Norwegian University of Life Sciences
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The Effect of Dietary Phytase Supplementation and Incubation in Soy Protein Concentrate based diet Fed to Nile Tilapia

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Master Thesis in Feed Manufacturing Technology

(30 credits)

By

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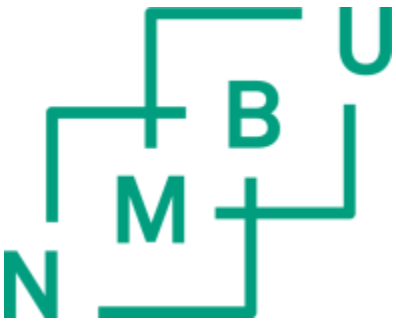

Contents

Abstract

Aquatic feed require high quality, low cost nutrients with increasing aquaculture production. Tilapia has become the third most important cultured fish species in the world, just after salmonids and carps. Soybean and its products are the most popular source of plant protein in compound aquatic feeds. In the existing plant protein sources phytate-P absorption and digestion is low in Nile Tilapia.

This experiment aimed to investigate the different effects on retention and utilization of minerals and growth performance between phytase supplemented feeds in the SPC-base diets and without phytase SPC-base diet for three different size groups of Nile tilapia. Three diets for Nile tilapia were prepared with 100% plant protein concentrate. Diet 1 was made without phytase as control group. In Diet 2, 500mg phytase was added per kilogram of soy protein concentrate in the feed. Incubated Phytase was added in Diet 3. Each diet contained 100 mg Y_2O_3 kg^{-1} for determining apparent digestibility.

In this experiment three different sizes of Nile tilapia with different numbers and same biomass in each tank were reared. Diet 3, with incubated phytase had not shown significant difference in growth performance, weight gain, feed intake and minerals retention except Zinc. Pretreated phytase decreased the mineral concentration in the fish body, plasma and feces. Pretreated Phytase increased the utilization and retention of minerals in Nile Tilapia during digestion and enhanced the apparent digestibility of minerals.

The optimum dose of phytase in Nile tilapia feed is not determined. There are many factors which can affect the availability of phytase in Nile tilapia in the feed processing and production.

Abbreviations

FAO = Food and Agriculture Organization

NSPs = Non-starch polysaccharides

Fe = ions

NSPase = Non-starch polysaccharidase

Zn = zinc

NRC = National Research Council

Cu = copper

Cd = Cadmium

P = Phosphorus

Al = Aluminum

g = gram

U / FTU / FYT = unit of phytase activity

SPC = soya protein concentrate

USSEC = United State Soybean Export Council

Fig. = Figure

AD = Apparent digestibility

MCP = Mono calcium phosphate

Y_2O_3 = Yttrium oxide

cm = centimeter

mmol = millimole

mg = milligram

MS-222 = Tricaine methanesulfonate

NMBU = Norwegian university life sciences

CO_2 = Carbon dioxide

IHA = Department of Aquaculture and Animal

mbar = millibar

EEC = European Economic Community

Mn = Manganese

ICP-MS = inductively coupled plasma mass spectroscopy

ASE = Accelerated Solvent Extractor

FCR = Feed conversion rate

ANF = Anti-nutritional factors

DM = Dry matter

FM = Fish meal

SGR = Specific growth rate

FI = Feed intake

Mg = Magnesium

FBW = finial body weight

IBW = initial body weight

s.e.m = standard error of the mean

GLM = Generalized linear model

SAS = Statistical Analysis System

kg = kilogram

MJ = mega joules

List of Figures

List of Tables

Acknowledgement

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1. Introduction

1.1 Aquatic Feed

Aquatic feeds are highly different for omnivorous species of fish (for example Tilapias, catfish, common carp, and milkfish), carnivorous fishes (such as salmon, trout, sea bass, yellowtail and Tuna) and crustacean species (marine and brackish-water shrimps, freshwater prawns, crabs and lobsters) (FAO, 2012). In 2008, the total aquaculture production of the world was 68.8 million tons, 52.9 million tons was aquatic animals and 15.9 tons was aquatic plants (Bene, 2010). According to the FAO statistics, around 46.1% of total world aquaculture production including aquatic plants (31.7 million tons) of fish and crustaceans was based on feed to animals in 2008, either farmed produced or factory manufactured feeds (Mora, 2009).

1.2 Tilapia

Generally, tilapias are divided into two genera: *Tilapia*, are macrophagous and substrate-spawners. *Oreochromis* are microphages and mouth-breeders (John, 2002). Tilapia has become increasingly important in the world's aquaculture, and has become the third most important cultured fish species in the world, just after salmonids and carps. Tilapia farming is also one of the fastest growing aquacultures in the world, with the average annual growth rate from 1970 to 2003 at13.6%. Tilapia is widely cultured in around 100 tropical and subtropical countries. The production of farmed tilapia was rapidly increased from 383654 tons in 1990 to worldwide production over 1.5 million tons in 2002, accounted about 6% of the total farmed fish production (Fessehaye, 2006). The production of farmed tilapia tripled from 703,086 tons in 1995 to 2,025,560 tons in 2005 (Fig. 1; FAO, 2007). As a result, tilapia has been described as the most important aquaculture species of the twenty-first century (Fitzsimmons, 2000).

Tilapias are natively from Africa, and usually live in freshwater, but also may survive in brackish and salt water. It has strong ability to adapt the living environment, even

in water with low dissolved oxygen levels (Bai, 2013). Thus, tilapias can survive in a wide range of environments and can tolerate stressful conditions (Rinchard, 2002).

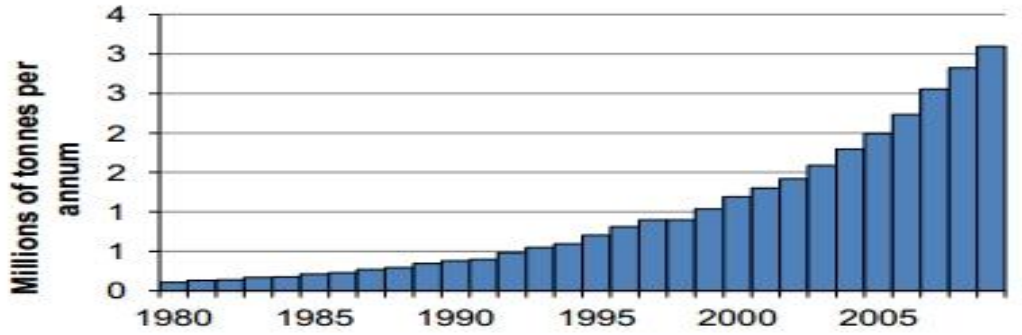

Fig. 1 The total world production of farmed tilapia (Seafish, 2011).

