known to bind nutrients in its complex and prevent their use by birds. Thus, the higher the PP content in the diet the lower the nutrient availability to the birds, which will explain why birds fed diets with the highest PP (3.3 g PP/kg) from rice bran had the lowest performance. However, the use of PhyG mitigated the negative impacts of PP on the growth performance of birds indicating its extra-phosphoric effects especially at high doses. Previous studies have shown that inclusion of phytase improves the growth performance of broiler chicks fed diets deficient in nutrients in the starter phase (Kiarie et al., 2015; Babatunde et al., 2019a,b; Dersjant-Li et al., 2020). Dietary phytase up to 4,000 FYT/kg improved BW gain and feed efficiency of broiler chicks comparably with birds fed the PC. This indicated that, PhyG was able to efficiently hydrolyze the phytate complex in rice bran, release most of the nutrients required by birds for growth and development (compensated nutrients down spec applied), and recover the performance of birds compared with a nutrient adequate diet. The mitigating effect of phytase is one of the reasons for its increased use in commercial broiler productions as its inclusion is immediately observed in the growth performance of birds.

The adverse effect of P deficiency on bone mineralization has been well documented in previous studies (Dersjant-Li et al., 2018; Babatunde et al., 2020a). There was a clear difference in the tibia ash weight and percentage between birds fed the PC and NC diets without phytase. Because the bones are mostly comprised of P and Ca, a deficiency of these minerals in the diet causes a resorption from the bones to support the P or Ca requirement of birds. Thus, birds fed the deficient NC diets had lower bone mineralization as compared with birds fed the PC diet, except for those NC diets supplemented with 4,000 FYT/kg. There was no effect of PP concentration on

tibia ash. However, birds may have utilized the available P from the diet for bone mineralization; and besides the obvious impact of low available P, there was no further negative effect of additional increase in the dietary PP level. The inclusion of PhyG at a low dose of 500 FYT/kg improved the tibia ash of birds by 20% as compared with birds fed the NC diets without phytase. A high dose of PhyG at 4,000 FYT/kg improved the tibia ash percentage of birds by up to 28% as compared with the PC. This observation agrees with previous studies where inclusion of phytase at either traditional or high doses improved bone mineralization considerably and supported the skeletal structure of broiler chickens (Olukosi et al 2013; Gautier et al 2018; Babatunde et al., 2020a). Moreover, the high dose of phytase may have improved the ratio of Ca to P from the diet thus increasing the absorption and utilization of both minerals for skeletal development. Thus, under the current experimental conditions and considering bone mineralization, it may be implied that PhyG at highest inclusion compares favorably with phytase from other microbial sources as previously reported in Babatunde et al (2020a).

As observed previously, birds fed NC diets (without phytase) deficient in minerals, energy and protein had lower AID of energy and nutrients as compared with birds fed the nutrient dense PC. Previous studies have shown that feeding nutrient deficient diets negatively impacted the utilization of these nutrients and the overall production of birds (Ravindran et al., 2001; Dilger et al., 2004). In particular, P deficiency has been known to negatively impact the utilization of energy and other nutrients as P is required in various biochemical reactions that support the metabolism of energy, protein, minerals and even vitamins (Ravindran et al., 2006; Babatunde et al., 2020a). This impact is however of varying degrees as an increase in the PP of diets did not influence the AID of DM, energy, nitrogen, and zinc but negatively impacted the AID of P and Ca. In contrast to the current study, Ravindran et al. (2000, 2006) observed negative effects of increasing PP from rice pollards and rice bran, respectively, on the AID of nitrogen. However, in both studies, samples

were collected from birds at day 21 and 25 post hatching, respectively, as compared with day 11 post hatching in the current study. Although, studies have shown that phytate is able to bind and hinder the utilization of energy, nitrogen, and minerals (Sebastian et al., 1996; Ravindran et al., 2006), it is possible that increased PP concentrations does not significantly impact their utilization in young broiler chickens. In the current study, P deficiency reduced the AID of nutrients, however an increase in the PP content only impacted the AID of P and Ca which is directly bound by phytate in the complex. Other nutrients may have been indirectly affected by the phytate content in the diets but not in a sufficient manner as to be impacted by a further increase in the PP content.

Previous work from our lab has shown that birds at d 13 or 14 are sensitive to low available P and high phytate content as observed with the AID of P and Ca (Babatunde et al., 2020b; Babatunde and Adeola, 2021). Hypothesis was that when the PP content is increased and nPP kept constant, the amount of P, Ca, as well as other minerals like Zn available to birds decreases significantly in the absence of phytase, thereby impacting the AID of these minerals. Surprisingly, the increasing PP content in diets did not affect the AID of Zn. The early age of birds, and consequently their sensitivity to the Zn content in the diet, absorption capacity, or requirement may have impacted the effect of increasing PP on the AID of Zn. Moreover, Zn is usually required in smaller amounts as compared with P or Ca, thus the impact of increasing PP on its utilization may be reduced. Inclusion of phytase improved the AID of DM, energy, nitrogen, P and Ca as have been observed in previous studies (Ravindran et al., 2000, 2006; Bello et al., 2019).

The AID of P was increased up to 88% when PhyG was included at 4,000 FYT/kg as compared with 0 FYT/kg and matched the AID of P in birds fed the PC. Although there was no interaction between PP and phytase, the percentage difference in the AID of P within each NC diet increased with elevated phytase levels. In the NC3 diets, which had the highest PP content, inclusion of phytase at 4,000 FYT/kg improved the AID of P in birds by up to 137% when

compared with birds fed the NC3 without phytase. This observation supported the assumption that with the increase in the phytate substrate pool, the efficacy of phytase was improved in relation to P release. It also indicates that the novel PhyG was very efficacious in releasing most of the P in the phytate rich, rice bran based-diets such that it compares favorably with the PC diet containing inorganic P. Therefore, including PhyG in diets at sufficient doses, could effectively replace all the inorganic P sources including monocalcium phosphate in the diet without compromising on the digestibility of P in birds (Marchal et al., 2021). In contrast with Babatunde et al. (2020a), phytase had no effect on the AID of Zn in the current study.

Feeding diets low in available P has not shown consistent effects on the AID of AA in broiler chickens (Dilger et al., 2004). However, the reduction in the nPP and Lys content of the NC2 diet, negatively impacted the AID of some dispensable and indispensable AA such as His, Met, Trp, and Tyr in broiler chickens. Similarly, increasing the PP content of diets did not affect all AA although, the AID of some AA was low when diets contained 2.8 g PP/kg as compared with 2.3 or 3.3 g PP/kg. This was different from observations in Ravindran et al. (2006) where increasing PP content hindered the AID of most AA. In the current study, it seemed reasonable to note the decrease in the AID of AA when PP content was increased from 2.3 to 2.8 g PP/kg as a higher phytic acid content may bind AA in the gut of chickens and prevent their utilization. However, we could not readily explain the increase in the AID of AA when the PP was further increased from 2.8 to 3.3 g PP/kg. It could be that observations were as a result of changes in the feed composition, fiber content, analyzed P and Ca levels or other analyzed nutrients between the NC diets. The beneficial effects of PhyG in broiler chickens was evident considering the improvements with the AID of all AA and in agreement with previous studies (Amerah et al., 2014; Babatunde et al 2020a). It suggests that phytase is able to degrade PP before it forms protein-phytate complexes in the gut, reduce the negative impact of phytate on protease function, or

improve the Na-K pump function thus, increasing the utilization of AA in diets, and preventing the loss of nitrogen into the environment.

The utilization of energy from diets is important because all biochemical reactions in the body of broiler chickens require energy to function. In the current study, the slight deficiency in the energy content of NC diets as compared with the PC negatively impacted the AME and AMEn of broiler chickens. The deficiency of P would have also played a role in the low energy utilization of the NC diets. In agreement with Ravindran et al. (2000), increasing the PP content had no impact on the utilization of energy. Phytase inclusion improved the AID of energy in the NC diets of the current study and in agreement with previous studies (Olukosi et al., 2008; Woyengo and Wilson, 2019). Observations with the TTR of nutrients followed a similar pattern with the AID of nutrients discussed previously except that the response of TTR of Ca to increasing phytase supplementation was greater than with AID of Ca. Birds fed NC diets without phytase were able to digest Ca to a reasonable extent however, due to the low TTR of P, the excessive Ca released was not retained but excreted. Phytase inclusion improved the retention of Zn which is required for immune health and as a cofactor for many essential biological reactions in broiler chickens (Yi et al., 1996). The disparity in the effect of phytase on Zn digestibility and retention in the current study may have been due to the differences in the site of sample collection.

In conclusion, results from the current trial showed that increasing the PP content of diets negatively impacted the utilization of nutrients by broiler chickens. However, regardless of the PP content of diets in this trial, PhyG improved the growth performance, bone mineralization, and utilization of energy, AA, and minerals in broiler chickens. From this trial, phytase supplementation especially at high doses elicited extra-phosphoric effects that alleviated the negative impact of high phytate and the dietary nutrient deficiency while supporting the productivity of broiler chickens. Therefore, the use of high phytate ingredients in addition to PhyG

may be considered when formulating diets for broiler chicks to potentially reduce feed costs as well as P and N emissions, with consequences on sustainability. Lastly, results in this trial reveal that broiler chickens in the starter phase respond to the PP content of diets. Similarly, broiler chickens are able to increase their productivity when fed nutrient deficient diets supplemented with a novel consensus bacterial phytase variant.

4.6 References

Adedokun, S. A., A. Owusu-Asiedu, D. Ragland, P. Plumstead, and O. Adeola. The efficacy of a new 6-phytase obtained from *Buttiauxella spp.* expressed in *Trichoderma reesei* on digestibility of amino acids, energy, and nutrients in pigs fed a diet based on corn, soybean meal, wheat middlings, and corn distillers' dried grains with solubles. J. Anim. Sci. 2015.93:168–175.

Adeola, O., and A. J. Cowieson. 2011. Opportunities and challenges in using exogenous enzymes to improve non ruminant animal production. J. Anim. Sci. 89:3189–3218.

Almeida, F. N., M. Vazquez-Anon, and J. Escobar. 2017. Dose-dependent effects of a microbial phytase on phosphorus digestibility of common feedstuffs in pigs. Asian-Australas J. Anim. Sci. 30:985–993.

Amerah, A. M., P. W. Plumstead, L. P. Barnard, and A. Kumar. 2014. Effect of calcium level and phytase addition on ileal phytate degradation and amino acid digestibility of broilers fed corn-based diets. Poult. Sci. 93:906–915.

AOAC International. 2000. Official Methods of Analysis, 17th ed. AOAC Int., Gaithersburg, MD.

AOAC. 2006. Official Methods of Analysis of AOAC International. 18th ed. AOAC. Arlington, VA.

Babatunde, O. O., A. J. Cowieson, J. W. Wilson, and O. Adeola. 2019a. Influence of age and duration of feeding low-phosphorus diet on phytase efficacy in broiler chickens during the starter phase. Poult. Sci. 98:2588–2597.

Babatunde, O. O., A. J. Cowieson, J. W. Wilson, and O. Adeola. 2019b. The impact of age and feeding length on phytase efficacy during the starter phase of broiler chickens. Poult. Sci. 98:6742–6750.

Babatunde, O. O., J. A. Jendza, P. Ader, P. Xue, S. A. Adedokun, and O. Adeola. 2020a. Response of broiler chickens in the starter and finisher phases to three sources of microbial phytase. Poult. Sci. 99:3997–4008.

Babatunde, O. O., S. O. Osho, C. S. Park, and O. Adeola. 2020b. Additivity of apparent and standardized ileal digestibility of phosphorus in mixed diets containing corn and soybean meal fed to broiler chickens. Poult. Sci. 99:6907–6913.

Babatunde, O. O. and O. Adeola. 2021. Additivity of apparent and standardized ileal digestibility of phosphorus in corn and canola meal mixed diets; basal endogenous loss of phosphorus responses to phytase and age in broiler chickens. Br. Poult. Sci. 62:244–250.

Batal, A. B., and C. M. Parsons. 2002. Effects of age on nutrient digestibility in chicks fed different diets. Poult. Sci. 81:400–407.

Bello, A., Y. Dersjant-Li, and D. R. Korver. 2019. The efficacy of 2 phytases on inositol phosphate degradation in different segments of the gastrointestinal tract, calcium and phosphorus digestibility, and bone quality of broilers. Poult. Sci. 98:5789–5800.

Cabahug, S., V. Ravindran, P. H. Selle, and W. L. Bryden. 1999. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus contents. I. Effects on bird performance and toe ash. Br. Poult. Sci. 40:660–666.

Christensen, T., Y. Dersjant-Li, V. Sewalt, R. Mejldal, S. Haaning, S. Pricelius, I. Nikolaev, R. A. Sorg, and A. de Kreij. 2020. In vitro characterization of a novel consensus bacterial 6-Phytase and one of its variants. Curr. Biochem. Eng. 6:156–171.

Cobb. 2013. Cobb 500 Broiler Performance and Nutrition Supplement. Cobb-Vantress, Siloam Springs, Arkansas, USA.

Coelho, M. B. and E. T. Kornegay. 1999. Phytases in Animal Nutrition and Management: Practical Reference Manual. 2nd rev. ed. BASF Monograph Series. BASF Corp., Parsippany, NJ.

Dersjant-Li, Y., G. Archer, A. M. Stiewert, A. A. Brown, E. B. Sobotik, A. Jasek, L. Marchal, A. Bello, R. A. Sorg, T. Christensen, H. S. Kim, R. Mejldal, I. Nikolaev, S. Pricelius, S. Haaning, J. F. Sørensen, A. de Kreij, and V. Sewalt. 2020. Functionality of a next generation biosynthetic bacterial 6-phytase in enhancing phosphorus availability to broilers fed a corn-soybean meal-based diet. Anim. Feed Sci. Tech. 264:114481.

Dersjant-Li, Y., Evans, C., Kumar, A., 2018. Effect of phytase dose and reduction in dietary calcium on performance, nutrient digestibility, bone ash and mineralization in broilers fed corn-soybean meal-based diets with reduced nutrient density. Anim. Feed Sci. Tech. 242:95–110.

Dilger, R. N., E. M. Onyango, J. S. Sands, and O. Adeola. 2004. Evaluation of microbial phytase in broiler diets. Poult. Sci. 83:962–970.

Engelen, A. J., F. C. van der Heeft, P. H. G. Randsdorf, and E. L. C. Smit. 1994. Simple and rapid determination of phytase activity. J. AOAC Int. 77:760 -764.

Gautier, A. E., C. L. Walk, and R. N. Dilger. 2018. Effects of a high level of phytase on broiler performance, bone ash, phosphorus utilization, and phytate dephosphorylation to inositol. Poult. Sci. 97:211–218.

Kiarie, E., Woyengo, T., Nyachoti, C.M., 2015. Efficacy of new 6-phytase from *Buttiauxella spp.* on growth performance and nutrient retention in broiler chickens fed corn soybean meal-based diets. Asian-Australas. J. Anim. Sci. 28:1479–1487.

Leske, K. L., and C. N. Coon. 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. Poult. Sci. 78:1151–1157.

Liu, N., Y. J. Ru, A. J. Cowieson, F. D. Li, and X. CH. Cheng. Effects of phytate and phytase on the performance and immune function of broilers fed nutritionally marginal diets. Poult. Sci. 87:1105–1111.

Marchal, L., A. Bello, E. B. Sobotik, G. Archer, and Y. Dersjant-Li. 2021. A novel consensus bacterial 6-phytase variant completely replaced inorganic phosphate in broiler diets, maintaining growth performance and bone quality: data from two independent trials. Poult. Sci. 100: doi:10.1016/j.psj.2020.12.059.

Namkung, H., and S. Leeson. 1999. Effect of phytase enzyme on dietary nitrogen-corrected apparent metabolizable energy and the ileal digestibility of nitrogen and amino acids in broiler chicks. Poult. Sci. 78:1317–1319.

Ogunwole, O. A., O. O. Babatunde, R. A. Faboyede, B. S. Adedeji, and F. O. Jemiseye. 2017. Calcium and phosphorus retention by broiler chickens fed groundnut cake based-diet supplemented with l-lysine and dl- methionine. J. Anim. Prod. Res. 29:240–248.

Olukosi, O. A., C. Kong, F. Fru-Nji, K. M. Ajuwon, and O. Adeola. 2013. Assessment of a bacterial 6-phytase in the diets of broiler chickens. Poult. Sci. 92:2101–2108.

Olukosi, O. A., A. J. Cowieson, and O. Adeola. 2008. Energy utilization and growth performance of broilers receiving diets supplemented with enzymes containing carbohydrase or phytase activity individually or in combination. Br. J. Nutr. 99:682–690.

Ravindran, V., S. Cabahug, G. Ravindran, P. H. Selle, and W. L. Bryden. 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorous levels: Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. Br. Poult. Sci. 41:193–200.

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

Ravindran, V., P. C. H. Morel, G. G. Partridge, M. Hruby, and J. S. Sands. 2006. Influence of an *Escherichia coli* derived phytase on nutrient utilization in broiler starters fed diets containing varying concentrations of phytic acid. Poult. Sci. 85:82–89.

Sebastian, S., S. P. Touchburn, E. R. Chavez, and P. C. Lague. 1996. 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. Poult. Sci. 75:729–736.

Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 135:1–41.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Tech. 59:215–221.

Walk, C. L. and O. A. Olukosi. 2019. Influence of graded concentrations of phytase in high-phytate diets on growth performance, apparent ileal amino acid digestibility, and phytate concentration in broilers from hatch to 28 D post-hatch. Poult. Sci. 98:3884–3893.

Woyengo, T. A., and J. Wilson. 2019. Enhancing nutrient utilization in maize for broiler chickens by superdosing phytase. Anim. Feed Sci. Tech. 252:34–40.

Yi, Z., E. T. Kornegay, and D. M. Denbow. 1996. Supplemental microbial phytase improves zinc utilization in broilers. Poult. Sci. 75:540–546.

Zhang, F., and O. Adeola. 2017. Techniques for evaluating digestibility of energy, amino acids, phosphorus, and calcium in feed ingredients for pigs. Anim. Nutr. 3:344–352.

4.7 Tables & Figure

Table 4-1. Ingredient composition of experimental diets fed to broiler chickens at starter phase (day 1-11 post hatching), g/kg as-fed basis

	Diet[1,2]			
Item	PC	NC1	NC2	NC3
Ingredients, g/kg				
Corn	504.3	438.1	434.8	433.6
Soybean meal, 480g/kg CP	322.8	308.0	302.7	297.2
Soybean oil	10.4	5.0	5.0	5.0
Rice, Polished	33.0	80.8	49.4	15.9
Rice bran	34.6	3.1	42.5	82.0
Soy hulls	13.6	46.1	47.7	49.1
Meat and bone meal	21.2	22.4	21.2	20.2
Limestone	9.8	8.8	9.1	9.3
Monocalcium phosphate	10.2	0.0	0.0	0.0
Salt	3.7	2.5	2.5	2.5
Vitamin-mineral premix[3]	3.0	3.0	3.0	3.0
DL-Methionine	3.6	3.0	3.1	3.1
L-Lysine.HCl	3.2	2.7	2.7	2.7
Threonine	1.7	1.3	1.3	1.2
L-Tryptophan	0.0	0.1	0.1	0.1
Phytase premix[4]	-	50.0	50.0	50.0
Titanium dioxide premix[5]	25.0	25.0	25.0	25.0
Total	1,000	1,000	1,000	1,000
Calculated nutrients and energy, g/kg				
CP	215.2	209.7	209.7	209.7
ME, kcal/kg	2950.0	2862.2	2862.2	2862.2
Ca	9.0	7.2	7.2	7.2
P	7.1	4.6	5.1	5.6
Phytate-P	2.8	2.3	2.8	3.3
Non-phytate P	4.3	2.3	2.3	2.3
Na	1.7	1.2	1.2	1.2
dig. Lys	12.2	11.4	11.4	11.4
dig. Met	6.4	5.7	5.8	5.8
dig. Thr	8.3	7.6	7.6	7.6

[1]PC = positive control; NC = negative control

[2]Each NC diet had 5 levels of phytase including 0, 500, 1,000, 2,000, and 4,000 phytase units (FYT)/kg.

[3]Supplied the following quantities per kg of diet: vitamin A, 5,484 IU; vitamin D_3, 2,643 ICU; vitamin E, 11 IU; menadione sodium bisulfite,4.38 mg; riboflavin, 5.49 mg; D-pantothenic acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B_{12}, 13.2 µg; biotin, 55.2 µg; thiamine mononitrate, 2.2 mg; folic acid, 990 µg; pyridoxine hydrochloride, 3.3mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 µg.

[4]Each premix contained 1 g of phytase product prepared with 99g of corn. Adding 50 g of premix/kg of NC diet supplied 0, 500, 1,000, 2,000, or 4,000 FYT, respectively.

[5]Prepared as 1 g titanium dioxide added to 4 g corn.

Table 4-2. Analyzed energy and nutrients of experimental diets fed to broiler chickens at starter phase (day 1-11 post hatching), g/kg as-fed basis

	Diet[1,2]			
Item	PC[3]	NC1[4]	NC2[5]	NC3[6]
Energy and nutrients, g/kg				
DM	890	888	889	884
GE, kcal/kg	4,016	3,940	3,942	3,958
CP	209.1	206.5	206.9	205.8
Phytate P	2.7	2.0	3.0	3.7
P	7.2	4.6	5.2	5.6
Ca	10.0	8.5	8.5	8.6
Zn	0.01	0.01	0.01	0.01
Arg	13.3	13.9	13.7	13.6
His	5.2	5.3	5.3	5.2
Ile	9.0	9.3	9.2	9.0
Leu	16.7	17.2	16.9	16.8
Lys	13.4	13.5	13.6	13.3
Met	6.0	5.9	5.7	5.6
Phe	10.0	10.3	10.1	10.1
Thr	8.7	8.7	8.6	8.6
Trp	2.5	2.6	2.6	2.7
Val	10.0	10.3	10.2	10.1
Ala	10.0	10.4	10.4	10.4
Asp	20.0	21.1	20.8	20.5
Cys	3.2	3.2	3.2	3.1
Glu	34.7	35.9	35.1	34.6
Gly	9.1	9.9	9.7	9.9
Pro	11.2	11.5	11.3	11.4
Ser	8.2	8.6	8.5	8.5
Tyr	6.7	7.1	6.9	6.8
Total AA	201.0	208.3	205.4	203.9

[1]PC = positive control; NC = negative control

[2]NC1, NC2, and NC3 had 5 levels of phytase inclusion (0, 500, 1,000, 2,000, and 4,000 phytase units (FYT)/kg); analyzed values for each NC are average of 5 diets.

[3]PC had an analyzed phytase activity of 226 phytase units/kg.

