EFFECT OF SUPERPOSING DIETARY PHYTASE ON GROWTH, MINERAL UTILIZATION, AND AMINO ACID DIGESTIBILITY OF CHANNEL CATFISH (*ICTALURUS PUNCTATUS*) AND RED DRUM (*SCIAENOPS OCELLATUS*)

A Dissertation

by

KEQUAN CHEN

Submitted to the Graduate and Professional School of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Chair of Committee,	Delbert M. Gatlin III
Committee Members,	Christopher A. Bailey
	Robert J. Taylor
	Rosemary Walzem
Head of Department,	Kirk Winemiller

December 2021

Major Subject: Ecology and Conservation Biology

ABSTRACT

Two indoor feeding trials were conducted to investigate the effects of dietary superdosing of Quantum Blue® phytase on the growth performance, as well as mineral and amino acid utilization of juvenile channel catfish (initial weight of 8.02 ± 0.18 g) and red drum (initial weight of 4.99 ± 0.09 g) over 8 and 9 weeks, respectively. Both feeding trials had a negative control diet formulated to contain adequate nutrients but deficient in available phosphorus based on the established requirement of each species. Another four diets were produced from the negative control formulation with Quantum Blue® phytase supplemented at either 1000, 2000, 4000, or 8000 FTU/kg dry diet. A positive control diet also was prepared with supplemental monocalcium phosphate (MCP) to meet the available phosphorus requirement of each species. Both channel catfish and red drum fed the phytase-supplemented diets had significantly ($P < 0.05$) improved weight gain and feed efficiency compared to fish fed the negative control diet. In addition, dietary phytase supplementation significantly enhanced bone concentrations and whole-body retention of various minerals, although slightly differences were found between the two species. Furthermore, dietary phytase supplementation significantly increased apparent availability of phosphorus from the posterior intestinal region, and reduced excretion of nitrogen and various minerals. Additionally, apparent digestibility coefficients (ADCs) for most indispensable and dispensable amino acids were promoted by dietary phytase. Based on regression analysis of weight gain data from channel catfish and red drum feeding trials, the

optimal dietary phytase dosage was estimated to be $5{,}492^{5050}_{5773}$ and $5{,}520^{5150}_{6175}$ FTU phytase /kg of dry diet, respectively.

In addition, a pond feeding trial was carried out with channel catfish (initial weight of 39.8 ± 3.46 g) reared in cages to evaluate the effect of dietary phytase supplementation of a commercial diet and the interaction between dietary MCP and phytase supplementation. The results shown that neither dietary MCP nor phytase supplementation increased growth performance of channel catfish when the diet had adequate available phosphorus. Besides that, although both dietary MCP and phytase supplementation increased bone concentration of some minerals, only dietary phytase supplementation increased the retention of these minerals.

DEDICATION

This dissertation is dedicated to my parents and family members, whose continuous love and encouragement have kept me pursuing.

ACKNOWLEDGEMENTS

First of all, I would like to thank Dr. Peng Li and professor Yeyuan Tu, who provided the chance for me to know and visit Fish Nutrition Laboratory at Texas A&M University in 2014. The whole journey would not have happened if they did not introduce Dr. Delbert Gatlin III to me.

Next, I would like to convey my heartfelt appreciation to my committee chair Dr. Delbert Gatlin III for pursuing, guiding, and supporting my Ph.D. during the past five years at Texas A&M University. I really appreciate for all the time, effort, and patience he has spent to revise my work, correct my mistakes, and help me to consistently improve myself.

Also, I would like to thank my committee members: Dr. Christopher A. Bailey, Dr. Robert J. Taylor, and Dr. Rosemary Walzem, for their great help in my research progress. Dr. Bailey gave me the general idea about the animal nutrition and precise formula. Dr. Walzem reopened the mineral course to give me the chance to learn the functions and pathways of various minerals. And Dr. Taylor helped me to analyze the mineral composition of various samples. Without all of these means of assistance from my committee members, I could not complete my dissertation without a hitch.

Next, I appreciate all the help and support from my lab mates, Alejandro, Alton, Blaine, Brittany, Bryan, Clement, Grayson, Sergio, Yamamoto, and Zena. Besides that, I would thank our farm manger Mr. Brian Ray and ex farm assistant Mr. Fernando Campero, who helped

maintain our Aquacultural Research and Teaching Facility (ARTF).

Last but not least, I would like to thank my parents, family members, and friends, for all their support, understanding, and encouragements during the past five years.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

These feeding trials were supported by a dissertation committee containing Dr. Delbert Gatlin III (advisor) of the Department of Ecology and Conservation Biology (home department), Dr. Christopher A. Bailey and Dr. Rosemary Walzem of the Department of Poultry Science (outside department), and Dr. Robert J. Taylor of the Department of Veterinary Integrative Biosciences (outside department).

Staffs from the Fish Nutrition Laboratory at Texas A&M University and AB Vista assisted with the termination of feeding trials and part of the sample analyses. All the other work regarding the dissertation was conducted and completed by the student independently.

Funding Source

These studies were supported by Texas A&M AgriLife Research and AB Vista.

NOMENCLATURE

Al	Aluminum
Ala	Alanine
Arg	Arginine
Asp	Aspartic acid
Ca	Calcium
Cu	Copper
Fe	Iron
Glu	Glutamic acid
His	Histidine
Ile	Isoleucine
Leu	Leucine
Lys	Lysine
Met	Methionine
Mg	Magnesium
Mn	Manganese
N	Nitrogen
P	Phosphorus
Phe	Phenylalanine
Pro	Proline

S	Sulfur
Ser	Serine
Tau	Taurine
Thr	Threonine
Tyr	Tyrosine
Val	Valine
Zn	Zinc

TABLE OF CONTENTS

Page

LIST OF TABLES

CHAPTER I

INTRODUCTION AND RESEARCH GOAL

I.1 Introduction and justification

I.1.1 Phytate

Phytate (hexakisphosphates of myo-inositol), the salt form of phytic acid, is the primary phosphate storage compound in plant seeds (Kumar et al., 2012). Phytate has been documented to bind phosphorus in up to 60, 71, 76, and 77% of the total phosphorus content of plant feedstuffs such as soybean meal, wheat, wheat bran, and cottonseed meal, respectively (Kumar et al., 2012; Selle et al., 2010). During seed germination, the phytate will be enzymatically hydrolyzed and liberated as inorganic phosphate for seed growth and development (Urbano et al., 2000). However, dietary phytate is unfriendly to monogastric animals including various fish species due to their lack of the phytase enzyme (Liener, 1989; Woyengo & Nyachoti, 2013), thus resulting in anti-nutritional effects which typically include reduction in the absorption of certain dietary minerals and protein.

Phytate can chelate with positively charged di- and tri-valent mineral ions, such as Ca^{2+}, Mg^{2+}, Zn^{2+}, and Fe^{3+}, to form stable complexes in the gastrointestinal (GI) tract. These chelates are unavailable for absorption (Angel et al., 2002; D'Mello et al., 1991; Erdman, 1979), and may create marginal or overt dietary deficiencies of these essential nutrients. Moreover, other effects of phytate such as reduced protein digestibility leading to reduced growth performance and feed

efficiency of commonly cultured fish species also have been reported (Francis et al., 2001). For example, Atlantic salmon (*Salmo salar*) exhibited reduced feed intake and growth performance when fish were fed phytate-supplemented diets, although negative effects were not observed in fish fed dietary phytate levels between 4.7 and 10.0 g/kg (Denstadli et al., 2006). These findings are supported by the results of Sajjadi and Carter (2004b), who found no difference in feed intake and feed efficiency between Atlantic salmon fed a control diet, which used casein and fishmeal as the main protein sources, and an experimental diet to which 10.0 g phytate/kg was added. Laining et al. (2010) noticed that Japanese flounder (*Paralichthys olivaceus*) fed a control diet, based on casein and fishmeal as the main protein sources, without phytate supplementation had greater weight gain and feed intake compared to fish fed diets supplemented with 13.5 and 20.6 g phytate/kg. Liu et al. (2014) reported that grass carp (*Ctenopharyngodon Idella*) had lower growth performance when fed a phytate-supplemented diet, in association with increased expression of neuropeptide Y (NPY) and ghrelin in the brain. They concluded that these changes in gene expression were consistent with reduced appetite, and so feed intake was reduced when fish were fed phytate-supplemented diets.

As mentioned previously, monogastric animals have limited ability to utilize the phosphorus from phytate, thus commercially, extra inorganic form of phosphorus may be added to animal feeds. However, this leads to the undigested phytate, and unutilized phosphorus is excreted into the environment. According to the data regarding the amount of phosphorus transferred between global fisheries and aquaculture from 1950 to 2016, summarized by Huang et al. (2020), the

global P harvest (wild + aquaculture) has increased almost five times from $0.21^{0.24}_{0.19}$ (mean and interquartile range) in 1950 to $1.10^{1.14}_{1.04}$ Tg P yr^{-1} in 2016. Meanwhile, P input (aquaculture feeds and fertilizer) has been raised more than 200-fold from $0.01^{0.015}_{0.007}$ to $2.04^{3.09}_{1.59}$ Tg P yr^{-1}. The aquaculture industry expanded dramatically in the 1980s which was associated with increasing P input. However, P use efficiency did not improve as fast as P input, resulting in a net flux of P from land-human systems to aquatic ecosystems. In addition, the turning point of global P net from positive to negative occurred around 2004^{2008}_{1997}. Nowadays, the global P net is still negative and up to $-0.95^{-0.50}_{-1.99}$ Tg P yr^{-1}. Besides that, freshwater aquaculture contributes around 84-94% of the total aquaculture P input. Within freshwater aquaculture, the majority of P input is from the finfish sector (95-100%). Additionally, the share from crustaceans slowly increased with time, which reached 5.31% in 2010. In the marine aquaculture sector, more than 90% of P input was from finfish aquaculture during 1950-1970. However, crustacean culture contributed more than 50% of marine aquaculture P input since 1990.

Thus, due to human disruption, the global P cycle has changed, in which considerable amounts of P have flowed from phosphate rock into the aquatic ecosystem. According to the idealized scenario built by Huang et al. (2020), in order to rebalance the anthropogenic P flux, the efficiency of aquaculture phosphorus (the ratio of harvested to input P) needs to improve from the current 20% to at least 48% by 2050. Consequently, the primary conflict of the aquaculture industry today is how to balance the continuous growth of aquacultural production while simultaneously minimizing its environmental influence (Vandenberg & Koko, 2006).

Aquatic wastes are composed principally of fecal material, uneaten feed, and metabolic wastes (Tovar et al., 2000; Wu, 1995), in which the majority of P in aquaculture effluent is directly excreted by aquatic animals. Although adequate dietary P level is required to ensure the normal growth of fish, the surplus dietary P supply and unavailable dietary P are excreted into the environment. As such, phytases can play a vital role in the liberation of inorganic phosphorus from organic phosphorus in the aquatic biosphere. According to Herbes et al. (1975), phytase hydrolyzed more than 50% of organic P collected from condensed lake water to orthophosphate. Similar results were derived by De Groot and Golterman (1993), who found that up to 34% of residual organic phosphorus from sediment P can be hydrolyzed by phytase. However, only around 5% of organic P was freed by alkaline phosphatase.

Usually, inorganic P is the least available among the nutrients required in large quantity (macronutrients) by photosynthetic or heterotrophic organisms; thus P is the primary nutrient which limits the growth of fish (Schindler, 1977). However, as mentioned previously, nowadays, a large amount of phytate is released from the aquaculture industry, provided a tremendous amount of phosphorus to the aquatic environment. The excessive phosphorus can be consumed by blue-green algae (BGA) and lead to eutrophication (Stewart et al., 1978). Although nitrogen and phosphorus are usually considered as the two most critical elements on BGA blooms, some BGAs, such as *Anabaena flos-aquae* and *Aphanizomenon flos-aquae*, can directly derive and fix nitrogen from the air. In addition, P plays an important role in the proliferation and growth of BGA, because it can promote the fixation of nitrogen (Bisoyi & Singh, 1988) Thus, the

concentration of aquatic P is more important to control BGA blooms (Chen et al., 2009).. Blooms of BGA can deplete oxygen in the water after the decomposition of massive amounts of dead BGA (Abeliovich, 1969) and they also can release toxins, which can lead to severe negative impacts on the growth and health of other organisms. According to various research studies, BGA can produce three major toxic compounds including hepatotoxins, which are cyclic peptides including a unique hydrophobic amino acid (ADDA: 3-amino-9-methoxy-10-phenyl-2,6,8-trimethyldeca-4,6-dienoic acid). Hepatotoxins can be absorbed through the gastrointestinal (GI) tract without being degraded and eventually concentrated in liver cells of animals, via a bile acid-type carrier mechanism (Falconer, 2012; Falconer & Yeung, 1992); Cytotoxins are another major group of toxic compounds including various antimetabolites produced by BGA. Fortunately, a defensive barrier can be formed in the GI tract with digestive enzymes by hydrolyzing or not absorbing these substances that could cause danger (Falconer, 1999). Neurotoxins are a third group of toxins that have been recognized for several centuries as a trigger of human shellfish poisonings (Falconer, 2012). Humpage et al. (1994) determined that the paralytic shellfish poisons were also a cause of livestock poisoning by BGA, which killed lots of sheep and cattle in Australia during the summer of 1990. Kao (1993) determined that these neurotoxins could block the sodium channels in nerve axons, leading to severe paralysis and death of animals due to asphyxia. Besides these toxic compounds mentioned above, BGA also can release other compounds which will cause off-flavor and off-odors in fish. Leger (1910) assumed that a substance released by the BGA *Oscillatoria tenuis* caused a muddy flavor

in rainbow trout *Oncorhynchus mykiss* and was credited as the first person to disclose the reason for earthy-musty flavor problems in fish. Today, the most common preharvest off-flavors in aquaculture are caused by geosmin and 2-methylisoborneol, which are earthy-musty metabolites of BGA (Tucker, 2000).

Not only can the released phosphorus from phytate cause so many environmental issues as mentioned above, but also the freed inositol may also impact fish produced in aquaculture. Inositol widely exists in plants and animals, mainly as a structural component in phospholipids of biological membranes (Chang et al., 2001). In addition, inositol is one of the essential nutrients for most aquatic animals (Michael & Koshio, 2008), and is considered a vitamin-like nutrient (Shiau & Su, 2005). However, if dietary phytate cannot be utilized by aquatic animals, the inositol from phytate may be excreted and consumed by *Aeromonas hydrophila,* which is an opportunistic bacterial pathogen existing in freshwater environments and the cause of diseases in many species, including amphibians, fish, reptiles, and mammals (Janda & Abbott, 2010). Pang et al. (2015) reported that virulent *A. hydrophila* strains were identified to have three different pathways for metabolizing myo-inositol, sialic acid, and L-fucose. Besides that, Li et al. (2018) showed the myo-inositol metabolic pathway played a fairly important role in the virulence of A. *hydrophila* NJ-35, compared to the other two utilization pathways. Thus, the excessive inositol in the aquatic environment can be utilized as the main energy source for *A. hydrophila* strains and may result in epidemics worldwide (Hossain et al., 2014). Motile *Aeromonas* septicemia (MAS), a disease caused by mesophilic *A. hydrophila*, impacts a variety of primarily freshwater fish species,

including carp, catfish, salmon, and tilapia (Joseph & Carnahan, 1994). Several epidemic outbreaks caused by *A. hydrophila* occurred in the late 1980s as well as in the carps farmed in China during the past decades (Hossain et al., 2014; Zhang et al., 2013). In the summer of 2009, an outbreak of MAS caused by highly virulent *A. hydrophila* began in commercially raised catfish of western Alabama (Hemstreet, 2010).

I.1.2 Phytase

Phytase, known as myo-inositol hexakisphosphate phosphohydrolase, is a phosphatase enzyme that can catalyze the stepwise removal of inorganic phosphorus from the inositol ring of phytate (Wyss et al., 1999; Yu et al., 2012). According to Bohn et al. (2008), phytase has been divided into three categories, 3-phytase (EC 3.1.3.8), 5-phytase (EC 3.1.3.72), and 4/6 phytase (EC 3.1.3.26), depending on the site where the hydrolysis of the phytate molecule is initiated. The hydrolysis process operates by the stepwise removal of inorganic P from the inositol ring of a fully phosphorylated phytic acid (IP6), to generate a chain of lower phosphoric esters as intermediates (penta- IP5, tetra- IP4, tri- IP3, di- IP2, and mono- IP1) (Baruah et al., 2004; Debnath et al., 2005). Table 1 summarizes the results of the major studies conducted to date concerning the effects of different types of phytases on farmed aquatic species.

I.1.2.1 Improvement in phosphorus bioavailability

As one of the essential nutrients of fish as with various terrestrial animals, phosphorus is

critically important for growth and bone development/mineralization (Åsgård & Shearer, 1997) as well as a reproduction (Hardy & Shearer, 1985) and a variety of other physiological processes (NRC, 2011). Due to the negligible amounts of phosphorus that fish can absorb from water, dietary phosphorus is the primary source to fulfill their metabolic requirements. Because phosphorus is the rate-limiting nutrient in most lentic and lotic environments, excessive waterborne phosphorus can be a critical pollutant in these water bodies, and directly stimulates algal bloom or eutrophication, leading to negative effects in aquatic ecosystems as well as rendering the water undesirable for human or animal use (Chowdhury et al., 2017; Correll, 1999). Therefore, supplementation of dietary phytase can not only improve the bioavailability of phytate phosphorus in the diet to fish but also reduce the amount of phosphorus released into the environment.

Many previous studies have reported positive effects of phytase supplementation on dietary phosphorus availability in various fish species as reviewed by (Kumar et al., 2012) and summarized in Table 1. For example, Schäfer et al. (1995) reported that adding 500 and 1000 (FTU) of phytase/kg to a soybean-meal-based diet respectively released 20% and 40% of the phytate-phosphorus to common carp (*Cyprinus carpio*). Yu (2000) claimed that crucian carp (*Carassius carassius*) fed soybean-meal-based diets containing 500 and 1000 FTU of phytase /kg could release 60% and 80% of the dietary phytate-phosphorus, respectively, compared to the basal diet without any phytase added. Channel catfish fed diets containing more than 1000 FTU of phytase/kg had significantly increased phosphorus concentration in bone and whole-body

(Yan et al., 2002). Similar evidence also was revealed in other fish species such as Nile tilapia (*Oreochromis niloticus*) (Cao et al., 2008; Furuya et al., 2001), Atlantic salmon (Sajjadi & Carter, 2004a), and rainbow trout (*Oncorhynchus mykiss*) (Sugiura et al., 2001; Vielma et al., 2004), demonstrating that supplementation of plant-protein-based diets with phytase could promote the bioavailability of phytate-phosphorus and decrease the discharge of phosphorus to the environment.

I.1.2.2 Enhancement of growth performance

Evaluations into the effects of different types of phytases on growth performance of various fish species fed phytate-containing diets also have been conducted. Several studies have proven that dietary phytase can release the phosphorus from phytate and improve growth performance of channel catfish (*Ictalurus punctatus*) (Jackson et al., 1996), rainbow trout (Vielma et al., 2000), striped bass (*Morone saxatilis*) (Papatryphon & Soares, 2001), Nile tilapia (Nwanna, 2005), and common carp (Nwanna et al., 2007). However, positive growth performance responses have been observed inconsistently, most likely due to the available phosphorus level in the basal diet receiving phytase supplementation. For example, Forster et al. (1999) found no positive effects on growth performance of rainbow trout fed diets based on low-glucosinylate canola protein concentrate and supplemented with phytase. Similar results also were observed in Atlantic salmon fed diets containing the low-glucosinylate canola protein concentrate and supplemented with phytase (Sajjadi & Carter, 2004a). Yoo et al. (2005) claimed that no significant

improvement in weight gain was noticed when Korean rockfish (*Sebastes schlegeli*) were fed phytase-supplemented diets containing 35% of soybean meal. Based on these examples and other studies described in Table 1, the potential influence of dietary phytase supplementation relates to the type/activity of phytase, specific diet formulation including phosphorus concentration, fish species (gastric or agastric), and development status of the fish.

I.1.2.3 Promotion of protein digestibility

In pigs, there is evidence showing that phytase can improve the utilization of protein and amino acids by breaking down the phytin-protein complex (Kornegay & Qian, 1996). Protein digestibility also has been shown to increase in some fish species fed plant-protein-based diets supplemented with phytase. For example, in rainbow trout, a phytase-supplemented diet based on soybean meal as the main protein source increased protein digestibility by 1.2% to 3.2%, but not lysine utilization, compared to the fish fed the basal diet without phytase addition (Vielma et al., 2004). Cheng and Hardy (2003) showed that compared to rainbow trout fed a diet containing raw soybeans without phytase, those fed a diet including expelled (heat treated) soybeans with phytase had a significantly higher apparent digestibility coefficient (ADC) for crude protein. However, positive effects of phytase supplementation on protein and amino acid availability in fish have been inconsistently reported. Papatryphon et al. (1999) found no positive effects of phytase supplementation on ADC of protein in striped bass. Similarly, no significant differences in the ADC of protein were observed when Atlantic salmon (Storebakken et al., 1998) were fed a

diet based on phytase-treated soy protein concentrate as the main protein feedstuff, or rainbow trout (Lanari et al., 1998) were fed a phytase- supplemented diet with herring meal and soybean meal as the main protein sources, or tilapia (Riche et al., 2001) fed phytase-supplemented diet based on herring meal and solvent-extracted soybean meal. Riche et al. (2001) claimed that the potential reason for the neutral or negative interaction between phytase and amino acids, especially methionine and lysine, was because the removal of phytate may promote the activity of other anti-nutritional factors and inhibit the hydrolysis of amino acids or increase the leaching of water-soluble components.

I.1.2.4 Absorption of minerals

Phytase supplementation can hydrolyze phytate, and so reduce mineral chelation. The phytase-induced increase in mineral availability can improve uptake and concentrations of minerals such as magnesium, calcium, manganese, and zinc in plasma, bone, and whole-body tissues of various animals including fish (Vielma et al., 1998). Sugiura et al. (2001) claimed that rainbow trout fed low-ash diets supplemented with phytase had significantly increased apparent absorption of Ca, Mg, Cu, Fe, Sr, and Zn. Yan et al. (2002) also reported that channel catfish fed a diet supplemented with 1000 FTU of phytase/kg had significantly increased Ca, Mg, and Mn concentrations in bone, but a positive effect on zinc was not observed until the dietary phytase concentration was increased to 8000 FTU/kg. In another study with channel catfish, Li et al. (2004) reported the addition of 250 FTU of phytase/kg increased bone ash and P concentrations.

Nwanna et al. (2005) claimed that African catfish (*Clarias gariepinus*) fed a raw soybean-based diet containing phytase at 8000 FTU/kg had improved feed conversion ratio, whole-body P, Ca, and Mn concentrations, but not growth performance, whole-body Mg or Zn concentrations. Baruah et al. (2005) found that compared to rohu (*Labeo rohita*) fingerlings fed a diet without phytase, those fed a diet containing phytase had significantly higher bone ash, P, and Ca, and the peak was observed in fish fed the diet supplemented with phytase at 750 FTU/kg. Thus, the utilization and retention of certain dietary minerals is likely species specific and also dependent on diet formulation and phytase concentration.

Several investigations into the effects of phytase on channel catfish performance have reported variable and somewhat contentious findings. Jackson et al. (1996) reported in a laboratory feeding trial that channel catfish fingerlings (6.5 g/fish initial weight) fed diets containing graded phytase levels from 0 to 4000 FTU/kg had significantly increased weight gain, feed consumption, bone ash, and P concentration. Similar results were reported by Li and Robinson (1997), who claimed that channel catfish fingerlings (6.8 g/fish) fed a diet with the addition of 250 FTU of phytase/kg or above had increased feed intake, weight gain, and improved bioavailability of phytate phosphorus. In contrast, Yan et al. (2002) reported positive effects of phytase supplementation on mineral concentrations in bone, but they did not find any beneficial effects on growth performance in a laboratory trial in which larger fingerling channel catfish (12 g/fish) was fed plant-protein-based diets supplemented with phytase. In addition, Robinson et al. (2002) reported no positive effects on growth performance or bone

mineralization when channel catfish (23 g/fish) were fed diets containing phytase under pond culture conditions. Thus, the different responses to phytase supplementation in the same species likely relate to differences in diet formulations, including phytate and inorganic phosphorus levels, as well as development status of the fish, and the culture environment.

In contrast to the channel catfish, the red drum (*Sciaenops ocellatus*), is a carnivorous marine fish, which has been produced in aquaculture for several decades for both seafood and stock enhancement (Gatlin III, 2002). However, to date there have been no studies evaluating the effects of dietary phytase supplementation on growth performance, nutrient digestibility, or mineral composition of this species. It is of interest to investigate the effects of dietary phytase on red drum and compare the results with that of the omnivorous channel catfish whose diets typically contain higher levels of plant feedstuffs.

I.2 Objective:

The objectives of this project are two-fold: 1) determine potential effects of graded levels of dietary phytase supplementation on growth performance, mineral utilization, and nutrient digestibility from different regions of the gastrointestinal tract of channel catfish and red drum fingerlings and 2) estimate the optimal dietary phytase dosage for both fish species.

Table 1. Reported beneficial effects of phytase supplementation to diets on animal performance and other aspects

Species	Protein source	Phytase source and level (FTU/kg)	Fish size and trial length	Growth performance	Other improvements	Reference
African catfish (*Clarias gariepinus*)	Fishmeal, soybean meal (raw, oven-dried, cooked, toasted, or soaked)	*Aspergilus oryzae* 0, 8000	7 g 77 d	Improved Specific growth rate (SGR) and feed conversion ratio (FCR)	Improved whole-body Ca, P, Zn, and Mn content. And reduced fecal Ca, Mg, P, Zn, and Mn concentration	Nwanna *et al*. (2005)
Atlantic salmon (*Salmo salar*)	Canola meal and casein	*A. niger* 0, 2000	100.7 g 84 d	No significant differences	A positive interaction effect between phytase and mono calcium phosphate (MCP) on bone ash, bone P, and whole-body P content. Similar trend was found in P digestibility, P retention, and P load.	Sajjadi and Carter (2004a)
	Casein and fishmeal	*A. niger* 0, 2000	28.9 g 84 d	Phytase increased weight gain. Positive interaction between phytate and phytase on FE	Positive interaction between phytate and phytase on ADC of crude protein (CP).	Sajjadi and Carter (2004b)
	Fish meal and soy protein concentrate (SPC) (pretreated or untreated)	*A. niger* NS	100 g 84 d	Pretreated SPC improved FCR	Pretreated SPC improved protein digestibility, protein retention, and reduced metabolic N-excretion. Increased whole-body Ca, Mg, and Zn level, and ADC of Ca, Mg, and Zn.	Storebakken *et al*. (1998)
Channel catfish (*Ictalurus punctatus*)	Soybean meal (dehulled)	*A. niger* 0, 500, 1000, 2000, 4000	6.5 g 70 d	Increased weight gain, feed consumption, and decreased FCR	Increased bone ash and bone P content. The fecal P content decreased linearly as phytase supplementation increased	Jackson *et al*. (1996)
	Soybean meal(dehulled)	*A. niger* 0, 250, 500, 750	6.8 g 84 d	Higher feed consumption and weight gain, and lower FCR	Higher bone ash and P content and lower fecal P concentration	Li *et al*. (1997)
	Soybean meal	*A. niger* 0,250, 500	23 g May to October	No significant difference	Phytase can replace the dicalcium phosphate supplement in channel catfish diet	Robinson *et al*. (2002)