Nile tilapia is commonly known as "African carp" and it is one species of the *Tilapia* genus. In the wild, Nile tilapia can eat a wide variety of foods, such as zooplanktons, algae, and aquatic plants. The farmed tilapias sometimes lack elements which will affect the growth, nutritional value or body composition. Nile tilapias require the same essential amino acids as other fishes and land animals. The quantitative requirements for these essential amino acids are important for growth.

1.3 Plant protein use as feed ingredient in Tilapia feed

Nile tilapia can efficiently utilize fishmeal free feeds (Shiau, 1990; Goda, 2007). Soybean meal is the most popular source of plant protein in compound aquatic feed, and the feeds used for fish species of herbivorous and omnivorous and crustaceans will normally contain 15-45% soybean meal. Soya protein concentrate (SPC) is water- or alcohol-washed defatted soybean. The production process of SPC is presented in Fig. 3. Soybean with high quality is first selected and cleaned. The second step is to dehulled the soybeans and exact the oil. Then the defatted "white flakes" in the residue is used to make the soybean meal. The SPC is then made by extracting the white flakes with aqueous alcohol. This exaction removes the soluble carbohydrates as well as slower the levels of lectins, trypsin inhibitors, glycinin, β-conglycinin and saponins. Phytic acid is, however concentrated in this process, from 1.2 to 1.3% in

soybean meal to 2% or more in SPC (USSEC, 2012). Thus, SPC may be a suitable ingredient for decreasing the negative effects of phytase treatment in feeds for aquatic animals.

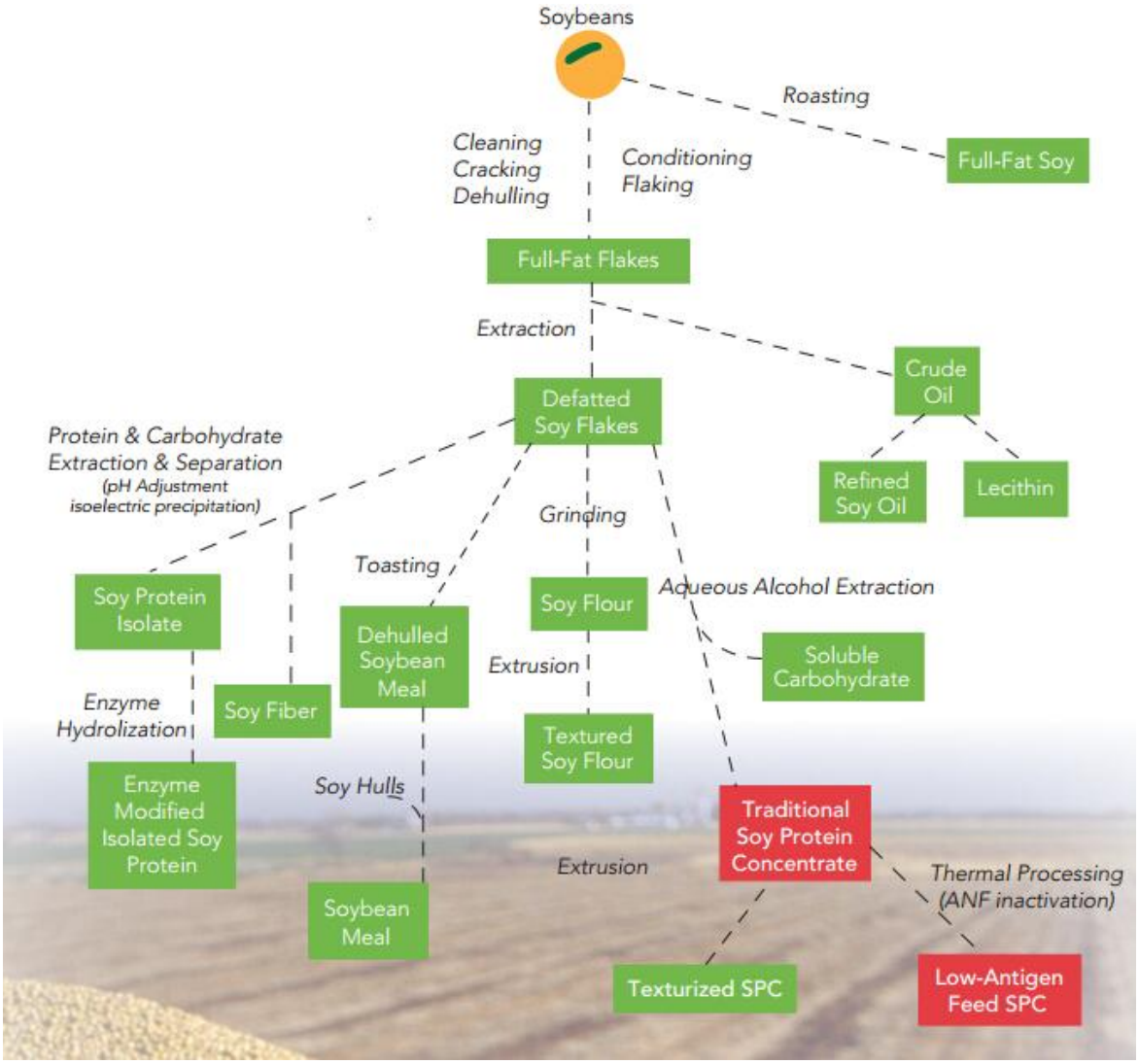

Fig.2 SPC processing (USSEC, 2012)

1.4 Phytate

Recently, using plant protein (such as soybean meal, soy protein concentrate) to replaced fish meal in fish diet is a hot topic in the aquaculture area (Fontainhas, 1999; Mabahinzireki, 2001). The phosphorous concentration in plants or plant products is shown in Table 1. Almost two third of the total phosphorous in plant ingredients formed as phytate-P (Cao, 2008; Frank, 2007). Phosphorus is an important mineral for fish and it is main component of nucleic acids, cell membranes and bones. Low content of P in the natural water in fish has low absorbability from natural water, so fish gets phosphorus mainly from feed (Wu, 2011). The absorption of divalent and trivalent cationic elements such as zinc, magnesium, calcium and iron is negatively affected by the formation of insoluble chelate complexes with phytate (Papatryphon, 1999; [2]Liu, 2009). Phytate, in addition may reduce the utilization efficiency, activity and digestibility of protein and vitamins by reducing the activities of digestive enzymes ([1]Liu, 1998; Nie, 1999).