[4]NC1 diets had analyzed phytase activities of 200, 849, 1,332, 2,349, and 4,493 FYT/kg respectively

[5]NC2 diets had analyzed phytase activities of 184, 879, 1,244, 2,004, and 4,517 FYT/kg respectively

[6]NC3 diets had analyzed phytase activities of 202, 714, 1,070, 3,093, and 5,143 FYT/kg respectively

Table 4-3. Effect of phytate and phytase concentrations on growth performance and bone mineralization of broiler chickens fed experimental diets at starter phase (day 1-11 post hatching)

Diet[1]	Phytate P, g/kg	Phytase, FYT/kg	Final BW (g)	BW gain, g/bird	Feed intake, g/bird	G:F, g/kg	Tibia ash weight, mg/bone	Tibia ash, %	No. of replicates
PC[2]	2.8	0	267	214	272	785	420	48.1	6
NC2	2.8	0	215	168	238	704	297	38.3	6
Main effect phytate									
	2.3		247	197	265	743	389	44.6	30
	2.8		243	194	260	745	395	45.1	30
	3.3		236	187	254	737	374	44.1	30
Main effect phytase									
		0	214	166	236	703	289	37.2	18
		500	237	190	258	735	377	44.5	18
		1,000	245	194	262	742	397	46.0	18
		2,000	251	202	267	758	412	47.1	18
		4,000	262	213	276	771	453	47.9	18
SEM[3]			5.02	5.40	5.13	14.58	14.66	0.70	
***P* values**									
PC vs NC2 (0 FYT/kg)			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	
Phytate x Phytase			1.00	1.00	1.00	1.00	0.98	0.35	
Phytate Linear			<0.01	0.01	<0.01	0.49	0.10	0.32	
Phytate Quadratic			0.62	0.45	0.73	0.50	0.10	0.07	
Phytase Linear			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	
Phytase Quadratic			0.02	0.07	0.03	0.36	<0.01	<0.01	

[1]PC= positive control, NC= negative control formulated without inorganic P, with reduction of 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 g/kg available P, 1.8 g/kg Ca and 0.5 g/kg Na vs PC diet

[2]PC contains 2.8 g/kg phytate and 0 FYT/kg, formulated with higher ME, dig AA, adequate in P and Ca

[3]SEM = standard error of mean (for the interaction)

Table 4-4. Effect of phytate and phytase concentrations on apparent ileal digestibility (%) of DM, energy, and nutrients in broiler chickens fed experimental diets at starter phase (day 1-11 post hatching)

Diet[1]	Phytate P, g/kg	Phytase, FYT/kg	DM	Energy	Nitrogen	P	Ca	Zn	No. of replicates
PC[2]	2.8	0	67.6	71.2	71.3	62.2	68.5	27.8	6
NC2	2.8	0	60.5	63.9	64.7	35.2	54.5	22.1	6
Main effect phytate									
	2.3		62.5	66.8	69.7	55.6	64.5	25.3	30
	2.8		62.9	65.7	68.2	49.8	59.2	25.5	30
	3.3		67.6	65.6	68.7	42.7	50.6	24.0	30
Main effect phytase									
		0	61.7	64.5	65.9	33.0	52.2	22.7	18
		500	62.8	65.4	67.8	45.9	56.5	24.5	18
		1,000	63.0	66.2	69.8	50.5	58.7	25.1	18
		2,000	64.1	67.0	70.1	55.2	61.4	26.2	18
		4,000	63.7	67.1	70.8	62.2	61.6	26.2	18
SEM[3]					2.05	3.24	2.54	6.68	
***P* values**									
PC vs NC2 (0 FYT/kg)			<0.01	<0.01	0.03	<0.01	<0.01	0.54	
Phytate x Phytase			0.87	1.00	1.00	0.86	0.94	1.00	
Phytate Linear			0.33	0.16	0.46	<0.01	<0.01	0.75	
Phytate Quadratic			0.25	0.47	0.38	0.72	0.22	0.81	
Phytase Linear			0.05	<0.01	<0.01	<0.01	<0.01	0.47	
Phytase Quadratic			0.49	0.63	0.35	0.10	0.16	0.84	

[1]PC= positive control, NC= negative control formulated without inorganic P, with reduction of 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 g/kg available P, 1.8 g/kg Ca and 0.5 g/kg Na vs PC diet

[2]PC contains 2.8 g/kg phytate and 0 FYT/kg, formulated with higher ME, dig AA, adequate in P and Ca

[3]SEM = standard error of mean (for the interaction)

Table 4-5. Effect of phytate and phytase concentrations on apparent ileal digestibility (%) of indispensable AA in broiler chickens fed experimental diets at starter phase (day 1-11 post hatching)

Diet[1]	Phytate P, g/kg	Phytase, FYT/kg	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	No. of replicates
PC[2]	2.8	0	80.3	75.2	72.0	73.2	76.2	86.8	73.7	68.6	77.9	67.7	6
NC2	2.8	0	78.3	70.6	68.0	69.9	72.4	80.9	70.5	62.0	74.3	62.6	6
Main effect phytate													
	2.3		82.1	74.4	73.2	74.1	76.9	86.1	75.0	67.2	77.0	68.4	30
	2.8		80.7	73.2	71.6	72.8	75.6	84.6	73.4	65.4	76.4	66.4	30
	3.3		81.7	74.7	72.6	74.1	76.2	85.6	74.7	67.7	79.2	67.7	30
Main effect phytase													
		0	79.7	72.0	70.1	71.3	74.1	82.6	71.9	64.4	75.4	65.4	18
		500	80.4	73.1	71.1	72.5	75.2	84.4	72.9	65.9	75.0	66.4	18
		1,000	81.6	74.1	72.8	73.8	76.4	86.3	74.7	66.9	77.5	67.8	18
		2,000	82.3	75.0	73.5	74.8	76.9	86.7	75.6	67.4	79.3	68.5	18
		4,000	83.5	76.2	74.7	75.9	78.6	87.2	76.9	69.3	80.7	69.5	18
SEM[3]					1.51	1.44	1.53	0.94	1.38	1.71	1.24	1.73	
***P* values**													
PC vs NC2 (0 FYT/kg)			0.20	0.02	0.07	0.10	0.08	<0.01	0.10	0.01	0.04	0.04	
Phytate x Phytase			0.65	0.60	0.40	0.68	0.73	0.44	0.58	0.98	0.48	0.41	
Phytate Linear			0.63	0.75	0.55	1.00	0.48	0.37	0.72	0.63	0.01	0.57	
Phytate Quadratic			0.06	0.06	0.13	0.09	0.26	0.02	0.06	0.04	0.01	0.09	
Phytase Linear			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	
Phytase Quadratic			0.84	0.94	0.85	0.87	0.91	0.05	0.92	0.92	0.25	0.86	

[1]PC= positive control, NC= negative control formulated without inorganic P, with reduction of 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 g/kg available P, 1.8 g/kg Ca and 0.5 g/kg Na vs PC diet

[2]PC contains 2.8 g/kg phytate and 0 FYT/kg, formulated with higher ME, dig AA, adequate in P and Ca

[3]SEM = standard error of mean (for the interaction)

Table 4-6. Effect of phytate and phytase concentrations on apparent ileal digestibility (%) of dispensable and total AA in broiler chickens fed experimental diets at starter phase (day 1-11 post hatching)

Diet[1]	Phytate P, g/kg	Phytase, FYT/kg	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr	Total AA	No. of replicates
PC[2]	2.8	0	71.1	70.9	61.0	79.1	66.1	73.1	68.6	74.8	73.5	6
NC2	2.8	0	67.5	68.4	49.8	77.1	61.9	68.8	66.1	71.0	69.8	6
Main effect phytate												
	2.3		72.0	73.0	57.4	80.5	66.7	72.9	70.0	75.4	74.2	30
	2.8		70.4	71.2	55.9	79.2	64.6	71.5	68.5	73.9	72.8	30
	3.3		72.1	72.3	58.7	80.0	67.2	73.2	70.2	75.3	74.0	30
Main effect phytase												
		0	69.5	70.2	53.2	78.2	64.3	70.5	67.4	72.4	71.5	18
		500	70.8	70.8	55.3	78.8	66.2	72.1	68.1	73.6	72.6	18
		1,000	71.4	72.4	57.4	80.0	66.1	72.4	69.2	75.0	73.9	18
		2,000	72.4	73.0	59.1	80.7	66.6	73.6	70.7	76.1	74.5	18
		4,000	73.5	74.5	61.7	81.7	67.5	74.3	72.5	77.2	75.7	18
SEM[3]					2.03	1.10	1.64	1.34	1.57	1.28	1.40	
***P* values**												
PC vs NC2 (0 FYT/kg)			0.12	0.20	<0.01	0.20	0.07	0.02	0.25	0.04	0.07	
Phytate x Phytase			0.58	0.39	0.53	0.59	0.27	0.66	0.82	0.61	0.68	
Phytate Linear			0.92	0.46	0.30	0.49	0.66	0.70	0.81	0.94	0.80	
Phytate Quadratic			0.07	0.05	0.06	0.11	0.01	0.04	0.06	0.04	0.09	
Phytase Linear			<0.01	<0.01	<0.01	<0.01	0.02	<0.01	<0.01	<0.01	<0.01	
Phytase Quadratic			0.97	0.77	0.90	0.88	0.71	0.76	0.44	0.87	0.88	

[1]PC= positive control, NC= negative control formulated without inorganic P, with reduction of 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 g/kg available P, 1.8 g/kg Ca and 0.5 g/kg Na vs PC diet

[2]PC contains 2.8 g/kg phytate and 0 FYT/kg, formulated with higher ME, dig AA, adequate in P and Ca

[3]SEM = standard error of mean (for the interaction)

Table 4-7. Effect of phytate and phytase concentrations on total tract retention (%) of DM, energy, and nutrients in broiler chickens fed experimental diets at starter phase (day 1-11 post hatching)

Diet[1]	Phytate P, g/kg	Phytase, FYT/kg	DM	AME, kcal/DMI	AMEn, kcal/DMI	Nitrogen	P	Ca	Zn	No. of replicates
PC[2]	2.8	0	73.2	3,432	3,224	67.4	56.2	48.0	27.4	6
NC2	2.8	0	71.4	3,292	3,091	65.3	36.0	26.3	19.5	6
Main effect phytate										
	2.3		71.7	3,303	3,101	66.1	56.9	50.2	28.4	30
	2.8		72.4	3,321	3,113	67.7	54.8	43.2	27.4	30
	3.3		72.7	3,345	3,136	68.1	49.4	33.7	26.7	30
Main effect phytase										
		0	71.3	3,289	3,088	65.4	38.2	27.4	18.9	18
		500	71.8	3,324	3,119	66.8	50.9	39.9	26.0	18
		1,000	72.8	3,339	3,131	67.6	57.0	45.2	28.5	18
		2,000	72.6	3,358	3,148	68.3	60.4	48.2	31.5	18
		4,000	72.9	3,306	3,096	68.3	61.7	51.2	32.6	18
SEM[3]					3.74	1.44	3.20	3.20	5.29	
***P* values**										
PC vs NC2 (0 FYT/kg)			0.22	0.02	0.01	0.30	<0.01	<0.01	0.29	
Phytate x Phytase			0.96	1.00	0.99	0.99	0.80	0.71	1.00	
Phytate Linear			0.14	0.11	0.14	0.03	<0.01	<0.01	0.61	
Phytate Quadratic			0.65	0.88	0.80	0.43	0.36	0.47	0.95	
Phytase Linear			0.03	0.37	0.51	0.01	<0.01	<0.01	<0.01	
Phytase Quadratic			0.49	0.06	0.05	0.33	<0.01	<0.01	0.32	

[1]PC= positive control, NC= negative control formulated without inorganic P, with reduction of 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 g/kg available P, 1.8 g/kg Ca and 0.5 g/kg Na vs PC diet

[2]PC contains 2.8 g/kg phytate and 0 FYT/kg, formulated with higher ME, dig AA, adequate in P and Ca

[3]SEM = standard error of mean (for the interaction)

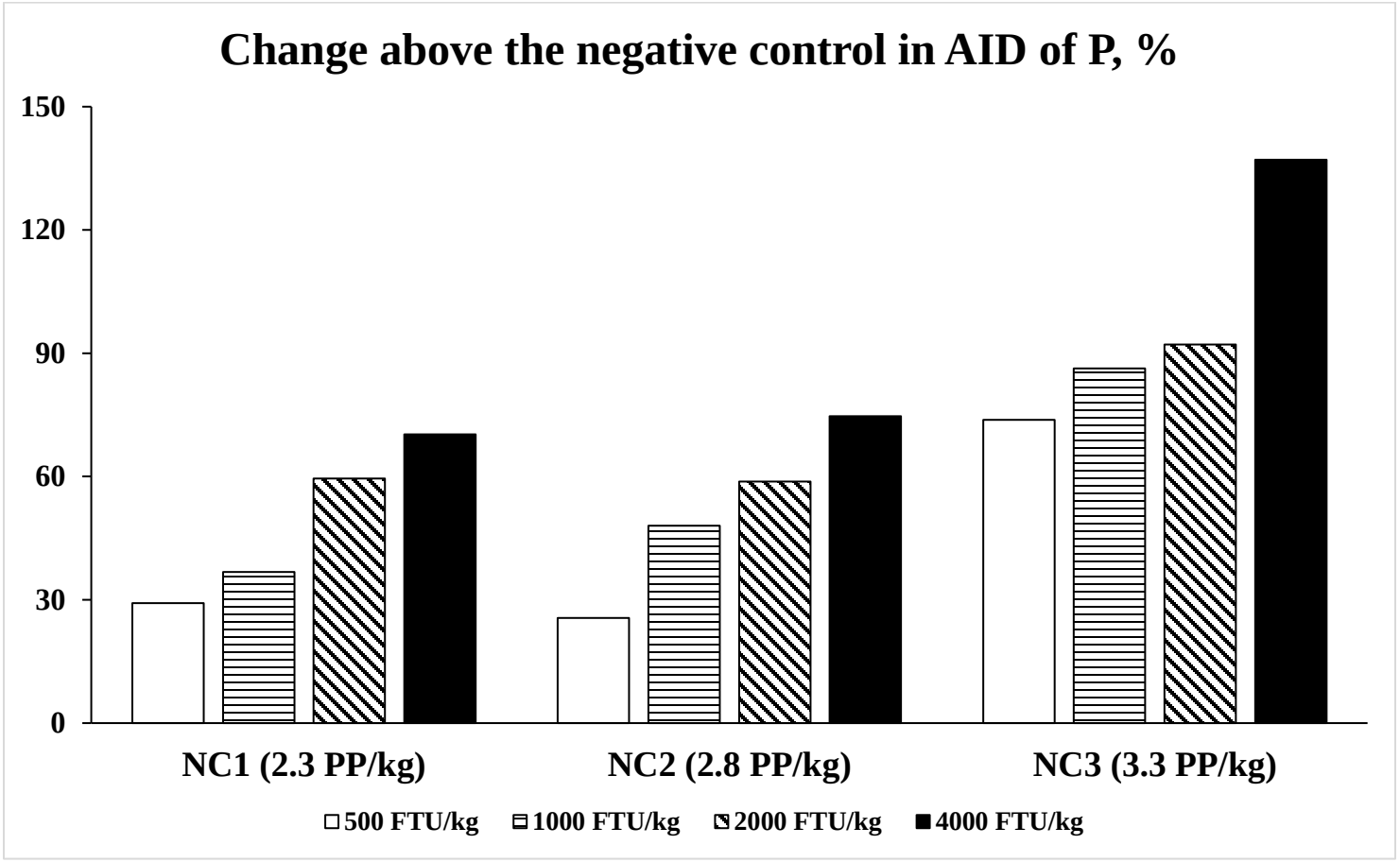


Figure 4-1. The efficacy of phytase (PhyG) on the apparent ileal digestibility (AID) of P relative to the phytate P (PP) concentration in each of the NC diets. The percentage difference values were derived by subtracting the AID of P in the NC diets (i.e. NC1, NC2, or NC3) with 0 FYT/kg from 500, 1,000, 2,000, or 4,000 FYT/kg diet, in each block within each NC diet.

CHAPTER 5. EVALUATION OF THE RESPONSES OF BROILER CHICKENS TO VARYING CONCENTRATIONS OF PHYTATE PHOSPHORUS AND PHYTASE. II. GROWER PHASE (DAY 12-23 POST HATCHING)

5.1 Abstract

A randomized complete block design study used 768 male broiler chickens to investigate the effects of phytate P (PP) and a novel consensus bacterial phytase variant (PhyG) concentration on growth performance, bone mineralization, apparent ileal digestibility (AID) and total tract retention (TTR) of nutrients in broiler chickens. Treatments were arranged in a 1 + 3 × 5 factorial with a nutrient-adequate positive control diet (PC) with 2.8 g PP/kg, 3 nutrient-reduced negative control diets (NC: PC minus 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 g/kg available P, 2.0 g/kg Ca and 0.5 g/kg Na) with varying PP (g/kg) levels, mainly from rice bran, at 2.3 (NC1), 2.8 (NC2), or 3.3 (NC3) and 5 PhyG doses at 0, 500, 1,000, 2,000, or 4,000 FYT/kg. All treatments had 6 replicate cages with 8 birds/cage. A commercial starter diet was fed from day 0 to 11 and the experimental diets from day 12 to 23 post hatching. Birds fed the NC2 diet without phytase had lower ($P < 0.01$) BW, BW gain, and feed intake (FI) as compared with birds fed the PC with the same PP level. With increasing phytate, there was a decrease ($P < 0.05$) in BW, BW gain, and FI. Phytase increased ($P < 0.01$) BW and feed efficiency of broiler chickens. An interaction ($P < 0.05$) between PP and phytase concentrations was observed on the AID of Met, Cys and Thr. Linear decrease ($P < 0.01$) in the AID and TTR of P and Ca with increasing PP concentrations were observed. Phytase supplementation increased ($P \leq 0.05$) the AID of P, Ca, and all AA. The TTR of P, Ca, and Zn was linearly increased ($P < 0.01$) by 112, 123, and 46%, respectively, when birds fed NC diets with 0 and 4,000 FYT/kg were compared. In conclusion, phytate reduced the growth

performance and nutrient utilization of broiler chickens from day 12 to 23 post hatching while phytase ameliorated these negative effects.

Key words: broiler chickens, growth performance, nutrient utilization, phytase, phytate

5.2 Introduction

Phytate phosphorus (PP) is the main form of P in most cereal grains and oilseeds fed to poultry. However, it is not well utilized by birds due to poor digestion and hydrolysis of the bonds that hold P tightly in the phytate complex. Similarly, the negatively charged phytate forms complexes with some positively charged molecules in the intestinal tract thus reducing their digestibility (Woyengo and Nyachoti, 2013). Exogenous phytase is widely used in poultry diets to hydrolyze PP and release P and other nutrients for use by birds (Dilger et al., 2004; Gautier et al., 2017; Babatunde et al., 2020a). Several fungal and bacterial phytases have been produced and used commercially. However, there is continuous research on creating improved phytases with better efficiency in utilizing most of the P available in feedstuffs, as well as improving the utilization of other nutrients. To test the efficacy of these phytase products in poultry production, trials must be carried out to determine the nutrient matrix in birds. The evaluation of parameters such as growth performance, bone mineralization, and nutrient utilization have proved valuable in determining the efficiency of phytase in improving P bioavailability and utilization of other nutrients such as AA and energy for broiler chickens (Ravindran et al., 2000, 2001; Babatunde et al., 2020a). The information from these trials will help to determine the contribution of available nutrients from phytase and the knowledge can be used in the formulation of diets that accurately meet the requirements of broiler chickens while reducing the waste of nutrients to the environment and encouraging sustainability.

Varying concentrations of phytate is a factor that can be used to estimate the efficiency of a new phytase product since PP is the substrate on which phytase acts on. From previous studies, we know that the PP in rice bran is more difficult to hydrolyze by phytase as compared to other feedstuffs such as canola meal, wheat, or soybean meal (Leske and Coon, 1999; Almeida et al., 2017). Thus, it seems appropriate to test a new phytase product using the most challenging PP source, hereby giving an opportunity to properly estimate its efficacy. Its ability to efficiently hydrolyze the PP from rice bran may encourage the use of other high PP ingredients in broiler chicken nutrition.

Commercial broiler chicken production generally occurs in 3 growth phases that differ mainly in the nutrient requirements of birds and in the biological and physiological states of birds. The starter phase of broiler chickens is characterized by the rapid growth and development of tissues, organs, and the skeletal system. Thus, the evaluation of phytase efficacy in this phase is of great importance (Babatunde et al., 2019a). Previous work from our lab evaluated the responses of birds to varying levels of a novel consensus bacterial phytase variant (PhyG) and PP in broiler chickens in the starter phase (Babatunde et al., 2021). However, it is also important to evaluate the responses of broiler chickens to this novel phytase in the grower phase. The age of birds has been known to influence the utilization of nutrients and efficacy of phytase in broiler chickens (Batal and Parsons, 2002; Babatunde et al., 2019a,b). Similarly, the grower phase prepares broiler chickens for the rapid deposition of meat in the finisher phase. Thus, a proper evaluation of PhyG in this phase allows nutritionists to determine the nutrient contribution of the phytase enzyme especially in the presence of varying concentrations of PP.

Therefore, this study aimed to evaluate the responses of broiler chickens in the grower phase (day 12 to 23 post hatching) to varying levels of PP concentrations and a novel consensus

bacterial phytase variant (PhyG) using growth performance, bone mineralization, apparent ileal digestibility (AID), and total tract retention (TTR) of energy and nutrients as the response criteria. The null hypothesis tested in this study was that there was no effect of PP and phytase on responses of broiler chickens in the grower phase.

5.3 Materials and Methods

All protocols of animal experiments were reviewed and approved by the Purdue University Animal Care and Use Committee.

5.3.1 Birds, Experimental Design, and Diets

From day 0 to 12 post hatching, male Cobb 500 birds were individually tagged, housed in temperature-controlled battery cages (model SB 4T, Alternative Design Manufacturing, Siloam Springs, AR) and fed a commercial starter diet formulated to meet or exceed the requirements of broiler chickens (NRC, 1994). On day 12 post hatching, birds were weighed and allotted to one of 16 dietary treatments with 6 replicate cages and 8 birds per cage for a total of 768 birds. Treatments were arranged as a 1 + 3 × 5 factorial in a randomized complete block design with BW as the blocking factor. Experimental diets consisted of a nutrient-adequate positive control diet (PC) with 2.8 g PP/kg, 3 nutrient-reduced negative control (NC: PC minus 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 g/kg available P, 2.0 g/kg Ca and 0.5 g/kg Na) diets with varying PP (g/kg) levels, mainly from rice bran, at 2.3 (NC1), 2.8 (NC2), or 3.3 (NC3) and 5 PhyG doses at 0, 500, 1,000, 2,000, or 4,000 FTU/kg (Table 1). All diets were formulated based on analyzed nutrient values in feed ingredients. The PC diet had the same PP concentration as the NC2 diets. The phytate concentrations in diets were adjusted through the addition of rice bran and polished rice. The PhyG phytase (Danisco Animal Nutrition (IFF), The Netherlands) used in the NC diets was expressed in *Trichoderma*

reesei and developed to have a broad pH profile, higher intrinsic thermostability, and faster hydrolysis in the upper gastrointestinal tract. The phytase was prepared as a premix with ground corn and included at 50 g/kg to provide the targeted phytase doses. Titanium dioxide, which is an indigestible marker, was included into all diets at 5 g/kg but in a corn premix at 25 g/kg. All experimental diets were fed ad libitum to broiler chickens from day 12 to 23 post hatching. Water was freely available. Mortality and general health of birds were monitored daily.

5.3.2 Sample Collection and Chemical Analyses

On day 23 post hatching, BW of all birds and feed intake (FI) per cage were recorded and used to determine the BW gain and gain to feed ratio. Birds were euthanized by CO_2 asphyxiation and dissected to collect the digesta from the distal two-thirds of the ileum which is that section of the intestines from Merkel's diverticulum to the ileocecal junction. The ileal digesta was flushed with distilled water into plastic containers, pooled by cage and stored at −20°C until lyophilized. Excreta, collected during the last 3 days of the experimental period, was dried in a forced air oven at 56°C for 7 d. The left tibia of 4 median weight birds per cage were collected and processed to determine percentage bone ash and ash weight per bone as described by Ogunwole et al. (2017). Dried ileal digesta was ground using a coffee grinder while diet and dried excreta samples were ground using a centrifugal grinder (Retsch ZM 200 GmbH, Haan, Germany). All ground samples were passed through a 0.5-mm screen. Diets, ileal digesta and excreta samples were analyzed for dry matter (DM) by placing in a drying oven for 24 h at 105°C (The Precision Scientific Co., Chicago, IL; method 934.01; AOAC, 2006). An isoperibol bomb calorimeter (Parr 1261; Parr 105 Instrument Co., Moline, IL) was used to determine the gross energy in diets, ileal digesta and excreta samples using benzoic acid as the calibration standard. The nitrogen content of diets, ileal, and excreta samples were determined by the combustion method (TruMac N; LECO Corp., St.