	Soybean meal	*A. niger* 0, 500, 1000, 2000, 4000, 8000	12 g 98 d	No significant difference	Significantly increased bone ash, Ca, P, and Mn concentration-phytase ≥500 FTU; Significantly increased bone Mg concentration- phytase ≥1000 FTU; Significantly increased bone Zn concentration- phytase ≥8000 FTU; Phytase significantly increased the rate of phytate dephosphorylation in the stomach.	Yan *et al*. (2002)
Common carp (*Cyprinus carpio*)	Plant feedstuffs (maize gluten, isolated soy protein, wheat, and maize)	*A. niger and A. oryza* 0, 4000	30.9 g 84 d	No significant difference among fish fed diet containing un-incubated plant feedstuffs and with or without both kind of phytase. Improved feed intake, SGR, and FCR was noticed when fish fed diet containing incubated plant feedstuffs with either kind of phytase.	Similar trend was found in ADC of organic matter, ash, P, and Mg and bone and scale P, Ca, Mg, Zn, and Mn content, and plasma P content. Opposite trend was shown in fecal P, Ca, Mg, Zn, Mn, and Cu concentration	Nwanna *et al*. (2007)
	Soybean meal	*A. niger* 0, 500, 1000	40 g 63 d	Increase weight gain	Higher whole-body ash and P content; Improved utilization of native P and reduced the P excretion by 30%.	Schäfer *et al*. (1995)
Korean rockfish (*Sebastes schlegeli*)	White fishmeal and soybean meal	*A. niger* 0, 1000, 2000	7.25 g 56 d	No significant difference	Phytase increased ADC of dry matter and P	Yoo *et al*. (2005)
Nile tilapia (*Oreochromis niloticus*)	Soybean meal (Oil-extracted)	NS 0, 1000	0.7-0.8 g 60d	Decreased the FCR from 1.85 to 1.35 after adding phytase to the basal diet without extra (MCP); Did not reduce growth performance until the diet contained less than 12.5 g/kg MCP, compared to the basal diet which contained 25	Compared to the fish fed basal diet without MCP, fish fed phytase-supplemented diet had higher whole-body ash (10%), crude protein (3.9), and P (26.3%); Increased the ADC of phosphorus from 28.2 to 69.5%.	Cao *et al*. (2008)

				g/kg MCP.		
	Soybean meal	*A. niger* 0, 500, 1500, 3000	8.88 g 45 d	Based on the broken line, FCR was significantly increased in fish fed diet containing 700 FTU/kg phytase	Based on the broken line, bone Ca, P, Zn, Mg, and Fe, and digestibility of CP, Ca, and P were significantly increased when fish fed diet contained 700 FTU/kg phytase	Furuya *et al*. (2001)
	Soybean meal (toasted)	NS 0, 2000, 4000, 6000, 8000, 10000	6.21 g 63 d	No significant difference	Higher ADC of protein (44.7%), lower fecal Ca (41.2%), P (33.9%), Mg (15.1%), K (44.9%), Zn (10.5%), and Mn (41.9%) content.	Nwanna *et al*. (2005)
	Herring meal and soybean meal (phytase pretreated or untreated)	*A. niger* NS	68 g 10 d	NS		Riche *et al*. (2001)
Pangas catfish (*Pangasius Pangasius*)	Soybean meal	*A. niger* 0, 150, 250, 350, 500, 1000, 2000	1.97-2.05 g 60 d	NS	Increasing ADC of Ca (104.5%), P (28.4%), Mg (32.7%), Mn (73.1%), Zn (6.6%), Fe (82.5%), K (2.8%), Cu (22.3%), and Co (25.5%); Increasing whole-body Ca (49.3%), P (3.2%), Mg (13.3%), Zn (20.7%), Fe (44.2%), Cu (43.3%), and Co (500%); Increasing bone Ca (14.2%), P (5.8%), Mg (35%), Mn (34.3%), Fe (64.8%), K (34.6%), Cu (240.9%), and Co (162.7%).	Debnath *et al*. (2005)
Rainbow trout (*Onchorhyncus mykiss*)	Casein and full-fat soybean meal	*A. niger* 0, 200, 400, 600, 800, 1000	170.8 g 14 d	NS	Nutrient digestibility increased with dietary phytase content up to 400 FTU/kg: P (>100%), Mn (>100%), Zn (>100%), and Mg (35%); Up to 800 FTU/kg: digestibility of threonine, alanine, aspartic acid, glutamic acid and glycine increased by 1-2%.	Cheng *et al*. (2003)
	Anchovy meal Canola protein concentrate	*A. niger* 0, 500, 1500, 4500	17.9 g 84 d	No significant difference	Increasing lipid-free bone Ca (15.7%), P (13.8%), Mg (4.2%), and Zn (18.7%)	Forster *et al*. (1999)
	Herring meal and soybean meal (defatted and dehulled)	*A. niger* 0, 1000	115.3 g 49 d	Improved daily weight gain and FCR	Increased whole-body P content, whole-body protein, ash, and P retention. Also, reduced P released to water.	Lanari *et al*. (1998)

	Herring meal and soybean meal (untreated, phytase treated)	*A. oryzae* 0, 500, 1000, 2000, 4000	153 g NS	NS	Phytase supplementation increased ADC of P, protein, ash, Ca, Mg, Cu, Fe, Sr, and Zn.	Sugiura *et al*. (2001)
	Soy protein concentrate	*A. niger* 0, 1500	51.6 g 84 d	Higher weight gain	Improved ADC of P and bone ash and plasma and whole-body P concentration	Vielma *et al*. (1998)
	Fishmeal and soy-derived protein (soy protein concentrate:soybean meal = 4:1)	*A. niger* 0, 1200	250 g 168 d	No significant difference	No significant difference	Vielma *et al*. (2000)
	Soybean meal	*A. oryzae* 0, 2000	20 g 84 d	Phytase increased weight gain from 243% (basal diet-P deficient) to 459% (basal diet+phytase)	Phytase decreased fecal phytic acid content from 35 to 5; Increased ADC of P from 23% to 83%; Increase ADC of protein by 3.2%.	Vielma *et al*. (2004)
Rohu (*Labeo rohita*)	Soybean meal	NS 0, 500	12.61-13.72 g 60 d	NS	Bone mineral content increased: Na (15%), Ca (12.1%), K (17.4%), P (9.2%), and Fe (40.7%)	Baruah *et al*. (2005)
Striped bass (*Morone saxatilis*)	Soybean meal and corn gluten meal	*A. niger* 0, 500, 1000, 2000	24.5 g 112 d	Lower FCR when fish fed diet with phytase higher than 1000 FTU	Higher vertebral and scale ash content, and P retention when fish fed diet with phytase higher than 1000 FTU. Increased ADC of P (72.1%) when fish fed diet with phytase. Increased ADC of Ca (428.8%) and Zn (166.5%) when fish fed diet with phytase up to 2000 FTU.	Papatryphon *et al*. (1999)
	Isolated soy protein, soybean meal, corn gluten meal, or wheat middling	*A. niger* 0, 1000	3-year-old 28 d	NS	Increased ADC of P.	Papatryphon *et al*. (2001)

* NS, not specified

CHAPTER II

THE EFFECTS OF SUPERDOSING DIETARY PHYTASE ON GROWTH, MINERAL UTILIZATION, AND AMINO ACID DIGESTIBILITY OF CHANNEL CATFISH (*Ictalurus punctatus*)

II.1. Introduction

The increasing of the fishmeal supply can't catch up the global fishmeal demand due to the fast expansion of the aquaculture industry. Thus, nowadays, to maintain the price and quality of aqua-feeds, plant-derived protein ingredients were commonly used in aquaculture field (FAO 2020). Although these plant-derived protein sources are lower in price and improved in sustainability, they usually contain lots of antinutritional factors, such as protease inhibitors, phytate, lectins, or gossypol (Francis et al., 2001). Phytate (hexakisphosphates of Myo-inositol), the salt form of phytic acid, had been proven to reduce the growth performance, mineral absorption, and protein utilization of different fish species (Vikas Kumar et al., 2012). Phytase is the enzyme commonly used to degrade phytate in poultry and swine diets. In addition, the positive effects of phytase on aquatic animals had been well summarized by several researchers (Cao et al., 2007; Dersjant-Li et al., 2015; Lemos & Tacon, 2017; Lie et al., 1999).

However, outcomes from the several investigations into the effects of phytase on channel catfish performance were variable and contentious. Jackson et al. (1996) reported in a laboratory feeding trial that channel catfish fingerlings (6.5 g/fish initial weight) fed diets containing graded

levels of phytase from 0 to 4000 FTU/kg had significantly increased weight gain, feed consumption, bone ash, and bone P concentration compared to fish fed the diet with inadequate available phosphorus. Similar results were reported by Li and Robinson (1997), who claimed that channel catfish fingerlings (6.8 g/fish initial weight) fed a diet with the addition of 250 FTU of phytase/kg or above had increased feed intake, weight gain, and improved bioavailability of phytate phosphorus, compared to the fish fed a diet deficient in available phosphorus. Besides that, Robinson et al. (2002) reported no positive effects on growth performance or bone mineralization when channel catfish (23 g/fish initial weight) were fed diets containing phytase up to 500 FTU under pond culture conditions, compared to fish was fed the diet supplied with extra 0.75% of dicalcium phosphate. In contrast to that study, Yan et al. (2002) reported positive effects of dietary phytase supplementation on mineral concentrations in bone of channel catfish fingerlings (12 g/fish initial weight) in a laboratory trial but they did not find any beneficial effects on growth performance. Thus, the different responses to phytase supplementation in the same species likely relate to differences in diet formulations, including phytate and inorganic phosphorus levels, as well as developmental status of the fish, and the experimental environment. However, almost all of the previous studies with channel catfish exclusively focused on growth performance and phosphorus utilization. The phytase levels used in those studies were usually between 250 to 4000 FTU/kg. It has been reported in poultry that super-dosing, that is going to higher levels of phytase, can give additional positive effects on the digestibility and utilization of nutrients (Cowieson et al., 2011; Fernandes et al., 2019; Woyengo & Wilson, 2019). Therefore,

the objectives of this study were to confirm and evaluate the effects of superdosing dietary phytase on growth performance, mineral utilization, and amino acid availability from different regions of the gastrointestinal tract of channel catfish to estimate the optimal dietary dosage.

II.2. Materials and methods

II.2.1 Diet formulations

Two control diets were designed to provide 31% crude protein primarily from soybean meal (Table 2). One diet without added monocalcium phosphorate (MCP) was used as the negative control diet (NC) and contained 0.11% available phosphorus (0.61% of total phosphorus) less than the established requirement for channel catfish at 0.33% of dry diet (Wilson et al., 1982). The positive control diet (PC) was supplemented with 1% MCP to achieve the minimum available phosphorus requirement. Another four experimental diets were formulated by adding phytase (Quantum Blue Phytase, AB Vista) at 1000 (F1000), 2000 (F2000), 4000 (F4000), or 8000 (F8000) FTU/kg to the NC diet. One FTU of phytase represents the amount of phytase that liberates inorganic phosphorus from a 5.1mM solution of sodium phytate at a rate of 1 μmol per min at pH 5.5 and 37 °C. All diets contained 0.1% of yttrium oxide as an inert marker for the digestibility calculation. The dietary phytase activity was analyzed by AB Vista using an enzyme-linked immunosorbent assay (ELISA) and supplemental levels were confirmed to be within 13% of formulated levels.

A V-mixer (Blendmaster Lab Blender; Patterson-Kelly, Stroudsburg, PA, USA) was used to blend all ingredients for 30 min. Then, an industrial food mixer (Model A-200; Hobart, Troy, OH, USA) was used to mix the dry ingredients with menhaden oil and water sequentially, each for approximately 15 min. Thereafter, diets were pelleted by a commercial meat grinder attachment via a 3-mm die plate. The pellets were air-dried at room temperature with forced ventilation for 24 h and ground to appropriate size to match the mouth gape of channel catfish. Pellets were stored at -20 °C but smaller aliquots were maintained at 4°C prior to weighing and feeding.

Table 2. Formulation and proximate composition of experimental diets (% dry matter).

Ingredients	Negative Control	Positive Control	F1000	F2000	F4000	F8000
Soybean meal[a]	46.65	46.65	46.65	46.65	46.65	46.65
Poultry byproduct meal[b]	6.00	6.00	6.00	6.00	6.00	6.00
Cottonseed flour[c]	2.00	2.00	2.00	2.00	2.00	2.00
Wheat flour[d]	13.90	13.90	13.90	13.90	13.90	13.90
Menhaden oil[e]	2.96	2.96	2.96	2.96	2.96	2.96
Vitamin premix[f]	3.00	3.00	3.00	3.00	3.00	3.00
Mineral premix[g]	4.00	4.00	4.00	4.00	4.00	4.00
Monocalcium phosphate[h]	0.00	1.00	0.00	0.00	0.00	0.00
Dextrinized corn starch[h]	16.00	16.00	16.00	16.00	16.00	16.00
Carboxymethyl cellulose[h]	3.00	3.00	3.00	3.00	3.00	3.00
DL-Methionine[i]	0.50	0.50	0.50	0.50	0.50	0.50
Yttrium oxide[j]	0.10	0.10	0.10	0.10	0.10	0.10
Cellulose[k]	1.89	0.89	1.79	1.69	1.49	1.09
Phytase premix[l]	0.00	0.00	0.10	0.20	0.40	0.80
Total	100.00	100.00	100.00	100.00	100.00	100.00
Analyzed composition %						
Crude protein	30.7	31.8	30.9	31.1	31.2	31.2
Crude lipid	5.26	5.53	5.16	5.07	5.48	5.33
Ash	8.62	8.64	8.59	8.63	8.56	8.38
Available phosphorus[m]	0.11	0.29	0.19	0.10	0.12	0.09
Total phosphorus	0.61	0.80	0.69	0.60	0.62	0.59
Phytase activity (FTU/kg)[n]	< 50	< 50	1130	2100	4380	7960

[a] Producers Cooperative Association, Bryan, Texas (crude protein [CP] = 53.14%; lipid = 3.34% on dry-matter basis).

[b] Tyson Foods, Springdale, AR, USA

[c] ADM, Decatur, IL, USA

[d] Rangen, Inc. Angelton, TX, USA

[e] Omega Protein, Reedville, VA, USA.

[f] Moon and Gatlin (1991)

[g] Mineral premix (contains as g/kg of dry weight): Calcium lactate 348.553, Ferrous sulfate 5, Magnesium sulfate heptahydrate 132, Sodium chloride 45, Aluminum chloride 0.084, Potassium iodide 0.15, Cupric sulfate 0.5, Manganous sulfate 0.7, Cobalt chloride 1, Zinc sulfate heptahydrate 3, Sodium selenite 0.0127, Cellulose 464.

[h] MP Biomedicals, Solon, OH, USA.

[i] Ajinomoto North America, Inc.

[j] SIGMA-ALDRICH, Co., St. Louis, MO, USA

[k] U.S. Biochemical, Cleveland, OH, USA.

[l] Enhanced *Escherichia coli* phytase (Quantum Blue; AB Vista, Marlborough, UK)

[m] Available P was computed based on the difference between total P and Phytate-P. All the P concentrations were measured via near infrared spectroscopy (NIR) by AB Vista.

[n] The phytase activity was analyzed by AB Vista via an enzyme-linked immunosorbent assay (ELISA)

II.2.2 Fish and experimental conditions

The feeding trial was carried out at the Texas A&M University Aquacultural Research and Teaching Facility (ARTF) of the Texas A&M University System (Burleson County, TX). Juvenile channel catfish with an initial weight of 8.02 ± 0.18 g (mean ± S.D.) were obtained from ponds at the ARTF. All fish was acclimatized for 2 weeks in the recirculating aquaculture system, consisting of 110-L glass aquaria with a settling chamber, biological and mechanical filtration, as well as UV-filtration prior to the feeding trial. Groups of 20 catfish were stocked into each of 24 aquaria, and all dietary treatments were randomly assigned to quadruplicate aquaria. Meanwhile, 15 fish was collected as initial whole-body samples. Fish were fed their assigned diet twice daily according to a percentage of body weight which approached apparent satiation but without wastage. Feeding rate initially started at 6% of total body weight per day for all treatments and was gradually reduced over time to maintain feeding levels close to apparent satiation without overfeeding. Water quality and other environmental parameters were monitored and maintained within the appropriate ranges for catfish culture (pH: 7.6-8, total nitrite – nitrogen < 0.010 ml/L, total ammonia – nitrogen < 0.15 mg/L, dissolved oxygen > 6.5 mg/L, temperature: 26 ± 0.5). The photoperiod was set at 12h :12h via fluorescent lights controlled by a timer.

II.2.3 Sample collection and analysis

At the end of the 8-week feeding trial, production parameters such as weight gain (WG), feed efficiency ratio (FE), and survival were recorded for each aquarium. Three fish per

aquarium were randomly selected and euthanized with an overdose of tricaine methane sulphonate (MS-222) at 300 mg/L (Topic Popovic et al., 2012) to measure the condition indices: hepatosomatic index (HSI), intraperitoneal fat (IPF) ratio, and muscle yield (MY) as previously described (Wu & Gatlin III, 2014). In addition, the vertebrae samples from those same three fish per tank were isolated and analyzed for ash and various minerals, using similar methods as described by Savolainen and Gatlin III (2010). Another three fish per aquarium were homogenized as a composite sample for measurement of proximate composition along with the experimental diets and the initial whole-body samples using the following established methods: the Dumas protocol for crude protein (6.25 × N)(AOAC, 2005), chloroform : methanol extraction for crude lipid (Folch et al., 1957), and heating samples at 650 °C in the muffle furnace for 3 h for ash (AOAC, 1990). Organic matter was computed as dry matter minus ash (inorganic matter). Whole-body protein retention (PR) and lipid retention (LR) also were computed as previously described (de Cruz et al., 2020). In addition, the homogenized whole-body samples were analyzed for nitrogen, calcium, magnesium, phosphorus, copper, iron, manganese, and zinc by the Department of Veterinary Integrative Biosciences at Texas A&M University following digestion with nitric and hydrochloric acids according to protocol 1638 of the Environmental Protection Agency. The samples were analyzed by ICP-MS on a Perkin Elmer DRC 2 instrument in standard mode, using Rh as an internal standard. The whole-body retention of various minerals was computed according to established procedures (Chen et al., 2020) based on the whole-body mineral concentrations and amount of minerals consumed by fish fed the

various experimental diets. In addition, the excretion of various minerals also was calculated according to established procedures.

Additionally, three days after the trial termination, five fish per aquarium were dissected and digesta samples from stomach, anterior and posterior intestinal regions were collected approximately 7 hours after feeding. Amino acid composition of these digesta samples were measured via an Ultra Performance Liquid Chromatography (UPLA ACQUITY system; Waters), according to the procedures described by Castillo et al. (2015). The concentrations of various minerals were also determined in these digesta samples as described previously. Then, the digestibility of amino acids and availability of various minerals was calculated based on the concentrations of various nutrients and yttrium.

The remaining fish in each aquarium were maintained on their respective experimental diets for an additional week prior to measuring post-prandial plasma phosphorus concentrations. All the fish were fasted for 36 h prior to feeding to satiation, after which 2 fish per tank were bled from the caudal vasculature with 1.0-ml heparinized syringes at 0, 2, 4, 6, 8, 10, 12 and 24 h after feeding. Plasma was separated from the whole blood by centrifugation at 10,000 rpm for 10 minutes and deproteinized according the method described by Castillo et al. (2015) prior to the phosphorus analysis. Afterward, plasma phosphorus concentration was measured by the phosphate assay kit (Sigma Aldrich Corporation).

II.2.4 Calculations and statistical analysis

The following variables were calculated as follows:

Weight gain (WG) = 100 × (FW - IW) / IW

Feed efficiency (FE) = (weight gain / dry feed offered)

Survival = 100 × (final fish number) / (initial fish number)

Hepatosomatic index (HSI) = 100 × liver weight / body weight

Intraperitoneal fat (IPF) ratio = 100 × IPF weight / body weight

Nutrient retention = 100 × (final body nutrient – initial body nutrient) / total nutrient fed

Nutrient excretion = (total nutrient fed – (final body nutrient – initial body nutrient)) / (final body weight – initial body weight)

Where, FW is final body weight, IW is initial body weight.

Apparent digestibility / availability Coefficient = 100 × (1 - Yttrium in feed / Yttrium in feces × Nutrient content of feces / Nutrient content of feed)

All data were validated for homogeneity of variances (Levene's test) and subjected to analysis of variance and orthogonal polynomial contrasts to verify the patterns that most adequately described the results. Linear orthogonal contrast analyses also were performed to detect significant differences ($P < 0.05$) among the following treatment groups (NC – PC; NC – (F1000 to F8000); PC – (F1000 to F8000)), performed using JMP 15.2.0 Pro (SAS Institute Inc., Cary, NC, USA). In addition, due to some values showing negative apparent availability values, only the minerals that had all positive apparent availability results were analyzed via regression and linear orthogonal contrast analysis.

II.3. Results

II.3.1 Fish growth performance and condition indices

Channel catfish fed all of the experimental diets for 8 weeks achieved an increase of over 300% in initial body weight with survival over 98% (Table 3). According to the contrast analysis, catfish fed the NC diet had the lowest weight gain and feed efficiency values which were significantly ($P < 0.05$) reduced compared to those fed the PC and all phytase-supplemented diets. Although the quadratic model between dietary phytase doses and WG or FE was well established, the quadratic element had a higher P value. Thus, the relationship between the dietary phytase doses and WG or FE was better explained as an ascending linear model. The same justification was used whenever both linear and quadratic models were significant, but the linear model was chosen. Body condition indices including HSI, IPF ratio and MY did not vary considerably by treatment, although catfish fed the NC diet had significantly higher MY than fish fed the phytase-supplemental diets (Table 3).

Fish fed the NC diet had significantly lower ($P < 0.05$) whole-body moisture and ash contents, as well as PR compared to fish fed the other diets (Table 4). In addition, whole-body moisture, ash, and PR had an upward quadratic relationship with increasing dietary phytase doses, in which the optimal dosage was estimated to be 4850, 5491, and 5356 FTU/kg diet, respectively. On the other hand, the relationship between dietary phytase dose and whole-body lipid or LR was explained as a downward quadratic regression.

Table 3. Weight gain (WG), feed efficiency (FE), survival, hepatosomatic index (HSI), intraperitoneal fat (IPF) ratio, and muscle yield (MY) of channel catfish fed experimental diets including those with different levels of phytase for 8 weeks[a]

Diet	WG (%)	FE	Survival (%)	HSI	IPF ratio	MY (%)
Negative control	305	0.67	98.8	1.39	3.12	29.1
Positive control	390	0.76	98.8	1.43	3.12	26.7
F1000	399	0.77	100.0	1.42	3.36	26.1
F2000	438	0.81	98.8	1.46	3.36	26.7
F4000	383	0.76	100.0	1.38	3.06	25.2
F8000	447	0.81	98.8	1.42	3.13	26.3
PSE[b]	19.9	0.019	1.31	0.064	0.344	0.966
Orthogonal contrast (Pr > F)[c]						
Linear	0.02	0.02	0.8	1	0.7	0.2
Quadratic	0.03	0.02	0.6	1	0.9	0.07
Regression						
Model[d]	L	L	NOS	NOS	NOS	NOS
Pr > F	0.02	0.02	-	-	-	-
R^2	0.27	0.25	-	-	-	-
Optimal dosage[e]	-	-	-	-	-	-
Contrast (Pr > F)						
NC – PC	0.006	0.005	0.6	0.7	1	0.1
NC – (F1000 to F8000)	<0.0001	<0.0001	0.3	0.7	0.8	0.01
PC – (F1000 to F8000)	0.3	0.2	0.7	0.9	0.8	0.6

[a] Values are means of three fish from each of four replicate groups (n=4). Significance probability associated with the *P*-value.

[b] Pooled standard error.

[c] Orthogonal contrast analysis was only run among treatment NC and all phytase-supplemental treatments, which needed to be all positive results. If statistical significance (P<0.05) were detected, the model which fit best with the data was chosen.

[d] L = linear model; Q = quadratic model; NOS = no structure.

[e] Upward quadratic model was used to derive the optimal dosage.

Table 4. Whole-body proximate composition (% of fresh weight), protein retention (PR), and lipid retention (LR) of channel catfish fed experimental diets including those with different levels of phytase for 8 weeks[a]

Diet	Moisture	Protein	Lipid	Ash	PR	LR
Negative control	69.7	15.1	12.3	2.3	32.5	168
Positive control	70.9	15.6	9.7	2.8	38.1	133
F1000	70.9	16.0	9.9	2.8	39.7	142
F2000	70.7	15.8	10.0	2.9	41.5	149
F4000	71.9	15.8	8.9	2.9	39.2	124
F8000	70.9	15.6	10.0	3.0	41.0	151
PSE[b]	0.41	0.20	0.34	0.06	1.26	7.6
Orthogonal contrast (Pr > F)[c]						
Linear	0.09	0.75	0.05	0.004	0.04	0.35
Quadratic	0.002	0.21	<0.0001	0.0002	0.02	0.007
Regression						
Model[d]	Q	NOS	Q	Q	Q	Q
Pr > F	0.002	-	<0.0001	0.0002	0.02	0.007
R^2	0.51	-	0.70	0.63	0.38	0.45
Optimal dosage[e]	4850	-	-	5491	5356	-
Contrast (Pr > F)						
NC – PC	0.04	0.11	<0.0001	<0.0001	0.006	0.004
NC – (F1000 to F8000)	0.01	0.01	<0.0001	<0.0001	<0.0001	0.005
PC – (F1000 to F8000)	0.75	0.47	0.98	0.48	0.13	0.31

[a] Values are means of composite samples of three fish from each of four replicate groups (n=4). Significance probability associated with the *P*-value.

[b] Pooled standard error

[c] Orthogonal contrast analysis was only run among treatment NC and all phytase-supplemental treatments, which needed to be all positive results. If statistical significance (P<0.05) were detected, the model which fit best the data was chosen.

[d] L = linear model; Q = quadratic model; NOS = no structure

[e] Upward quadratic model was used to derive the optimal dosage.

In addition, significantly reduced ($P < 0.05$) whole-body protein was observed in fish fed the NC diet compared to those fed the phytase-supplemented diets. Catfish fed NC diet also had the highest ($P < 0.05$) whole-body lipid content and LR compared to fish fed all other experimental diets.

The whole-body mineral retention of catfish was affected by various diets (Table 5). Catfish fed the NC diet had significantly lower ($P < 0.05$) Ca, Mg, P and Mn retention compared to fish fed the PC diet. However, a significantly decreased ($P < 0.05$) Cu and Zn retention was observed in catfish fed PC diet compared to those fed all other experimental diets. Compared to catfish fed the NC diet, those fed the phytase-supplemented diets had significantly increased ($P < 0.05$) Fe and Mn retention. Based on the contrast analysis, catfish fed the phytase-supplemented diets had significantly higher Ca, Mg, P, and Zn retention than those fed the NC or PC diets ($P < 0.05$). All mineral retention data, except the whole-body Cu, had an upward quadratic relationship with dietary phytase dose, with the optimal dosage estimated via these models to range from 4764 to 6845 FTU/kg diet. On the other hand, a descending linear relationship was detected between dietary phytase dose and whole-body Cu retention.

Table 5. Whole-body mineral retention (%) of channel catfish fed experimental diets including those with different levels of phytase for 8 weeks[a]

Diet	Ca	Mg	P	Cu	Fe	Mn	Zn
Negative control	33.8	5.6	34.9	1.5	2.2	2.2	18
Positive control	69.4	9.1	49.6	1.1	2.5	5.9	11.9
F1000	75.5	10.9	57.5	1.5	2.1	4.7	37.5
F2000	92.1	9.7	72.2	1.7	3.6	6.6	41.8
F4000	87.4	9.4	66.1	1.2	3.5	6.1	37.7
F8000	103.3	9.8	83.3	1.5	2.9	6.8	36.5
PSE[b]	4.97	0.34	3.10	0.05	0.25	0.40	1.38
Orthogonal contrast (Pr > F)[c]							
Linear	0.0004	0.1	<0.0001	<0.0001	0.1	0.001	0.08
Quadratic	<0.0001	0.026	<0.0001	<0.0001	0.006	<0.0001	0.0007
Regression							
Model[d]	Q	Q	Q	L	Q	Q	Q
Pr > F	<0.0001	0.026	<0.0001	<0.0001	0.006	<0.0001	0.0007
R^2	0.7	0.35	0.72	0.84	0.46	0.7	0.58
Optimal dosage[e]	5982	5020	6845	-	4764	5647	4819
Contrast (Pr > F)							
NC – PC	<0.0001	<0.0001	0.004	<0.0001	0.4	<0.0001	0.006
NC – (F1000 to F8000)	<0.0001	<0.0001	<0.0001	0.4	0.009	<0.0001	<0.0001
PC – (F1000 to F8000)	0.002	0.04	<0.0001	<0.0001	0.09	0.8	<0.0001

[a] Values are means of composite samples of three fish from each of four replicate groups (n=4). Significance probability associated with the *P*-value.

[b] Pooled standard error

[c] Orthogonal contrast analysis was only run among treatment NC and all phytase-supplemental treatments, which needed to be all positive results. If statistical significance (P<0.05) were detected, the model which fit best the data was chosen.

[d] L = linear model; Q = quadratic model; NOS = no structure

[e] Upward quadratic model was used to derive the optimal dosage.

Based on contrast analysis, catfish fed the NC diet had significantly higher ($P < 0.05$) excretion of N, Ca, Mg, and Mn than fish fed the other diets. In addition, significantly higher ($P < 0.05$) excretions of P and Cu were observed in catfish fed the NC diet compared to fish fed the phytase-supplemented diets. A downward quadratic model was well established between dietary phytase dose and the excretion of N, Ca, P, and Zn, where the optimal dosages were estimated to be from 4703 to 6504 FTU/kg diet (Table 6). Nonetheless, the highest Zn excretion occurred in catfish fed the PC diet. Compared to catfish fed the PC diet, fish fed the phytase-supplemented diets had a significantly decreased excretion of Ca, P, Cu, and Zn. However, catfish fed the PC diet had a significantly lower Mn excretion than fish fed the phytase-supplemented diets.

According to contrast analysis, significantly reduced ($P < 0.05$) bone ash, Ca, Mg, P, and Mn content was observed in catfish fed the NC diet compared to fish fed any of the other experimental diets. Moreover, catfish fed the phytase-supplemented diets had significantly higher ($P < 0.05$) bone Ca, Mg, P, Zn concentrations than fish fed the NC or PC diets. However, a significantly lower ($P < 0.05$) bone Zn content was found when catfish were fed the PC diet compared to the NC diet. The bone ash, Ca, Mg, P, Mn, and Zn concentrations demonstrated upward quadratic relationships with dietary phytase dose, in which the optimal dosages ranked from 5050 to 5773 FTU/kg diet (Table 7).

Table 6. Estimated excretion of various minerals (g/kg of body weight) of channel catfish fed experimental diets including those with different levels of phytase for 8 weeks[a]

Diet	N	Ca	Mg	P	Cu	Fe	Mn	Zn
Negative control	50.5	6.6	4.0	6.0	0.021	0.19	0.06	0.07
Positive control	41.0	3.2	3.2	5.3	0.022	0.20	0.05	0.09
F1000	39.5	2.4	2.7	3.8	0.016	0.27	0.07	0.04
F2000	36.0	0.6	3.0	2.0	0.014	0.16	0.04	0.04
F4000	40.1	1.1	3.3	2.8	0.019	0.17	0.05	0.05
F8000	36.3	-0.2	3.4	1.2	0.016	0.20	0.05	0.05
PSE[b]	1.84	0.46	0.09	0.31	0.0005	0.006	0.002	0.002
Orthogonal contrast (Pr > F)[c]								
Linear	0.02	0.0005	0.8	<0.0001	0.4	0.5	0.04	0.2
Quadratic	0.01	<0.0001	0.1	<0.0001	0.5	0.3	0.06	0.002
Regression Model[d]	Q	Q	NOS	Q	NOS	NOS	NOS	Q
Pr > F	0.01	<0.0001	-	<0.0001	-	-	-	0.002
R^2	0.41	0.71	-	0.72	-	-	-	0.53
Optimal dosage[e]	5572	5825	-	6504	-	-	-	4703
Contrast (Pr > F)								
NC – PC	0.002	<0.0001	<0.0001	0.2	0.09	0.8	<0.0001	<0.0001
NC – (F1000 to F8000)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.2	0.02	<0.0001
PC – (F1000 to F8000)	0.2	0.0004	0.3	<0.0001	<0.0001	0.4	0.0007	<0.0001

[a] Values are means of composite samples of three fish from each of four replicate groups (n=4). Significance probability associated with the *P*-value.