Table 1 Phytate concentration in plants or plant products (Ling, 2007)

	Total P (g/kg)	Phytate-P (g/kg)	Proportion (%
Cereals			
Wheat grain	3.07	2.19	71.6
Oat	3.60	2.10	59.0
Corn grain	2.62	1.88	71.6
Barley grain	3.21	1.96	61.0
Sorghum grain	3.01	2.18	72.6
Rye	3.05	1.95	63.9
Oilseed meals			
Canola meal	9.72	6.45	66.4
Cottonseed meal	10.02	7.72	77.1
Corn glutton meal	4.24	2.67	63.0
Rapeseed meal	9.60	6.34	66.0
Soybean meal	6.49	3.88	59.9
By-products			
Rice bran	17.82	14.17	79.5
Wheat bran	10.96	8.36	76.3

1.5 Different enzymes as ingredient in fish feed

The main raw materials in tilapia feeds include soybean meal, grains, fish meal, and several low-priced feed ingredients. Through feed processing, Feed producers can increase digestibility and nutritional value, remove some unnecessary impurities, improve the feed intake, and reduce dust and other factors that influence feed hygienic quality. Depending on the different periods of fish growing, the feed will have different requirements, because the digestibility and ability for absorption, as well as needs for protein and energy are different in periods of life of the fish. The optimal usage of the feed is also affected by the water temperature, water flow rate, water environment, bacteria, fungi, plankton production in the rearing system.

There are some anti-nutritional factors in wheat such as arabinoxylans. Soluble arabinoxylans will affect the digestibility and the ability of absorption of nutrients, especially lipid (Nie, 2009). Other water soluble non-starch polysaccharides (NSPs), such β-glucans act in a similar manner, by increasing the viscosity of the gut contents. NSPs are divided into two kinds: water-soluble NSPs and water- insoluble NSPs. Starch usually accounts for 30-40% of the feed for tilapias, while the lipid content is low (usually less than 8%), meaning that NSP's are not commonly used in feed for this species.

1.6 Phytase and utilization in tilapia feed

Ruminants can produce phytase in rumen by phytate hydrolysis but monogastric animals don't have phytase available during digestion (NRC, 1993). Thus, most of phytate-P will excrete in the water, and may give rise to algal pollution in freshwater production system (Frank, 2005; Wyss, 2009). The phytase (myo-inositol hexakisphosphate phosphohydrolase) is a phosphate enzyme that catalyzes the hydrolysis of phytic acid (myo-inositol hexakisphosphate) and releases inorganic phosphorus (Edward, 2000). Phytase hydrolyzed phytate- P one by one and the finial products are free phosphate and inositol, so that reduces the need for inorganic P

supplementation to the feed (Nelson, 1967). The mechanism of action for phytase is shown in Fig.3.

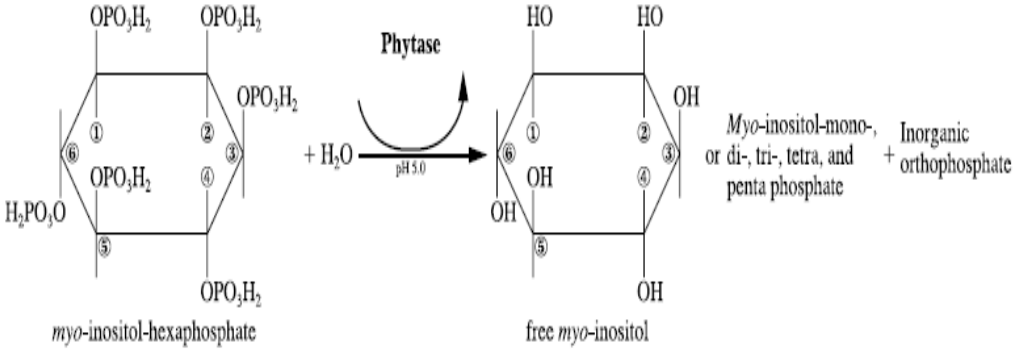

Fig. 3 Mechanism of action for phytase ([1]Liu, 1998)

Phytase activity is influenced by many factors, such as pH, temperature, reaction time, inhibitor and activator. Phytase likes other enzymes has optimum pH value and temperature for the enzymatic action. Generally the optimum pH range is 4.5-6.0 and the optimum temperature range is 45 – 60 ^{0}C. Phytase is sensitive to high temperature and pressure. The optimum temperature of microbial phytase is around 56 ^{0}C. Phytase will loss big part of activity when the temperature is higher than 65^{0}C and completed loss over 80 ^{0}C (Qi, 2004).

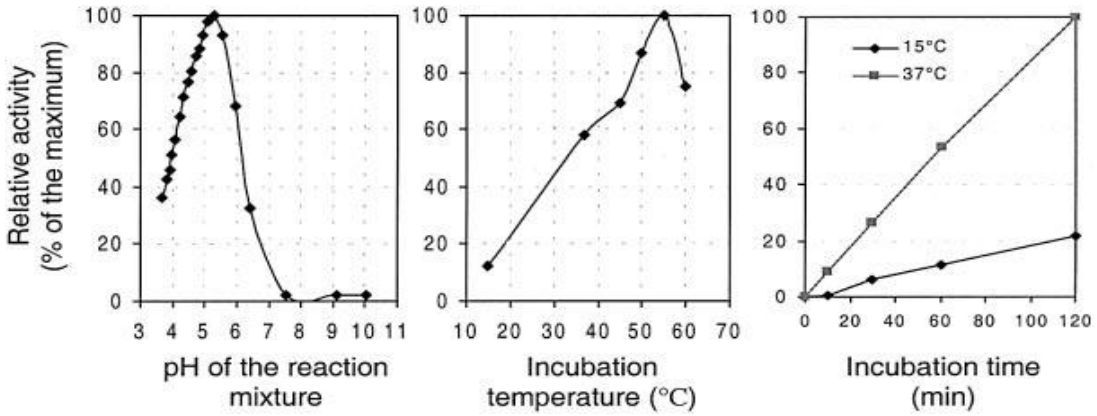

Fig. 4 Relative activity of phytase at pH, incubation temperature and incubation time (Sugiura, 2001).

The Fig. 4 is shows that the relative activity of phytase was increased rapidly fast, the pH of the reaction mixture increased at the same time. The activity was arrived the top at pH 5.3 and then decreased until pH 7.5. The activity of phytase was increased together with incubation temperature increased until at 57 ^{o}C. The high iucubation temperature has higher relative activity of phytase.

Phytase is also influenced by metal cations like Fe^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+} , Al^{3+} and other multivalent ions which can react with phytic acid (complexation), formulation of insoluble complex, reducing the effective concentration of phytic acid, reflecting the reduced activity. In the aquatic feeds, the research of phytase is left behind than the feed of poultry and swine (Wang, 2005).