Joseph, MI, USA; method 990.03; AOAC, 2000) using EDTA as a calibration standard. Nitrogen values were multiplied by a factor of 6.25 to estimate the CP contents. The University of Missouri Experiment Station Chemical Laboratories (Columbia, MO) carried out the amino acid (AA) analyses for the ingredients, diet, and ileal digesta samples using methods described by the AOAC [method 982.30 E (a, b, c); 2006]; and Ti analysis in diets, ileal digesta and excreta samples (Short et al., 1996). Phosphorus, and Ca concentrations in the diets, ileal and excreta samples were determined after wet ash digestion with nitric and hydrochloric acid using methods described by Babatunde et al. (2019a). While Zn concentrations in the samples were determined by flame atomic absorption spectrometry using a Varian Spectr. AA 220FS (Varian Australia Pty Ltd., Victoria, Australia) with absorbance read at 214 nm. Phytase activity in diets was analyzed by DuPont Feed Technical Service (Brabrand, Denmark) using methods previously described by Engelen et al. (1994).

5.3.3 Calculation and Statistical Analyses

The AID and TTR of nutrients in the ileal digesta and excreta were determined using the index method described by Adeola (2001);

$$\text{AID or TTR, \%} = 100 - [(Ti_I/Ti_O) \times (N_O/N_I) \times 100]$$

Where Ti_I is Ti concentration in the diets, Ti_O is the Ti concentration in the ileal digesta or excreta, N_O is the concentration of a nutrient in the ileal digesta or excreta and N_I is the concentration of a nutrient in the diets. The concentration of Ti and nutrients in this equation was expressed as g/kg of DM. The AID of energy and the apparent metabolizable energy (AME; kcal/kg DM) of the diet was calculated as a product of the coefficient and gross energy concentrations (kcal/kg) in the diet.

The nitrogen corrected AME (AMEn) was calculated by correcting for zero N retention using a factor of 8.22 kcal/g N.

Data were analyzed using the general linear model procedure of SAS (SAS Inst. Inc., Cary, NC) as 1 + 3 × 5 factorial arrangements of treatments with PC, phytate, phytase, and their interactions as fixed effects and replicate blocks as random effects. Polynomial contrasts were used to compare the PC and NC2 (0 FYT/kg) diets which had similar PP content, and to determine the linear and quadratic effects of PP and phytase doses in the NC diets. Cage served as the experimental unit for all analyses. Statistical significance was set at $P \leq 0.05$.

5.4 Results

The analyzed nutrients and phytase activity in experimental diets were similar with calculated values and within acceptable ranges (Table 2). All birds were healthy throughout the experimental period and only a 0.4 % mortality was recorded in this trial. There was a decrease ($P < 0.01$) in the BW, BW gain, FI, and gain to feed ratio of birds fed the NC2 without phytase as compared with birds fed the PC (Table 3). There was no interaction between phytate and phytase dose concentrations on growth performance indices in broiler chickens. However, there was a linear reduction ($P < 0.05$) in the BW and FI of birds with increasing PP contents in the NC diets. The respective reduction in BW gain and feed efficiency of broiler chickens were 6 and 3.3% as PP increased from 2.3 to 3.3 g/kg. There was a quadratic increase ($P < 0.01$) in BW, BW gain, FI, and feed efficiency of birds with phytase supplementation from 0 to 4,000 FTU/kg. The BW gain was increased by 25.6% while the feed efficiency was increased by 11.9 % with phytase supplementation from 0 to 4,000 FTU/kg.

There was a decrease ($P < 0.01$) in the percentage tibia ash and the ash weight per bone of birds fed the NC2 without phytase versus the PC (Table 3). There was no interaction between

phytate and phytase and no effect of phytate on tibia ash properties. However, there was a quadratic response ($P < 0.01$) of phytase on tibia ash properties with a difference of 642 mg ash/bone between birds fed the NC without phytase and birds fed the NC with 4,000 FYT/kg. Similarly, percent tibia ash was increased by 23% with phytase supplementation. Compared with birds fed the PC, there was a decrease ($P < 0.05$) in the AID of DM, energy, P, and Ca of birds fed the NC2 without phytase (Table 4). There was no interaction between phytate and phytase on the AID of DM, energy, nitrogen, P, Ca, and Zn. There was a linear reduction ($P < 0.01$) in the AID of DM, energy, and P by 2.9, 2.9, and 28.8%, respectively, as PP increased from 2.3 to 3.3 g/kg in the NC diets. Phytase mitigated the effects of phytate on DM, energy, and P by increasing ($P < 0.01$) their AID by 3.8, 3.6, and 96%, respectively. Similar increases ($P < 0.05$) in the AID of nitrogen, Ca, and Zn was also observed in birds fed diets with added phytase. Within birds fed each NC diet, the inclusion of phytase from 500 to 4,000 FYT/kg increased the AID of P by 31-83, 37-105, and 39-105% over each respective NC1, NC2, and NC3 diets without phytase (Figure 1).

The AID of some indispensable AA such His, Phe, Thr, and Trp were lower ($P \leq 0.05$) in birds fed the NC2 without phytase as compared with the PC (Table 5). There was an interaction ($P < 0.05$) between phytate and phytase on the AID of Met and Thr with linear increases in birds fed NC1 and NC2 diets with phytase but a quadratic increase in birds fed the NC3 with phytase. An interaction was also observed for AID Cys ($P < 0.01$), there were linear increments in birds fed the NC diets containing 2.3 and 2.8 g PP/kg with added phytase, and an increase, which plateaued at 2,000 FTU/kg, in the NC diet containing 3.3 g PP/kg. There was a quadratic response ($P \leq 0.05$) in the AID of Lys and Phe with increases in phytate levels. With phytase addition, there was a linear increase ($P < 0.01$) in the AID of all indispensable AA. When comparing birds fed the NC2 without phytase and the PC, there was a decrease ($P < 0.01$) in the AID of the total AA and all

dispensable AA except Asp (Table 6). There was a quadratic response ($P \leq 0.05$) in the AID of total AA and all dispensable AA except Ala, Pro, Tyr with increases in the PP concentration. Linear increase ($P < 0.01$) in the AID of Ala, Asp, Cys, Glu, Gly, Tyr, and total AA, and a quadratic response ($P < 0.05$) with the AID of Pro and Ser with phytase supplementation were noted.

A difference ($P < 0.05$) in the AID of P, Ca, and Zn in birds fed the NC2 without phytase versus birds fed the PC was observed (Table 7). There was no interaction between phytate and phytase concentrations on the TTR of nutrients and the metabolizable energy. A linear decrease ($P \leq 0.01$) in the TTR of DM, nitrogen, P, and Ca was observed in birds fed diets with increasing PP content. With phytase supplementation, the AME and AMEn were linearly increased ($P < 0.01$) by 113 and 110 kcal/kg DM intake, respectively. Similarly, the TTR of DM and Zn were linearly increased ($P < 0.01$) by 2.1 and 45.5%, respectively, while a quadratic response ($P < 0.01$) was observed with the TTR of P and Ca with phytase supplementation.

5.5 Discussion

The importance of P in the nutrition of broiler chickens cannot be overemphasized due to its role in skeletal development and in several biochemical reactions necessary to support life (Leeson and Summers, 2001). Inadequate supply of P in the diets of broiler chickens will usually result in reduced growth and development of birds as observed in the current study. The inability of broiler chickens to utilize the P in PP and the absence of inorganic P in the NC2 diet resulted in the reduced BW, BW gain, FI and feed efficiency of birds fed the NC2 diet without phytase. This observation is consistent with previous studies where insufficient available P in the NC diet resulted in reduced growth performance (Walters et al., 2019; Babatunde et al., 2020a). However, it should also be noted that the NC2 diet without phytase was deficient in non-phytate P (nPP), energy and other nutrients such as crude protein, AA, Ca, and Na as compared with the PC. This

deficiency in addition to the inadequate available P would have contributed to the lowered growth observed in birds fed the NC2 without phytase. The negative effect of increasing concentrations of phytate on growth performance was expected and has been reported by Cabahug et al. (1999). In addition, feeding the low PP diet (NC1) without phytase supplementation to birds resulted in reduced growth performance. This diet will be typical of a corn-soybean meal-based commercial diets that has been formulated to be low in P however, with additional P-supplying ingredients such as soyhulls, polished rice, rice bran, and meat and bone meal. In the current trial we aimed to test the efficacy of the new phytase enzyme in birds fed diets of commercial or practical importance which explains why diets from low to high PP were included in the trial.

Broiler chickens are known to have difficulties in hydrolyzing the phytic acid bonds that tightly bind P in most cereals and oilseeds (Babatunde et al., 2020b). Phytate also forms complexes with other divalent minerals such as Ca and Zn, AA, and even enzymes such as carbohydrases and proteases necessary to digest starch and proteins, respectively (Selle and Ravindran, 2007; Cowieson et al., 2011). Thus, increasing the concentration of PP in the diets without phytase supplementation should reduce the availability of several nutrients necessary to support the growth of broiler chickens. In addition, PP from rice bran has been reported to be particularly difficult to hydrolyze as compared to other feed ingredients (Almeida et al., 2017), hereby reducing the accessibility of nutrients to birds. The inclusion of phytase in the diets mitigated the negative effects of low nPP on growth performance in birds regardless of the PP content and as observed with several studies (Ravidran et al., 2008; Powell et al., 2011; Leyva-Jimenez et al., 2019). Phytase increases the bioavailability of P and other nutrients by hydrolyzing the phytate complex and releasing nutrients necessary for growth in broiler chickens.

When comparing birds fed the PC and the NC2 without phytase but with similar PP levels, we observe the impact of available P on bone mineralization. Broiler chickens were able to utilize the inorganic P in the PC for mineral deposition to the bones as compared with birds fed the NC2 with limited access to the P in phytate. There was no effect of increasing PP on tibia ash properties. This observation was surprising as we expected that the increase in relatively unavailable PP will adversely affect the deposition of P and Ca in bones particularly as birds grow older. However, the PP effect was across all phytase dose levels and not just the NC without phytase hence, phytase would have increased the available P and tibia ash thus negating the debilitating effect of phytate. Similarly, birds may have utilized the available P in the diets to meet their bone mineralization requirements. It is possible that on day 23, birds may still be young enough such that the requirement of P and Ca for skeletal development may have been met despite the absence of inorganic P and the increase in PP content of the NC diets. Furthermore, birds were fed P-sufficient diets from days 0-11 and may have had P reserves that prevented the depression of the P status of birds and the decrease of tibia ash significantly in the short duration of the experimental period. Subsequently, further studies may be required to evaluate the effect of increasing PP on the bone mineralization of much older birds i.e. at the finishing phase. The positive effect of PhyG on tibia ash in birds on day 23 post hatching was similar to the previous study with birds on day 11 post hatching (Babatunde et al., 2021). However, when comparing both studies, the effect of the NC diet without phytase on tibia ash seemed to be more evident in the younger birds as compared with the older birds (37.2 vs. 40.5%). Similarly, the efficacy of phytase on tibia ash in terms of percentage improvement was more evident in the younger birds as compared with birds in the current study when phytase was included at 4,000 FYT/kg (23 vs 29%). Therefore, we speculate that younger birds may be more sensitive to P deficiency for bone mineralization as well as to

phytase supplementation. However, this does not dispute the positive influence of PhyG or other phytases on bone mineralization regardless of the growth phase of broiler chickens (Augspurger and Baker, 2004; Manobhavan et al., 2016; Babatunde et al., 2020a).

Differences in the concentration of nPP and other nutrients between the PC and the NC2 diet without phytase was responsible for the lowered AID of DM, energy, P and Ca observed in broiler chickens. In the current study, increased PP concentrations hindered the AID of DM, energy and P but had no effect on the AID of nitrogen, Ca, and Zn. From previous studies, we know that phytate is able to hinder the utilization of several nutrients (Ravindran et al., 2000; Babatunde et al., 2019b) however, the sensitivity of birds to the presence of phytate and its effect on digestibility of nutrients may be influenced by several factors including age of birds, phytate source and solubility, etc. Batal and Parsons (2002) previously reported that age of broiler chickens could influence the utilization of nutrients. Similarly, previous work from our lab has reported the impact of age on the AID of P and Ca in broiler chickens, with younger birds being more sensitive to P deficiency (Babatunde et al., 2019a, b). It is possible that the increased demand for energy and nutrients in older birds impacted the sensitivity of birds to the higher phytate content in the diet thus, reducing the digestibility of DM and energy in addition to P.

The addition of phytase improved the AID of DM, energy, nitrogen, P and Ca in agreement with previous studies (Dersjant-Li and Kwakernaak, 2019; Dersjant-Li et al., 2020; Babatunde and Adeola, 2021). In addition, PhyG improved the AID of Zn which was not the case in younger birds from the previous study (Babatunde et al., 2021). Age of birds may have played a role in the response of birds with regards to Zn absorption and utilization in the presence of phytase. Although Zn is required in minor quantities as compared with other minerals, birds may require more Zn to support biochemical reactions and the immune system as they grow older. The efficacy of phytase

on the AID of P was apparent and seemed to be dependent on the PP content of the diet. When comparing birds fed the NC1 diet (2.3 g PP/kg) and the NC3 diet (3.3 g PP/kg) with similar phytase concentrations (4,000 FYT/kg), the AID of P was improved by 83 and 105%, respectively. This indicates that an increase in the PP content of diets potentially increases the substrate pool from which phytase could act upon. Thus, increasing the bioavailability of nutrients particularly P and Ca to broiler chickens. Even though the P in rice bran is more tightly bound as compared to other ingredients, PhyG was able to extract the P in the PP complex even as the phytate concentrations increased. Therefore, this should encourage the use of feed ingredients with high PP content in the grower phase of broiler chickens if diets are properly supplemented with PhyG. In addition, the effect of high doses of phytase was evident as the AID of P was improved by over 100% in some instances. This agrees with several studies where higher doses of phytase allowed for an increased degradation of PP and supported the growth of broiler chickens beyond the use of the low 500 FYT/kg dose (Lee et al., 2017; Sommerfeld et al., 2018; Dersjant-Li and Kwakernaak, 2019).

The effect of phytase on the AID of Met, Thr, and Cys was influenced by the phytate content of the diet resulting in an interaction. At the highest PP levels, the AID of most AA was lower in the NC as compared with the NC in the lower PP levels. However, the improvement of phytase above the NC on some digestible AA such as Met and Cys was larger at high PP levels as compared with lower PP levels. Generally, phytase improved the digestibility of all AA both linearly and quadratically. However, significant linear responses were observed on the AID of most AA when phytase was added from 500 to 4,000 FYT/kg in diets, while quadratic responses were observed with digestible Met, Ser, and Pro. As observed with previous studies, the extra-phosphoric effect of PhyG on AA utilization was evident as phytase improved not just the P utilization but was able to counter the anti-nutritive effects of PP on digestible AA (Truong et al.,

2015; Christensen et al., 2020; Dersjant-Li et al., 2020). In agreement with previous studies, phytase inclusion improved the AID of all dispensable and indispensable AA (Dersjant-Li and Kwakernaak, 2019; Babatunde et al., 2020a).

There was an effect of phytate on the TTR of nitrogen, P and Ca in birds at the grower phase but not on the AME and AMEn. We cannot readily explain the increase in the TTR of nitrogen with increasing PP content mainly because there was no effect of phytate on the AID of nitrogen. However, we can speculate that with increasing PP content, a significant amount of unhydrolyzed phytate and its derivatives may have reached the caecum of birds. This may have supported the degradation of phytate by microbes and increased the amount of nutrients such as microbial nitrogen released and retained in birds. An estimation of the microbial population and activity in the post-ileal region of the gut of broiler chickens in response to increasing phytate concentrations may be important information in further studies. In agreement with other trials (Ravindran et al., 2000; Ravindran et al., 2001; Santos et al., 2008) there was an increase in the AME of birds with phytase inclusion. The age of birds may have an impact the effect of phytase on energy utilization. Older birds may require more energy in supporting growth and development and may be more sensitive to the activity of phytase in energy deficient diets. Similarly, phytase improved the TTR of DM, P, Ca, and Zn in agreement with Sebastian et al (1996) and Babatunde et al. (2019a,b).

In conclusion, broiler chickens in the grower phase generally responded negatively to the presence of phytate in the diets. However, the inclusion of PhyG in diets of broiler chickens increased growth performance, bone mineralization, digestibility and retention of energy, AA, P, Ca, and other nutrients in the grower phase regardless of phytate levels. Lastly, inclusion of high doses of PhyG extends extra-phosphoric benefits on broiler production beyond the traditional

dosage and may be considered during the grower phase of production if benefits outweigh the financial implications.

5.6 References

Adeola, O. 2001. Digestion and balance techniques in pigs. In: A. J. Lewis, and L. L. Southern, editors, Swine nutrition. 2nd ed. CRC Press, Washington, DC. p. 903–916.

Almeida, F. N., M. Vazquez-Anon, and J. Escobar. 2017. Dose-dependent effects of a microbial phytase on phosphorus digestibility of common feedstuffs in pigs. Asian-Australas J. Anim. Sci. 30:985–993.

AOAC International. 2000. Official Methods of Analysis, 17th ed. AOAC Int., Gaithersburg, MD.

AOAC. 2006. Official Methods of Analysis of AOAC International. 18th ed. AOAC. Arlington, VA.

Augspurger, N. R., and D. H. Baker. 2004. High dietary phytase levels maximize phytate-phosphorus utilization but do not affect protein utilization in chicks fed phosphorus or amino acid-deficient diets. J. Anim. Sci. 82:1100–1107.

Babatunde, O. O., A. J. Cowieson, J. W. Wilson, and O. Adeola. 2019a. Influence of age and duration of feeding low-phosphorus diet on phytase efficacy in broiler chickens during the starter phase. Poult. Sci. 98:2588–2597.

Babatunde, O. O., A. J. Cowieson, J. W. Wilson, and O. Adeola. 2019b. The impact of age and feeding length on phytase efficacy during the starter phase of broiler chickens. Poult. Sci. 98:6742–6750.

Babatunde, O. O., J. A. Jendza, P. Ader, P. Xue, S. A. Adedokun, and O. Adeola. 2020a. Response of broiler chickens in the starter and finisher phases to three sources of microbial phytase. Poult. Sci. 99:3997–4008.

Babatunde, O. O., S. O. Osho, C. S. Park, and O. Adeola. 2020b. Additivity of apparent and standardized ileal digestibility of phosphorus in mixed diets containing corn and soybean meal fed to broiler chickens. Poult. Sci. 99: 6907–6913.

Babatunde, O. O. and O. Adeola. 2021. Additivity of apparent and standardized ileal digestibility of phosphorus in corn and canola meal mixed diets; basal endogenous loss of phosphorus responses to phytase and age in broiler chickens. Br. Poult. Sci. 62:244–250.

Babatunde, O. O., A. Bello, Y. Dersjant-Li, and O. Adeola 2021. Evaluation of the responses of broiler chickens to varying concentrations of phytate phosphorus and phytase. I. Starter phase (d 1-11 post hatching). Poult. Sci. 100:101396.

Batal, A. B., and C. M. Parsons. 2002. Effects of age on nutrient digestibility in chicks fed different diets. Poult. Sci. 81:400–407.

Cabahug, S., V. Ravindran, P. H. Selle, and W. L. Bryden. 1999. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus contents. I. Effects on bird performance and toe ash. Br. Poult. Sci. 40:660–666.

Christensen, T., Y. Dersjant-Li, V. Sewalt, R. Mejldal, S. Haaning, S. Pricelius, I. Nikolaev, R. A. Sorg, and A. de Kreij. 2020. In vitro characterization of a novel consensus bacterial 6-Phytase and one of its variants.Curr. Biochem. Eng. 6:156–171.

Cowieson, A. J., P. Wilcock, and M. R. Bedford. 2011. Super-dosingeffects of phytase in poultry and other monogastrics. World Poult.Sci. J. 67:225–236.

Dersjant-Li, Y., G. Archer, A. M. Stiewert, A. A. Brown, E. B. Sobotik, A. Jasek, L. Marchal, A. Bello, R. A. Sorg, T. Christensen, H. S. Kim, R. Mejldal, I. Nikolaev, S. Pricelius, S. Haaning, J. F. Sørensen, A. de Kreij, and V. Sewalt. 2020. Functionality of a next generation biosynthetic bacterial 6-phytase in enhancing phosphorus availability to broilers fed a corn-soybean meal-based diet. Anim. Feed Sci. Tech. 264:114481.

Dersjant-Li, Y., and C. Kwakernaak. 2019. Comparative effects of two phytases versus increasing the inorganic phosphorus content of the diet, on nutrient and amino acid digestibility in broilers. Anim. Feed Sci. Tech. 253:166–180.

Dilger, R. N., E. M. Onyango, J. S. Sands, and O. Adeola. 2004. Evaluation of microbial phytase in broiler diets. Poult. Sci. 83:962–970.

Engelen, A. J., F. C. van der Heeft, P. H. G. Randsdorf, and E. L. C. Smit. 1994. Simple and rapid determination of phytase activity. J. AOAC Int. 77:760 -764.

Gautier, A. E., C. L. Walk, and R. N. Dilger. 2018. Effects of a high level of phytase on broiler performance, bone ash, phosphorus utilization, and phytate dephosphorylation to inositol. Poult. Sci. 97:211–218.

Lee, S. A., D. Nagalakshmi, M. V. L. N. Raju, S. V. Rama Rao, and M. R. Bedford. 2017. Effect of phytase superdosing, myo-inositol and available phosphorus concentrations on performance and bone mineralisation in broilers. Anim. Nutr. 3:247–251.

Leeson, S., and J. D. Summers. 2001. Minerals. In: Scott´s Nutrition of the chicken. 4th ed. University Books, Guelph, Ontario, Canada. p 358–363.

Leske, K. L., and C. N. Coon. 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. Poult. Sci. 78:1151–1157.

Leyva-Jimenez, H., A. M. Alsadwi, K. Gardner, E. Voltura, and C. A. Bailey. 2019. Evaluation of high dietary phytase supplementation on performance, bone mineralization, and apparent ileal digestible energy of growing broilers. Poult. Sci. 98:811–819.

Ogunwole, O. A., O. O. Babatunde, R. A. Faboyede, B. S. Adedeji, and F. O. Jemiseye. 2017. Calcium and phosphorus retention by broiler chickens fed groundnut cake based-diet supplemented with l-lysine and dl- methionine. J. Anim. Prod. Res. 29:240–248.

Olukosi, O. A., C. Kong, F. Fru-Nji, K. M. Ajuwon, and O. Adeola. 2013. Assessment of a bacterial 6-phytase in the diets of broiler chickens. Poult. Sci. 92:2101–2108.

Manobhavan, M., A. V. Elangovan, M. Sridhar, D. Shet, S. Ajith, D. T. Pal, and N. K. S. Gowda. 2016. Effect of super dosing of phytase on growth performance, ileal digestibility and bone characteristics in broilers fed corn-soya-based diets. J. Anim. Physiol. Anim. Nutr. 100:93–100.