[b] Pooled standard error

[c] Orthogonal contrast analysis was only run among treatment NC and all phytase-supplemental treatments, which needed to be all positive results. If statistical significance (P<0.05) were detected, the model which fit best the data was chosen.

[d] L = linear model; Q = quadratic model; NOS = no structure

[e] Downward quadratic model was used to derive the optimal dosage.

Table 7. Ash and mineral composition (dry-matter basis) of bone from channel catfish fed experimental diets including those with different levels of phytase for 8 weeks[a]

Diet	Ash (%)	Ca (g/kg)	Mg (g/kg)	P (g/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)
Negative control	38	143	1.9	7.1	12.6	29.2	114
Positive control	45	161	2.6	8.1	10.8	50.9	83
F1000	45	165	2.6	8.4	11.2	48.2	180
F2000	46	172	2.8	8.7	9.2	51.7	183
F4000	46	169	2.8	8.5	11.7	52.6	194
F8000	47	175	3	8.9	11.7	50.6	203
PSE[b]	0.5	2.8	0.05	1.35	0.01	2.24	4.88
Orthogonal contrast (Pr > F)[c]							
Linear	0.006	0.002	0.0004	0.002	0.9	0.02	0.0006
Quadratic	0.0001	0.0002	<0.0001	0.0002	0.6	0.0001	<0.0001
Regression Model[d]	Q	Q	Q	Q	NOS	Q	Q
Pr > F	0.0001	0.0002	<0.0001	0.0002	-	0.0001	<0.0001
R^2	0.65	0.63	0.76	0.63	-	0.65	0.75
Optimal dosage[e]	5324	5748	5733	5773	-	5050	5670
Contrast (Pr > F)							
NC – PC	<0.0001	0.0002	<0.0001	<0.0001	0.3	<0.0001	0.0003
NC – (F1000 to F8000)	<0.0001	<0.0001	<0.0001	<0.0001	0.2	<0.0001	<0.0001
PC – (F1000 to F8000)	0.2	0.01	0.001	0.004	0.9	1	<0.0001

[a] Values are means of composite samples of three fish from each of four replicate groups (n=4). Significance probability associated with the *P*-value.

[b] Pooled standard error

[c] Orthogonal contrast analysis was only run among treatment NC and all phytase-supplemental treatments, which needed to be all positive results. If statistical significance (P<0.05) were detected, the model which fit best the data was chosen.

[d] L = linear model; Q = quadratic model; NOS = no structure

[e] Upward quadratic model was used to derive the optimal dosage.

II.3.2 Apparent availability of various minerals

In the digestibility trial, the apparent availability of different minerals in the stomach, anterior and posterior intestinal regions of channel catfish are presented in Tables 8, 9, and 10, respectively. For digesta taken from the stomach, catfish fed the phytase-supplemented diets had significantly higher ($P < 0.05$) apparent availability of P than fish fed either NC or PC diets (Table 8). In addition, catfish fed PC had a significantly improved ($P < 0.05$) apparent availability of P than fish fed the NC diet. In addition, the highest ($P < 0.05$) apparent availability of Zn was observed in catfish fed the PC diet compared to fish fed all other experimental diets. No significant differences were found on the apparent availability of Ca, Mg, and Mn among fish fed various experimental diets. Only the apparent availability of P had an upward quadratic relationship with dietary phytase dose, where the optimal dosage was estimated to be 5186 FTU/kg diet.

Table 8. Apparent availability (%) of different minerals in digesta from the stomach of channel catfish fed experimental diets including those with different levels of phytase for 8 weeks[a]

Diet	Ca	Mg	P	Cu	Fe	Mn	Zn
Negative control	55.3	78.1	40.6	15.3	-27.9	63.5	58.9
Positive control	55.6	75.4	48.3	40.2	14.2	61.0	67.6
F1000	49.6	73.2	55.6	29.2	49.2	75.0	57.5
F2000	46.7	75.2	53.1	-54.3	21.9	60.7	55.4
F4000	59.9	77.0	60.5	31.2	17.9	63.1	56.2
F8000	52.2	77.3	57.0	-17.9	28.1	65.6	56.2
PSE[b]	4.24	1.86	2.46	26.58	13.04	2.51	2.16
Orthogonal contrast (Pr > F)[c]							
Linear	0.8	0.5	0.02	-	-	0.6	0.5
Quadratic	0.9	0.7	0.001	-	-	0.7	0.6
Regression							
Model[d]	NOS	NOS	Q	NOS	NOS	NOS	NOS
Pr > F	-	-	0.001	-	-	-	-
R^2	-	-	0.56	-	-	-	-
Optimal dosage[e]	-	-	5186	-	-	-	-
Contrast (Pr > F)[f]							
NC – PC	1	0.3	0.04	-	-	0.5	0.01
NC – (F1000 to F8000)	0.5	0.3	<0.0001	-	-	0.4	0.3
PC – (F1000 to F8000)	0.5	0.9	0.008	-	-	0.08	0.0002

[a] Values are means of composite samples of three fish from each of four replicate groups (n=4). Significance probability associated with the *P*-value.

[b] Pooled standard error

[c] Orthogonal contrast analysis was only run among treatment NC and all phytase-supplemental treatments, which needed to be all positive results. If statistical significance (P<0.05) were detected, the model which fit best the data was chosen.

[d] L = linear model; Q = quadratic model; NOS = no structure

[e] Upward quadratic model was used to derive the optimal dosage.

[f] The contrast analysis was only applied to the groups that had all positive results. If any groups contained negative data, "-" will be shown in the cell

Table 9. Apparent availability (%) of different minerals in digesta from the anterior intestinal region of channel catfish fed experimental diets including those with different levels of phytase for 8 weeks[a]

Diet	Ca	Mg	P	Cu	Fe	Mn	Zn
Negative control	18.2	14.3	44.7	-39.3	17.4	0.3	-9.5
Positive control	12.2	21.6	55.4	-5.2	23.7	46.9	18.7
F1000	29.8	-16.2	73.4	-9.7	50.8	59.7	-2.3
F2000	36.1	20.2	79.6	-34.7	27.0	15.9	-0.4
F4000	34.8	27.3	82.7	-5.9	12.1	20.9	-7.2
F8000	17.4	19.0	82.8	-70.2	32.2	8.8	-23
PSE[b]	10.32	11.20	3.85	34.60	3.99	9.82	14.81
Orthogonal contrast (Pr > F)[c]							
Linear	-	-	0.005	-	0.9	-	-
Quadratic	-	-	<0.0001	-	0.7	-	-
Regression							
Model[d]	NOS	NOS	Q	NOS	NOS	NOS	NOS
Pr > F	-	-	<0.0001	-	-	-	-
R^2	-	-	0.68	-	-	-	-
Optimal dosage[e]	-	-	5335	-	-	-	-
Contrast (Pr > F)[f]							
NC – PC	-	-	0.06	-	0.3	-	-
NC – (F1000 to F8000)	-	-	<0.0001	-	0.009	-	-
PC – (F1000 to F8000)	-	-	<0.0001	-	0.1	-	-

[a] Values are means of composite samples of three fish from each of four replicate groups (n=4). Significance probability associated with the *P*-value.

[b] Pooled standard error

[c] Orthogonal contrast analysis was only run among treatment NC and all phytase-supplemental treatments, which needed to be all positive results. If statistical significance (P<0.05) were detected, the model which fit best the data was chosen.

[d] L = linear model; Q = quadratic model; NOS = no structure

[e] Upward quadratic model was used to derive the optimal dosage.

[f] The contrast analysis was only applied to the groups that had all positive results. If any groups contained negative data, "-" will be shown in the cell

In the anterior intestinal region, based on the contrast analysis, catfish fed the NC diet had significantly lower ($P < 0.05$) apparent availability of P and Fe than fish fed the phytase-supplemental diets (Table 9). Similar to digesta from the stomach, only the apparent availability of P was observed to have an upward relationship with dietary phytase doses, in which the optimal dosage was estimated to be 5335 FTU/kg diet. Catfish fed the phytase-supplemented diets had significantly increased ($P < 0.05$) apparent availability of P compared to fish fed the PC diet.

In the posterior intestinal region, according to the contrast analysis, catfish fed the PC diet had significantly lower ($P < 0.05$) apparent availability of Ca, Fe, and IM than fish fed all the other experimental diets (Table 10). Moreover, a significantly higher ($P < 0.05$) apparent availability of P was observed in catfish fed the phytase-supplemental diets compared to those fed the NC and PC diets. The apparent availability of P was observed to have an upward quadratic relationship with dietary phytase dose, where the optimal dosage was estimated to be 5903 FTU/kg diet. In addition, an ascending linear relationship was found between the dietary phytase doses and apparent availability of Mg.

Table 10. Apparent availability (%) of different minerals as well as organic matter (OM) and inorganic matter (IM) in digesta from the posterior intestinal region of channel catfish, fed experimental diets including those with different levels of phytase for 8 weeks[a]

Diet	Ca	Mg	P	Cu	Fe	Mn	Zn	OM	IM
Negative control	44.4	44.4	52.5	39.9	46.1	25.9	24.6	61.7	68.7
Positive control	35.9	41.7	57.9	14.2	35.6	38.9	16.6	62.4	62.4
F1000	55.8	32.5	73.4	47.9	58.6	59.2	34.2	56.1	69.9
F2000	55.2	44.8	79.6	34.7	50.6	43.4	31.4	56.7	73.5
F4000	46.8	46.9	82.1	45.4	46.7	17.3	24.2	61.4	72.7
F8000	54.8	53.9	87.4	20.2	48.7	42.0	25.6	62.0	70.8
PSE[b]	2.60	3.93	2.49	11.64	2.12	9.50	6.54	1.87	2.30
Orthogonal contrast (Pr > F)[c]									
Linear	0.5	0.02	0.0002	-	0.6	-	0.5	0.2	0.6
Quadratic	0.8	0.06	<0.0001	-	0.9	-	0.8	0.3	0.4
Regression Model[d]	NOS	L	Q	NOS	NOS	NOS	NOS	NOS	NOS
Pr > F	-	0.02	<0.0001	-	-	-	-	-	-
R^2	-	0.28	0.76	-	-	-	-	-	-
Optimal dosage[e]	-		5903	-	-	-	-	-	-
Contrast (Pr > F)[f]									
NC – PC	0.03	0.6	0.1	-	0.003	0.4	-	0.8	0.04
NC – (F1000 to F8000)	0.001	1	<0.0001	-	0.3	-	0.6	0.2	0.3
PC – (F1000 to F8000)	<0.0001	0.5	<0.0001	-	0.004	-	-	0.1	0.002

[a] Values are means of composite samples of three fish from each of four replicate groups (n=4). Significance probability associated with the *P*-value.

[b] Pooled standard error

[c] Orthogonal contrast analysis was only run among treatment NC and all phytase-supplemental treatments, which needed to be all positive results. If statistical significance (P<0.05) were detected, the model which fit best the data was chosen.

[d] L = linear model; Q = quadratic model; NOS = no structure

[e] Upward quadratic model was used to derive the optimal dosage.

[f] The contrast analysis was only applied to the groups that had all positive results. If any groups contained negative data, "-" will be shown in the cell

II.3.3 Apparent digestibility of various amino acids

The apparent digestibility coefficients (ADCs) of indispensable amino acids (AAs) in digesta obtained from stomach, anterior and posterior intestinal regions of catfish are presented in Table 11, 12, and 13, respectively.

Due to some of the negative ADCs, only the indispensable AAs having all positive ADCs were analyzed via regression and linear orthogonal contrast analysis. No regression relationships were found between ADCs for the various indispensable AAs in digesta from the stomach and the dietary phytase doses (Table 11). Although the P value of the model between ADC of lysine and the dietary phytase doses was 0.03, the P value of the quadratic parameter was 0.0595. Thus, the quadratic model between ADC of lysine and dietary phytase dose was invalid. According to the contrast analysis, no significant differences of ADCs of any indispensable AA in digesta from the stomach were found among channel catfish fed any experimental diets.

In digesta from the anterior intestinal region, ADC values were much higher than observed in digesta from the stomach (table 12). Only the ADC of threonine had a downward quadratic relationship with dietary phytase dose. In addition, channel catfish fed the PC diet had significantly lower ($P < 0.05$) ADC of methionine compared to fish fed the phytase-supplemented diets.

Table 11. Apparent digestibility coefficients (ADCs; %) of indispensable amino acids from the stomach of channel catfish fed experimental diets including those with different levels of phytase for 8 weeks[a]

Diet	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
Negative control	0.5	-8.2	-14	0.1	7.5	31.5	-9.2	9.5	-12.3
Positive control	14.4	4.9	-6.1	8.1	9.2	39.7	4.7	18.4	-4.4
F1000	-0.4	-5.0	-14.7	0.4	7.0	26.2	-8.1	10.4	-11.6
F2000	14.5	6.7	-0.5	12.8	22.6	51.2	4.2	22.2	2.2
F4000	15.7	7.6	2.5	12.6	21.9	41.3	5.6	20.9	4.2
F8000	13.4	1.5	-3.0	10.1	20.7	43.8	-2.5	19.3	0.3
PSE[b]	4.62	6.24	5.44	5.17	4.93	3.87	6.18	4.73	5.66
Orthogonal contrast (Pr > F)[c]									
Linear	-	-	-	-	0.05	0.1	-	0.2	-
Quadratic	-	-	-	-	0.03	0.1	-	0.1	-
Regression									
Model[d]	NOS	NOS	NOS	NOS	NOS	NOS	NOS	NOS	NOS
Pr > F	-	-	-	-	-	-	-	-	-
R^2	-	-	-	-	-	-	-	-	-
Optimal dosage[e]	-	-	-	-	-	-	-	-	-
Contrast (Pr > F)[f]									
NC – PC	-	-	-	-	-	0.2	-	0.2	-
NC – (F1000 to F8000)	-	-	-	-	0.08	0.06	-	0.1	-
PC – (F1000 to F8000)	-	-	-	-	-	0.8	-	1	-

[a] Values are means of composite samples of three fish from each of four replicate groups (n=4). Significance probability associated with the *P*-value.

[b] Pooled standard error

[c] Orthogonal contrast analysis was only run among treatment NC and all phytase-supplemental treatments, which needed to be all positive results. If statistical significance (P<0.05) were detected, the model which fit best the data was chosen.

[d] L = linear model; Q = quadratic model; NOS = no structure

[e] Upward quadratic model was used to derive the optimal dosage.

[f] The contrast analysis was only applied to the groups that had all positive results. If any groups contained negative data, "-" will be shown in the cell

Table 12. Apparent digestibility coefficients (ADCs, %) of indispensable amino acids from the anterior intestinal region of channel catfish fed experimental diets including those with different levels of phytase for 8 weeks[a]

Diet	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val	∑[b]
Negative control	91.0	86.1	85.2	87.2	88.9	94.2	87.4	84.5	84.5	87.7
Positive control	91.7	86.3	85.0	87.1	90.0	95.3	87.4	85.2	84.6	86.4
F1000	91.7	86.9	86.7	88.5	90.4	94.1	88.8	85.2	85.8	88.5
F2000	93.0	88.1	87.0	89.1	90.8	94.7	89.7	85.9	86.1	89.3
F4000	87.5	81.2	80.0	83.1	85.9	91.5	83.2	79.1	79.5	83.3
F8000	93.7	88.1	88.8	90.3	93.0	92.7	90.2	88.0	87.9	90.4
PSE[b]	0.97	1.06	1.32	1.07	0.98	0.84	1.04	1.02	1.23	1.01
Orthogonal contrast (Pr > F)[c]										
Linear	0.5	0.9	0.6	0.6	0.3	0.09	0.7	0.5	0.6	0.6
Quadratic	0.2	0.2	0.1	0.1	0.08	0.2	0.2	0.04	0.09	0.1
Regression										
Model[d]	NOS	NOS	NOS	NOS	NOS	NOS	NOS	Q	NOS	NOS
Pr > F	-	-	-	-	-	-	-	0.04	-	-
R^2	-	-	-	-	-	-	-	0.41	-	-
Optimal dosage[e]	-	-	-	-	-	-	-	-	-	-
Contrast (Pr > F)										
NC – PC	0.6	0.9	0.9	1	0.5	0.4	1	0.6	1	0.4
NC – (F1000 to F8000)	0.7	1	0.8	0.7	0.4	0.3	0.6	1	0.8	0.9
PC – (F1000 to F8000)	0.8	0.9	0.7	0.6	1	0.049	0.6	0.6	0.8	0.2

[a] Values are means of composite samples of three fish from each of four replicate groups (n=4). Significance probability associated with the *P*-value.

[b] Pooled standard error

[c] Orthogonal contrast analysis was only run among treatment NC and all phytase-supplemental treatments, which needed to be all positive results. If statistical significance (P<0.05) were detected, the model which fit best the data was chosen.

[d] L = linear model; Q = quadratic model; NOS = no structure

[e] Upward quadratic model was used to derive the optimal dosage.

In digesta from the posterior intestinal region, no regression relationships were found between the dietary phytase doses and various indispensable AAs or the sum of indispensable AAs (Table 13). Based on the contrast analysis, channel catfish fed the NC diet had significantly higher ($P < 0.05$) ADC of phenylalanine and the sum of indispensable AAs compared to the fish fed PC diet. Besides that, channel catfish fed the phytase-supplemental diets had significantly higher ($P < 0.05$) ADC of all the indispensable AAs and their sum compared to fish fed NC or PC diets, except for histidine, methionine, or threonine.

Table 13. Apparent digestibility coefficients (ADCs, %) of indispensable amino acids from the posterior intestinal region of channel catfish fed experimental diets including those with different levels of phytase for 8 weeks[a]

Diet	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val	∑[b]
Negative control	95.6	92.1	91.5	92.6	93.4	95.8	92.6	91.0	90.7	92.8
Positive control	95.3	91.9	90.9	91.8	93.3	96.7	91.8	90.7	90.2	91.4
F1000	96.9	93.5	93.9	94.8	95.3	96.5	94.7	92.6	92.7	94.5
F2000	96.8	93.4	93.7	94.4	96.6	99.2	94.7	92.5	92.7	94.6
F4000	96.1	91.1	92.6	93.1	94.2	96.0	93.3	90.9	91.1	93.2
F8000	96.0	92.2	92.9	93.6	94.0	95.6	94.6	90.8	91.5	93.4
PSE[c]	0.27	0.34	0.39	0.34	0.32	0.51	0.26	0.42	0.39	0.28
Orthogonal contrast (Pr > F)[d]										
Linear	0.7	0.2	0.8	0.9	0.5	0.4	0.8	0.1	0.7	0.6
Quadratic	0.4	0.4	0.4	0.6	0.2	0.2	0.5	0.2	0.6	0.4
Regression Model[e]	NOS	NOS	NOS	NOS	NOS	NOS	NOS	NOS	NOS	NOS
Pr > F	-	-	-	-	-	-	-	-	-	-
R^2	-	-	-	-	-	-	-	-	-	-
Optimal dosage[f]	-	-	-	-	-	-	-	-	-	-
Contrast (Pr > F)										
NC – PC	0.6	0.6	0.3	0.2	0.8	0.2	0.048	0.7	0.5	0.005
NC – (F1000 to F8000)	0.01	0.3	0.001	0.003	0.002	0.1	0.0003	0.2	0.01	0.004
PC – (F1000 to F8000)	0.003	0.1	0.0001	<0.0001	0.001	0.8	<0.0001	0.07	0.002	<0.0001

[a] Values are means of composite samples of three fish from each of four replicate groups (n=4). Significance probability associated with the *P*-value.

[b] Sum of all indispensable AAs in posterior intestinal region.

[c] Pooled standard error

[d] Orthogonal contrast analysis was only run among treatment NC and all phytase-supplemental treatments, which needed to be all positive results. If statistical significance (P<0.05) were detected, the model which fit best the data was chosen.

[e] L = linear model; Q = quadratic model; NOS = no structure

[f] Upward quadratic model was used to derive the optimal dosage.

The ADCs of dispensable AAs and their sum in digesta obtained from the stomach, anterior and posterior intestinal regions of catfish are presented in Tables 14, 15, and 16, respectively. In the stomach, an upward quadratic relationship was disclosed between the ADC of glutamate and dietary phytase dose, with the optimal dosage estimated to be 5217 FTU/kg diet (Table 14). However, no other significant differences were observed for the ADC of all other dispensable AAs among catfish fed the various experimental diets.

In the anterior intestinal region, no significant regression relationships were observed between dietary phytase dose and the ADCs of all the dispensable AAs or the ADC of the sum of dispensable AAs (Table 15). Besides that, there were no significant differences observed for the ADC of any dispensable AAs or their sum among catfish fed the various experimental diets.

In the posterior intestinal region, similar to the ADCs of dispensable AAs in the anterior intestinal region, there were no significant regression relationships observed between dietary phytase dose and ADC values (Table 16). However, compared to catfish fed PC diet, fish fed the NC diet had a significantly higher ($P < 0.05$) ADC for the sum of dispensable AAs. Besides that, catfish fed the phytase-supplemented diets had significantly enhanced ($P < 0.05$) ADCs of all dispensable AAs and their sum compared to fish fed the NC or PC diets, except for the ADC of taurine and tyrosine. In addition, channel catfish fed the phytase-supplemented diet had the significantly increased ($P < 0.05$) ADC of tyrosine compared to fish fed the NC diet.

Table 14. Apparent digestibility coefficients (ADCs, %) of dispensable amino acids from the stomach of channel catfish fed experimental diets including those with different levels of phytase for 8 weeks[a]

Diet	Ala	Asp	Glu	Pro	Ser	Tau	Tyr
Negative control	6.2	11.8	22.9	13.6	15.9	15.5	-17.3
Positive control	14.2	17.4	28.3	20.5	23.1	32.7	6.2
F1000	12.6	15.8	26.5	17.8	19.2	8.4	-5.2
F2000	22.7	24.7	34.9	27.6	29.7	15.4	6.7
F4000	23.4	25.1	36.9	28.4	28.6	32.5	4.3
F8000	22.3	23.5	33.7	26.5	26.7	8.5	11.4
PSE[b]	5.58	4.95	4.53	5.45	5.09	6.41	7.16
Orthogonal contrast (Pr > F)[c]							
Linear	-	0.1	0.1	0.1	0.2	-	-
Quadratic	-	0.05	0.03	0.05	0.07	-	-
Regression							
Model[d]	NOS	NOS	Q	NOS	NOS	NOS	NOS
Pr > F	-	-	0.03	-	-	-	-
R^2	-	-	0.44	-	-	-	-
Optimal dosage[e]	-	-	5217	-	-	-	-
Contrast (Pr > F)[f]							
NC – PC	-	0.4	0.4	0.4	0.3	0.08	-
NC – (F1000 to F8000)	-	0.1	0.07	0.08	0.1	-	-
PC – (F1000 to F8000)	0.4	0.4	0.4	0.5	0.6	-	-

[a] Values are means of composite samples of three fish from each of four replicate groups (n=4). Significance probability associated with the *P*-value.

[b] Pooled standard error

[c] Orthogonal contrast analysis was only run among treatment NC and all phytase-supplemental treatments, which needed to be all positive results. If statistical significance (P<0.05) were detected, the model which fit best the data was chosen.

[d] L = linear model; Q = quadratic model; NOS = no structure

[e] Upward quadratic model was used to derive the optimal dosage.

[f] The contrast analysis was only applied to the groups that had all positive results. If any groups contained negative data, "-" will be shown in the cell

Table 15. Apparent digestibility coefficients (ADCs, %) of dispensable amino acids and their sum from the anterior intestinal region of channel catfish fed experimental diets including those with different levels of phytase for 8 weeks[a]

Diet	Ala	Asp	Glu	Pro	Ser	Tau	Tyr	∑[b]
Negative control	87.1	83.0	88.9	86.8	87.5	-2608	84.9	87.2
Positive control	87.4	83.4	89.4	86.7	87.3	-962	86.2	85.9
F1000	87.9	84.8	90.5	87.6	88.4	-657	86.2	88.4
F2000	88.3	85.4	90.9	88.5	89.1	-1289	87.4	89.0
F4000	83.1	77.8	86.1	83.1	82.8	-2047	79.0	83.2
F8000	88.3	88.5	92.8	90.2	90.9	-2057	87.8	90.9
PSE[c]	1.05	1.32	1.05	1.11	1.11	506.9	1.26	1.04
Orthogonal contrast (Pr > F)[d]								
Linear	0.9	0.3	0.3	0.4	0.5	-	0.8	0.4
Quadratic	0.2	0.06	0.1	0.1	0.07	-	0.2	0.08
Regression								
Model[e]	NOS	NOS	NOS	NOS	NOS	NOS	NOS	NOS
Pr > F	-	-	-	-	-	-	-	-
R^2	-	-	-	-	-	-	-	-
Optimal dosage[f]	-	-	-	-	-	-	-	-
Contrast (Pr > F)[g]								
NC – PC	0.9	0.8	0.8	0.9	0.9	-	0.5	0.4
NC – (F1000 to F8000)	0.9	0.5	0.4	0.7	0.8	-	0.9	0.6
PC – (F1000 to F8000)	0.7	0.6	0.6	0.6	0.7	-	0.5	0.1

[a] Values are means of composite samples of three fish from each of four replicate groups (n=4). Significance probability associated with the *P*-value.

[b] Sum of all dispensable AAs in anterior intestinal region, except taurine due to its negative value.

[c] Pooled standard error

[d] Orthogonal contrast analysis was only run among treatment NC and all phytase-supplemental treatments, which needed to be all positive results. If statistical significance (P<0.05) were detected, the model which fit best the data was chosen.

[e] L = linear model; Q = quadratic model; NOS = no structure

[f] Upward quadratic model was used to derive the optimal dosage.

[g] The contrast analysis was only applied to the groups that had all positive results. If any groups contained negative data, "-" will be shown in the cell

Table 16. Apparent digestibility coefficients (ADCs) of dispensable amino acids and their sum from the posterior intestinal region of channel catfish fed experimental diets including those with different levels of phytase for 8 weeks[a]

ADC %	Ala	Asp	Glu	Pro	Ser	Tau	Tyr	∑[b]
Negative control	91.0	90.0	93.9	91.5	92.5	-276	91.3	92.2
Positive control	90.8	90.1	93.6	91.2	92.2	-48	91.4	91.0
F1000	92.9	92.8	95.8	93.3	94.3	-253	92.9	94.3
F2000	93.2	92.9	95.9	93.3	94.3	-128	93.1	94.2
F4000	91.6	91.3	94.6	92.1	93.2	-111	91.4	93.1
F8000	91.7	91.7	95.4	93.0	93.0	-199	92.6	93.4
PSE[c]	0.41	0.43	0.39	0.50	0.25	86.5	0.39	0.30
Orthogonal contrast (Pr > F)[d]								
Linear	0.6	0.8	0.5	0.4	0.4	-	0.4	0.9
Quadratic	0.4	0.4	0.6	0.6	0.2	-	0.5	0.5
Regression Model[e]	NOS	NOS	NOS	NOS	NOS	NOS	NOS	NOS
Pr > F	-	-	-	-	-	-	-	-
R^2	-	-	-	-	-	-	-	-
Optimal dosage[f]	-	-	-	-	-	-	-	-
Contrast (Pr > F)[g]								
NC – PC	0.7	0.9	0.6	0.7	0.4	-	0.9	0.01
NC – (F1000 to F8000)	0.01	0.001	0.005	0.02	0.001	-	0.049	0.001
PC – (F1000 to F8000)	0.005	0.001	0.001	0.01	0.0001	-	0.07	<0.0001

[a] Values are means of composite samples of three fish from each of four replicate groups (n=4). Significance probability associated with the *P*-value.

[b] Sum of all dispensable AAs in anterior intestinal region, except taurine due to its negative value.

[c] Pooled standard error

[d] Orthogonal contrast analysis was only run among treatment NC and all phytase-supplemental treatments, which needed to be all positive results. If statistical significance (P<0.05) were detected, the model which fit best the data was chosen.