Recent Researchers have studied phytase application for fish feed focused on optimal condition and addition into feed, and some have studied phytase activity in the different period of same fish. In the dose- response study, phytase addition of 250–1500 U/kg is usually considered feasible in many fish species as shown in the Table 2. The optimum dose effects by many factors such as fish species, different phytase sources, diet formulation (amount of substrate for phytase) and selected response parameters (Cao, 2007).

Table 2 Optimum dose of phytase addition in diets of different fish species (Cao, 2007)

Fish species	Optimam dose of phytase addition (U/kg)
Channel catfish	250–500
African catfish	250–500
Stripped bass	1000
Nile tilapia	500–1500
Crucian carp	500
Common carp	800–1000
Korean rockfish	1000
Pangasius pangasius	500

Nile tilapia needs available P in diet between 5-9 g/kg and young fish need more P than adults, but juvenile tilapia utilization of phytate is lower than adult fish (NRC, 1993; Cao, 2007; Wu, 2011). Tilapia diets are mainly composed of vegetable protein sources, and microbial phytase is an enzyme which is becoming increasingly used as an feed additive into the tilapia feeds, since it improves P availability and reduces P excretion (Frank, 2007). On the other side, high phytase activity in the diet had no significant effect on P utilization in the fish described by Frank (2007).

1.7 The objective of the experiment

The objectives of the experiment include several parts. First is to find the effects of dietary phytase supplementation in soy protein concentrate – base feed for Nile tilapia at growth performance (weight gain, feed intake), utilization of minerals. Second part is to find the effect of phytase working in the different period for Nile tilapia and also to find the difference between phytase with incubation and phytase without incubation fed to different size groups of Nile tilapia.

2. Materials and methods

2.1 Feed formulation

Three diets were formulated in this experiment (Table 3). Diet 1 was made without addition of phytase. Diet 2 had 500 mg phytase added per kg SPC (5000 FYT). Unit of phytase activity is defined as release of one micromole of inorganic phosphate per minutes under certain conditions (pH is 5.5, 37 ^{o}C and substrate concentration is 5.1 mmol/l) (Frank, 2005). Diet 3 was incubated with phytase (500 mg phytase preparation mixed into each kg of SPC) before adding water (60 ^{o}C) and incubating for half hour before mixing with other dry ingredients. Phytase (Ronozymes P5000 (CT)) is supplied by Novozymes from Denmark. Novozymes P5000 (CT) is a fungal phytase extracted from *Peniophora lyci* which is found in dead trees in nature (Nielsen, 2007). According to analysis done by the producer, this phytase preparation contained more than 5000 phytase units (FYT) per g.

Only vitamin premix was used. No additional minerals were supplemented. Neither micro-elements nor calcium phosphate were added, in order to maximize the effects of phytase supplementation on mineral availabilities.

The diets were processed by mixing the ingredients, adding 30% cold tap water to Diets 1 and 2, and same amount of hot water and temperature was kept around 60 ^{o}C for the SPC used in Diet 3. The formulation of the diets is presented in Table 3.

Table 3 Composition of the basal diet for Nile tilapia

Ingredient [a]	g/kg feed
Soy protein concentrate (SPC) [b]	494
Threonine	4.5
Methionine	9
Lysine	4
Tryptophan	1
Taurine	1.45
Gelatinized potato starch	405
Rapeseed oil	50
Mono calcium phosphate (MCP)	10
Vitamin premix [c]	1.5
Yttrium oxide (Y_2O_3)	0.08
Vit-C 35%	0.1
Sodium alginate	20
Total	1000

[a]For details, see Shizari (2014). [b]SPC, The Solae company. Typical protein level: 66.5%. Lysine: 4.2%. Composition proprietary to Ewos Innovation, Sandnes, Norway.

2.2 Experimental diets preparation

The diets were produced in the feed laboratory at IHA, NMBU; 8 kg of each diet was produced. All the ingredients were weighed and then were mixed in the mixer (Moretti Forni Grain, Italy). All the dry ingredients with or without phytase were mixed uniformly, and the liquid ingredients like rapeseed oil and water for Diets 1 and 2 were added slowly while mixing. Mixing continued for at least 10 minutes. For Diet 3 production, dry ingredients mixed uniformly as same as other 2 diets, hot water was added into mixer for incubated phytase for half an hour. The feed mixes were then transferred to a pasta extruder (P55DV, Carasco, Italy), conditioned by 2 rounds of production through the extruder, and were finally shaped into pellets through 3 mm

diameter dies during the third round through the extruder. The pellets were then dried at 55 ^{o}C in a hot air dryer for five hours. After that all diets were cooled to room temperature, packaged in plastic bags and stored frozen at -20 ^{o}C. Before putting into the bags, diet moisture was tested and confirmed to be lower than 5%. Samples of 100 g were taken from each diet for chemical composition analysis.

2.3 Fish, rearing units and feeding

The experiment was conducted at the fish laboratory at NMBU. Three different size groups of fish were used (average initial weights were 27.9 g, 40.2 g and 59.5 g). Each size group was allocated to 3 tanks. Indoor tanks with dimensions 70×50×50 cm and water level of 45cm were used. The Biomass for each tank was approximately 750g. The tanks were supplied with water from a recirculation system.

Water temperature, dissolved oxygen and light were controlled during the experimental period by Monitoring System daily. The water flow rate was between 6 to 8 L/min. 24 hours of light was maintained during the whole experiment. The temperature and pH in each tank (average temperature was 26.8 ^{0}C and pH was 6.9) in the bearable range for the Nile tilapia. The optimum temperature and pH for tilapia is 26 -28 ^{0}C and the pH value is 6.5 to 7 (Bergheim, 2007). After feeding every day, the outlet water pipes were cleaned by the brush so that the solids on the bottom of the tank could be removed with the outlet water. The dissolved oxygen (7.2 mg / l) of the feeding trial was in agreement with as reported by Giovani Sampaio Gonçalves (2005).

The diets were fed 3 times per day at 7:00, 14:00, and 21:00. The feed was offered for 45 minutes at each meal, using automatic feeders. Each diet was fed to triplicate tanks of fish for 28 days. Uneaten feed was collected during feeding from the sieves fitted outside the water outlet of tanks and was also collected after 15 minutes by the end of each feeding time. The water pipes were kept clean to ensure the better water flow by

using the brush in the end of last feeding of the day. The feed for next day was filled up in the feeders. The uneaten feed was dried in the hot air oven at 105 ^{0}C overnight and dried feed was weighed the next morning. The amount of feed for next day was adjusted every day based on the data of uneaten feed.