NRC. 1994. Nutrient requirements of poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Powell, S., T. D. Bidner, and L. L. Southern. 2011. Phytase supplementation improved growth performance and bone characteristics in broilers fed varying levels of dietary calcium. Poult. Sci. 90:604–608

Ravindran, V., S. Cabahug, G. Ravindran, P. H. Selle, and W. L. Bryden. 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorous levels: Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. Br. Poult. Sci. 41:193–200.

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

Santos, F. R., M. Hruby, E. E. M. Pierson, J. C. Re-mus, and N. K. Sakomura. 2008. Effect of phytase supplementation in diets on nutrient digestibility and performance in broiler chicks. J. Appl. Poult. Res. 17:191–201.

Sebastian, S., S. P. Touchburn, E. R. Chavez, and P. C. Lague. 1996. 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. Poult. Sci. 75:729–736.

Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 135:1–41.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Tech. 59:215–221.

Sommerfeld, V., S. Kunzel, M. Schollenberger, I. Kuhn, and M. Rodehutscord. 2018. Influence of phytase or myo-inositol supplements on performance and phytate degradation products in the crop, ileum, and blood of broiler chickens. Poult. Sci. 97:920–929.

Truong, H. H., R. M., Bold, S. Y. Liu, and P. H. Selle. 2015. Standard phytase inclusion in maize-based broiler diets enhances digestibility coefficients of starch, amino acids, and sodium in four small intestinal segments and digestive dynamics of starch and protein. Anim. Feed Sci. Tech. 209:240–248.

Walters, H. G., M. Coelho, C. D. Coufal, and J. T. Lee. 2019. Effects of increasing phytase inclusion levels on broiler performance, nutrient digestibility, and bone mineralization in low-phosphorus diet. J. Appl. Poult. Res. 28: 1210–1225.

Woyengo, T. A., and C. M. Nyachoti. 2013. Review: anti-nutritional effects of phytic acid in diets for pigs and poultry- current knowledge and directions for future research. Can. J. Anim. Sci. 93: 9–21.

5.7 Tables

Table 5-1. Ingredient composition of experimental diets fed to broiler chickens at grower phase (day 12-23 post hatching), g/kg as-fed basis

	Diet[1,2]			
Item	PC	NC1	NC2	NC3
Ingredients, g/kg				
Corn	531.1	479.0	476.0	473.0
Soybean meal, 480g/kg CP	325.9	276.2	272.8	269.7
Soybean oil	15.8	9.1	10.0	8.0
Rice, Polished	26.6	80.4	45.0	18.1
Rice bran	30.5	7.2	46.4	84.5
Soy hulls	1.4	38.8	41.5	39.4
Meat and bone meal	11.0	12.2	11.0	9.8
Limestone	11.0	9.6	9.9	10.2
Monocalcium phosphate	9.9	0.0	0.0	0.0
Salt	3.1	2.7	2.7	2.7
Vitamin-mineral premix[3]	3.0	3.0	3.0	3.0
DL-Methionine	3.0	2.9	2.9	3.0
L-Lysine.HCl	2.1	2.8	2.8	2.7
Threonine	0.7	0.9	0.9	0.8
L-Tryptophan	0.0	0.2	0.1	0.1
Phytase premix[4]	-	50.0	50.0	50.0
Titanium dioxide premix[5]	25.0	25.0	25.0	25.0
Total	1,000	1,000	1,000	1,000
Calculated nutrients and energy, g/kg				
CP	209.1	192.4	193.0	193.6
ME, kcal/kg	3050.0	2962.2	2962.2	2962.2
Ca	8.4	6.4	6.4	6.4
P	6.6	4.1	4.6	5.1
Phytate-P	2.8	2.3	2.8	3.3
Non-phytate P	3.8	1.8	1.8	1.8
Na	1.7	1.2	1.2	1.2
dig. Lys	11.2	10.5	10.5	10.5
dig. Met	5.8	5.4	5.5	5.5
dig. Thr	7.3	6.7	6.7	6.7

[1]PC = positive control; NC = negative control

[2]Each NC diet had 5 levels of phytase including 0, 500, 1,000, 2,000, and 4,000 phytase units (FYT)/kg.

[3]Supplied the following quantities per kg of diet: vitamin A, 5,484 IU; vitamin D_3, 2,643 ICU; vitamin E, 11 IU; menadione sodium bisulfite,4.38 mg; riboflavin, 5.49 mg; $_D$-pantothenic acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B_{12}, 13.2 µg; biotin, 55.2 µg; thiamine mononitrate, 2.2 mg; folic acid, 990 µg; pyridoxine hydrochloride, 3.3mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 µg.

[4]Each premix contained 1 g of phytase product prepared with 99 g of corn. Adding 50 g of premix/kg of NC diet supplied 0, 500, 1,000, 2,000, or 4,000 FYT, respectively.

[5]Prepared as 1 g titanium dioxide added to 4 g corn.

Table 5-2. Analyzed energy and nutrients of experimental diets fed to broiler chickens at grower phase (day 12-23 post hatching), g/kg as-fed basis

	Diet[1,2]			
Item	PC[3]	NC1[4]	NC2[5]	NC3[6]
Energy and nutrients, g/kg				
DM	900	885	886	891
GE, kcal/kg	4,090.1	3,890.0	3,956.3	3,987.1
CP	202.6	182.9	185.9	182.2
Phytate P	2.6	2.0	3.1	3.7
P	6.8	4.2	4.6	5.2
Ca	9.5	7.3	7.3	7.2
Zn	0.01	0.01	0.02	0.02
Arg	13.4	12.1	12.7	12.8
His	5.2	4.8	5.0	5.0
Ile	8.9	8.3	8.6	8.6
Leu	16.7	15.7	16.3	16.1
Lys	13.0	11.8	13.0	12.2
Met	5.1	5.3	5.8	5.6
Phe	10.1	9.2	9.6	9.6
Thr	8.2	7.4	7.7	7.6
Trp	2.5	2.4	2.3	2.4
Val	9.7	9.2	9.5	9.6
Ala	10.0	9.4	9.8	9.8
Asp	20.3	18.7	19.5	19.1
Cys	3.0	3.0	3.1	3.1
Glu	35.1	32.2	33.5	33.0
Gly	8.9	8.4	8.7	8.6
Pro	11.0	10.3	10.7	10.7
Ser	8.6	7.8	8.1	7.9
Tyr	7.0	6.3	6.6	6.6
Total AA	199.8	185.5	193.9	191.4

[1]PC = positive control; NC = negative control

[2]NC1, NC2, and NC3 had 5 levels of phytase inclusion (0, 500, 1,000, 2,000, and 4,000 phytase units (FYT)/kg); analyzed values for each NC are average of 5 diets.

[3]PC had an analyzed phytase activity of 233 phytase units/kg.

[4]NC1 diets had analyzed phytase activities of 181, 817, 1,420, 2,393, and 4,170 FYT/kg respectively

[5]NC2 diets had analyzed phytase activities of 200, 623, 1,219, 1807, and 5,060 FYT/kg respectively

[6]NC3 diets had analyzed phytase activities of 210, 684, 1,403, 2,486, and 4,641 FYT/kg respectively

Table 5-3. Main effects of phytate and phytase concentrations on growth performance and bone mineralization of broiler chickens fed experimental diets at grower phase (day 12-23 post hatching)

Diet[1]	Phytate P, g/kg	Phytase, FYT/kg	Final BW (g)	BW gain, g/bird	Feed intake, g/bird	G:F, g/kg	Tibia ash weight, mg/bone	Tibia ash, %	No. of replicates
PC[2]	2.8	0	1021	704	971	725	2,192	48.7	6
NC2	2.8	0	845	531	873	608	1,517	42.2	6
Main effect phytate									
	2.3		958	644	941	684	1,945	47.0	30
	2.8		933	620	930	666	1,959	47.3	30
	3.3		920	606	916	661	1,905	46.3	30
Main effect phytase									
		0	850	536	862	621	1,508	40.5	18
		500	934	620	930	668	1,889	46.5	18
		1,000	952	638	941	680	2,003	48.5	18
		2,000	963	650	945	688	2,132	49.1	18
		4,000	986	673	968	695	2,150	49.8	18
SEM[3]			11.94	11.90	17.47	12.44	36.56	0.75	
***P* values**									
PC vs NC2 (0 FYT/kg)			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	
Phytate x Phytase			0.33	0.32	0.48	0.48	0.62	0.17	
Phytate Linear			<0.01	<0.01	0.02	<0.01	0.09	0.14	
Phytate Quadratic			0.38	0.41	0.88	0.34	0.09	0.14	
Phytase Linear			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	
Phytase Quadratic			<0.01	<0.01	0.01	<0.01	<0.01	<0.01	

[1]PC= positive control, NC= negative control: formulated without inorganic P, with reduction of 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 available P, 2.0 g/kg Ca, and 0.5 g/kg Na vs PC diet

[2]PC contains 2.8 g/kg phytate and 0 FYT/kg, formulated with higher ME, dig AA, adequate in P and Ca

[3]SEM = standard error of mean (for the interaction)

Table 5-4. Main effects of phytate and phytase concentrations on apparent ileal digestibility (%) of DM, energy, and nutrients in broiler chickens fed experimental diets at grower phase (day 12-23 post hatching)

Diet[1]	Phytate P, g/kg	Phytase, FYT/kg	DM	Energy	Nitrogen	P	Ca	Zn	No. of replicates
PC[2]	2.8	0	67.0	71.8	76.2	60.7	50.3	30.6	6
NC2	2.8	0	63.5	66.4	73.0	32.1	33.3	20.5	6
Main effect phytate									
	2.3		66.0	69.3	75.3	58.6	43.7	26.7	30
	2.8		64.4	67.5	75.9	50.0	41.7	24.3	30
	3.3		64.1	67.3	74.8	41.7	37.9	22.7	30
Main effect phytase									
		0	63.8	66.9	72.4	32.7	33.2	21.1	18
		500	64.5	67.3	74.5	44.2	37.8	24.0	18
		1,000	65.3	68.0	75.3	52.1	42.1	24.9	18
		2,000	64.5	68.6	76.6	57.4	44.9	26.0	18
		4,000	66.2	69.3	77.9	64.1	47.5	27.0	18
SEM[3]			0.95	0.89	1.25	3.10	5.01	3.72	
***P* values**									
PC vs NC2 (0 FYT/kg)			0.01	<0.01	0.08	<0.01	0.02	0.06	
Phytate x Phytase			0.91	1.00	0.61	0.94	1.00	1.00	
Phytate Linear			<0.01	<0.01	0.49	<0.01	0.07	0.10	
Phytate Quadratic			0.19	0.11	0.20	0.94	0.75	0.85	
Phytase Linear			0.01	<0.01	<0.01	<0.01	<0.01	0.05	
Phytase Quadratic			0.91	0.80	0.68	0.07	0.61	0.65	

[1]PC= positive control, NC= negative control: formulated without inorganic P, with reduction of 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 available P, 2.0 g/kg Ca, and 0.5 g/kg Na vs PC diet

[2]PC contains 2.8 g/kg phytate and 0 FYT/kg, formulated with higher ME, dig AA, adequate in P and Ca

[3]SEM = standard error of mean (for the interaction)

Table 5-5. Effect of phytate and phytase concentrations on apparent ileal digestibility (%) of indispensable AA in broiler chickens fed experimental diets at grower phase (day 12-23 post hatching)

Diet[1]	Phytate P, g/kg	Phytase, FYT/kg	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	No. of replicates
PC[2]	2.8	0	84.4	79.8	76.6	78.5	81.3	89.6^{DEFG}	78.7	72.6^{BCD}	82.8	72.9	6
NC1	2.3	0	82.7	74.7	73.5	75.1	78.6	88.0^{G}	75.6	66.6^{GH}	80.1	69.3	6
		500	83.7	76.8	75.5	77.5	79.4	89.0^{EFG}	77.7	69.0^{EFG}	80.9	71.3	6
		1,000	86.6	79.3	78.2	79.7	82.3	89.2^{EFG}	80.1	71.7^{CDE}	83.1	73.9	6
		2,000	86.5	78.9	78.8	80.1	81.9	89.7^{DEFG}	80.7	71.8^{CDE}	82.3	73.9	6
		4,000	88.0	81.4	81.3	82.0	83.3	91.2^{ABCD}	83.3	75.4^{AB}	84.5	77.0	6
NC2	2.8	0	83.0	75.7	74.3	75.8	80.0	88.4^{FG}	76.0	68.0^{FG}	79.1	70.3	6
		500	84.1	77.2	75.9	77.8	81.1	90.6^{BCDE}	77.8	70.2^{DEF}	79.7	71.6	6
		1,000	85.5	79.2	77.8	79.8	82.2	89.1^{EFG}	80.1	71.0^{CDEF}	81.3	73.0	6
		2,000	87.3	81.5	79.8	81.5	83.8	91.9^{ABC}	81.9	75.7^{AB}	82.4	75.9	6
		4,000	89.4	83.2	82.7	83.6	86.0	92.0^{AB}	84.2	75.8^{AB}	84.8	78.6	6
NC3	3.3	0	80.3	73.4	71.2	73.3	74.9	84.7^{H}	72.8	63.9^{H}	77.8	66.8	6
		500	82.5	76.0	73.6	75.8	78.4	89.3^{EFG}	75.4	66.5^{GH}	81.1	70.0	6
		1,000	84.8	79.2	77.6	79.0	80.4	90.2^{CDEF}	79.0	71.7^{CDE}	83.6	73.4	6
		2,000	88.5	83.5	82.1	82.9	84.2	92.6^{A}	83.5	77.2^{A}	84.1	78.3	6
		4,000	87.3	81.3	79.4	81.1	82.5	91.2^{ABCD}	81.6	74.3^{ABC}	84.3	75.6	6
Main effect of phytate													
	2.3		85.5	78.2	77.4	78.9	81.1	89.4	79.5	70.9	82.2	73.1	30
	2.8		85.9	79.4	78.1	79.7	82.6	90.4	80.0	72.1	81.5	73.9	30
	3.3		84.7	78.7	76.8	78.4	80.1	89.6	78.5	70.7	82.2	72.8	30
Main effect of phytase													
		0	82.0	74.6	73.0	74.7	77.8	87.0	74.8	66.1	79.0	68.8	18
		500	83.4	76.7	75.0	77.0	79.6	89.6	77.0	68.6	80.6	71.0	18
		1,000	85.7	79.3	77.9	79.5	81.6	89.5	79.8	71.5	82.6	73.4	18
		2,000	87.5	81.3	80.3	81.5	83.3	91.4	82.0	74.9	82.9	76.0	18
		4,000	88.2	81.9	81.1	82.2	83.9	91.5	83.1	75.2	84.5	77.1	18
SEM[3]			0.76	0.97	1.09	1.00	1.01	0.65	0.96	1.25	0.88	1.19	

Table 5-5 continued

***P* values**										
PC vs NC2 (0 FYT/kg)	0.18	<0.01	0.13	0.06	0.36	0.19	0.05	0.01	<0.01	0.12
Phytate x Phytase	0.11	0.11	0.14	0.36	0.12	<0.01	0.14	0.04	0.35	0.12
Phytate Linear	0.10	0.46	0.33	0.46	0.12	0.68	0.09	0.81	0.99	0.75
Phytate Quadratic	0.07	0.09	0.10	0.06	<0.01	0.01	0.05	0.06	0.14	0.17
Phytase Linear	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Phytase Quadratic	0.29	0.11	0.23	0.09	0.22	0.03	0.18	0.16	0.39	0.41

[A-H]Simple effect means within a column with different superscripts differ ($P < 0.05$).

[1]PC= positive control, NC= negative control: formulated without inorganic P, with reduction of 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 available P, 2.0 g/kg Ca, and 0.5 g/kg Na vs PC diet

[2]PC contains 2.8 g/kg phytate and 0 FYT/kg, formulated with higher ME, dig AA, adequate in P and Ca

[3]SEM = standard error of mean (for the interaction)

Table 5-6. Effect of phytate and phytase concentrations on apparent ileal digestibility (%) of dispensable and total AA in broiler chickens fed experimental diets at grower phase (day 12-23 post hatching)

Diet[1]	Phytate P, g/kg	Phytase, FYT/kg	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr	Total AA	No. of replicates
PC[2]	2.8	0	77.4	75.1	66.0^{DE}	83.3	71.2	77.5	78.1	79.9	78.2	6
NC1	2.3	0	73.0	71.9	56.5^{H}	80.5	63.4	72.0	70.7	74.8	74.2	6
		500	75.9	74.0	60.8^{FG}	82.0	66.5	75.3	73.8	76.8	76.3	6
		1,000	78.0	77.3	63.2^{EF}	84.6	70.3	77.4	76.8	80.0	78.8	6
		2,000	78.3	77.6	63.6^{EF}	84.7	70.4	77.4	76.9	79.6	78.9	6
		4,000	79.9	80.2	71.1^{ABC}	86.3	73.0	79.9	79.8	82.3	81.3	6
NC2	2.8	0	74.4	73.0	58.2^{GH}	81.1	65.6	73.6	72.2	76.3	75.2	6
		500	76.1	74.3	63.2^{EF}	82.4	67.4	75.2	75.0	78.0	76.8	6
		1,000	78.0	77.0	65.7^{DE}	84.4	70.2	77.7	77.3	79.4	78.6	6
		2,000	79.5	79.1	69.4^{BCD}	85.8	72.3	79.1	80.5	81.0	80.7	6
		4,000	82.0	81.1	73.4^{AB}	87.6	75.1	81.0	81.1	83.3	82.7	6
NC3	3.3	0	71.8	69.7	55.7^{H}	78.6	62.3	71.3	67.4	73.3	72.0	6
		500	75.0	71.2	58.3^{GH}	80.7	66.4	74.1	72.0	76.0	74.8	6
		1,000	77.4	76.1	68.0^{CD}	83.7	69.4	77.4	76.6	79.2	78.2	6
		2,000	80.9	80.8	74.4^{A}	87.0	74.7	81.1	80.9	83.3	82.3	6
		4,000	79.5	78.1	69.2^{CD}	85.3	71.9	79.6	79.5	81.7	80.3	6
Main effect of phytate												
	2.3		77.0	76.2	63.0	83.6	68.7	76.4	75.6	78.7	77.9	30
	2.8		78.0	76.9	66.0	84.2	70.1	77.3	77.2	79.6	78.8	30
	3.3		76.9	75.2	65.1	83.0	68.9	76.7	75.3	78.7	77.5	30
Main effect of phytase												
		0	73.0	66.1	56.8	80.0	63.8	72.3	70.1	74.8	73.8	18
		500	75.7	68.6	60.8	81.7	66.8	74.9	73.6	76.9	76.0	18
		1,000	77.8	71.5	65.6	84.2	70.0	77.5	76.9	79.6	78.5	18
		2,000	79.6	74.9	69.1	85.8	72.5	79.2	79.4	81.3	80.6	18
		4,000	80.5	75.2	71.2	86.4	73.3	80.2	80.1	82.4	81.4	18
SEM[3]			1.10	0.98	1.50	0.74	1.21	0.94	1.04	0.93	0.96	

Table 5-6 continued

***P* values**									
PC vs NC2 (0 FYT/kg)	0.05	0.14	<0.01	0.04	<0.01	<0.01	<0.01	0.01	0.03
Phytate x Phytase	0.60	0.07	<0.01	0.12	0.23	0.29	0.06	0.10	0.13
Phytate Linear	0.87	0.11	0.03	0.22	0.76	0.62	0.63	0.99	0.51
Phytate Quadratic	0.09	0.03	0.02	0.03	0.05	0.15	<0.01	0.08	0.04
Phytase Linear	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Phytase Quadratic	0.12	0.12	0.13	0.06	0.06	0.04	0.01	0.15	0.13

[A-H]Simple effect means within a column with different superscripts differ (P < 0.05).

[1]PC= positive control, NC= negative control: formulated without inorganic P, with reduction of 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 available P, 2.0 g/kg Ca, and 0.5 g/kg Na vs PC diet

[2]PC contains 2.8 g/kg phytate and 0 FYT/kg, formulated with higher ME, dig AA, adequate in P and Ca

[3]SEM = standard error of mean (for the interaction)

Table 5-7. Main effects of phytate and phytase concentrations on total tract retention (%) of DM, energy, and nutrients in broiler chickens fed experimental diets at grower phase (day 12-23 post hatching)

Diet[1]	Phytate P, g/kg	Phytase, FYT/kg	DM	AME, kcal/kg DMI	AMEn, kcal/kg DMI	Nitrogen	P	Ca	Zn	No. of replicates
PC[2]	2.8	0	73.9	3,377	3,183	70.9	59.3	42.6	32.7	6
NC2	2.8	0	72.4	3,294	3,108	68.2	25.4	20.4	22.2	6
Main effect of phytate										
	2.3		73.5	3,335	3,149	68.1	58.4	46.6	30.3	30
	2.8		72.9	3,359	3,169	69.3	47.1	36.3	31.0	30
	3.3		72.4	3,350	3,158	70.5	36.9	30.9	27.4	30
Main effect of phytase										
		0	72.1	3,291	3,104	68.2	27.6	21.3	22.4	18
		500	72.6	3,316	3,128	68.8	43.9	35.1	29.2	18
		1,000	72.7	3,358	3,169	69.3	51.7	40.8	31.1	18
		2,000	73.6	3,372	3,179	70.6	55.4	45.0	32.5	18
		4,000	73.6	3,403	3,214	69.4	58.7	47.5	32.6	18
SEM[3]			0.72	3.14	2.83	1.41	2.17	2.95	3.43	
***P* values**										
PC vs NC2 (0 FYT/kg)			0.14	0.07	0.07	0.19	<0.01	<0.01	0.03	
Phytate x Phytase			0.99	0.91	0.84	1.00	0.07	0.97	1.00	
Phytate Linear			0.01	0.46	0.65	<0.01	<0.01	<0.01	0.18	
Phytate Quadratic			0.89	0.36	0.31	0.98	0.64	0.14	0.26	
Phytase Linear			<0.01	<0.01	<0.01	0.11	<0.01	<0.01	<0.01	
Phytase Quadratic			0.91	0.82	0.90	0.35	<0.01	<0.01	0.06	

[1]PC= positive control, NC= negative control: formulated without inorganic P, with reduction of 88 kcal/kg ME, 0.8 g/kg dig. Lys, 2.0 available P, 2.0 g/kg Ca, and 0.5 g/kg Na vs PC diet

[2]PC contains 2.8 g/kg phytate and 0 FYT/kg, formulated with higher ME, dig AA, adequate in P and Ca

[3]SEM = standard error of mean (for the interaction)

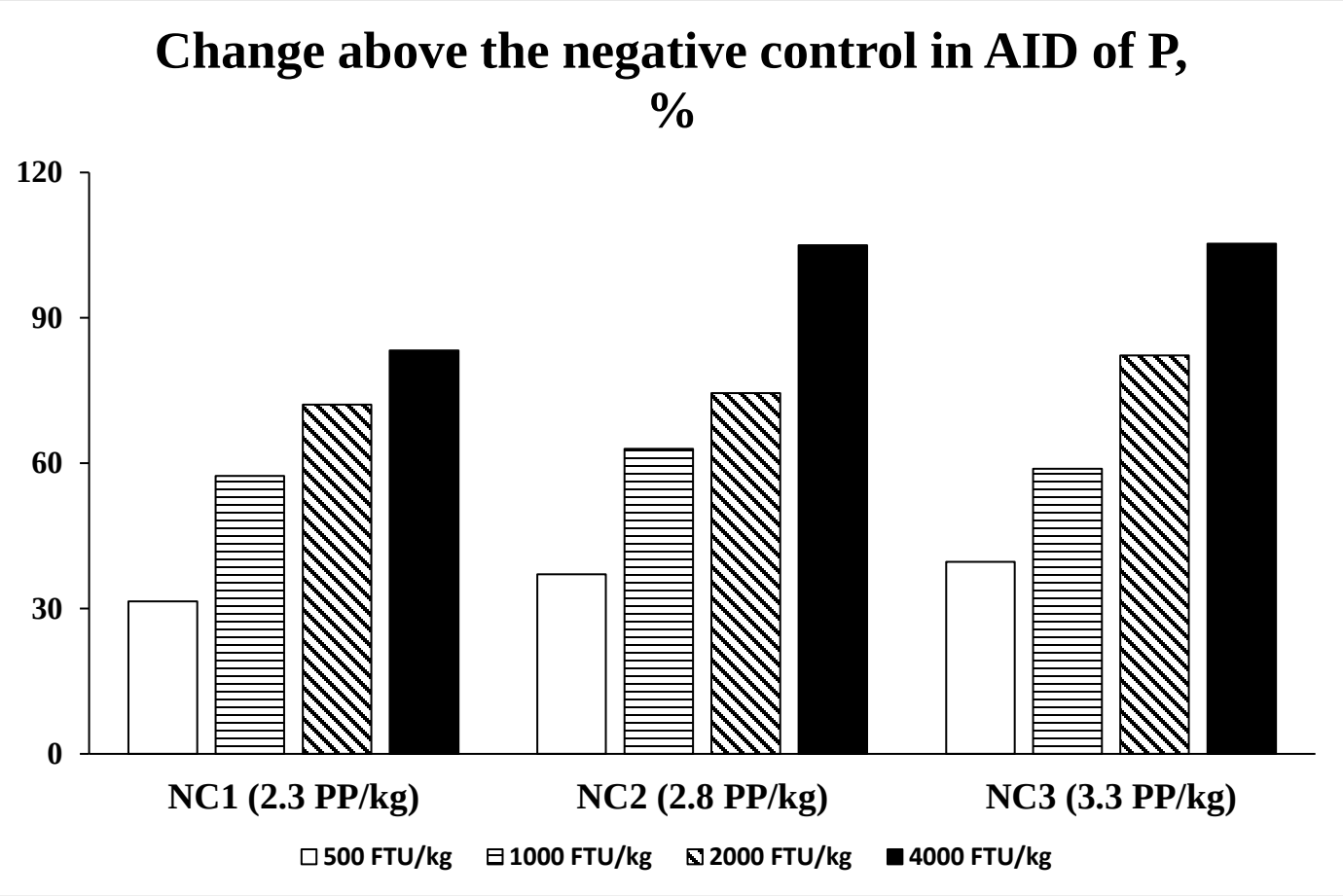


Figure 5-1. The efficacy of phytase (PhyG) on the apparent ileal digestibility (AID) of P relative to the phytate P (PP) concentration in each of the NC diets. The percentage difference values were derived by subtracting the AID of P in the NC diets (i.e. NC1, NC2, or NC3) with 0 FYT/kg from 500, 1,000, 2,000, or 4,000 FYT/kg diet, in each block within each NC diet.