[e] L = linear model; Q = quadratic model; NOS = no structure

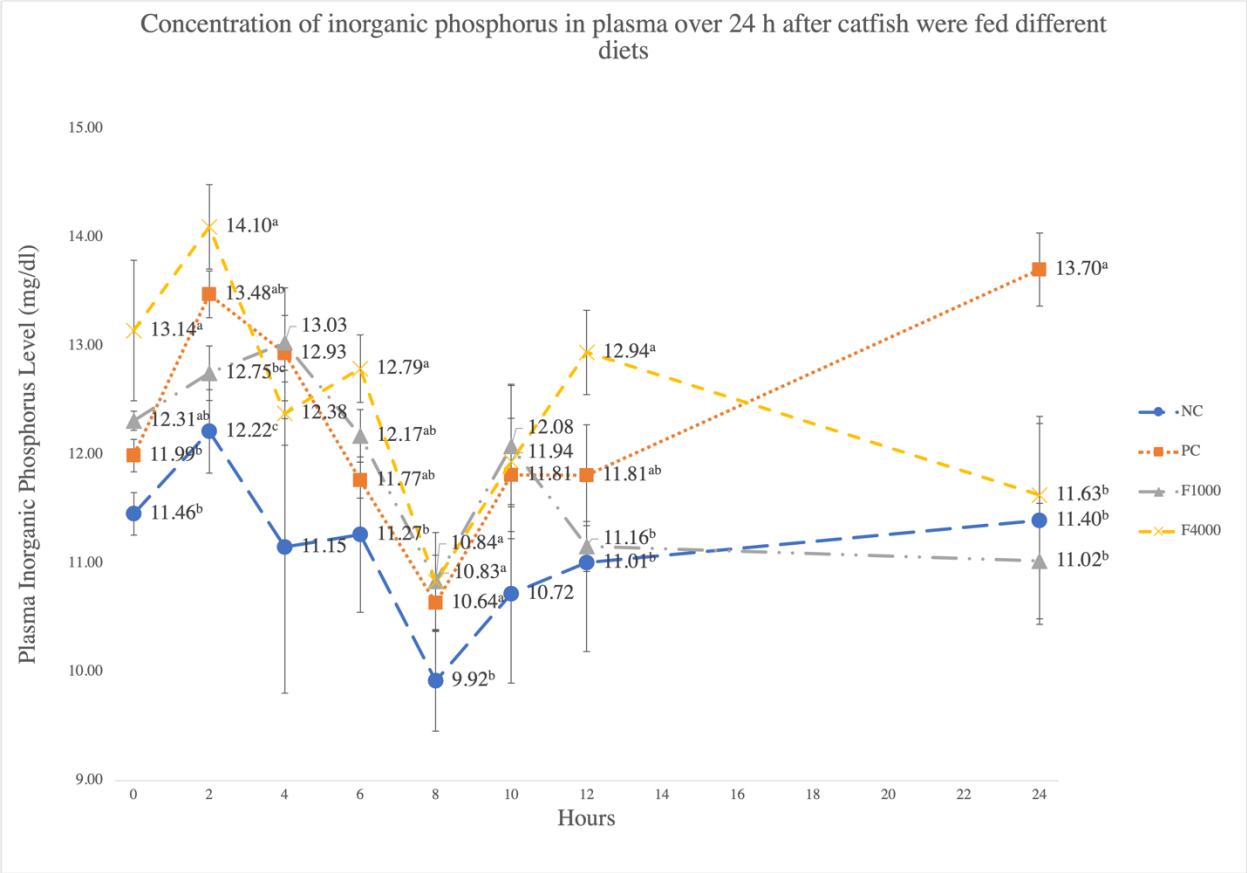
[f] Upward quadratic model was used to derive the optimal dosage.

[g] The contrast analysis was only applied to the groups that had all positive results. If any groups contained negative data, "-" will be shown in the cell

II.3.4 Postprandial plasma phosphorus levels

The plasma phosphorus level of catfish fed the four experimental diets declined to the lowest level at 8 h after feeding (Fig. 1). Catfish fed the F4000 diet had significantly increased ($P < 0.05$) plasma phosphorus level, compared to fish fed the NC diet at most time points, except 4, 10, and 24 h. However, catfish fed the PC diet had significantly higher ($P < 0.05$) plasma phosphorus level compared to fish fed all the other experimental diets at 24 h. In addition, catfish fed with the PC diet was the only one that had a higher plasma phosphorus level at 24 h than the initial plasma phosphorus level. At 36 h since the last feeding, channel catfish fed the F4000 diet still had a significantly higher ($P < 0.05$) plasma phosphorus level than fish fed the NC or PC diets.

Figure 1. Post-prandial pattern of plasma phosphorus from channel catfish fed diets NC, PC, F1000 and F4000[a]



[a] Values are means of composite samples of three fish from each of four replicate groups (n=4). Values within the same time point with different letters are significantly different (P<0.05).

II.4. Discussion

II.4.1 The effect of dietary phytase on the growth performance and condition indices of channel catfish

The growth performance results from the current research are somewhat similar to previous studies with channel catfish in that fish fed the phytase-supplemented diets had significantly improved WG and FE compared to fish fed the NC diet containing deficient levels of available phosphorus based on the established minimum dietary requirement of this species (Chen et al., 2019; Jackson et al., 1996; Li & Robinson, 1997). In addition, the WG and FE had an ascending linear relationship with dietary phytase dose. However, Yan et al. (2002) reported that dietary phytase supplementation did not improve WG of channel catfish fed an all-plant-protein diet. But, in this case, although Yan et al. (2002) formulated a phosphorus deficient diet (0.16% of available phosphorus) with adequate total phosphorus level (0.57%), the dietary zinc level (5.3 mg/kg of available zinc and 53.37 mg/kg of total zinc) was much lower than the zinc requirement of channel catfish established 20 mg/kg in a semi-purified diet (Gatlin III & Wilson, 1983). Due to the impact of dietary phytate level (Satoh et al., 1989), the optimal dietary zinc supplementation level for a practical channel catfish diet was 150 mg/kg (Gatlin III & Wilson, 1984). Thus, it is assumed that the deficient zinc level of the all-plant-protein diet designed by Yan et al. (2002) was the main reason for the negligible effect of dietary phytase on channel catfish performance. Additionally, Li and Robinson (1997) claimed there were no significant differences in growth performance of channel catfish fed phytase- supplemented diets compared

to a control diet containing extra dicalcium phosphorus because phytase could be used to replace inorganic phosphorus supplementation of diets for channel catfish. This statement was supported by the current feeding trial. Therefore, the positive effect of dietary phytase supplementation on the growth performance of fish only occurred when fish were fed diet containing sufficient nutrients, except available phosphorus.

In regard to the body condition and composition of catfish fed phytase-supplemented diets, a significantly lower MY, similar to the PC, was observed in the present study compared to catfish fed the NC diet. This may have been caused by the deposition of more visceral mass and skeletal tissues, which lowered the relative percentage of muscle for the larger fish resulting from the greater availability of P released from phytate degradation by phytase or MCP supplementation. In addition, although significantly higher lipid retention (LR) was observed in channel catfish fed the NC diet compared to those fed the phytase-supplemented diets, no significant difference in HSI and IPF ratio were seen. Significantly lower whole-body lipid concentration and lipid retention were found in the present study when channel catfish were fed the PC and diets supplemented with phytase, compared to channel catfish fed the NC. This is a common observation caused by cellular hypoxia and inhibition of oxidative phosphorylation when fish are phosphorus deficient (Sugiura et al., 2004). In addition, Sugiura et al. (2011) found that the phosphorus-deficient diet downregulated the mRNA expression of CPT1 (carnitine palmitoyltransferase-I), a rate-limiting enzyme in lipolysis, and upregulated the expression of ACC1 (acetyl-CoA carboxylase), a rate-limiting enzyme in lipogenesis.

II.4.2 The effect of dietary phytase on the utilization of minerals by channel catfish

Numerous previous studies have reported that dietary supplementation of phytase can improve the deposition of various minerals in whole-body and bone (Liebert & Portz, 2005; Nwanna et al., 2007; Olugbenga et al., 2017; Sajjadi & Carter, 2004a), decrease the excretion of several minerals (Lee et al., 2020; Nwanna, 2005; Yang et al., 2011), and increase the apparent availability of some minerals (Hussain et al., 2016; Hussain et al., 2015; Morales et al., 2016; Vandenberg et al., 2012). These effects of dietary phytase supplementation are due to the phytase degrading dietary phytate and thus improving the bioavailability of various di-and tri- valent mineral ions. In the present study, channel catfish fed the PC and phytase-supplemented diets had significantly increased whole-body and bone ash, compared to the fish was fed the NC diet. Thus, the available phosphorus level in the NC diet was lower than the requirement of channel catfish, and dietary phytase increased the bioavailability of this and other minerals. The P retention of channel catfish fed the phytase-supplemented diets was increased up to 48.4 and 33.7% compared to fish fed the NC and PC diets, respectively. In addition, the P excretion of channel catfish fed the NC or PC diets was decreased from 5.3 and 6 g/kg, respectively, to 2.45 g/kg on average for fish fed the phytase-supplemented diets. What's more, channel catfish fed the phytase-supplemented diets increased bone P concentrations up to 25.4% and 9.9% compared to fish fed the NC and PC diets, respectively. Although fish can absorb P from the water, the dietary P is the main source for fish (Lall, 2003). Morales et al. (2011) and Zhu et al. (2016) have reported that phytase had the highest activity in the stomach of fish due to its low pH.

Furthermore, Zhu et al. (2016) reported that dietary phytase (1000 to 8000 FTU/kg) could break down 63 to 97% of the total phytate in the catfish's stomach at 6 h after feeding. According to the results of this study with regard to P apparent availability in different GI tract regions, dietary phytase supplementation significantly increased the P apparent availability in all three regions, but the stomach was the main site of P absorption, removing 40 to 60% of dietary P. Besides that, from the stomach to the anterior intestinal region, the P apparent availability of channel catfish fed the NC and PC diets only increased 10.1% and 14.7%, respectively, compared to the P apparent availability of channel catfish fed the phytase-supplemented diets which increased between 32 to 49.9%. Thus, results from the current trial confirmed that the catfish stomach is the optimum active site for dietary phytase, and the phosphorus released from phytate-P was mainly absorbed in the anterior intestinal region.

Differences in Ca metabolism was also observed among catfish fed the various diets in the present study. Channel catfish was fed the diets containing supplemental phytase on average has Ca retention increased up to 55.8 and 20.2 %, compared to catfish fed the NC and PC diets, respectively. In addition, the Ca excretion of channel catfish fed the phytase-supplemented diets decreased considerably to as low as 0 g/kg, compared to fish fed the NC and PC diets. Besides that, the bone Ca concentration of channel catfish fed the phytase-supplemented diets was on average 22.4% and 8.7% higher than the fish was fed the NC and PC diets, respectively. The results of apparent availability of Ca in the posterior intestinal region in our current study also supported these results above. Previous research claimed that, in the freshwater, fish can directly

absorb Ca from water via the gills (Ichii & Mugiya, 1983). In addition, the Ca absorbed from the water can contribute up to 50 to 80% of the total body Ca uptake, with the remainder coming from the diet (Berg, 1968; Perry & Wood, 1985; SIMKISS, 1974). This likely explains why the Ca retention was higher than 100% and excretion was lower than 0 g/kg in the current trial. According to the Ca apparent availability results from different GI tract regions, the stomach was the most important site of Ca absorption, in which roughly 50% of the dietary Ca was absorbed. Similar results was reported by Bucking and Wood (2007), who also found about 50% of the ingested dietary Ca was absorbed by the stomach of rainbow trout. In addition, the net secretion of Ca in the anterior intestinal region in their feeding trial was similar to observed in the present trial in which the apparent availability of Ca in the anterior intestinal region was lower than the stomach. Their assumption was that the extra Ca in the anterior intestine was caused by the bile, pancreatic and intestinal secretions (Bucking & Wood, 2006), containing enzymes and other ligands that can bind with Ca. This assumption also explained why the apparent Ca availability in the posterior intestinal region was slightly lower than the stomach, regardless of diet.

Channel catfish fed the phytase-supplemented diets also had significantly increased Mg retention and bone Mg concentration, compared to catfish fed the NC and PC diets. In addition, channel catfish fed the phytase-supplemented diets had decreased Mg excretion of up 48.1% compared to fish fed the NC diet. The results of apparent availability of Mg from different GI tract regions showed the absorption ability of Mg was ranked as stomach > posterior intestine > anterior intestine. Previous studies have shown that, although fish can absorb Mg from water via

gill (Shearer & Åsgård, 1992), the Mg contributed by diet was up to 80% (Flik et al., 1993). Thus, unlike the apparent availability of Ca, the Mg retention was less than 100% and Mg excretion was positive. According to the results obtained by Bucking and Wood (2007), the fish stomach can absorb up to 80% of the dietary Mg, and a net excretion of Mg was detected in the anterior intestine, which was similar to results of the current study.

The current results with regard to Cu were unexpected. Although a decreased Cu excretion was noticed in channel catfish fed the phytase-supplemented diets, no difference in Cu retention was observed between fish fed the NC and phytase-supplemented diets. Moreover, all of Cu apparent availability results in the anterior intestinal region were negative, which mean a net Cu excretion occurred in anterior intestine. Nadella et al. (2006) found that the major absorption sites of Cu in rainbow trout was in the middle and posterior intestine, which explains how the Cu apparent availability changed from negative to positive from the anterior to posterior intestinal region in the present experiment. In addition, other studies have shown that excess Cu is mainly excreted via the bile (Grosell et al., 1997; Grosell et al., 2000) and gills (Grosell et al., 2001; Handy, 1996) of teleost fish, and very little through urine (Grosell et al., 1998). Thus, the net Cu excretion in the anterior intestinal region was likely caused by the additional Cu-containing fluids from the bile. In addition, the minimum dietary Cu requirement of channel catfish was determined to be approximately 5 mg/kg diet (Gatlin III & Wilson, 1986), which was lower than the dietary copper levels (11 to 14 ppm) in our present trial. Thus, it is assumed that the

unexpected Cu apparent availability results in the posterior intestinal region was due to excretion of the excessive dietary Cu via bile.

Channel catfish fed with the phytase-supplemented diets also were observed to have an increased Fe retention compared to catfish was fed the NC diet. However, the Fe excretion and bone Fe concentration were not affected by the dietary phytase supplementation in the present trial. According to previous studies, Fe is mainly stored in the liver and gill tissues of fish (Çoğun et al., 2005; Honda et al., 1983), possibly explaining why the Fe retention was higher, but no difference was found in the excretion and bone Fe concentrations. Based on the Fe apparent availability results in the present trial, the main Fe absorption site was in the posterior intestinal region, as reported by Bury et al. (2001) in the European flounder (*Platichthys flesus*). In addition, Bury et al. (2001) also claimed that Fe absorption was upregulated in the posterior intestinal region of fish in response to prior hemoglobin depletion. A significantly lower Fe apparent availability in the posterior intestinal region was noticed when channel catfish were fed the PC diet compared to the fed all the other experimental diets. The absorption of Fe had been shown to be inhibited by dietary Ni, Pb, Cd, Cu, and Zn (Kwong & Niyogi, 2009). However, the only difference between the NC and PC diets in the present study was PC diet had an extra 1% MCP, whereas none of the minerals mentioned above had different concentrations in the NC and PC diets. Limited research has been done on fish regarding the interaction between dietary Fe and Ca or P. However, it has been reported in humans that dietary Ca has a negative influence on the absorption of Fe (Hallberg et al., 1991). In addition, Ca significantly decreased the absorption

of heme-Fe, indicating the negative effect of Ca was related to the mucosal transfer of Fe. However, the Fe whole-body retention and excretion were similar between channel catfish was fed the NC and PC diets, which means fish was fed the PC diet had another Fe source other than the dietary Fe. Andersen (1997) and Roeder and Roeder (1966) demonstrated that fish have the ability to absorb the Fe ion directly from the water via gills, although, the absorption of Fe by the gills decreased when fish received adequate Fe in diet (Segner & Storch, 1985).

Significantly higher Mn retention and bone Mn concentrations were caused by dietary phytase supplementation as observed in the present trial. Previous research reported that dietary phytase significantly increased Mn apparent availability in pangas catfish (Debnath et al., 2005) and rainbow trout (Cheng & Hardy, 2003). However, no difference in Mn apparent availability was found in the three regions of the GI tract of channel catfish fed any of the experimental diets. Although Srivastava and Agrawal (1983) demonstrated the absorption of waterborne Mn by fish, it is generally agreed that fish have better absorption of Mn via the diet. Based on Mn apparent availability results in the trial, the stomach of channel catfish absorbed 60.7 to 75% of dietary Mn; thus, the stomach appears to be the main absorption site for dietary Mn. It had to be determined in calves that excessive dietary Mn is excreted in the bile (Abrams et al., 1977). Fish may excrete Mn similarly, which may explain the decreased Mn apparent availability in the anterior and posterior intestinal regions compared to the stomach.

Dietary phytase supplementation significantly increased Zn retention and Zn bone concentrations in the present study. In addition, a significantly lower Zn excretion was observed

when channel catfish were fed the phytase-supplemented diets compared to catfish fed the NC and PC diets. Besides that, it was observed that compared to fish fed NC diet, fish fed the PC diet had a lower Zn retention and Zn bone concentration and higher Zn excretion. Similar results were reported by Gatlin III and Phillips (1989) in which higher dietary Ca level did not affect Zn bioavailability, but the combination of dietary phytate and calcium significantly reduced Zn bone concentration. Bury et al. (2003) claimed that the GI tract of fish was the dominant site for absorption of Zn in the natural environment. However, increased gill uptake of Zn has been reported with decreasing dietary Zn levels, especially when the Zn level of waterborne was high (Spry et al., 1988). Additionally, according to the Zn apparent availability results from the three different GI tract regions, it appeared that the stomach was the dominate site for Zn uptake, in which up to 67.6% of dietary Zn was absorbed. Previous studies have shown that zinc was mainly excreted through the bile (Handy, 1996), intestinal sloughing (Handy, 1996) or gill (Hardy et al., 1987). Although fish can excrete Zn via urine, the amount is very little (Spry & Wood, 1985). Thus, the decreased or negative Zn apparent availability results in the anterior intestinal region may be due to surplus Zn present in the bile.

The significantly lower IM apparent availability was found when channel catfish was fed the PC diet, compared to fish received the NC and phytase-supplemented diets. There were two premises: first, the higher dietary Ca level in the PC diet likely impacted the absorption of other minerals, such as Fe and Zn, in the present experiment; second, with the high dietary minerals concentrations, the total mineral absorption increased, but the utilization or bioavailability of

minerals or the fractional absorption reached the maximum when the concentrations met the metabolic requirements of fish and decreased at higher values (Pimentel-Rodrigues & Oliva-Teles, 2007).

The lower apparent availability of various minerals in the posterior intestinal region in the present study, compared to some others could be caused by different fecal collection methods. Vandenberg and De La Noüe (2001) claimed that feces stripping method usually led to a significantly lower ADC results compared to passive feces collection via sedimentation or sieving, due to the unabsorbed nutrients in stripped feces.

Overall, both the stomach and posterior intestinal region are the absorption sites of minerals for channel catfish. Dietary phytase supplementation generally improved bone minerals concentrations, whole-body mineral retention, and reduced the excretion of various minerals. However, the only positive results on apparent availability of minerals from the posterior intestinal region was for phosphorus.

II.4.3 The effect of dietary phytase on the protein digestibility and amino acid availability of channel catfish

The ability of phytic acid binding with proteins within the digestive system is well known (Noureddini & Dang, 2009). Wise (1983) claimed that phytic acid preferred to bind proteins rather than minerals at low acidic pH. Binary insoluble complexes were formed by the electrostatic linkages with amino acids, like arginine, histidine, and lysine, at low pH. In

addition, ternary complexes IP6 (myo-inositol hexaphosphate) - Ca^{+2} - proteins were formed when pH approached the isoelectric point of proteins. Besides that, once the IP6 binary complexes were formed, they were less susceptible to phytase, which were shielded by aggregated proteins (Rajendran & Prakash, 1993). Similar conclusions were also derived from the *in vitro* studies conducted by Morales et al. (2011), who reported that IP6 - protein complexes were less subject to attack by proteolytic enzymes than the protein itself due to the insoluble of IP6- protein complexes. The IP6 - protein complexes reduced by 82% the activity of pepsin and by 58% the activity of acid protease of fish extract, respectively. Furthermore, these results are in aggrement with Camus et al. (1976) who found that pepsin activity was inhibited by binary IP6-protein binding. Additionally, phytate reduced trypsin activity of porcine pancreatin by 20.8% and trypsin activity from fish extract by 86.4%. Caldwell (1992) concluded that the negative effect on trypsin by IP6 - Ca complexes was caused by the reduction of the activation of trypsinogen rather than directly worked on the trypsin. However, Sajjadi and Carter (2004b) found that additional phytate didn't affect the trypsin activity in pyloric caeca of Atlantic salmon. In addition, although Morales et al. (2011) reported that IP6-protein complexes reduced the activity of all of the gastric and intestinal proteases of rainbow trout, except for chymotrypsin, they proposed that fish proteases may be less vulnerable to form ternary IP6 – Ca - protein complexes than mammal proteases. Morales et al. (2014) also claimed that the activity of total protease in stomach of fish fed the phytase-supplemented diet was almost 60% higher than fish receiving the control diet. However, the activity of total protease, trypsin, or chymotrypsin from

proximal and distal intestine were not improved by the dietary phytase.

The potential mechanisms by which may phytase increase the availability of amino acids in the gut are not completely established. There were four theories including directly freeing proteins from the IP6-protein complexes, avoiding formation of *de novo* binary and ternary IP6 - protein complexes in GI tract, diminishing inhibition of digestive proteases (Selle et al., 2000), and reducing the excretion of mucin and enzymes which contain endogenous amino acids (Cowieson et al., 2004). Unfortunately, we don't have enough information from the present study to discuss these hypotheses. But some interesting results were obtained for the ADCs of both indispensable and dispensable amino acids from digesta taken from the stomach, anterior and posterior intestinal regions.

In the present study, dietary phytase supplementation significantly increased protein retention and reduced N excretion. However, in the stomach, unexpected negative ADCs of several amino acids were detected independent of the dietary treatments. It was assumed that this abnormal phenomenon was caused by gastric and intestinal secretions that comprise pepsinogen, pepsin, lipase, and amylase, and mucus. Thus, these secretions contain proteins and would contribute to the amino acid pool whenever the hydrolyzed amino acids from the stomach digesta were measured. Besides that, the negative ADC of taurine was also revealed in the anterior and posterior intestinal regions. This could be explained by the high taurine content in bile acids. One of the main excretion pathways of taurine is to bind to bile acids in fish liver (Sampath et al., 2020). Then, taurine is excreted into the intestine to emulsify lipid and become associated with

the absorption of lipids and fat-soluble vitamins with bile acids (Haslewood, 1967). Because the dietary taurine level was close to zero, it appears that channel catfish have the ability to biosynthesize taurine unlike most marine and some freshwater fish which have been studied to date and shown to require dietary taurine supplementation (Wei et al., 2019).

Almost all the amino acids were mainly absorbed in the anterior intestinal region, except methionine, which was noticed to be absorbed up to 51.2% in the stomach. However, no significant differences in ADCs of various amino acids were detected among channel catfish fed the different experimental diets. Bakke-McKellep et al. (2000) claimed that a declining trend of nutrient absorption rates were observed from proximal to distal intestine in Atlantic salmon. In addition, in their in vitro trial, 84 to 92% of the protein hydrolysis was absorbed via ceca and proximal intestine of Atlantic salmon. The distal intestine of teleost fish also has absorption ability but principally for endocytosing intact proteins (Sire & Vernier, 1992). Based on the ADCs of various amino acids from the posterior intestinal region, channel catfish absorbed roughly 5% of amino acids out of the total dietary amino acids. However, significantly higher ADCs of amino acids were observed when channel catfish were fed the phytase-supplemented diets, compared to fish fed the NC or PC diets, except for the ADCs of histidine, methionine, threonine, and taurine. In the present study, it also was noticed that channel catfish fed the NC diet had a significantly higher ADCs of total amino acids and N excretion than fish fed the PC diet. As previously mentioned, channel catfish fed the phosphorus-deficiency diet had reduced utilization of dietary lipid for energy; therefore, fish fed the NC diet appeared to have higher

protein utilization for energy.

Overall, dietary phytase supplementation improved the utilization of most amino acids by channel catfish. Difference in ADCs of amino acids only happened in the posterior intestinal region.

II.4.4 The effect of dietary phytase on the postprandial plasma phosphorus level of channel catfish

Plasma level of P was considered as important index of mineral status in fish (Antony Jesu Prabhu et al., 2013; Sugiura et al., 2000). In addition, postprandial plasma levels of P are used to evaluate dietary phosphorus utilization of fish (Hossain et al., 2020; Prabhu et al., 2014). In the present study, channel catfish fed the NC diet had a significantly lower plasma P level at 0, 2, 6, 8 and 12 h after feeding than fish fed the diet supplemented with 4000 FTU/kg phytase. Plasma P level of channel catfish fed all the experimental diets reached a peak at 2 h and decreased to the lowest at 8h. Similar trends were reported by Prabhu et al. (2014), who found that 4 h after feeding rainbow trout (203.1 - 230 g) plasma P level reached the peak and declined to the lowest at 12 h. Those authors claimed that the trend of plasma P level was caused by the rapid absorption of a fraction of dietary inorganic sources, and the rest was absorbed relatively slowly. However, Hossain et al. (2020) found that the plasma P level of rainbow trout (63.49 - 71.34 g) was highest at 1.5 h after feeding diets containing MCP or monoammonium phosphate, but the lowest points were also at 12 h. The different peak times also may be caused by the different

sizes of fish, because smaller fish had faster metabolism (Fonds et al., 1992). The other hypothesis for the decreased plasma, which was even lower than the baseline, can be explained by the digestion process. It had been reported that serum phosphorus had a negative relationship between blood glucose (Bansal, 1990; Izzo, 1956). In addition, according to the data collected by Tiemeier and Deyoe (1973), stomach of channel catfish processed 66.5 to 81.5% of the food during the first 8 hours after they received food. And only another 2 to 3% of food was processed by their stomach during the following 2 hours. Thus, we assumed that most of food was digested by channel catfish within 8h after they was fed and the blood glucose level changed with the digestion process of fish, which also supported our results that the plasma P level was lowest at 8h. The only unexpected result happened at 24h, at which plasma P level of fish fed the PC diet reached another peak and was significantly higher than fish fed the NC or phytase-supplemented diets. Phosphorus can be excreted by fish via feces and urine, and the non-fecal phosphorus excretion pathway only becomes the priority when fish receive surplus dietary phosphorus (Rodehutscord et al., 2000). Because the PC diet had the highest total dietary phosphorus level in our current study, the extra dietary phosphorus was excreted via urine and caused the higher plasma P level at 24 h. A higher plasma P level at 24 h compared to the 12 h also was observed when rainbow trout were fed a complete plant-ingredient-based diet containing extra di-calcium phosphate (Prabhu et al., 2014).

II.4.5 The optimal dosage of dietary phytase of channel catfish

Dose-response studies concerning dietary phytase and fish were well reviewed by Cao et al. (2007), and they claim that the optimum dose changed along with many factors, like fish species, diet formulation, phytase sources and selected response parameters. In addition, the activity of phytase also depends on many aspects, such as optimal pH range, the resistance to endogenous protease, temperature, and other feed additives (Dersjant-Li et al., 2015; V Kumar et al., 2012). Thus, there are no exact guidelines regarding optimal phytase addition for all the species. The optimal dose may even change in the same species with different phytase or diet formulations. Most of the previous studies only tested the dietary phytase level between 0 to 4000 FTU/kg phytase of diet, and the standard phytase dose in typically from 250 to 1500 FTU/kg phytase of diet (Cao et al., 2007; Li et al., 2019). However, in poultry, several studies have shown more positive effects of higher doses of phytase on nutrient digestibility and utilization (Cowieson et al., 2011; Fernandes et al., 2019; Woyengo & Wilson, 2019). In the current feeding trial, it was estimated that the optimal phytase dosage of channel catfish fed diets containing 46% of soybean meal to be between 4703 to 6845 FTU/kg phytase of diet based on orthogonal contrasts of all response parameters measured in the study. The average of these optimal dosages was 5492 FTU/kg phytase of diet, and 50% of them were between 5050 and 5773 FTU/kg phytase of diet. Thus, it was assumed that the optimal dosage of Quamtum Blue phytase for channel catfish was 5492 $^{5050}_{5773}$ FTU/kg phytase of diet (mean and interquartile range).

In conclusion, the present study showed that dietary phytase supplementation enhanced the

growth performance of channel catfish when the diet was deficient in available phosphorus. In addition, phytase addition increased bone ash, Ca, Mg, P, Mn, and Zn concentration, promoted whole-body Ca, Mg, P, Fe, Mn, and Zn retention, and reinforced the apparent availability of P to channel catfish. Besides that, channel catfish fed the phytase-supplemented diets had improved ADCs of amino acids, with significant differences mainly evident in the posterior intestinal region. Furthermore, it was calculated that, under the conditions of the current study, the optimal dosage of dietary phytase was 5492 $^{5050}_{5773}$ FTU/kg of diet for channel catfish fed diets containing mostly plant protein feedstuffs.

CHAPTER III

THE EFFECTS OF SUPERDOSING DIETARY PHYTASE ON GROWTH AND MINERAL UTILIZATION OF RED DRUM (*Sciaenops ocellatus*)

III.1. Introduction

Fishmeal is one of the best protein feedstuffs used in the diets of various aquaculture organisms. However, with the continuing expansion of global aquaculture and finite supply of fishmeal, the aqua-feed industry has diversified in using more diverse protein feedstuffs including plant proteins (Fontainhas-Fernandes et al., 1999). Phosphorus (P) is one of the most important essential minerals for fish as with terrestrial animals, playing several key structural and metabolic roles in skeletal tissues, cell membranes, nucleic acids, and directly participating in the generation of ATP (Pimentel-Rodrigues & Oliva-Teles, 2001). Phosphorus from fishmeal has generally been considered to be readily available and easily absorbed by fish. Nevertheless, most of the P from plant protein feedstuffs is stored in the form of phytate (V Kumar et al., 2012), which has been determined to be poorly utilized by fish like other monogastric animals. What's more, phytate can also reduce the utilization of other minerals and protein (Liener, 1989; Woyengo & Nyachoti, 2013), and lead to eutrophication of water by increasing excretion of phytate-P into the environment (Stewart et al., 1978). Phytase is the enzyme which can release the phytate-P and other minerals and protein which may bind to the phytate molecule (Yu et al., 2012). The benefits of phytase on aquaculture animal have been well summarized, including

improvement in growth performance, promotion of protein digestibility, and enhancement in the bioavailability of various minerals (Cao et al., 2007; Dersjant-Li et al., 2015; Lemos & Tacon, 2017; Lie et al., 1999). However, most of the published studies to date with aquatic species only investigated dietary phytase levels between 250 to 4000 FTU/Kg. It has been reported in poultry that dietary super-dosing phytase supplementation can achieve additional positive effects on digestibility and utilization of various nutrients (Cowieson et al., 2011; Fernandes et al., 2019; Woyengo & Wilson, 2019). Therefore, the present study investigated phytase supplementation up to 8000 FTU/kg in the diet of red drum.