2.4 Sampling procedures

Before starting the feed experiment, no feed was offered for one day. MSN-222 0.1 g/l of water was used to anesthetize fish before individually weighing them. Five fish were taken from each size group and were kept in the freezer at -20 ^{0}C as initial samples for the chemical analysis of the body composition and nutrient retention analysis. The fish samples for the body composition were first cut down to small pieces and then were mixed in the small mixer with CO_2- ice and were grinded again at high speed for short time and then were weighed 100 g for freeze drying at -56 ^{0}C and 25 mbar for four days. The samples were dried and were fined with the help of pestle and mortar to analyze for the body composition.

Three fish were taken for blood sampling. The blood samples were taken by using anti-coagulated syringes. The needle was inserted under the lateral lines. After encountered spine, the blood sample was collected in tail vein. The blood samples were kept inside two Eppendorff vials per tank. After that, the blood samples were centrifuged in 20 minutes at 3000* G to extracted plasma then the plasma was taken to the plasma analysis.

After taking blood samples, abdominal cavity was dissected from the tail with a scalpel, and feces were carefully collected by gently squeezing with the help of tweezers from the last 10 centimeters of the intestine. The feces were kept in the plastic containers to freeze-dried first and then the samples were taken for analysis of chemical composition of minerals and yttrium oxide. And later the results used to calculate the nutrient retention and apparent digestibility.

2.5 Chemical analysis

Chemical compositions of diets are given in Table 3. Dry matter was determined as mass loss at 105 ^{o}C overnight. The dry matter was then combusted at 550 ^{o}C in muffle furnace, the end product was ash content. The procedure was according to Commission dir. 71/250/EEC. Crude proteins of the diet, feces and fish body were tested by Kjeldahl method (Commission dir. 93/28/EEC). Yttrium oxide and five other minerals (Ca, Mg, Mn, Zn and P) concentration in feed, feces and fish body were determined by inductively coupled plasma mass spectroscopy (ICP-MS). Crude fat was measured by Accelerated Solvent Extractor (ASE 200, Dionex, USA) in the laboratory at IHA-NMBU according to described methods in Commission dir. 98/64/EC.

2.6 Fish growth performance

Following equations were used for the weight gain, feed intake, feed conversion ratio, specific growth rate and specific feeding rate respectively.

Weight gain (WG) = finial body weight (FBW, g) – initial body weight (IBW, g)

Feed intake (FI) = cumulative feed intake (dry matter, g) / Fish amount

Feed conversion ratio (FCR) = feed intake (dry matter, g) / weight gain (wet weight, g)

Specific growth rate (SGR) = 100* {ln [finial body weight (FBW, g)] – ln [initial body weight (IBW, g)]} / feeding days

Specific feeding rate (SFR) = specific growth rate (SGR) * feed conversion ratio (FCR)

Minerals retention = 100* [finial body weight (FBW, g) * mineral weight in finial weight (MFW) –initial body weight (IBW, g) * mineral weight in initial weight (MIW)] / [feed intake (FI) * minerals in feces (MIF)]

2.7 Calculations and Statistical analysis

After finishing 28 days experiment, we took 5 fish from each tank and squeezed the intestine by tweezers to collect the feces from the last 10 cm of the intestines for the digestibility testing. Mineral and yttrium oxide (Y_2O_3) contents were tested from feces and feed.

Following equation was used for, measuring apparent digestibility.

Apparent digestibility= 100 – 100 * [nutrient in feces (%) / nutrient in feed (%) * Yttrium in feed (%) / Yttrium in feces (%)]

The results were analyzed by the GLM procedure in the SAS computer software by a factorial analysis of variance, where factors were diet (1,…,3) and fish size class (1,…,3). The level of significance was chosen at $P< 0.05$, and the results are presented as means ± s.e.m (standard error of the mean).

3. Results and discussion

3.1 Chemical composition of experimental diets

During the 28 days experiment, there was no mortality in all nine tanks. In this experiment, three different feeds were fed to three different sizes of fish.

The chemical composition of experimental diets is shown in Table 4. The content of dry matter, crude protein, crude fat, ash and energy in the three diets were almost same. Also it showed no notable difference between the mineral compositions of all experimental diets. Diet with phytase or without phytase had no notable difference on the chemical composition of experimental diets. Also there were no significant differences between Diet 2 and Diet 3.

Chemical composition	Diet 1	Diet 2	Diet 3
Dry matter, g/kg	907	911	905
Crude protein, g/kg	316.7	316.8	313.6
Crude fat, g/kg	35.8	37.0	36.9
Ash, g/kg	44	44	43
Energy, MJ/kg	17.7	17.8	17.7
Minerals g/kg:			
Calcium	4.2	4.2	4.2
Magnesium	1.9	1.9	1.9
Manganese	0.031	0.031	0.031
Phosphorous	7.3	7.4	7.4
Yttrium oxide	0.064	0.069	0.074
Zinc	0.025	0.024	0.025