CHAPTER 6. A TIME-SERIES EFFECT OF PHYTASE SUPPLEMENTATION ON PHOSPHORUS UTILIZATION IN GROWING AND FINISHING PIGS FED A LOW-PHOSPHORUS DIET

6.1 Abstract

Two experiments (Exp) were carried out to determine a time-series effect of phytase on phosphorus (P) utilization in growing and finishing pigs using growth performance, apparent total tract digestibility (ATTD) of nutrients, P excretion, and plasma concentrations of minerals as the response criteria for evaluation. In both Exp., treatments were arranged as a 3 × 4 factorial in a randomized complete block design with 3 corn-soybean meal based diets including a P-adequate positive control (PC), a low-P negative control (NC; no inorganic P), and NC supplemented with phytase at 1,000 FYT/kg (NC + 1,000); and 4 sampling time points at d 7, 14, 21, and 28 in Exp. 1, and d 14, 26, 42, and 55 in Exp. 2. In both trials, 96 growing pigs with average BW of 19.8 ± 1.16 kg and 49.8 ± 3.21 kg, respectively, were allocated to the 3 diets with 8 replicates pens (4 barrows and 4 gilts) and 4 pigs per pen. In Exp. 1, pigs fed the PC had higher ($P < 0.01$) body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F) as compared with pigs fed the NC. There was an interaction ($P < 0.01$) between time and diet on the BW and ADG of pigs while a linear and quadratic increase ($P < 0.01$) was observed with the ADFI and G:F, respectively, over time. Phytase supplementation improved ($P < 0.01$) all growth performance responses. Pigs fed the PC had greater ($P < 0.01$) ATTD of P and Ca than pigs fed the NC. There was no interaction effect on the ATTD of nutrients. Phytase addition improved the ATTD of P and Ca over pigs fed the NC. There was an interaction ($P < 0.01$) between diet and time on the total and water-soluble P excreted. There was a quadratic decrease ($P < 0.01$) in plasma concentration of Ca in pigs over time. In Exp. 2, there was a quadratic increase ($P <$

0.01) in BW, ADG, and G:F of pigs over time. Similarly, the inclusion of phytase improved ($P < 0.05$) all growth performance parameters except ADFI. A linear increase ($P < 0.05$) in the ATTD of DM, P and Ca occurred over time. Phytase inclusion improved ($P < 0.01$) the ATTD of P and Ca. Plasma concentrations of P was improved by phytase addition. Phytase supplementation of the NC reduced water-soluble P excretion by 45, 32, and 35 % over the growing, finishing, and entire grow-finish period, respectively. In conclusion, phytase improves the utilization of P in growing and finishing pigs however, the magnitude of effect on responses may vary over time.

Key words: apparent total tract digestibility, growth performance, phosphorus, phytase, pigs, time

6.2 Introduction

Phosphorus (P) remains one of the most widely researched nutrients in monogastric nutrition due to its importance in several biochemical reactions and its debilitating effects on the environment if not properly managed. The swine industry, which is one of the fastest growing areas in the meat industry, produced over 100 million metric tons of pork products worldwide in 2020 (Shahbandeh, 2021). This was accompanied by vast amounts of waste in the form of manure that while beneficial for agronomic purposes, could contain high amounts of nutrients such P and nitrogen with environmental consequences when improperly managed. The use of phytase in the diets of pigs is common as it has been proven to improve growth performance, nutrient utilization, and bone mineralization of pigs at different physiological stages, while reducing the amount of P lost into the environment (Harper et al., 1997; Jendza et al., 2005; Kim et al., 2017). This is because phytase can hydrolyze the phytic bonds from phytate present in most feed ingredients from the cereals and oilseeds families thus, releasing P and other nutrients, and increasing its utilization in pigs or poultry (Adedokun et al., 2015; Babatunde et al., 2019a).

The growth phase of pigs is usually differentiated into weanling, growing, and finishing phases, and each phase is peculiar when the growth curve of pigs and the utilization of nutrients are considered. The growing and finishing phases are characterized by the high consumption of feed by pigs which results in rapid growth and muscle deposition. Thus, investigating the utilization of nutrients such as P in these phases are of paramount importance. Although several studies have investigated the effects of phytase in growing and finishing pigs (Kemme at al., 1999; Kim et al., 2017; Wensley et al., 2020), there is little information on the effects of phytase on P utilization at different time points within each growth phase of pigs. It is important to have this information because it helps researchers and commercial producers pinpoint the times at which phytase is most efficacious during each growth phase. This information could also play a role in deciding when to alter phytase doses in swine diets during a particular time point in the growing or finishing phases due to the objectives of the producer or researcher. Lastly, the loss of P into the environment from swine production, particularly in its soluble form, has been a cause of concern to researchers. Some studies have investigated the effects of phytase on the loss of soluble and insoluble P in the manure of pigs (Angel et al., 2005; Powers et al., 2006; Jendza et al., 2009). However, there is little or no information on how phytase may affect the form or quantity of P being lost into the environment at different time points within the growing and finishing phases of pigs.

Thus, the objective of this study was to investigate a time-series effect of phytase supplementation on P utilization in growing and finishing pigs fed a low-P diet. Two independent trials were conducted and growth performance, nutrient utilization, P excretion, and plasma concentration of minerals were the evaluation criteria. We hypothesized that there will be no impact of time on the effects of phytase on growing and finishing pig responses.

6.3 Materials and Methods

All protocols used in this study were approved by the Purdue University Animal Care and Use Committee.

6.3.1 Experiment 1

A total of 96 growing pigs (48 barrows and 48 gilts) with an average initial body weight (BW) of 19.8 ± 1.16 kg were assigned to 3 treatments. Four replicate pens (1.7 × 3.0 m) each of barrows and gilts with 4 pigs per pen were used in a randomized complete block design. Treatments were arranged as a 3 × 4 factorial with 3 diets and 4 sampling time points. Dietary treatments consisted of a positive control (PC) with adequate supply of all nutrients including total calcium (Ca) and available P at 7.05 and 3.3 g/kg, respectively; a negative control (NC), similar to the PC but with all the inorganic P removed, hence a reduced available P of 1.5 g/kg; and a NC supplemented with phytase at 1,000 phytase units (FYT)/kg (RONOZYME® HiPhos, DSM Nutritional Products, Switzerland). All diets were in mash form and formulated to meet the nutrients requirements of 25- to 50-kg growing pigs as recommended by NRC (2012) except for P and Ca in the NC diets (Table 1). Calcium to available P ratio was maintained at 2:1 in all diets. Titanium dioxide was included in all diets at 5 g/kg as an indigestible marker. Pigs were blocked by weight and sex and assigned to pens such that the average weight across all treatments were similar. Pigs had *ad libitum* access to water and experimental diets for 28 d with BW and feed intake (FI) recorded at 4 time points (d 7, 14, 21, and 28) to determine the average daily gain (ADG) and gain to feed ratio (G:F). Blood was collected via the anterior vena cava into EDTA tubes at d 0 from one median weight pig per pen, and at each time point (i.e., d 7, 14, 21, and 28) from the same pig. Plasma, used in analyzing the concentrations of P and Ca, was obtained by centrifugation of blood samples at 3,000 x g for 15 min at 4˚C (Babatunde et al., 2019a) and stored at −80˚C until

further analyses. Similarly, fecal samples were collected at the same time points via rectal palpations from the same pig that had blood drawn. Fecal samples were stored in −20˚C until further analysis and determination of the apparent total tract digestibility (ATTD) of nutrients.

6.3.2 Experiment 2

A total of 96 finishing pigs (48 barrows and 48 gilts) with an average initial BW of 49.8 ± 3.21 kg were assigned to 3 treatments in a randomized complete block design with 8 replicate pens (split evenly between barrows and gilts) and 4 pigs per pen. Treatments were arranged as a 3 × 4 factorial with 3 diets and 4 sampling time points. Experimental diets were similar to those in Exp. 1 but were fed in 2 phases as determined by the nutrient requirements of pigs (NRC, 2012). Diets in phases 1 and 2 were formulated to meet the nutrient requirements of 50- to 75-kg kg and 75- to 100-kg growing pigs, respectively, except for the NC diets with reduced available P at 1.37 g/kg and 1.22 g/kg, respectively (Table 1). Calcium to available P ratio was maintained at 2:1 in all diets. Titanium dioxide was included in all diets at 5 g/kg as an indigestible marker. Pigs had *ad libitum* access to water and experimental diets until d 26 in phase 1 and then phase 2 diets were introduced and fed until d 55. Initially, pigs were supposed to be fed phase 1 diets until d 28 and phase 2 diets until d 56, but the experimental period had to be slightly reduced because of the nationwide lockdowns mandated during the corona virus pandemic. Samples were collected using the same methodology as Exp. 1. Body weight and feed consumption were recorded at 4 time points (d 14, 26, 42, and 55) to determine the average daily gain and feed efficiency. Fecal samples, used to determine the ATTD of nutrients, was collected at the same time points from 1 pig per pen previously assigned at d 0. Blood samples, used to determine the plasma concentration of P and Ca, were collected from the same pig per pen at d 0 and at 2 time points (d 26 and 55).

6.3.3 Chemical Analyses

Fecal samples were dried in a forced air oven at 56˚C for 7 d. Diet and dried fecal samples were finely ground through a 0.5-mm screen in a centrifugal grinder (Retsch ZM 200; Retsch GmbH, Haan, Germany). Dry matter (DM) concentration was determined in the diets and fecal samples by drying in a forced-air drying oven for 24 h at 105°C (Precision Scientific Co., Chicago, IL; method 934.01; AOAC, 2006). Gross energy of diet samples was determined by an isoperibol bomb calorimeter using benzoic acid as the calibration standard (Parr 1261; Parr 105 Instrument Co., Moline, IL). Nitrogen concentrations in the diet samples were determined using the combustion method (TruMac N; LECO Corp., St. Joseph, MI; method 984.13A-D; AOAC, 2006), using EDTA as a calibration standard and values were multiplied by a factor of 6.25 to estimate the CP contents. Titanium concentrations in the diet and fecal samples were determined using methods previously described by Short et al. (1996). Calcium and P concentrations in diets and fecal samples were determined using methods previously described by Babatunde et al. (2021). Water-soluble P (WSP) in feces was determined as described by Jendza and Adeola, (2009). Plasma concentrations of P and Ca were determined in methods previously described by Sands et al. (2001).

6.3.4 Calculation and Statistical Analyses

The ATTD (%) of nutrients in the experimental diets were determined using the following equations (Adeola, 2001):

$$\text{ATTD, \%} = 100 - [(Ti_I/Ti_O) \times (N_O/N_I) \times 100]$$

Where Ti_I and Ti_O are the concentrations of titanium (g/kg DM) in diets and feces, respectively; N_i and N_o are the concentration of nutrients (g/kg DM) in diets and feces, respectively. In Exp. 1

and 2, the estimated total P excreted (g/period) in growing pigs fed the PC was calculated as follows:

$$\text{Total P excreted, g/period} = ADFI_{time} \times D_{time} \times PI \times [1- PRet_{time}]$$

Where $ADFI_{time}$ is the average daily feed intake for each time point, D_{time} is the number of days during each time point, PI is the analyzed P of the intake in g/kg at each phase of the current study, $PRet_{time}$ is the proportion of P retained at each time point, determined by multiplying the coefficient of the ATTD of P with a factor of 99.9%. This factor is the average retention of digested P from a P balance trial carried out in grower and finisher pigs fed experimental diets similar to the current study (Jendza and Adeola, 2009).

The estimated total P excretion (g/period) for growing pigs fed either the NC or NC + 1,000 diets was calculated using the following formula:

$$\text{Total P excreted, g/period} = [(ADFI_{time} \times D_{time}) + (Diff_{BW}/G{:}F_{time})] \times PI \times [1- PRet_{time}]$$

Where $Diff_{BW}$ is the BW of pigs fed the PC minus the BW of pigs fed either the NC or NC +1,000 diets at each time point while $G{:}F_{time}$ is the gain to feed ratio at each time point; and $ADFI_{time}$, D_{time}, PI, and $PRet_{time}$ are as defined above. The extra complexity in the calculation of the estimated total P excreted is because the BW of pigs fed the NC, or the NC + 1,000 diets were slightly different from that of the pigs fed the PC at each time point. The modifications to the equation account for the extra days and FI required for the pigs fed the NC or phytase supplemented NC to attain parity with the BW of pigs fed the PC at each time point. Thus, it accounts for BW and FI differences among treatments. Estimates for WSP excreted by growing pigs at each time point in both Exp. was determined by multiplying the total P excretion estimates described above by the percentage of total P in the WSP form.

All Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) as a 3 × 4 factorial with 3 diets, 4 sampling time points, and their interaction as a fixed variables except for the plasma mineral concentrations in Exp. 2 which was analyzed as a 3 × 2 factorial with 3 diets and 2 sampling time points. Blocks by BW and sex served as random variables while pen was the experimental unit for all analyses. Contrasts were used to examine the effects of diets (PC vs. NC; PC vs. NC + 1,000; and NC vs. NC + 1,000) and the linear and quadratic effects of time on pig responses. Statistical significance was declared at $P \leq 0.05$ and a trend was set at $0.05 < P \leq 0.1$.

6.4 Results

All pigs were healthy and readily consumed diets throughout the duration of both experiments.

6.4.1 Experiment 1

The average initial BW of pigs was 19.8 ± 1.16 kg. Growing pigs fed the PC had a greater ($P < 0.01$) BW, ADG, average daily feed intake (ADFI), and G:F as compared to pigs fed the NC (Table 2). As pigs grew from d 0 until 28, the difference in BW of pigs fed the NC as compared with pigs fed PC or NC + 1000 diets increased with time thus, resulting in a time × diet interaction ($P < 0.01$). A 17 or 22 % difference was observed between the ADG of pigs fed the NC and PC, or the NC and phytase supplemented NC at d 7, respectively. In addition, a 37 or 38 % difference in ADG was observed for both groups, respectively, at d 28 resulting in an interaction ($P < 0.01$) between time and diet. A linear increase ($P < 0.01$) in ADFI was observed over time with pigs consuming 0.85 kg/d at d 7 and up to 1.64 kg/d at d 28. Similarly, pigs fed the NC + phytase diets had increased ($P < 0.01$) ADFI as compared with pigs fed the NC diets (1.17 vs. 1.32kg). There was a quadratic decrease ($P < 0.01$) in feed efficiency as pigs grew from d 0 to 28 while an overall

improvement in G:F ($P < 0.01$) was observed when comparing pigs fed the NC and the phytase supplemented NC. There were no differences in the growth performance of pigs fed the PC and the phytase-supplemented NC diets.

There was no difference in the ATTD of DM among diets fed to pigs (Table 3). There was no interaction between time and diets on the ATTD of DM however, there was a linear increase ($P < 0.01$) in the ATTD of DM over time. Pigs fed the NC had lower ($P < 0.01$) ATTD of P and Ca as compared to pigs fed the PC however, the supplementation of phytase improved ($P < 0.01$) the ATTD of P and Ca. There was no effect of time on P and Ca digestibility. The effects of diets on total P excretion increased with time thus, resulting in a time × diet interaction ($P < 0.01$). A similar interaction between time and diet occurred with the WSP excreted. Pigs fed phytase supplemented NC diets had a lower ($P < 0.01$) amount of WSP excreted per day as compared with pigs fed the PC or NC diets. Pigs fed the PC had an increased ($P < 0.01$) plasma concentration of P but not Ca when compared with pigs fed the NC (Fig. 1). There was no interaction between time and diet on the plasma concentrations of P and Ca in pigs, but the inclusion of phytase improved ($P < 0.01$) the plasma concentration of P in pigs as compared with those fed the NC diets (Fig. 1A). There was a quadratic trend ($P < 0.1$) on the plasma concentrations of P over time with increases from d 7 to 14 and a peak at d 21. Meanwhile, a quadratic decrease ($P < 0.01$) was observed on the plasma concentrations of Ca over time (Fig. 1B).

6.4.2 Experiment 2

The average initial BW of pigs in this trial was 49.8 ± 3.21 kg. A significant difference ($P < 0.05$) was observed for all growth performance parameters between pigs fed the PC and NC in Exp. 2 (Table 4). A trend ($P < 0.1$) was observed in the interaction between time and diet on the BW and ADG of pigs, as the magnitude of differences within the BW and ADG of pigs fed the PC

and NC, and the NC and NC + phytase, respectively increased over time. However, a quadratic increase ($P < 0.01$) in the BW and ADG of pigs was observed over time while phytase improved ($P < 0.05$) both parameters in pigs fed phytase supplemented NC diets as compared to pigs fed the NC diets. There was a linear increase ($P < 0.01$) in the ADFI of pigs over time however, phytase inclusion did not affect the ADFI. As pigs grew from d 0 to 55, the feed efficiency was reduced quadratically ($P < 0.01$) by approximately 15 % however, the inclusion of phytase improved the feed efficiency of pigs by 11 % as compared with pigs fed the NC.

As observed in Exp. 1, there was no interaction or effect of diet on the ATTD of DM however, a linear increase ($P < 0.01$) was observed over time. Pigs fed the NC had lower ($P < 0.01$) ATTD of P and Ca as compared with pigs fed the PC diets (Table 5). Although, there was no interaction between time and diets on the ATTD of P and Ca, there was a linear increase ($P < 0.05$) in the digestibility of both minerals over time. Phytase supplementation in the NC diet also improved ($P < 0.01$) the ATTD of P and Ca by 135 and 46 %, respectively, as compared with pigs fed the NC diets without phytase. There were interactions ($P < 0.01$) between time and diets on the total P and WSP excretion with changes to the effects of diets on P excreted at each time point. The ratio of water soluble to total P in the feces differed among pigs fed experimental diets with pigs fed the PC having the lowest values while pigs fed the phytase diets having the highest values. However, this ratio reduced over time resulting in an interaction effect ($P < 0.01$). Phytase supplementation of the NC reduced total P excretion by 49, 41, or 42 % over the growing, finishing, and entire grow-finish period, respectively(Fig. 2A). Similarly, a 45, 32, and 35 % reduction in WSP excretion was observed between pigs fed the PC and NC + 1,000 diets at the growing, finishing, and entire grow-finish period, respectively (Fig. 2B). Pigs fed the PC had a higher ($P < 0.01$) plasma P as compared with pigs fed the NC (Fig. 3A). Although the interaction between time

and diet was not significant, a trend was observed ($P < 0.1$) as the concentration of P in the plasma of pigs fed the PC and NC + phytase diets increased slightly over time but reduced in pigs fed the NC diets during the same period. However, there was an increase ($P < 0.05$) in plasma P from d 28 to 55 and phytase inclusion improved ($P = 0.04$) the concentration of plasma P in pigs. There was no difference in the concentrations of Ca in the plasma of pigs fed the PC and NC, nor was there an interaction between diet and time (Fig. 3B). However, phytase inclusion increased ($P < 0.03$) the plasma concentrations of Ca over the pigs fed the PC.

6.5 Discussion

The use of phytase in monogastric nutrition is widespread due to its proven benefits in improving productivity and reducing manure P. Phytase is known to hydrolyze the phytate complex present in most cereals and oilseeds thus releasing P and other nutrients in the upper section of the gastrointestinal tract (Adeola and Cowieson, 2011). Pigs are unable to effectively hydrolyze the phytate compounds in feed ingredients due to an inadequacy of endogenous enzymes capable of breaking the phytic bonds hence, the increased use of exogenous phytase in swine diets. Although there is a lot of information on the effects of phytase on the performance and nutrient utilization of pigs (Almeida et al., 2013; Humer et al., 2015), there is little information that reveals the effects of phytase on responses of pigs at different time points within the growing and finishing phases. Even though it is common practice to include phytase in the diets of pigs until market weight, it may be important to know if there are changes in the effects of phytase on P utilization at various time points during each phase as it could help farmers decide when or how much phytase to include at a particular time for optimum productivity. It could also be that there are slight changes in the P release capacity of phytase, or in the amount of P being lost into the environment

by pigs over intermittent periods of time. Thus, information from this study will contribute to the literature, and may provide more insight on the action of phytase on P utilization.