Red drum, due to its rapid growth and wide tolerance of different environmental conditions (Castillo & Gatlin III, 2018; Chamberlain et al., 1990), is a popular marine species cultured in the United States and several Asian countries, such as China (FAO, 2020). However, to date no studies have been conducted regarding the effects of dietary phytase supplementation on this species. Thus, the objectives of this study were evaluating the effect of superdosing dietary phytase on growth performance and utilization of various minerals by juvenile red drum and estimating the optimal dietary phytase dosage.

III.2. Materials and methods

III.2.1 Diet formulations

Two basal diets were formulated having 44% crude protein mainly from dehulled, solvent-extracted soybean meal, menhaden fishmeal, soy protein concentrate, and red drum muscle meal

(Table 17). Red drum muscle meal was used to balance the dietary amino acid composition and limit the available P level in the diets. The negative control diet (NC) was designed to contain only 0.9% phosphorus from the dietary ingredients, in which the available phosphorus (P) was computed to be 0.57%, less than established minimum dietary available P requirement for red drum which was established at 0.7% (Davis & Robinson, 1987). On the other hand, the positive control diet (PC) was supplemented with 1.9% of monocalcium phosphorate (MCP) to meet the minimum dietary available P requirement of red drum. Four phytase-supplemented diets were made by adding graded levels of phytase (Quantum Blue Phytase, AB Vista) at 1000 (F1000), 2000 (F2000), 4000 (F4000), and 8000 (F8000) FTU/kg to the NC diet. One FTU of phytase represents the amount of phytase freeing inorganic P from a 5.1 mM solution of sodium phytate at a rate of 1 μmol per min at pH 5.5 and 37 °C. The phytase activity of diets were analyzed by AB Vista via an enzyme-linked immunosorbent assay (ELISA) and supplemental levels were confirmed to be within 20% of designed levels. All diets contained 0.1% of yttrium oxide as an inert marker for determination of mineral availability.

The same diet processing method was used in the feeding trial, which has been mentioned in Chapter II, materials and methods section.

Table 17. Formulation and proximate composition of experimental diets (% dry matter).

Ingredients	Negative Control	Positive Control	F1000	F2000	F4000	F8000
Soybean meal[a]	40.00	40.00	40.00	40.00	40.00	40.00
Menhaden meal[b]	6.00	6.00	6.00	6.00	6.00	6.00
Soy protein concentrate[c]	7.00	7.00	7.00	7.00	7.00	7.00
Red drum meal[d]	12.51	12.51	12.51	12.51	12.51	12.51
Menhaden oil[e]	7.38	7.38	7.38	7.38	7.38	7.38
Vitamin premix[f]	3.00	3.00	3.00	3.00	3.00	3.00
Mineral premix[g]	4.00	4.00	4.00	4.00	4.00	4.00
Monocalcium phosphate[h]	0.90	1.90	0.90	0.90	0.90	0.90
Dextrinized corn starch[h]	14.50	14.50	14.50	14.50	14.50	14.50
Glycine[i]	1.00	1.00	1.00	1.00	1.00	1.00
Taurine[i]	0.70	0.70	0.70	0.70	0.70	0.70
DL-Methionine[i]	0.75	0.75	0.75	0.75	0.75	0.75
Yttrium oxide[j]	0.10	0.10	0.10	0.10	0.10	0.10
Cellulose[k]	2.16	1.16	2.06	1.96	1.76	1.36
Phytase premix[l]	0.00	0.00	0.10	0.20	0.40	0.80
Total	100.00	100.00	100.00	100.00	100.00	100.00
Analyzed composition (% dry weight)						
Crude protein	44.2	44.1	43.9	44.0	44.4	44.1
Crude lipid	9.4	9.8	9.5	9.5	9.4	9.6
Ash	10.1	10.1	10.0	9.9	10.0	10.0
Available phosphorus[m]	0.57	0.73	0.65	0.56	0.61	0.61
Total phosphorus	0.90	1.09	1.01	0.92	0.99	0.97
Phytase activity (FTU/kg)[n]	< 50	< 50	1200	2270	4270	8430

[a] Producers Cooperative Association, Bryan, TX, USA (crude protein [CP] = 53.14%; lipid = 3.34% on dry-matter basis).

[b] Omega Protein Corp., Houston, TX, USA (CP = 67.93%; lipid = 11.96% on dry-matter basis).

[c] Solae LLC, St. Louis, MO, USA (CP = 70.85%; lipid = 0.88% on dry-matter basis).

[d] Contained 86% CP and 11.5% lipid (dry-matter basis).

[e] Omega Protein, Reedville, VA, USA.

[f] Moon and Gatlin (1991)

[g] Mineral premix (contains as g/kg of dry weight): Calcium lactate 348.553, Ferrous sulfate 5, Magnesium sulfate heptahydrate 132, Sodium chloride 45, Aluminum chloride 0.084, Potassium iodide 0.15, Cupric sulfate 0.5, Manganous sulfate 0.7, Cobalt chloride 1, Zinc sulfate heptahydrate 3, Sodium selenite 0.0127, Cellulose 464.

[h] MP Biomedicals, Solon, OH, USA.

[i] Ajinomoto North America, Inc.

[j] SIGMA-ALDRICH, Co., St. Louis, MO, USA.

[k] U.S. Biochemical, Cleveland, OH, USA.

[l] Enhanced *Escherichia coli* phytase (Quantum Blue; AB Vista, Marlborough, UK)

[m] Available P was computed based on the difference between total P and Phytate-P. All the P concentrations were measured via near infrared spectroscopy (NIR) by AB Vista.

[n] The phytase activity was analyzed by AB Vista via an enzyme-linked immunosorbent assay (ELISA)

III.2.2 Fish and experimental conditions

The feeding experiment was conducted at the Texas A&M University Aquacultural Research and Teaching Facility (ARTF) of the Texas A&M University System (Burleson County, TX). Red drum juveniles (*Sciaenops ocellatus)* were obtained from the Texas Parks and Wildlife Department Marine Development Center (Corpus Christi, Texas). All fish was maintained in a recirculating aquaculture system consisting of 110-L glass aquaria with a settling chamber, as well as biological, mechanical and UV filters for a 2-week acclimation. After the conditioning period, groups of 20 red drum with an initial weight 4.99 ± 0.09 g (mean ± S.D.) were stocked into each 24 aquaria, and all experimental diets were randomly assigned to quadruplicate aquaria. In addition, 20 fish were collected as initial whole-body sample prior to the initiation of the feeding trial. Red drum in each aquarium were fed diet twice daily based on a percentage of their total biomass. Each aquarium of fish was group-weighed weekly and feeding rate adjusted accordingly. The feeding rate was initially set at 6% of total body weight per day for all treatments and gradually decreased over time to 2%, maintaining feeding levels close to apparent satiation without wastage. Water quality and other environmental parameters were monitored twice weekly and maintained within the appropriate ranges for red drum (pH: 7.2-8; total nitrite – nitrogen < 0.015 ml/L; total ammonia – nitrogen < 0.10 mg/L; dissolved oxygen > 6.8 mg/L; temperature: 26.4 ± 0.5; salinity: 7-8 ppt). The photoperiod was maintained at 12 hlight:12 h dark via fluorescent lights controlled by a timer.

III.2.3 Sample collection and analysis

At the end of the 9-week feeding experiment, growth performance parameters were recorded for fish in every aquarium, including weight gain percentage (WG), feed efficiency (FE) and survival. Three fish per aquarium were randomly chosen and euthanized with an overdose of tricaine methane sulphonate (MS-222) at 300 mg/kg after which they were individually weighed along with the liver, intraperitoneal fat, and fillet to compute the condition indices: hepatosomatic index (HSI), intraperitoneal fat (IPF) ratio, and muscle yield (MY). Besides that, the vertebrae samples from these fish were also separated using the methods described by Savolainen and Gatlin III (2010) for determination of ash concentration and mineral composition. In addition, another three fish per aquarium were randomly selected and homogenized as a composite sample for determination of whole-body proximate composition. Those samples along with the experimental diets and the initial whole-body sample was analyzed by the following previously established methods: crude protein by measuring nitrogen by the Dumas protocol and multiplying by 6.25 (AOAC, 2005); crude lipid was extracted via chloroform : methanol (Folch et al., 1957); and ash was obtained in a muffle furnace at 650 °C for 3 h (AOAC, 1990). Whole-body protein retention (PR) and lipid retention (LR) also were computed as previously described (de Cruz et al., 2020).

Additionally, all the remaining fish were fed with their assigned diet for another week, after which five fish from each aquarium were selected and dissected to collect digesta from their gastrointestinal (GI) tract, approximately 6 hours after feeding. The digesta samples from the

stomach, anterior and posterior intestinal regions were collected via stripping the contents with tweezers. The digesta samples, homogenized whole-body samples, bone samples and experimental diets were analyzed for calcium, magnesium, phosphorus, copper, iron, manganese, and zinc by the Department of Veterinary Integrative Biosciences at Texas A&M University via the method described by Chen et al. (2020). In addition, the whole-body retention of various minerals was computed according to established procedures (Chen et al., 2020) based on the whole-body mineral concentrations and amount of minerals consumed by fish fed the various experimental diets. In addition, the digestibility of various minerals in digesta from different sections of the GI tract was calculated based on the concentration of minerals and yttrium in the diets and digesta according to the following formula:

Furthermore, the excretion of various minerals also was calculated according to established procedures (Lee et al., 2020) in which the difference between nutrient fed and nutrient retained in fish body was determined based on the weight gain.

III.2.4 Calculations and statistical analysis

Same formulas and statistical analysis method were used in this feeding trial, which has been descried detailly in Chapter II, calculations and statistical analysis section.

III.3. Results

III.3.1 Fish growth performance and condition indices

Production parameters and condition indices of red drum fed the different diets are shown in Table 18. According to the results of contrast analysis, red drum fed the NC diet had significantly ($P < 0.05$) lower WG and FE than fish fed all the other experimental diets. However, a significantly lower survival was noticed for red drum fed the PC diet compared to fish fed all the other experimental diets. No significant differences among fish fed the various diets were found for HSI, IPF ratio, or MY. The effects of incremental doses of dietary phytase on WG were best explained as a quadratic function. Besides that, the upward quadratic relationship between dietary phytase dose and WG revealed the optimal dosage to be 6175 FTU phytase /kg of diet. Although, the quadratic relationship between survival and dietary phytase doses was significant, the *P* value of the quadratic element was higher than 0.05. Thus, the relationship between survival and dietary phytase doses was better explained as a descending linear model.

Red drum fed the NC diet had significantly ($P < 0.05$) lower whole-body ash and PR than fish fed all the other experimental diets (Table 19). In addition, whole-body ash had an upward quadratic relationship with dietary phytase dose, in which the optimal dosage was estimated at 5263 FTU/kg diet. The relationship between dietary phytase dose and PR was explained as an ascending linear model.

Table 18. Weight gain (WG), feed efficiency (FE), survival, hepatosomatic index (HSI), intraperitoneal fat (IPF) ratio, and muscle yield (MY) of red drum fed diets with different levels of phytase for 9 weeks[a]

Diets	WG (%)	FE	Survival (%)	HSI	IPF ratio	MY (%)
Negative Control	891	0.83	93.8	2.4	0.58	34.6
Positive Control	1065	0.91	81.3	2.1	0.37	33.0
F1000	1123	0.95	95.0	2.2	0.56	34.2
F2000	1089	0.92	93.8	2.2	0.39	33.1
F4000	1203	0.94	87.5	2.3	0.44	33.7
F8000	1239	0.95	87.5	2.3	0.50	35.4
PSE[b]	55.7	0.028	3.13	0.12	0.099	0.80
Orthogonal contrast (Pr > F)[c]						
Linear	0.002	0.06	0.01	0.7	0.6	0.1
Quadratic	0.002	0.06	0.04	0.8	0.4	0.2
Regression						
Model[d]	Q	NOS	L	NOS	NOS	NOS
Pr > F	0.002	-	0.01	-	-	-
R^2	0.54	-	0.29	-	-	-
Optimal dosage[e]	6175	-	-	-	-	-
Contrast (Pr > F)						
NF0 – PF0	0.04	0.04	0.01	0.08	0.2	0.6
NF0 – (F1000 to F8000)	0.0004	0.002	0.43	0.3	0.4	0.6
PF0 – (F1000 to F8000)	0.1	0.4	0.01	0.2	0.4	0.2

[a] Values are means of three fish from each of four replicate groups (n=4). Significance probability associated with the *P*-value.

[b] Pooled standard error

[c] Orthogonal contrast analysis was only run among treatment NC and all phytase-supplemental treatments, which needed to be all positive results. If statistical significance (P<0.05) were detected, the model which fit best the data was chosen.

[d] L = linear model; Q = quadratic model; NOS = no structure

[e] Upward quadratic model was used to derive the optimal dosage.

Table 19. Whole-body proximate composition (% of fresh weight), protein retention percentage (PR), and lipid retention percentage (LR) of red drum fed diets with different levels of phytase for 9 weeks[a]

Diets	Moisture	Protein	Lipid	Ash	PR	LR
Negative Control	73.7	17.8	5.2	3.4	33.0	49.4
Positive Control	73.1	17.8	5.2	3.9	37.1	52.5
F1000	73.1	18.4	5.0	3.7	40.4	54.7
F2000	73.8	17.9	4.7	3.7	38.2	51.4
F4000	72.7	17.8	4.9	3.9	38.3	51.7
F8000	72.5	18.2	5.4	3.8	40.5	60.0
PSE[b]	0.46	0.19	0.33	0.07	1.11	4.15
Orthogonal contrast (Pr > F)[c]						
Linear	0.07	0.5	0.5	0.02	0.04	0.1
Quadratic	0.2	0.7	0.4	0.001	0.06	0.3
Regression						
Model[d]	NOS	NOS	NOS	Q	L	NOS
Pr > F	-	-	-	0.001	0.04	-
R^2	-	-	-	0.56	0.22	-
Optimal dosage[e]	-	-	-	5263	-	-
Contrast (Pr > F)						
NF0 – PF0	0.4	0.9	0.9	<0.0001	0.02	0.6
NF0 – (F1000 to F8000)	0.2	0.2	0.6	<0.0001	<0.0001	0.3
PF0 – (F1000 to F8000)	0.9	0.2	0.7	0.2	0.09	0.7

[a] Values are means of composite samples of three fish from each of four replicate groups (n=4). Significance probability associated with the *P*-value.

[b] Pooled standard error

[c] Orthogonal contrast analysis was only run among treatment NC and all phytase-supplemental treatments, which needed to be all positive results. If statistical significance (P<0.05) were detected, the model which fit best the data was chosen.

[d] L = linear model; Q = quadratic model; NOS = no structure

[e] Upward quadratic model was used to derive the optimal dosage.

The whole-body mineral retention of red drum was impacted by various diets (Table 20). According to the contrast analysis, red drum fed the NC diet had significantly ($P < 0.05$) reduced retention of Ca, Mg, P, and Fe among fish fed the various experimental diets. Besides that, significantly higher Cu and Zn retention was observed when red drum were fed the phytase-supplemented diets, compared to fish fed either the NC or PC diets. Furthermore, red drum fed the PC diet had highest Mn retention that was significantly different than those fed other diets. The whole-body retention of Mg, P, and Zn were detected to have an upward quadratic relationship with dietary phytase dose, where the optimal dosages were estimated to be 5390, 6574, and 6835 FTU/kg diet, respectively. Additionally, the relationship between Ca retention and dietary phytase doses was best explained as an ascending linear model.

Based on contrast analysis, red drum fed the NC diet had significantly higher excretion of all the measured minerals, except for P when compared to fish fed the PC diet (Table 21). In addition, the lowest excretion of Mg, P, and Cu were noticed in red drum fed the phytase-supplemented diets although they had a significantly higher Fe excretion than fish fed the PC diet. A descending linear relationship was disclosed between dietary phytase dose and excretion of N, Ca, Cu, and Zn. Besides that, the exertion of Mg and P presented a downward quadratic model with dietary phytase dose, in which the optimal dosages were estimated at 5393 and 5655 FTU/kg diet, respectively.

Table 20. Whole-body mineral retention (%) of red drum fed diets with different levels of phytase for 9 weeks[a]

Diets	Ca	Mg	P	Cu	Fe	Mn	Zn
Negative Control	71.2	7.0	51.9	2.9	2.4	20.4	7.4
Positive Control	91.7	10.2	60.5	2.3	3.1	29.5	10.2
F1000	84.6	9.8	61.5	5.7	3.3	20.3	17.6
F2000	92.2	10.8	66.1	5.3	3.3	25.6	17.5
F4000	87.5	11.7	64.7	3.7	3.0	21.6	18.7
F8000	95.4	11.4	67.4	5.7	3.2	21.2	23.2
PSE[b]	3.89	0.30	2.45	0.49	0.17	2.12	1.18
Orthogonal contrast (Pr > F)[c]							
Linear	0.006	0.0007	0.01	0.1	0.1	1	0.0001
Quadratic	0.007	<0.0001	0.004	0.4	0.1	0.6	<0.0001
Regression							
Model[d]	L	Q	Q	NOS	NOS	NOS	Q
Pr > F	0.006	<0.0001	0.004	-	-	-	<0.0001
R^2	0.35	0.86	0.49	-	-	-	0.67
Optimal dosage[e]	-	5390	6574	-	-	-	6835
Contrast (Pr > F)							
NF0 – PF0	0.002	<0.0001	0.02	0.4	0.008	0.007	0.1
NF0 – (F1000 to F8000)	0.0004	<0.0001	0.0002	0.0008	0.0003	0.5	<0.0001
PF0 – (F1000 to F8000)	0.7	0.05	0.1	<0.0001	0.5	0.006	<0.0001

[a] Values are means of composite samples of three fish from each of four replicate groups (n=4). Significance probability associated with the *P*-value.

[b] Pooled standard error

[c] Orthogonal contrast analysis was only run among treatment NC and all phytase-supplemental treatments, which needed to be all positive results. If statistical significance (P<0.05) were detected, the model which fit best the data was chosen.

[d] L = linear model; Q = quadratic model; NOS = no structure

[e] Upward quadratic model was used to derive the optimal dosage.

Table 21. Estimated excretion of various minerals (g/kg) of red drum fed diets with different levels of phytase for 8 weeks[a]

Diets	N	Ca	Mg	P	Cu	Fe	Mn	Zn
Negative Control	59.3	4.0	4.3	5.6	0.014	0.23	0.032	0.087
Positive Control	49.3	1.1	3.3	4.8	0.016	0.19	0.024	0.065
F1000	44.3	1.9	3.2	4.1	0.009	0.20	0.028	0.064
F2000	47.4	0.9	3.0	3.4	0.009	0.20	0.024	0.068
F4000	46.7	1.6	2.8	3.8	0.012	0.22	0.028	0.066
F8000	43.6	0.5	2.8	3.3	0.009	0.21	0.026	0.053
PSE[b]	2.39	0.55	0.12	0.39	0.0004	0.006	0.0014	0.0028
Orthogonal contrast (Pr > F)[c]								
Linear	0.03	0.009	0.002	0.02	0.04	0.8	0.1	0.0002
Quadratic	0.03	0.01	<0.0001	0.01	0.1	0.8	0.1	0.0006
Regression Model[d]	L	L	Q	Q	L	NOS	NOS	L
Pr > F	0.03	0.009	<0.0001	0.01	0.04	-	-	0.0002
R^2	0.24	0.32	0.76	0.44	0.2	-	-	0.54
Optimal dosage[e]	-	-	5393	5655	-	-	-	-
Contrast (Pr > F)								
NF0 – PF0	0.008	0.002	<0.0001	0.1	0.003	0.001	0.001	<0.0001
NF0 – (F1000 to F8000)	<0.0001	0.0003	<0.0001	0.0003	<0.0001	0.01	0.003	<0.0001
PF0 – (F1000 to F8000)	0.2	0.8	0.009	0.02	<0.0001	0.03	0.1	0.5

[a] Values are means of composite samples of three fish from each of four replicate groups (n=4). Significance probability associated with the *P*-value.

[b] Pooled standard error

[c] Orthogonal contrast analysis was only run among treatment NC and all phytase-supplemental treatments, which needed to be all positive results. If statistical significance (P<0.05) were detected, the model which fit best the data was chosen.

[d] L = linear model; Q = quadratic model; NOS = no structure

[e] Downward quadratic model was used to derive the optimal dosage.

Significantly ($P < 0.05$) lower bone ash, Ca, Mg, and P concentration was observed in red drum fed the NC diet compared to those fed all of the other experimental diets (Table 22). In addition, red drum fed the phytase-supplemented diets also achieved the highest bone Zn content and lowest Mn content, compared to fish fed either NC or PC diets. An ascending linear model was observed between the dietary phytase dose and bone ash, Ca, Mg, and P concentrations. On another hand, the relationship between bone Zn concentration and dietary phytase doses was an upward quadratic relationship, in which the optimal dosage was estimated to be 5632 FTU phytase /kg of diet.

Table 22. Ash and mineral composition of bone from red drum fed diets with different levels of phytase for 9 weeks[a]

Diets	Ash (%)	Ca (g/kg)	Mg (g/kg)	P (g/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)
Negative Control	42.2	157	2.3	78.4	8.6	135	29.3
Positive Control	46.4	171	2.8	87.8	9.4	137	32.5
F1000	45.8	166	2.7	85.0	9.2	112	73.6
F2000	44.7	165	2.6	84.5	11.7	117	72.0
F4000	45.7	165	2.7	85.8	8.8	113	80.9
F8000	47.0	170	2.7	87.3	8.8	112	85.4
PSE[b]	0.51	3.5	0.07	1.77	0.77	8.4	2.38
Orthogonal contrast (Pr > F)[c]							
Linear	0.001	0.04	0.02	0.02	0.6	0.2	0.001
Quadratic	0.002	0.1	0.01	0.02	0.4	0.2	<0.0001
Regression							
Model[d]	L	L	L	L	NOS	NOS	Q
Pr > F	0.001	0.04	0.02	0.02	-	-	<0.0001
R^2	0.46	0.22	0.26	0.27	-	-	0.74
Optimal dosage[e]	-	-	-	-	-	-	5632
Contrast (Pr > F)							
NF0 – PF0	<0.0001	0.01	<0.001	0.002	0.4	0.9	0.3
NF0 – (F1000 to F8000)	<0.0001	0.02	<0.001	0.002	0.2	0.03	<0.001
PF0 – (F1000 to F8000)	0.3	0.3	0.3	0.3	0.8	0.02	<0.001

[a] Values are means of composite samples of three fish from each of four replicate groups (n=4). Significance probability associated with the *P*-value.

[b] Pooled standard error

[c] Orthogonal contrast analysis was only run among treatment NC and all phytase-supplemental treatments, which needed to be all positive results. If statistical significance (P<0.05) were detected, the model which fit best the data was chosen.

[d] L = linear model; Q = quadratic model; NOS = no structure

[e] Upward quadratic model was used to derive the optimal dosage.

III.3.2 Apparent availability of various minerals

The apparent availability of different minerals in the stomach, anterior and posterior intestinal regions are presented in Tables 23, 24, and 25. Due to some of the negative apparent availability results, only the minerals that had all positive results were analyzed via regression and linear orthogonal contrasts. According to the contrast analysis results from the stomach, red drum fed the PC diet had significantly ($P < 0.05$) lower apparent availability of Ca than fish fed all other experimental diets (Table 23). In addition, fish fed the phytase-supplemental diets had significantly improved apparent availability of P than fish fed either the NC or PC diets. In addition, red drum fed the PC had significantly reduced apparent availability of Fe compared to fish fed the phytase-supplemental diets. However, red drum fed the NC had significantly higher apparent availability of Mg than fish fed either the PC or phytase-supplemental diets. Only the relationship between dietary phytase dose and apparent availability of Mg and Fe were evident and explained by downward quadratic and ascending linear models, respectively.

Table 23. Apparent availability of different minerals in the stomach of red drum fed diets with different levels of phytase for 9 weeks[a]

Diets	Ca	Mg	P	Cu	Fe	Mn	Zn
Negative Control	17.9	46.9	20.4	-6.5	22.3	4.2	26.5
Positive Control	4.5	38.9	16.2	36.8	17.8	-5.7	16.7
F1000	12.6	43.3	29.9	-7.8	24.7	6.9	33.3
F2000	9.7	33.2	19.6	-30.6	20.9	-9.2	22.5
F4000	26.4	34.0	29.2	3.2	28.2	5.9	36.8
F8000	16.5	38.7	28.3	-19.2	29.2	4.4	23.8
PSE[b]	1.71	1.48	1.53	14.05	2.05	2.48	6.24
Orthogonal contrast (Pr > F)[c]							
Linear	0.9	0.08	0.07	-	0.007	-	0.8
Quadratic	0.7	<0.0001	0.2	-	0.03	-	0.6
Regression							
Model[d]	NOS	Q	NOS	NOS	L	NOS	NOS
Pr > F	-	<0.0001	-	-	0.007	-	-
R^2	-	0.72	-	-	0.34	-	-
Optimal dosage[e]	-	-	-	-	-	-	-
Contrast (Pr > F)[f]							
NF0 – PF0	<0.0001	0.001	0.07	-	0.14	-	-
NF0 – (F1000 to F8000)	0.6	<0.0001	0.002	-	0.16	-	0.7
PF0 – (F1000 to F8000)	<0.0001	0.4	<0.0001	-	0.003	-	-

[a] Values are means of composite samples of three fish from each of four replicate groups (n=4). Significance probability associated with the *P*-value.

[b] Pooled standard error

[c] Orthogonal contrast analysis was only run among treatment NC and all phytase-supplemental treatments, which needed to be all positive results. If statistical significance (P<0.05) were detected, the model which fit best the data was chosen.

[d] L = linear model; Q = quadratic model; NOS = no structure

[e] Upward quadratic model was used to derive the optimal dosage.

[f] The contrast analysis was only applied to the groups that had all positive results. If any groups contained negative data, "-" will be shown in the cell

In the anterior and posterior intestinal regions, similar results were observed. In both regions, red drum fed the phytase-supplemental diets had significantly higher apparent availability of P than fish fed either the NC or PC diets (Table 24, 25). In addition, the relationship between apparent availability of P and dietary phytase doses was explained as upward quadratic models, in which the optimal dosages were estimated to be 4667 and 5150 FTU phytase /kg of diet, for the anterior and posterior intestine, respectively. Additionally, red drum fed the phytase-supplemental diets had the significantly higher apparent availability of P in both regions. Furthermore, an upward quadratic relationship was evident between the apparent availability of Zn and dietary phytase doses in the posterior intestinal region, in which the optimal dosage was estimated to be 3986 FTU/kg phytase of diet.

Table 24. Apparent availability of different minerals in the anterior intestinal region of red drum fed diets with different levels of phytase for 9 weeks[a]

Diets	Ca	Mg	P	Cu	Fe	Mn	Zn
Negative Control	7.9	21.6	56.7	-21.2	21.0	47.8	25.6
Positive Control	-2.2	14.9	55.5	6.7	11.5	47.4	6.1
F1000	5.0	4.4	67.3	-3.9	10.9	32.7	14.3
F2000	-1.5	-11.3	73.3	-88.4	6.0	26.6	7.3
F4000	-4.7	-29.7	69.0	-60.7	11.1	13.6	1.4
F8000	-42.1	-61.0	69.6	-140.1	-13.7	-2.9	-50.5
PSE[b]	11.85	13.96	2.94	32.87	6.13	10.72	8.34
Orthogonal contrast (Pr > F)[c]							
Linear	-	-	0.11	-	-	-	-
Quadratic	-	-	0.02	-	-	-	-
Regression							
Model[d]	NOS	NOS	Q	NOS	NOS	NOS	NOS
Pr > F	-	-	0.02	-	-	-	-
R^2	-	-	0.36	-	-	-	-
Optimal dosage[e]	-	-	4667	-	-	-	-
Contrast (Pr > F)[f]							
NF0 – PF0	-	0.74	0.08	-	-	0.98	-
NF0 – (F1000 to F8000)	-	-	0.001	-	-	-	-
PF0 – (F1000 to F8000)	-	-	0.0004	-	-	-	-

[a] Values are means of composite samples of three fish from each of four replicate groups (n=4). Significance probability associated with the *P*-value.

[b] Pooled standard error

[c] Orthogonal contrast analysis was only run among treatment NC and all phytase-supplemental treatments, which needed to be all positive results. If statistical significance (P<0.05) were detected, the model which fit best the data was chosen.

[d] L = linear model; Q = quadratic model; NOS = no structure

[e] Upward quadratic model was used to derive the optimal dosage.