Table 4 Chemical composition of experimental diet

3.2 Feed intake and Growth performance

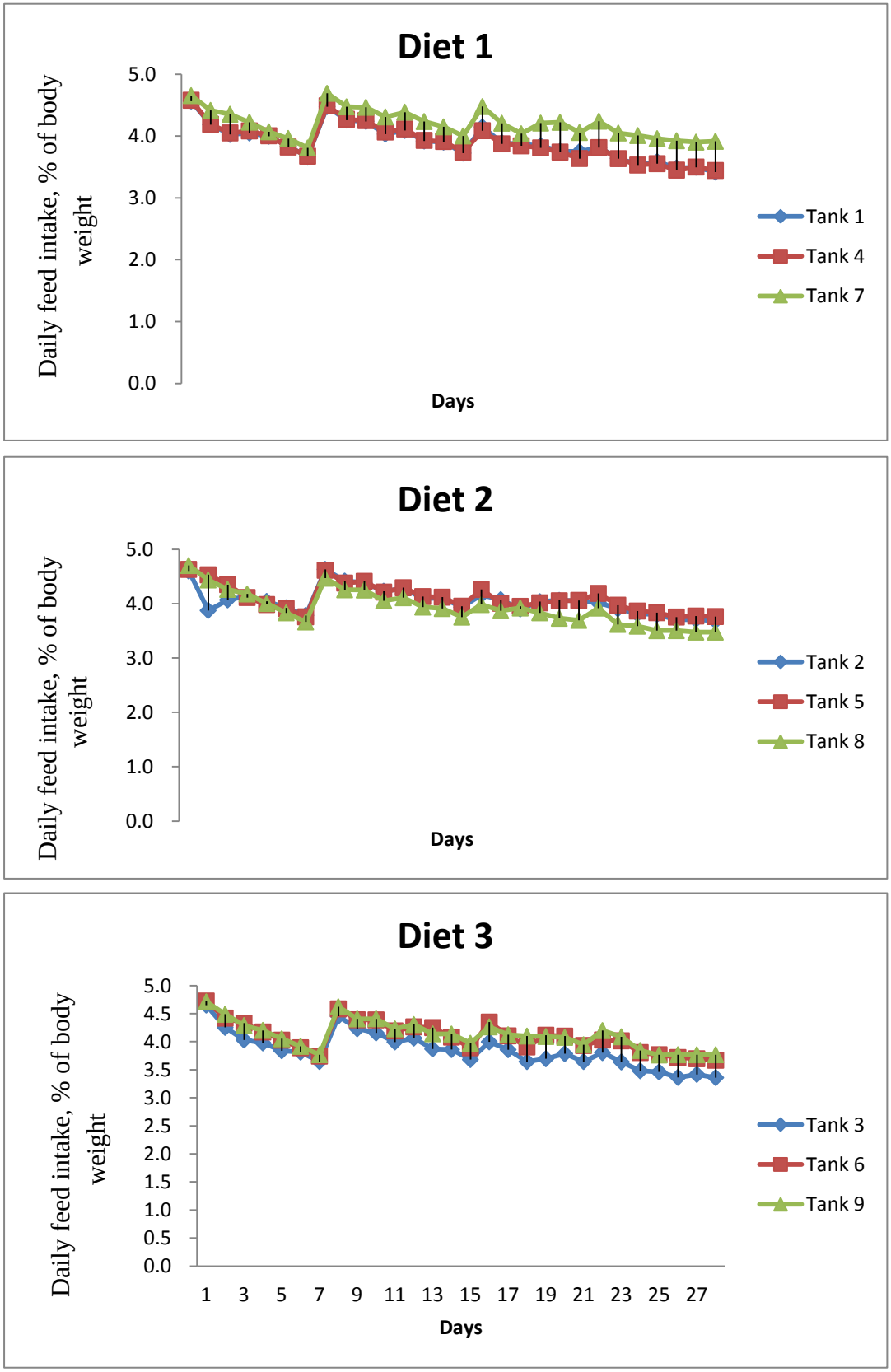

Fig.5 Feed intake % of body weight per day the same diet fed to different period of tilapia.

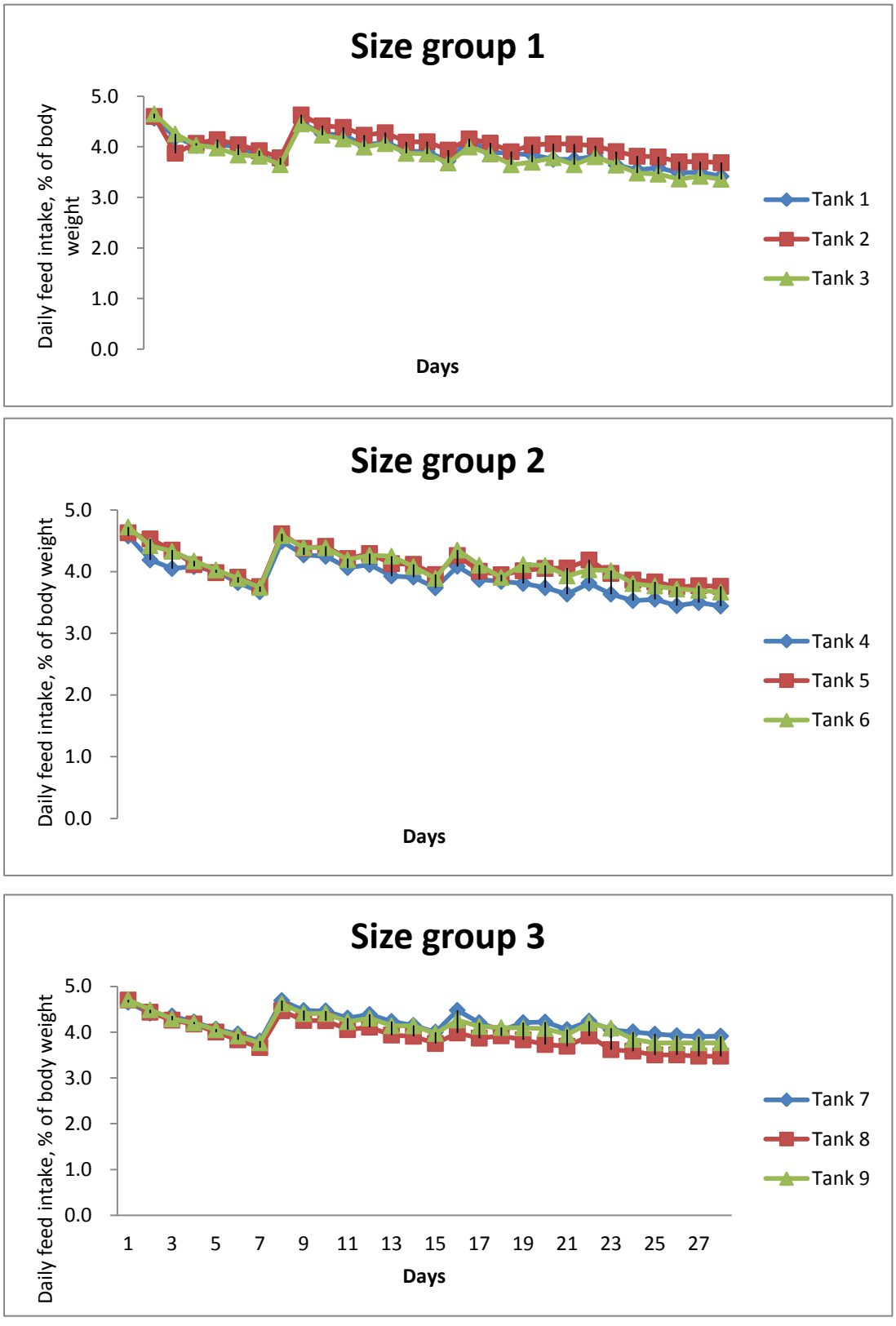

Fig. 6 Feed intake % of body weight per day in Size Group 1, Size Group 2 and Size Group 3 fed with 3 different diets.

From the Fig. 5 and Fig. 6, the feed intake of body weight decreased during the start feeding experiment. After 7 days later, the dry intake rate increased and then decreased slowly until finish of the feeding experiment. The same diet fed to different period of tilapia is shown in Fig. 5. From the Fig. 5, adults' tilapia has higher feed intake than tilapia juveniles. Phytase supplementation in tilapia diets was increased feed intake obviously in the bigger size group. From Fig. 6, incubated phytase has high efficient at the Size group 2 and Size group 3. In the Table 5, FI of Diet 3 was high than Diet 1 and Diet 2 during then experimental period.