Growth performance is often used as an indicator of phytase effects on P utilization particularly when P deficient diets are fed to pigs (Jones et al., 2010). Pigs fed the low-P diets in the grower or finisher phases had reduced performance as compared to pigs fed P-adequate diets which indicates the importance of P bioavailability to the productivity of pigs and as observed with previous studies (Jendza et al., 2005; Brana et al., 2006; Blavi et al., 2019). There were interactions between time and diets on BW and ADG in pigs at the grower phase and tendencies for interactions in pigs at the finisher phase. It was observed that the impact of P deficiency on growth performance seemed to increase at each time point in both trials. For instance, in Exp. 1 and 2, there was approximately a 17 and 15 % difference between the ADG of pigs fed the PC and NC diets at d 7 and 14, respectively, while at d 28 and 55, the difference had increased to 37 and 26 %, respectively. Similarly, the impact of phytase in ameliorating the effects of the P deficiency on growth performance seemed to increase at each time point in both trials. When examining the growth curve of pigs, the growing and finishing phases are characterized by rapid growth and development which requires that adequate nutrients be supplied to meet these physiological needs. A disturbance in the supply of a nutrient such as P, either due to the presence of phytate or the absence of inorganic P (IP), could negatively impact the growth curve of pigs even when other nutrients are in adequate supply. hereby indicating the important role P plays in several biochemical processes necessary to sustain life (Jendza et al., 2005; Brana et al., 2006; Blavi et al., 2019). This means that as pigs grow older, the impact of P deficiency only gets worse as pigs struggle to meet their physiological needs and catch up with other pigs fed P adequate diets.

It is logical that the impact of phytase on growth performance would follow similar trends as the absence of available P since the release of P and other nutrients by phytase would have met the increasing demand of pigs as they grew older. From the current trials, the impact of P deficiency and the efficacy of phytase on growth performance were more evident during the grower phase (Exp. 1) than the finisher phase (Exp. 2). This suggests that age may impact the efficacy of phytase on growth performance as have been observed with previous trials in pigs (Cambra-Lopez et al., 2020) and in broiler chickens (Babatunde et al., 2019a,b). It was also evident that supplementing pig diets with phytase while completely removing inorganic P supported the growth performance of pigs as compared with pigs fed diets with inorganic P in both trials.

Several studies have used the ATTD of nutrients particularly P and Ca as indicators of phytase efficiency in swine (Kerr et al., 2010; Almeida et al., 2013; Wensley et al., 2020). Although there were no interactions between time and diet on the ATTD of nutrients in both experiments, there were effects of either diet or time on the ATTD of nutrients. As observed by Tsai et al. (2020), there was no effect of P status in diets or phytase supplementation on the ATTD of DM in both trials however, the ATTD of DM increased over time in both trials. This could be indicative of the increased demand of nutrients and the efficiency in utilizing the nutrients present in diets as pigs grew older. There was an effect of diets (P deficiency and phytase supplementation) on the ATTD of P and Ca in pigs at both phases and as observed in previous trials (Olukosi et al., 2007; Arredondo et al., 2019). The presence of phytate has been known to hinder the digestibility of P, Ca, and other nutrients in pigs as they bind tightly to the phytic hexose structures, thus preventing them from being hydrolyzed and utilized by pigs (Selle and Ravindran., 2008). However, the presence of phytase in diets was able to break the phytate bonds thus, increasing the digestibility of both P and Ca in pigs at both growth phases. There was no effect of time on the

ATTD of P and Ca in pigs at the growing phase (Exp. 1), however, there was an increase in the ATTD of both nutrients in pigs at the finishing phase (Exp. 2). We speculate that the demand for P and Ca in adolescent pigs at the growing phase were very similar and being that the pigs were fed the experimental diets for only 28 d, there may have been no changes in the digestion of P and Ca from week to week. However, in the older pigs which were on the experimental diets for 55 d, the efficiency at which nutrients such as P and Ca were digested may have improved as pigs grew older and as observed with the ATTD of DM as previously observed (Olukosi et al., 2007) and in the current study. Although there was no interaction between time and diets, it may be possible to tap into the increased digestive potential of pigs in the finisher phase by supplying an increased dose of phytase. This may further boost the digestion of nutrients, subsequently getting pigs to market weight faster, while reducing the amount of P lost to the environment. However, further studies are required to confirm this hypothesis.

Although we did not conduct a P balance trial in the current study, we considered the amount of P lost from pigs through feces particularly in the water-soluble form as an important tool in determining phytase effects on P utilization over time. Previous P balance studies in pigs have indicated that more than 99% of absorbed P is retained (Jendza et al., 2009; Sorensen et al., 2018). Thus, the amount of P lost in the urine is minimal when compared with the feces (Sorensen et al., 2018) and since all the P in the urine will be soluble, we assume that the contribution of urinary P to the WSP excreted would also be minimal. Water-soluble P is of more importance when the impact of waste from the livestock industry on the environment is evaluated (Powers et al., 2006). It has been established that WSP contribute to the run-off of nutrients from manure-applied soils into water courses thereby causing issues such as eutrophication (Smith et al., 2004).

From both trials, phytase supplementation reduced the total P and WSP excreted per period by an average of 44 and 37 %, respectively when compared with pigs fed the PC diets.

To blunt the disparity in BW of pigs fed the PC and NC diets at each time point, we accounted for the extra days and FI required by pigs fed the NC or NC + 1,000 diets to attain similar BW with pigs fed the PC. This correction resulted in meaningful assessment of the environmental cost savings of phytase as compared with feeding IP to pigs because of the adjustment for differences in growth performance and P retention. This environmental savings is broadly defined in terms of P loss that may contribute to eutrophication, the resulting damage that may have an unquantifiable cost implication, as well as interventions that will be required to repair the damage to the environment. Although pigs fed the phytase-supplemented NC diet may have required some extra feed and days to catch up with the BW of pigs fed the PC, the excretion of WSP by pigs that received the PC was 80 % (18.5 vs. 10.3 g) or 46 % (64.7 vs. 44.3 g) more than pigs that received the phytase-supplemented NC diet during the grower or finisher phases, respectively. The ramifications of this observation becomes more vivid when one considers that the values of 74 vs. 41 g in the grower period and 259 vs. 177 g in the finisher period were the estimated amounts of WSP excreted by a group of 4 pigs/pen fed either the PC or NC + 1,000 diets, respectively. Therefore, a group of 4 pigs raised from 20 to 100 kg and fed either the PC or NC + 1,000 diets for 83 days would excrete an estimated 333 or 218 g of WSP, respectively. When phytase completely replaces the inclusion of IP in pig diets, the amount of savings, in terms of WSP lost to the environment, becomes more apparent in commercial herds with thousands of pigs. Since WSP is of greater importance to the environment due to its role in nutrient runoff and eutrophication (Powers et al., 2006), the implication is that phytase inclusion at 1,000 units/ kg reduces the environmental impact of pig production by approximately 40 %. If the environmental

issues, arising from the loss of nutrients such as P, from commercial swine production are to be addressed, then the complete replacement of IP with phytase should be considered commercially. From the current data, it was clear that regardless of the age of pigs or the form of P, the amount of P lost increased over time, however this could be related to the increase in FI over time. In agreement with Powers et al. (2006), we also observed that the WSP as a percentage of total fecal P was higher in pigs fed the phytase supplemented diets as compared with those fed the PC. This may be due to the action of microbes in the feces or the presence of undigested IP and unhydrolyzed phytate in the feces of pigs fed the NC or PC (Angel et al., 2005; Jendza et al., 2009). However, this did not reduce the impact of phytase on WSP loss as the quantity of P in terms of mass (g) lost from the pigs fed the PC was significantly higher than pigs fed the NC + 1,000 diet. With information from these studies, it is possible to project how much P is lost from pigs per time during the grow-finish phase thereby supporting the use of time-sensitive interventions that could reduce nutrient losses and protect the environment.

The P and Ca status of animals can usually be evaluated by measuring their concentrations in the blood (Oster et al., 2016). Regardless of the absence of interactions on plasma P concentrations in the younger pigs (Exp. 1), the P status of the diets influenced the plasma concentration of P with pigs fed the NC having lower levels of P in the plasma as compared to pigs fed either the PC or the NC + phytase. Similar observations have been made with previous studies where P deficiency led to low concentrations of P in the blood however, the inclusion of phytase mitigates these effects (Adeola et al., 2004; Jendza et al., 2005; Madrid et al., 2013). The hydrolysis of phytate by phytase ensures that the released P in the gut is absorbed into the bloodstream by the pigs. Although the body regulates P homeostasis during deficiency by reducing P wastage through the kidneys and increasing resorption from the bones, this occurs slowly and

cannot compensate for the low quantities being absorbed from the gastrointestinal tract. However, in the first study, we observed that there was a tendency for the plasma P concentrations to increase weekly indicating that the P regulating mechanisms strived to increase the circulating levels of P in response to increasing demands by pigs within that growth phase. In Exp. 2, there was a tendency for interaction between time and diets with pigs fed the NC diets having the lowest circulating levels of P as compared with pigs fed the PC and the phytase supplemented NC until d 28. However, at d 55, there was an upregulation in the circulating levels of P across all the dietary treatments but more so in the pigs fed the NC and this may be connected to the increase in ATTD of P with time regardless of the P status of diets. It is also possible that the body of pigs fed the NC were able to increase the P levels in the blood over a longer time through various regulatory mechanisms such as increasing the resorption from the bones.

There were no interaction effects on plasma Ca levels in both trials however, there was an effect of time in Exp. 1 but not in Exp. 2 and this could be due to age or physiological state of pigs. Concentrations of circulating Ca decreased over time in the younger pigs and may have been due to the rapid deposition of minerals on the bones as pigs grew exponentially during the growing phase or the reduced requirements of minerals as pigs grow older (Xu et al., 2002). However, in the finisher phase, pigs are at a mature stage, and the mechanism for regulating Ca should be well developed such that it is not necessarily impacted by time. Furthermore, blood was sampled at 5 time points in Exp. 1, but at 3 time points in Exp. 2. In agreement with Madrid et al. (2013), there was no effect of diets on the plasma Ca levels in the pigs at Exp. 1. However, in Exp. 2, pigs fed the phytase supplemented diets had the highest concentrations of Ca in the blood as compared with pigs fed the PC. Calcium is more tightly regulated than P in the body (Votterl et al., 2021) and may explain why there was no effects of diets on plasma Ca levels in younger pigs (Exp. 1) despite

the time effects observed. However, pigs fed the NC + phytase in Exp. 2 were digesting and absorbing Ca at a level much higher than in pigs fed the PC (approximately 21 % more) thus, it may explain the difference in circulating Ca levels regardless of the time at which samples were collected.

In conclusion, the positive effects of phytase on the responses of growing and finishing pigs fed diets without IP are irrefutable. However, the magnitude of these phytase effects on responses vary over time. Therefore, time-sensitive application of phytase may be carried out with pigs depending on the aim of the farmer or researcher. Similarly, the cost saving effects of phytase to the environment is established when the amount of WSP lost from commercial swine production is considered.

6.6 References

Adedokun, S. A., A. Owusu-Asiedu, D. Ragland, P. Plumstead, and O. Adeola. 2015. The efficacy of a new 6-phytase obtained from *Buttiauxella* spp. expressed in *Trichoderma reesei* on digestibility of amino acids, energy, and nutrients in pigs fed a diet based on corn, soybean meal, wheat middlings, and corn distillers' dried grains with solubles. J. Anim. Sci. 93:168–175.

Adeola, O. 2001. Digestion and balance techniques in pigs. In: A. J. Lewis, and L. L. Southern, editors, Swine nutrition. 2nd ed. CRC Press, Washington, DC. p. 903–916.

Adeola, O., and A. J. Cowieson. 2011. Board-invited review: opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. J. Anim. Sci. 89:3189–3218.

Adeola, O., Sands, J. S., Simmins, P. H. and H. Schulze. 2004.The efficacy of an Escherichia coli-derived phytase preparation. J. Anim. Sci. 82:2657-2666.

Almeida, F. N., R. C. Sulabo, and H. H. Stein. 2013. Effects of a novel bacterial phytase expressed in Aspergillus oryzae on digestibility of calcium and phosphorus in diets fed to weanling or growing pigs. J. Anim. Sci. Biotechnol. 4:8.

Angel C. R., W. J. Powers, T. J. Applegate, N. M. Tamim, and M. C. Christman. 2005. Influence of phytase on water-soluble phosphorus in poultry and swine manure. J. Environ. Qual. 34:563–571.

AOAC. 2006. Official methods of analysis. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Arredondo, M. A., G. A. Casas, and H. H. Stein. 2019. Increasing levels of microbial phytase increases the digestibility of energy and minerals in diets fed to pigs. Anim. Feed Sci. Technol. 248:27–36.

Babatunde, O. O., A. Bello, Y. Dersjant-Li, and O. Adeola. 2021. Evaluation of the responses of broiler chickens to varying concentrations of phytate phosphorus and phytase. I. Starter phase (d 1-11 post hatching). Poult. Sci. 100:101396.

Babatunde, O. O., A. J. Cowieson, J. W. Wilson, and O. Adeola. 2019a. Influence of age and duration of feeding low-phosphorus diet on phytase efficacy in broiler chickens during the starter phase. Poult. Sci. 98:2588–2597.

Babatunde, O. O., A. J. Cowieson, J. W. Wilson, and O. Adeola. 2019b. The impact of age and feeding length on phytase efficacy during the starter phase of broiler chickens. Poult. Sci. 98:6742–6750.

Blavi, L., C. J. Munoz, J. N. Broomhead, and H. H. Stein, 2019. Effects of a novel corn-expressed E. Coli phytase on digestibility of calcium and phosphorous, growth performance, and bone ash in young growing pigs. J. Anim. Sci. 97:3390–3398.

Brana, D. V., M. Ellis, E. O. Castaneda, J. S. Sands, and D. H. Baker, 2006. Effect of a novel phytase on growth performance, bone ash, and mineral digestibility in nursery and grower-finisher pigs. J. Anim. Sci. 84:1839–1849.

Cambra-López, M., A. Cerisuelo, P. Ferrer, L. Ródenas, R. Aligué, V. Moset, J. J. Pascual. 2020. Age influence on effectiveness of a novel 3-phytase in barley-wheat based diets for pigs from 12 to 108 kg under commercial conditions. Anim. Feed Sci. Tech. 267:114549.

Harper, A. F., Kornegay, E. T. and T. C. Schell. 1997. Phytase supplementation of low-phosphorus growing-finishing pig diets improves performance, phosphorus digestibility, and bone mineralization and reduces phosphorus excretion. J. Anim. Sci. 75:3174-3186.

Humer, E., C. Schwarz, and K. Schedle. 2015. Phytate in pig and poultry nutrition. J. Anim. Physiol. Anim. Nutr.(Berl). 99:605–625.

Jendza J. A. and O. Adeola. 2009. Water-soluble phosphorus excretion in pigs fed diets supplemented with microbial phytase. Anim. Sci. J. 80:296–304.

Jendza, J. A., R. N. Dilger, S. A. Adedokun, J. S. Sands, and O. Adeola. 2005. Escherichia coli phytase improves growth performance of starter, grower, and finisher pigs fed phosphorus-deficient diets. J. Anim. Sci., 83:1882-1889.

Jones, C. K., M. D. Tokach, S. S. Dritz, B. W. Ratliff, N. L. Horn, R. D. Goodband, J. M. DeRouchey, R. C. Sulabo, and J. L. Nelssen. 2010. Efficacy of different commercial phytase enzymes and development of an available phosphorus release curve for Escherichia coli-derived phytases in nursery pigs. J. Anim. Sci. 88:3631–3644.

Kemme, P. A., A. W. Jongbloed, Z. Mroz, J. Kogut, and A. C Beynen. 1999. Digestibility of nutrients in growing–finishing pigs is affected by Aspergillus niger phytase, phytate and lactic acid levels: 2. Apparent total tract digestibility of phosphorus, calcium and magnesium and ileal degradation of phytic acid. Livest. Prod. Sci. 58:119-127.

Kerr, B. J., T. E. Weber, P. S. Miller, and L. L. Southern. 2010. Effect of phytase on apparent total tract digestibility of phosphorus in corn-soybean meal diets fed to finishing pigs. J. Anim. Sci. 88:238–247.

Kim, J. W., S. P. Ndou, G. A. Mejicanos, and C. M. Nyachoti. 2017. Standardized total tract digestibility of phosphorus in flaxseed meal fed to growing and finishing pigs without or with phytase supplementation. J. Anim. Sci. 95:799–805.

Madrid, J., S. Martinez, C. Lopez, and F. Hernandez. 2013. Effect of phytase on nutrient digestibility, mineral utilization and performance in growing pigs. Livest. Sci. 154:144–151.

Olukosi O. A. J. S. Sands, and O. Adeola. 2007. Supplementation of carbohydrases or phytase individually or in combination to diets for weanling and growing-finishing swine. J. Anim. Sci.85:1702–1711.

Oster, M., F. Just, K. Busing, P. Wolf, C. Polley, B. Vollmar, E. Murani, S. Ponsuksili, and K. Wimmers. 2016. Toward improved phosphorus efficiency in monogastrics—interplay of serum, minerals, bone, and immune system after divergent dietary phosphorus supply in swine. Am. J. Physiol. Regul. Integr. Comp. Physiol. 310:917–925.

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Powers W. J., E. R. Fritz, W. Fehr, and R. Angel. 2006. Total and water-soluble phosphorus excretion from swine fed low-phytate soybean. J. Anim. Sci. 84:1907–1915.

Sands, J. S., D. Ragland, C. Baxter, B. C. Joern, T. E. Sauber, and O. Adeola. 2001. Phosphorus bioavailability, growth performance, and nutrient balance in pigs fed high available phosphorus corn and phytase. J. Anim. Sci. 79:2134–2142.

Selle, P.H., and V. Ravindran, 2008. Phytate-degrading enzymes in pig nutrition. Livest. Sci. 113:99–122.

Shahbandeh M. 2021. Global pork production 2020 and 2021, by country. https://www.statista.com/statistics/273232/net-pork-production-worldwide-by-country/

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Tech. 59:215–221.

Smith, D. R., P. A. Moore, Jr., C. V. Maxwell, B. E. Haggard, and T. C. Daniel. 2004. Reducing phosphorus runoff from swine manure with dietary phytase and aluminum chloride. J. Environ. Qual. 33:1048–1054.

Sorensen, K. U., M. C. Kruger, J. Hansen-Moller, and H. D. Poulsen. 2018. Bone biochemical markers for assessment of bone responses to differentiated phosphorus supply in growing-finishing pigs. J. Anim. Sci. 96:4693–4703.

Tsai, T. C., R. Dove, M. R. Bedford, and M. J. Azain. 2020. Effect of phytase on phosphorous balance in 20-kg barrows fed low or adequate phosphorous diets. Anim. Nutr. 6:9–15.

Votterl, J. C., J. Klinsoda, I. Hennig-Pauka, D. Verhovsek, and B. U. Metzler-Zebeli. 2021. Evaluation of serum parameters to predict the dietary intake of calcium and available phosphorus in growing pigs. Transl. Anim. Sci. 5:1-13.

Wensley, M. R., C. M. Vier, J. C. Woodworth, J. M. DeRouchey, M. D. Tokach, R. D. Goodband, S. S. Dritz, and J. R. Bergstrom. 2020. Assessing current phytase release values for calcium, phosphorus, amino acids, and energy in diets for growing-finishing pigs. Transl. Anim. Sci. 4:558–568.

Xu, H., L. Bai, J. F. Collins, and F. K. Ghishan. 2002. Age dependent regulation of rat intestinal type IIb sodium-phosphate cotransporter by 1,25-(OH)2 vitamin D3.Am. J. Physiol. Cell Physiol. 282:487–493.

6.7 Tables & Figures

Table 6-1. Ingredients and nutrient composition of experimental diets fed to growing pigs (20 kg) in Exp. 1 and finishing pigs (50 kg) in 2 phases of Exp. 2

	Exp. 1[1,2,]		Exp. 2[1,2]			
			Phase 1		Phase 2	
Item	PC	NC	PC	NC	PC	NC
Ingredients, g/kg						
Corn	641.0	670.9	717.7	717.7	780.2	780.6
Soybean meal	270.0	265.0	210.0	210.0	151.0	151.0
Soybean oil	27.0	17.5	20.0	20.0	18.0	18.0
Monocalcium phosphate	9.5	-	7.2	-	6.2	-
Limestone	12.3	6.4	10.7	5.9	9.7	5.5
Salt	4.0	4.0	4.0	4.0	4.0	4.0
Solka-floc[3]	-	-	-	12.0	-	10.0
Vitamin premix[4]	1.5	1.5	1.5	1.5	1.5	1.5
Mineral premix[5]	0.8	0.8	0.8	0.8	0.8	0.8
DL-Methionine	1.2	1.2	0.2	0.2	0.1	0.1
L-Lysine·HCl	5.2	5.2	2.1	2.1	2.4	2.4
L-Threonine	1.7	1.7	0.3	0.3	0.5	0.5
L-Tryptophan	0.3	0.3	-	-	0.1	0.1
Selenium premix[6]	0.5	0.5	0.5	0.5	0.5	0.5
Titanium dioxide premix[7]	25.0	25.0	25.0	25.0	25.0	25.0
Total	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0
Calculated nutrients and energy, g/kg						
CP	190.4	190.5	163.3	163.3	140.7	140.8
ME, kcal/kg	3,402.0	3,406.0	3,379.0	3,379.0	3,382.0	3,383.0
Ca	7.1	3.3	5.9	3.0	5.2	2.7
P	5.7	3.7	5.0	3.4	4.5	3.2
STTD P	3.3	1.5	2.7	1.4	2.4	1.2

Table 6-1 continued

Analyzed nutrients, g/kg						
DM	885	890	882	890	876	879
GE, kcal/kg	4,019	4,037	4,055	4,062	4,015	4,003
CP	182.1	183.0	158.2	157.8	134.2	135.1
Ca	7.2	3.5	5.4	2.8	5.0	2.4
P	5.6	3.8	4.4	3.0	4.0	2.8

[1]PC = positive control; NC = negative control.

[2]NC diets had phytase included at 1,000 phytase units (FYT)/kg as the third dietary treatment. Phytase was prepared as a premix with ground corn to contain 50 FYT per g corn and included into at diets at 20 g/kg at the expense of corn.

[3]International Fiber Corporation, North Tonawanda, NY.

[4]Provided the following quantities per kg of complete diet: vitamin A, 3,960 IU; vitamin D_3, 396 IU; vitamin E, 26.4 IU; menadione, 1.32 mg; riboflavin, 5.28 mg; $_D$-pantothenic acid, 13.2 mg; niacin, 19.8 mg; and vitamin B_{12}, 0.02 mg.

[5]Provided the following quantities per kg of complete diet: I, 0.26 mg; Mn, 12.0 mg; Cu, 6.33 mg; Fe, 136 mg; and Zn, 104 mg.

[6]Provided 0.24 mg Se/kg of complete diet.

[7]Prepared as 1 g titanium dioxide added to 4 g corn.

Table 6-2. Performance of growing pigs in response to experimental diets over time, Exp. 1

Time (d)	Diet[1]	Final BW[2], kg	ADG, kg/d	ADFI, kg/d	G:F, kg/kg	Replicates
7	PC	23.0	0.47	0.90	0.51	8
	NC	22.6	0.39	0.78	0.48	8
	NC + 1,000	23.2	0.48	0.86	0.55	8
14	PC	28.8	0.82	1.34	0.61	8
	NC	27.3	0.64	1.18	0.54	8
	NC + 1,000	29.2	0.86	1.31	0.66	8
21	PC	34.7	0.77	1.52	0.50	8
	NC	31.9	0.55	1.29	0.42	8
	NC + 1,000	35.0	0.77	1.43	0.54	8
28	PC	41.3	1.04	1.78	0.58	8
	NC	37.1	0.66	1.44	0.46	8
	NC + 1,000	41.3	0.90	1.71	0.54	8
7		22.9	0.44	0.85	0.52	24
14		28.4	0.78	1.28	0.61	24
21		33.9	0.69	1.41	0.49	24
28		39.9	0.87	1.64	0.53	24
	PC	32.0	0.77	1.39	0.55	32
	NC	29.7	0.56	1.18	0.48	32
	NC + 1,000	32.2	0.75	1.32	0.57	32
SEM[3]		0.46	0.03	0.04	0.02	
***P* values**						
PC vs NC		<0.01	<0.01	<0.01	<0.01	
Time x Diet		<0.01	<0.01	0.26	0.39	
Time Linear		<0.01	<0.01	<0.01	<0.01	
Time Quadratic		0.36	<0.01	0.13	<0.01	
PC vs. 1,000		0.83	0.69	0.10	0.57	
NC vs 1,000		<0.01	<0.01	<0.01	<0.01	

[1]Diets PC= positive control, NC= negative control, NC + 1,000 = NC + 1,000 phytase units (FYT)/kg.