[f] The contrast analysis was only applied to the groups that had all positive results. If any groups contained negative data, "-" will be shown in the cell

Table 25. Apparent availability of different minerals in the posterior intestinal region of red drum fed diets with different levels of phytase for 9 weeks[a]

Diets	Ca	Mg	P	Cu	Fe	Mn	Zn
Negative Control	5.8	22.9	53.7	-9.4	15.2	52.0	20.2
Positive Control	-5.8	13.0	57.6	26.3	7.4	55.3	9.2
F1000	-2.1	3.8	58.2	9.2	14.2	28.2	26.9
F2000	4.3	1.6	73.2	-86.7	4.8	35.1	27.0
F4000	18.6	7.3	78.8	-8.4	1.3	37.9	37.5
F8000	-3.3	5.6	72.9	-19.5	19.2	15.4	18.6
PSE[b]	9.0	7.05	4.39	26.75	10.78	10.14	5.19
Orthogonal contrast (Pr > F)[c]							
Linear	-	-	0.05	-	-	-	0.76
Quadratic	-	-	0.002	-	-	-	0.03
Regression							
Model[d]	NOS	NOS	Q	NOS	NOS	NOS	Q
Pr > F	-	-	0.002	-	-	-	0.03
R^2	-	-	0.52	-	-	-	0.34
Optimal dosage[e]	-	-	5150	-	-	-	3986
Contrast (Pr > F)[f]							
NF0 – PF0	-	0.33	0.53	-	-	-	-
NF0 – (F1000 to F8000)	-	-	0.001	-	-	-	0.22
PF0 – (F1000 to F8000)	-	-	0.005	-	-	-	-

[a] Values are means of composite samples of three fish from each of four replicate groups (n=4). Significance probability associated with the *P*-value.

[b] Pooled standard error

[c] Orthogonal contrast analysis was only run among treatment NC and all phytase-supplemental treatments, which needed to be all positive results. If statistical significance (P<0.05) were detected, the model which fit best the data was chosen.

[d] L = linear model; Q = quadratic model; NOS = no structure

[e] Upward quadratic model was used to derive the optimal dosage.

[f] The contrast analysis was only applied to the groups that had all positive results. If any groups contained negative data, "-" will be shown in the cell

III.4 Discussion

III.4.1 The effect of dietary phytase on growth performance and condition indices of red drum

Numerous evaluations into the effects of dietary supplementation of different types of phytases on growth performance of various fish species have been conducted. Jackson et al. (1996) reported that channel catfish (*Ictalurus punctatus*) fed diets based primarily on plant feedstuffs and containing more than 500 FTU phytase /kg had significantly increased feed intake and WG. In addition, Vielma et al. (2004) claimed that adding 2000 FTU phytase /kg diet containing 50% soybean meal significantly improved the WG of rainbow trout (*Onchorhyncus mykiss*). Besides that, Papatryphon et al. (1999) stated that striped bass (*Morone saxatilis*) fed diets supplemented more than 1000 FTU phytase /kg had significantly lower FCR compared to fish fed the basal diet. Moreover, the benefits of dietary phytase supplementation on weight gain also had been reported for African catfish (*Clarias gariepinus*) (Nwanna et al., 2005), Atlantic salmon (*Salmo salar*) (Sajjadi & Carter, 2004b), Nile tilapia (Cao et al., 2008), and common carp (Nwanna et al., 2007). Similar results were observed in the present study showing that red drum fed phytase-supplemental diets had significantly improved WG (22.2 to 39.1%) and FE (10.8 to 14.5%), compared to the fish fed the NC diet containing an inadequate level of available P. One contradictory study was that of Yan et al. (2002), who fed channel catfish all-plant-protein diets supplemented with phytase (0 to 8000 FTU/kg) but did not observe improved WG. In that study, although the basal diet was formulated to contain insufficient available phosphorus (0.16%) but adequate total phosphorus (0.57%), the dietary zinc level (available zinc: 5.3 mg/kg and total

zinc: 53.37 mg/kg) was deficient and thus may have limited the response to supplemental phytase. Based on the dietary zinc requirement of channel catfish established by Gatlin III and Wilson (1983), channel catfish require at least 20 mg Zn/kg in a semi-purified diet. In addition, because of the adverse effects of dietary phytate (Satoh et al., 1989), Gatlin III and Wilson (1984) claimed that the optimal dietary zinc level for a practical catfish diet was 150 mg/kg. Therefore, the inadequate dietary available zinc level of the basal diet formulated by Yan et al. (2002) rather than dietary phytase supplementation likely led to the negligible effects of phytase supplementation on growth performance of channel catfish. Moreover, previous research reported that channel catfish received phytase-supplemented diets had comparable growth as catfish fed a control diet containing extra dicalcium phosphate(Li & Robinson, 1997). Thus, Li and Robinson (1997) concluded that dietary phytase supplementation could replace the inorganic phosphorus supplementation of channel catfish diets. This observation was confirmed by the current research with red drum, in which no significant differences in growth performance were found between red drum fed the PC and phytase-supplemented diets. As such, the advantage of dietary phytase supplementation was that it enhanced the growth performance of red drum by making more of the dietary P available to the fish and thus eliminating the need to supplement with inorganic phosphorus.

No significant differences were observed in whole-body composition or lipid retention of red drum fed the various experimental diets. However, red drum fed the phytase- supplemented diets had significantly higher whole-body protein retention and reduced nitrogen excretion than

fish fed either the NC or PC diets. Thus, dietary phytase supplementation did significantly increase the utilization of dietary protein by red drum in the present study. Several studies have been conducted to evaluate the effect of dietary phytase supplementation on the protein availability. Similarly, Sajjadi and Carter (2004b) claimed a positive interaction between phytate and phytase on apparent digestibility of crude protein in Atlantic salmon fed diets based on casein and fishmeal. In addition, Storebakken et al. (1998) reported that Atlantic salmon fed a diet containing phytase pre-treated soy protein concentrate had significantly improved protein digestibility, whole-body protein retention, and reduced metabolic nitrogen excretion. Similar results were also found with Nile tilapia (Furuya et al., 2001; Nwanna, 2005), rainbow trout (Sugiura et al., 2001), and rohu (Baruah et al., 2005). Previous research showed that proteins were more easily bound with phytate than minerals at low (acidic) pH (Wise, 1983). In addition, the ternary complexes of myo-inositol hexaphosphate(IP6) – Ca – proteins were less subject to attack by phytase once they formed, due to the aggregated proteins covering them (Rajendran & Prakash, 1993). Previous studies also revealed negative effects of dietary phytate on the activities of pepsin (Camus et al., 1976), trypsin, trypsinogen (Caldwell, 1992), and total proteases (Morales et al., 2014) in various fish species. However, the potential mechanisms associated with the positive effects of dietary phytase on improving the digestibility of protein in the fish gut are not completely understood. However, there are four popular theories regarding the beneficial effects of phytase including: preventing the proteins binding with phytic acid in the fish GI tract; releasing the proteins bound to the ternary IP6-protein complexes, weakening the restriction of

phytate on digestive proteases (Selle et al., 2000); and, diminishing the secretion of mucin and the enzymes containing endogenous amino acids (Cowieson et al., 2004).

III.4.2 The effect of dietary phytase on the utilization of various minerals by red drum

The positive effects of dietary phytase on the utilization of minerals of various fish species have been well summarized by Cao et al. (2007) and V Kumar et al. (2012). Although some of these results varied due to different fish species, phytase dosages, and diet compositions, it was agreed that the advantages of dietary phytase supplementation included improving the whole-body retention and bone concentrations of various minerals, diminishing the excretion of some minerals, and enhancing the apparent digestibility coefficients (ADCs) of several minerals. However the ADC of minerals may be impacted by different sample collection methods and the salinity of water, which may limit comparison between different species. Vandenberg and De La Noüe (2001) compared ADCs of various nutrients in feces from rainbow trout obtained by three common feces collection methods (modified Guelph system, St-Pee system, and abdominal massage). They concluded that the abdominal massage (feces stripping method) had significantly lower ADC results than the other two passive feces collecting methods, due to unabsorbed nutrients in stripped digesta and the leaching of nutrients from the feces in water.

Mineral metabolism of fish may differ between seawater and freshwater species due to differences in osmotic regulation. Unlike the freshwater fish which remove excess body water by producing large quantity of dilute urine, marine fish species must drink water and absorb it in

their gut to compensate the lost fluids (Maetz, 1971; Wurts, 1998). In addition, the abundance of minerals in seawater may be absorbed by fish in their gut and then eliminated in their feces (Wurts, 1998). Approximately 20% of excessive divalent ions were reported to be excreted by the fish in urine by the kidney while the remaining 80% was excreted in the feces (Hickman Jr, 1968), which obviously will influence the concentrations of various minerals in the feces. This is a likely explanation for the negative ADC results observed in the current study. Similar results concerning mineral ADC values were found with Atlantic salmon (Denstadli et al., 2007; Storebakken et al., 1998).

Previous research reported that the whole-body mineral composition of fish was mainly influenced by osmotic regulation when fish were fed adequate minerals; however, one deficient mineral could impact the utilization of other minerals (Shearer, 1995; Storebakken et al., 1998). For instance, when Atlantic salmon were fed P-deficient diets, not only the was the whole-body P concentration decreased but also the whole-body Ca and Mg concentrations (Åsgård & Shearer, 1997; Baeverfjord et al., 1998). It was assumed that the significantly lower whole-body retention of Mg, Fe, and Mn by red drum fed the NC diet compared to those fed the PC diet was caused by the insufficient P level in the NC diet.

In the present study, red drum fed the PC and phytase-supplemental diets had significantly increased whole-body and bone ash concentrations compared to fish fed the NC diet. These observations confirmed that the available P level in the NC diet was lower than the dietary requirement of red drum, and dietary phytase supplementation increased mineral utilization of

red drum. The whole-body P retention of red drum fed the phytase-supplemented diets increased between 18.5 and 29.9%, compared to fish fed the NC diet. Likewise, the P excretion of red drum fed the NC and PC diets dropped from 5.6 and 4.8 g/kg, respectively, to an average of 3.65 g/kg for fish fed the phytase-supplemented diets. In addition, red drum fed the phytase-supplemented diets had up to 11.5% higher bone P concentration than fish fed the NC diet. Although marine fish had the ability to directly absorb P from water through the gills (Lall, 1991), their GI tract is considered the major site of absorption (Bakke et al., 2010). In addition, due to the low P level (0.02 mg/L) in seawater (Lall, 1991), water-borne P typically has a limited effect on the ADC of P (Storebakken et al., 1998). According to the ADC of P results from different regions of the GI tract in the present study, the dietary phytase supplementation significantly increased the utilization of P for red drum throughout the GI tract. Although, previous research reported that the highest activity of dietary phytase was detected in the stomach of fish because of the low pH (Zhu et al., 2016), there was only 16.2 to 29.9% of P absorbed in stomach. Meanwhile, there was up to another 53.7% of P removed in the anterior region of the intestine. Thus, results of the present study confirmed that dietary phytase supplementation can significantly enhance P utilization of red drum and the anterior intestinal region was the main site of P absorption.

Red drum fed the phytase-supplemented diets had up to 34% higher whole-body Ca retention than fish fed the NC diet. Besides that, the excretion of Ca by red drum fed diets with phytase supplementation was reduced to 0.5 g/kg, compared to 4 g/kg for fish fed the NC diet.

Additionally, the bone Ca concentration of red drum was increased from 157 g/kg to an average of 166.5 g/kg with phytase addition to the NC diet. Unlike freshwater fish which mainly absorbed Ca from the water via the gills (Ichii & Mugiya, 1983), the main site of Ca absorption in marine fish is the GI tract. Previous studies claimed that 20 to 70% of Ca was absorbed through the intestine of adult marine fish, with most of the Ca coming from seawater rather than diet (Guerreiro et al., 2002; Schoenmakers et al., 1993).

According to the ADC values for Ca in digesta taken from different GI tract regions, dietary phytase supplementation significantly increased the absorption of Ca in the stomach; however, the extra MCP in the PC diet decreased Ca absorption in the red drum stomach. Previous study showed that the net absorption of minerals increased with increasing dietary levels, however, after reaching the maximum requirement of these minerals, the excess minerals decreased the utilization or bioavailability value (Pimentel-Rodrigues & Oliva-Teles, 2007). This could be the reason why red drum fed the PC diet had significantly lower ADC of Ca in the stomach, compared to fish given the NC diet. In addition, the apparent availability of Ca in the anterior and posterior intestinal regions were affected by the water-borne Ca in the present study.

Red drum red the phytase-supplemented diets had significantly increased whole-body Mg retention of up to 40%, and decreased Mg excretion by at least 20%, compared to fish fed the NC diet. In addition, bone Mg concentration of red drum was enhanced from 2.3 to at most 2.7 g/kg after phytase was supplemented to the NC diet. However, the results of apparent availability of Mg in the stomach region showed that red drum fed the NC diet had significantly higher Mg

absorption than fish fed the PC and phytase-supplemented diets. The intestinal absorption of Mg by various fish species has not been well established to date. Bijvelds et al. (1996) and Sakamoto (1979) thought marine fish derived Mg mainly from the water and dietary uptake was only used to regulate the homeostasis of whole-body Mg. In addition, Lin et al. (2013) reported that the dietary Mg requirement of hybrid tilapia was decreased from 0.2 g/kg diet to less than 0.02 g/kg diet when the fish were moved from freshwater to seawater (32 ppt), which confirmed that water-borne Mg was the main resource for marine fish. However, there was no further information regarding Mg metabolism that could be derived from the current study.

Differences in Cu metabolism were also noticed among red drum given the various experimental diets in the current feeding trial. Red drum fed the phytase-supplemented diets had up to 97% and 148% higher whole-body Cu retention, compared to fish fed the NC and PC diets, respectively. In addition, the dietary phytase supplementation reduced up Cu excretion by up to 35.7%. The intestinal absorption of Cu by freshwater fish was discussed by Nadella et al. (2006), who concluded the major absorption sites for rainbow trout were in the middle and posterior intestinal regions. However, not many studies have been able to provide details regarding intestinal Cu absorption of marine fish. Berntssen et al. (1999) claimed that the intestine of Atlantic salmon may played an important role in regulating the uptake of dietary Cu. The Cu apparent availability results in the present study were unexpected. We assumed that the water-borne Cu severely impacted our Cu apparent availability results.

Red drum fed the phytase-supplemented diets had whole-body Fe retention up to 37.5%

higher than fish fed the NC diet. In addition, Fe excretion decreased 13% from 0.23 g/kg to 0.20 g/kg in red drum fed diets supplemented with phytase. However, no significant differences were found in the Fe bone concentrations among fish fed the various experimental diets. This result was not surprising, because the majority of Fe is stored in fish liver and gill rather than bone tissue (Çoğun et al., 2005; Honda et al., 1983). There was no useful information obtained from the Fe apparent availability data in the current experiment. Although the mechanism of intestinal Fe absorption of marine fish is not clearly established, studies have shown that the intestinal environment of marine fish is not friendly to iron absorption (Bury & Grosell, 2003; Bury et al., 2003). The high Ca and Mg ion concentrations of seawater lead to excess intestinal Ca and Mg ion concentrations of marine fish, which is believed to cause the intestine of marine fish to secrete bicarbonate ions to bind and reduce the intestinal Ca and Mg ion concentrations (Wilson, 1999; Wilson et al., 2002). In addition, the large quantity of bicarbonate ions may not only limit the intestinal Ca and Mg ion concentrations, but also bind Fe ion to form Fe carbonates and reduce the bioavailability of dietary iron (Wilson, 1999).

Results of the current study regarding Mn utilization of red drum was unexpected in that phytase supplementation did not increase Mn utilization. According to the results from previous studies, dietary phytase supplementation significantly increased whole-body Mn content of African catfish (Nwanna et al., 2005), bone Mn concentration of channel catfish (Yan et al., 2002), bone and scale Mn content of common carp (Nwanna et al., 2007), bone Mn content and apparent availability in pangas catfish (Debnath et al., 2005), and Mn apparent availability in

rainbow trout (Cheng & Hardy, 2003). However, no significant differences were found in whole-body Mn retention between red drum fed the NC and phytase-supplemented diets. Although red drum fed the phytase-supplemented diets had significantly lower Mn excretion than fish fed the NC diet, the significantly reduced Mn bone content was detected in fish fed the phytase-supplemented diets. According to the Mn apparent availability results from the three GI tract regions, most of the Mn was absorbed in the anterior intestinal region. Because this study is the first one to investigate the effects of dietary phytase on Mn utilization of red drum, these is not much information can explain the observed results and further research is required.

In regard to Zn metabolism of red drum, up to 214% of whole-body Zn retention and 193% of Zn bone increment were observed when red drum were fed phytase-supplemented diets compared to those fed the NC diet. In addition, the Zn excretion of red drum decreased from 0.087 g/kg to 0.063 g/kg on average by dietary phytase supplementation. Compared to the freshwater fish, it has been proposed that marine fish need higher dietary Zn to meet their minimal dietary requirement due to the high concentration of chloride in seawater, which may bind Zn (Antony Jesu Prabhu et al., 2016). In addition, both saturable and diffusive absorption of Zn in marine fish gut had been established (Glover et al., 2003; Shears & Fletcher, 1983). Unfortunately, the Zn apparent availability results from different regions of the GI tract did not provide further insight concerning Zn metabolism.

III.4.3 The optimal dosage of dietary phytase for red drum

Numerous previous studies have been conducted to estimate the optimal dosage of dietary phytase for various fish species. However, due to the different phytase products, fish species, diet formulations, and selected response parameters, there is no standard for determining the optimal dietary phytase dosage for all fish (Cao et al., 2007; Dersjant-Li et al., 2015; V Kumar et al., 2012). The most common standard of dietary phytase dosage recommended in diets of aquatic species has been between 250 to 1500 FTU/kg (Cao et al., 2007; Li et al., 2019). However, according to the results of the orthogonal contrasts of all reported parameters in the current trial, the optimal phytase dosage for red drum fed diets containing 40% of soybean meal was between 3986 and 6835 FTU/kg, which is much higher than other recommended phytase supplementation levels. In addition, the average of all these results was 5520 FTU/kg, with 50% of the data ranging from 5150 to 6175 FTU/kg. Thus, the optimal dietary Quamtum Blue phytase dosage for red drum was recommended as 5520 $^{5150}_{6175}$ FTU/kg (mean and interquartile range).

In summary, the current study showed that superdosing dietary phytase supplementation significantly improved WG and FE of red drum when diets were deficient in available P. Moreover, dietary phytase supplementation enhanced the whole-body retention of Ca, Mg, P, CU, Fe, and Zn ions, reduced the excretion of N, Ca, Mg, P, Cu, Fe, Mn, and Zn ions, and increased the bone ash, Ca, Mg, P, Mn, and Zn concentrations. In addition, the apparent availability of P was significantly promoted by dietary phytase supplementation, and according to the orthogonal contrast results, the optimal dosage of dietary phytase was estimated to be 5520

FTU/kg of diet for red drum given diets containing 40% soybean meal without supplemental inorganic P.

CHAPTER IV

THE EFFECTS OF DIETARY PHOSPHORUS AND PHYTASE SUPPLEMENTATION ON GROWTH PERFORMANCE AND UTILIZATION OF VARIOUS MINERALS IN CAGE-RAISED CHANNEL CATFISH

IV1. Introduction and justification

My previous channel catfish indoor feeding trial demonstrated the positive effects of dietary phytase supplementation on growth performance, mineral utilization, and amino acid digestibility of channel catfish, as presented in Chapter II. In the present experiment, I focused on applying the phytase to a commercial channel catfish diet and investigating the interaction between the dietary extra monocalcium phosphate (MCP) and phytase supplementation on growth performance and utilization of various minerals by cage-raised channel catfish.

IV.2. Materials and methods

IV.2.1 Diets

Four nutritionally complete, commercial channel catfish diets (33.2% protein and 4.2% lipid) were made by Alabama Catfish Feedmill LLC (Uniontown, AL, USA), and used to investigate the effects of dietary phytase supplementation and its potential interaction with dietary monocalcium phosphate (MCP) supplementation on the growth and mineral utilization of channel catfish raised in cages at the Texas A&M University Aquacultural Research and Teaching Facility (ARTF) of the Texas A&M University System (Burleson County, TX). The

basal diet was designed to contain neither extra MCP nor supplemental phytase and was designated -/-. The second diet (-/+) without supplemental MCP was top-coated at the feed mill with a target of 2500 FTU/kg of phytase (Quantum Blue Phytase, AB Vista) to the basal diet. One FTU of phytase is defined as the amount of phytase liberating inorganic phosphorus from sodium phytate solution (5.1 mM) at a rate of 1 μmol per min at pH 5.5 and 37 °C. The third diet (+/-) was supplemented with 0.1% of MCP to the basal diet but without phytase supplementation. The fourth diet was based on the basal diet supplemented with both phytase and extra MCP and designated +/+. All the diets were analyzed by AB Vista using an enzyme-linked immunosorbent assay (ELISA) for dietary phytase activity and near infrared spectroscopy (NIR) for proximate composition (Table 26).

Table 26. Proximate composition of experimental diets (% dry matter).

	Dietary supplementation (phosphorus/phytase)			
Analyzed composition %	-/-	-/+	+/-	+/+
Moisture %	9.98	9.91	8.94	9.15
Crude protein	33.10	32.30	33.50	33.10
Crude lipid	4.31	4.23	4.18	4.32
Ash	9.12	9.09	9.52	9.44
Available phosphorus[a]	0.55	0.61	0.67	0.80
Phytate-P	0.48	0.44	0.49	0.41
Total phosphorus	1.03	1.05	1.16	1.21
Phytase activity (FTU/kg)[b]	177.00	2200.00	242.00	4140.00

[a] Available P was computed based on the difference between total P and Phytate-P. All the P concentrations were measured via near infrared spectroscopy (NIR) by AB Vista.

[b] The phytase activity was analyzed by AB Vista via an enzyme-linked immunosorbent assay (ELISA)

IV.2.2 Fish and experimental conditions

Channel catfish with an initial weight of 39.8 ± 3.46 g (mean ± S.D.) were derived from ponds at the ARTF and stocked into twelve cages at100 fish per cage. Twenty fish was collected as an initial whole-body sample prior to initiating the experiment. Three ponds were used in this feeding trial, with each pond accommodating four cages which were assigned to one of the four dietary treatments. The feeding trial was designed as a randomized complete block design, using pond as the block to minimize variable environmental conditions. Fish from each cage were fed their assigned experimental diet for 5 mins until they stopped eating twice a day, to apparent satiation. Weight of each feeding containers was tracked every day to determine the feed intake of fish from each cage per day. Temperature and dissolved oxygen (DO) were measured daily in the morning and ranged from 18.5 to 30.5 °C and 3.07 to 10.63 mg/L, respectively, from April to September. No differences of temperature and DO were observed among the three ponds.

IV.2.3 Sample collection and analysis

At the end of the 20-week (136 days) feeding trial, all fish from each cage was counted and weighed as a group to compute weight gain (WG), feed efficiency (FE), and survival. Ten fish per cage was randomly selected and killed in an ice bath to subsequently measure whole-body mineral composition and mineral retention following established procedures (Chen et al., 2020). Another ten fish per cage also were sampled and their vertebrae columns were isolated and analyzed for ash and different mineral concentrations. The concentrations of various minerals of

whole-body and vertebrae samples were analyzed by AB Vista. Besides that, the excretion of various minerals of fish from each cage was calculated according to the established procedures (Lee et al., 2020), where the net retained minerals in fish was determined based on per gram of fish weight gained.

IV.2.4 Calculations and statistical analysis

The same formulas, which were described in Chapter II, were used for computing weight gain, feed efficiency, survival, nutrient retention, and nutrient excretion.

Treatment means for the various responses were analyzed using a 2 x 2 factorial design, in which the presence or absence of phytase and MCP were the main factors and pond was the blocking factor. Statistical significance was set at $P<0.05$. Tukey's HSD test was applied when significant ($P<0.05$) differences were detected via JMP Pro (SAS Institute Inc., Cary, NC, USA).

IV.3. Results

IV.3.1 Growth performance

After feeding channel catfish the experimental diets with or without extra MCP or phytase for 136 days, all fish grew at least nine times their initial weight with an overall mean weight of 417.5 ± 34.6 g (mean ± S.D.) g/fish. No significant differences due to diet were noticed with regard to weight gain, feed efficiency, or survival of channel catfish based on the main factors or their interaction (Table 27). However, a significant difference in weight gain was noticed based

on the block, indicating the different pond environments impacted the growth of channel catfish in the present trial. Although the FE results in the present trial were lower than the results from my indoor channel catfish feeding trial (0.76 to 0.81), they were still reasonable to a pond feeding trial.

Table 27. Growth performance results for channel catfish fed diets supplementing with (+) or without (-) MCP or phytase for 136 days[a]

Phosphorus	Phytase	Weight gain (%)	Feed efficiency	Survival
		Individual treatment means		
-	-	911	0.65	97
-	+	925	0.70	99
+	-	930	0.65	99
+	+	995	0.69	98
Pooled SE[b]		74.5	0.021	1.2
		Means of main effect		
Phosphorus	-	918	0.67	98
	+	963	0.67	99
Phytase	-	921	0.65	98
	+	960	0.70	99
		ANOVA: P-value		
Phosphorus		0.3	0.9	0.7
Phytase		0.4	0.08	0.7
Phosphorus × Phytase		0.6	0.9	0.2
Block		0.01	0.6	0.3

[a] Values are means of all fish from each of three replicate cages (n=3) initially averaging 39.8 ± 3.46 g (mean ± S.D.). Significance probability associated with the *P*-value.

[b] Pooled standard error.

IV.3.2 Utilization of various minerals

According to the analysis of bone ash and various mineral concentrations as shown in Table 28, the interaction between dietary MCP and phytase supplementation was found to significantly increase bone ash, Ca, Fe, P, and S concentrations. Furthermore, compared to channel catfish fed the basal diet, bone ash, Ca, and P contents were significantly increased in fish fed the experimental diets supplemented with phytase or MCP or both. Besides that, bone Fe content was only significantly enhanced when channel catfish received the diet containing both MCP and phytase. However, channel catfish fed the BF diet had significantly lower ($P<0.05$) bone S content than fish fed the basal diet. Bone Al and Zn content also were significantly increased ($P<0.05$) when channel catfish were given the diets containing phytase, independent of MCP level. Moreover, channel catfish fed diets containing either MCP or phytase had significantly higher bone Cu concentrations, but no significant interaction was found.

Table 28. Ash and various mineral concentrations (dry-matter basis) of bone from channel catfish fed diets supplemented with (+) or without (-) phosphorus or phytase for 136 days[a]

Phosphorus	Phytase	Ash(%)	Ca(g/kg)	Mg(g/kg)	P(g/kg)	S(g/kg)	Al(ppm)	Cu(ppm)	Fe(ppm)	Mn(ppm)	Zn(ppm)
Individual treatment means											
-	-	45.6[c]	171[b]	2.49	84.5[b]	1.75[a]	38.6	1.76	17.7[b]	15.9	135
-	+	47.9[a]	179[a]	2.67	88.6[a]	1.70[ab]	40.4	2.31	19.4[b]	15.1	146
+	-	46.7[b]	179[a]	2.66	89.0[a]	1.66[b]	39.4	2.19	17.8[b]	14.7	135
+	+	47.1[ab]	180[a]	2.68	89.6[a]	1.72[ab]	41.8	3.14	23.4[a]	14.7	148
Pooled SE[b]		0.24	1.7	0.064	0.88	0.025	0.81	0.131	0.50	0.43	2.5
Means of main effect											
Phosphorus	-	46.7	175	2.58	86.6	1.72	39.5	2.04[b]	18.6	15.5	141
	+	46.9	180	2.67	89.3	1.69	40.6	2.67[a]	20.6	14.7	142
Phytase	-	46.2	175	2.57	86.8	1.70	39.0[b]	1.98[b]	17.8	15.3	135[b]
	+	47.5	180	2.67	89.1	1.71	41.1[a]	2.73[a]	21.4	14.9	147[a]
ANOVA: P-value											
Phosphorus		0.6	0.02	0.1	0.006	0.2	0.2	0.002	0.009	0.1	0.6
Phytase		0.003	0.01	0.08	0.01	0.9	0.04	0.0008	0.0005	0.5	0.0008
Phosphorus × Phytase		0.01	0.049	0.08	0.04	0.047	0.7	0.2	0.01	0.5	0.7
Block		0.9	0.2	0.06	0.08	0.2	0.9	0.3	0.7	0.9	0.1

[a] Values are means of composite samples from each of three replicate groups (n=10). Significance probability associated with the *P*-value.

[b] Pooled standard error.

Table 29. Whole-body ash content (dry-matter basis) and whole-body mineral retention (%) of channel catfish fed diets supplemented with (+) or without (-) phosphorus or phytase for 136 days[a]

Phosphorus	Phytase	Ash	Ca	Mg	P	S	Al	Cu	Fe	Mn	Zn
Individual treatment means											
-	-	8.3	57.1	7.1	26.7	36.6	11.4[b]	0.93	3.6	2.0	8.5[d]
-	+	12.6	77.3	8.8	33.8	41.1	15.8[a]	1.03	4.1	2.1	12.7[a]
+	-	8.8	38.6	6.7	24.7	37.6	6.5[c]	0.43	3.3	1.5	9.4[c]
+	+	10.2	51.6	7.8	30.0	38.6	7.6[c]	1.30	3.4	1.7	11.1[b]
Pooled SE[b]		0.63	4.95	0.4	1.80	1.71	0.53	0.176	0.22	0.09	0.35
Means of main effect											
Phosphorus	-	10.5	67.2[a]	8.0	30.2	38.9	13.6	0.98	3.9	2.0[a]	10.6
	+	9.5	45.1[b]	7.3	27.3	38.1	7.1	0.87	3.4	1.6[b]	10.2
Phytase	-	8.6[b]	47.8[b]	6.9[b]	25.7[b]	37.1	9	0.68[b]	3.5	1.8	8.9
	+	11.4[a]	64.4[a]	8.3[a]	31.9[a]	39.9	11.7	1.17[a]	3.8	1.9	11.9
ANOVA: P-value											
Phosphorus		0.2	0.0002	0.1	0.1	0.7	<0.0001	0.5	0.06	0.005	0.1
Phytase		0.004	0.0008	0.009	0.008	0.2	0.0003	0.03	0.3	0.2	<0.0001
Phosphorus × Phytase		0.06	0.2	0.5	0.6	0.4	0.004	0.06	0.4	0.9	0.001
Block		0.4	0.01	0.04	0.2	0.8	0.04	0.3	0.3	0.7	0.02

[a] Values are means of composite samples from each of three replicate groups (n=10). Significance probability associated with the *P*-value.