Growth performance of the fish is shown in Table 5. The initial weight of the fish ($P<0.05$) and the finial weight fish ($P<0.05$) were significantly different in each size group in Table 6. The fish in 3 different size groups were grew from the initial average weight of 27.9g, 40.2g and 59.5g to the average final weight 73.8g, 121.5g and 175.2g. The ratio of gain was 180.29%, 201.74% and 194.45%. The weight gains of this experiment were not exactly as same as described by the Yu (2000) (phytase addition of 1000 units per kg diet can improve average weight to 25%), but it was also showed that the phytase added in diet improved the weight gain. Gaber (2005) demonstrated that high feed intake lead to high weight gain in juvenile Nile tilapia. It has been conducted to find the effects of various microbial phytase on growth performance of different fish species. Many studies shown that the addition of phytase to low-P diets has enhance growth performance (Cao, 2007). There are no notable differences on growth performance between diets with and without phytase in pond-raised channel catfish (Robinson, 2002). Phytase-pretreated improved FCR in Atlantic salmon (Storebakken, 1998).

Although no significant differences were observed in Feed Intake (FI), Weight Gain (WG), Specific Growth Rate (SGR), Specific feed rate (SFR) and Feed conversion ratio (FCR) among the three groups, the FCR of Diet 3 was higher than other two diets in the Table 5. Weight gain, Specific Growth Rate and Feed intake was lower than the feed without phytase when the phytase loss activity in the feed process. But

Feed conversion ratio was increased by the phytase supplement.

Table 5 Initial and final weight (mean ± SE), weight gain (mean ± SE) and FCR (mean ± SE) of tilapia in the 28-days feeding trial.

	Average start weight, g			P<F	Dietary enzyme treatment			P<F	Pooled
	21.4-37.4 g	39.5-41.7 g	53.6-62.5		None	Added	Incubated		s.e.m.
initialweight, g	27.9	40.2	59.5	0.0114	41.1	44.2	42.3	0.86	4.9
Final weight, g	73.8	121.5	175.2	<0.0001	122.9	120.7	126.9	0.50	14.7
Gain, g	50.3	81.1	115.7	0.0002	81.8	80.7	84.6	0.64	9.5
SGR	3.95	3.86	3.92	0.72	3.93	3.82	3.98	0.96	0.06
Feed intake, g DM	48.4	74.2	122.7	0.079	84.6	82.0	87.6	0.57	4.8
FCR g DM (g gain)$^{-1}$	0.92	0.97	1.00	0.54	0.975	0.990	0.999	0.97	0.03

3.3 Body composition

The minerals of the fish body composition are shown in Table 6. In the Table 6, Fe ($P<0.05$) was significantly different probably because of the fish size difference. Other minerals were not significantly different with the difference in fish size. The differences of minerals in the body composition are not really significant. But the P, Fe and Ca content in the fish which were fed Diet 3 was low than other two groups of fish. It means incubation of phytase reduced the minerals content in fish body, increased availability and digestibility of minerals. Pfeffer (1995) reported that utilization of P can increased by feeding 1000 U/kg phytase offered in soybean-base diet to rainbow trout. Pretreated phytase improved the availability of minerals in Nile tilapia.

Table 6.The body composition of the experimental fish with the different sizes and different diets

	Average start weight, g			P<f	Dietary enzyme treatment			P<f	Pooled
	21.4-37.4 g	39.5-41.7 g	53.6-62.5		None	Added	Incubated		s.e.m.
Ca	130.0	143.33	140.00	0.2689	136.67	143.33	133.33	0.4444	3.24
Mg	27.67	24.33	22.00	0.1010	25.33	27.00	21.67	0.1133	1.27
Mn	0.01	0.01	0.01	0.0735	0.01	0.01	0.01	0.1159	0.00
P	356.67	326.67	276.67	0.1000	330.00	336.67	293.33	0.3378	15.63
Zn	35.33	38.33	37.00	0.1184	38.67	36.00	36.00	0.1111	0.70

Fe	5.30	2.53	2.13	0.0308	3.13	3.87	2.97	0.5389	0.57

3.4 Mineral retention

The mineral retention of the fish body is shown in Table 7. In this table, the retention of Zn ($P<0.05$) in the Diet 3 was significantly different with other 2 diets. The retention of Ca ($P<0.05$), Mg ($P<0.05$), Mn ($P<0.05$), P ($P<0.05$) and Zn ($P<0.05$) were seen with notably varying in three different sizes in table 8. The minerals retention from the incubated phytase group was higher than the other 2 group. Phytase was helpful for the mineral retention and improved the availability of minerals in Nile tilapia. The incubated phytase was more effective than without incubated phytase in retention of minerals. Phytase pretreated in tilapia diet can increase the availability of minerals and improve mineral retention (Frank, 2005). Increasing the retention of minerals in fish body while reduce minerals excreted to water environment especially P which is lead to water pollution.

Table 7 Mineral retention of the body composition

	Average start weight, g			P<f	Dietary enzyme treatment			P<f	Pooled
	21.4-37.4 g	39.5-41.7 g	53.6-62.5		None	Added	Incubated		s.e.m.
Ca ret	121.33	138.07	261.3	0.0009	160.7	159.1	200.9	0.0613	23.43
Mg ret	10.9	13.43	24.33	0.0029	16.03	14.87	17.77	0.3325	2.16
Mn ret	1.17	1.03	2.83	0.0390	1.47	1.43	2.13	0.3763	0.34
P ret	50.0	58.7	105.27	0.0012	67.73	65.5	80.73	0.1033	9.05
Zn	69.43	71.0	136.33	0.000	85.5	86.5	104.7	0.045	11.57

ret				4	7			3	

Table 8 The Ca, P and Zn retention of the body composition with different diet

Least Squares Means for effect Diet Pr>｜t｜ for H_0: LS Means (i)= LS Means (j) Dependent Variable: Ca ret			
i/j	1	2	3
1		0.9119	0.0416
2	0.9119		0.0370
3	0.0416	0.0370	
Least Squares Means for effect Diet Pr>｜t｜ for H_0: LS Means (i)= LS Means (j) Dependent Variable: Zn ret			
i/j	1	2	3
1		0.8759	0.0270
2	0.8759		0.0315
3	0.0270	0.0315	
Least Squares Means for effect Diet Pr>｜t｜ for H_0: LS Means (i)= LS Means (j) Dependent Variable: P ret			
i/j	1	2	3
1		0.7133	0.0833
2	0.7133		0.0546
3	0.0833	0.0546	