[2]Initial bodyweight were similar for all treatments with an average of 19.8 ± 1.16 kg.

[3]SEM is for the simple effects

Table 6-3. Apparent total tract digestibility (ATTD) of nutrients and relative P excretion of growing pigs fed experimental diets over time, Exp. 1

Time (d)	Diet[1]	ATTD DM, %	ATTD P, %	ATTD Ca, %	Total P Excreted[2], g/period	WSP Excreted[3], g/period	WSP, % of total fecal P	Replicates
7	PC	84.3	54.5	60.5	16.1	12.9	80.4	8
	NC	83.8	40.5	51.3	15.0	13.2	88.5	8
	NC + 1,000	83.8	62.2	61.3	8.7	7.6	87.0	8
14	PC	84.3	55.9	67.6	23.2	18.3	78.9	8
	NC	83.5	41.6	52.7	24.4	20.6	84.4	8
	NC + 1,000	84.5	66.1	69.3	11.0	9.5	86.1	8
21	PC	84.7	58.5	66.4	24.6	19.0	76.9	8
	NC	84.0	43.2	53.2	33.2	27.3	82.4	8
	NC + 1,000	84.8	66.5	69.4	12.1	10.0	83.1	8
28	PC	85.7	54.8	66.4	32.1	23.9	74.5	8
	NC	85.6	43.5	53.3	43.6	35.1	80.5	8
	NC + 1,000	85.7	62.8	67.3	17.1	13.9	81.3	8
7		84.0	52.4	57.7	13.3	11.2	85.3	24
14		84.1	54.5	63.2	19.6	16.1	83.1	24
21		84.5	56.0	63.0	23.3	18.8	80.8	24
28		85.7	53.7	62.3	30.9	24.3	78.8	24
	PC	84.8	55.9	65.2	24.0	18.5	77.7	32
	NC	84.3	42.2	52.6	29.1	24.1	84.0	32
	NC + 1,000	84.7	64.4	66.8	12.2	10.3	84.4	32
SEM[4]		0.51	2.62	3.37	2.16	1.74	0.72	

Table 6-3 continued

***P* values**						
PC vs NC	0.28	<0.01	<0.01	0.03	<0.01	<0.01
Time x Diet	0.94	0.96	0.95	<0.01	<0.01	0.33
Time Linear	<0.01	0.69	0.74	<0.01	<0.01	<0.01
Time Quadratic	0.31	0.27	0.92	0.18	0.21	0.81
PC vs. 1,000	0.97	<0.01	0.85	<0.01	<0.01	<0.01
NC vs 1,000	0.39	<0.01	<0.01	<0.01	<0.01	0.68

[1]Diets PC= positive control, NC= negative control, NC + 1,000 = NC + 1,000 phytase units (FYT)/kg.

[2]Estimated total P excreted (g/period) in growing pigs fed the PC was calculated using the formula: $[ADFI_{time} \times D_{time} \times PI \times (1-PRet_{time})]$; Where $ADFI_{time}$ is the average daily feed intake for each time point; D_{time} is the number of days between each time point; PI is the analyzed P (g/kg) of the intake in Exp. 1; $PRet_{time}$ is the proportion of P retained at each time point, determined by multiplying the coefficient of the ATTD of P with a factor of 99.9%. This factor is the average retention of digested P retention of digested P from a P balance trial conducted in grower and finisher pigs fed experimental diets similar to the current study (Jendza and Adeola, 2009). The estimated total P excretion (g/period) for growing pigs fed either the NC or NC + 1,000 diets was calculated using the formula: $[(ADFI_{time} \times D_{time}) + (Diff_{BW}/G:F_{time})] \times PI \times [1- PRet_{time}]$; Where $Diff_{BW}$ is the BW of pigs fed the PC minus that of pigs fed either the NC or NC +1,000 diets at each time point; $G:F_{time}$ is the gain to feed ratio at each time point, and $ADFI_{time}$, D_{time}, PI, and $PRet_{time}$ are as defined above.

[3]Estimates for WSP excreted by growing pigs at each time point was determined by multiplying the total P excretion estimates by the percentage of total P in the WSP form.

[4]SEM is for the simple effects.

Table 6-4. Performance of finishing pigs in response to experimental diets over time, Exp. 2

Time (d)	Diet[1]	Final BW[2], kg	ADG, kg/d	ADFI, kg/d	G:F, kg/kg	Replicates
14	PC	62.9	0.93	2.40	0.39	8
	NC	60.8	0.79	2.31	0.34	8
	NC + 1,000	62.2	0.89	2.33	0.38	8
26	PC	74.7	0.98	2.65	0.37	8
	NC	71.7	0.91	2.56	0.36	8
	NC + 1,000	73.9	0.98	2.62	0.37	8
42	PC	91.3	0.87	2.91	0.30	8
	NC	86.9	0.80	2.78	0.29	8
	NC + 1,000	90.9	0.87	2.85	0.31	8
55	PC	105.5	1.13	3.35	0.34	8
	NC	97.3	0.84	3.13	0.27	8
	NC + 1,000	103.6	1.08	3.20	0.34	8
14		62.0	0.87	2.35	0.37	24
26		73.4	0.96	2.61	0.37	24
42		89.7	0.84	2.85	0.30	24
55		102.2	1.02	3.23	0.32	24
	PC	83.6	0.98	2.83	0.35	32
	NC	79.2	0.84	2.70	0.31	32
	NC + 1,000	82.6	0.95	2.75	0.35	32
SEM[3]		1.12	0.04	0.07	0.01	
***P* values**						
PC vs NC		<0.01	<0.01	0.03	<0.01	
Time x Diet		0.06	0.07	0.97	0.13	
Time Linear		<0.01	0.05	<0.01	<0.01	
Time Quadratic		<0.01	<0.01	0.18	<0.01	
PC vs. 1,000		0.66	0.62	0.25	0.97	
NC vs. 1,000		0.02	<0.01	0.47	<0.01	

[1]Diets PC= positive control, NC= negative control, NC + 1,000 = NC + 1,000 phytase units (FYT)/kg.
[2]Initial bodyweight were similar for all treatments with an average of 49.8 ± 3.21 kg.
[3]SEM is for the simple effects.

Table 6-5. Apparent total tract digestibility (ATTD) of nutrients and P excretion in finishing pigs fed experimental diets over time, Exp. 2

Time (d)	Diet[1]	ATTD DM, %	ATTD P, %	ATTD Ca, %	Total P Excreted[2], g/period	WSP Excreted[3], g/period	WSP, % of total fecal P	Replicates
14	PC	84.9	36.7	50.4	94.3	63.9	67.8	8
	NC	84.5	17.8	40.1	96.1	72.6	75.5	8
	NC + 1,000	85.1	45.3	60.7	57.0	44.9	78.7	8
26	PC	85.1	37.5	53.0	87.4	57.0	65.2	8
	NC	85.1	20.2	43.6	93.1	68.1	73.2	8
	NC + 1,000	85.5	48.2	64.6	53.5	41.1	76.9	8
42	PC	86.0	38.5	55.0	112.7	72.8	64.6	8
	NC	85.6	22.5	46.8	129.1	92.8	71.8	8
	NC + 1,000	85.9	52.5	66.8	63.0	46.7	74.0	8
55	PC	86.3	41.1	57.7	101.6	64.9	63.9	8
	NC	86.0	24.3	48.6	149.8	106.1	70.9	8
	NC + 1,000	86.7	54.1	69.7	61.4	44.7	73.0	8
14		84.8	33.3	50.4	82.5	60.4	74.0	24
26		85.3	35.3	53.7	78.0	55.4	71.8	24
42		85.8	37.8	56.2	101.6	70.7	70.1	24
55		86.3	39.8	58.6	104.2	71.9	69.3	24
	PC	85.6	38.5	54.0	99.0	64.7	65.4	32
	NC	85.3	21.2	44.8	117.0	84.9	72.9	32
	NC + 1,000	85.8	50.0	65.4	58.7	44.3	75.6	32
SEM[4]		0.44	2.58	2.74	6.27	4.46	0.21	

Table 6-5 continued

***P* values**						
PC vs NC	0.77	<0.01	<0.01	0.02	<0.01	<0.01
Time x Diet	0.99	0.97	1.00	<0.01	<0.01	<0.01
Time Linear	<0.01	0.04	0.03	<0.01	<0.01	<0.01
Time Quadratic	1.00	0.87	0.99	<0.01	<0.01	0.01
PC vs 1,000	0.79	<0.01	<0.01	<0.01	<0.01	<0.01
NC vs 1,000	0.39	<0.01	<0.01	<0.01	<0.01	<0.01

[1]Diets PC= Positive control, NC= Negative control, NC + 1,000 = NC + 1,000 phytase units (FYT)/kg.

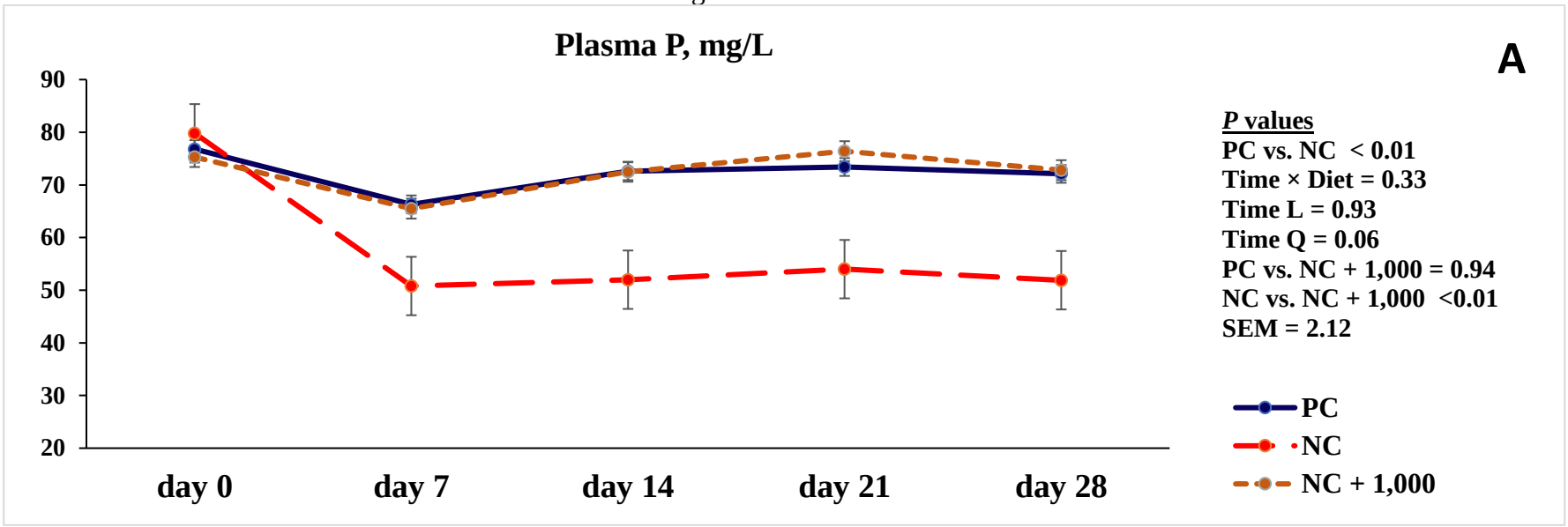
[2]Estimated total P excreted (g/period) in growing pigs fed the PC was calculated using the formula: $[ADFI_{time} \times D_{time} \times PI \times (1-PRet_{time})]$; Where $ADFI_{time}$ is the average daily feed intake for each time point; D_{time} is the number of days between each time point; PI is the analyzed P (g/kg) of the intake in Exp. 1; $PRet_{time}$ is the proportion of P retained at each time point, determined by multiplying the coefficient of the ATTD of P with a factor of 99.9%. This factor is the average retention of digested P retention of digested P from a P balance trial conducted in grower and finisher pigs fed experimental diets similar to the current study (Jendza and Adeola, 2009). The estimated total P excretion (g/period) for growing pigs fed either the NC or NC + 1,000 diets was calculated using the formula: $[(ADFI_{time} \times D_{time}) + (Diff_{BW}/G:F_{time})] \times PI \times [1- PRet_{time}]$; Where $Diff_{BW}$ is the BW of pigs fed the PC minus that of pigs fed either the NC or NC +1,000 diets at each time point; $G:F_{time}$ is the gain to feed ratio at each time point, and $ADFI_{time}$, D_{time}, PI, and $PRet_{time}$ are as defined above.

[3]Estimates for WSP excreted by growing pigs at each time point was determined by multiplying the total P excretion estimates by the percentage of total P in the WSP form.

[4]SEM is for the simple effects.

Figure 6-1. Plasma concentration (mg/L) of minerals in growing pigs (20kg) fed experimental diets over time (Exp. 1). Panel A represents the Time × Diet effect on plasma P concentrations in pigs fed experimental diets for 28 d; Average initial plasma concentration of P was 77.3 mg/L; Each data point represents 8 replicate pens. Panel B represents the main effect of Time on plasma Ca concentrations in pigs fed experimental diets for 28 d. Average initial plasma concentration of Ca was 102.8 mg/L; Each data point represents 24 replicate pens. PC = positive control; NC = negative control; NC + 1,000 = NC + 1,000 phytase units (FYT)/kg; Time L and Time Q = linear and quadratic effects of time; SEM is for the simple effects

Figure 6-1 continued



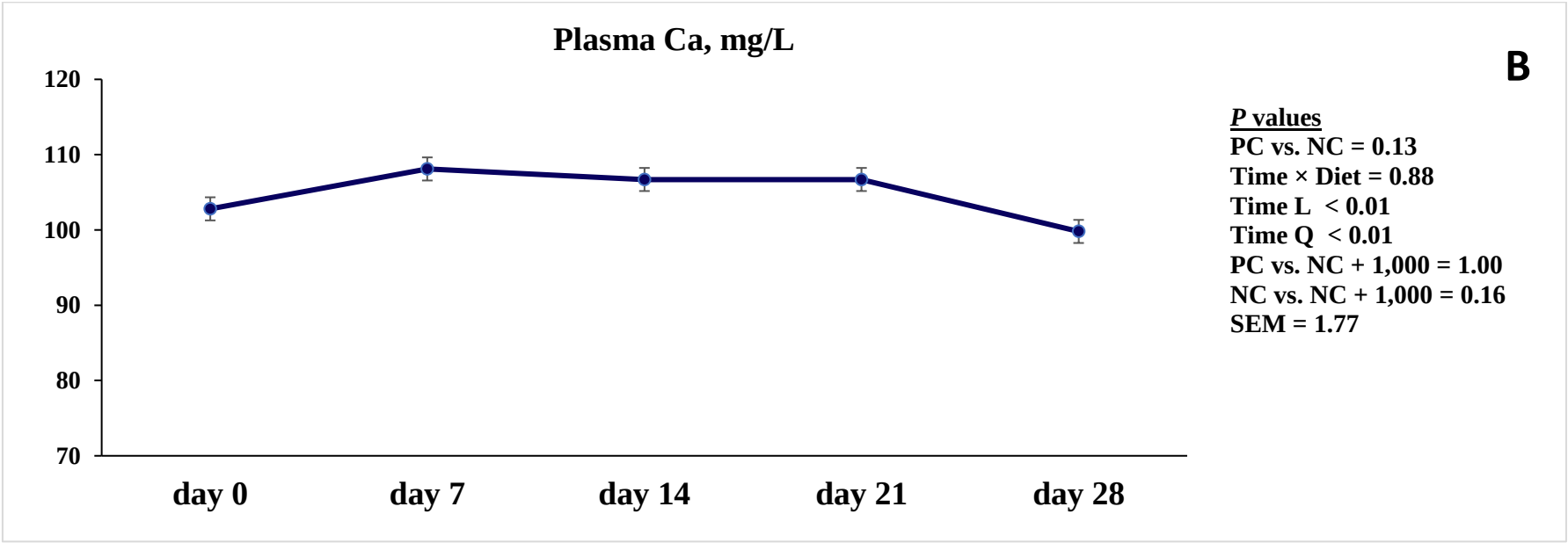
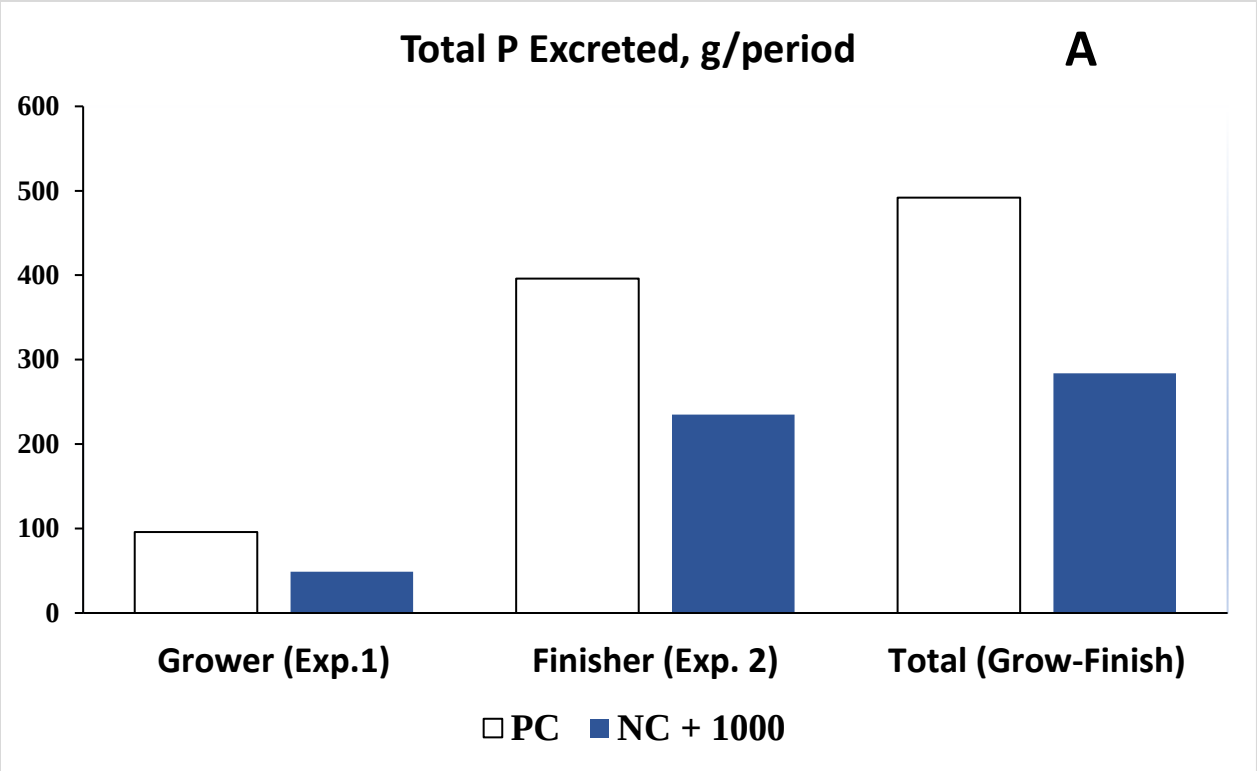


Figure 6-2. Sum of estimated P excretion (g/period) in growing pigs fed the PC and phytase supplemented NC (NC + 1,000) diets over each time point in Exp. 1 and 2. Panel A represents the sum of estimated total P excreted by pigs over the grower phase (28 days), finisher phase (55 days), and entire grow-finish phase (83 days). Panel B represents the sum of estimated water-soluble P (WSP) excreted by pigs over the grower phase (28 days), finisher phase (55 days), and entire grow-finish phase (83 days). Each bar represents a mean of 8 observations. Estimated total P excreted (g/period) in growing pigs fed the PC was calculated using the formula: [$ADFI_{time}$ × D_{time} × PI × (1- $PRet_{time}$)]; Where $ADFI_{time}$ is the average daily feed intake for each time point; D_{time} is the number of days between each time point; PI is the analyzed P (g/kg) of the intake in Exp. 1; $PRet_{time}$ is the proportion of P retained at each time point, determined by multiplying the coefficient of the ATTD of P with a factor of 99.9%. This factor is the average retention of digested P retention of digested P from a P balance trial conducted in grower and finisher pigs fed experimental diets similar to the current study (Jendza and Adeola, 2009). The estimated total P excretion (g/period) for growing pigs fed either the NC + 1,000 diets was calculated using the formula: [($ADFI_{time}$ × D_{time}) + ($Diff_{BW}$/G:F_{time})] × PI × [1- $PRet_{time}$]; Where $Diff_{BW}$ is the BW of pigs fed the PC minus that of pigs fed either the NC +1,000 diets at each time point; G:F_{time} is the gain to feed ratio at each time point, and $ADFI_{time}$, D_{time}, PI, and $PRet_{time}$ are as defined above. Estimates for WSP excreted by growing pigs at each time point was determined by multiplying the total P excretion estimates by the percentage of total P in the WSP form.