[b] Pooled standard error.

Whole-body ash and whole-body Mg, P, and Cu retention were significantly increased when channel catfish fed the diets supplemented with phytase, independent of dietary MCP level (Table 29). In addition, channel catfish fed the phytase-supplemented diets had significantly (P<0.05) increased whole-body Ca retention than fish fed diets without phytase. On the other hand, channel catfish fed the MCP-supplemented diets had significantly reduced whole-body Ca and Mn retention, independent of dietary phytase level. Furthermore, a significant interaction was disclosed between dietary MCP and phytase supplementation on whole-body Al and Zn retention, in which channel catfish fed the BF diet had the highest whole-body Al and Zn retention. Besides that, channel catfish fed the diets containing extra MCP had significantly lower whole-body Al retention and significantly higher whole-body Zn retention than fish fed the basal diet, respectively, independent of dietary phytase level. Additionally, a significant difference due to block was observed for whole-body Ca, Mg, Al, and Zn retention, which means the retention of these minerals was significantly affected by the different pond environments.

The results of mineral excretion are exhibited in Table 30. No significant differences or interaction between dietary MCP and phytase supplementation nor the block were found on the excretion of various minerals in the current feeding trial. A significantly enhanced excretion of Ca, P, Al, and Fe was observed when channel catfish were fed the MCP-supplementation diets, independent of dietary phytase level. However, channel catfish fed the diets containing phytase had significantly reduced excretion of Ca, P, Al, Cu, and Zn, independent of dietary MCP level.

Table 30. The excretion of various minerals (g/kg) by channel catfish fed diets supplemented with or without phosphorus or phytase for 136 days[a]

Phosphorus	Phytase	Ca	Mg	P	S	Al	Cu	Fe	Mn	Zn
					Individual treatment means					
-	-	5.7	3.7	11	2.8	0.04	0.022	0.33	0.092	0.21
-	+	2.8	3.3	9	2.3	0.03	0.019	0.27	0.081	0.19
+	-	9.4	3.7	12	2.7	0.07	0.023	0.39	0.091	0.20
+	+	7.6	3.5	11	2.6	0.07	0.020	0.38	0.088	0.18
Pooled SE[b]		0.62	0.16	0.5	0.17	0.002	0.0010	0.014	0.0038	0.009
					Means of main effect					
Phosphorus	-	4.3[b]	3.5	10[b]	2.6	0.03[b]	0.021	0.30[b]	0.086	0.19
	+	8.5[a]	3.6	12[a]	2.7	0.07[a]	0.022	0.39[a]	0.090	0.19
Phytase	-	7.5[a]	3.7	12[a]	2.8	0.06[a]	0.023[a]	0.36	0.092	0.21[a]
	+	5.2[b]	3.4	10[b]	2.4	0.05[b]	0.020[b]	0.33	0.085	0.17[b]
					ANOVA: P-value					
Phosphorus		0.0005	0.6	0.01	0.6	<0.0001	0.4	0.001	0.4	0.8
Phytase		0.009	0.1	0.03	0.08	0.04	0.03	0.06	0.1	0.008
Phosphorus × Phytase		0.5	0.5	0.4	0.3	0.7	0.8	0.2	0.3	0.2
Block		0.06	0.6	0.3	0.8	0.5	0.7	0.7	0.7	0.6

[a] Values are means of composite samples from each of three replicate groups (n=10). Significance probability associated with the *P*-value.

[b] Pooled standard error.

IV.4. Discussion

IV.4.1 The effects of dietary MCP and phytase supplementation on the growth performance of cage-raised channel catfish

In Chapter II, I reported the positive effects of dietary phytase supplementation on growth performance of channel catfish when phytase was added to the diets containing adequate nutrients except available phosphorus. Such response has been observed not only in indoor trials (Jackson et al., 1996; Li & Robinson, 1997) but also pond trials (Robinson et al., 2002; Yan et al., 2002). In the current pond feeding trial, the basal diet had 1.03% of total P and 0.55% of available P, which exceeded the established P requirement (0.33% of available P of dry diet) for channel catfish reported by Wilson et al. (1982). Thus, I was not surprised that there were no significant differences between channel catfish fed the basal diet and other experimental diets. Similar results were also found in my indoor feeding trial with channel catfish presented in Chapter II, in which channel catfish fed the phytase-supplemented diets had similar growth performance compared to fish fed the diet containing sufficient available P due to MCP supplementation. In addition, Li et al. (2019) also reported no positive growth results in pond-raised hybrid catfish (♀ *Ictalurus punctatus* × ♂ *Ictalurus furcatus*), in which fish was fed a commercial basal diet containing adequate available P and the same basal diet superdosed with phytase. Although the average FE result (0.67) in my current study was lower than the average FE result (0.78) from my previous indoor channel catfish feeding trial, the average weight of fish I used in the indoor trial was only 8.02 ± 0.18 g (mean ± S.D.), much smaller than the fish we

raised in the present trial. In addition, according to the growth data reported by Robinson et al. (2002), who also conducted a channel catfish pond trial with average fish weight of 23 g, the average FE was 0.69. Thus, I believe that the FE results in the present feeding trial were reasonable. In brief, I believe the positive effects of dietary phytase supplementation on growth performance will not be noticed if fish are fed a diet with sufficient available P.

IV.4.2 The effect of dietary MCP and phytase supplementation on the utilization of various minerals of cage-raised channel catfish

The mechanisms associated with the negative effects of dietary phytate and positive effects of dietary phytase supplementation on mineral utilization on various fish species have been discussed and summarized in Chapters II and III. The main benefits of dietary phytase supplementation on the utilization of various minerals by different fish species were well described by Cao et al. (2007) and V Kumar et al. (2012), such as increasing the whole-body retention and bone concentration of various minerals, and the reduced excretion of some minerals, as well as increased apparent availability of several minerals. However, some of these results differed owing to fish species, phytase dosages, and dietary ingredients. However, only a few studies have discussed the potential interaction effects between dietary MCP and phytase supplementation on the mineral utilization on fish. In my channel catfish indoor trial, the significantly increased bone ash was only noticed between fish fed the negative basal diet and other experimental diets, but not between the fish fed the positive control diet with phosphorus

supplementation and the phytase-supplemented diets. In addition, in the present study, the interaction between dietary MCP and phytase supplementation was detected to significantly increase the bone ash concentration. Thus, I conclude that adding phytase to a diet containing adequate nutrients and available P had limited positive effects on the channel catfish bone ash concentration. Similar result of the interaction between dietary MCP and phytase supplementation was also reported by Sajjadi and Carter (2004a). In their case, the basal diet even had an inadequate available P level (0.446 %), according to the P requirement for Atlantic salmon (0.6%) established by Ketola (1975).

In the present study, although the interaction between dietary MCP and phytase supplementation was noticed to significantly improve bone Ca and P concentration, the whole-body Ca retention and Ca excretion results revealed differences between dietary MCP and phytase supplementation. Adding the extra MCP to diet did significantly increase the net Ca and P absorption of channel catfish, but not percentage wise. Pimentel-Rodrigues and Oliva-Teles (2007) reported that the utilization of minerals was maximized when fish are fed various minerals closed to their requirement, then it drops when the dietary mineral level exceeds metabolic requirements. On the other hand, dietary phytase supplementation directly increased the bioavailability of Ca and P to channel catfish by liberating the minerals bound to phytate and making them available to the fish. Thus, the extra MCP supplementation caused the fish to excrete surplus Ca and P to the water and thus may lead to a higher chance of eutrophication (Stewart et al., 1978).

In the current study, dietary phytase supplementation significantly increased the bone Al concentration and reduced Al excretion. However, dietary MCP supplementation significantly increased Al excretion of channel catfish. In addition, there was an interaction between dietary MCP and phytase supplementation which significantly reduced the whole-body Al retention. Previous research has shown that fish can absorb water-borne Al via the gill (Gensemer & Playle, 1999) and dietary Al through the GI tract (Yu et al., 2017). However, very limited study had been done regarding the interaction between Al and other minerals. According to my data, there was a negative interaction between dietary MCP supplementation and Al absorption. Thus, further research is needed to investigate the negative effect of extra MCP supplementation on Al utilization of channel catfish.

Either dietary MCP or phytase supplementation significantly increased the bone Cu concentration, but the interaction between them was not significant. However, only dietary phytase supplementation increased the whole-body Cu retention and reduced Cu excretion. According to the results from the current study, I am unable to explain how MCP supplementation increased bone Cu concentration while not increasing the whole-body Cu retention or decreasing the Cu excretion. Similar unexplainable results regarding the absorption of Cu in channel catfish and red drum were found in my indoor trials.

Although the interaction between dietary MCP and phytase supplementation significantly increased bone Fe concentration, channel catfish fed the MCP-supplemented diet excreted 30% more Fe than fish fed the diets without extra MCP. It had been reported in human that the extra

dietary Ca has an inhibitory effect on iron bioavailability (Whiting, 1995). In addition, in marine fish it had been reported that in order to excrete the high concentration of water-borne Ca via the intestine, bicarbonate ions are also secreted. And the bicarbonate ions may not only target Ca ion, but also bind Fe ion to form Fe carbonates, which increase the excretion of Fe ions (Wilson, 1999; Wilson et al., 2002). Thus, the extra Ca from MCP may directly or indirectly decrease Fe utilization of fish.

In my indoor channel catfish feeding trial, fish fed the diet containing extra MCP had the lowest Zn utilization, compared to fish fed the diet without extra MCP or the phytase-supplemented diets. Gatlin III and Phillips (1989) reported that high dietary phytate and 2% of supplemental dietary calcium significantly reduced Zn bone concentration of channel catfish. In the present trial, dietary phytase supplementation significantly increased the bone Zn concentration and reduced the Zn excretion, independent of dietary MCP level. However, an interaction between dietary MCP and phytase supplementation was found to significantly increase whole-body Zn retention. I assumed that the lack of a negative effect of extra MCP supplementation on Zn utilization in the current trial was because only 0.1% of MCP was supplemented, which did not increase dietary calcium concentration much compared to the basal diet.

The significant block effect was found in the whole-body retention of several minerals, such as Ca, Mg, Al, and Zn. Unlike the indoor feeding trial conducted in one culture system which can limit the variability throughout the whole system, the outdoor pond trial had many uncontrollable

environmental factors, such as temperature, and water quality. Thus, we used the pond as the block factor to reduce the unexplained variability.

In conclusion, neither the dietary MCP nor phytase supplementation increased the growth performance of cage-raised channel catfish, because the basal diet contained adequate available phosphorus. In addition, although limited benefits were found due to extra MCP supplementation, dietary phytase supplementation directly increased the utilization of some minerals. On the other hand, the extra MCP supplementation increased the net absorption of Ca and P but decreased their bioavailability and increased their excretion.

CHAPTER V

SUMMARY AND CONCLUSIONS

The rapidly growing aquaculture industry has increased the demand for fishmeal and consequently its price; therefore, more plant protein ingredients are being used as fishmeal alternatives in aquatic feeds. However, the high phytate concentration in these plant protein ingredients has caused many negative impacts, not only nutritional but also environmental as excess phosphorus in the aquatic environment increases eutrophication. Phytase, as the enzyme to degrade phytate into available phosphorus and inositol, has been more commonly used in terrestrial monogastrics such as poultry and swine but to a more limited extent in aquacultured organisms. Therefore, this dissertation designed a series of feeding trials to evaluate the effects of superdosing dietary phytase on growth, mineral utilization, and amino acid availability of different fish species in different environments.

Both indoor feeing trials showed that dietary phytase supplementation significantly increased the growth performance of channel catfish and red drum, when phytase was added to the diet containing sufficient levels of all nutrients except for available phosphorus. Besides that, dietary phytase supplementation significantly improved bone concentration, whole-body retention, and availability of various minerals, although slight differences were found between the two fish species. For example, dietary phosphorus deficiency affected lipid utilization of channel catfish, but no negative impact was noticed in red drum. In addition, higher concentrations of ash, Ca, Mg, P, Mn, and Zn were present in bone, and greater whole-body Ca,

Mg, P, Fe, Mn, and Zn retention was observed in catfish fed the phytase-supplemented diets compared to those fed the negative control. On the other hand, red drum given the phytase-supplemented diets had significantly enhanced utilization of all measured mineral ions, except for whole-body retention of Mn and bone deposition of Fe, compared to fish fed the negative control diet. Due to the high ion concentration in brackish water, it was difficult to investigate the availability of various minerals in different sections of the gastrointestinal tract of red drum. Additionally, in the channel catfish indoor feeding trial, the apparent digestibility coefficients of most indispensable and dispensable amino acids were improved by dietary phytase supplementation, except for histidine, methionine, threonine, and taurine. What's more, this improvement only happened in the posterior intestinal region. It was also noted that channel catfish fed the diet containing 4000 FTU/kg phytase had the highest postprandial plasma phosphorus level most of the time, except 24 h after feeding. Based on the polynomial regression analysis, the optimal dietary phytase dosage for both species was roughly 5500 FTU/kg of dry diet.

Another trial in which cage-raised channel catfish were fed experimental diets in ponds supported the positive effect of dietary phytase supplementation on growth of fish bit only if the diet did not contain sufficient available phosphorus. In addition, dietary phytase supplementation not only increased the bone concentration of various minerals but also the whole-body retention. in contrast, dietary moncalcium phosphate (MCP) supplementation only enhanced the net absorption of Ca and P. Also, the pond environment with natural productivity compared to the

indoor trial significantly impacted the whole-body retention of Ca, Mg, Al, and Zn, independent of the different diets.

In conclusion, dietary phytase supplementation was able to replace MCP supplementation when diets for channel catfish and red drum contained adequate levels of all nutrients except for available phosphorus. In addition, dietary phytase supplementation was able to significantly increase the utilization of various minerals and bioavailability of most amino acids, although the results varied in different fish species and environments.

REFERENCES

Abeliovich, A. (1969). Water blooms of blue green algae and oxygen regime in fish ponds: With 8 figures and 1 table in the text. *Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen*, *17*(1), 594-601.

Abrams, E., Lassiter, J., Miller, W., Neathery, M., Gentry, R., & Blackmon, D. (1977). Effect of normal and high manganese diets on the role of bile in manganese metabolism of calves. *Journal of Animal Science*, *45*(5), 1108-1113.

Andersen, Ø. (1997). Accumulation of waterborne iron and expression of ferritin and transferrin in early developmental stages of brown trout (*Salmo trutta*). *Fish Physiology and Biochemistry*, *16*(3), 223-231.

Angel, R., Tamim, N., Applegate, T., Dhandu, A., & Ellestad, L. (2002). Phytic acid chemistry: influence on phytin-phosphorus availability and phytase efficacy. *Journal of Applied Poultry Research*, *11*(4), 471-480.

Antony Jesu Prabhu, P., Schrama, J., & Kaushik, S. (2013). Quantifying dietary phosphorus requirement of fish–a meta-analytic approach. *Aquaculture Nutrition*, *19*(3), 233-249.

Antony Jesu Prabhu, P., Schrama, J. W., & Kaushik, S. J. (2016). Mineral requirements of fish: a systematic review. *Reviews in Aquaculture*, *8*(2), 172-219.

AOAC. (1990). Official methods of analysis. *Assoc Anal Chem*.

AOAC. (2005). Official methods of analysis of the Association of Analytical Chemists

International. In: AOAC Gaithersburg, MD.

Åsgård, T., & Shearer, K. (1997). Dietary phosphorus requirement of juvenile Atlantic salmon, *Salmo salar L. Aquaculture Nutrition*, *3*(1), 17-23.

Baeverfjord, G., Asgard, T., & Shearer, K. (1998). Development and detection of phosphorus deficiency in Atlantic salmon, *Salmo salar L.*, parr and post-smolts. *Aquaculture Nutrition*, *4*(1), 1-12.

Bakke, A. M., Glover, C., & Krogdahl, Å. (2010). Feeding, digestion and absorption of nutrients. *Fish physiology* (Vol. 30, pp. 57-110).

Bakke-McKellep, A. M., Nordrum, S., Krogdahl, Å., & Buddington, R. (2000). Absorption of glucose, amino acids, and dipeptides by the intestines of Atlantic salmon (*Salmo salar L.*). *Fish Physiology and Biochemistry*, *22*(1), 33-44.

Bansal, V. K. (1990). Serum inorganic phosphorus. *Clinical methods: The History, Physical, and Laboratory Examinations. 3rd edition.*

Baruah, K., Pal, A. K., Sahu, N. P., Jain, K. K., Mukherjee, S. C., & Debnath, D. (2005). Dietary protein level, microbial phytase, citric acid and their interactions on bone mineralization of Labeo rohita (*Hamilton*) juveniles. *Aquaculture Research*, *36*(8), 803-812.

Baruah, K., Sahu, N., Pal, A., & Debnath, D. (2004). Dietary phytase: an ideal approach for a cost effective and low-polluting aquafeed. *NAGA, WorldFish Center Quarterly*, *27*(3-4), 15-19.

Berg, A. (1968). Studies on the metabolism of calcium and strontium in freshwater fish I:

relative contribution of direct and intestinal absorption. *Mem. Ist. Ital. Idrobiol.*, *23*, 161-196.

Berntssen, M. H., Hylland, K., Bonga, S. E. W., & Maage, A. (1999). Toxic levels of dietary copper in Atlantic salmon (*Salmo salar L.*) parr. *Aquatic Toxicology*, *46*(2), 87-99.

Bijvelds, M. J., Flik, G., Kolar, Z. I., & Bonga, S. E. W. (1996). Uptake, distribution and excretion of magnesium in *Oreochromis mossambicus*: dependence on magnesium in diet and water. *Fish Physiology and Biochemistry*, *15*(4), 287-298.

Bisoyi, R., & Singh, P. (1988). Effect of phosphorus fertilization on blue-green algal inoculum production and nitrogen yield under field conditions. *Biology and fertility of soils*, *5*(4), 338-343.

Bohn, L., Meyer, A. S., & Rasmussen, S. K. (2008). Phytate: impact on environment and human nutrition. A challenge for molecular breeding. *Journal of Zhejiang University Science B*, *9*(3), 165-191.

Bucking, C., & Wood, C. M. (2006). Water dynamics in the digestive tract of the freshwater rainbow trout during the processing of a single meal. *Journal of Experimental Biology*, *209*(10), 1883-1893.

Bucking, C., & Wood, C. M. (2007). Gastrointestinal transport of Ca 2+ and Mg 2+ during the digestion of a single meal in the freshwater rainbow trout. *Journal of Comparative Physiology B*, *177*(3), 349-360.

Bury, N., & Grosell, M. (2003). Iron acquisition by teleost fish. *Comparative Biochemistry and*

Physiology Part C: Toxicology & Pharmacology, *135*(2), 97-105.

Bury, N. R., Grosell, M., Wood, C., Hogstrand, C., Wilson, R., Rankin, J. C., Busk, M., Lecklin, T., & Jensen, F. B. (2001). Intestinal iron uptake in the European flounder (*Platichthys flesus*). *Journal of Experimental Biology*, *204*(21), 3779-3787.

Bury, N. R., Walker, P. A., & Glover, C. N. (2003). Nutritive metal uptake in teleost fish. *Journal of Experimental Biology*, *206*(1), 11-23.

Caldwell, R. A. (1992). Effect of calcium and phytic acid on the activation of trypsinogen and the stability of trypsin. *Journal of Agricultural and Food Chemistry*, *40*(1), 43-46.

Camus, M.-C., Laporte, J., & Pocholle, P. (1976). Inhibition de la protéolyse pepsique in vitro par le blé. Rôle de l'acide phytique des issues. Annales de Biologie Animale Biochimie Biophysique.

Cao, L., Wang, W., Yang, C., Yang, Y., Diana, J., Yakupitiyage, A., Luo, Z., & Li, D. (2007). Application of microbial phytase in fish feed. *Enzyme and microbial technology*, *40*(4), 497-507.

Cao, L., Yang, Y., Wang, W., Yakupitiyage, A., Yuan, D., & Diana, J. (2008). Effects of pretreatment with microbial phytase on phosphorous utilization and growth performance of Nile tilapia (*Oreochromis niloticus*). *Aquaculture Nutrition*, *14*(2), 99-109.

Castillo, S., & Gatlin III, D. (2018). Dietary requirements for leucine, isoleucine and valine (branched-chain amino acids) by juvenile red drum *Sciaenops ocellatus*. *Aquaculture Nutrition*, *24*(3), 1056-1065.

Castillo, S., Halligan, S., & Gatlin III, D. M. (2015). Growth responses of juvenile red drum *Sciaenops ocellatus* to dietary phenylalanine and tyrosine can be used to calculate the total aromatic amino acid requirement. *The Journal of Nutrition*, *145*(10), 2341-2346.

Chamberlain, G., Miget, R., & Haby, M. (1990). *Red Drum Aquaculture*. *Texas A & M University Sea Grant College Program*.

Chang, Y.-T., Rosania, G. R., & Chung, S.-K. (2001). Inositol phospholipid pathway inhibitors and regulators Inositol phospholipid pathway inhibitors and regulators. *Expert Opinion on Therapeutic Patents*, *11*(1), 45-59.

Chen, A., Liu, X., Cui, C., Yang, C., Wang, Y., Bu, X., & Yang, Y. (2019). An evaluation of phytase for Channel catfish (*Ictalurus punctatus*) fed all plant-protein diet: Growth performance, nutrient utilization and P equivalency value. *Aquaculture Nutrition*, *25*(1), 215-224.

Chen, K., Yamamoto, F. Y., & Gatlin III, D. M. (2020). Effects of inorganic and organic dietary copper supplementation on growth performance and tissue composition for juvenile red drum (*Sciaenops ocellatus L.*). *Aquaculture Nutrition*, *26*(3), 820-827.

Chen, S., Chen, X., Peng, Y., & Peng, K. (2009). A mathematical model of the effect of nitrogen and phosphorus on the growth of blue-green algae population. *Applied Mathematical Modelling*, *33*(2), 1097-1106.

Cheng, Z. J., & Hardy, R. W. (2003). Effects of extrusion and expelling processing, and microbial phytase supplementation on apparent digestibility coefficients of nutrients in

full-fat soybeans for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *218*(1-4), 501-514.

Chowdhury, R. B., Moore, G. A., Weatherley, A. J., & Arora, M. (2017). Key sustainability challenges for the global phosphorus resource, their implications for global food security, and options for mitigation. *Journal of Cleaner Production*, *140*, 945-963.

Çoğun, H., Yüzereroğlu, T., Kargin, F., & Firat, Ö. (2005). Seasonal variation and tissue distribution of heavy metals in shrimp and fish species from the yumurtalik coast of iskenderun gulf, mediterranean. *Bulletin of Environmental Contamination & Toxicology*, *75*(4).

Correll, D. (1999). Phosphorus: a rate limiting nutrient in surface waters. *Poultry science*, *78*(5), 674-682.

Cowieson, A., Acamovic, T., & Bedford, M. (2004). The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. *British poultry science*, *45*(1), 101-108.

Cowieson, A., Wilcock, P., & Bedford, M. (2011). Super-dosing effects of phytase in poultry and other monogastrics. *World's Poultry Science Journal*, *67*(2), 225-236.

D'Mello, J. F., Duffus, C. M., & Duffus, J. H. (1991). Toxic substances in crop plants. *Woodhead Publishing*.

Davis, D. A., & Robinson, E. H. (1987). Dietary phosphorus requirement of juvenile red drum *Sciaenops ocellatus*. *Journal of the World Aquaculture Society*, *18*(3), 129-136.

De Cruz, C. R., Yamamoto, F. Y., Ju, M., Chen, K., Velasquez, A., & Gatlin III, D. M. (2020). Efficacy of purified nucleotide supplements on the growth performance and immunity of hybrid striped bass *Morone chrysops x Morone saxatilis*. *Fish & shellfish immunology*, *98*, 868-874.

De Groot, C., & Golterman, H. (1993). On the presence of organic phosphate in some Camargue sediments: evidence for the importance of phytate. *Hydrobiologia*, *252*(1), 117-126.

Debnath, D., Sahu, N. P., Pal, A. K., Jain, K. K., Yengkokpam, S., & Mukherjee, S. C. (2005). Mineral status of Pangasius pangasius (*Hamilton*) fingerlings in relation to supplemental phytase: absorption, whole-body and bone mineral content. *Aquaculture Research*, *36*(4), 326-335.

Denstadli, V., Skrede, A., Krogdahl, Å., Sahlstrøm, S., & Storebakken, T. (2006). Feed intake, growth, feed conversion, digestibility, enzyme activities and intestinal structure in Atlantic salmon (*Salmo salar L.*) fed graded levels of phytic acid. *Aquaculture*, *256*(1-4), 365-376.

Denstadli, V., Storebakken, T., Svihus, B., & Skrede, A. (2007). A comparison of online phytase pre-treatment of vegetable feed ingredients and phytase coating in diets for Atlantic salmon (*Salmo salar L.*) reared in cold water. *Aquaculture*, *269*(1-4), 414-426.

Dersjant-Li, Y., Awati, A., Schulze, H., & Partridge, G. (2015). Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. *Journal of the Science of Food and Agriculture*, *95*(5), 878-896.

Erdman, J. W. (1979). Oilseed phytates: nutritional implications. *Journal of the American Oil Chemists' Society*, *56*(8), 736-741.

Falconer, I. R. (1999). An overview of problems caused by toxic blue–green algae (*cyanobacteria*) in drinking and recreational water. *Environmental Toxicology: An International Journal*, *14*(1), 5-12.

Falconer, I. R. (2012). Algal toxins in seafood and drinking water. *Elsevier*.

Falconer, I. R., & Yeung, D. S. (1992). Cytoskeletal changes in hepatocytes induced by Microcystis toxins and their relation to hyperphosphorylation of cell proteins. *Chemico-biological interactions*, *81*(1-2), 181-196.

Fernandes, J., Horn, D., Ronconi, E., Buzim, R., Lima, F., & Pazdiora, D. (2019). Effects of phytase superdosing on digestibility and bone integrity of broilers. *Journal of Applied Poultry Research*, *28*(2), 390-398.

Flik, G., Van Der Velden, J. A., Dechering, K. J., Verbost, P. M., Schoenmakers, T. J., Kolar, Z. I., & Bonga, S. E. W. (1993). Ca2+ and Mg2+ transport in gills and gut of tilapia, Oreochromis mossambicus: a review. *Journal of Experimental Zoology*, *265*(4), 356-365.

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem*, *226*(1), 497-509.

Fonds, M., Cronie, R., Vethaak, A., & Van der Puyl, P. (1992). Metabolism, food consumption and growth of plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*) in relation to fish size and temperature. *Netherlands Journal of Sea Research*, *29*(1-3), 127-143.

Fontainhas-Fernandes, A., Gomes, E., Reis-Henriques, M. A., & Coimbra, J. (1999). Replacement of fish meal by plant proteins in the diet of Nile tilapia: digestibility and growth performance. *Aquaculture International*, *7*(1), 57-67.

Forster, I., Higgs, D. A., Dosanjh, B. S., Rowshandeli, M., & Parr, J. (1999). Potential for dietary phytase to improve the nutritive value of canola protein concentrate and decrease phosphorus output in rainbow trout (*Oncorhynchus mykiss*) held in 11 C fresh water. *Aquaculture*, *179*(1-4), 109-125.

Francis, G., Makkar, H. P., & Becker, K. (2001). Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture*, *199*(3-4), 197-227.

Furuya, W. M., Gonçalves, G. S., Furuya, V. R. B., & Hayashi, C. (2001). Phytase as feeding for Nile tilapia (*Oreochromis niloticus*). Performance and digestibility. *Revista Brasileira de Zootecnia*, *30*(3), 924-929.

Gatlin III, D. M. (2002). Red drum, *Sciaenops ocellatus*. *Nutrient requirements and feeding of finfish for aquaculture*, 147-158.

Gatlin III, D. M., & Phillips, H. F. (1989). Dietary calcium, phytate and zinc interactions in channel catfish. *Aquaculture*, *79*(1-4), 259-266.

Gatlin III, D. M., & Wilson, R. P. (1983). Dietary zinc requirement of fingerling channel catfish. *The Journal of Nutrition*, *113*(3), 630-635.

Gatlin III, D. M., & Wilson, R. P. (1984). Zinc supplementation of practical channel catfish diets. *Aquaculture*, *41*(1), 31-36.

Gatlin III, D. M., & Wilson, R. P. (1986). Dietary copper requirement of fingerling channel catfish. *Aquaculture*, *54*(4), 277-285.

Gensemer, R. W., & Playle, R. C. (1999). The bioavailability and toxicity of aluminum in aquatic environments. *Critical reviews in environmental science and technology*, *29*(4), 315-450.

Glover, C. N., Balesaria, S., Mayer, G. D., Thompson, E. D., Walsh, P. J., & Hogstrand, C. (2003). Intestinal zinc uptake in two marine teleosts, squirrelfish (*Holocentrus adscensionis*) and gulf toadfish (*Opsanus beta*). *Physiological and Biochemical Zoology*, *76*(3), 321-330.

Grosell, M., Hansen, H., & Rosenkilde, P. (1998). Cu uptake, metabolism and elimination in fed and starved European eels (*Anguilla anguilla*) during adaptation to water-borne Cu exposure. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, *120*(2), 295-305.

Grosell, M., Hogstrand, C., & Wood, C. (1997). Cu uptake and turnover in both Cu-acclimated and non-acclimated rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology*, *38*(4), 257-276.

Grosell, M., McGeer, J., & Wood, C. (2001). Plasma copper clearance and biliary copper excretion are stimulated in copper-acclimated trout. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *280*(3), R796-R806.