3.5Apparent digestibility of the minerals

From the Table 9, the apparent digestibility of the minerals in the 3 different diets was not significantly different with each other (P>0.05). Apparent digestibility of P, Ca, Mg and Mn in the Diet 3 was higher than other 2 diets. Apparent digestibility of Zn in the Diet 3 was lower than other 2 diet. With addition of the phytase, the apparent digestibility of Zn was decreased. Phytase did not influenced absorption of Mg, Mn or Zn. Phytase influenced the apparent digestibility of minerals. Increasing dietary P and Ca levels can reduce Mg, Mn and Zn availability in fish (Hardy and Shearer, 1985; Satoh, 1992). Phytase addition in rainbow trout in feed with soybean as a main ingredient may improve the apparent digestibility of P, Ca, Mg, Mn, Sn and Zn. (Sigiura, 2001). The apparent digestibility of Ca, crude protein and P were enhanced by microbial phytase offered in diets (Frank, 2007). Similarly, phytase supplementation in the diets improved the apparent digestibility of Mg, total P, Mn and Zn in the fish body (Huang, 2010).

Table 9 Apparent digestibility of the minerals

	Dietary enzyme treatment			P>f	Pooled
	None	Added	Incubated		s.e.m.
AD Ca	-47.27	-32.99	-30.83	0.6823	7.46
AD Mg	55.60	57.18	73.95	0.2228	4.68
AD Mn	-57.96	-56.68	-48.71	0.5666	3.48
AD P	51.04	51.74	53.54	0.5745	0.91
AD Zn	-663.42	-762.59	-817.12	0.4335	45.62

3.6 The content of mineral in plasma

The content of mineral in plasma is shown in Table 10. At the same size group, phytase incubation has lowest mineral content in the plasma, except in Size group 2. Phytase supplementation increased the composition of minerals like Mg, Ca, Mn and Zn in plasma and whole body (Cao, 2007). Phytase increased the P retention in the tilapia body increased utilization rate of P and decreased content of P in the water which was excreted with feces (Cao, 2008).

Table 10 Plasma mineral compositions (mg l^{-1})

	Average start weight, g			P<f	Dietary enzyme treatment			P<f	Pooled
	21.4-37.4 g	39.5-41.7 g	53.6-62.5 g		None	Added	Incubated		s.e.m.
Ca	130	143	140	0.27	137	143	133	0.44	3.2
Mg	27.6	24.3	22.0	0.10	25.3	27.0	21.7	0.11	1.3
P	370	327	277	0.10	330	337	293	0.34	15.6
Zn	35.3	38.3	37.0	0.11	38.7	36.0	36.0	0.12	0.7

Table 11 The content of mineral in feces

	Mg g/kg	P g/kg	Ca g/kg	Mn g/kg	Zn g/kg
Tank 1	0.33	1.55	2.22	0.020	0.070
Tank 2	0.23	1.50	1.95	0.018	0.082

Tank 3	0.28	1.30	2.09	0.019	0.078
Tank 4	0.40	1.46	3.42	0.020	0.072
Tank 5	0.40	1.50	2.57	0.022	0.084
Tank 6	0.27	1.40	2.06	0.021	0.10
Tank 7	0.26	1.43	1.68	0.017	0.079
Tank 8	0.28	1.24	1.78	0.016	0.066
Tank 9	0.34	1.18	2.24	0.015	0.078

3.7 Factors affecting phytase activity

The phytase commercially available has different availability, so that the optimal amount of phytase used in the feed of tilapia is not exactly determined. 2500 U of microbial phytase was added in per kilogram of feed in this experiment. Growth performance, protein digestibility and mineral availability improved by added microbial phytase between 500 and 1500 U/kg feed of tilapia (Furuya, 2001). Microbial phytase offered 1000 U/kg at tilapia feed also improved the growth performance and mineral availability (Frank, 2005). Phytase supplementation from 1000 to 2000 FTU/kg in diets improved growth performance more efficiently than other levels in tilapia (Furuya, 2001).

Phytase is sensitive to temperature and pH value and the temperature in the feed processing is affected by the activity of phytase. Most phytase lost activity when the temperature was over than 60 ^{o}C. The purified phytase lost 60% activity by treating at 68 ^{o}C (Huang, 2010). Phytate-P is digested and absorbed at the end of small intestine (Frank, 2007). Organic acid added in the diets can enhance the activity of microbial phytase because most microbial phytase have highest activity at acidic pH (Cao 2007). The different methods of phytase supplementation affect growth performance. Phytase sprayed onto the surface of soy protein-base at 0, 500, 1500 or 4500 U/kg diet not enhanced growth performance in rainbow trout (Forster, 1999). Oilva-Teles (1998) reported that phytase at 2000 U/kg added in the diet before pelleting has not affected

growth performance and P utilization. Phytase pretreatment might avoid activity loss (Lei, 2000).

In this study, the utilization of minerals increased with growing fish. The bigger fish need more nutrients and energy to maintain their growth. The digestibility, metabolism and absorption ability improved with increase in growth. The length of digestive tract also affected the utilization of minerals in different size groups of fish perhaps because of long stay of digesta in the digestive tract. The incubated phytase significantly improved the retention of Zn and also increased other minerals retention slightly. The phytase addition in the Diet 2 may have been affected due to the high temperature in the drying process of feed. It could be the possible reason of not having a huge impact in the mineral retention. It may have been that the recirculation system produced sufficient phytase for hydrolysis of phytic acid in the SPC and so that the fish may have got this by drinking the water from the tank, or by eating the biofilm.

The negative values for apparent digestibility of minerals have been observed in Table 9. This is probably due to excess minerals in the water, taken up by the fish. The ratio between Ca and P was also affected the digestibility of minerals in Nile tilapia. The levels of total Ca and P, Ca and P ratio in the diets may have been also affected by the activity of phytase in the fish. High level of Ca leads to increased pH value in intestine and formed phytate-Ca or Ca-Phytate-protein complexes, reducing the activity of phytase (Huang, 2010).

4. Conclusion

In this study, phytase gave positive effect on feed intake, specific growth rate, and feed conversion ratio and weight gain of the Nile Tilapia. Phytase pretreatment may improve retentions of minerals in blood, bone and body of tilapia, increase utilization and absorption of minerals for tilapia. The different size of fish has affected utilization of minerals for Nile tilapia more than diet difference, indicating that there was naturally occurring phytase in the rearing water. Temperature, pH value, adding method of phytase also affected the utilization of minerals in Nile Tilapia. Optimum dose of phytase addition in diets of Nile tilapia and how to increase activity of phytase in fish body need deeply research in the future.

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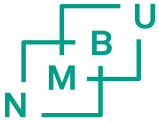

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