Figure 6-2 continued



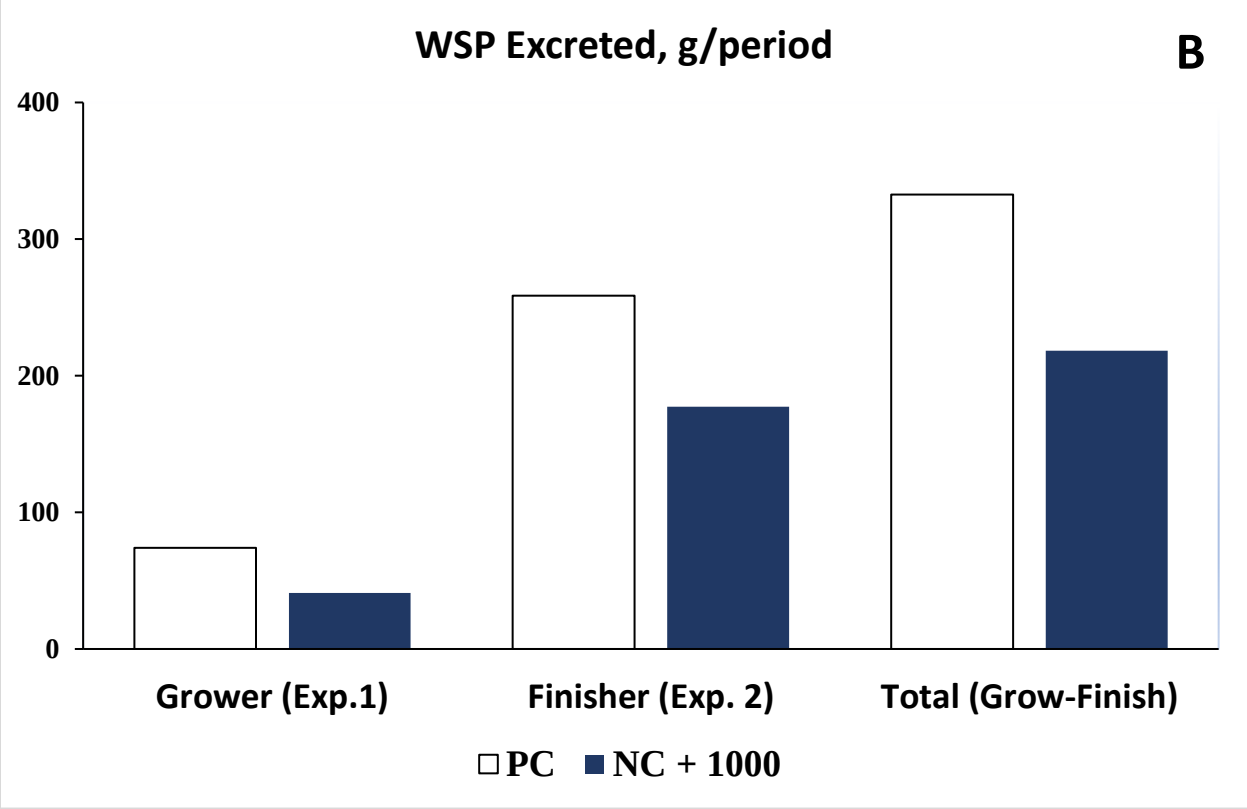
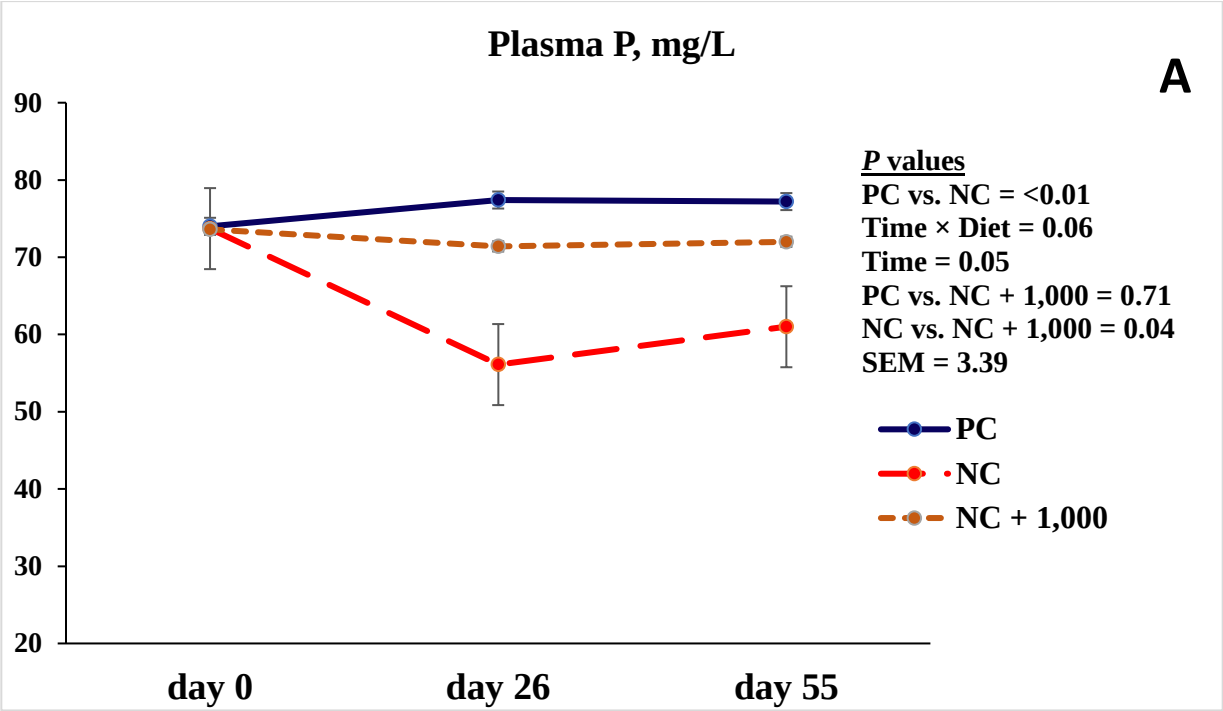
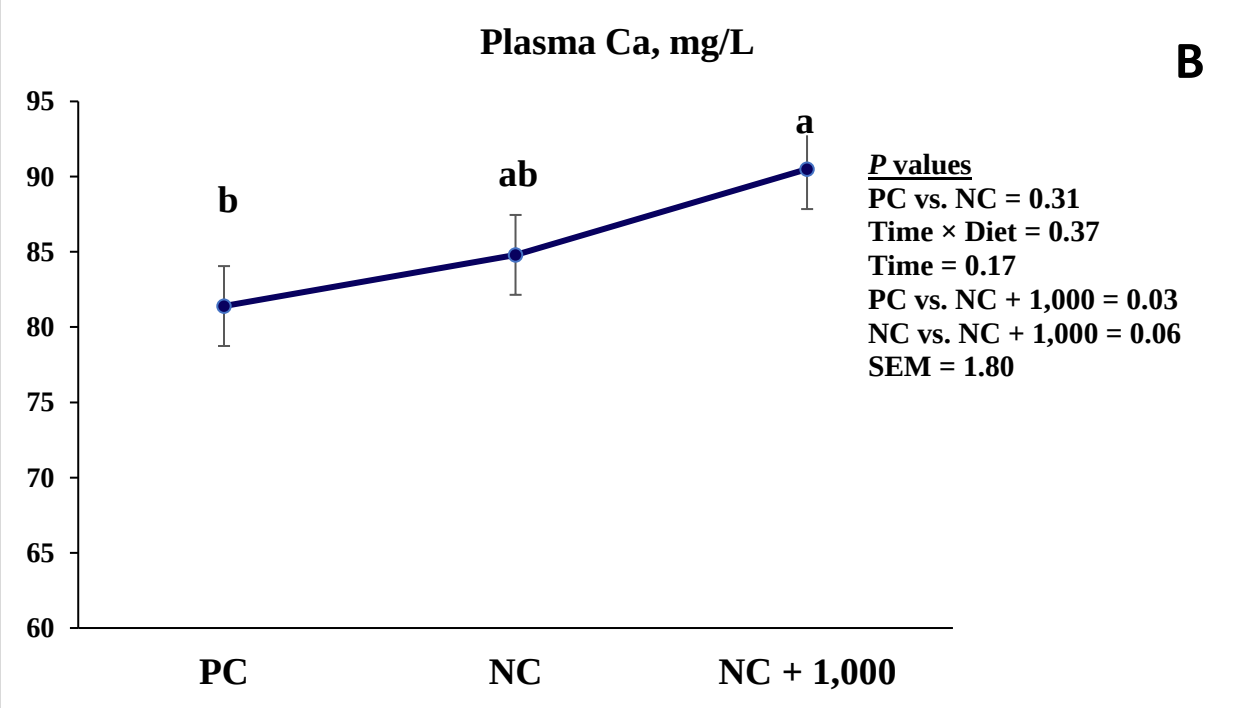


Figure 6-3. Plasma concentration (mg/L) of minerals in growing pigs (50kg) fed experimental diets over time (Exp 2). Panel A represents the Time × Diet effect on plasma P concentrations in pigs fed experimental diets for 55 d; Average initial plasma concentration of P was 73.8 mg/L; Each data point represents 8 replicate pens. Panel B represents the main effect of diet on plasma Ca concentrations in pigs fed experimental diets for 55 d. Main effect means of diet with different superscripts differ ($P < 0.05$). Average initial plasma concentration of Ca was 100.1 mg/L; Each data point represents 32 replicate pens. PC = positive control; NC = negative control; NC + 1,000 = NC + 1,000 phytase units (FYT)/kg; SEM is for the simple effects.

Figure 6-3 continued





CHAPTER 7. SUMMARY

7.1 Summary

Considering the importance of Phosphorus (P) to the sustenance of life and the numerous biochemical reactions P is involved in, it is imperative to optimize its level in the diets of monogastric animals is encouraged. When P is considered in monogastric nutrition, it is common to regard phytate and phytase alongside due to the inability of poultry and pigs to optimally utilize P from plant materials. Although a lot of research has been carried out on P, phytate, and phytase utilization in poultry and pigs, there are still a lot of grey areas to be considered if optimization, reduction of waste, and sustainable practices are to be achieved. In this research, we investigated the basal endogenous loss (BEL) of P as well as the additivity of digestible P in broiler chickens. In addition, we evaluated a new generation consensus bacterial phytase enzyme in broiler chickens as well as the time effects of phytase on P utilization in growing and finishing pigs.

In chapter 1, previous information on the utilization of P, phytase, and phytase in monogastric nutrition was reviewed while areas that required further investigation were identified. Phosphorus is one of the most abundant minerals in the body of animals as it is found in the skeletal system as well as in almost every cell of the body. It plays a role in utilization of energy and in several biochemical reactions necessary for life (Boling et al., 2000). Due to its importance and its intrinsic ability to interact with other components in the body, its regulation is closely monitored and controlled through various mechanisms. The main storage form of P in most plant materials is phytate but its utilization is hindered in the gut of poultry and pigs due to the inadequacy of endogenous phytase capable of hydrolyzing the phytic bonds. The inclusion of exogenous phytase in monogastric diets has become common due its ability to release P from the phytate complex, increase the utilization of P and other nutrients, while reducing nutrient loss (Selle et al., 2000).

However, phytase utilization in monogastric animals can be influenced by several factors which may be intrinsic, diet, or animal- related.

Chapter 2 was a study conducted in broiler chickens to investigate the additivity of apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of P in mixed diets containing corn and soybean meal (SBM) with or without phytase. Seven dietary treatments were fed to broiler chickens including 4 semi-purified diets containing either corn or SBM as the sole source of P with or without phytase inclusion at 1,000 FYT/kg, 2 mixed diets containing corn and SBM with or without phytase inclusion, and a P-free diet (PFD) to estimate the BEL of P. The AID and SID of P in corn and SBM with and without phytase inclusion was determined. The predicted and determined AID and SID of P in the mixed diets with and without phytase inclusion were compared. Results from this study validated the assumption of additivity of the AID and SID of P in mixed diets containing corn and SBM with or without the addition of phytase in broiler chickens.

In chapter 3, the additivity of the AID and SID of P in broiler chickens was further investigated in mixed diets containing corn and canola meal (CCM) in the presence and absence of phytase. Canola meal is known to be high in phytate P (PP; NRC, 2012). Furthermore, the effects of age on the BEL of P as well as the additivity of digestible P was investigated. Dietary treatments were arranged as a 2 × 3 × 2 factorial with 2 ages (day 13 and 21), 3 diets (corn, canola meal, and CCM), and 2 levels of phytase (0 and 1,000 FYT/kg) in a randomized complete block design. A PFD was fed to determine the BEL of P at both ages. The AID and SID of P in corn and canola meal with or without phytase and at both ages were determined. Similarly, the predicted and determined AID and SID of P in CCM with or without phytase at both ages were compared. Results from this study also validated the additivity of AID and SID of P in the CCM diets regardless of the age of birds or the presence or absence of phytase. In addition, age of birds

influenced the BEL of P with a higher loss in younger birds (197 mg/kg dry matter intake) as compared with birds at day 21 post hatching (159 mg/kg dry matter intake).

In chapter 4, an experiment was conducted to investigate the responses of broiler chickens in the starter phase (day 1 to 11 post hatching) to varying concentrations of PP and a new consensus bacterial 6-phytase variant (PhyG). Dietary treatments were arranged as a 3 × 5 + 1 factorial with a nutrient-adequate positive control (PC; 2.8 g PP/kg) and 15 nutrient-reduced negative control (NC) diets with 3 levels of phytate (NC1, NC2, and NC3 with 2.3, 2.8, and 3.3 g PP/kg respectively), and 5 levels of PhyG (0, 500, 1,000, 2,000, and 4,000 FYT/kg). Rice bran was the main source of PP in the experimental diets. Birds fed the PC had greater ($P < 0.05$) growth performance, nutrient utilization, and bone mineralization as compared with birds fed the NC2 without phytase. There was no interaction effect between PP and PhyG on responses of birds. Increasing levels of PP reduced ($P < 0.05$) the growth performance, P and Ca utilization of broiler chickens but had no effect on bone mineralization. Increasing levels of PhyG improved ($P < 0.05$) all responses of broiler chickens although to varying extents. In particular, broiler chickens fed NC3 diet with PhyG supplemented at 4,000 FYT/kg had improved AID of P (up to 137%) as compared with birds fed the NC3 without phytase.

Chapter 5 describes an experiment that was similar to that described in chapter 4 except that it was carried out with broiler chickens in the grower phase (day 12 to 23 post hatching). Results from this study indicated that birds fed the PC had lower ($P < 0.05$) responses as compared with birds fed the NC2 without phytase. There was no interaction effect between PP and PhyG on most responses of broiler chickens in this phase except ($P < 0.05$) for the AID of Met, Thr, and Cys where the effect of PhyG in improving the AA digestibility was higher in NC diets containing high PP as compared with NC diets with low PP concentrations. Increasing levels of PP reduced

($P < 0.05$) the growth performance and the utilization of some nutrients in birds while the inclusion of PhyG improved ($P < 0.05$) the growth performance, bone mineralization, and the utilization of energy and nutrients in birds at the grower phase.

Chapter 6 included two experiments (Exp) that investigated the time effects of phytase on the utilization of P in growing and finishing pigs fed low-P diets. Growth performance, nutrients utilization, plasma mineral concentrations, and total-P and water-soluble P (WSP) excretion were the evaluation parameters. In both Exp, treatments were arranged as a 3 × 4 factorial in a randomized complete block design with 3 corn-SBM based diets (a P-adequate PC, a low-P NC, and NC + 1,000 FYT/kg) and 4 sampling time points at days 7, 14, 21, and 28 in Exp 1 and days 14, 26, 42, and 55 in Exp 2. In both trials, 96 growing pigs were used with an average body weight of 19.8 ± 1.16 kg in Exp 1 and 49.8 ± 3.21 kg in Exp 2. In addition, treatments had 4 replicates each of barrows and gilts with 4 pigs per pen. Results from both trials indicated that phytase was effective in improving ($P < 0.05$) the growth performance and nutrient utilization of pigs in the growing and finishing phases while reducing ($P < 0.05$) the loss of both total-P and WSP. However, phytase effects on responses of pigs were sometimes affected by time with greater improvements of phytase on growth performance in older pigs as compared with younger pigs in the growing phase. The excretion of WSP in pigs at the growing, finishing, and entire grow-finish period was reduced by 45, 32, and 35 %, respectively, with phytase supplementation.

The main goal of monogastric nutritionists is ensuring the optimum utilization of nutrients in feed ingredients by animals while reducing wastage of nutrients that may become harmful to the environment. Strategies utilized by nutritionists include formulating diets that adequately supply the exact nutrients required by animals or utilizing dietary interventions that improve the digestive efficiency of animals such that wastage is minimized. This research is a combination of

both strategies that aims to improve the utilization of P in both poultry and pigs. There was limited information in literature on the BEL of P in broiler chickens as determined by feeding a PFD since previous studies utilized the regression method to determine the BEL of P (Dilger et al., 2006). However, the regression method may be prone to errors hence the use of nutrient-free diets to determine the BEL of nutrients as have been done with amino acids (AA) in previous trials. Similarly, the additivity of the AID and SID of P in mixed diets had not been well established in broiler chickens. Thus, in chapters 2 and 3, we demonstrated that the AID and SID of P in mixed diets containing ingredients that were either high or low in PP and with or without phytase were additive. Similarly, the age of birds had no effect on the additivity of AID and SID of P however, there were age effects on the BEL of P in broiler chickens. Considering that most commercial broiler chicken diets contain phytase, this information becomes relevant as it supports the effective formulation of diets that will supply the exact amount of P required by broiler chickens. Digestibility values from this research can also be included in diet formulation programs to effectively represent broiler chicken P requirements and reduce the wastage of P to the environment.

The use of phytase as a dietary intervention to improve P utilization in broiler chickens has become common, however, the need to evaluate new generation phytase continuously arise. This is because current phytase products cannot totally hydrolyze the phytate present in ingredients of plant origins. Therefore, it becomes imperative that new phytase products that are more effective be developed and thoroughly evaluated in broiler chickens using adequate testing protocols. Results from chapters 4 and 5 indicate the efficacy of a new consensus bacteria 6-phytase in broiler chickens at the starter and grower phases in improving growth performance and nutrient utilization by birds. Both phases are critical for the initial growth and development of birds required for the

increased deposition of meat in the finisher phase. Thus, adequate utilization of nutrients, particularly from unconventional feed ingredients such as rice bran, helps to maximize productivity, reduce nutrient wastage, and encourage sustainable practices.

Likewise, phytase is increasingly used in swine diets to improve growth performance and P utilization (Blavi et al., 2019). However, due to the high amount of waste from commercial swine production, research that supports the reduction of P loss through manure is required. Trials from chapter 6 indicate that phytase is effective in reducing P loss particularly in the soluble form which is of more importance to environmental and soil researchers (Angel et al., 2005). However, the effects of phytase on pig responses are sometimes time-based and may hereby result in the need for time-dependent phytase supplementation strategies that target a particular response depending on the aim of the farmer or researcher. Both trials in chapter 6 also present information on the utilization and loss of P per time during the growing and finishing phases. This may become relevant to support other interventions, asides or in combination with phytase supplementation, targeted at improving the utilization of P in pigs or reducing the loss P from commercial production into the environment.

Although this research has gone further to explore the area of P, phytate, and phytase in monogastric nutrition, there is still more to be done to ensure the optimal utilization of P in poultry and pigs. Studies that investigate the additivity of the AID and SID of P in mixed diets supplemented with phytase is required for pigs since phytase is increasingly being included in commercial swine diets. Furthermore, factors such as dietary P, fiber, concentration of phytate, types of index markers, gut health, or site of collection which may affect the flow of endogenous P in broiler chickens or pigs need to be investigated as have been done with AA (Adedokun et al., 2011). Likewise, studies that investigate the SID of P in feed ingredients for broiler chickens need

to be carried out to support the use of standardized ileal digestible P requirements for broiler chickens instead of non-phytate P as have been done with pigs (NRC, 2012). Additional evaluations of new phytase products for broiler chickens need to be carried out in the finisher phase of broiler chickens as well as investigating any interactions between phytase, phytate, and the microbial populations in the gut of broiler chickens during their lifecycle. Lastly, pig trials carried out in this research did not investigate the time effects of phytase on bone mineralization and this may be important if diets supplemented with phytase but without inorganic sources of P are to be fed to pigs during the entire grow-finish period.

7.2 References

Adedokun, S.A., O. Adeola, C. M. Parsons, M. S. Lilburn, and T. J. Applegate. 2011. Factors affecting endogenous amino acid flow in chickens and the need for consistency in methodology. Poult. Sci. 90:1737–1748.

Angel, C. R., W. J. Powers, T. J. Applegate, N. M. Tamim, and M. C. Christman. 2005. Influence of phytase on water-soluble phosphorus in poultry and swine manure. J. Environ. Qual. 34:563–571.

Blavi, L., C. J. Munoz, J. N. Broomhead, and H. H. Stein. 2019. Effects of a novel corn-expressed E. Coli phytase on digestibility of calcium and phosphorous, growth performance, and bone ash in young growing pigs. J. Anim. Sci. 97:3390–3398.

Boling, S. D., M. W. Douglas, M. L. Johnson, X. Wang, C. M. Parsons, K. W. Koelkebeck, and R. A. Zimmerman. 2000a. The effects of dietary available phosphorus levels on performance of young and older laying hens. Poult. Sci. 79:224–230.

Dilger, R. N., and O. Adeola. 2006. Estimation of true phosphorus digestibility and endogenous phosphorus loss in growing chicks fed conventional and low-phytate soybean meals. Poult. Sci. 85:661–668.

National Research Council (NRC). 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC, USA.

Selle, P. H., V. Ravindran, A. Caldwell, and W. L. Bryden. 2000. Phytate and phytase: consequences for protein utilisation. Nutr. Res. Rev. 13:255–278.

VITA

Olufemi Babatunde
Graduate School, Purdue University

Education

B.Agric., Animal Sciences, 2014, University of Ibadan, Ibadan, Nigeria

M.S., Animal Nutrition, 2018, Purdue University, West Lafayette, Indiana, USA

Ph.D., Animal Nutrition, 2022, Purdue University, West Lafayette, Indiana, USA

Research Interests:

Mineral Utilization in Monogastric Animals.

Nutritional Evaluation of Enzymes and other Feed Additives.

Nutrient Evaluation of Feed Ingredients.

PUBLICATIONS

Babatunde O.O., A. Bello, Y. Dersjant-Li, and O. Adeola. 2022. Evaluation of the responses of broiler chickens to varying concentrations of phytate phosphorus and phytase. II. Grower phase (day 12-23 post hatching). Poultry Science (In press).

Dersjant-Li, Y., A. Bello, T. Stormink, M.R. Abdollahi, V. Ravindran, **O.O. Babatunde**, O. Adeola, M. Toghyani, S.Y. Liu, P.H. Selle and L. Marchal. 2022. Modeling digestible amino acids improvement by a novel consensus bacterial 6-phytase variant in broilers using 13 data sets from 4 digestibility trials encompassing varied diets, dietary phytate levels and bird ages. Poultry Science (In press).

Babatunde O.O., and O. Adeola. 2021. Time-series effects of phytase on phosphorus utilization in growing and finishing pigs fed a low-phosphorus diet. Journal of Animal Science (In press).

Babatunde O.O., A. Bello, Y. Dersjant-Li, and O. Adeola. 2021. Evaluation of the responses of broiler chickens to varying concentrations of phytate phosphorus and phytase. I. Starter phase (day 1-11 post hatching). Poultry Science 100:101396.

Babatunde O.O., C.S. Park, and O. Adeola. 2021. Nutritional potentials of atypical feed ingredients for broiler chickens and pigs. Animals 11:1196.

Babatunde O.O., A.S. Aderibigbe, and O. Adeola. 2021. Contributions of enzyme technology to poultry and swine nutrition. Proceedings for the Animal Nutrition Conference of Canada, May 10 – 14, 2021; pp 36-50.

Babatunde O.O., and O. Adeola. 2021. Additivity of apparent and standardised ileal digestibility of phosphorus in corn and canola meal mixed diets; basal endogenous loss of phosphorus responses to phytase and age in broiler chickens. British Poultry Science 62:244-250.

Babatunde O.O., S.O. Osho, C.S. Park, and O. Adeola. 2020. Additivity of apparent and standardized ileal digestibility of phosphorus in mixed diets containing corn and soybean meal fed to broiler chickens. Poultry Science 99:6907-6913.

Babatunde O.O., J.A. Jendza, P. Ader, P. Xue, S.A. Adedokun, and O. Adeola. 2020. Response of broiler chickens in the starter and finisher phases to three sources of microbial phytase. Poultry Science 99:3997-4008.

Babatunde O.O., A. Cowieson, J.W. Wilson, and O. Adeola. 2019. Impact of age and feeding length on phytase efficacy during the starter phase of broiler chickens. Poultry Science 98:6742-6750.

Babatunde O.O., A. Cowieson, J.W. Wilson, and O. Adeola. 2019. Influence of age and duration of feeding low-phosphorus diet on phytase efficacy in broiler chickens during the starter phase. Poultry Science 98:2588-2597

Osho, S.O., **O.O. Babatunde,** and O. Adeola. 2019. Additivity of apparent and standardized ileal digestibility of amino acids in wheat, canola meal, and sorghum distillers dried grains with solubles in mixed diets fed to broiler chickens. Poultry Science 98:1333-1340.

Ogunwole O.A., **O.O. Babatunde**, R.A. Faboyede, B.S. Adedeji, F.O. Jemiseye. 2017. Calcium and phosphorus retention by broiler chickens fed groundnut cake based-diet supplemented with L-lysine and DL-methionine. Journal of Animal Production Research 29: 240-248.