Grosell, M., O'donnell, M., & Wood, C. (2000). Hepatic versus gallbladder bile composition: in

vivo transport physiology of the gallbladder in rainbow trout. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *278*(6), R1674-R1684.

Guerreiro, P., Fuentes, J., Canario, A. V., & Power, D. (2002). Calcium balance in sea bream (*Sparus aurata*): the effect of oestradiol-17 beta. *Journal of Endocrinology*, *173*(2), 377-385.

Hallberg, L., Brune, M., Erlandsson, M., Sandberg, A.-S., & Rossander-Hulten, L. (1991). Calcium: effect of different amounts on nonheme-and heme-iron absorption in humans. *The American journal of clinical nutrition*, *53*(1), 112-119.

Handy, R. (1996). Dietary exposure to toxic metals in. *Toxicology of aquatic pollution: physiological, molecular and cellular approaches*, *57*, 29.

Hardy, R. W., & Shearer, K. D. (1985). Effect of dietary calcium phosphate and zinc supplementation on whole body zinc concentration of rainbow trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences*, *42*(1), 181-184.

Hardy, R. W., Sullivan, C. V., & Koziol, A. M. (1987). Absorption, body distribution, and excretion of dietary zinc by rainbow trout (*Salmo gairdneri*). *Fish Physiology and Biochemistry*, *3*(3), 133-143.

Haslewood, G. A. D. (1967). Bile salts. *Angewandte Chemie*, *80*(8), 328-328.

Hemstreet, B. (2010). An update on Aeromonas hydrophila from a fish health specialist for summer 2010. *Catfish J*, *24*(4).

Herbes, S. E., Allen, H. E., & Mancy, K. H. (1975). Enzymatic characterization of soluble

organic phosphorus in lake water. *Science*, *187*(4175), 432-434.

Hickman Jr, C. P. (1968). Ingestion, intestinal absorption, and elimination of seawater and salts in the southern flounder, *Paralichthys lethostigma*. *Canadian journal of zoology*, *46*(3), 457-466.

Honda, K., Sahrul, M., Hidaka, H., & Tatsukawa, R. (1983). Organ and tissue distribution of heavy metals, and their growth-related changes in Antarctic fish, *Pagothenia borchgrevinki*. *Agricultural and Biological Chemistry*, *47*(11), 2521-2532.

Hossain, M. J., Sun, D., McGarey, D. J., Wrenn, S., Alexander, L. M., Martino, M. E., Xing, Y., Terhune, J. S., & Liles, M. R. (2014). An Asian origin of virulent Aeromonas hydrophila responsible for disease epidemics in United States-farmed catfish. *MBio*, *5*(3).

Hossain, M. S., Chance, A. B., El Kertaoui, N., Wattiez, X., Houndji, A., Mandiki, S. N., & Kestemont, P. (2020). Dietary inorganic monophosphates in high plant ingredient-based diets influence nutrient digestibility, postprandial macro-mineral status and immune functions of juvenile rainbow trout, *Oncorhynchus mykiss*. *Aquaculture Nutrition*, *26*(6), 2178-2194.

Huang, Y., Ciais, P., Goll, D. S., Sardans, J., Peñuelas, J., Cresto-Aleina, F., & Zhang, H. (2020). The shift of phosphorus transfers in global fisheries and aquaculture. *Nature communications*, *11*(1), 1-10.

Humpage, A., Rositano, J., Bretag, A., Brown, R., Baker, P., Nicholson, B., & Steffensen, D. (1994). Paralytic shellfish poisons from Australian cyanobacterial blooms. *Marine and*

Freshwater Research, *45*(5), 761-771.

Hussain, S., Afzal, M., Nasir, S., Javid, A., Makhdoom, S., Jabeen, F., Azmat, H., Hussain, M., & Shah, S. (2016). Efficacy of phytase supplementation in improving mineral digestibility in *Labeo rohita* fingerlings fed on canola meal-based diets. *Iranian Journal of Fisheries Sciences*, *15*(2), 645-661.

Hussain, S. M., Shahzad, M. M., Afzal, M., Javid, A., Mubarik, M. S., Shah, S. Z. H., Hussain, M., Ahmad, S., Arsalan, M., & Manzoor, R. (2015). Efficacy of phytase enzyme for increasing mineral digestibility of Cirrhinus mrigala fingerlings fed on soybean meal-based diet. *Pakistan J. Zool*, *47*(6), 1807-1816.

Ichii, T., & Mugiya, Y. (1983). Effects of a dietary deficiency in calcium on growth and calcium uptake from the aquatic environment in the goldfish, *Carassius auratus*. *Comparative biochemistry and physiology. A, Comparative physiology*, *74*(2), 259-262.

Izzo, J. L. (1956). Interrelationships of glucose and inorganic phosphorus in blood and urine of patients with diabetes mellitus. *Proceedings of the Society for Experimental Biology and Medicine*, *91*(3), 373-377.

Jackson, L. S., Li, M. H., & Robinson, E. H. (1996). Use of Microbial Phytase in Channel Catfish *Ictalurus punctatus* Diets to Improve Utilization of Phytate Phosphorus 1. *Journal of the World Aquaculture Society*, *27*(3), 309-313.

Janda, J. M., & Abbott, S. L. (2010). The genus Aeromonas: taxonomy, pathogenicity, and infection. *Clinical microbiology reviews*, *23*(1), 35-73.

Joseph, S. W., & Carnahan, A. (1994). The isolation, identification, and systematics of the motile Aeromonas species. *Annual Review of Fish Diseases*, *4*, 315-343.

Kao, C. (1993). Algal toxins in seafood and drinking water. *Falconer, IR, Ed*, 75-86.

Ketola, H. (1975). Requirement of Atlantic salmon for dietary phosphorus. *Transactions of the American Fisheries Society*, *104*(3), 548-551.

Kornegay, E., & Qian, H. (1996). Replacement of inorganic phosphorus by microbial phytase for young pigs fed on a maiz–soyabean-meal diet. *British Journal of Nutrition*, *76*(4), 563-578.

Kumar, V., Sinha, A., Makkar, H., De Boeck, G., & Becker, K. (2012). Phytate and phytase in fish nutrition. *Journal of animal physiology and animal nutrition*, *96*(3), 335-364.

Kwong, R. W., & Niyogi, S. (2009). The interactions of iron with other divalent metals in the intestinal tract of a freshwater teleost, rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *150*(4), 442-449.

Laining, A., Traifalgar, R. F., Thu, M., Komilus, C. F., Kader, M. A., Koshio, S., Ishikawa, M., & Yokoyama, S. (2010). Influence of dietary phytic acid on growth, feed intake, and nutrient utilization in juvenile Japanese flounder, *Paralichthys olivaceus*. *Journal of the World Aquaculture Society*, *41*(5), 746-755.

Lall, S. (1991). Digestibility, metabolism and excretion of dietary phosphorus in fish. *Nutritional Strategies and Aquaculture Waste. First International Symposium on Nutritional*

Strategies in Management of Aquatic Wastes. *University of Guelph, Guelph, Ontario*. 1991.

Lall, S. P. (2003). The minerals. In *Fish nutrition*, 259-308.

Lanari, D., D'agaro, E., & Turri, C. (1998). Use of nonlinear regression to evaluate the effects of phytase enzyme treatment of plant protein diets for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *161*(1-4), 345-356.

Lee, S. A., Lupatsch, I., Gomes, G. A., & Bedford, M. R. (2020). An advanced Escherichia coli phytase improves performance and retention of phosphorus and nitrogen in rainbow trout (*Oncorhynchus mykiss*) fed low phosphorus plant-based diets, at 11° C and 15° C. *Aquaculture*, *516*, 734549.

Leger, L. (1910). Le gout de vase chez les poissons d'eau douce. *Trav*. *Lab*. *Piscicult*. *Univ*. *Grenoble*, *2*, 1-4.

Lemos, D., & Tacon, A. G. (2017). Use of phytases in fish and shrimp feeds: a review. *Reviews in Aquaculture*, *9*(3), 266-282.

Li, M. H., Manning, B., & Robinson, E. H. (2004). A Summary of phytase studies for channel carfish. MISSISSIPPI, Estados Unidos : EXPERIMENT STATION, *23*(13), 5p.

Li, M. H., & Robinson, E. H. (1997). Microbial Phytase Can Replace Inorganic Phosphorus Supplements in Channel Catfish *Ictalurus punctatus* Diets 1. *Journal of the World Aquaculture Society*, *28*(4), 402-406.

Li, M. H., Wise, D. J., Mischke, C. C., Aarattuthodiyil, S., Tiwari, A., Lucas, P. M., Tucker, C.

S., Torrans, E. L., & Perera, T. (2019). Pond-raised Hybrid Catfish, ♀ *Ictalurus punctatus*× ♂ *Ictalurus furcatus*, Do Not Respond to Microbial Phytase Superdosing. *Journal of the World Aquaculture Society*, *50*(1), 78-86.

Li, S., Pang, M., Dong, Y., Liu, J., Yang, Y., Lu, C., & Liu, Y. (2018). Effects of myo-inositol, sialic acid and L-fucose metabolic pathways on pathogenicity of Aeromonas hydrophila strain NJ-35. *Acta Microbiol. Sin*, *58*, 1465-1474.

Lie, G., Li, X., & Qiao, Z. (1999). Application of phytase in feed industry. *Journal of Guangdong Feed*, *12*, 36-38.

Liebert, F., & Portz, L. (2005). Nutrient utilization of Nile tilapia Oreochromis niloticus fed plant based low phosphorus diets supplemented with graded levels of different sources of microbial phytase. *Aquaculture*, *248*(1-4), 111-119.

Liener, I. (1989). Antinutritional factors in legume seeds: state of the art. *Recent advances of research in antinutritional factors in legume seeds*, 6-13.

Lin, Y.-H., Ku, C.-Y., & Shiau, S.-Y. (2013). Estimation of dietary magnesium requirements of juvenile tilapia, *Oreochromis niloticus*× *Oreochromis aureus*, reared in freshwater and seawater. *Aquaculture*, *380*, 47-51.

Liu, L., Liang, X.-F., Li, J., Yuan, X., Zhou, Y., & He, Y. (2014). Feed intake, feed utilization and feeding-related gene expression response to dietary phytic acid for juvenile grass carp (*Ctenopharyngodon idellus*). *Aquaculture*, *424*, 201-206.

Maetz, J. (1971). Fish gills: mechanisms of salt transfer in fresh water and sea water.

Philosophical Transactions of the Royal Society of London. B, Biological Sciences, *262*(842), 209-249.

Michael, F. R., & Koshio, S. (2008). Biochemical studies on the interactive effects of dietary choline and inositol in juvenile Kuruma shrimp, *Marsupenaeus japonicus* Bate. *Aquaculture*, *285*(1-4), 179-183.

Morales, G. A., Denstadli, V., Collins, S., Mydland, L., Moyano, F., & Øverland, M. (2016). Phytase and sodium diformate supplementation in a plant-based diet improves protein and mineral utilization in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition*, *22*(6), 1301-1311.

Morales, G. A., Márquez, L., Saenz de Rodrigañez, M., Bermúdez, L., Robles, R., & Moyano, F. (2014). Effect of phytase supplementation of a plant-based diet on phosphorus and nitrogen bioavailability in sea bream *Sparus aurata*. *Aquaculture Nutrition*, *20*(2), 172-182.

Morales, G. A., Moyano, F. J., & Marquez, L. (2011). In vitro assessment of the effects of phytate and phytase on nitrogen and phosphorus bioaccessibility within fish digestive tract. *Animal Feed Science and Technology*, *170*(3-4), 209-221.

Nadella, S. R., Bucking, C., Grosell, M., & Wood, C. M. (2006). Gastrointestinal assimilation of Cu during digestion of a single meal in the freshwater rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *143*(4), 394-401.

Noureddini, H., & Dang, J. (2009). Degradation of phytates in distillers' grains and corn gluten feed by Aspergillus niger phytase. *Applied biochemistry and biotechnology*, *159*(1), 11-23.

NRC, 2011. Nutrient Requirements of Fish and Shrimp, National Research Council. The National Academies Press, Washington, D.C. 376 pp.

Nwanna, L. (2005). Toasted Soybean Meal Based Diets Supplemented with Phytase Enhanced Growth and Reduced Faecal Mineral Deposition by Nile Tilapia. *Journal of Food Technology*, *3*(2), 232-237.

Nwanna, L., Eisenreich, R., & Schwarz, F. (2007). Effect of wet-incubation of dietary plant feedstuffs with phytases on growth and mineral digestibility by common carp (*Cyprinus carpio L*). *Aquaculture*, *271*(1-4), 461-468.

Nwanna, L., Fagbenro, O., & Adeyo, A. (2005). Effects of different treatments of dietary soybean meal and phytase on the growth and mineral deposition in African catfish *Clarias gariepinus*. *J. Anim. Vet. Adv*, *4*, 980-987.

Olugbenga, O., Falaye, A. E., & Kareem, O. K. (2017). Effect of phytase supplementation on the growth, mineral composition and phosphorus digestibility of African catfish (*Clarias gariepinus*) juveniles. *Animal Research International*, *14*(2), 2741–2750-2741–2750.

Pang, M., Jiang, J., Xie, X., Wu, Y., Dong, Y., Kwok, A. H., Zhang, W., Yao, H., Lu, C., & Leung, F. C. (2015). Novel insights into the pathogenicity of epidemic Aeromonas hydrophila ST251 clones from comparative genomics. *Scientific Reports*, *5*(1), 1-15.

Papatryphon, E., Howell, R. A., & Soares Jr, J. H. (1999). Growth and mineral absorption by striped bass *Morone saxatilis* fed a plant feedstuff based diet supplemented with phytase. *Journal of the World Aquaculture Society*, *30*(2), 161-173.

Papatryphon, E., & Soares, J. (2001). The effect of phytase on apparent digestibility of four practical plant feedstuffs fed to striped bass, *Morone saxatilis*. *Aquaculture Nutrition*, *7*(3), 161-168.

Perry, S., & Wood, C. (1985). Kinetics of branchial calcium uptake in the rainbow trout: effects of acclimation to various external calcium levels. *Journal of Experimental Biology*, *116*(1), 411-433.

Pimentel-Rodrigues, A., & Oliva-Teles, A. (2007). Phosphorus availability of inorganic phosphates and fish meals in European sea bass (*Dicentrarchus labrax L.*) juveniles. *Aquaculture*, *267*(1-4), 300-307.

Pimentel-Rodrigues, A., & Oliva-Teles, A. (2001). Phosphorus requirements of gilthead sea bream (*Sparus aurata L.*) juveniles. *Aquaculture Research*, *32*, 157-161.

Prabhu, P. A. J., Schrama, J., Mariojouls, C., Godin, S., Fontagne-Dicharry, S., Geurden, I., Surget, A., Bouyssiere, B., & Kaushik, S. (2014). Post-prandial changes in plasma mineral levels in rainbow trout fed a complete plant ingredient based diet and the effect of supplemental di-calcium phosphate. *Aquaculture*, *430*, 34-43.

Rajendran, S., & Prakash, V. (1993). Kinetics and thermodynamics of the mechanism of interaction of sodium phytate with. alpha.-globulin. *Biochemistry*, *32*(13), 3474-3478.

Riche, M., Trottier, N., Ku, P., & Garling, D. (2001). Apparent digestibility of crude protein and apparent availability of individual amino acids in tilapia (*Oreochromis niloticus*) fed phytase pretreated soybean meal diets. *Fish Physiology and Biochemistry*, *25*(3), 181.

Robinson, E. H., Li, M. H., & Manning, B. B. (2002). Comparison of microbial phytase and dicalcium phosphate for growth and bone mineralization of pond-raised channel catfish, *Ictalurus punctatus*. *Journal of Applied Aquaculture*, *12*(3), 81-88.

Rodehutscord, M., Gregus, Z., & Pfeffer, E. (2000). Effect of phosphorus intake on faecal and non-faecal phosphorus excretion in rainbow trout (*Oncorhynchus mykiss*) and the consequences for comparative phosphorus availability studies. *Aquaculture*, *188*(3-4), 383-398.

Roeder, M., & Roeder, R. H. (1966). Effect of iron on the growth rate of fishes. *The Journal of Nutrition*, *90*(1), 86-90.

Sajjadi, M., & Carter, C. (2004a). Dietary phytase supplementation and the utilisation of phosphorus by Atlantic salmon (*Salmo salar L.*) fed a canola-meal-based diet. *Aquaculture*, *240*(1-4), 417-431.

Sajjadi, M., & Carter, C. (2004b). Effect of phytic acid and phytase on feed intake, growth, digestibility and trypsin activity in Atlantic salmon (*Salmo salar, L.*). *Aquaculture Nutrition*, *10*(2), 135-142.

Sakamoto, S. & Yone Y. (1979). Requirement of red sea bream for dietary trace elements. *Bulletin of the Japanese Society of Scientific Fisheries*, 44(12), 1341-1344.

Sampath, W., Rathnayake, R., Yang, M., Zhang, W., & Mai, K. (2020). Roles of dietary taurine in fish nutrition. *Marine Life Science & Technology*, 2, 360-375.

Satoh, S., Poe, W. E., & Wilson, R. P. (1989). Effect of supplemental phytate and/or tricalcium phosphate on weight gain, feed efficiency and zinc content in vertebrae of channel catfish. *Aquaculture*, *80*(1-2), 155-161.

Savolainen, L. C., & Gatlin III, D. M. (2010). Evaluation of sulfur amino acid and zinc supplements to soybean-meal-based diets for hybrid striped bass. *Aquaculture*, *307*(3-4), 260-265.

Schäfer, A., Koppe, W., Meyer-Burgdorff, K., & Günther, K. (1995). Effects of a microbial phytase on the utilization of native phosphorus by carp in a diet based on soybean meal. *Water Science and technology*, *31*(10), 149.

Schindler, D. W. (1977). Evolution of phosphorus limitation in lakes. *Science*, *195*(4275), 260-262.

Schoenmakers, T. J., Verbost, P., Flik, G., & Bonga, S. W. (1993). Transcellular intestinal calcium transport in freshwater and seawater fish and its dependence on sodium/calcium exchange. *Journal of Experimental Biology*, *176*(1), 195-206.

Segner, H., & Storch, V. (1985). Influence of water-borne iron on the liver of *Poecilia reticulata* (Peters 1859). *Journal of Applied Ichthyology*, *1*(1), 39-47.

Selle, P., Ravindran, V., Caldwell, A., & Bryden, W. (2000). Phytate and phytase: consequences for protein utilisation. *Nutrition Research Reviews*, *13*(2), 255-278.

Selle, P., Ravindran, V., Cowieson, A., & Bedford, M. (2010). Phytate and phytase. *Enzymes in farm animal nutrition. 2nd ed. Wallingford (UK): Centre for Agriculture and Biosciences International*, 160-205.

Shearer, K. D. (1995). The use of factorial modeling to determine the dietary requirements for essential elements in fishes. *Aquaculture*, *133*(1), 57-72.

Shearer, K. D., & Åsgård, T. (1992). The effect of water-borne magnesium on the dietary magnesium requirement of the rainbow trout (*Oncorhynchus mykiss*). *Fish Physiology and Biochemistry*, *9*(5-6), 387-392.

Shears, M., & Fletcher, G. (1983). Regulation of Zn2+ uptake from the gastrointestinal tract of a marine teleost, the winter flounder (*Pseudopleuronectes americanus*). *Canadian Journal of Fisheries and Aquatic Sciences*, *40*(S2), s197-s205.

Shiau, S.-Y., & Su, S.-L. (2005). Juvenile tilapia (*Oreochromis niloticus*× *Oreochromis aureus*) requires dietary myo-inositol for maximal growth. *Aquaculture*, *243*(1-4), 273-277.

SIMKISS, K. (1974). Calcium metabolism of fish in relation to ageing. *Ageing of fish*.

Sire, M. F., & Vernier, J.-M. (1992). Intestinal absorption of protein in teleost fish. *Comparative Biochemistry and Physiology Part A: Physiology*, *103*(4), 771-781.

Spry, D., Hodson, P., & Wood, C. (1988). Relative contributions of dietary and waterborne zinc in the rainbow trout, *Salmo gairdneri*. *Canadian Journal of Fisheries and Aquatic Sciences*, *45*(1), 32-41.

Spry, D. J., & Wood, C. M. (1985). Ion flux rates, acid–base status, and blood gases in rainbow

trout, *Salmo gairdneri*, exposed to toxic zinc in natural soft water. *Canadian Journal of Fisheries and Aquatic Sciences*, *42*(8), 1332-1341.

Srivastava, A., & Agrawal, S. (1983). Changes induced by manganese in fish testis. *Experientia*, *39*(11), 1309-1310.

Stewart, W., Pemble, M., & Al-Ugaily, L. (1978). Nitrogen and phosphorus storage and utilization in blue-green algae: With 4 plates, 14 figures and 3 tables in the text. *Internationale Vereinigung für Theoretische und Angewandte Limnologie: Mitteilungen*, *21*(1), 224-247.

Storebakken, T., Shearer, K., & Roem, A. (1998). Availability of protein, phosphorus and other elements in fish meal, soy-protein concentrate and phytase-treated soy-protein-concentrate-based diets to Atlantic salmon, *Salmo salar*. *Aquaculture*, *161*(1-4), 365-379.

Sugiura, S., Dong, F., & Hardy, R. (2000). Primary responses of rainbow trout to dietary phosphorus concentrations. *Aquaculture Nutrition*, *6*(4), 235-245.

Sugiura, S., Gabaudan, J., Dong, F., & Hardy, R. (2001). Dietary microbial phytase supplementation and the utilization of phosphorus, trace minerals and protein by rainbow trout [*Oncorhynchus mykiss (Walbaum)*] fed soybean meal-based diets. *Aquaculture Research*, *32*(7), 583-592.

Sugiura, S., Hardy, R., & Roberts, R. (2004). The pathology of phosphorus deficiency in fish–a review. *Journal of fish diseases*, *27*(5), 255-265.

Sugiura, S., Higashitani, A., & Sasaki, T. (2011). Effects of dietary phosphorus restriction on

fillet fat deposition and hepatic lipid metabolism in rainbow trout (*Oncorhynchus mykiss*) and crucian carp (*Carassius auratus grandoculis*). *Aquaculture Science*, *59*(1), 109-122.

Tiemeier, O., & Deyoe, C. (1973). Digestion and gut evacuation by channel catfish given pelleted feeds. *Transactions of the Kansas Academy of Science (1903)*, 254-260.

Topic Popovic, N., Strunjak-Perovic, I., Coz-Rakovac, R., Barisic, J., Jadan, M., Persin Berakovic, A., & Sauerborn Klobucar, R. (2012). Tricaine methane-sulfonate (MS-222) application in fish anaesthesia. *Journal of Applied Ichthyology*, *28*(4), 553-564.

Tovar, A., Moreno, C., Mánuel-Vez, M. P., & Garcı´a-Vargas, M. (2000). Environmental impacts of intensive aquaculture in marine waters. *Water Research*, *34*(1), 334-342.

Tucker, C. S. (2000). Off-flavor problems in aquaculture. *Reviews in Fisheries Science*, *8*(1), 45-88.

Urbano, G., Lopez-Jurado, M., Aranda, P., Vidal-Valverde, C., Tenorio, E., & Porres, J. (2000). The role of phytic acid in legumes: antinutrient or beneficial function? *Journal of physiology and biochemistry*, *56*(3), 283-294.

Vandenberg, G., & De La Noüe, J. (2001). Apparent digestibility comparison in rainbow trout (*Oncorhynchus mykiss*) assessed using three methods of faeces collection and three digestibility markers. *Aquaculture Nutrition*, *7*(4), 237-245.

Vandenberg, G., Scott, S., & De La Noüe, J. (2012). Factors affecting nutrient digestibility in rainbow trout (*Oncorhynchus mykiss*) fed a plant protein–based diet supplemented with microbial phytase. *Aquaculture Nutrition*, *18*(4), 369-379.

Vandenberg, G. W., & Koko, G. K. D. (2006). Phosphorus and feeding fish: issues and perspectives. Aquaculture Canada OM 2004–Proceedings of Contributed Papers–Canadian Freshwater Aquaculture Symposium. Part of the 21st Annual Meeting of the Aquaculture Association of Canada, Québec City, Québec, October 17-20, 2004. OM Aquaculture Canada is an Official Mark of the Aquaculture Association of Canada and may not be. 2006: 53

Vielma, J., Lall, S. P., Koskela, J., Schöner, F.-J., & Mattila, P. (1998). Effects of dietary phytase and cholecalciferol on phosphorus bioavailability in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *163*(3-4), 309-323.

Vielma, J., Mäkinen, T., Ekholm, P., & Koskela, J. (2000). Influence of dietary soy and phytase levels on performance and body composition of large rainbow trout (*Oncorhynchus mykiss*) and algal availability of phosphorus load. *Aquaculture*, *183*(3-4), 349-362.

Vielma, J., Ruohonen, K., Gabaudan, J., & Vogel, K. (2004). Top-spraying soybean meal-based diets with phytase improves protein and mineral digestibilities but not lysine utilization in rainbow trout, *Oncorhynchus mykiss (Walbaum)*. *Aquaculture Research*, *35*(10), 955-964.

Wei, Y., Liang, M., Xu, H., & Zheng, K. (2019). Taurine alone or in combination with fish protein hydrolysate affects growth performance, taurine transport and metabolism in juvenile turbot (*Scophthalmus maximus L.*). *Aquaculture Nutrition*, *25*(2), 396-405.

Whiting, S. J. (1995). The inhibitory effect of dietary calcium on iron bioavailability: a cause for

concern? *Nutrition reviews*, *53*(3), 77-80.

Wilson, R. (1999). A novel role for the gut of seawater teleosts in acid-base balance. Seminar Series-Society for Experimental Biology,

Wilson, R. P., Robinson, E. H., Gatlin III, D. M., & Poe, W. E. (1982). Dietary phosphorus requirement of channel catfish. *The Journal of Nutrition*, *112*(6), 1197-1202.

Wilson, R. W., Wilson, J. M., & Grosell, M. (2002). Intestinal bicarbonate secretion by marine teleost fish—why and how? *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1566*(1-2), 182-193.

Wise, A. (1983). Dietary factors determining the biological activities of phytate. Nutrition Abstracts and Reviews (series A), 1983, 53: 791-806

Woyengo, T., & Nyachoti, C. (2013). anti-nutritional effects of phytic acid in diets for pigs and poultry–current knowledge and directions for future research. *Canadian Journal of Animal Science*, *93*(1), 9-21.

Woyengo, T., & Wilson, J. (2019). Enhancing nutrient utilization in maize for broiler chickens by superdosing phytase. *Animal Feed Science and Technology*, *252*, 34-40.

Wu, R. (1995). The environmental impact of marine fish culture: towards a sustainable future. *Marine pollution bulletin*, *31*(4-12), 159-166.

Wu, X., & Gatlin III, D. M. (2014). Effects of altering dietary protein content in morning and evening feedings on growth and ammonia excretion of red drum (*Sciaenops ocellatus*). *Aquaculture*, *434*, 33-37.

Wurts, W. A. (1998). Why can some fish live in freshwater, some in salt water, and some in both. *World Aquaculture*, *29*(1), 65.

Wyss, M., Brugger, R., Kronenberger, A., Rémy, R., Fimbel, R., Oesterhelt, G., Lehmann, M., & van Loon, A. P. (1999). Biochemical characterization of fungal phytases (myo-inositol hexakisphosphate phosphohydrolases): catalytic properties. *Appl. Environ. Microbiol.*, *65*(2), 367-373.

Yan, W., Reigh, R. C., & Xu, Z. (2002). Effects of fungal phytase on utilization of dietary protein and minerals, and dephosphorylation of phytic acid in the alimentary tract of channel catfish *Ictalurus punctatus* fed an all-plant-protein diet. *Journal of the World Aquaculture Society*, *33*(1), 10-22.

Yang, Y.-H., Wang, Y.-Y., Lu, Y., & Li, Q.-Z. (2011). Effect of replacing fish meal with soybean meal on growth, feed utilization and nitrogen and phosphorus excretion on rainbow trout (*Oncorhynchus mykiss*). *Aquaculture International*, *19*(3), 405-419.

Yoo, G.-Y., Wang, X., Choi, S., Han, K., Kang, J.-C., & Bai, S. C. (2005). Dietary microbial phytase increased the phosphorus digestibility in juvenile Korean rockfish *Sebastes schlegeli* fed diets containing soybean meal. *Aquaculture*, *243*(1-4), 315-322.

Yu, F. (2000). Effects of supplemental phytase on growth and the utilization of phosphorus by crucian carp *Carassius carassius*. *J. Fish Sci. Chin.*, *7*, 106-109.

Yu, L., Zhai, Q., Zhu, J., Zhang, C., Li, T., Liu, X., Zhao, J., Zhang, H., Tian, F., & Chen, W. (2017). Dietary Lactobacillus plantarum supplementation enhances growth performance

and alleviates aluminum toxicity in tilapia. *Ecotoxicology and environmental safety*, *143*, 307-314.

Yu, S., Cowieson, A., Gilbert, C., Plumstead, P., & Dalsgaard, S. (2012). Interactions of phytate and myo-inositol phosphate esters (IP1-5) including IP5 isomers with dietary protein and iron and inhibition of pepsin. *Journal of Animal Science*, *90*(6), 1824-1832.

Zhang, X., Yang, W., Li, T., & Li, A. (2013). The genetic diversity and virulence characteristics of Aeromonas hydrophila isolated from fishponds with disease outbreaks in Hubei province. *Acta Hydrobiologica Sinica*, *37*(3), 458-466.

Zhu, W., Zhong, G., Hua, X., Ju, M., Chen, X., & Du, Z. (2016). The optimum active site of acidic phytase in the gastrointestinal tract of channel catfish (*Ictalurus punctatus*): in vitro and in vivo studies. *Aquaculture Nutrition*, *22*(4), 881-889.